Clinical Pediatric Endocrinology

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Foreword

It is an honour to write this foreword. *Clinical Pediatric Endocrinology*, first edited solely by Charles Brook, has deservedly acquired a reputation as an essential textbook for everyone concerned with the care of children and adolescents with endocrine disease. The fifth edition, which Charles Brook co-edits with Peter Clayton and Rosalind Brown, maintains the very high standard of previous editions and also presents what I believe to be the most authoritative account of the scientific and clinical 'state of the art' related to pediatric endocrinology.

The book opens with three outstanding chapters on the scientific basis of current clinical practice. These are up-to-date, readable, and extremely informative. The description of disorders in the broad field of pediatric endocrinology are well organized and the international contributors are universally recognized for expertise in their respective areas. Again, there is attention to clear presentation and readability, the hallmarks of good writing and editorial rigor.

In particular, I was impressed by the accounts of topics of rapidly evolving scientific interest such as the development of the reproductive systems, fetal programing, and monogenic obesity, all now clearly embraced by the field of pediatric endocrinology.

This publication does not rely on its previous reputation, but enhances it as a modern textbook of exceptional quality. Despite the availability of a massive amount of online information, a detailed textbook, with the clear aim of contributing to the care of the child with endocrine disease, offers a far more useful source of authoritative information. The fifth edition of *Clinical Pediatric Endocrinology* emphatically achieves this aim.

Martin O. Savage

Preface to the fifth edition

Looking back over the quarter century since I embarked on the first edition of this book, I am impressed more by what has not changed in the clinical practice of pediatric endocrinology than by the advances. Clinical assessment remains the core function and, although the advances in molecular biology have illumined some of the mechanisms of disease, their clinical applicability is limited. Perhaps genomics and proteomics will serve us better. Some diagnostic and therapeutic advances, for example the hypothalamic peptides, have changed clinical practice, but we still debate the uses and abuses of steroids, thyroxine, and growth hormone.

The book has once again been almost entirely rewritten with the intention of reflecting the themes of 2005, and my co-editors and I have been served very well by the contributors. The choice of whom to invite was based on the wise advice of Rosalind Brown and Peter Clayton and it was due to their input that only one author actually failed to deliver – 'twas ever thus. These co-editors oversaw the science in the book but I take entire responsibility for the errors of commission and omission in the editing of the text.

It is a pleasure to acknowledge my continuing friendship with Blackwell Publishing, this time represented by Alison Brown, but the quantum leap in book production is due to Gillian Whytock of Prepress Projects Ltd. Gillian's charm, patience, and courtesy have been a continual comfort to all involved in completing the project.

It is fitting that the book will be launched at another joint meeting of the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. It is to celebrate the contribution of Martin Savage to the former that we have invited him to write the foreword on this occasion. It is a matter of great pleasure that other pediatric endocrine societies now join our meetings to make them truly a world endeavor to bring endocrinology to its proper place in the care of children.

C.G.D. Brook

Preface to the first edition

Endocrine problems are not uncommon in paediatric practice and are mostly, *faute de mieux*, rather badly managed by non-specialists. This is especially true in England, where paediatric specialities are relatively newly defined. The same is certainly less true of Europe as a whole and this book has its origins in the friendship of the European Society for Paediatric Endocrinology, which has acted as a focus for the subject and which benefits greatly from its transatlantic corresponding members. If the book were to have a dedication, it would be to the health of the Society, coupled with a toast to its American counterpart, the Lawson Wilkins Society.

I hope that the book will be of service to general paediatric departments and of help and interest to departments of (adult) endocrinology in their dealings with patients who are still growing. In a book of this size, there may well be sins of omission and commission and for these I alone can take responsibility and I apologize for them in advance. If any readers were to take the trouble to let me know about such sins for future reference, I would be very grateful.

In the completion of my editorial task I have been greatly assisted by Miss Lynette Napper and Mrs Sue Shorvon, my secretaries at The Middlesex Hospital, and by Mr Jony Russell and Mr Peter Saugman at Blackwell Scientific Publications. My co-authors and I thank them for assisting at the birth of our work.

C.G.D. Brook

Clinical Pediatric Endocrinology, Fifth Edition
Edited by Charles G.D. Brook, Peter E. Clayton, Rosalind S. Brown

1 Principles of hormone action

Melissa Westwood

Introduction

Hormones elicit their effects on target cell function by interacting with specific receptors. These are located either at the cell surface or within intracellular compartments, such as the cytoplasm and nucleus. Receptor location, which forms the basis of their classification into subgroups (Fig. 1.1), reflects ligand characteristics. Receptors for hydrophilic hormones, such as the pituitary-derived proteins, insulin, and the catecholamines, are present at the plasma membrane; lipid-soluble steroid and thyroid hormones cross this barrier to access intracellular binding sites.

Receptors are specific and usually have a high affinity for their particular ligand, but the forces involved in ligand/receptor binding (ionic attractions, van der Waal's forces, hydrogen bonding, or hydrophobic interactions) are weak. The reaction is therefore reversible, and the receptor can be reused. K_d values, the concentration of ligand at which half the receptors are occupied, approximate the physiological concentration of the hormone (usually ranging between pico- and nanomolar concentrations), so that the receptor is sensitive to changes in hormone concentrations.

The concentration of each receptor can vary, and a cell may become more or less sensitive to a given extracellular concentration of ligand. Sensitization can occur by increasing the number of binding sites available. This is achieved through a combination of increased receptor synthesis and decreased degradation. Cells can become refractory (desensitized) to ligand by altering receptor localization (e.g. by internalizing cell surface receptors), reducing receptor levels, or recruiting molecules that deactivate intracellular signaling pathways. Internalization of cell-surface receptors involves endocytosis: the receptors relocate into clusters within the membrane, which then invaginates to form first a pit and then an endosomic vesicle. Once part of an endosome, the signal is terminated because the receptor/ligand complex dissociates as a result of the acidic pH within this compartment. Degradation usually involves the ubiquitin–proteosome pathway.

Cell-surface receptors

There are two major groups of cell-surface receptors linked to intracellular signals. The first relies upon tyrosine kinase for the initiation of signaling. The second group tends to activate serine or threonine kinases by coupling to G-proteins. However, there is an underlying structural unity in all cell-surface receptors because each is made up of three segments, an extracellular domain, a transmembrane region, and a cytoplasmic domain (Fig. 1.2).

The N-terminus of the protein forms the extracellular component of the receptor, and this domain is responsible for hormone recognition and binding. The extracellular domain is heavily glycosylated and comparatively rich in cysteine residues. These are necessary for disulfide bond formation and correct protein folding, but the functional significance of the oligosaccharide moieties is not known. The extracellular domain of some receptors [e.g. the growth hormone receptor and the receptor for thyroid-stimulating hormone (TSH)] can be cleaved from the plasma membrane so that it forms a separate entity that can be detected in the circulation, where it may function as hormone-binding proteins (see below).

The transmembrane region varies in structure from a simple linear stretch of approximately 25 hydrophobic amino acids to a more complex arrangement that threads the plasma membrane crossing it seven times. This segment of the receptor is often regarded as a passive lipid anchor, but there is evidence to suggest that it can influence receptor function as, for example, mutations in the transmembrane region of the fibroblast growth factor (FGF) receptor are associated with achondroplasia.

The cytoplasmic C-terminus of the receptor generally forms the effector region of the molecule because it initiates an intracellular signaling cascade, often involving protein phosphorylation, that eventually results in the cellular response.

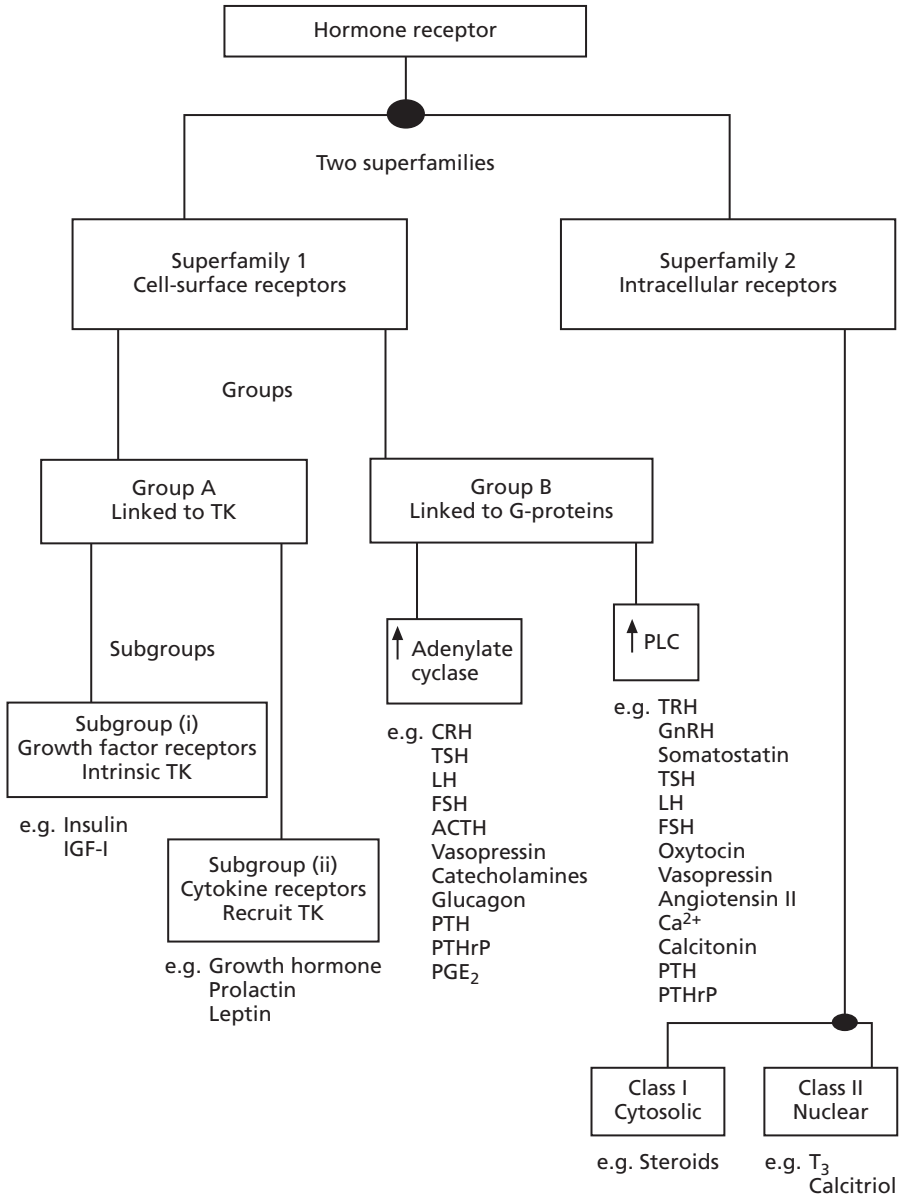

Fig. 1.1. A composite diagram showing the different classes of hormone receptors. Receptors for some hormones can occur in more than one grouping. For example, different types of PTH receptors link to different G-proteins and therefore couple to either adenylate cyclase or phospholipase C (PLC). TK, tyrosine kinase.

Protein phosphorylation

The amino acids serine, threonine, and tyrosine each carry a polar hydroxyl group that can be exchanged for a phosphate group from adenosine triphosphate (ATP) by enzymes collectively referred to as protein kinases. The energy generated during this reaction leads to a conformational change in the tertiary structure of the phosphorylated protein and, once activated in this way, many molecules within signaling pathways relay the signal by acting as protein kinases themselves. Each protein may be phosphorylated on more than one residue, and a protein may be the substrate for more than one kinase, in many cases allowing the convergence of several signaling molecules. The target sequence of most kinases has been identified, although the presence of a consensus motif within a protein's primary sequence does not mean that the protein will automatically be phosphorylated as the tertiary structure may prevent kinase access.

Kinases are grouped according to which amino acid they target. Serine/threonine kinases account for approximately 350 of the known phosphorylating enzymes and are responsible for the majority of the 10% of proteins that are phosphorylated at any given time in a mammalian cell.

Tyrosine kinases account for only 0.05% of the phosphoamino acid content of a cell, but they are key regulators of many cellular signaling pathways. In addition to protein activation, phosphorylation of tyrosine residues generates binding sites necessary for subsequent protein–protein interactions.

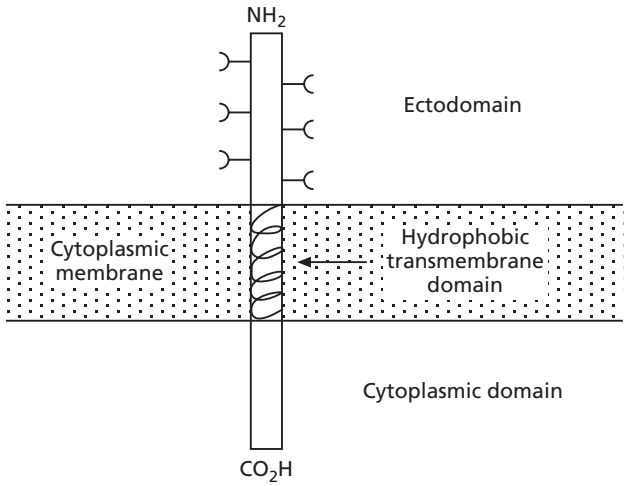

Fig. 1.2. Schematic representation of a membrane-spanning cell-surface receptor with three clearly identifiable domains: the extracellular domain is bridged by a membrane-spanning component to the intracellular cytoplasmic domain. Each domain has characteristic structural features that reflect its location and function.

The relatively long side-chain of phosphotyrosine enables it to dock with proteins containing "deep pockets" resulting from the presence of one or more consensus sequences (approximately 100 amino acids) known as the Src homology (SH2 or SH3) domain. Phosphorylation is a reversible process, and this molecular switch can be rapidly overturned through the action of enzymes termed phosphatases.

Tyrosine kinase-linked cell-surface receptors

These have a relatively simple transmembrane domain and either possess intrinsic tyrosine kinase activity or recruit such enzymes after activation by ligand binding.

Receptors with intrinsic tyrosine kinase activity

This family contains the insulin receptor, the structurally related type 1 insulin-like growth factor (IGF) receptor, and the receptors for epidermal growth factor (EGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF). They are often referred to as growth factor receptors.

The structure of these receptors is shown in Figure 1.2. Some ligands, for example FGF and EGF, stimulate dimerization of two adjacent monomeric receptors, whereas others, for example insulin and IGF-I, bind to receptors that exist as dimers in their unoccupied state. Either way, ligand/receptor coupling results in activation of the tyrosine kinase located in the cytosolic domain of the receptor.

Insulin/type 1 IGF receptors

Both receptors are heterotetrameric structures comprising two extracellular α-subunits linked to two membrane-spanning β-subunits by disulfide bonds [1,2] (Fig. 1.3). The α-subunits confer specificity for their cognate ligand, whereas the β-subunits possess the motifs required for recruiting the major signaling adaptor proteins and a tyrosine kinase domain, which is essential for the catalytic activity of the receptor. As a result of the considerable homology between the insulin and type 1 IGF receptors, cells expressing both can form a hybrid of an insulin αβ-hemireceptor coupled to an IGF-I αβ-hemireceptor [3]. The functional significance of this phenomenon has yet to be determined.

Insulin signaling pathways

Following autophosphorylation of tyrosine residues on the receptor β-subunit, a cascade involving more than 50 enzymes is activated; this includes primarily members of the insulin receptor substrate (IRS) family of proteins [4] (Fig. 1.3). Four mammalian IRS proteins have been identified, and evidence from transgenic mice suggests that they may display tissue and functional specificity [5], as IRS-1 seems to be important for somatic cell growth and insulin action in muscle and adipose tissue, whereas IRS-2 appears to be the main signaling molecule in the liver and is necessary for β-cell survival. Phosphorylation of IRS creates docking sites for proteins with Src homology 2 (SH2) domains. These include the regulatory (p85) subunit of phosphatidylinositol (PI) 3-kinase and growth factor receptor-binding protein 2 (Grb2). The effect of insulin depends on which of these effector molecules are expressed and recruited and which signaling pathways are activated as a result.

Stimulation of PI 3-kinase leads to the generation of PI 3-phosphate, by phosphorylation of phosphatidylinositol lipids at the D-3 position of the inositol ring and then activation of PI 3-dependent kinases (PDK) [6]. PDKs activate protein kinase B (PKB; also known as Akt) via phosphorylation of a critical serine and threonine residue, which results in the translocation of glucose transporters, predominantly GLUT-4, to the plasma membrane and the initiation of glycogen synthesis through activation of glycogen synthase (Fig. 1.3).

The mitogenic effects of insulin are mediated via Grb2. This adaptor protein links tyrosine-phosphorylated receptors or cytoplasmic tyrosine kinases to the guanine nucleotide exchange factor, SOS (son of sevenless protein) and, along with Ras, Raf, and MEK, is part of the pathway that leads to the activation of mitogen-activated protein kinase (MAPK; Fig. 1.3) [7]. This acts on multiple proteins to result in cytoplasmic and nuclear responses. The latter lead to stimulation of gene expression, protein synthesis, and cell growth. The MAPK pathway can be stimulated independently of IRS, because Shc, which is a substrate of the activated insulin receptor, can also associate with Grb2 (Fig. 1.3) [7].

IGF-I signaling pathways

Insulin and IGF-I have overlapping roles in metabolism, cell growth, differentiation, and cell survival, and their receptors

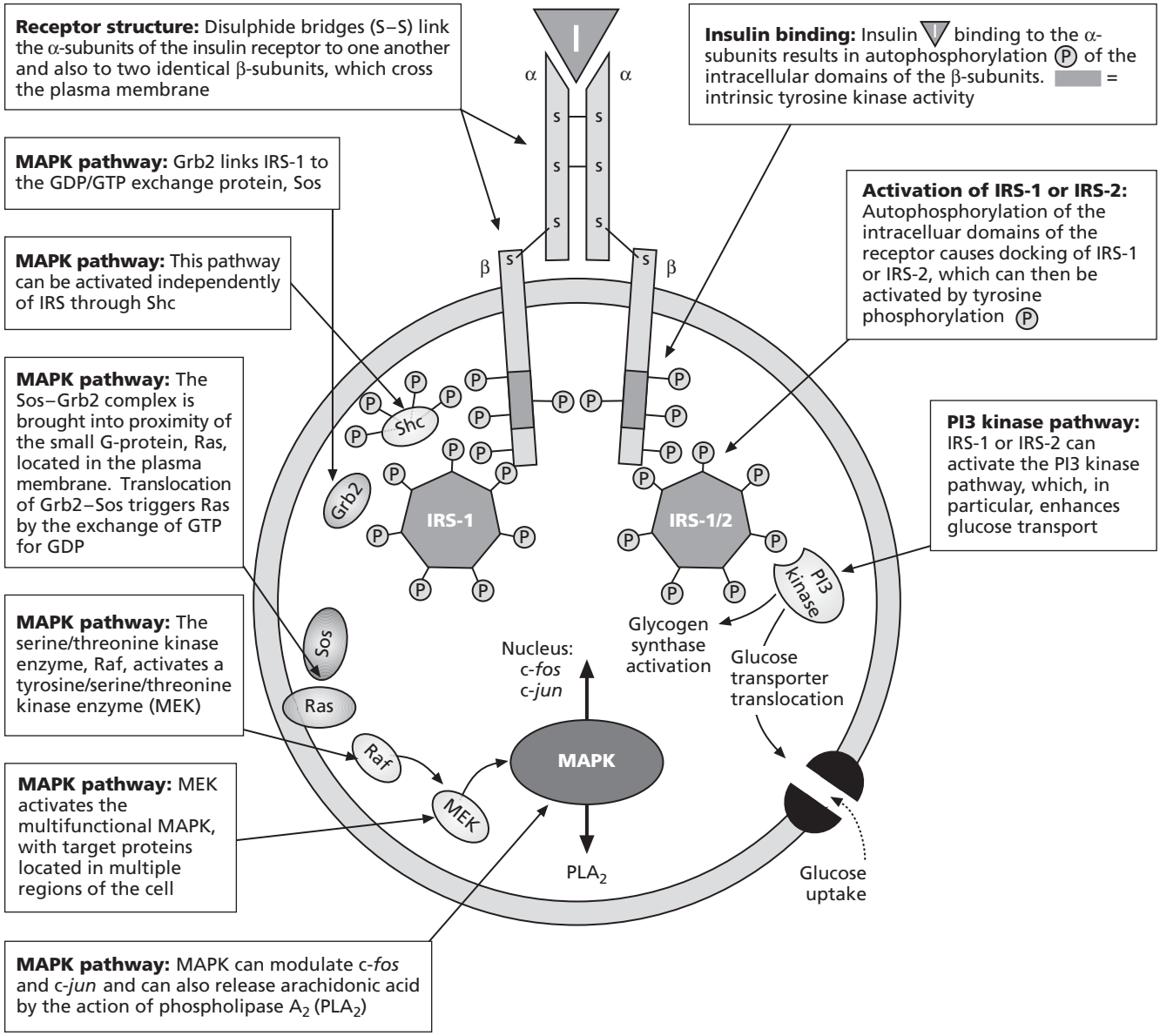

Fig. 1.3. Signaling pathways initiated in response to activation of the insulin receptor, an example of a receptor with intrinsic tyrosine kinase activity. The type 1 IGF receptor shares many of these pathways.

have structural similarity, so it is not surprising that activation of the type 1 IGF receptor activates many of the intracellular signaling events described above. IRS proteins, Grb2, and Shc are all involved in the cellular response to IGF-I (Fig. 1.3) [8]. However, it is thought that the two receptors can elicit distinct biological responses by using specific or preferential substrates, adaptor molecules, or signaling pathways. For example, through PI 3-kinase and generation of PI 3-phosphate, insulin activates protein kinase C (PKC) to stimulate proliferation of murine keratinocytes, whereas PKC is not involved in the proliferative response to IGF-I in these cells [9].

Desensitization

Protein tyrosine phosphatases (PTPs) play a key role in terminating the signal generated through the insulin or type 1 IGF receptor. This family of enzymes includes PTPα, SHP2, LAR, and PTP1B, but current evidence favors the last, particularly with regard to the negative regulation of insulin signaling pathways [10]. PTP1B *in vitro* dephosphorylates activated insulin receptors, IRS proteins, and possibly other downstream molecules as well. PTP1B-deficient mice display enhanced insulin sensitivity and increased insulin-stimulated phosphorylation of the insulin receptor in muscle and glucose. PTP1B gene variants in humans are associated with changes

in insulin sensitivity, which has prompted an interest in developing specific PTP1B inhibitors for the treatment of type 2 diabetes. Such compounds may also prove to be useful in cancer therapy as inappropriate activation of the type 1 IGF receptor has been linked to cellular transformation [11].

Defects

Mutations in the gene coding for the insulin receptor are associated with syndromes of severe insulin resistance, namely type A insulin resistance, Rabson–Mendenhall syndrome, and leprechaunism. All have impaired glucose metabolism in association with raised insulin levels, but only patients with leprechaunism completely lack functional insulin receptors, and they rarely survive beyond the first year of life [5]. Some patients with type A insulin resistance are reported to have normal insulin receptors, and these may harbor as yet unidentified mutations in any of other of the critical insulin signaling molecules described above. Mice deficient in the gene for IRS-1 display marked pre- and postnatal growth failure, insulin resistance, impaired glucose tolerance, and other features of the metabolic syndrome, but they do not develop diabetes, unlike the IRS-2 knockout animals [5]. This has led to the suggestion that IRS-2 is a diabetes-predisposing gene, although this has not been substantiated by clinical studies.

There have been no reports of humans completely lacking the gene for the type 1 IGF receptor, but knockout mice show severe growth failure, widespread developmental defects, and usually die at birth as a result of respiratory failure [12]. A recent study of children with intrauterine and/or postnatal growth restriction revealed a number of mutations associated with reduced receptor number and function [13].

Receptors that recruit tyrosine kinase activity

This group of receptors is referred to as the cytokine receptor superfamily. From an endocrine perspective, the most important members are the receptors for growth hormone (GH) and prolactin (PRL), which form the class 1 subgroup along with the receptors for erythropoietin, granulocyte–macrophage colony-stimulating factor (GM-CSF), leptin, and the interleukins (IL) 2–7, IL-9, IL-11, and IL-12 [14].

Like the tyrosine kinase receptors, cytokine receptors are expressed at the cell surface and are composed of a ligand-binding extracellular domain, a transmembrane region, and an intracellular carboxy tail (Fig. 1.2). Here the similarity ends, because members of the cytokine receptor superfamily do not possess enzymatic activity in their cytoplasmic domain. Instead, these receptors couple physically and functionally with non-receptor tyrosine kinases.

GH receptor activation

Early crystallographic studies revealed that ligand and receptor exist in a complex of one GH molecule and two molecules of receptor [15]. Subsequent work involving mutational analysis of residues within the ligand demonstrated that each molecule of GH has two distinct sites, a high-affinity "site 1" and a lower affinity "site 2," both of which are capable of binding to the extracellular domain of a GH receptor. The unoccupied receptor has not been crystallized, and it is not known whether dimerization occurs before or after GH binding, although there is evidence to suggest the latter. Hence, it is thought that GH initially binds to GHR via site 1 and that this then facilitates site 2 binding with a second receptor molecule (Fig. 1.4) [16]. Such homodimerization has also been reported for PRL [17] and erythropoietin [18], but members of the other subgroups of the cytokine receptor superfamily form heterodimers and oligomers.

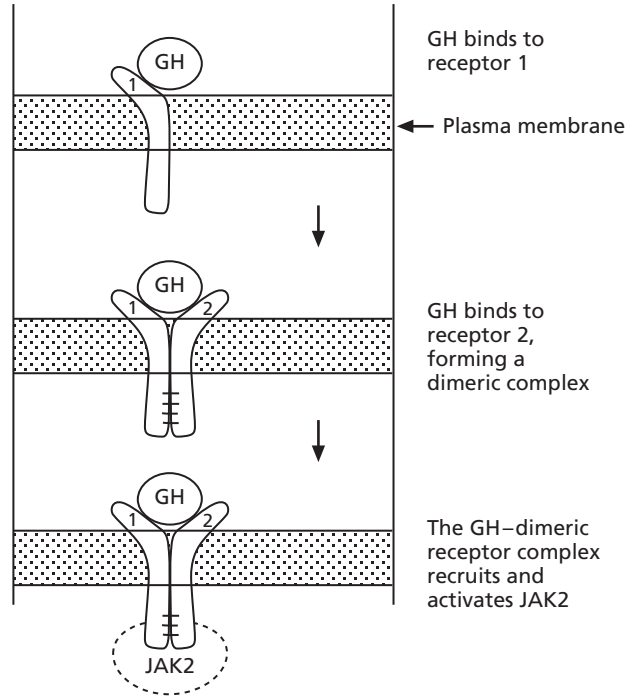

Fig. 1.4. Diagrammatic representation of GH binding to its cell-surface receptors and, via the formation of receptor dimers, subsequently recruiting Janus-associated kinase 2 (JAK2). The two receptors depicted (1 and 2) have identical structures.

Tyrosine kinase recruitment

Recent data suggest that the receptor undergoes a conformational change to the status required for tyrosine kinase recruitment and initiation of signaling following GH binding. Thirty-two mammalian non-receptor tyrosine kinases have been identified, which are classified into 11 groups based on sequence similarity in their Src homology (SH) 1, SH2, and SH3 domains. Each has the ability to bind to the intracellular motif of different receptor molecules. Early studies of GH signaling pathways used cross-linking and immunoprecipitation techniques to demonstrate that the activated GH receptor recruits predominantly members of the Janus family of tyrosine kinases [19,20].

Janus-associated kinases (JAK)

Four evolutionarily conserved members of the JAK family have been identified, JAK1, JAK2, JAK3, and Tyk2. JAK3 is expressed primarily in hematopoietic cells, although the others are found in most cell types. GH usually recruits JAK2 (Fig. 1.4), although GH-induced phosphorylation of JAK1 and JAK3 has also been reported. They all possess seven conserved JH regions (JH1–7), of which JH1 is the functional domain and JH2 a pseudokinase domain necessary to regulate the catalytic domain negatively so that the enzyme is inactive in the absence of a stimulus [21].

Although JAKs associate constitutively with the receptor through a proline-rich site known as Box 1 in the receptor's intracellular domain, they are spatially positioned and/or conformationally modified upon receptor activation so that transphosphorylation and activation of the kinase domain occur. This results in phosphorylation of the intracellular domain of the GH receptor and provision of docking sites for a variety of signaling molecules that contain SH2 or other phosphotyrosine-binding (PTB) motifs. These include SHC, IRS, and members of the signal transducer and activator of transcription (STAT) family of proteins (Fig. 1.5) [22].

STATs

In mammals, there are seven STAT family members, STAT1–4, STAT5A, STAT5B, and STAT6. Only STAT1, STAT3, and the STAT5 molecules are usually recruited in response to GH-induced JAK2 activation.

STAT proteins are localized in the cytoplasm in unstimulated cells, but they are rapidly recruited to the intracellular domain of the receptor after ligand/receptor coupling through binding between STAT SH2 domains and phosphorylated tyrosine residues on the receptor [23]. This interaction is highly specific and represents a critical step in determining the specificity of receptor-mediated STAT activation. Once bound, the STATs become phosphorylated. This leads to the formation of STAT homo- and heterodimers, which translocate rapidly to the nucleus for DNA binding (Fig. 1.5). Most STAT dimers recognize an 8- to 10-bp inverted repeat DNA element with a consensus sequence of 5′-TT($N_{4–6}$)AA-3′, usually referred to as a GAS element as a result of its initial characterization as a γ-interferon activation sequence recognized by STAT1 homodimers.

Following DNA binding, activated STAT dimers initiate the transcription of immediate early response genes that regulate

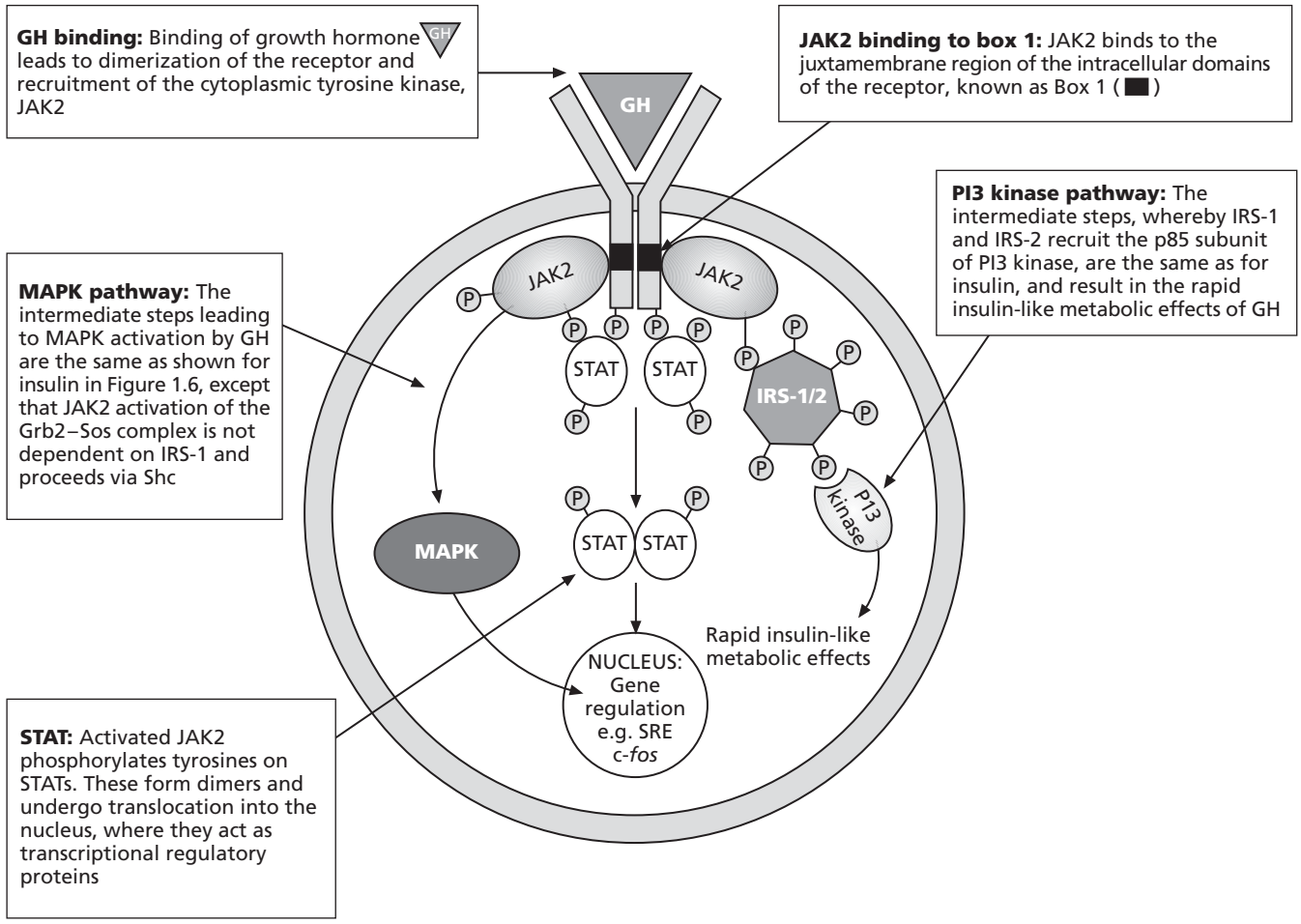

Fig. 1.5. Signaling pathways initiated in response to activation of the growth hormone receptor, an example of a receptor that recruits tyrosine kinase activity.

proliferation of more specific genes that determine the functional status of the cell [23].

Desensitization

Following activation, there is a rapid attenuation of receptor responsiveness to GH. This process is achieved by removal of the receptor from the cell surface (internalization) and degradation of the GH receptor/JAK complex [24]. In addition, JAK/STAT signaling pathways are inhibited by at least three families of proteins: phosphatases, suppressors of cytokine signaling (SOCS), and protein inhibitors of activated STATs (PIAS).

In vitro evidence for the involvement of protein tyrosine phosphatases (PTP) comes from the demonstration of prolonged GH-stimulated JAK2 and STAT5 phosphorylation in the presence of phosphatase inhibitors. Furthermore, in mice deficient in the enzyme SHP1, these signaling molecules are superactivated in response to GH [25].

Activation of the JAK/STAT pathway also induces expression of the SOCS proteins; these interact with JAK and also with the GH receptor to result in proteosomal degradation [26]. Finally, PIAS have been shown to regulate signal transduction negatively in response to prolactin, although less is known about the involvement of PIAS in GH regulation of STAT-mediated transcription [27].

Alternative pathways

JAK/STAT pathways are important in the cellular response to GH and prolactin, but there is evidence to suggest that other non-receptor tyrosine kinases may also mediate signal transduction of the cytokine receptor superfamily [28].

Members of the Src family of kinases (s-Src and c-Fyn) are activated by GH–receptor coupling, and this may lead to phosphorylation of focal adhesion kinase (FAK), recruitment of Grb2, and stimulation of the MAPK pathway. c-Fyn has also been implicated in the activation of PI 3-kinase by prolactin. Furthermore, Src kinases can associate with STAT1, STAT3, and STAT5, and so it is possible that this pathway is also involved in the transcriptional events regulated by GH or PRL. The use of signaling molecules from the transduction pathways associated with insulin may explain the acute insulin-like effects of GH. In addition, GH and prolactin have been reported to increase intracellular free calcium through activation of phospholipase C (see below).

Defects in GH and PRL signaling

Abnormalities in GH signal transduction result in GH resistance and severe growth impairment despite normal or elevated levels of circulating GH (Laron syndrome). Such patients have exceptionally low levels of IGF-I and its principal carrier protein, IGF binding protein-3 (IGFBP-3), and these cannot be elevated by the administration of exogenous GH. This observation gave the first clue that GH resistance resulted from non-responsive receptor or signaling pathways, mainly as a result of mutations in the gene for the GH receptor [29].

Deletions, nonsense, missense, splice, and frameshift mutations have all been detected in the exons of the GH receptor that code for the extracellular domain (exons 2–7). Some affect the ability of the receptor to bind GH, whereas others result in reduced GH-stimulated dimerization. Mutations in exons 8–10, which code for the transmembrane and intracellular domains, can lead to defective GH receptor–JAK coupling. Some patients, however, have no apparent defect in their GH receptor, suggesting that the problem must lie in genes further downstream. Indeed, STAT5b knockout mice fail to respond effectively to GH, and GH activation of STAT5 and MAP kinase is defective in fibroblasts isolated from patients with Laron syndrome [30]. There has been a report of one patient with severe intrauterine growth retardation followed by postnatal growth failure, sensorineural deafness, mild mental retardation, and GH resistance in whom there appears to be a partial deletion in the gene coding for IGF-I [31]. Clearly, this would render the GH–IGF-I axis ineffective.

There have been no reports of human disease resulting from gene defects in the prolactin receptor. This suggests that either mutations of the PRL receptor have no detectable effect *in vivo* or such mutations are lethal [17]. Evidence from PRL receptor knockout mice supports the former hypothesis, as these animals are viable but they do display a number of reproductive, behavioral, and bone abnormalities.

Circulating receptors

The extracellular domain of the GH receptor can be cleaved by an enzyme thought to be the metalloprotease TACE (tumor necrosis factor alfa converting enzyme) to form a circulating binding protein with high affinity for GH (GHBP) [32]. The physiological significance of GHBP is poorly understood, but "receptor decapitation" presents an alternative mechanism for receptor desensitization. The binding protein itself has the potential to modulate GH function either by prolonging its half-life and providing a circulating reservoir or by competing with GH for GH binding and inhibiting GH signaling through the formation of non-functional GHBP/GH receptor heterodimers.

Serum GHBP levels approximate GH receptor expression and are therefore used as a reflection of GH receptor status: for example, 75–80% of patients with Laron syndrome have low or undetectable levels of GHBP.

G-protein-coupled receptors

G-protein-coupled receptors (GPCRs) form a superfamily of more than 1000 membrane proteins that accounts for approximately 1% of the genes found within mammalian genomes. These receptors have a diverse range of ligands and, in addition to transducing hormonal signals, they also mediate the

cellular response to neurotransmitters, lipids, including prostaglandins and leukotrienes, nucleotides, ions, and sensory stimuli, such as light, smell, and taste [33].

As their name suggests, activation of GPCRs generally leads to the recruitment of intracellular G (guanine)-proteins and then the generation of second messengers, for example cyclic adenosine monophosphate (cAMP) and inositol 1,4,5-triphosphate (IP_3). Some of these receptors can signal through G-protein-independent pathways.

Structure

Although GPCRs have the same basic design as the tyrosine kinase-linked receptors, in that they possess extracellular, transmembrane, and intracellular domains, they can be defined structurally by their more elaborate "serpentine" transmembrane region [33]. This contains seven α-helices linked by alternating intracellular and extracellular loops, which can be arranged to form a hydrophobic pore (Fig. 1.6a and b). In general, each of the transmembrane segments consists of 20–27 amino acids. The extracellular N-terminal segment, loops, and intracellular C-terminal domain are much more variable in size; consequently, GPCRs range from the gonadotropin-releasing hormone receptor, at only 337 amino acid residues, to the calcium-sensing receptor, which has 1085 residues. The latter has a disproportionately long N-terminus (> 600 amino acids), because, in general, the length of the N-terminal segment is weakly correlated with ligand size. It has been suggested that this domain, along with the extracellular loops and transmembrane pore, has an important role in ligand recognition. Like the tyrosine kinase-linked receptors, the intracellular domains (both loops and C-terminus) are necessary for interaction with intracellular signaling partners.

GPCRs can be grouped into three families, A, B, and C (Table 1.1), on the basis of sequence similarity within the transmembrane region. There is little similarity between the groups, apart from the characteristic tertiary structure facilitated by the seven transmembrane helices [33]. Group A, which is the largest family and contains the receptors for light and adrenaline, has a putative fourth intracellular loop due to palmitoylation of cysteine residues in the C-terminal domain. Group B includes receptors for a variety of hormones and neuropeptides and is characterized by a long amino-terminus containing several cysteine residues, which presumably form a network of disulfide bridges. Group C includes the receptors for glutamate, γ-aminobutyric acid, and calcium.

G-proteins

Upon binding ligand, the conformation of the transmembrane domain, particularly the third and sixth helices, is

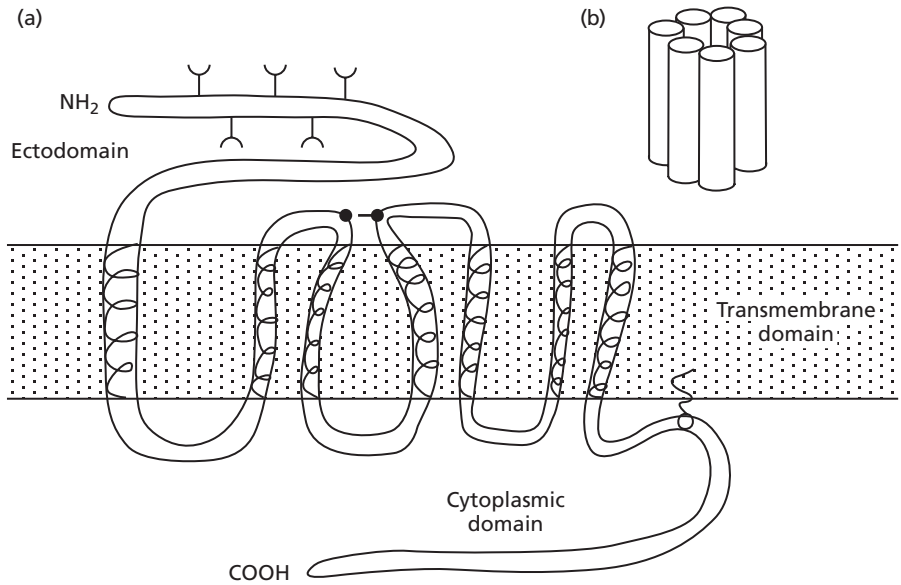

Fig. 1.6. A schematic representation of G-protein-coupled receptors (GPCRs) showing the seven transmembrane domains. (a) The structure is an elaborate variation of the three-segment design depicted in Figure 1.2. The size of the N-terminal extracellular domain is generally in proportion to the size of the cognate ligand. Homology of this region, which is obviously important for ligand binding, is less than that of the transmembrane and cytoplasmic domains. This region can be heavily glycosylated, and the carbohydrate moieties may contribute as much as 40% of its mass. The transmembrane domain has a characteristic heptahelical structure, most of which is embedded in the plasma membrane and provides a hydrophobic core. Conserved cysteine residues may form a disulfide bridge between the second and third extracellular loops. The cytoplasmic domain links the receptor to the signal-transducing G-proteins. Evidence from the β-adrenergic receptor suggests that specific regions in the third intracellular loop and sections of the C-terminal tail are critical for G-protein coupling. A fourth intracellular loop may be formed by a cysteine residue in the C-terminal tail, which could be palmitoylated in some GPCRs. (b) The hydrophobic pore formed by the seven transmembrane α-helices of the GPCR.

Table 1.1. Examples of GPCRs and their associated G-proteins/second messengers (AC, adenylate cyclase; PLC, phospholipase C). For somatostatin, vasopressin, calcitonin, and PTH/PTHrP, different receptor subtypes determine α-subunit specificity, and there may be differential tissue distributions of these receptor subtypes. This phenomenon provides opportunities to develop selective therapeutic antagonists.

Family	Characteristics	Examples	G-protein	Second messenger
A	Disulfide bridge connecting second and third extracellular loop Putative fourth intracellular loop	TRH receptor GnRH receptor Oxytocin	Gqα	PLC
		Biogenic amine receptors FSH receptor LH receptor TSH receptor Vasopressin	Gsα/Gqα	AC/PLC
		Somatostatin	Giα/Gqα	AC/PLC
		Melanocortin receptor	Gsα	AC
B	Disulfide bridge connecting second and third extracellular loop Long amino-terminus containing several cysteine residues	Calcitonin receptor	Gsα/Giα/Gqα	AC/PLC
		CRH receptor Glucagon receptor	Gsα	AC
		PTH receptor PTHrP receptor	Gsα/Gqα	AC/PLC
C	Very long (>600 amino acids) amino-terminus Very short and highly conserved third intracellular loop	Calcium receptors	Gqα/Giα	AC/PLC
		Glutamate receptors	Gsα/Gqα	AC/PLC

altered. This leads to a conformational change in the intracellular domains to uncover binding sites for heterotrimeric G-proteins previously masked. G-proteins consist of α-, β-, and γ-subunits. The β- and γ-subunits associate with such high affinity that G-proteins are usually described as having two functional units, Gα and Gβγ; to date, 23 α-subunits, six β-, and 12 γ-subunits have been described [34].

Activation by GPCRs induces a conformational change in the α-subunit, which results in the exchange of a molecule of GDP for a molecule of GTP and dissociation of the α-subunit from both the receptor and the βγ-dimer. Both the GTP-bound α-subunit and the βγ-dimer independently regulate a number of downstream signaling pathways.

Based on their primary effector molecules, the α-subunits can be grouped into four families. $G\alpha_s$ and $G\alpha_i$ activate or inhibit adenylate cyclase (AC) respectively. $G\alpha_q$ activates phospholipase C (PLC). Less is known about the $G\alpha_{12}$-subunits, although it appears that their effects are mediated through members of the Rho family of GTPases [35]. In addition to the effectors used by the α-subunits, Gβγ-dimers are known to target ion channels and protein kinases, and the list continues to increase [34].

The existence of numerous G-protein subunits in combination with a variety of downstream effectors enables the diversity and selectivity of intracellular signals in response to GPCR activation. Each receptor has the possibility of interacting with many G-proteins (Table 1.1). Recruitment of a particular Gα-subunit depends on many factors [36], including receptor subtype (Fig. 1.7a), structural features of the cytoplasmic domain, and the concentration of the ligand. For example, at low concentrations, TSH, calcitonin, and luteinizing hormone (LH)/human chorionic gonadotropin (hCG) receptors activate adenylate cyclase through $G\alpha_s$, whereas at higher concentration, $G\alpha_q$ is recruited to activate PLC. Further complexity is introduced by the potential for receptors simultaneously or successively to couple with distinct G-proteins (Fig. 1.7b) and the ability of a particular G-protein to activate multiple intracellular signaling cascades (Fig. 1.7c).

Intracellular second messengers

cAMP

Activation of membrane-bound adenylate cyclase catalyzes the conversion of ATP to the potent second messenger cAMP (Fig. 1.8) [37]. This cyclic nucleotide activates the heterotetrameric protein kinase A (PKA) by binding to repressive regulatory subunits (R), which then dissociate from the two catalytic subunits (C) so that phosphorylation of serine/threonine residues in proteins containing the consensus sequence Arg-Arg-X-Ser/Thr-X can occur. These include intermediaries of lipolysis, glycogenolysis, and steroidogenesis (for example, glycogen synthase, hormone-sensitive lipase, cholesterol ester hydrolase) as well as the transcription factor CREB (cAMP response element binding protein). Phosphorylated CREB translocates to the nucleus where it binds to a short palindromic sequence, the CRE or cAMP response

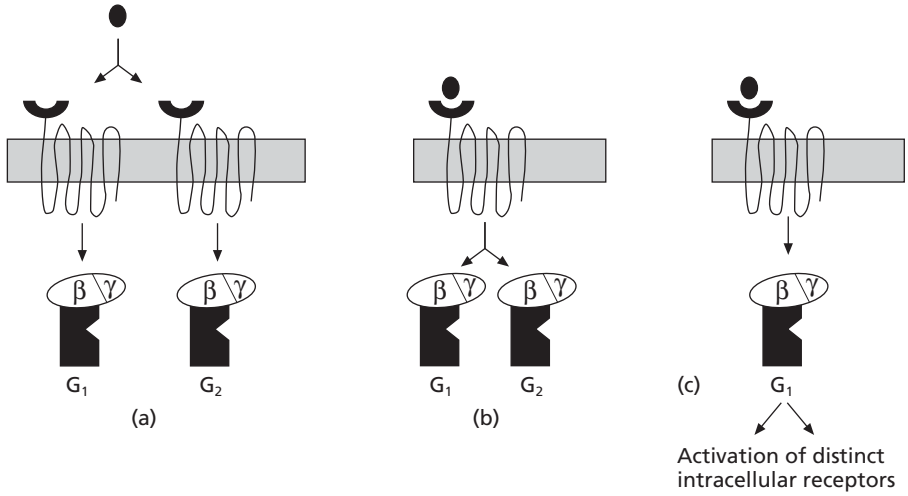

Fig. 1.7. Various mechanisms of G-protein selection and subsequent activation of intracellular second messengers. Adapted from Hermans E. Biochemical and pharmacological control of the multiplicity of coupling at G-protein coupled receptors. *Pharmacol Ther* 2003; 99: 25–44 [36], with permission from Elsevier.

element, of cAMP-regulated genes (e.g. somatostatin). In this way, the generation of cAMP can have a direct effect on gene transcription.

cAMP does not act exclusively through PKA, and there is a growing list of alternative cAMP targets [38]. The physiological effects of cAMP are also produced by direct regulation of monovalent and divalent cation channels and the ubiquitous guanine exchange factors Epac 1 and 2.

The cAMP-mediated signal is terminated by members of the phosphodiesterase (PDEs) family of proteins. These hydrolyze cAMP rapidly to the inactive 5′-AMP in response to phosphorylation by PKA and other mechanisms.

Diacylglycerol and Ca^{2+}

Occupancy of numerous GPCRs, including TRH, GnRH, and oxytocin, results in G-protein activation of the enzyme phospholipase C (PLC; Fig. 1.9) [39]. This leads to the hydrolysis of phospholipids, specifically phosphatidylinositol-4,5-bisphosphate (PIP_2), which resides in the inner leaflet of the plasma membrane, to yield diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). DAG, together with a cofactor phosphatidylserine, recruits another protein kinase, the membrane-bound PKC, which, in the presence of calcium, phosphorylates a wide variety of proteins and peptides to bring about the cellular response. Ca^{2+} is provided by IP_3, which diffuses through the cytoplasm to bind receptors on the endoplasmic reticulum, causing Ca^{2+} mobilization and a rapid increase in cytosolic free Ca^{2+}. In addition to PKC, the rise in intracellular Ca^{2+} also activates the protein kinase calmodulin and phospholipase A_2. Phospholipase A_2 liberates arachidonate from phospholipids and thereby generates potent local tissue activators known collectively as eicosanoids. These include thromboxanes, leukotrienes, and prostaglandins. Prostaglandins are well-recognized paracrine and autocrine mediators that may amplify or prolong the response to the original hormone stimulus. Intracellular Ca^{2+} concentrations are restored to resting levels by several mechanisms including Ca^{2+} pumps and deactivation of G-proteins.

Receptor dimerization

Receptor dimerization is required for signal transduction through some types of receptor, but models that describe the activation of GPCRs are usually based on the assumption that GPCRs exist as monomers and that they couple to G-proteins in a 1:1 molar ratio. Increasing evidence from *in vitro* studies suggests that these models may need to be refined [33]. Homodimers of the β_2-adrenergic receptor, the δ-opioid receptor, and the dopamine D_1, D_2, and D_3 receptors have been described. Intriguingly, ligand appears to regulate dimer formation, which suggests that homodimerization could have a role either in the receptor activation mechanism or in the subsequent desensitization and internalization process.

The possibility of heterodimerization between closely related receptor subtypes has been proposed. This process may be important for targeting functional receptors to the cell surface, and it is possible that heterodimerization could generate novel ligand binding and signal transduction pathways to result in functional properties distinct from those of either of the receptors. The physiological significance of homo- and heterodimers remains to be determined.

Non-G-protein pathways

GPCRs do not always signal through G-proteins. There is a rapidly growing list of new GPCR effector molecules [40]. In some cases, these are known receptor-interacting proteins, such as the arrestins, which, in addition to their well-established role in receptor desensitization, appear to link GPCRs into MAP kinase pathways. In other cases, novel binding partners have been identified, and the challenge will be to understand how the classical and new effector pathways are integrated to achieve specificity of GPCR signal transduction.

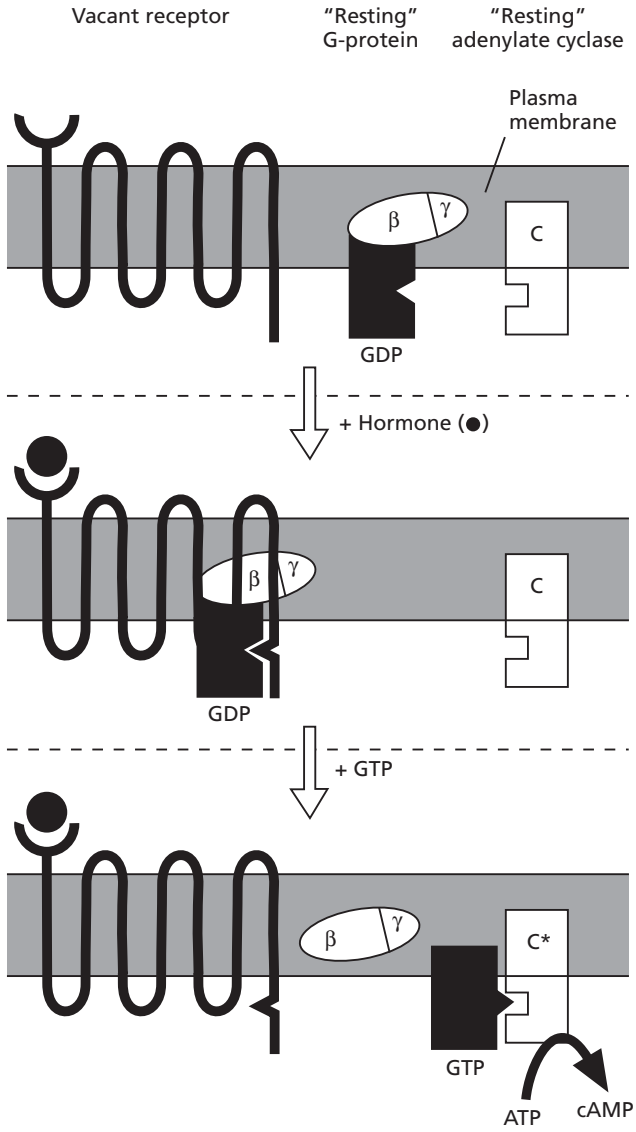

Fig. 1.8. A representation of G-protein-modulated activation of a membrane-bound enzyme such as adenylate cyclase. A hormone, e.g. adrenaline, binds to the extracellular region of the receptor. The third intracellular loop and the C-terminus of the receptor associate with a G-protein, for example $G\alpha_s\beta\gamma$. This leads to displacement of GDP by GTP and dissociation of $G\alpha_s$ from the βγ-dimer. The α-subunit diffuses in the lipid bilayer and activates the catalytic subunit (C*) to generate many molecules of cAMP.

GPCR desensitization

GPCR desensitization results from changes to either the receptor or the intracellular G-proteins [41]. The extent varies from complete termination of signaling, which occurs in the sensory systems, to a reduction in the potency of ligand, as is observed with the β-adrenergic receptors.

Internalization of receptors to intracellular compartments and reduced expression as a result of decreased mRNA and protein synthesis both lead to desensitization, but this can be achieved more rapidly (in seconds rather than minutes or hours) by uncoupling the receptor from G-protein-mediated signaling pathways. It is widely accepted that both second messenger-dependent protein kinases [e.g. protein kinase A (PKA) and PKC] and G-protein-coupled receptor kinases (GRK) are responsible for uncoupling GPCRs from G-proteins by phosphorylating serine and threonine residues within the intracellular loop and carboxy-terminal tail domains of the receptor. GRK phosphorylation of GPCRs also promotes the binding of cytosolic cofactor proteins known as arrestins, which target GPCR for endocytosis by clathrin-coated vesicles.

GPCR signals can also be terminated at the G-protein level. Gα-subunits possess intrinsic GTPase activity, which can cleave phosphate from GTP to result in GαGDP. This process can be enhanced by a family of proteins called regulators of G-protein signaling (RGS), which accelerate the rate of hydrolysis of GTP bound to both $G\alpha_i$ and $G\alpha_q$ to dampen $G\alpha_i$- and $G\alpha_q$-mediated signaling pathways. Hydrolysis of GTP allows the Gα-subunit to associate with a Gβγ-dimer again, and the heterotrimeric complex returns to the G-protein pool so that it can be activated by subsequent receptor occupation by ligand.

Defects

Given their numerous and varied ligands, it is not surprising that mutations in GPCRs or their interacting G-proteins are associated with endocrine disease [42]. Mutations that alter the extracellular (ligand-binding) domains of the receptor lead to hormone resistance (e.g. the TSH receptor), whereas aberrations in the transmembrane region of the receptor can result in altered receptor function.

Germline mutations in Xq28, which codes for the vasopressin V2 receptor, cause receptor misfolding and loss of receptor function so that circulating vasopressin, despite being present at very high levels, cannot increase urine concentration, and nephrogenic diabetes insipidus results. Some cases of early-onset severe obesity may be explained by functional defects in the melanocortin-4 receptor [43].

Activating mutations are also detrimental, presumably by altering crucial helix–helix interactions so that the receptor is active even in the absence of ligand. Familial male precocious puberty (testotoxicosis) is the result of such a mutation in the gene coding for the LH receptor, and activating mutations in the transmembrane domain of the TSH receptor have been reported in association with neonatal hyperthyroidism and toxic thyroid adenomas in adults.

Mutations resulting in the loss of $G\alpha_s$ function are linked to pseudohypoparathyroidism (Albright's hereditary osteodystrophy). If the mutation is maternally transmitted, resistance to the multiple hormones that activate $G\alpha_s$ in their target tissues occurs. Mutations resulting in the constitutive activation of $G\alpha_s$ cause McCune–Albright syndrome and some cases of acromegaly.

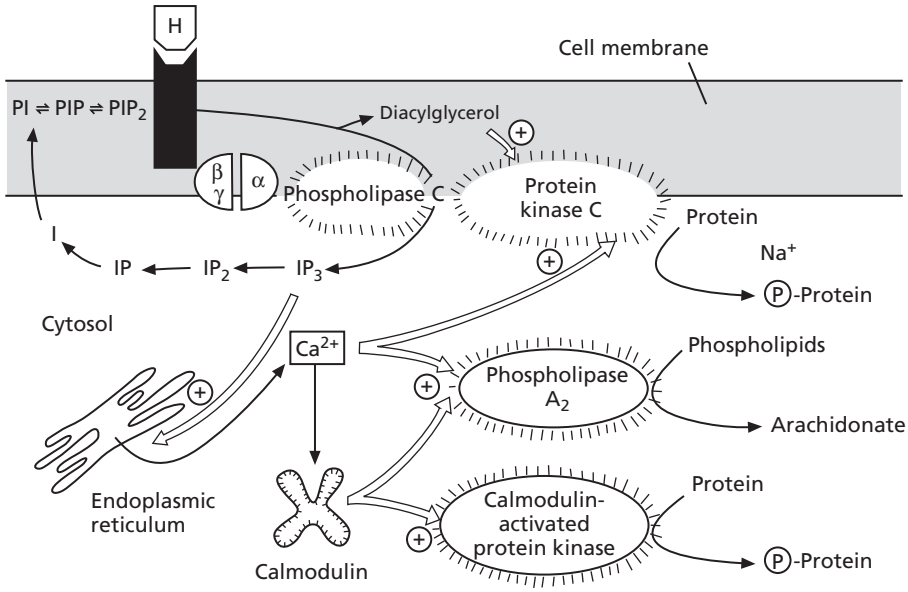

Fig. 1.9. A representation of hormone-stimulated phospholipid turnover and calcium metabolism as a result of G-protein-coupled receptors activating phospholipase C.

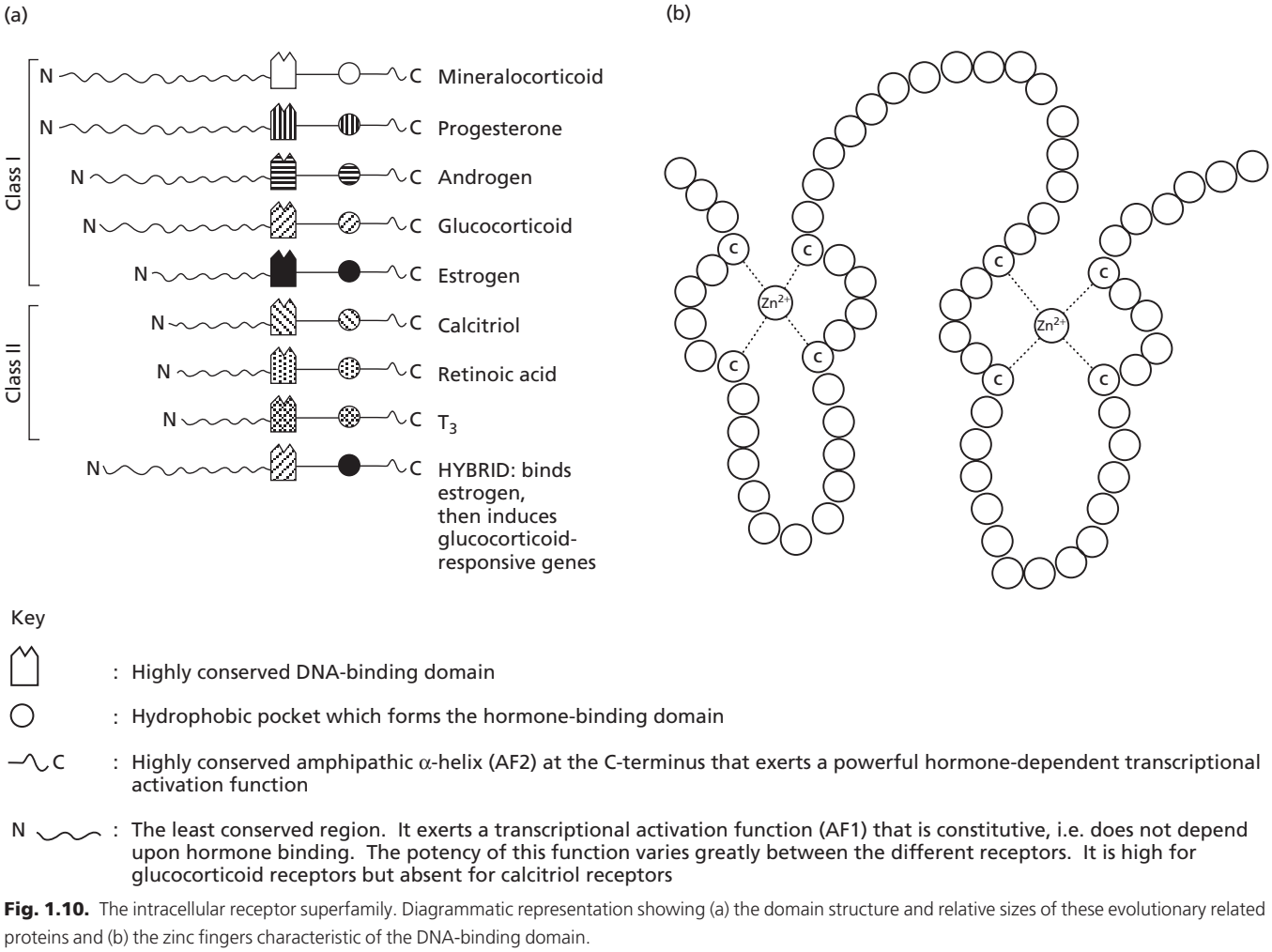

Fig. 1.10. The intracellular receptor superfamily. Diagrammatic representation showing (a) the domain structure and relative sizes of these evolutionary related proteins and (b) the zinc fingers characteristic of the DNA-binding domain.

Intracellular receptors

Receptors for hydrophobic hormones such as the sex steroids, glucocorticoids, thyroxine, and aldosterone are part of a large family of receptors (> 150 members) that are located inside the cell (Fig. 1.1). These receptors function as hormone-regulated transcription factors and control the expression of specific target genes by interacting with regions close to the gene promoters. Consequently, the cellular response to these hormones takes longer than the quickfire cell surface receptor/second-messenger systems described above.

Receptor structure

All intracellular receptors consist of three major regions (Fig. 1.10a) [44]. There is a highly variable N-terminal domain, which has a role in transcription activation because of a region known as AF1 (activation function) in some receptors. There is also a DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD), although their molecular weights vary from 46 to 100 kDa. The DNA-binding domain shows the highest degree of homology across the receptor family. It is characterized by two polypeptide loops, each of approximately 20 amino acids, which are known as "zinc fingers" as a result of their formation from the co-ordination of four cysteine or two cysteine/two histidine residues by a single atom of zinc (Fig. 1.10b). These distinctive fingers are necessary for interlocking with target DNA sequences in the nucleus.

Based on the crystal structure reported for the LBD of the estrogen and progesterone receptors, this region is thought to be composed of 12 α-helices that are folded to create a hydrophobic pocket for the ligand [45]. Following occupation by ligand, there is a conformational change such that the 12th helix is repositioned to "seal" the pocket like a lid. In the glucocorticoid receptor, point mutations of the DNA coding for the LBD result in altered ligand binding affinity and receptor specificity. Complete deletion of this region gives rise to a constitutively active receptor, which suggests that the LBD in the unoccupied state acts as a negative regulator of receptor function [46].

Receptor activation and DNA binding

Unoccupied "class I" receptors (Fig. 1.1) shuttle between the cytoplasm and the nucleus. Although some reside mainly in the former (e.g. the glucocorticoid receptor), others (e.g. the androgen receptor) are predominantly nuclear. In either case, the receptors are associated with "chaperone" molecules, such as heat shock protein 90 (hsp90), in a large heteromeric complex that obscures the zinc fingers of the DBD, thereby preventing the interaction with target sequences in the nucleus [44]. As a result of hormone binding, the inhibitory complex dissociates, the receptor becomes hyperphosphorylated, and, if necessary, hormone–receptor complexes translocate to the nucleus. Activated receptors homodimerize here and then bind through the zinc fingers to DNA sequences that are specific for each receptor and known as the hormone response element (HRE; Fig. 1.11a). The glucocorticoid receptor binds to genes containing a glucocorticoid response element (GRE), the estrogen receptor to the estrogen response element (ERE), and so on. Targeting of the hormone–receptor complex to the HRE is directed by remarkably few amino acids in the DBD. These occur in a region called the P-box, which is usually located at the base of the first zinc finger. Each zinc finger recognizes a sequence of approximately six nucleotide basepairs,

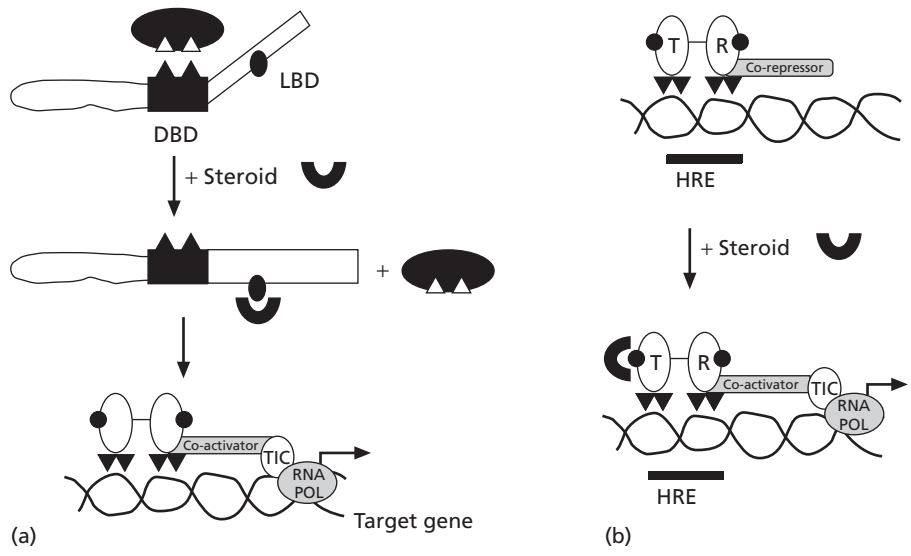

Fig. 1.11. Activation of steroid hormone receptors. (a) Unoccupied class I receptors are associated with inhibitory molecules such as hsp90. Following hormone binding to the ligand-binding domain (LBD), the inhibitory protein dissociates, and the two zinc fingers characteristic of the DNA-binding domain (DBD) are exposed. The receptor forms homodimers, interacts with the hormone response element (HRE) of target genes, and, with the help of co-activator proteins, initiates transcription. TIC, transcriptor initiation complex; POL, polymerase. (b) Class II receptors, e.g. T_3 receptor (T), are constitutively bound to DNA target sequences, usually as heterodimers with the retinoid X receptor (R). In the unoccupied state, they are transcriptionally inactive as a result of interaction with co-repressor molecules. Following ligand binding, the co-repressors are replaced by co-activators, and RNA polymerase is activated.

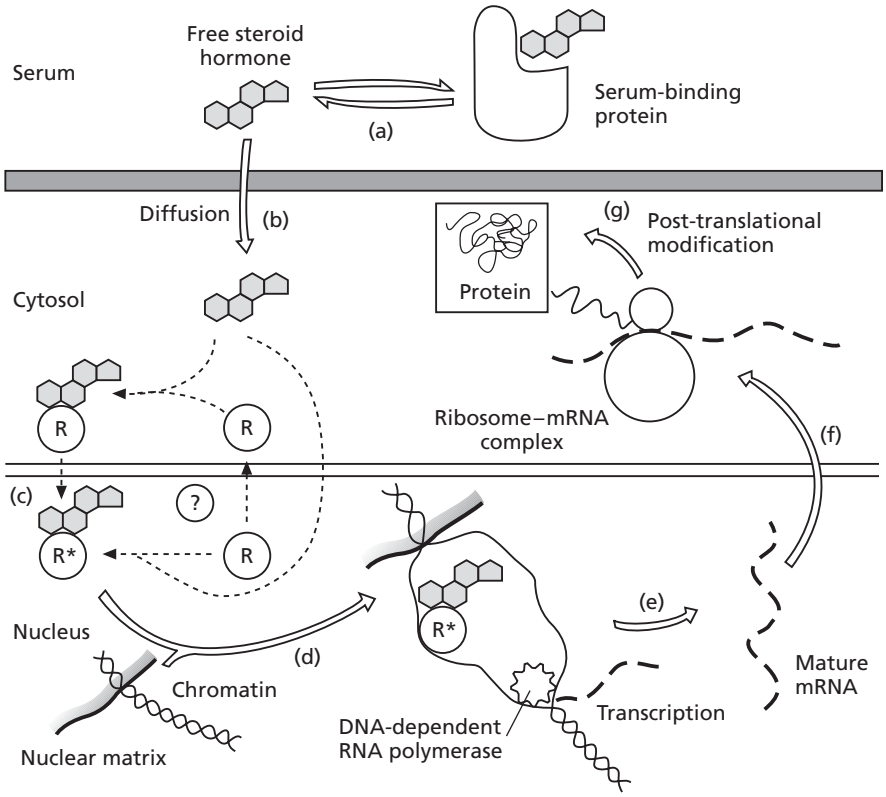

Fig. 1.12. Mechanism of steroid hormone action. Free steroid hormone in equilibrium with bound hormone (a) diffuses across the target cell membrane (b) and binds to the steroid hormone receptor protein in the cytoplasm or in the cell nucleus. The hormone–receptor complex (c) interacts with chromatin and binds to a receptor site of one DNA strand associated with a particular gene (d). This region is the hormone response element (HRE). The promoter region permits DNA-dependent RNA polymerase to start transcription to yield messenger RNA (e), which passes out of the nucleus (f) after post-transcriptional modification. Peptides are formed by translation of the message on ribosomes attached to the endoplasmic reticulum, and modification of the proteins gives the final gene product (g).

and the HRE often consists of a palindromic or tandemly repeated sequence. The four steroid hormone receptors (glucocorticoid, mineralocorticoid, androgen, and progesterone) bind to an imperfect palindrome that consists of two hexamers repeated in reverse orientation and separated by three nucleotides (5′-GGTACAnnnTGTTCT-3′) [47]. Despite the receptors recognizing the same target sequence, a specific hormonal response is achieved by the recruitment of auxiliary molecules known as co-activators or co-repressors.

Class II receptors (Fig. 1.1), for example the receptors for the thyroid hormones, are located exclusively in the nucleus where they are constitutively bound as homodimers or heterodimers [usually with an unoccupied retinoid X receptor (RAR)] to their DNA target sequence (Fig. 1.11b). In general, unoccupied receptors "silence" basal promoter activity, probably by associating with a co-repressor; ligand binding leads to a conformational change in the receptor and exchange of the inhibitory molecules for proteins necessary for the activation of transcription [48].

Co-activators and co-repressors

The hormone response element is usually upstream of the promoter region for the target gene. The promoter region also contains a consensus sequence, the TATA-box, for RNA polymerase. Through the recruitment of co-activators or co-repressors, which may be dependent on the phosphorylation status of the receptor, the hormone–receptor complex can direct the binding and activity of this enzyme to enhance or suppress transcription [49,50]. Following transcription, the "genomic" pathway is completed by translation of the newly generated mRNA into the proteins that ultimately result in the cellular response to hormone stimulation (Fig. 1.12).

Co-activator proteins facilitate transcription by remodeling the chromatin environment so that it is more accessible to RNA polymerase or by coupling ligand-occupied receptors to the basal transcription apparatus. They include creb binding protein (CBP/p300) and steroid receptor co-activator-1 (SRC-1), both of which possess histone acetyltransferase activity. Co-repressors such as NcoR (nuclear receptor co-repressor) and SMRT (silencing mediator of retinoid and thyroid hormone receptors) also function by modifying chromatin, usually by recruiting histone deacetylase to the vicinity of the receptor.

Desensitization

As with the receptors expressed at the cell surface, ligand binding of intracellular receptors results in rapid desensitization. A ligand-dependent reduction in mRNA levels has been demonstrated for many of the steroid receptors. Other mechanisms for limiting hormone responsiveness include diminished receptor half-life, degradation via the ubiquitin/

Table 1.2. Examples of defects in intracellular receptors that are associated with endocrine disease.

Receptor	Clinical effects	Molecular defects reported to date
Androgens (ARs)	Partial or complete androgen insensitivity syndromes	↓Receptor number ↓Androgen binding ↓AR dimerization
	Kennedy syndrome	Expanded CAG repeat in N-terminus
	Breast cancer Prostate cancer	↓AR dimerization ↓AR responds to progesterone
Glucocorticoid	Generalized inherited glucocorticoid resistance	↓Hormone binding ↓GR number ↓DNA binding
Oestrogen (ER)	Usually lethal Estrogen resistance	↓Hormone binding ↓DNA binding
T_3 (TR)	Resistance to thyroid hormone	↓TRβ gene defects ↓T_3 binding
Calcitriol (VDR)	Calcitriol-resistant rickets	↓VDR dimerization

proteosome pathway, and transfer to alternative intracellular compartments.

Non-genomic actions of steroids

The ability of intracellular receptors to act as hormone-regulated transcription factors is commonly accepted as the mechanism of steroid hormone action. However, steroids have a number of physiological effects, for example aldosterone activation of the Na^+/H^+ exchanger, that cannot be attributed to activation of the genome because they occur over too short a timeframe. Uncovering the mechanisms involved in mediating the "non-genomic" actions of steroids is an emerging focus of research. The role of novel vs. classic receptors is currently the subject of much controversy [51,52].

Evidence from knockout mice and pharmacological studies suggests the existence of non-classical steroid receptors. For example, animals lacking the mineralocorticoid receptor have a similar aldosterone-stimulated rise in intracellular calcium and cAMP levels to their wild-type littermates, and many of the effects of aldosterone cannot be blocked by spironolactone, a known inhibitor of the mineralocorticoid receptor. The identity of such receptors remains to be determined, although glucocorticoids, progesterone, and testosterone are all thought to have membrane-bound binding sites.

Conversely, overexpression of the estrogen receptor augments the non-genomic response to this hormone, which demonstrates that at least some of these actions are mediated via the classic receptor.

Defects

Mutations in the genes coding for intracellular receptors are responsible for numerous endocrinopathies as they can result in hormone resistance due to reduced ligand binding, impaired receptor dimerization, and decreased interaction with the HRE (summarized in Table 1.2). Recent studies of the glucocorticoid receptor suggest that defects resulting in abnormal interactions with co-activator molecules, and indeed problems with the co-activators themselves, may also be the cause of hormone resistance syndromes [53].

Target tissue metabolism

Some of the hormones that work through intracellular receptors are converted by enzymes expressed in their target cells to metabolites that are more potent because of their higher affinity for the receptor. For example, tissue-specific 5′-deiodinases convert T_4 to T_3, 5α-reductase metabolizes testosterone to dihydrotestosterone, and 1α-hydroxylase in the mitochondria of cells in the renal tubule converts 25-OH-vitamin D to calcitriol. These "activation" steps offer a way of achieving a range of effects, and various disorders can result from defects in target tissue metabolism. The best known example is androgen insensitivity. Conversely, 11β-hydroxysteroid dehydrogenase (11β-HSD), which is expressed by aldosterone-responsive cells in the kidney, converts cortisol to cortisone to prevent the overstimulation of the mineralocorticoid receptor that would otherwise occur as a result of the high concentration of cortisol in relation to the circulating levels of aldosterone [54]. Deficiency or

impaired function of this enzyme leads to the hypertension and hypokalemia characteristic of the apparent mineralocorticoid excess (AME) syndrome.

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2 Genetics, genomics, proteomics, and bioinformatics

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The understanding of the molecular basis of many endocrine and non-endocrine disorders has grown impressively during the last decade (Table 2.1). With the exception of simple trauma, virtually every disease has a genetic component. In *monogenic disorders* such as congenital adrenal hyperplasia (CAH), the genetic component serves as the major etiologic factor. In *complex disorders*, multiple genes, in conjunction with environmental and lifestyle factors, contribute to the pathogenesis; hence their designation as *polygenic* or *multifactorial* disorders. In other instances, genetic factors influence the manifestation of disease indirectly by defining the host's susceptibility and resistance as, for example, in an environmental disease such as infection.

Genetics can be defined as the science of heredity and variation. *Medical genetics*, the clinical application of genetics, has historically focused on chromosomal abnormalities and inborn errors of metabolism, because of readily recognizable phenotypes and techniques to diagnose the conditions. Analysis of the transmission of human traits and disease within families, together with the study of the underlying molecular basis, has culminated in understanding many *monogenic* or *Mendelian* disorders, which has led to a significant modification in the diagnostic process for some of them. Many major health care problems, such as diabetes mellitus type 2, obesity, hypertension, heart disease, asthma, and mental illnesses, are complex disorders, and we are still at the early stages of unraveling the specific genetic alterations predisposing to these disorders, which are significantly influenced by exogenous factors. It is important to recognize that phenotype can also be influenced by genetic and environmental *modifiers* in monogenic disorders.

Cancer can also be viewed as a genetic disease, as *somatic* mutations in genes controlling growth and differentiation are key elements in its pathogenesis. Moreover, many cancers are associated with a predisposition conferred by hereditary *germline* mutations.

The term *genome*, introduced before the recognition that DNA is the genetic material, designates the totality of all genes on all chromosomes in the nucleus of a cell. *Genomics* refers to the discipline of mapping, sequencing, and analyzing genomes (Fig. 2.1). Because of the rapidly growing list of mapped and sequenced genomes of numerous organisms, genomics is currently undergoing a transition with increasing emphasis on functional aspects.

Genome analysis can be divided into *structural genomics* and *functional genomics*. The analysis of differences among genomes of individuals of a given species is the focus of *comparative genomics*. The complement of messenger RNAs (mRNAs) transcribed by the cellular genome is called the *transcriptome*, and the generation of mRNA expression profiles is also referred to as *transcriptomics*.

The term *proteome* has been coined to describe all the proteins expressed and modified following expression by the entire genome in the lifetime of a cell. *Proteomics* refers to the study of the proteome using techniques of large-scale protein separation and identification. The emerging field of *metabolomics* aims at determining the composition and alterations of the *metabolome*, the complement of low-molecular-weight molecules. The relevance of these comprehensive analyses lies in the fact that proteins and metabolites function in *modular networks* rather than linear pathways. Hence, any physiological or pathological alteration may have many effects on the proteome and metabolome.

The growth of biological information has required computerized databases to store organize, annotate, and index the data. This has led to the development of *bioinformatics*, the application of informatics to (molecular) biology. Computational and mathematical tools are essential for the management of nucleotide and protein sequences, the prediction and modeling of secondary and tertiary structures, the analysis of gene and protein expression, and the modeling of molecular pathways, interactions, and networks. Numerous continuously evolving databases provide easy access to the expanding information about the genome of man and other species, genetic disease, and genetic testing (Table 2.2). The integration of data generated by transcriptomic, proteomic, and metabolomic analyses through informatics, *systems biology*, is an emerging discipline aimed at understanding phenotypic

Table 2.1. Selected genetic disorders with relevance for pediatric endocrinology.

2.1.1. Selected chromosomal disorders with endocrine manifestations.

Disorder/phenotype	Chromosomal defect	OMIM
Klinefelter syndrome Hypogonadism, tall stature	47,XXY	
Turner syndrome Ovarian failure, short stature, autoimmune thyroid disease	45,XO	
Down syndrome Autoimmune thyroid disease, diabetes mellitus type 1	Trisomy 21, mosaicism	190685
Prader–Willi syndrome Short stature, obesity, hypogonadism	del15q11–13 (paternal copy) Maternal uniparental disomy	176270
Williams–Beuren syndrome Hypercalcemia, elfin facies, supravalvular aortic stenosis, mental impairment, growth retardation	del7q11.23 (contiguous gene syndrome), mutations in ELN (elastin) gene	194050
DiGeorge syndrome Hypoparathyroidism, thymus hypoplasia, cardiac defects	Del 22q11.2, mutations in TBX1	188400

2.1.2. Selected hypothalamic and pituitary disorders.

Disorder/phenotype	Gene	Inheritance	OMIM
CPHD (GH, PRL, TSH, LH, FSH)	PROP1	AR	601538
CPHD (GH, PRL, TSH)	POU1F1	AR, AD	173110
CPHD (GH, PRL, TSH, LH, FSH) with rigid spine	LHX3	AR	600577
CPHD with septo-optic dysplasia, agenesis of corpus callosum	HESX1	AR	601802
CPHD with various constellations of pituitary hormone deficiencies	HESX1	Monoallelic mutations	601802
Kallmann syndrome: hypogonadotrophic hypogonadism, anosmia, renal agenesis	KAL1	X	308700
Kallmann syndrome: hypogonadotrophic hypogonadism, anosmia	FGFR1		136350
Hypogonadotrophic hypogonadism, delayed puberty	GnRHR	AR	138850
Hypogonadotrophic hypogonadism, adrenal insufficiency	DAX1 (NROB1)	X	300473
Hypogonadotrophic hypogonadism	FSHβ	AR	136530
Hypogonadotrophic hypogonadism	LHβ	AR	152780
Obesity	Leptin receptor	AR	601007
Obesity	MC4R	AR	155541
Obesity adrenal insufficiency, red hair	POMC	AR	176830
Central adrenal insufficiency, CRH deficiency	CRH	AR	122560
Central adrenal insufficiency, ACTH deficiency	TBX19	AR	604614
Short stature	GHRHR	AR	139191
Short stature	GH	AR, AD	139250
Central hypothyroidism	TRHR	AR	188545
Central hypothyroidism	TSHβ	AR	188540
Neuurohypophyseal diabetes insipidus	AVP-NPII	AD, AR	192340

2.1.3. Selected defects in thyroid development, thyroid hormone synthesis, transport, and action.

Disorder/phenotype	Gene	Inheritance	OMIM
Congenital hypothyroidism, thyroid hypoplasia	PAX8	AD	167415
Congenital hypothyroidism, thyroid hypoplasia	TSHR	AR (inactivating mutations)	603372
Bamforth–Lazarus syndrome: congenital hypothyroidism, cleft palate, spiky hair	TTF2 (FOXE1)	AR	602617
Congenital hypothyroidism, impaired iodide uptake	NIS (SLC5A5)	AR	601843
Congenital hypothyroidism, impaired organification	TPO	AR	606765
Congenital hypothyroidism, impaired organification	THOX2	AR	606759
Transient congenital hypothyroidism, impaired H_2O_2	THOX2	Monoallelic mutations	606759
Pendred's syndrome: sensorineural deafness, impaired organification	PDS (SLC26A4)	AR	274600
Congenital hypothyroidism, thyroglobulin defects	TG	AR	188450
Thyroid dysfunction, respiratory distress, choreoathetosis	TTF1 (NKX2.1)	Monoallelic deletions or mutations	600635
Congenital non-autoimmune hyperthyroidism	TSHR	AD (activating mutations)	603372
Resistance to thyroid hormone	THRB	AD, (AR)	190160
Elevated T_3, decreased T_4, quadriplegia, hypotonia	MCT8	X	300095
Consumptive hypothyroidism due to overexpression of deiodinase type 3 in hemangiomas	DIO3	S	601038
Familial dysalbuminemic hyperthyroxinemia	ALB	AD	103600
Euthyroid hyperthyroxinemia	TBG	X	314200
Euthyroid hyperthyroxinemia, amyloid polyneuropathy	TTR	AD	176300

Table 2.1. (*continued*)

2.1.4. Selected parathyroid and bone disorders.

Disorder/phenotype	Gene	Inheritance	OMIM
Familial hypoparathyroidism	CASR	AD	601199
Familial hypoparathyroidism	PTH	AD, AR	168450
Hyperparathyroidism, jaw fibromas	HRPT2	AD	145001
Hyperparathyroidism	Fusion of PTH regulatory region with cyclin D1	Somatic mutation (PRAD1 rearrangement)	168461
Albright's hereditary osteodystrophy	GNAS1	AD	103580
Familial benign hypocalcuric hypercalcemia	CASR	AD	601199
Severe neonatal hyperparathyroidism	CASR	AR (AD)	601199
Vitamin D-dependent rickets type 1	CYP27B1	AR	264700
Vitamin D-dependent rickets type 2: vitamin D resistance	VDR	AR	601769
Hypophosphatemic rickets	PHEX	X	307800
Hypophosphatemic rickets	FGF23	AD	605380
Jansen metaphyseal chondrodysplasia	PTHR	AD	168468

2.1.5. Selected disorders of adrenal hormone synthesis and action.

Disorder/phenotype	Gene(s)	Inheritance	OMIM
Adrenal hypoplasia congenital, hypogonadism	DAX1 (NROB1)	X	300473
Lipoid adrenal hyperplasia, adrenal insufficiency, ambiguous genitalia	STAR	AR	600617
Congenital hypoaldosteronism, adrenal insufficiency	CYP11B2	AR	124080
X-linked adrenoleukodystrophy	ABCD1	X	300371
Neonatal adrenoleukodystrophy	PEX1, PEX10, PEX13, PXR1		202370
Congenital adrenal hyperplasia, 3β-dehydrogenase II	HSD3B2	AR	109715
Congenital adrenal hyperplasia, 11β-hydroxylase	CYP11B1	AR	202010
Congenital adrenal hyperplasia, 17-hydroxylase	CYP17	AR	202110
Congenital adrenal hyperplasia, 21-hydroxlyase	CYP21	AR	201910
Glucocorticoid-remediable aldosteronism	CYP11B2–CYP11B1 fusion gene	AD	103900
Glucocorticoid resistance	GCCR	AD	138040
Aldosterone resistance (pseudohypoaldosteronism)	MR (NR3C2)	AD	600983

2.1.6. Selected disorders of β-cell dysfunction and pancreas development.

Disorder/phenotype	Gene	Inheritance	OMIM
MODY 1	HNF4α	AD	125850
MODY 2	GCK	AD (inactivating mutations)	125851
MODY 3	HNF1α	AD	600496
MODY 4, renal cysts	IPF1	AD	606392
MODY 5	HNF1β	AD	604284
MODY 6	NEUROD1	AD	606394
Pancreas agenesis	IPF1	AR	600733
Rabson–Mendenhall syndrome	INSR	AR	262190
Leprechaunism	INSR	AR	246200
Nesidioblastosis	SUR1 or KCNJ11	AR	256450
Hyperinsulinism	GCK	AD (activating mutations)	602485
Hyperproinsulinemia	INS	AD	176730

Table 2.1. (*continued*)

2.1.7. Selected disorders of gonadal development, hormone synthesis, and action.

Disorder/phenotype	Gene	Inheritance	OMIM
Persistent Müllerian duct	AMH	AR	600957
Persistent Müllerian duct	AMHR2	AR	600956
XY sex reversal, adrenal failure	SF1 (NR5A1)	AD, AR	184757
Androgen insensitivity, androgen receptor inactivation	AR	AR	300068
Androgen insensitivity, 5α-reductase deficiency	SRD5A2	AR	607306
Azoospermia	DAZ	Y	400003
Estrogen resistance	ERα (ESR1)	AR	133430
Leydig cell hypoplasia, male pseudohermaphroditism	LHCGR	AR (inactivating mutations)	152790
Male limited precocious puberty	LHR	AD (activating mutations)	152790
Premature ovarian failure	FSHR	AR	233300
Aromatase deficiency, female genitalia with masculinization during puberty	CYP19A1	AR	107910
Frasier syndrome: male pseudohermaphroditism, streak gonads	WT1	AD	136680
XX male	SRY translocation	X	480000
XY female	SRY mutations	Y	278850

2.1.8. Selected disorders of water and salt metabolism.

Disorder/phenotype	Gene(s)	Inheritance	OMIM
Nephrogenic diabetes insipidus	AVPR2	X	304800
Nephrogenic diabetes insipidus	AQP2	AR, AD	107777
Liddle syndrome: hypokalemic metabolic acidosis, hypertension	SCNN1B or SCNN1G	AD	177200
Gitelman syndrome: hypokalemic metabolic alkalosis, hypocalcuria, hypomagnesemia	SLC12A3	AR	263800
Bartter syndrome: hypokalemic metabolic alkalosis, hypercalcuria, hypovolemia	SLC12A1, KCNJ1, CLCNKB, BSND	AR	241200

2.1.9. Selected defects in fat metabolism.

Disorder/phenotype	Gene	Inheritance	OMIM
Obesity	LEP	Leptin	164160
Familial hypercholesterinemia	LDLR	AD	606945
Familial hypobetalipoproteinemia	APOB	AD	107730
Congenital generalized lipodystrophy	AGPAT2	AR	608594

2.1.10. Tumor syndromes with endocrine manifestations.

Disorder/phenotype	Gene	Inheritance	OMIM
Multiple endocrine neoplasia 1: parathyroid adenoma, pituitary adenoma, pancreas tumors	MEN1	AD	131100
Multiple endocrine neoplasia 2A: medullary thyroid cancer, pheochromocytoma, parathyroid hyperplasia	RET	AD	171400
Multiple endocrine neoplasia 2B: medullary thyroid cancer, pheochromocytoma, ganglioneuromas	RET	AD	162300
Familial medullary thyroid cancer	RET	AD	155240
Cowden syndrome: multiple hamartomas, thyroid tumors	PTEN	AD	158350
Gardner syndrome: familial colon polyposis, papillary thyroid carcinoma, adrenal cancer	APC	AD	175100
Carney complex: lentigines, pituitary adenomas, pigmented nodular adrenocortical disease with atypical Cushing syndrome	PRKAR1A	AD	188830
Peutz–Jeghers syndrome: mucosal pigmentation, gastrointestinal cancers, thyroid and Leydig cell tumors, other malignancies	STKII	AD	175200
Von Hippel–Lindau disease: renal carcinomas, pheochromocytomas, other tumors	VHL	AD	193300

Table 2.1. (*continued*)

2.1.11. Syndromes with complex endocrine manifestations.

Disorder/phenotype	Gene	Inheritance	OMIM
Autoimmune polyglandular syndrome type 1: adrenal insufficiency, hypoparathyroidism, candidiasis	AIRE	AR	240300
McCune–Albright syndrome: precocious puberty, fibrous dysplasia, café-au-lait spots, hyperthyroidism	GNAS1	Mosaic mutations	174800
Pseudohypoparathyroidism IA: Albright's hereditary osteodystrophy, hypoparathyroidism, hypothyroidism, hypogonadism	GNAS1	Inactivating mutations in maternal allele	103580
Cystic fibrosis: pulmonary obstruction, exocrine and endocrine pancreas dysfunction, infertility, congenital aplasia of vas deferens	CFTR	AR	602421

2.1.12. Selected endocrine disorders with a polygenic/multifactorial etiology.

Disorder/phenotype	Genes/loci	OMIM
Diabetes mellitus type 1	HLA DR3/4-DQ201/0302, HLA DR4/4-DQ0300/03022, HLA DR3/3-DQ0201/0201 Insulin VNTR, NEUROD, CTLA4 Multiple others	222100
Diabetes mellitus type 2	CPN10, PPARγINS, SUR1, IPF1, IRS-1 Multiple others	125853
Hashimoto's thyroiditis	HLA DR3, HLA DR4, HLA DR5, CTLA4, TG Others	140300
Autoimmune polyglandular syndrome type 2: Adrenal insufficiency, autoimmune thyroid disease, diabetes mellitus type 1, other autoimmune disorders	HLA DR3-DQ2, HLA DR4-DQ8 Others	269200

AD, autosomal dominant; AR, autosomal recessive; X, X-chromosomal; Y, Y-chromosomal; CPHD, combined pituitary hormone deficiency.
For further details, see Online Mendelian Inheritance in Man (OMIM) http://www.ncbi.nlm.nih.gov/omim/

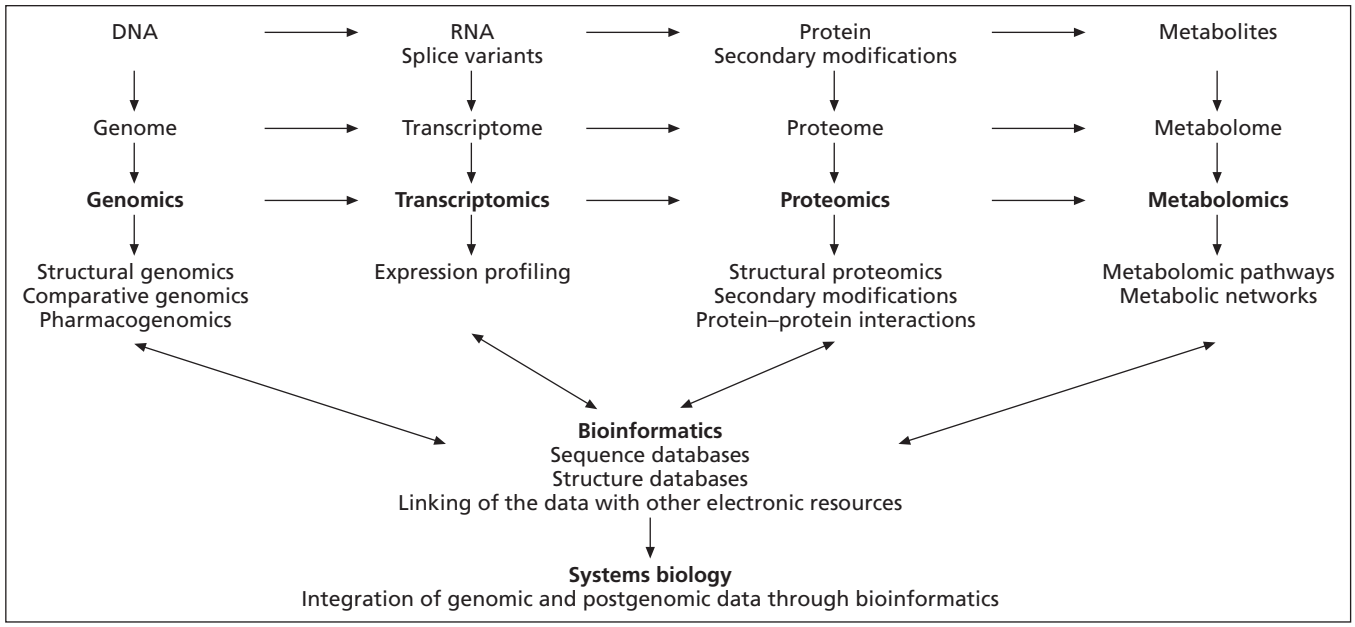

Fig. 2.1. Overview on genomics, transcriptomics, proteomics, metabolomics, bioinformatics, and systems biology.

variations and creating comprehensive models of cellular organization and function. These efforts are based on the expectation that an understanding of the complex and dynamic changes in a biological system may provide insights into pathogenic processes and the development of novel therapeutic strategies and compounds.

Table 2.2. Selected databases relevant for genomic medicine.

Site	Content	URL
National Center for Biotechnology Information (NCBI)	Portal with extensive links to genomic databases, PubMed, OMIM. Links to educational online resources including guidelines for the use of genomic databases	http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM)	Catalog of human genetic disorders	http://www.ncbi.nlm.nih.gov/omim/
National Human Genome Research Institute	Information about the human genome sequence, genomes of other organisms, and genomic research	http://www.genome.gov/
European Bioinformatics Institute (EBI)	Portal to numerous databases and tools for the analysis of sequences and structures	http://www.ebi.ac.uk
DNA Database of Japan	Portal to numerous databases and tools for the analysis of sequences and structures	http://www.ddbj.nig.ac.jp/
UCSC Genome Bioinformatics	Reference sequence of the human and other genomes. Multiple tools for sequence analysis	http://genome.ucsc.edu/
SwissProt	Protein sequence database with description of protein function, domain structure, post-translational modifications, and variants	http://www.ebi.ac.uk/swissprot/index.html
American College of Medical Genetics	Access to databases relevant for the diagnosis, treatment, and prevention of genetic disease	http://www.acmg.net/
Genecards	A database of human genes, their products, and involvement in diseases	http://bioinformatics.weizmann.ac.il/cards/
GeneTests·GeneClinics	Directory of laboratories offering genetic testing	http://www.genetests.org/
Chromosomal Variation in Man	Catalog of chromosomal disorders	http://www.wiley.com/legacy/products/subject/life/borgaonkar/access.html
Mitochondrial disorders	Catalog of disorders associated with mtDNA mutations	http://www.neuro.wustl.edu/neuromuscular/mitosyn.html
DNA repeat sequences and disease	Catalog of disorders associated with DNA repeats	http://www.neuro.wustl.edu/neuromuscular/mother/dnarep.htm
National Organization for Rare Disorders	Catalog of rare disorders including clinical presentation, diagnostic evaluation, and treatment	http://www.rarediseases.org/

DNA, genes, and chromosomes

Structure of DNA

The recognition that DNA carries the genetic information (1944; Avery, MacLeod, McCarty) was followed by the deduction of its structure in 1953 (Watson and Crick). DNA is a double-stranded helix. Each strand consists of a back-bone formed by a deoxyribose-phosphate polymer (Fig. 2.2). Four different nitrogen-containing bases are attached to the sugar ring. They include the purines, adenine (A) and guanine (G), and the pyrimidines, cytosine (C) and thymidine (T). The two strands of DNA are complementary and held together by hydrogen bonds pairing adenine with thymidine and guanine with cytosine. The double-stranded nature of DNA and its strict basepair complementarity permit faithful replication during cell division, as each strand can serve as a template for the synthesis of a new complementary strand referred to as *semiconservative* replication. The complementary structure of the two strands is also of importance as a defense against DNA damage. Damage or loss of a base on the opposite strand can be repaired using the intact strand as a template.

The presence of four different bases provides surprising genetic diversity. In the protein-coding regions of genes, the DNA bases are arranged into codons, triplets of bases that encode one of the 20 different amino acids, or a stop codon. Combinatorial arrangement of the four bases creates 64 different triplets (4^3). Many amino acids, as well as the stop of translation, can be specified by several different codons. Because there are more codons than amino acids, the genetic code is said to be degenerate. Arranging the codons in different combinations of various length permits the generation of a tremendous diversity of polypeptides.

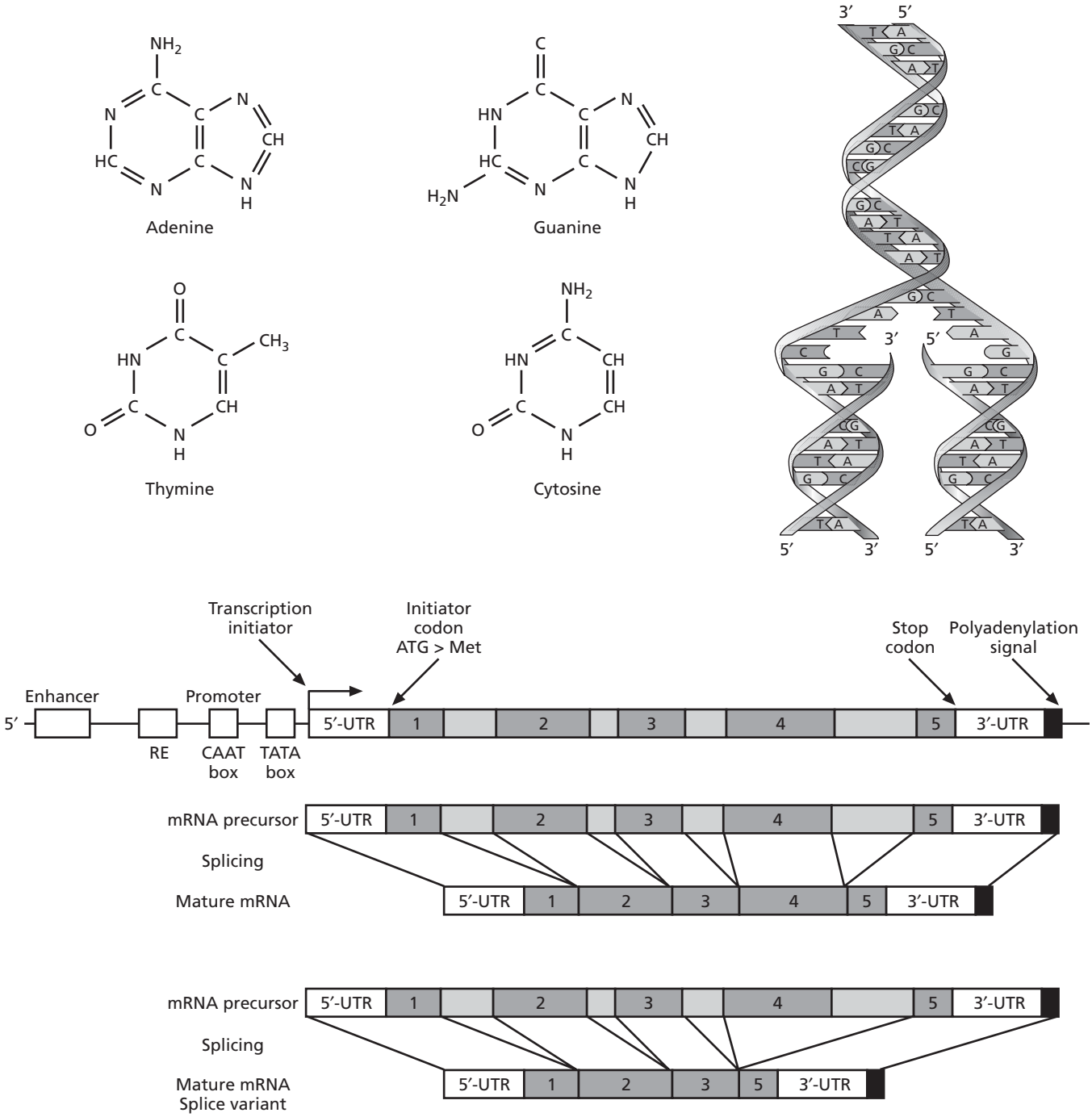

Fig. 2.2. *Top*: The four bases of DNA and the DNA helix. Semiconservative replication generates two identical daughter molecules, each composed of one parental strand and one newly synthesized strand. *Bottom*: General structure of a gene. The 5′ regulatory regions contain enhancer elements, response elements (RE), and often a CAAT box and a TATA box. The exons (dark gray) are separated by introns (light gray). Alternative splicing may generate distinct mRNA products from a given gene and is an important mechanism generating diversity at the protein level. TATA box, TATA-binding protein box; UTR, untranslated region; Met, methionine.

The human genome

The *Human Genome Project*, launched in the 1980s, first led to the creation of genetic and physical maps. A *genetic map* describes the order of genes and defines the position of a gene relative to other loci on the same chromosome. It is constructed by assessing how frequently two markers are inherited together, i.e. *linked*, by linkage studies. Distances of the genetic map are expressed in recombination units or centimorgans (cM). One centimorgan corresponds to a

recombination frequency of 1% between two polymorphic markers and corresponds to approximately 1 Mb of DNA. *Physical maps* indicate the position of a locus or gene in absolute values. Sequence-tagged sites (STSs), any site in a chromosome or genome that is identified by a known unique DNA sequence, are used for physical mapping and, after cloning of DNA fragments, they serve as landmarks for arranging overlapping cloned DNA fragments in the same order as they occur in the genome. These overlapping clones allow the characterization of contiguous DNA sequences (*contigs*). This approach led to high-resolution physical maps by cloning the whole genome into overlapping fragments. The complete DNA sequence of each chromosome provides the highest resolution physical map and, after publication of a first draft of the whole genome in 2000, its sequence analysis was largely completed in 2003.

Human DNA consists of about 3 billion basepairs (bp) of DNA per haploid genome contained in the 23 chromosomes. The smallest chromosome (chromosome 21) contains approximately 50 million bp, the largest (chromosome 1) 250 million bp. The human genome is estimated to contain about 30 000–40 000 *genes*. This number is smaller than the original estimates (up to 100 000 genes), which were derived from the large diversity of proteins. This observation indicates that *alternative splicing* of genes is an important mechanism generating protein diversity.

Historically, genes were identified because they conferred specific traits that are transmitted from one generation to the next. Genes can be defined as functional units that are regulated by transcription and encode RNA (Fig. 2.2). The majority of RNA transcripts consists of mRNA, which is subsequently translated into protein. Other RNA transcripts exert specialized functions, such as transfer of amino acids for polypeptide synthesis (tRNA), contribute to ribosome structure (rRNA), or regulate transcription. Genes account for 10–15% of the genomic DNA. Much of the remaining DNA consists of highly repetitive sequences, the function of which remains poorly understood. These repetitive DNA regions, along with non-repetitive sequences that do not encode genes, may be involved in the packaging of DNA into chromatin and chromosomes or in the regulation of gene expression. Genes are unevenly distributed across the various chromosomes and vary in size from a few hundred to more than 2 million basepairs. The vast majority of genes is located in nuclear DNA, but a few are found in mitochondrial DNA (mtDNA).

A major goal of human genetics aims at understanding the role of common genetic variants in susceptibility to common disorders. This involves identifying, cataloging, and characterizing gene variants, followed by performing association studies. The variants include short repetitive sequences in regulatory or coding regions and single-nucleotide polymorphisms (SNPs). SNPs occur roughly every 300 bp, and most are found outside coding regions. SNPs within a coding sequence can be synonymous (i.e. not altering the amino acid code) or non-synonymous. There are roughly 3 million differences between the DNA sequences of any two copies of the human genome, and current efforts focus on increasing the density of the SNP map of the human genome.

Structure and function of genes

The structure of a typical gene consists of regulatory regions followed by exons and introns and downstream untranslated regions (Fig. 2.2). The regulatory regions controlling gene expression most commonly involve sequences upstream (5′) of the transcription start site, although there are examples of control elements located within introns or downstream of the coding region of a gene. Exons designate the regions of a gene that are eventually spliced together to form the mature mRNA. Introns refer to the intervening regions between the exons that are spliced out of precursor RNAs during RNA processing. A gene may generate various transcripts through the use of alternative promoters and/or alternative splicing of exons (Fig. 2.2). These mechanisms contribute to the diversity of proteins and their functions.

The regulatory DNA sequences of a gene, which are typically located upstream of the coding region, are referred to as the *promoter*. The promoter region contains specific sequences, *response elements*, that bind transcription factors. Some of these are ubiquitous; others are cell specific. Gene expression is controlled by additional regulatory elements, *enhancers* and *locus control regions*, that may be located far away from the promoter region. The transcription factors that bind to the promoter and enhancer sequences provide a code for regulating transcription that is dependent on developmental state, cell type, and endogenous and exogenous stimuli. Transcription factors interact with other nuclear proteins, *co-activators*, and *co-repressors*, and generate large regulatory complexes that ultimately activate or repress transcription.

In the eukaryotic cell nucleus, DNA is packaged by histones into nucleosomes. This packaging inhibits transcription by impeding the binding of transcriptional activators to their cognate DNA sites. Therefore, alterations in chromatin structure typically precede gene transcription. Repression is often associated with histone deacetylation. Conversely, activation of transcription may involve histone acetylation, which results in the remodeling of chromatin and subsequent binding of *trans*-acting factors to DNA (Fig. 2.3). Once bound to DNA, the transcription factor complexes recruit proteins that form the basal transcription complex including RNA polymerase. Gene transcription occurs with the synthesis of RNA from the DNA template by RNA polymerase. mRNA is encoded by the coding strand of the DNA double helix and is translated into proteins by ribosomes. The transcriptional termination signals reside in the 3′ region of a gene. A polyadenylation signal encodes a poly-A tail, which influences mRNA export to the cytoplasm, stability, and translation efficiency.

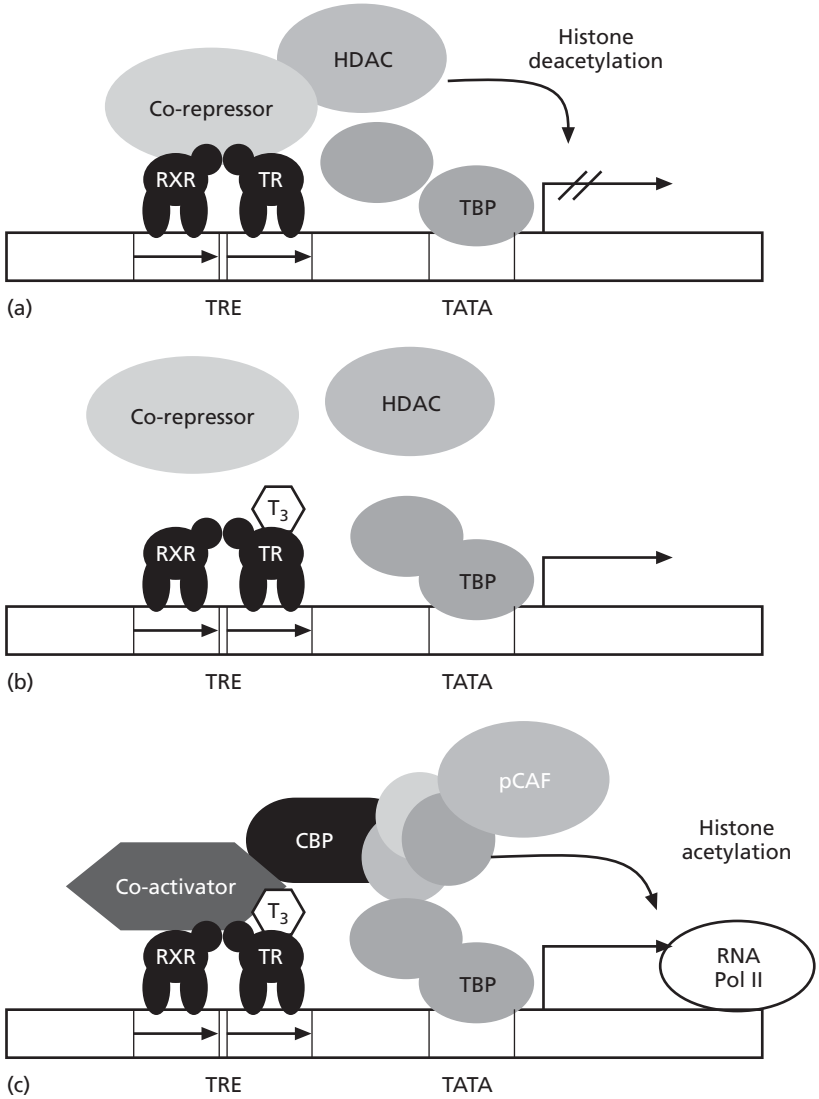

Fig. 2.3. Control of gene transcription by the ligand-dependent thyroid hormone receptor (TR) interacting with co-repressors and co-activators. In the absence of triiodothyronine (T_3), the TR binds to a thyroid hormone response element (TRE) in conjunction with the retinoid X receptor (RXR). Co-repressors associated with the TR recruit histone deacetylases (HDAC), and transcription is silenced. After binding of T_3, the TR undergoes a conformational alteration, the co-repressors dissociate, and co-activators can interact with the receptor. This, in turn, leads to binding of histone acetylases such as pCAF that modify chromatin structure and enable transcription. In the case of resistance to thyroid hormone, mutations in TRβ do not permit release of the co-repressors and lead to silencing of gene transcription in target genes. TBP, TATA-binding protein; CBP, CREB-binding protein (CREB, cyclic AMP response element-binding protein); RNA Pol II, RNA polymerase II.

Transcription factors account for about 30% of all expressed genes. Mutations in transcription factors cause a significant number of endocrine and non-endocrine genetic disorders. Because a given set of transcription factors may be expressed in various tissues, it is not uncommon to observe a syndromic phenotype. The mechanism by which transcription factor defects cause disease often involves *haploinsufficiency*, a situation in which a single copy of the normal gene is incapable of providing sufficient protein production to assure normal function. Biallelic mutations in such a gene may result in a more pronounced phenotype. For example, monoallelic mutations in the transcription factor HESX1 (RPX) result in various constellations of pituitary hormone deficiencies, and the phenotype is variable among family members with the same mutation. Inactivating mutations of both alleles of HESX1 cause familial septo-optic dysplasia and combined pituitary hormone deficiency (Table 2.1.2).

Gene expression is also influenced by *epigenetic events*, such as X-inactivation and imprinting, i.e. a marking of genes that results in monoallelic expression depending on their parental origin. In this situation, DNA methylation leads to silencing, i.e. suppression of gene expression. Genomic imprinting plays an important role in the pathogenesis of several genetic disorders such as Prader–Willi syndrome and Albright hereditary osteodystrophy (AHO).

Chromosomes

The normal diploid number of chromosomes in humans is 46, consisting of two homologous sets of 22 autosomes (chromosomes 1 to 22) and a pair of sex chromosomes. Females have two X chromosomes (XX), whereas males have one X and one Y chromosome (XY). As a consequence of *meiosis*, germ cells – sperm or oocytes – are haploid and contain one set of

22 autosomes and one of the sex chromosomes. At the time of fertilization, the pairing of the homologous chromosomes from the mother and father results in reconstitution of the diploid genome. With each cell division, i.e. *mitosis*, chromosomes are replicated, paired, segregated, and divided into two daughter cells.

Replication of DNA, mitosis, and meiosis

Genetic information in DNA is transmitted to daughter cells during two different types of cell division, *mitosis* and *meiosis*. Somatic cells divide by mitosis, allowing the diploid ($2n$) genome to replicate itself during cell division. The formation of germ cells, sperm and ova, requires meiosis, a process that leads to the reduction of the diploid ($2n$) set of chromosomes to the haploid state ($1n$).

Before mitosis, cells exit the resting or G_0 state and enter the cell cycle. After traversing a critical checkpoint in G_1, cells undergo DNA synthesis (S phase), during which the DNA in each chromosome is replicated, yielding two pairs of sister chromatids ($2n$ to $4n$). The process of DNA synthesis requires strict fidelity in order to avoid transmitting errors to subsequent generations of cells. Therefore, genetic abnormalities of enzymes that are involved in DNA mismatch repair predispose to neoplasia because of the rapid acquisition of additional mutations (e.g. xeroderma pigmentosa, Bloom syndrome, ataxia telangiectasia, and hereditary non-polyposis colon cancer). After completion of DNA synthesis, cells enter G_2 and progress through a second checkpoint before entering mitosis. Subsequently, the chromosomes condense and are aligned along the equatorial plate at metaphase. The two identical sister chromatids, held together at the centromere, divide and migrate to opposite poles of the cell. After the formation of a nuclear membrane around the two separated sets of chromatids, the cell divides forming two daughter cells with a diploid ($2n$) set of chromosomes.

Meiosis occurs only in germ cells of the gonads. It involves two steps of cell division that reduce the chromosome number to the haploid state. *Recombination*, the exchange of DNA between homologous paternal and maternal chromosomes during the first cell division, is essential for generating genetic diversity. Each chromosome pair forms two sister chromatids ($2n$ to $4n$). This is followed by an exchange of DNA between homologous chromosomes through the process of *crossover*. In most instances, there is at least one crossover on each chromosomal arm. This recombination process occurs more frequently in female meiosis than in male meiosis. Subsequently, the chromosomes segregate randomly. As there are 23 chromosomes, this can generate 2^{23} (> 8 million) possible combinations of chromosomes. Together with the genetic exchanges that occur during recombination through crossover, chromosomal segregation generates tremendous diversity, and therefore each gamete is genetically unique. The processes of recombination and independent segregation of chromosomes provide the foundation for performing linkage analyses, in which the inheritance of *linked* genes is correlated with the presence of a disease or genetic trait. After the first meiotic division, which results in two daughter cells ($2n$), the two chromatids of each chromosome separate during a second meiotic division to yield four gametes with a haploid chromosome set ($1n$). Through fertilization of an egg by a sperm, the two haploid sets are combined, thereby restoring the diploid state ($2n$) in the zygote.

Analysis of chromosomes and DNA

Analyses of large alterations in the genome are possible using cytogenetics, fluorescent *in situ* hybridization (FISH), and Southern blotting. More discrete sequence alterations rely heavily on the use of the *polymerase chain reaction* (PCR) (Fig. 2.4). PCR permits rapid genetic testing and mutational analysis with small amounts of DNA extracted from solid tissues, nucleated blood cells, leukocytes, buccal cells, or hair roots. Reverse transcription PCR (RT-PCR) transcribes RNA into a complementary DNA strand, which can then be amplified by PCR. RT-PCR can be used for sequence analyses of the coding regions and to detect absent or reduced levels of mRNA expression resulting from a mutated allele.

Screening for point mutations can be performed by numerous methods, such as sequencing of DNA fragments amplified by PCR, recognition of mismatches between nucleic acid duplexes, or electrophoretic separation of single- or double-stranded DNA. The majority of traditional diagnostic methods are gel based. Novel techniques for the analysis of mutations, genetic mapping, and mRNA expression profiles are being developed. Chip techniques allow hybridization of DNA or RNA to hundreds of thousands of probes simultaneously. Microarrays are being used clinically for mutational analysis of several human disease genes, for the identification of viral sequence variations, and for large-scale analyses of transcripts. These techniques provide the foundation to expand from a focus on single genes to analyses at the scale of the genome. A variety of mass spectrometry-based techniques is emerging for high-throughput applications, such as sequencing, genotyping, and analyses of proteins and proteomes.

Cloning of DNA

Cloning refers to the isolation of a DNA fragment that can be propagated indefinitely. Cloning is essential for DNA sequencing, nucleic acid hybridization studies, expression of recombinant proteins, and functional characterization of mutated genes. The cloning of DNA involves the insertion of a DNA fragment into a cloning vector, followed by the propagation of the recombinant DNA in a host cell. The process of DNA insertion relies heavily on the use of restriction

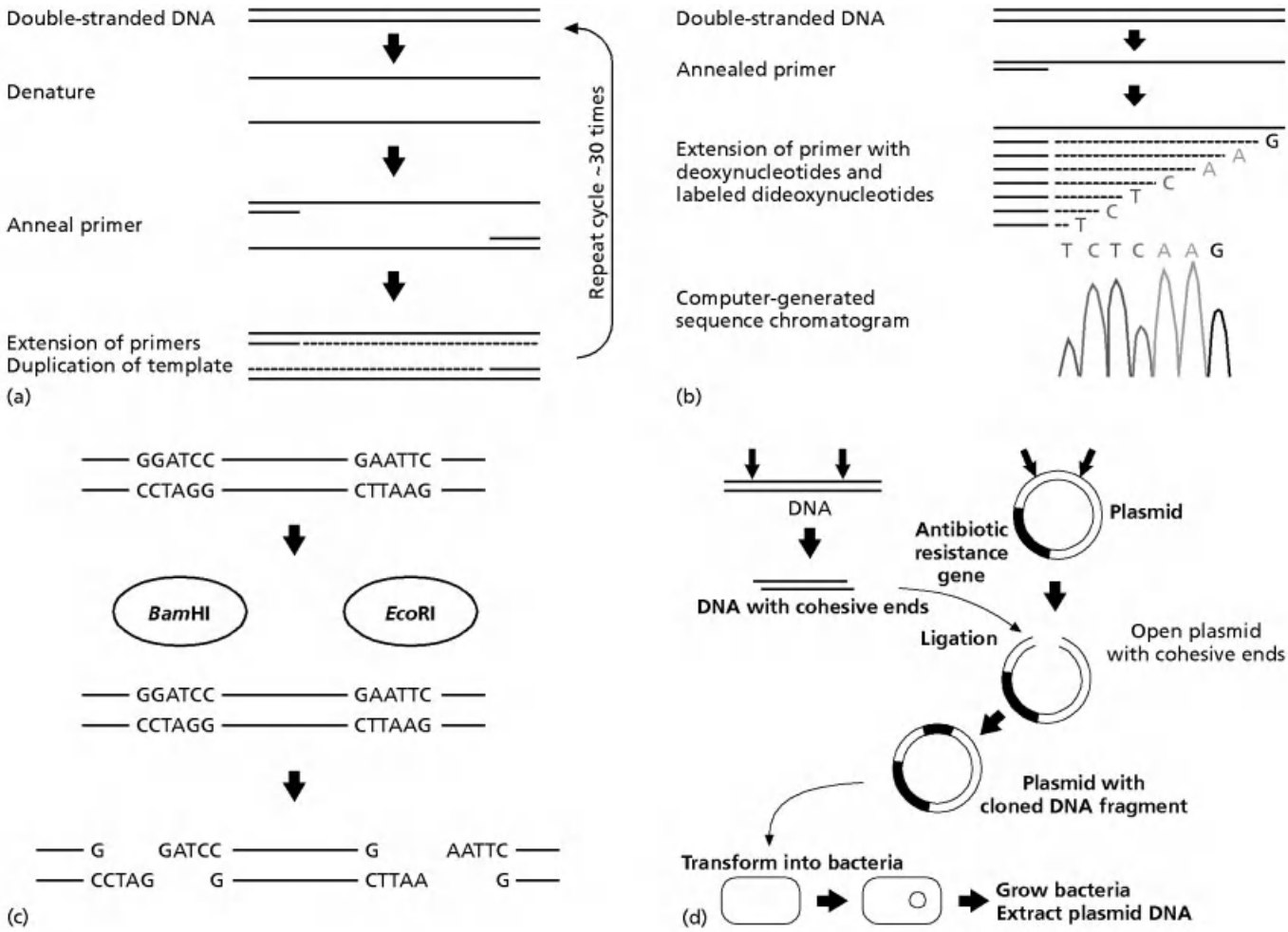

Fig. 2.4. Selected techniques used for the analysis of DNA. *Upper left*: The polymerase chain reaction (PCR) generates multiple copies of a DNA segment. After denaturing the double-stranded DNA, complementary synthetic oligonucleotide primers are annealed on each side of the region to be amplified. A heat-stable polymerase then extends the oligonucleotides and synthesizes complementary strands. This cycle is repeated about 30 times and results in the doubling of the copies after every cycle. *Lower left*: Sequencing of DNA with the Sanger method. A primer complementary to one of the strands is extended with a polymerase. In addition to deoxynucleotides, the reaction also contains dideoxynucleotides (ddATP, ddCTP, ddGTP, ddTTP). Every time a dideoxynucleotide gets incorporated instead of a deoxynucleotide, the synthesis of the molecule stops. This generates extension products of variable length. The four dideoxynucleotides can be distinguished by using different fluorophores, and the sequence can be deciphered after gel or capillary electrophoresis. The computer generates a chromatogram for easy visualization of the sequence. *Upper right*: Digestion of DNA with restriction endonucleases. Restriction enzymes recognize specific sequences, usually palindromes of 4–6 bp. Cleavage of DNA with a particular enzyme digests the DNA into a characteristic collection of fragments. These fragments can be cloned into a variety of vectors. Restriction analysis can also be used for the identification of certain variants (restriction fragment length polymorphisms, RFLPs). *Lower right*: Cloning of a DNA fragment into a plasmid vector. The DNA fragment to be cloned and the plasmid vector are digested with the same restriction enzymes. The cohesive ends are then ligated, and the plasmid, which typically contains an antibiotic resistance gene, is inserted into bacteria. After replication of the bacteria, the plasmid DNA can be harvested in large amounts and can be used for further analysis or manipulation.

enzymes, which cleave DNA at highly specific sequences (usually 4 to 6 bp in length). Restriction enzymes can generate complementary sequences at the ends of the DNA fragment, which allow them to be ligated efficiently to the plasmid vector.

The most commonly used cloning strategy involves inserting a DNA fragment into bacterial plasmids (Fig. 2.4). Plasmids are small, autonomously replicating, circular DNA molecules that propagate independently from the chromosome in bacterial cells. Because plasmids contain genes that confer resistance to antibiotics, their presence in the host cell can be used for selection and DNA amplification. A variety of other vectors and hosts is now used for cloning. Many are used for creating *libraries*, large collections of DNA clones.

A *genomic library* represents clones derived from genomic DNA. These overlapping DNA fragments represent the entire genome and can be arranged according to their linear order. *cDNA libraries* reflect clones derived from mRNA, typically from a particular tissue source. A cDNA library from the thyroid gland, for example, contains predominantly copies of mRNA expressed specifically in thyroid follicular

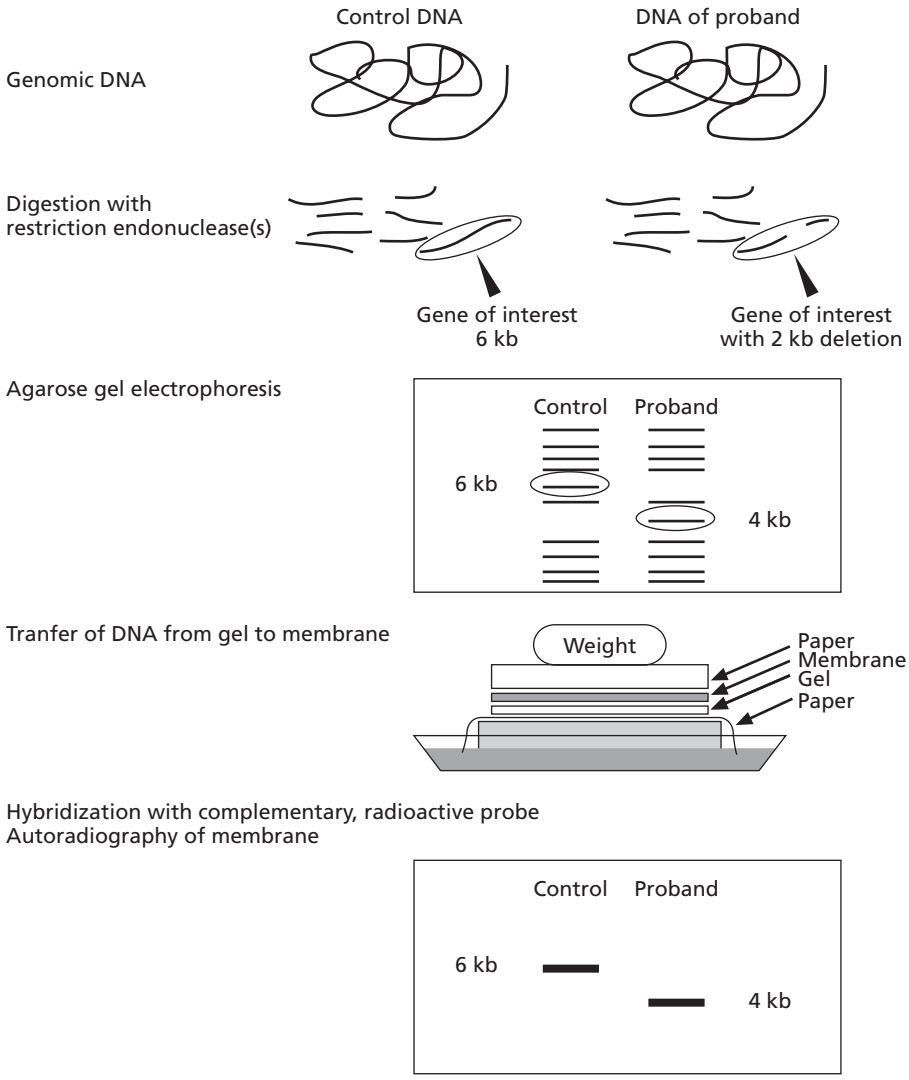

Fig. 2.5. Southern blot analysis. Southern blot analysis can be used for the detection of RFLPs or deletions/insertions in genomic DNA. In this example, the proband has a large deletion in the gene of interest. After digestion of the genomic DNA with restriction endonucleases, the fragments are separated on an agarose gel and then transferred to a membrane. This membrane is hybridized to a complementary, labeled probe recognizing the gene of interest. The difference in size on the autoradiography reveals a fragment that is only 4 kb in the proband, indicating the presence of a 2 kb deletion.

cells in addition to those that are expressed ubiquitously. For this reason, a thyroid cDNA library will be enriched with thyroid-specific gene products and will differ from cDNA libraries generated from other tissues.

Nucleic acid hybridization

Hybridization of nucleic acids takes advantage of the fact that the two complementary strands of nucleic acids hybridize to one another with high specificity. Using a complementary probe, a specific nucleic acid sequence (DNA or RNA) can be detected in a complex background of other sequences. This technique is used for the detection of DNA and RNA through Southern and Northern blotting screening of libraries and has led to the development of microarray DNA chips.

Southern blotting is used to analyze whether genomic regions or genes have been deleted or rearranged (Fig. 2.5). It is also used to detect restriction fragment length polymorphisms (RFLPs), polymorphisms that can be distinguished through the use of endonuclease restriction enzymes. After digestion of genomic DNA with restriction enzymes, the DNA fragments are transferred to a membrane and detected by hybridization with specific radioactively labeled DNA probes.

Northern blots are used to analyze patterns and levels of gene expression in different tissues. The mRNA is separated on a gel and transferred to a membrane. The transcripts of interest are then detected using specific radiolabeled DNA probes. This technique has, in part, been replaced by more sensitive and comprehensive methods such as RT-PCR and gene expression microarrays. Microarrays consist of hundreds or thousands of hybridization probes spotted on glass slides (Fig. 2.6). They can consist of DNA oligonucleotides or cDNA probes derived from EST (expressed sequence tag) databases. An EST is a unique DNA sequence derived from a cDNA library; it thus represents a sequence that has been transcribed in a given tissue at a specific (developmental) stage.

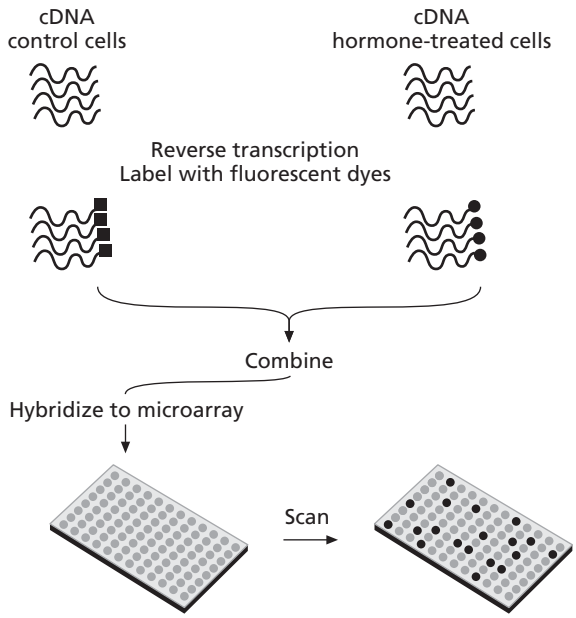

Fig. 2.6. Principle of a microarray experiment. A large number of probes, cDNA sequences, or DNA oligonucleotides are spotted on a glass slide. RNA is extracted from a control sample and a test sample, for example a tumor or treated cells. After differential labeling of the two RNA populations with fluorescent probes, the samples are mixed and hybridized to the probes on the slide. The slide is then scanned once for each fluorophore. The intensity of the emitted signal on each spot permits calculation of the ratios of each RNA that binds to the respective probe.

For a microarray experiment, RNA pools from two or more cellular phenotypes (e.g. normal tissue compared with cancerous tissue) are used to generate cDNAs that are differentially labeled. These are then purified, pooled, and hybridized to the arrays. After hybridization, the slides are scanned, and independent images are obtained for the distinct groups. This method permits comprehensive analyses of gene regulation. The detection of differentially regulated genes provides insights into developmental mechanisms or metabolic alterations and generates fingerprints of different types of malignancies that can be useful for classification, prognosis, and treatment.

Cytogenetics and FISH analysis

Chromosome smears can be prepared easily from amniotic cells, blood, bone marrow, or fibroblasts that undergo cell division during short-term cell culture. After chemical or enzymatic treatment, which reveals the characteristic bands, the number of chromosomes and gross structural abnormalities can be determined. The advent of FISH has changed the field of cytogenetics because it permits the detection of more subtle abnormalities, such as microdeletions and small translocations. FISH takes advantage of the complementarity of DNA. By labeling the probe with a fluorescent marker, it can be detected easily (see Plate 1, facing p. 148). This allows staining of specific DNA segments *in situ* on a metaphase chromosome spread or within a cell nucleus. FISH is widely used for diagnostic cytogenetic analyses and in the mapping of genes. FISH probes can consist of a single contiguous genomic sequence or a mixture of two or multiple probes. This permits staining of specific segments of a chromosome, e.g. a chromosome arm, or whole chromosomes. The staining of chromosomes with probe mixtures is often referred to as "chromosome painting." *Spectral karyotyping* (SKY), the use of 24 distinct chromosome painting probe mixtures, allows individual chromosomes to be stained and, because each chromosome is easily identified by its emission of a distinct wavelength, structural rearrangements are easily detectable.

Comparative genome hybridization is used to measure differences in copy numbers of a specific chromosomal segment. DNA from two different sources is labeled with different fluorescent dyes, mixed, and used for painting normal metaphase chromosomes. The painting with the two dyes will be equal over all regions of the chromosomes present in equal amounts in the two DNA probes. If a segment of a chromosome is duplicated or deleted in one DNA sample compared with the other, the relative intensity of hybridization will shift from the expected balanced ratio (1:1). Comparative genome hybridization is particularly useful for the detection of somatic chromosomal abnormalities in neoplasias.

The polymerase chain reaction (PCR)

PCR has changed the way in which DNA analyses are performed and has become a cornerstone of molecular biology and genetic analysis (K. Mullis, Nobel prize 1998). PCR provides a rapid way of selectively amplifying specific DNA fragments *in vitro* (Fig. 2.4). In a first step, DNA is denatured by heat. This is followed by annealing of oligonucleotides (primers). Primers are complementary to the opposite strands and flank the DNA region to be amplified. A heat-resistant polymerase then extends the primers. Repeated cycles of this process lead to an exponential amplification of the region of interest. This *amplicon* can then be used for further analyses, for example sequence analysis.

PCR can be used to amplify DNA from very small samples, including single cells, and from a variety of tissue sources, including blood samples, biopsies, surgical or autopsy specimens, or cells from hair or saliva. PCR can also be used to study mRNA. In this case, reverse transcriptase (RT) is used first to convert the RNA to DNA, which can then be amplified by PCR. This procedure, commonly known as RT-PCR, is useful as a quantitative measure of gene expression.

PCR provides a key component of molecular diagnosis. It provides a strategy for the rapid amplification of DNA or mRNA to search for mutations by several techniques, including DNA sequencing. PCR is also used for the amplification of highly polymorphic di- or trinucleotide repeat sequences,

microsatellites, which allow various polymorphic alleles to be traced in genetic linkage or association studies. PCR is used increasingly to diagnose various microbial pathogens.

DNA sequencing

DNA sequencing is automated. The most commonly used strategy is based on the Sanger method, in which dideoxynucleotides are used to terminate DNA polymerization randomly at each of the four bases (A, G, T, C) (Fig. 2.4). After separating the array of terminated DNA fragments using high-resolution gel or capillary electrophoresis, it is possible to deduce the DNA sequence by examining the progression of fragment lengths generated in each of the four nucleotide reactions. The use of fluorescently labeled dideoxynucleotides allows automated detection of the different bases and direct computer analysis of the DNA sequence. Efforts are under way to develop faster, more cost-effective DNA and comprehensive sequencing techniques using, among others, mass spectrometry and DNA chips.

Genetic linkage and association

There are two primary strategies for mapping genes that cause or increase susceptibility to human disease, *linkage* and *association* studies.

Genetic linkage refers to the fact that genes are physically connected, i.e. *linked*, to one another along the chromosomes (see Plate 2, facing p. 148). Two principles are essential for understanding the concept of genetic linkage. First, when two genes are close together on a chromosome, they are usually transmitted together, unless a recombination event separates them. Secondly, the odds of a crossover or recombination event between two linked genes is proportional to the distance that separates them. Thus, genes that are further apart are more likely to undergo a recombination event than genes that are very close together. The detection of chromosomal loci that segregate with a disease by linkage has been widely used to identify the gene responsible for the disease by *positional cloning*, a technique of isolating a gene from the knowledge of its map location. It has also been used to predict the odds of disease gene transmission in genetic counseling.

Polymorphisms are essential for linkage studies because they provide a means to distinguish the maternal and paternal chromosomes in an individual. On average, one out of every 300 bp varies from one person to the next. Although this degree of variation seems low (99.9% identical), it means that more than 3 million sequence differences exist between any two unrelated individuals, and the probability that the sequence at such loci will differ on the two homologous chromosomes is high (often > 70–90%). These sequence variations include a variable number of tandem repeats (VNTRs), microsatellites [also referred to as short tandem repeats (STRs)], and SNPs. Most microsatellite markers consist of di-, tri-, or tetranucleotide repeats that can be measured readily using PCR and primers that reside on either side of the repeat sequences (Plate 2, facing p. 148). Analyses of SNPs using DNA chips provide a promising means for rapid analysis of genetic variation and linkage.

In order to identify a chromosomal locus that segregates with a disease, it is necessary to determine the genotype or haplotype of DNA samples from one or several pedigrees. A haplotype designates a group of alleles that are closely linked, i.e. in close proximity on a chromosome, and that is usually inherited as a unit. After characterizing the alleles, one can assess whether certain marker alleles co-segregate with the disease. Markers closest to the disease gene are less likely to undergo recombination events and therefore receive a higher linkage score. Linkage is expressed as a *lod* (*logarithm of odds*) *score*, i.e. the ratio of the probability that the disease and marker loci are linked rather than unlinked. Lod scores of +3 (1000:1) are generally accepted as supporting linkage.

Allelic association refers to a situation in which the frequency of an allele is significantly increased or decreased in a particular disease. Linkage and association differ in several respects. Genetic linkage is demonstrable in families or sibships. Association studies compare a population of affected individuals with a control population. Association studies are often performed as case–control studies that include unrelated affected individuals and matched controls, or as family-based studies that compare the frequencies of alleles that are transmitted to affected children. Allelic association studies are useful for identifying susceptibility genes in complex disorders. When alleles at two loci occur more frequently in combination than would be predicted based on known allele frequencies and recombination fractions, they are said to be in *linkage disequilibrium*.

Medical genetics

Mutations and human disease

Structure of mutations

Mutations are an important cause of genetic diversity as well as disease. A *mutation* can be defined as any change in the nucleotide sequence of DNA regardless of its functional consequences (Fig. 2.7). Mutations are structurally diverse. They can affect one or a few nucleotides, consist of gross numerical or structural alterations in individual genes or chromosomes, or involve the entire genome. Mutations can occur in all domains of a given gene. Large deletions may affect a portion of a gene or an entire gene or, if several genes are involved, they may lead to a *contiguous gene syndrome*. Occasionally, mispairing of homologous sequences leads to *unequal crossover*. This results in gene duplication on one of the chromosomes and gene deletion on the other chromosome.

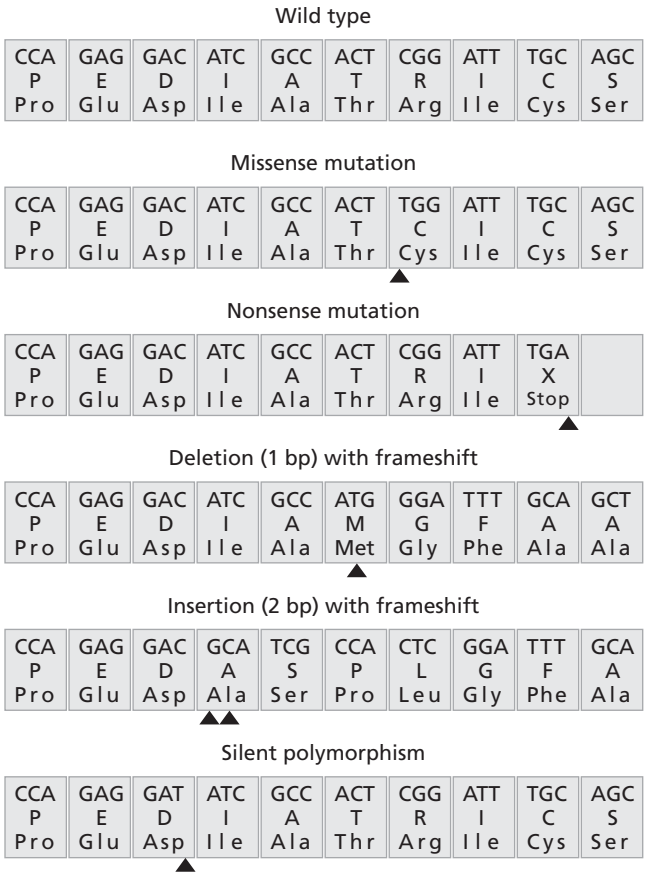

Fig. 2.7. Examples of mutations. The coding strand is shown with the encoded amino acid sequence in the one-letter code and the three-letter code.

For example, a significant fraction of growth hormone (*GH*) gene deletions involves unequal crossing-over (Table 2.1.2). The *GH* gene is a member of a large gene cluster that includes a growth hormone variant gene as well as several structurally related chorionic somatomammotrophin genes and *pseudogenes*, which are highly homologous but functionally inactive relatives of a normal gene. Because such gene clusters contain multiple homologous DNA sequences arranged along the same chromosome, they are particularly prone to undergo recombination and, consequently, gene duplication or deletion.

Unequal crossing-over between homologous genes can result in fusion gene mutations, as illustrated, for example, by glucocorticoid-remediable aldosteronism (GRA). GRA is caused by a rearrangement involving the genes that encode aldosterone synthase (*CYP11B2*) and steroid 11β-hydroxylase (*CYP11B1*), normally arranged in tandem on chromosome 8q (Table 2.1.5). Because these two genes are 95% identical, they are predisposed to undergo unequal recombination. The rearranged gene product contains the regulatory regions of 11β-hydroxylase upstream to the coding sequence of aldosterone synthetase. The latter enzyme is then expressed in the adrenocorticotrophic hormone (ACTH)-dependent zona fasciculata of the adrenal gland, resulting in overproduction of mineralocorticoids and hypertension.

Gene conversion refers to a non-reciprocal exchange of homologous genetic information by which a recipient strand of DNA receives information from another strand having an allelic difference. The original allele on the recipient strand is converted to the new allele as a consequence of this event. These alterations may range from a few to several thousand nucleotides. Gene conversion often involves exchange of DNA between a gene and a related pseudogene. For example, the 21-hydroxylase gene (*CYP21A*) is adjacent to a non-functional pseudogene. Many of the nucleotide substitutions found in the *CYP21A* gene in patients with congenital adrenal hyperplasia correspond to sequences present in the pseudogene, suggesting gene conversion as the underlying mechanism of mutagenesis. In addition, mitotic gene conversion has been suggested as a mechanism to explain revertant mosaicism in which an inherited mutation is "corrected" in certain cells.

Trinucleotide repeats may be unstable and expand beyond a critical number. Mechanistically, the expansion is thought to be caused by unequal recombination and slipped mispairing. A premutation represents a small increase in trinucleotide copy number. In subsequent generations, the expanded repeat may increase further in length. This increasing expansion is referred to as *dynamic mutation*. It may be associated with an increasingly severe phenotype and earlier manifestation of the disease (*anticipation*). Trinucleotide expansion was first recognized as a cause of the fragile X syndrome, one of the most common causes of mental retardation. Malignant cells are also characterized by genetic instability, indicating a breakdown in mechanisms that regulate DNA repair and the cell cycle.

Mutations involving single nucleotides are referred to as *point mutations* (see Plate 3, facing p. 148). Substitutions are called *transitions* if a purine is replaced by another purine base (A to G) or if a pyrimidine is replaced by another pyrimidine (C to T). Changes from a purine to a pyrimidine or vice versa are referred to as *transversions*. Certain DNA sequences, such as successive pyrimidines or CG dinucleotides, are particularly susceptible to mutagenesis. Therefore, certain types of mutations (C to T or G to A) are relatively common. Moreover, the nature of the genetic code results in over-representation of certain amino acid substitutions. If the DNA sequence change occurs in a coding region and alters an amino acid, it is called a *missense mutation*. Depending on the functional consequences of such a missense mutation, amino acid substitutions in different regions of the protein can lead to distinct phenotypes. Small deletions and insertions alter the reading frame if they do not represent a multiple of three bases. Such "*frameshift*" mutations lead to an entirely altered carboxy-terminus. Mutations may also be found in the regulatory sequences of genes and result in reduced gene

transcription. Mutations in intronic sequences or in exon junctions may destroy or create splice donor or splice acceptor sites.

Some mutations are lethal, some have less deleterious yet recognizable consequences, and some confer evolutionary advantage. Mutations occurring in germ cells can be transmitted to the progeny. Alternatively, mutations can occur during embryogenesis or in somatic tissues. Mutations that occur during development lead to *mosaicism*, a situation in which tissues are composed of cells with different genetic constitutions, as illustrated by Turner syndrome or McCune–Albright syndrome (Tables 2.1.1 and 2.1.11). If the germline is mosaic, a mutation can be transmitted to some progeny but not others, which sometimes leads to confusion in assessing the pattern of inheritance. Other somatic mutations are associated with neoplasia because they confer a growth advantage to cells by activating (proto)oncogenes or inactivating tumor suppressor genes. Epigenetic events, heritable changes that do not involve changes in gene sequence (e.g. altered DNA methylation), may also influence gene expression or facilitate genetic damage.

Polymorphisms are sequence variations that have a frequency of at least 1% and do not usually result in an overt phenotype. Often they consist of single basepair substitutions that do not alter the protein coding sequence because of the degenerate nature of the genetic code, although some might alter mRNA stability, translation, or the amino acid sequence. Silent base substitutions and SNPs are encountered frequently during genetic testing and must be distinguished from true mutations that alter protein expression or function. Some SNPs or combinations of SNPs may, however, play a pathogenic role in complex disorders by conferring susceptibility for the development of the disease.

Functional consequences of mutations

Mutations can broadly be classified as gain-of-function and loss-of-function mutations. The consequences of an altered protein sequence often need experimental evaluation *in vitro* to determine that the mutation alters protein function. The appropriate assay depends on the properties of the protein and may, for example, involve enzymatic analyses, electromobility shift experiments, or reporter gene assays (Plate 3, facing p. 148).

Gain-of-function mutations are typically dominant and result in phenotypic alterations when a single allele is affected. Inactivating mutations are usually recessive, and an affected individual is homozygous or compound heterozygous (i.e. carrying two different mutant alleles) for the disease-causing mutations. Mutation in a single allele can result in *haploinsufficiency*, a situation in which one normal allele is not sufficient to maintain a normal phenotype. Haploinsufficiency is a commonly observed mechanism in diseases associated with mutations in transcription factors. For example, monoallelic mutations in the transcription factor TTF1 (NKX2.1) are associated with transient congenital hypothyroidism, respiratory distress, and ataxia (Table 2.1.3).

The clinical features among patients with an identical mutation in a transcription factor often vary significantly. One mechanism underlying this variability consists of the influence of modifying genes. Haploinsufficiency can affect the expression of rate-limiting enzymes. For example, in MODY 2 heterozygous glucokinase, mutations result in haploinsufficiency with a higher threshold for glucose-dependent insulin release and mild hyperglycemia (Table 2.1.6).

Mutation of a single allele can result in loss-of-function due to a dominant-negative effect. In this case, the mutated allele interferes with the function of the normal gene product by several different mechanisms. The mutant protein may interfere with the function of a multimeric protein complex, as illustrated by Liddle syndrome, which is caused by mutations in the β- or γ-subunit (SCCN1B, SCCN1G) of the renal sodium channel (Table 2.1.8). In thyroid hormone resistance, mutations in the thyroid hormone receptor β (TRβ,THRB) lead to impaired T_3 binding; the receptors cannot release co-repressors, and they silence transcription of target genes (Table 2.1.3). The mutant protein can be cytotoxic, as in autosomal-dominant neurohypophyseal diabetes insipidus, in which abnormal folding leads to retention in the endoplasmic reticulum and degeneration of the AVP-secreting neurons (Table 2.1.2).

An increase in dosage of a gene product may also result in disease. For example, duplication of the *DAX1* (*NR0B1*) gene results in dosage-sensitive sex reversal.

Genotype and phenotype

An observed trait is referred to as a *phenotype*. The genetic information defining the phenotype is called the *genotype*. Alternative forms of a gene or a genetic marker are referred to as alleles, which may be polymorphic variants of nucleic acids that have no apparent effect on gene expression or function. In other instances, these variants may have subtle effects on gene expression, thereby conferring adaptive advantages or increased susceptibility. Commonly occurring allelic variants may reflect mutations in a gene that clearly alter its function, as illustrated, for example, by the ΔF508 deletion in the cystic fibrosis conductance regulator (CFTR).

Because each individual has two copies of each chromosome, an individual can have only two alleles at a given locus. However, there can be many different alleles in the population. The normal or common allele is usually referred to as *wild type*. When alleles at a given locus are identical, the individual is *homozygous*. Inheriting such identical copies of a mutant allele occurs in many autosomal-recessive disorders, particularly in circumstances of consanguinity. If the alleles are different, the individual is *heterozygous* at this locus. If two different mutant alleles are inherited at a given locus, the individual is referred to as a *compound heterozygote*.

Hemizygous is used to describe males with a mutation in an X-chromosomal gene or a female with a loss of one X-chromosomal locus.

A *haplotype* refers to a group of alleles that are closely linked together at a genomic locus. Haplotypes are useful for tracking the transmission of genomic segments within families and for detecting evidence of genetic recombination, if the crossover event occurs between the alleles.

Allelic and phenotypic heterogeneity

Allelic heterogeneity refers to the fact that different mutations in the same genetic locus can cause an identical or similar phenotype. *Phenotypic heterogeneity* occurs when more than one phenotype is caused by allelic mutations. For example, different mutations in the androgen receptor can result in a wide phenotypic spectrum. In some cases, the receptor is deleted or mutated in a manner that inactivates it completely. In a karyotypic male, this leads to *testicular feminization*. In contrast, the phenotype may be milder if the androgen receptor is only partially inactivated. In these patients, the phenotype may include infertility, gynecomastia, or epispadias. Allelic heterogeneity is explained by the fact that many different mutations are capable of altering protein structure and function. Allelic heterogeneity creates a significant problem for genetic testing because one must often examine the entire genetic locus for mutations, as these can differ in each patient.

Locus or non-allelic heterogeneity and phenocopies

Non-allelic or *locus heterogeneity* refers to the situation in which a similar disease phenotype results from mutations at different genetic loci. This often occurs when more than one gene product produces different subunits of an interacting complex or when different genes are involved in the same genetic cascade or physiological pathway. For example, congenital hypothyroidism associated with dyshormonogenesis can arise from mutations in several genes (*NIS*, *TG*, *TPO*, *PDS*, *THOX2*) located on different chromosomes (Table 2.1.3). The effects of inactivating mutations in these genes are similar because the protein products are all required for normal hormone synthesis. Similarly, the genetic forms of diabetes insipidus can be caused by mutations in several genes. Mutations in the AVP-NPII gene cause autosomal-dominant or -recessive forms of neurohypophyseal diabetes insipidus (Table 2.1.2). The nephrogenic forms can be caused by mutations in the X-chromosomal AVPR2 receptor gene, whereas mutations in the aquaporin 2 (AQP2) gene cause either autosomal-recessive or -dominant nephrogenic diabetes insipidus (Table 2.1.8).

Recognition of non-allelic heterogeneity is important because the ability to identify disease loci in linkage studies is reduced by including patients with similar phenotypes but different genetic disorders. Genetic testing is more complex because several different genes need to be considered along with the possibility of different mutations in each of the candidate genes.

Phenocopies designate a phenotype that is identical or similar but results from non-genetic or other genetic causes. For example, obesity may be due to several Mendelian defects or have a primarily behavioral origin. As in non-allelic heterogeneity, the presence of phenocopies has the potential to confound linkage studies and genetic testing. Patient history, subtle differences in clinical presentation, and rigorous testing are key in assigning the correct phenotype.

Variable expressivity and incomplete penetrance

Penetrance and *expressivity* are two different yet related concepts that are often confused. Penetrance is a qualitative notion designating whether a phenotype is expressed for a particular genotype. Expressivity is a quantitative concept describing the degree to which a phenotype is expressed. It is used to describe the phenotypic spectrum in individuals with a particular disorder. Thus, expressivity is dependent on penetrance.

Penetrance is complete if all carriers of a mutant express the phenotype, whereas it is said to be *incomplete* if some individuals do not have any features of the phenotype. Dominant conditions with incomplete penetrance are characterized by skipping of generations with unaffected carriers transmitting the mutant gene. For example, hypertrophic obstructive cardiomyopathy (HCM) caused by mutations in the *myosin-binding protein C* gene is a dominant disorder with clinical features in only a subset of patients who carry the mutation. Incomplete penetrance in some individuals can confound pedigree analysis. In many conditions with postnatal onset, the proportion of gene carriers who are affected varies with age. Therefore, it is important to specify age when describing penetrance. Variable expressivity is used to describe the phenotypic spectrum in individuals with a particular disorder.

Some of the mechanisms underlying expressivity and penetrance include modifier genes (*genetic background*), gender, and environmental factors. Thus, variable expressivity and penetrance illustrate that genetic and/or environmental factors do not influence only complex disorders, but also "simple" Mendelian traits. This has to be considered in genetic counseling, because one cannot always predict the course of disease, even when the mutation is known.

Sex-influenced phenotypes

Certain mutations affect males and females quite differently. In some instances, this is because the gene resides on the X or Y sex chromosomes. As a result, the phenotype of mutated X-linked genes will usually be expressed fully in males but variably in heterozygous females, depending on the degree of X inactivation and the function of the gene. Because only

males have a Y chromosome, mutations in genes such as *SRY* (which causes male-to-female sex reversal) or *DAZ* (*deleted in azoospermia*, which causes abnormalities of spermatogenesis) are unique to males.

Other diseases are expressed in a sex-limited manner because of the differential function of the gene product in males and females. Activating mutations in the luteinizing hormone receptor (LHR) cause dominant male-limited precocious puberty in boys (Table 2.1.7). The phenotype is unique to males because activation of the receptor induces testosterone production in the testis, whereas it is functionally silent in the immature ovary. Homozygous inactivating mutations of the follicle-stimulating hormone (FSH) receptor cause primary ovarian failure in females because the follicles do not develop in the absence of FSH action (Table 2.1.7). In contrast, affected males have a more subtle phenotype, because testosterone production is preserved, allowing sexual maturation, and spermatogenesis is only partially impaired. In congenital adrenal hyperplasia, most commonly caused by 21-hydroxylase deficiency, cortisol production is impaired, and ACTH stimulation of the adrenal gland leads to increased production of androgenic precursors (Table 2.1.5). In females, the increased androgen concentration causes ambiguous genitalia, which can be recognized at birth. In males, the diagnosis may be made on the basis of adrenal insufficiency at birth because the increased adrenal androgen level does not alter sexual differentiation, or later in childhood because of the development of precocious puberty.

Approach to the patient

Clinical and biochemical evaluation is the first step in any attempt to unravel underlying pathogenic mechanisms. The family history is important to recognize the possibility of a hereditary component. For this purpose, it is useful to draw a pedigree of the nuclear and, in some cases, of the extended family. This should include information about ethnic background, age, health status, and (infant) deaths. The physician should explore whether other individuals within the family are affected by the same or a related illness as the index patient. This should be followed by a survey for the presence of commonly occurring disorders.

Because of the possibility of age-dependent expressivity and penetrance, the family history may need continuous updating on subsequent encounters. If the family history or other findings suggest a genetic disorder, the clinician has to assess whether some of the patient's relatives may be at risk of carrying or transmitting the disease. This information may become of practical relevance for carrier detection, genetic counseling, or early intervention and prevention of a disease in relatives of the propositus.

Where a diagnosis at the molecular level may be available, the physician faces several challenges. Genetic testing in children poses distinct ethical issues. In general, it should be limited to situations in which it has an impact on the medical management, and it requires informed consent by the parents. If there is no apparent benefit, the general recommendation is to defer testing until the patient can consent independently. This is particularly relevant in devastating disorders that manifest only later in life, such as Huntington disease.

If genetic testing is considered an option, the physician will have to identify an appropriate laboratory to perform the test (Table 2.2). If a disease-causing mutation is expected in all cells as a result of germline transmission, DNA can be collected from any tissue, most commonly nucleated blood cells or buccal cells, for cytogenetic and mutational analyses. In the case of somatic mutations, which are limited to neoplastic tissue, an adequate sample of this lesion will serve for the extraction of DNA or RNA. For the detection of pathogens, the material to be analyzed will vary and may include blood, cerebrospinal fluid, solid tissues, sputum, or fluid obtained through bronchioalveolar lavage.

New findings on the genetic basis of endocrine disorders are published in numerous scientific journals, books, and databases. The continuously updated Online Mendelian Inheritance in Man (OMIM) catalog lists several thousand genetic disorders and provides information about the clinical phenotype, molecular basis, allelic variants, and pertinent animal models (Tables 2.1 and 2.2). Hyperlinks to other electronic resources, e.g. Medline, GenBank, or databases compiling gene mutations, enable useful information that is relevant for both clinicians and basic scientists.

Chromosomal disorders

Chromosomal (cytogenetic) disorders are caused by numerical or structural aberrations in chromosomes (Table 2.1.1). Molecular cytogenetics has led to the identification of more subtle chromosome abnormalities referred to as microdeletion and imprinting syndromes.

Errors in meiosis and early cleavage divisions occur frequently. Some 10–25% of all conceptions harbor chromosomal abnormalities, which often lead to spontaneous abortion in early pregnancy. Numerical abnormalities are much more common than structural defects, especially trisomy, which is found in about 25% of spontaneous abortions and 0.3% of newborns. Trisomy 21, the most frequent cause of Down syndrome, occurs in 1:600–1000 live births. Trisomies 13 and 18 are also frequent.

Numerical abnormalities in sex chromosomes are relatively common (Table 2.1.1). Males with a 47,XXY karyotype have Klinefelter syndrome, and females with trisomy 47,XXX may be subfertile. Autosomal monosomies are usually incompatible with life, but 45,XO is present in 1–2% of all conceptuses but leads to spontaneous abortion in 99% of all cases. Mosaicism (e.g. 45,XO/45XX, 45XO/45XXX), partial deletions, isochromosomes, and ring chromosomes can also

cause Turner syndrome. Sex chromosome monosomy usually results from loss of the paternal sex chromosome. The 47,XXY can result from paternal or maternal non-disjunction, while the autosomal trisomies are most commonly caused by maternal non-disjunction during meiosis I, a defect that increases with maternal age. Trisomies are typically associated with alterations in genetic recombination.

Structural rearrangements involve breakage and reunion of chromosomes. Rearrangements between different chromosomes, *translocations*, can be *reciprocal* or *Robertsonian*. Reciprocal translocations involve exchanges between any of the chromosomes; Robertsonian rearrangements designate the fusion of the long arms of two acrocentric chromosomes. Other structural defects include deletions, duplications, inversions, and the formation of rings and isochromosomes. Deletions affecting several tightly clustered genes result in *contiguous gene syndromes*, disorders that mimic a combination of single gene defects. They have been useful for identifying the location of new disease-causing genes. Because of the variable size of gene deletions in different patients, a systematic comparison of phenotypes and locations of deletion breakpoints allows the positions of particular genes to be mapped within the critical genomic region. Structural chromosome defects can be present in a "balanced" form without an abnormal phenotype. They can, however, be transmitted in an "unbalanced" form to offspring and thus cause a hereditary form of chromosome abnormality.

Paternal deletions of chromosome 15q11–13 cause Prader–Willi syndrome (PWS), while maternal deletions are associated with the Angelman syndrome (Tables 2.1.1 and 2.5). The difference in phenotype results from the fact that this chromosomal region is imprinted, i.e. differentially expressed on the maternal and paternal chromosome.

Acquired somatic abnormalities in chromosome structure are often associated with malignancies and are important for diagnosis, classification, and prognosis. Deletions can lead to loss of tumor suppressor genes or DNA repair genes. Duplications, amplifications, and rearrangements, in which a gene is put under the control of another promoter, can result in gain-of-function of genes controlling cell proliferation. For example, rearrangement of the 5′ regulatory region of the *parathyroid* (*PTH*) gene located on chromosome 11q15 with the *cyclin D1* gene from 11q13 creates the *PRAD1* oncogene, resulting in overexpression of cyclin D1 and the development of parathyroid adenomas (Table 2.1.4).

Monogenic Mendelian disorders

Monogenic human diseases are often called *Mendelian disorders* because they obey the rules of genetic transmission defined by Gregor Mendel. The mode of inheritance for a given phenotypic trait or disease is determined by pedigree analysis. About 65% of human monogenic disorders are autosomal dominant, 25% are autosomal recessive, and 5% are X-linked. Genetic testing now available for many of these disorders plays an increasingly important role in clinical medicine.

Autosomal-dominant disorders

In autosomal-dominant disorders, mutations in a single allele are sufficient to cause the disease, in contrast to recessive disorders, which are the consequence of biallelic loss-of-function mutations. Various disease mechanisms are involved in dominant disorders. They include gain-of-function, a dominant-negative effect, and haploinsufficiency. In autosomal-dominant disorders, individuals are affected in successive generations, and the disease does not occur in the offspring of unaffected individuals. Males and females are affected with equal frequency because the defective gene resides on one of the 22 autosomes. Because the alleles segregate randomly at meiosis, the probability that an offspring will be affected is 50%. Children with a normal genotype do not transmit the disorder. The clinician must be aware that an autosomal-dominant disorder can be caused by *de novo* germline mutations, which occur more frequently during later cell divisions in gametogenesis, explaining why siblings are rarely affected. New germline mutations occur more frequently in fathers of advanced age. The clinical manifestations of autosomal-dominant disorders may be variable as a result of differences in *penetrance* or *expressivity*. Because of these variations, it is sometimes difficult to determine the pattern of inheritance.

Autosomal-recessive disorders

The clinical expression of autosomal-recessive disorders is more uniform than in autosomal-dominant disorders. Most mutated alleles lead to a partial or complete loss-of-function. They frequently involve receptors, proteins in signaling cascades, or enzymes in metabolic pathways. The affected individual, who can be of either sex, is homozygous or compound heterozygous for a single gene defect. In most instances, an affected individual is the offspring of heterozygous parents. In this situation, there is a 25% chance that the offspring will have a normal genotype, a 50% probability of a heterozygous state, and a 25% risk of homozygosity for the recessive alleles. In the case of one unaffected heterozygous and one affected homozygous parent, the probability of disease increases to 50% for each child. In this instance, the pedigree analysis mimics an autosomal-dominant mode of inheritance (*pseudodominance*). In contrast to autosomal-dominant disorders, new mutations in recessive alleles usually result in an asymptomatic carrier state without apparent clinical phenotype.

Many autosomal-recessive diseases are rare and occur more frequently in isolated populations in the context of parental consanguinity. A few recessive disorders, such as sickle cell anemia, cystic fibrosis, and thalassemia, are relatively frequent

in certain populations, probably because the heterozygous state confers a selective biological advantage. Although heterozygous carriers of a defective allele are usually clinically normal, they may display subtle differences in phenotype that become apparent only with more precise testing or in the context of certain environmental influences.

X-linked disorders

Because males have only one X chromosome, a female individual always inherits her father's X chromosome in addition to one of the two X chromosomes of her mother. A son inherits the Y chromosome from his father and one maternal X chromosome. The characteristic features of X-linked inheritance are therefore the absence of father-to-son transmission and the fact that all daughters of an affected male are obligate carriers of the mutant allele. The risk of developing disease due to a mutant X-chromosomal gene differs in the two sexes. Because males have only one X chromosome, they are hemizygous for the mutant allele. Consequently, they are more likely to develop the mutant phenotype, regardless of whether the mutation is dominant or recessive. A female may be either heterozygous or homozygous for the mutant allele, which may be dominant or recessive, and the terms *X-linked dominant* or *X-linked recessive* apply only to the expression of the mutant phenotype in women. In females, the expression of X-chromosomal genes is influenced by X chromosome inactivation. This can confound the assessment as skewed X inactivation may lead to a partial phenotype in female carriers of an X-linked recessive defect, such as inactivating mutations of the AVPR2 receptor, the cause of X-linked nephrogenic diabetes insipidus.

Y-linked disorders

The Y chromosome harbors relatively few genes. Among them, the sex region-determining Y factor (*SRY*), which encodes the testis-determining factor (TDF), is essential for normal male development (Table 2.1.7). Because the *SRY* region is closely adjacent to the pseudoautosomal region, a chromosomal segment on the X and Y chromosomes with a high degree of homology, crossing-over can occasionally involve the *SRY* region. Translocations can result in XY females, with the Y chromosome lacking the *SRY* gene or XX males harboring the *SRY* gene on one of the X chromosomes. Point mutations in the *SRY* gene may result in individuals with an XY genotype and an incomplete female phenotype. Men with oligospermia/azoospermia frequently have microdeletions of the AZF (azoospermia factor) regions on the long arm of the Y chromosome, which contain several genes involved in the control of spermatogenesis (Table 2.1.7). They may have point mutations in the transcription factor DAZ (deleted in azoospermia), which is located in this chromosomal region.

Exceptions to simple Mendelian inheritance

Mitochondrial disorders

Mendelian inheritance refers to the transmission of genes encoded by DNA in nuclear chromosomes. In addition, each mitochondrion contains several copies of a circular chromosome. Mitochondrial DNA (mtDNA) is small (16.5 kb) and encodes transfer and ribosomal RNAs, and 13 proteins that are part of the respiratory chain involved in oxidative phosphorylation and ATP generation. In contrast to the nuclear chromosomes, the mitochondrial genome does not recombine and is inherited through the maternal line because sperm does not contribute significant cytoplasmic components to the zygote. The D-loop, a non-coding region of the mitochondrial chromosome, is highly polymorphic. This property, together with the absence of recombination of mtDNA, makes it an invaluable tool for studies tracing human migration and evolution and for specific forensic applications.

Inherited mitochondrial disorders are transmitted in a matrilineal fashion. All children from an affected mother inherit the disease, but it will never be transmitted from an affected father to his offspring [except in intracytoplasmic sperm injection (ICSI)]. Alterations in the mtDNA affecting enzymes required for oxidative phosphorylation lead to reduction of ATP supply, generation of free radicals, and induction of apoptosis. Several syndromic disorders arising from mutations in the mitochondrial genome are known in humans, and they affect both protein-coding and tRNA genes (Table 2.3). The pleiotropic clinical spectrum often involves (cardio)myopathies and encephalopathies because of the high dependence of these tissues on oxidative phosphorylation. Many of them may present with endocrine features (Table 2.3). The age of onset and the clinical course are variable because of the unusual mechanisms of mtDNA replication. MtDNA replicates independently from nuclear DNA and, during cell replication, the proportion of wild-type and mutant mitochondria can drift among different cells and tissues. The resulting heterogeneity in the proportion of mitochondria with and without a mutation is referred to as *heteroplasmia* and underlies the phenotypic variability that is characteristic of mitochondrial diseases. Nuclear genes that encode proteins that are important for normal mitochondrial function can cause mitochondrial dysfunctions associated with autosomal-dominant or -recessive forms of inheritance.

Acquired somatic mutations in mitochondrial genes are thought to be involved in several age-dependent degenerative disorders involving muscle and the peripheral and central nervous system. Because of the high degree of polymorphisms in mtDNA and the phenotypic variability of these disorders, it is difficult to establish that a mtDNA alteration is causal for a clinical phenotype.

Table 2.3. Selected mitochondrial disorders with or without endocrine manifestations.

Disorder/phenotype	OMIM
Kearns–Sayre syndrome (KSS): ophthalmoplegia, pigmental degeneration of the retina, cardiomyopathy, increased risk for diabetes, hypothyroidism, hypoparathyroidism	530000
DIDMOAD, mitochondrial form: diabetes insipidus, diabetes mellitus, optic atrophy, deafness	598500
Pearson syndrome (PEAR): bone marrow failure, exocrine and endocrine pancreas failure	557000
MELAS syndrome: mitochondrial myopathy with encephalopathy, lactacidosis and stroke, diabetes (mutation in tRNA for leucine)	540000 540050
Leber's optic atrophy: hereditary optical neuropathy	535000

For detailed overview on mitochondrial disorders, see http://www.neuro.wustl.edu/neuromuscular/mitosyn.html.

Table 2.4. Selected trinucleotide repeat disorders with or without endocrine manifestations.

Disorder/phenotype	Involved gene/protein	Repeat	Inheritance	OMIM
X-chromosomal spinobulbar muscular atrophy (SBMA) Partial androgen resistance insensitivity, reduced fertility, gynecomastia	AR/androgen receptor	CAG	X	313200
Dystrophia myotonica (DM) Hypogonadism with testicular atrophy, insulin resistance Females: frequent miscarriages	DM/myotonin protein kinase	CTG	AD, variable penetrance	160900
Fragile X syndrome (FRAXA) Macroorchidism	FMR1	CGG	XR	309550
Fragile X syndrome (FRAXE)	FMR2	GCC	XR	309548
Huntington disease (HD)	HD/huntingtin	CAG	AD	143100
Friedreich's ataxia (FRDA1) Higher risk for diabetes with longer repeat expansions	FRDA/frataxin	GAA	AR	229300
Spinocerebellar ataxia type 1 (SCA1)	SCA1/ataxin 1	CAG	AD	164400

For detailed overview on trinucleotide disorders, see http://www.neuro.wustl.edu/neuromuscular/mother/dnarep.htm.

Trinucleotide expansion disorders

Several diseases are associated with an increase in the number of trinucleotide repeats above a certain threshold (Table 2.4). In some instances, the repeats are located within the coding region of the genes. For example, an expansion in a CAG repeat in the androgen receptor, which encodes a polyglutamine motif in its amino-terminus, leads to the X-linked form of spinal and bulbar muscular atrophy (SBMA, Kennedy syndrome). Similarly, an expansion in the *huntingtin* (*HD*) gene is the cause of Huntington disease. In other instances, the repeats are located in regulatory sequences. If an expansion is present, the DNA fragment is unstable and tends to expand further during cell division; hence the designation *dynamic mutation*. The length of the nucleotide repeat often correlates with the severity of the disease. When repeat length increases from one generation to the next, disease manifestations may worsen or appear at an earlier age, a phenomenon referred to as *anticipation*. In Huntington disease, for example, there is a correlation between age of onset and length of the triplet codon expansion.

Mosaicism

Mosaicism refers to the presence of two or more genetically distinct cell lines in the tissues of an individual. It results from a mutation that occurs during embryonic, fetal, or extrauterine development. The developmental stage at which the mutation arises will determine whether germ cells and/or somatic cells are involved. Chromosomal mosaicism results from non-disjunction at an early embryonic mitotic division, leading to the persistence of more than one cell line, as exemplified by some patients with Turner syndrome (Table 2.1.1). Somatic mosaicism is characterized by a patchy distribution of genetically altered somatic cells that occurs early in development. This is best illustrated by the McCune–Albright syndrome, which is caused by activating mutations in the *GNAS1* gene encoding the stimulatory G-protein α (Gsα)

(Table 2.1.11). The clinical phenotype varies depending on the tissue distribution of the mutation. Manifestations include ovarian cysts that secrete sex steroids and cause precocious puberty, polyostotic fibrous dysplasia, café-au-lait skin pigmentation, growth hormone-secreting pituitary adenomas, and, among others, hypersecreting autonomous thyroid nodules.

X-inactivation, imprinting, and uniparental disomy

According to traditional Mendelian principles, the parental origin of a mutant gene is irrelevant for the expression of the phenotype. There are, however, important exceptions to this rule. X inactivation prevents the expression of most genes on one of the two X chromosomes in every cell of a female (*Lyonization*). Gene inactivation also occurs on selected chromosomal regions of autosomes. This phenomenon, referred to as *genomic imprinting*, leads to preferential expression of an allele depending on its parental origin. It is of pathophysiologic importance in disorders in which the transmission of disease is dependent on the sex of the transmitting parent and plays an important role in the expression of certain genetic disorders (Table 2.5). The two classic examples are the Prader–Willi syndrome and Angelman syndrome (Tables 2.1.1 and 2.5). Prader–Willi syndrome is characterized by diminished fetal activity, obesity, hypotonia, mental retardation, short stature, and hypogonadotrophic hypogonadism. Deletions in the Prader–Willi syndrome occur exclusively on the paternal chromosome 15. Patients with Angelman syndrome present with mental retardation, seizures, ataxia, and hypotonia, and have deletions at the same site of chromosome 15; they are, however, located on the maternal chromosome 15. These two syndromes may also result from *uniparental disomy*, i.e. by the inheritance of either two maternal chromosomes 15 (Prader–Willi syndrome) or two paternal chromosomes (Angelman syndrome).

Another example of importance for pediatric endocrinology concerns the *GNAS1* gene encoding the Gsα subunit. Heterozygous loss-of-function mutations in the *GNAS1* gene lead to Albright hereditary osteodystrophy (AHO) with its characteristic features including short stature, obesity, round face, brachydactyly, subcutaneous ossifications, and mental deficits. Paternal transmission of *GNAS1* mutations leads to the AHO phenotype alone (*pseudo*pseudohypoparathyroidism) (Table 2.1.4), while maternal transmission leads to AHO in combination with resistance to several hormones such as PTH, TSH, and gonadotropins, which act through transmembrane receptors coupling to Gsα (pseudohypoparathyroidism type IA) (Table 2.1.11). These phenotypic differences result from a tissue-specific imprinting of *GNAS1*, which is expressed primarily from the maternal allele in tissues such as the proximal renal tubule and the thyroid. In most other tissues, however, it is expressed biallelically. Disrupting mutations in the maternal allele lead to loss of Gsα expression in proximal tubules and, therefore, loss of PTH action in the kidney, while mutations in the paternal

Table 2.5. Selected disorders involving mutations of imprinted loci or uniparental disomy.

Disorder/phenotype	Involved locus and mechanisms	OMIM
Prader–Willi syndrome Short stature, obesity, hypogonadism	15q11–13 Deletion in paternal chromosome Maternal uniparental disomy	176270
Angelman syndrome Mental and motor retardation, ataxia, hypotonia, epilepsy, microbrachycephaly, paroxysmal laughter, absent speech	15q11–13 Deletion in maternal chromosome Paternal uniparental disomy	105830
Russell–Silver syndrome Short stature, asymmetry, triangular face, cardiac defects, hypospadia, GH deficiency, hypoglycemia	7p11.2 Maternal uniparental disomy	180860
Beckwith–Wiedemann syndrome Generalized overgrowth, hemihypertrophy, macroglossia, omphalocele, hypoglycemia. Predisposition to neoplasms: Wilms' tumor, hepatoblastoma, adrenal carcinoma, gonadoblastoma	11p15.5 Paternal uniparental disomy Deletion in maternal chromosome Mutations in the cyclin-dependent kinase inhibitor 1C gene (CDKN1C)	130650
Transient neonatal diabetes mellitus	6q24 Paternal uniparental disomy	601410
Short stature, hypotonia, hyperextensible joints, scoliosis, facial dysmorphism, developmental delay, precocious puberty	14 Maternal uniparental disomy	–
Mental retardation, short-limb dwarfism, thorax deformities	14 Paternal uniparental disomy	608149

allele have little effect on PTH action. In patients with isolated renal resistance to PTH (pseudohypoparathyroidism type IB), an imprinting defect of GNAS1 leads to decreased $G_{s\alpha}$ expression in proximal tubules of the kidney.

Somatic mutations

Acquired mutations that occur in somatic rather than germ cells are called *somatic mutations*. This creates a chimeric situation and, if the cells proliferate, a neoplastic lesion. Therefore, cancer can be defined as a genetic disease at the cellular level. Cancers are monoclonal, indicating that they have arisen from a single precursor cell that has acquired one or several mutations in genes controlling growth and/or differentiation. These mutations are somatic, i.e. restricted to the tumor and its metastases, but not found in surrounding normal tissue. The molecular alterations include dominant gain-of-function mutations in oncogenes, recessive loss-of-function mutations in tumor suppressor genes and DNA repair genes, gene amplification, and chromosome rearrangements. Rarely, a single mutation in certain genes may be sufficient to transform a normal cell into a malignant cell, but the development of a malignant phenotype in most cancers requires several genetic alterations for the gradual progression from a normal to a cancerous cell, a process termed *multistep carcinogenesis*.

In many cancer syndromes, there is an inherited *predisposition* to tumor formation. In these instances, a germline mutation is inherited in an autosomal-dominant fashion. This germline alteration affects one allele of an autosomal tumor suppressor gene. If the second allele is inactivated by a somatic mutation in a given cell, this will lead to neoplastic growth (*Knudson hypothesis* or *two-hit model*). In this instance, the defective allele in the germline is transmitted in a dominant way, whereas the tumorigenic mechanism results from a recessive loss of the tumor suppressor gene in affected tissues. The classic example to illustrate this phenomenon is retinoblastoma, which can occur as a sporadic or hereditary tumor. In sporadic retinoblastoma, both copies of the retinoblastoma (*RB*) gene are inactivated through two somatic events. In hereditary retinoblastoma, one mutated or deleted *RB* allele is inherited in an autosomal-dominant manner, and the second allele is inactivated by a subsequent somatic mutation. This "two-hit" model applies to other inherited cancer syndromes, such as multiple endocrine neoplasia type 1 (MEN-1), which is caused by mutations in the tumor suppressor gene menin (Table 2.1.10).

Inherited defects in enzymes involved in DNA replication and repair can lead to a significant increase in mutations and are associated with several disorders predisposing to cancer.

Complex disorders

Many disorders have a complex etiology involving multiple genes (*polygenic disorders*), often in combination with environmental and lifestyle factors (*multifactorial disorders*). The major health care problems, cardiovascular disease, hypertension, diabetes, obesity, asthma, and psychiatric disorders, fall into this category, but they also include certain developmental abnormalities, such as cleft palate, congenital heart defects, and neural tube defects.

Compared with single gene defects, complex disorders have a low heritability and do not fit a Mendelian pattern of inheritance. Twin studies are particularly helpful in demonstrating the importance of both genetic and environmental factors. For example, first-degree relatives of patients with diabetes type 1 are about 15 times more likely to develop diabetes. The concordance rate for developing diabetes is about 50% in monozygotic twins and about 8% in dizygotic twins. The discordance rate in monozygotic twins illustrates the significant requirement for environmental factors. In addition, some of the susceptibility genes, a designation indicating that the carrier is susceptible to develop the disease, have a low penetrance. Susceptibility genes or loci can be mapped using several methods including linkage analyses, association studies, and affected sib-pair analyses. Current efforts aim at identifying these genes by establishing correlations between SNPs or SNP haplotypes and complex disorders in large populations. The results may, in part, depend on ethnicity and ascertainment criteria. The study of rare monogenic diseases may also provide insights into genetic and molecular mechanisms that are important for the understanding of complex disorders. For example, the identification of the genetic defects underlying the various autosomal-dominant forms of MODY (maturity-onset diabetes of the young) have defined them as candidate genes contributing to the pathogenesis of diabetes mellitus type 2 (Tables 2.1.6 and 2.1.12).

Genomics and post-genomic techniques

Broadly defined, genomics designates the discipline of mapping, sequencing, and analyzing genomes. The completion of the structural analysis of the human genome (*structural genomics*) is currently followed by a rapid emergence of "post-genomic" disciplines focusing on biological function of the gene products (*functional genomics*). These disciplines are concerned with analyses of gene transcripts (*transcriptomics*), proteins and their secondary modifications and interactions (*proteomics*), and metabolites and their networks (*metabolomics*). The ultimate goal is the integration of these complementary data into a *systems biology* that permits a comprehensive definition of the phenotype and pathophysiological perturbations.

What can be expected from this technology? Genotyping may become important for stratifying patients according to disease risk and for predicting the response to certain drugs. Gene expression studies can be used for the assessment of prognosis and for guiding therapy. Proteomic studies may allow diagnosis of early stages of malignancy. Because most

drug targets are proteins, proteomics will be important for drug discovery and development. These technologies, individually and in combination, will certainly contribute to the elucidation of complex disorders. Genomic approaches may have further impact on health care as a result of a thorough understanding of the genomes and proteins of infectious agents, e.g. *Plasmodium falciparum* or *Mycobacterium tuberculosis*, which may lead to the development of novel therapeutic strategies and compounds.

Comparative genomics

Comparative genomics involves the analysis of two or more genomes to identify the extent of similarity or large-scale screening of a genome to identify sequences present in another genome. Applications involve comparisons of prokaryotic and eukaryotic genomes to infer evolutionary relationships. The detection of high evolutionary conservation can be used as a screen for regulatory elements within otherwise poorly conserved non-coding DNA. Moreover, electronic screening of EST databases can identify homologs of genes in other species. For example, systematic screening of the *dbEST* database of ESTs has revealed many relevant human homologs of *Drosophila* genes known to be loci for mutant phenotypes.

Pharmacogenomics

Broadly defined, the scope of pharmacogenetics and pharmacogenomics is to define how the genome influences the response of an individual to a drug. Although many non-genetic factors influence the effects of medications, genetic polymorphisms in receptors, transporters, channels, and enzymes can result in variable absorption, distribution, metabolism, and excretion of a drug that ultimately lead to differential response or toxic concentrations. For example, a polymorphism in the thiopurine methyltransferase (TMT) inactivates the enzyme and is associated with hematopoietic toxicity of mercaptopurine. Determination of the TMT genotype is therefore important for choosing a safe dose of the medication. In other instances, drug effects will be influenced by polymorphisms in multiple genes.

Further development of genotyping and pharmacogenomics may improve the safety of medical therapy by choosing appropriate medications and dose levels, thereby decreasing the number of adverse drug reactions. It is also expected that this discipline will have an impact on drug development because screenings of SNPs can be used to enroll or exclude subgroups of patients.

Transcriptomics

mRNA expression of one or a few genes has usually been determined by Northern blot analyses, a technique that has been largely replaced by semiquantitative RT-PCR, but both techniques permit only the analysis of the expression pattern of a limited number of genes. Paralleling the characterization of the genomic sequences of humans and other organisms, as well as the genes that they encode, various *expression profiling* techniques have been developed. These analyses enable surveys of gene expression patterns for thousands of genes in a single assay (Fig. 2.6 and Plate 4, facing p. 148). These profiles are useful for the understanding of gene regulation and interactions in normal and pathologic tissues. As the complement of mRNAs transcribed by the cellular genome is also referred to as the *transcriptome*, the generation of mRNA expression profiles is now also referred to as *transcriptomics*.

The most widely used techniques for expression profiling include microarrays and serial analysis of gene expression (SAGE). After hybridization with the labeled probes, the microarrays are scanned, and special software allows analysis of the fluorescence intensities for each spot (Fig. 2.6 and Plate 4, facing p. 148). The limitations of microarray technology include high cost, special equipment, and the inability to detect novel transcripts. SAGE is a powerful tool that allows a comprehensive analysis of gene expression patterns without the requirement of pre-existing probes or sophisticated equipment.

The simplest way to identify genes of potential interest by expression profiling is to search for those that are consistently upregulated or downregulated. The identification of *patterns* of gene expression and regulated *classes* of genes may, however, provide more informative insights into their biological function and relevance. Genes that are part of a particular pathway or that respond to a common endogenous or exogenous stimulus are expected to be co-regulated and should consequently show similar patterns of expression. Several computational techniques, such as hierarchical clustering, self-organizing maps, and mutual information, are used for the analysis of gene expression data. The choice of the appropriate algorithm(s) for these analyses is a crucial element of the experimental design, and the methods that are used to analyze the data can have a profound influence on the interpretation of the results.

Proteomics

The term *proteome* designates the complete set of proteins expressed by the genome. *Proteomics* includes studies focusing on the expression and function of the proteome, as well as aspects of structural biology. The study of the proteome is difficult because it is so dynamic. Apart from the expression of isoforms, proteins undergo a plethora of secondary modifications and protein–protein interactions (*interactome*), and they form higher order complexes.

Comprehensive analyses of the proteome relied initially on protein separation by two-dimensional gel electrophoresis with subsequent mass spectrometric identification of protein spots. This approach is limited, as it is constrained to the most

abundant proteins in the sample. Studies of the proteome are now performed predominantly with direct mass spectrometric analyses, which have undergone technological refinement and can now identify ever smaller amounts of proteins from complex mixtures.

The classic methodology for studying protein–protein interactions was the yeast two-hybrid system. Currently, various protein- and antibody-based arrays are emerging to study protein activities, secondary modifications, and interactions. *Structural proteomics* has the ambitious goal of systematically understanding the structural basis for protein interactions and function.

Clinical proteomics is a new subdiscipline with high promise for disease detection and surveillance by monitoring proteomic pattern diagnostics. In this approach, high-throughput mass spectrometry generates a proteomic fingerprint of a diagnostic sample such as serum, fine-needle, or nipple fluid aspirate (see Plate 5, facing p. 148). Bioinformatic pattern recognition algorithms can then be applied to identify patterns of protein alterations that can discriminate benign from malignant tissues. Importantly, the specific pattern itself is diagnostic, and the underlying identities of the proteins that comprise the patterns do not need to be known. As well as identifying novel diagnostic and prognostic biomarkers for human cancer, proteomics is also expected to have an impact on drug discovery and action, given that most drugs target proteins and subsequently modify intracellular networks.

Inspired by the success of the Human Genome Project, the Human Proteome Organization (HUPO) aims at coordinating proteomic research. Major current goals in proteomics include definition of the plasma proteome, analyses of specific cell types, mapping of organelle compositions, generation of antibodies to all human proteins, generation of protein interaction maps, and analyses of important model and pathogenic organisms.

Metabolomics

The *metabolome* can be defined as the quantitative complement of all the low molecular weight molecules present in cells in a particular physiological or developmental state. While metabolomics is complementary to transcriptomics and proteomics, there are several attractive reasons for analyzing the metabolome. Relative to alterations in the transcriptome and the proteome, changes in the metabolome are often amplified. Moreover, alterations in metabolic fluxes are not regulated by gene expression alone and, reflecting the activities of the cell at a functional level, affect the concentrations of numerous individual metabolites. High-throughput analyses of metabolites can be performed with tools such as nuclear magnetic resonance spectroscopy and mass spectroscopy. Because of the complexity and heterogeneity of metabolites and the fact that the metabolic complement is even more dynamic than the proteome, the technical and computational challenges are substantial, and this discipline is still in its early stages. It has, however, already had a profound conceptual impact because of the ongoing shift from simple *metabolic pathways* to *metabolic networks*.

Bioinformatics

The enormous amounts of diverse biological data generated by recent biotechnological advances have led to the development and evolution of *bioinformatics*, in which biology and information technology converge. Initially, bioinformatics focused on the development and creation of nucleotide and protein databases and methods for the analysis of the deposited sequences. The application of bioinformatics for analysis of nucleotide and polypeptide sequences is well established and widely used. The largest of these sequence databases include GenBank at the National Center for Biotechnology Information (NCBI), Ensembl at the European Molecular Biology Laboratory (EMBL), the DNA Data Bank of Japan, and SwissProt, among others (Table 2.2). They permit rapid retrieval of sequence information of genomic DNA, mRNA, ESTs, SNPs, or polypeptides for a rapidly growing number of species. The evolution of these databases has been accompanied by expanding capabilities to annotate sequences, linking the data with other electronic resources and more sophisticated tools for analysis of nucleotide and protein sequences. It is crucial that bioinformatics software development is linked at an early stage through agreed documentation, standardized rules for structuring web forms (eXtensible Markup Language, XML), and controlled vocabularies that allow different tools to exchange primary data sets.

Navigation in this web of continuously evolving databases is often intimidating, but the high degree of interconnection between the multitude of databases permits relatively easy exploration of this knowledge. User guides and the online NCBI handbook (Table 2.2) provide helpful instructions to questions such as the following:

1 How does one find a gene of interest and determine the structure of this gene?
2 How can one find information about SNPs?
3 How can one find all the members of a human gene family?
4 For a given protein, how can one determine whether it contains any functional domains of interest?
5 What other proteins contain the same functional domains as this protein?
6 How can one determine whether there is a similarity to other proteins, not only at the sequence level, but also at the structural level?

Sequence alignments are performed most easily with the BLAST (Basic Local Alignment Search Tool), which compares a DNA or polypeptide sequence of interest with nucleotide or protein databases. In addition to determining the identity of an isolated nucleotide or protein fragment, this approach can

detect similar related sequences in one or several organisms. This type of comparison is the basis for the development of gene and protein families and unravels evolutionary relationships. The sensitivity and specificity of a BLAST search can be modified in such a way that even discrete homologies can be unraveled. By referring to databases of known regulatory element sequences, computer programs can inspect genomic sequences for the presence of regulatory elements. Motifs (i.e. specific amino acid patterns associated with defined functions) can be identified by submitting a polypeptide to computational analysis. This may permit assignment of a protein to functional and structural families and making predictions on the functional role of newly isolated proteins.

As more protein structures are identified, the relationship between structure and function becomes easier to predict. The development of more accurate algorithms for predicting and modeling secondary and tertiary structures is, in part, moving out of the laboratory and into the hands of bioinformaticists.

The field of bioinformatics is now challenged to integrate the data generated by the various "-omic" techniques (genomics, transcriptomics, proteomics, metabolomics, phenomics) with the hope of elucidating the functional relationships between genotype and observed phenotype, thereby permitting a system-wide analysis from genome to phenome.

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3 Measuring hormones, molecular tests, and their clinical application

Jan M. Wit and Marcel Karperien

General principles of diagnostic procedures

Introduction

The general aim of investigation is to increase or decrease the probability of a diagnosis, to monitor the natural history of a condition or the response to treatment. It can thus reshuffle the order of likelihood of the differential diagnosis based on the clinical presentation. The definition of a diagnosis is arbitrary and subject to changing views. For example, it is open to discussion whether symptoms and signs should define it, or whether it is defined by biochemical, anatomical, or pathological similarities, or by genetic markers. Furthermore, in each patient, a medical condition has a somewhat different expression, probably because of variation in genetic and environmental influences.

In the area of endocrinology, part of the fuzziness of diagnostic labels is caused by the uncertainty about the biological effect of circulating hormones. From its origin, endocrinology has focused on hormone levels in serum and urine, but little is known about the sensitivity to hormones. In the field of growth, the variation in growth patterns is determined not only by growth hormone (GH) secretion but also by GH sensitivity [1] (Fig. 3.1). Sensitivity to hormones can vary greatly, which is important if hormone levels are used as pathognomic markers of a diagnosis.

In most instances, establishing the diagnosis leads to decisions on management. Where diagnostic procedures are used in common disorders, evidence-based guidelines can be developed, but their number is small. In cases with an unusual presentation, diagnostic procedures are used not only for detecting or excluding known disorders, but also for seeking novel causes of disease through investigations for pathophysiological mechanisms.

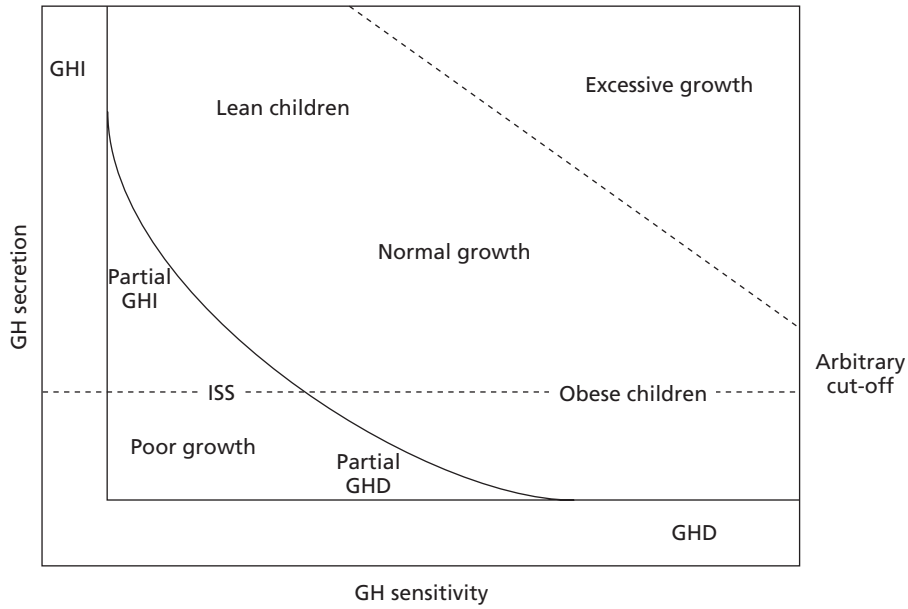

Fig. 3.1. Hypothetical model describing the relationship between GH secretion and GH sensitivity in growth regulation (from [1] with permission). GHI, growth hormone insufficiency; GHD, growth hormone deficiency; ISS, idiopathic short stature.

Table 3.1. Criteria for evaluating the value of a diagnostic test.

Validity

1 Was there a valid reference test (gold standard)?
2 Was there an independent (blind) comparison of the index test with the reference test?
3 Was the reference test applied regardless of the diagnostic test result?
4 Was the index test carried out independently of other relevant information about the health status of the patient?
5 Was the test evaluated in an appropriate spectrum of patients (like those in whom one would use it in practice)?
6 Was the test validated in a second, independent group of patients?

Importance

1 Diagnostic value of the index test
2 Precision of the estimated diagnostic parameters

Applicability

1 Is the diagnostic test available, affordable, accurate, and precise in one's own setting?
2 Can one generate a clinically sensible estimate of the patient's pretest probability? (from personal experience, prevalence statistics, practice databases, or primary studies; from an estimation of the similarity of the described patients to one's own practice; from an estimate of whether the disease possibilities have changed since the evidence was gathered)
3 Will the resulting post-test probabilities affect one's management and help one's patient? (could it move us across the test–treatment threshold?; would the patient be a willing partner in carrying it out?; would the consequences of the test help our patient reach his or her goals in all of this?)
4 Does the severity of the disease, and the possibilities for treatment, as well as the possible hazards and side-effects of the test, and the risk of false-positive and false-negative results warrant the use of this test?

Adapted from [3] and [2].

The value of a diagnostic test

A diagnostic test should not be used before its value is clearly documented. For a proper assessment of the value of a diagnostic test, three aspects have to be considered, validity, importance, and applicability [2,3] (Table 3.1).

Validity

A number of points have to be established for test validity:
1 The test should be compared with a valid reference test, a "gold standard" test that can demonstrate a disease with maximal certainty. Gold standards are lacking in pediatric endocrinology.
2 The index and reference tests should be assessed blindly (independently). If this is not done, it will usually cause an artificially increased agreement between the two tests (review bias).
3 Both tests should be carried out in all patients. If, for example, only the index test-positive patients were referred for the reference test, this would lead to so-called workup bias.
4 The index test should be independent of other relevant information about the clinical condition of the patient.
5 The value of the index test should be investigated in a population that is relevant to the situation in which the test is to be carried out. In general, the spectrum of disease characteristics should be broad. The group that does not have the pertinent disorder should consist of persons with medical conditions that can easily be confused with the disorder, and who are similar to those in whom one would use it in practice.
6 The test should be validated in a second, independent group of patients.

Importance

If the test is valid, one has to determine its importance, i.e. to what extent and how precisely the index test can predict the presence or absence of the suspected condition. The key principle is probability (likelihood) as an (inverse) expression of diagnostic uncertainty. In order to quantify this, one should decide first whether the test result can be treated as dichotomous (positive or negative) or continuous. In the latter, one has to perform an analysis including a series of cutoff points resulting in a so-called receiver operating characteristic (ROC) curve (see below).

Various parameters are available for measuring the power of a test. An example of the general scheme, a hypothetical diagnostic test for GH deficiency, and a definition of the various test characteristics is shown in Table 3.2 (the 2×2 table) [4].

The best known parameters are sensitivity and specificity. The sensitivity of a test is the proportion of positive index test results among the diseased (20 of 25). In the remaining patients (5 of 25), the test result is negative (a false-negative result). The specificity of a test is the proportion of negative index test results among the non-diseased. In the remaining persons (in our example 13%), the positive test would wrongly suggest disease (a false-positive result). When a test has a high sensitivity, a negative result rules *out* the diagnosis. Similarly, when a test has a high specificity, a positive

Table 3.2. The classical 2 × 2 table (A), a hypothetical example of test results in 100 individuals with respect to a diagnosis of growth hormone (GH) deficiency (B), and calculation of parameters for quantitating the value of a diagnostic test (C).

A

		Target disorder		Totals
		Present	Absent	
Test result	Positive	a	b	a+b
	Negative	c	d	c+d
Totals		a+c	b+d	a+b+c+d

B

		GH deficiency		Totals
		Present	Absent	
GH test result	Positive	20	10	30
	Negative	5	65	70
Totals		25	75	100

C

Parameter	Calculation	Outcome in example
Sensitivity (se) = proportion of positive index test results (true positives) among the diseased	a/(a+c)	20/25 = 0.80
Specificity (sp) = proportion of negative index test results (true negatives) among the non-diseased	d/(b+d)	65/75 = 0.87
Prevalence of disease (pretest probability of disease)	(a+c)/(a+b+c+d)	25/100 = 0.25
Prevalence of non-disease (pretest probability of non-disease)	(b+d)/(a+b+c+d)	75/100 = 0.75
Positive predictive value = proportion diseased among the persons with a positive result on the index test = post-test probability of disease	a/(a+b)	20/30 = 0.67
Negative predictive value = proportion non-diseased among the persons with a negative result on the index test = post-test probability of non-disease	d/(c+d)	65/70 = 0.93
Likelihood ratio for a positive result (LR+) = the ratio between the probability of a positive test result in diseased and in non-diseased	(a/(a+c))/(b/(b+d)) = se/(1–sp)	80/13.3 = 6.0
Likelihood ratio for a negative result (LR–) = the ratio between the probability of a negative test result in diseased and in non-diseased	(c/(a+c))/(d/(b+d)) = (1–se)/sp	20/87 = 0.23
Pretest odds = prevalence/(1–prevalence)		0.25:0.75 = 0.33
Post-test odds = pretest odds × likelihood ratio		0.33 × 6 = 2:1 = 2
Post-test probability = post-test odds/(post-test odds + 1)		2/(2+1) = 0.67

Sources: [2–4].

Note: for calculating test characteristics and their 95% confidence intervals, see for example http://www.cebm.net/toolbox.asp, or http://araw.mede.uic.edu/cgi-alansz/testcalc.pl.

result rules *in* the diagnosis. Because testing normal children is difficult, sufficient data are rarely available for an accurate assessment of specificity.

Sensitivity and specificity have only limited value for the practicing clinician. What one wishes to know is the positive and negative predictive value of the test, i.e. the proportion of patients with the condition among patients with a positive test result and the proportion without the condition among the patients with a negative test result. These values can be considered as post-test probabilities of the presence or absence of disease. In our example, the pretest probability of GH deficiency (prevalence) was 25%; with a positive test result, the post-test probability increased to 67%. The pretest probability of an absence of GH deficiency was 75%; with a negative test result, the post-test probability of absence of GH deficiency increased to 93%.

The predictive value depends on the prevalence of the condition. For example, if the data in Table 3.2 are reworked with a lower presumed prevalence, a positive test result increases the probability of GH deficiency from 5% to 25%, and a negative test result increases the probability of non-GH deficiency from 95% to 99% (Table 3.3). The relationship between pretest probability (prevalence) and post-test probability is shown in Figure 3.2. The largest diagnostic benefit can be obtained in situations where the prevalence is between 30% and 70%. If the prevalence is lower or higher, a test result does not add much to the clinical (un)certainty.

A test characteristic that is independent of the prevalence is the likelihood ratio (LR) for a positive (LR+) or negative (LR–) test. The LR+ is the proportion between the probability of a positive test result in people with or without the condition. A test with a LR+ of 1 is not informative but gets more informative as the LR+ increases: an infinite LR+ is pathognomic for the disease. The LR– is the proportion between the probability of a negative test result in people with or without the condition, so a test with a LR– of 0 excludes the condition. With the LR, a pretest probability (prevalence) can be transformed to a post-test probability. This transformation goes via the ratio between the probability of the occurrence of something and the probability that it does not occur. The equations were

Table 3.3. Results of a test with identical test characteristics as the test in Table 3.2, but with a prevalence of 5% instead of 25%.

		GH deficiency		Totals
		Present	Absent	
GH test result	Positive	20	63	80
	Negative	5	412	417
Totals		25	475	500

Sensitivity = 20/25 = 80%.
Specificity = 412/475 = 87%.
Pretest probability of GH deficiency = 25/500 = 0.05.
Pretest probability of absence of GH deficiency = 475/500 = 0.95.
Positive predictive value = post-test probability of disease = 20/80 = 0.25.
Negative predictive value = post-test probability of non-disease = 412/417 = 0.99.
Likelihood ratio for a positive test result = se/(1–sp) = 0.80/0.133 = 6.0.
Likelihood ratio for a negative test result = (1–se)/sp = 0.20/0.87 = 0.23.
Source: [4].

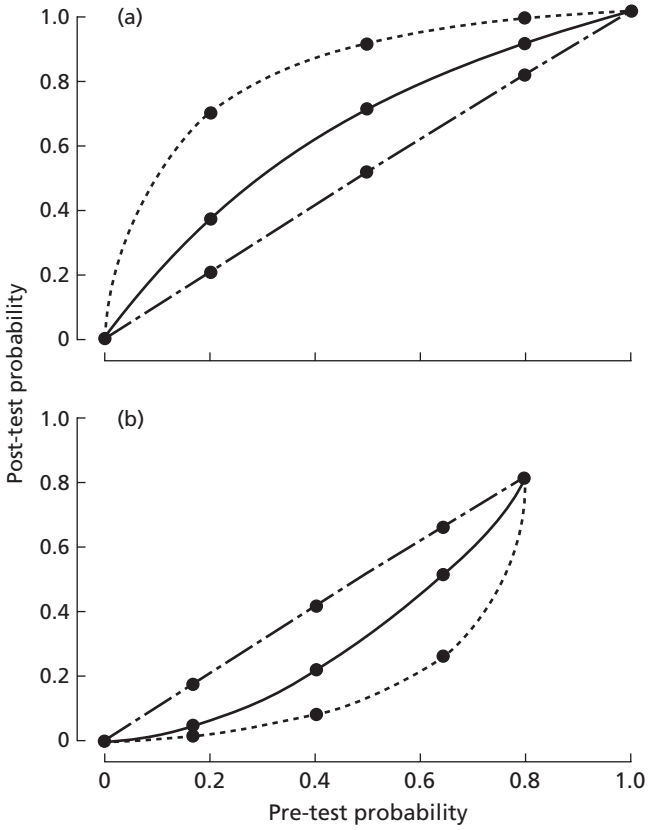

Fig. 3.2. The relation between pretest and post-test probability of disease. The data were constructed using Bayes' theorem with a test sensitivity and specificity of either ——, 70% or ------, 90%. (a) The post-test probability if the test were positive; (b) the post-test probability if the test were negative. If the post-test probability were the same as the pretest probability, then the relation would be given by the 45° line.

first described by Bayes (Bayes' theorems). For this calculation, one needs the following equations:

Pretest odds = pretest probability/(1–pretest probability)

Post-test odds = LR × pretest odds

Post-test probability = post-test odds/(post-test odds + 1)

Alternatively, one can use the nomogram depicted in Figure 3.3.

Dividing test results into normal and abnormal is a gross simplification. In reality, the test result is assessed and interpreted in more detail; some abnormal results are pathognomic for disease, while a less extreme value is not. For example, the interpretation of a serum thyroid-stimulating hormone (TSH) of 6 is quite different from the interpretation of a serum TSH of > 500 mU/L, although both are above the cutoff level of approximately 5 mU/L (depending on the laboratory method).

In most cases, there is an overlap of test results in diseased and non-diseased persons, which obviously leads to imperfect sensitivity and specificity. In Table 3.4, data are shown for various ranges of serum glucose in 300 persons with and 700 persons without diabetes mellitus [3]. If the cutoff point were set at 10.5 mmol/L, there would be no non-diabetic persons above that limit (specificity 100%), but a considerable

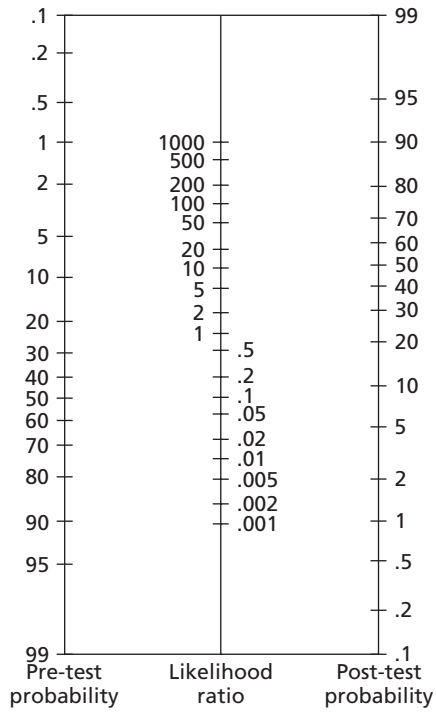

Fig. 3.3. A likelihood ratio nomogram. With this nomogram, the post-test probability for a disease can be calculated from the likelihood ratio and the pretest probability. Draw a line from the pretest probability on the left axis to the likelihood ratio on the middle axis. Extrapolate this line toward the right axis to indicate the post-test probability. From [3] with permission.

Table 3.4. Serum glucose levels (mmol/L) in 300 persons with and 700 persons without diabetes, and sensitivity and specificity of serum glucose for diabetes mellitus for various cutoff points.

Serum glucose	Diabetes	No diabetes	Sensitivity	Specificity
≥ 11.0	66	0	0.22	1.00
10.5–10.9	31	0	0.32	1.00
10.0–10.4	29	1	0.42	1.00
9.5–9.9	25	1	0.50	1.00
9.0–9.4	16	3	0.56	0.99
8.5–8.9	19	4	0.62	0.99
8.0–8.4	10	5	0.65	0.98
7.5–7.9	16	20	0.71	0.95
7.0–7.4	20	30	0.77	0.91
6.5–6.9	18	52	0.83	0.83
6.0–6.4	13	111	0.88	0.68
5.0–5.9	16	166	0.93	0.44
4.0–4.9	11	155	0.97	0.22
< 4.0	10	152		
Total	300	700		

Source: [3].

number of diabetic patients would not be detected (low sensitivity, high percentage of false-negative results). Within the zone of overlap, each cutoff would be associated with a certain sensitivity and specificity. In this example, there is no range where there would be absolutely no diabetic patients, so the sensitivity never reaches 100%.

The ROC curve can express the relationship between sensitivity and specificity. The ROC curve belonging to the data in Table 3.4 is shown in Figure 3.4. Sensitivity is plotted on the *y*-axis and 1–specificity on the *x*-axis. The optimal cutoff point, the best combination of sensitivity and specificity, is the point located as close as possible to the upper left corner of the diagram. Using this cutoff point, the number of false-positive and false-negative values is minimal. The better the diagnostic power of the test, the bigger the surface between the curve and the diagonal (area under the curve). Thus, a test with a ROC curve close to the diagonal is not discriminative.

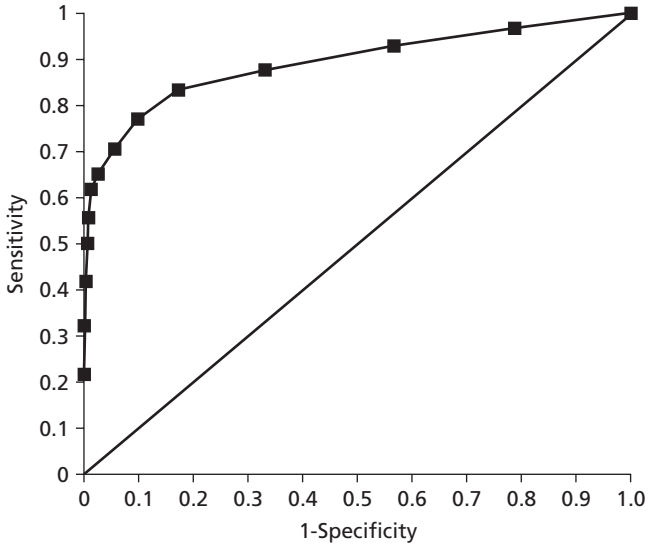

Fig. 3.4. Receiver operating characteristic (ROC) curve of the relationship between sensitivity and (1–specificity) of serum glucose determinations for the diagnosis of diabetes mellitus at 14 different cutoff points (data from Table 3.4). Each dot represents a cutoff point. From [3] with permission.

The precision of the estimated diagnostic parameters is usually expressed as the 95% confidence interval. The bigger the number of patients, the smaller the 95% confidence interval gets. Equations can be found in books on evidence-based medicine [2] and on the internet (see footnote to Table 3.2).

Applicability

Before using a test, one should assess whether four conditions are met: first, whether the test is available, affordable, accurate, and precise and whether the characteristics of the patient(s) in whom the test is to be used are sufficiently similar to the patients in whom the test has been described. Secondly, one should estimate the pretest probability (prevalence) of the condition. Thirdly, using the nomogram in Figure 3.3, it can be seen whether the test results may cause a substantial change in the probability of disease and thereby change decisions on treatment. In this context, the concepts of "test threshold" and "treatment threshold" are helpful [2]. If the probability of disease is (or would become) lower than the test threshold, one would do no more testing. If the probability of disease is or gets higher than the treatment threshold, one would abandon further testing and treatment. Only if the diagnostic test result would leave the clinician stranded between the test and the treatment thresholds would one pursue the initial diagnosis by performing other tests. Finally, the decision to use a test should depend not only on the expected change in probability and its consequences for treatment, but also on the severity of the disease and the possibilities for treatment, as well as the possible hazards and side-effects of the test and the risk of false-positive and false-negative results.

The decision to stop investigation and to treat or not depends on how convinced the clinician is of the diagnosis, the benefits and risks of therapy, and the potential yield and risks of further tests. In such circumstances, the clinician can conduct another test or use a more sophisticated analysis rather than a simple positive or negative. The strategy of using two tests for the diagnosis of GH deficiency is common. The additional value of a second test is illustrated in Table 3.5. The highest positive probability and the highest likelihood ratio for a positive test result can be reached with the combination of two tests if both tests are positive. The highest negative predictive value, and lowest likelihood ratio for a negative test result, can be reached when neither test is positive. The next step is to decide the level of probability required for the decision to start treatment.

Table 3.5. Results of applying two tests assessing growth hormone (GH) secretion (insulin-induced hypoglycemia and clonidine) to patients with or without GH insufficiency (assuming the availability of a gold standard).

		GH deficiency		Totals
		Present	Absent	
Insulin-induced hypoglycemia	Positive	70	35	105
	Negative	30	65	95
Totals		100	100	200

Sensitivity = 70/100 = 0.70.
Specificity = 65/100 = 0.65.
Pretest probability of GH deficiency = 100/200 = 0.50.
Pretest probability of absence of GH deficiency = 100/200 = 0.50.
Positive predictive value = post-test probability of disease = 70/105 = 0.67.
Negative predictive value = post-test probability of non-disease = 65/95 = 0.68.
Likelihood ratio for a positive test result = se/(1–sp) = 0.70/0.35 = 2.0.
Likelihood ratio for a negative test result = (1–se)/sp = 0.30/0.65 = 0.46.

		GH deficiency		Totals
		Present	Absent	
Clonidine test	Positive	65	15	80
	Negative	35	85	120
Totals		100	100	200

Sensitivity = 65/100 = 0.65.
Specificity = 85/100 = 0.85.
Positive predictive value = post-test probability of disease = 65/80 = 0.81.
Negative predictive value = post-test probability of non-disease = 85/120 = 0.71.
Likelihood ratio for a positive test result = se/(1–sp) = 0.65/0.15 = 4.3.
Likelihood ratio for a negative test result = (1–se)/sp = 0.35/0.85 = 0.41.

		GH deficiency		Totals
		Present	Absent	
Both tests combined	Both positive	55	10	65
	One positive	25	30	55
	Both negative	20	60	80
Totals		100	100	200

If one demands that both tests are positive:
Sensitivity = 55/100 = 0.55.
Specificity = 90/100 = 0.90.
Positive predictive value = post-test probability of disease = 55/65 = 0.85.
Negative predictive value = post-test probability of non-disease = 90/135 = 0.67.
Likelihood ratio for a positive test result = se/(1–sp) = 0.55/0.10 = 5.5.
Likelihood ratio for a negative test result = (1–se)/sp = 0.45/0.90 = 0.5.
If one demands that one or two tests are positive:
Sensitivity = 80/100 = 0.80.
Specificity = 60/100 = 0.60.
Positive predictive value = post-test probability of disease = 80/120 = 0.67.
Negative predictive value = post-test probability of non-disease = 60/80 = 0.75.
Likelihood ratio for a positive test result = se/(1–sp) = 0.80/0.40 = 2.0.
Likelihood ratio for a negative test result = (1–se)/sp = 0.20/0.60 = 0.3.
Source: [4].

Diagnostic strategy

Confronted with a child with a given set of symptoms and signs, an experienced clinician will summarize the problems, make a differential diagnosis, and list the diagnostic procedures to be performed. The list is usually a compromise of rational, economic, and social considerations. The rational approach would dictate that laboratory tests should aim to confirm or refute the most likely condition(s) in a stepwise manner and, at the same time, check for rarer conditions for which timely diagnosis and intervention are important. Economic considerations would urge the clinician to use only tests that offer a reasonable chance of shedding light on the diagnosis at a minimum cost. Social considerations would lead to minimizing the burden for the child by limiting the number of venipunctures, preferably to one. In many cases, it is useful to store part of the serum from the first venipuncture so that additional tests can be performed based on the findings from initial analyses. For this purpose, however, a well-organized storage system is needed.

An aspect of diagnostic tests that is often overlooked is the timing of the blood sampling. With respect to the time of day, some hormone concentrations show a strong diurnal variation (e.g. cortisol), but plasma testosterone and estradiol also show diurnal variation in early puberty.

Measurements of hormones in blood, blood serum or plasma, urine, and other body fluids

The pediatric endocrinologist is heavily dependent on the laboratory to make a proper diagnosis and for patient management. Therefore, some basic knowledge of hormone assays and a good relationship with the pathologist or clinical chemist is essential. In this collaboration, it is important to acknowledge that the physician is responsible for the choice of laboratory parameters to be examined and the test circumstances (e.g. timing, influence of nutrition, medication), for the appropriate transport of the sample to the laboratory, and for the clinical evaluation. The laboratory is responsible for the technical validity and reproducibility of the hormone measurements through standardized laboratory procedures and quality control [5,6].

The concentration of hormones in biological fluids is remarkably small, down to the lower pmol/L range for free thyroxine. Therefore, assays used to measure hormones must be exceptionally sensitive. Presently, most hormones are easily detected with fast and sensitive immunoassays. However, in some specific situations, other methods, such as bioassays, radioreceptor assays, and *in situ* methods, are used.

Before the discovery of the radioimmunoassay, bioassays were the only methods to determine hormone concentrations. Presently, bioassays are only used in three areas: (1) the

standardization of hormone preparations, for example by reference laboratories of the World Health Organization; (2) for testing the biological effectiveness of newly developed drugs; and (3) to test the biological activity of hormones in the serum of patients in whom there appears to be a discrepancy between the immunoassayable concentration and the biological effect. An example of the last is the recently developed test of biological activity of insulin growth factor (IGF)-I [7].

With radioreceptor assays (RRAs), the specific binding of the hormone to a membrane receptor is examined, which obviously does not need to be identical to the biological activity. For this purpose, lymphocytes are mostly used. Immunofunctional assays (IFAs) involve binding of the ligand to the binding site of its natural receptor and subsequent recognition of the ligand bound by a specific monoclonal antibody that serves for quantitative detection [6,8].

In situ methods are mainly used in basic research. They use labeled antibodies and antibody sandwiches for detecting immobilized antigens in microtome sections of tissues (immunohistochemistry), in cells (cytochemistry), and after transfer (blotting) on to carrier membranes (dot-blot, Western immunoblot) [6].

Immunoassays

Immunoassays make use of specific, high-affinity bonding between antibodies and their antigens and are usually divided into two main groups, competitive (reagent-limited) and non-competitive (reagent-excess), assays [5,6].

The principle of the competitive immunoassay (IA) is that, after mixing the antigen (the hormone in the sample) with antibodies and a labeled antigen (tracer), an equilibrium is created between the bound tracer and the bound antigen that reflects their relative concentrations in the assay mixture (Fig. 3.5). By subsequently removing the unbound component, the amount of bound antigens can be estimated. Using a calibration curve describing the strength of the signal as a function of the analyte concentration, the concentration in the sample is calculated. The initial amount of antigen in the sample is inversely proportional to the signal of the bound tracer. Competitive assays are most commonly used for the measurement of steroids and other very small molecules with only one epitope available for antibody binding [6].

Non-competitive immunometric assays (IMAs) use a labeled antibody for signal generation. The most common format is the sandwich technique, which uses a solid-phase bound antibody as a capture antibody [9] (Fig. 3.6). This antibody binds a proportion of the hormone in the serum. The second antibody (tracer), to which the signal-generating label is attached, is added in excess so that it quickly binds to the captured hormone. Reactions can be quite short because only enough hormone, sufficient to produce a detectable signal when the tracer is bound, is required. In IMAs, the bound signal is proportional to the amount of antigens in the sample.

Over the years, many improvements have been implemented. Originally, the tracer (in IA and IMA) was labeled with radionuclides, but enzymes, fluorescent, and chemoluminescent labels are now used. These labels may be coupled directly or indirectly through biotin–streptavidin coupling. Owing to the catalytic effect in enzyme-based assays, it is possible for small amounts of bound enzyme to metabolize large quantities of substrate [6]. The most common endpoint used in both IAs and IMAs is chemoluminescence.

The separation of the unbound components in IA was mostly done by precipitation and subsequent centrifugation of the antigen–antibody complex with the help of a secondary antibody and/or the addition of a precipitation reagent. An improvement comes from the use of solid-phase bound capture antibodies. Usually, this solid phase is the wall of a polystyrene tube, but polystyrene beads or magnetic particles are also used. Excess tracer can thus be removed easily by decanting or suction, and washing away aspecifically bound tracer is also achieved in the same way. Examples include the microparticle-capture enzyme immunoassay (MEIA) and the enzyme-linked immunosorbent assay

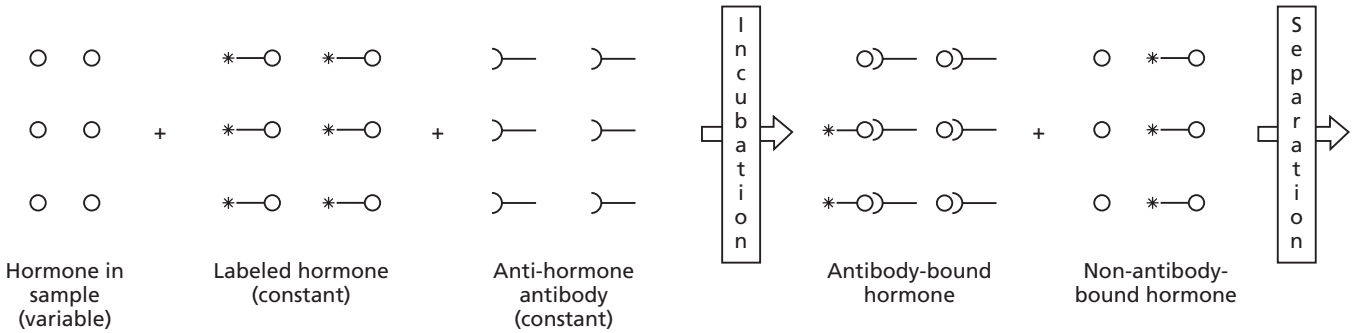

Fig. 3.5. A schematic representation of a competitive immunoassay. Hormone in the patient sample and a fixed amount of labeled hormone compete for a fixed, limited number of antibody binding sites. A variety of methods are used to separate antibody-bound hormone and unbound hormone. The amount of bound labeled hormone is then determined. From [5].

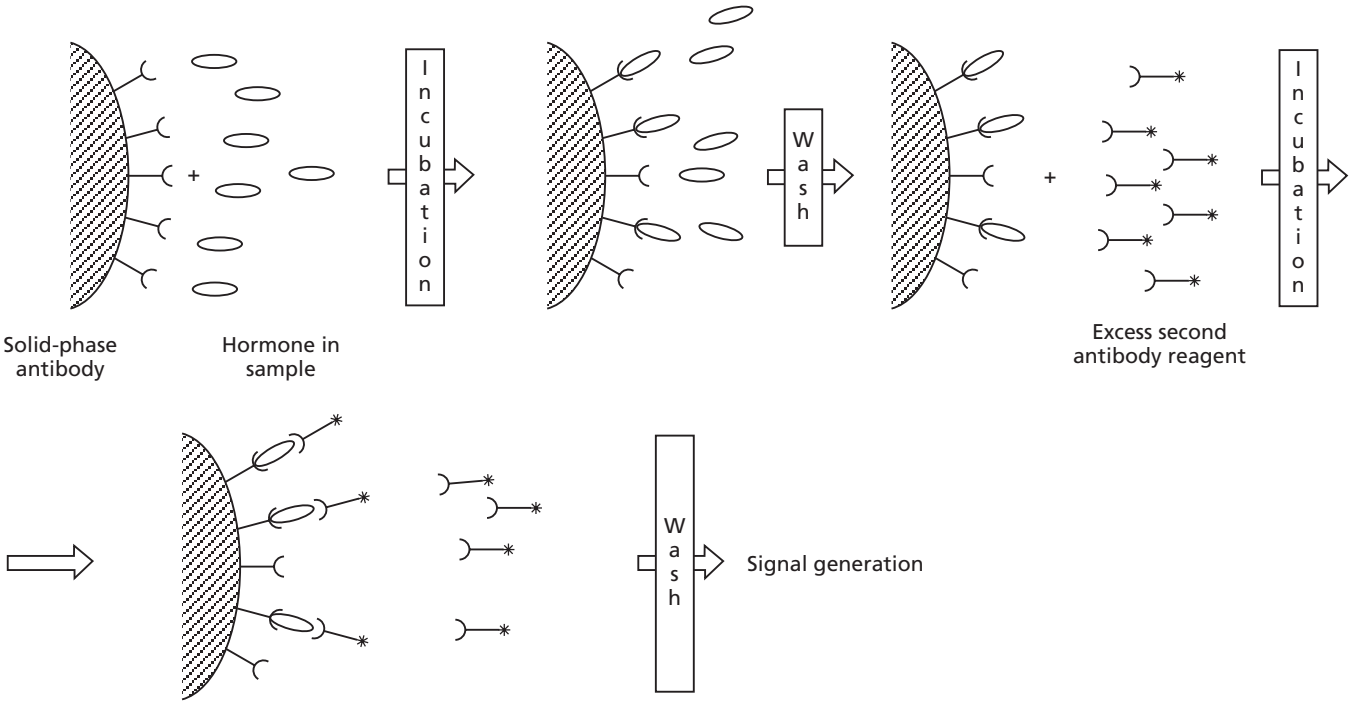

Fig. 3.6. A schematic representation of a sandwich-type immunometric assay. Hormone in the sample binds to a capture antibody, usually attached to a solid phase. Excess hormone is aspirated away, and a second labeled antibody is added in excess. After a short incubation time, excess labeled antibody is aspirated away. The amount of labeled antibody bound is then measured. From [5].

(ELISA), in which immobilization takes place mostly on 96-well microtitration plates.

Antibodies were formerly raised by repeated immunizations of animals with biomaterials. They are now usually raised to recombinant antigens of high purity, and polyclonal antibodies have been substituted for monoclonal antibodies.

One of the most significant changes in immunoassay technology has been the development of fully automated analyzers [5]. These may enable one technician to provide the results for as many as 20 different analytes in a single day. In the near future, new measuring techniques based on biosensorics are expected. In these techniques, binding of the analyte to the immobilized biosensor on a chip directly produces a physical signal that can be detected and processed electronically [6]. Similarly, various physical, biosensor-based measuring techniques are being developed using amperometric, potentiometric, mass detection, or optical biosensors. The microarray chip technology, which is presently mainly used for DNA analysis, can also be used for hormone measurements in protein arrays.

Besides immunoassays, mass spectrometry has also been used for the measurement of small molecules such as steroids. The main use of this technique in routine hormone analysis is in the measurement of steroids in urine [10]. Hormone concentrations can also be measured in body fluids other than blood and urine. In pediatrics, hormone measurements in saliva specimens, which can be gained non-invasively, are used increasingly, for example for cortisol, 17α-hydroxyprogesterone, and androstenedione.

There are four requirements for a good assay. It must be specific (i.e. measure only the particular analyte of interest and not others), sensitive (so that even low concentrations can be measured), accurate (i.e. the result should be close to the target value), and precise (i.e. the result should be reproducible). The result should be compared with an appropriate reference range.

Specificity

A constant challenge to the immunoassayist is specificity. The structures of steroid hormones are very similar, and it may be difficult to differentiate one steroid from another. Protein hormones circulate in different forms: as fragments with small pieces of protein removed, as subunits, or as macromolecular forms [11]. The measurement of the biologically active form may be very difficult because more than one form of the hormone may bind to the hormone receptor. Many hormones, both proteins and steroids, circulate in blood bound to a binding protein, and decisions have to be made whether the total hormone or the non-protein-bound hormone is measured. If the non-protein-bound fraction is measured, the concentration may only be 1/100th of the concentration of the total hormone (e.g. T_4, T_3, testosterone) [5]. The ectodomain of receptors may circulate as a binding

protein [e.g. GH binding protein (GHBP), TSH receptor fragments, leptin-soluble receptor]. Intracellular conversion of some hormones takes place to a more potent metabolite, for example T_4 to T_3, T to DHT, and 25-OH-vitamin D to calcitriol. Local conversion of a potent hormone to a less potent one (cortisol to cortisone in the kidney by 11β-hydroxylase) also occurs.

The ability to produce monoclonal antibodies [12] has led to increased specificity, particularly for peptide hormones. A monoclonal antibody recognizes a single epitope on a molecule. This may not be enough to provide absolute specificity because that epitope may also be present on circulating subunits and fragments of the same hormone or even a different hormone. Greater specificity is imbued by the use of a second monoclonal antibody that recognizes another unique epitope on the hormone. If the two monoclonal antibodies bind to epitopes at different ends of the molecule, then only the intact molecule will be captured, and fragments and subunits are excluded.

Another way to increase specificity (and to remove interference) is to carry out a purification or separation step before immunoassay. Common examples are adsorption, solvent extraction, and high-performance liquid chromatography (HPLC). Alternatively, for protein assays, large concentrations of a substance that has minimal or no cross-reaction with the antibody but binds to the binding protein can be added to the assay reagents to displace the hormone from the binding proteins.

An objective parameter of specificity is cross-reaction, which describes the amount of an analyte similar to the one being measured that will be measured in an assay in percentage terms. The cross-reaction is usually calculated from the virtual analyte concentrations that are detected in samples containing the cross-reacting analyte expressed as a percentage of the given concentration of the cross-reacting analyte.

Sensitivity

Sensitivity may be described as either analytical or functional sensitivity. Analytical sensitivity is the lowest concentration of analyte that is significantly different from zero and is usually the sensitivity quoted for an assay if the two terms are not used separately. Twenty replicate analyses of the zero standard are usually carried out, and the standard deviation (SD) of the responses is calculated. The concentration on the standard curve equivalent to either 2 or 2.5 SD from the zero response is taken as the sensitivity of the assay. Because of variation in the determination of analytical sensitivity and criticism of its usefulness [13], functional sensitivity is more meaningful. The functional sensitivity is defined as the lowest concentration above the analytical sensitivity threshold at which the interassay precision is < 20%, which can be determined from the between-assay precision profile (see below) [5].

Accuracy

Accuracy, or the absence of bias, relates to the closeness of a result to a target value or the systematic error of measurement. This is probably of more concern to the assayist than to the clinician because, as long as reference ranges have been determined properly for the assay in use and no change in the assay occurs, the clinician can determine whether or not a patient has a "normal" result.

It is important to know that the results produced from one assay to another are consistent. Large changes in the bias of an assay can result from errors in reagent preparation, deterioration of reagents, pipetting errors, changes in temperature, and several other variables. To ensure results are consistent, several quality control (QC) procedures may be followed. The standard procedure is to include QC samples. These are commonly commercially produced preparations, usually provided in lyophilized form, that are included in every assay.

A common approach is to run QC specimens at the beginning of the day to check that the machine is operating properly and that the calibration curve is giving the correct results. The QC specimens are then analyzed again at the end of the day. No matter what system is used, the results of the QC specimens must meet predetermined criteria. The most common approach is to plot the results on a Shewart or Levy Jennings graph (Fig. 3.7). To set up this chart, the QC specimens should be analyzed in 20 separate assays. The mean and SD are calculated and plotted. The SD should reflect the interassay precision of the assay determined during the development of the assay or as stated by the manufacturer. Examining the QC plot will indicate whether the assay is acceptable. However, the SD calculated from QC specimens may overestimate true precision, as lyophilization and reconstitution may introduce additional imprecision.

Precision

Precision represents the reproducibility of the measurement of an analyte at different concentrations. Usually the intra-assay or within-assay precision (reproducibility of measurement in a single assay) and the interassay or between-assay precision (reproducibility of measurement between separate assays) are quoted for assays. However, precision is affected by differences between operators, the lot numbers of kits, and temperature, and it also varies with analyte concentration so that a single figure for precision is not possible. Instead, the precision of an assay can be calculated across a wide range of concentrations, and the data are plotted to give a precision profile (Fig. 3.8). The intra-assay precision will usually be better than the interassay precision. Therefore, specimens from studies examining changes in one individual on several occasions should be analyzed in a single assay to improve the detection of small but important changes in concentration.

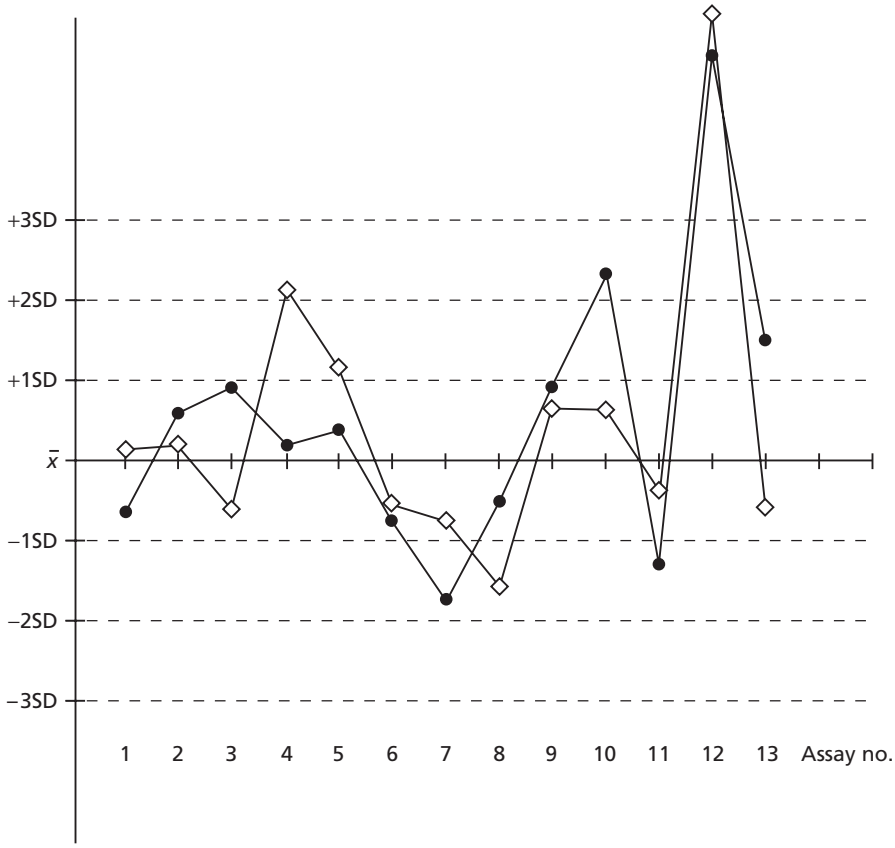

Fig. 3.7. A typical plot of a quality control (QC) specimen run at the beginning (●) and at the end (◇) of the assay. Drift would be indicated by the latter having a constant bias to the former. Assay 12 should be rejected and the patient specimens reanalyzed. There would be at least two, and often three, QCs of different concentrations run in every assay. From [5].

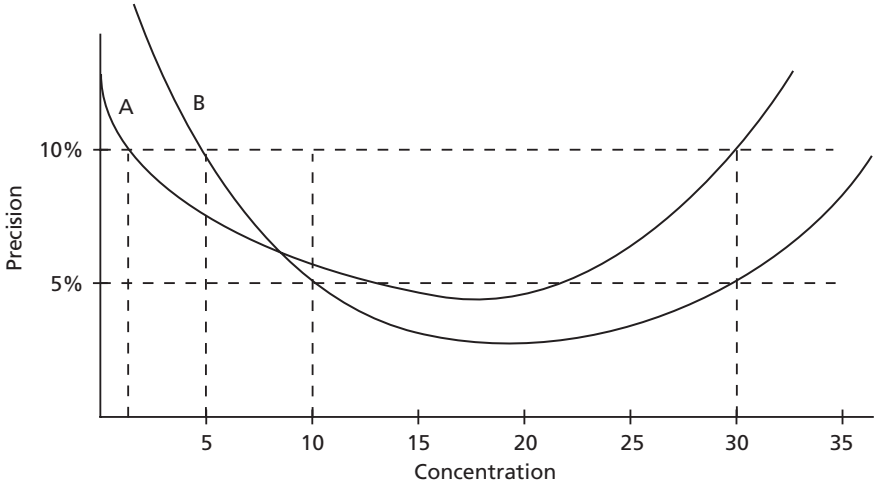

Fig. 3.8. The precision profiles for two methods, A and B. Method A has a greater precision (< 10%) at low concentrations, whereas method B shows better precision than method A at higher concentrations. Choice of method would depend on the range of concentration to be encountered in clinical samples. From [5].

The working range of an assay is derived from the precision profile and is the range of concentration over which the intra-assay precision is less than a chosen amount, usually < 10%. Examination of Figure 3.8 shows that, using this convention, method A has a working range of 1–30 nmol/L and method B a working range of 5 to > 35 nmol/L. Method A thus shows greater sensitivity and is better suited to measure low concentrations, but method B is superior to method A over the range 10–30 nmol/L because it achieves a precision of < 5%. It also shows better precision than method A at higher concentrations.

The most important information of an assay for the clinician is the between-assay precision (representing 1 SD) as specimens are usually sent over a period of days, weeks, or months. As a rough guide, within-assay precision is usually about 1–2% less than between-assay imprecision, so that an assay with a between-assay precision of 5% will have a within-assay precision of 3–4%.

Table 3.6. Physiological parameters associated with changes in hormone concentration.

Parameter	Hormones
Circadian rhythm	Growth hormone, adrenal steroids
Sleep	Growth hormone, prolactin
Puberty	LH, FSH, gonadal steroids, adrenal steroids, growth hormone
Stress and exercise	Growth hormone, cortisol, prolactin
Food	Insulin, glucagon
Age	Estradiol, testosterone, SHBG, IGF-I, DHEAS
Sex	Estradiol, testosterone, SHBG
Menstrual cycle	LH, FSH, estradiol, progesterone
Low and high body weight	Reproductive hormones, growth hormone, leptin

SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate.
Adapted from [5].

Reference range

Having developed an assay that is capable of measuring the hormone of interest in its clinically most relevant form, interpretation of results is further complicated by physiological variables (Table 3.6). Some hormones demonstrate a marked circadian rhythm, which may develop during puberty (e.g. luteinizing hormone (LH) [14,15]), and this has been examined as a possible diagnostic tool in the investigation of delayed puberty in children. Many hormones, both steroids and proteins, are increased during times of stress, which may confuse the interpretation of a result but, again, has been exploited clinically (e.g. the GH response to exercise in the investigation of GH deficiency in children [16]).

The *reference range* or reference interval is the range of concentrations of an analyte found in 95% of a defined population with no apparent pathology. Therefore, by definition, 5% of this population has analyte concentrations outside this range. Laboratories may use the range quoted by a manufacturer if using a commercial kit, but manufacturers usually state that the reference range data are provided only as a guide. Alternatively, a range quoted in the literature can be used, but one should avoid using a reference range established using another method. Ideally, a range determined by analyzing samples using the same laboratory assay should be established in a population clearly defined by sex, age range, and time of day. As shown in Table 3.7, the concentration of a number of hormones changes throughout life. In addition, the investigation of some diseases requires dynamic tests, and reference ranges for the response are also required. Reference ranges do not necessarily reflect health or disease. For example, high insulin values within the reference range for the general population may reflect subclinical insulin resistance in obese individuals. Therefore, evidence-based target values may sometimes be more appropriate.

Endocrine tests (profiles, stimulation tests, suppression tests)

Assessment of endogenous secretion

Many hormones are secreted in pulses or have specific oscillatory activity. The time course over which these regularly occurring cycles of hormone secretion take place is variable. For example, while insulin has a dominant periodicity of 13 min, GH pulses appear on average once every 3 h, and cortisol has a diurnal rhythm with superimposed smaller pulses. In the case of a diurnal rhythm, at least two blood samples have to be drawn. This minimal sampling protocol is often used to estimate diurnal variation of cortisol secretion.

From a clinical perspective, the main reason for performing multiple measurements of a hormone over time is to estimate its secretion. This primarily applies to GH profiles in pediatric endocrinology. A second reason is to assess the pulsatile pattern, either for diagnostic reasons (e.g. a nocturnal LH profile to estimate whether puberty has started) or to improve our understanding of the physiological role of pulsatile patterns. There is strong evidence that pulsatile secretion in animals may act as a biological signal for tissue-specific responses [17,18].

In discussing methods of pulse analysis, it is worth considering why we should analyze them. From a statistical point of view, a glance at an individual data array is enough to demonstrate the existence of oscillation. However, where multiple data sets are available and when statements need to be made about group data, it becomes important to be able to extract attributes of pulsatility, which can then be pooled to provide a generic description.

Blood sampling

Two methods have been devised to obtain hormone profiles, discrete (single spot samples) and integrated sampling, where blood is withdrawn continuously over periods of varying length. Both techniques have advantages and disadvantages but, as long as the proper time interval is chosen (e.g. 20 min for GH), the results are similar [18,19].

To define rhythm, sampling must take place over more than one cycle. It is important with any sampling technique to consider also the effect of the sampling interval on the possible results obtained. Inappropriately long sampling intervals can lead to spurious results and failure to detect the oscillation of a hormone concentration. A minimum of five or six samples per cycle is required to prevent the mismatching of infrequent sampling intervals to the predominant period of pulsatility that is being observed. This mismatching is known as aliasing and is illustrated in Figure 3.9 [20].

The sampling interval used determines the cycle frequency that can be detected. The lower the frequency of interest, the

Table 3.7. Situations that require separate reference ranges for the hormones affected.

Parameter	Hormones affected	Changes encountered
Neonate	17α-Hydroxyprogesterone	Rapid changes after delivery
	Testosterone in males	Rises after first 2 weeks of life and then falls at about 8–10 weeks
Children	Reproductive hormones	Low prepubertally and increase during puberty
	Adrenal androgens	
	IGF-I	
Aging adult	Gonadotrophins in women	Increase in post-menopausal women
	IGF-I	Decrease with age
	DHEAS and DHEA	
	Testosterone in men	
	SHBG	Increase as age increases
Menstrual cycle	Gonadotrophins	Concentrations are different in the follicular, mid-cycle, and luteal phases
	Estradiol	
	Progesterone	
	Inhibins	
	17α-Hydroxyprogesterone	
Circadian rhythm	ACTH	Higher levels in the morning than in afternoon and evening
	Cortisol and other adrenal steroids	
	Testosterone in men	
Sleep	GH	Higher levels at night
	Prolactin	
Posture	Renin	Increase in concentrations moving from supine to standing
	Aldosterone	

DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate.
Source: [5].

longer the time period over which measurements can be taken; conversely, the higher the frequency, the more frequently observations must be made.

Analysis of profiles

For clinical purposes, a single profile requires little analysis. Routine parametric statistics can be used to establish the mean and SD of the data. There is no advantage in calculating the area under the curve, because it is identical to the mean multiplied by the total duration of the sampling. Another parameter that can be read directly from the raw data is the number and amplitude of the peaks and the maximum peak.

Where statements need to be made about populations or subpopulations, or the changes within pathological states, or following treatment intervention, or when other attributes of pulsatile systems (such as periodicity, regularity, trough values, shape of peaks, rate of change of hormone concentrations, frequency, and/or amplitude modulation) are to be investigated, more sophisticated techniques are required. These techniques fall under the general heading of "time series analysis," which involves techniques for analyzing regularly sampled data [20].

Pulse identification

The goals of pulse detection include uncovering the underlying pattern in the profile and serving as a tool to evaluate and compare profiles from different groups of patients. Several computerized peak detection algorithms have been developed for this purpose, each trying to pinpoint criteria of what constitutes a peak and what is biological noise. Two programs, PULSAR [21] and Cluster [22], have been used most widely.

The principle of PULSAR consists of a moving window placed over part of the data series. Within the window, baseline secretion is calculated. Peaks are then defined as data points exceeding the baseline by a preset multiple of the standard deviation. The peaks recognized within the first pass through the series are then removed for the next calculation of the baseline to take place. After several iterative passes, the baseline remains stable, and peaks are identified.

The underlying algorithm of Cluster [22] tests the hormone profile for significant upstrokes by performing a *t*-test between groups of data points and their successors. In a second step, downstrokes are localized in a similar way. Peaks are then defined as hormone levels preceded by an upstroke and followed by a downstroke.

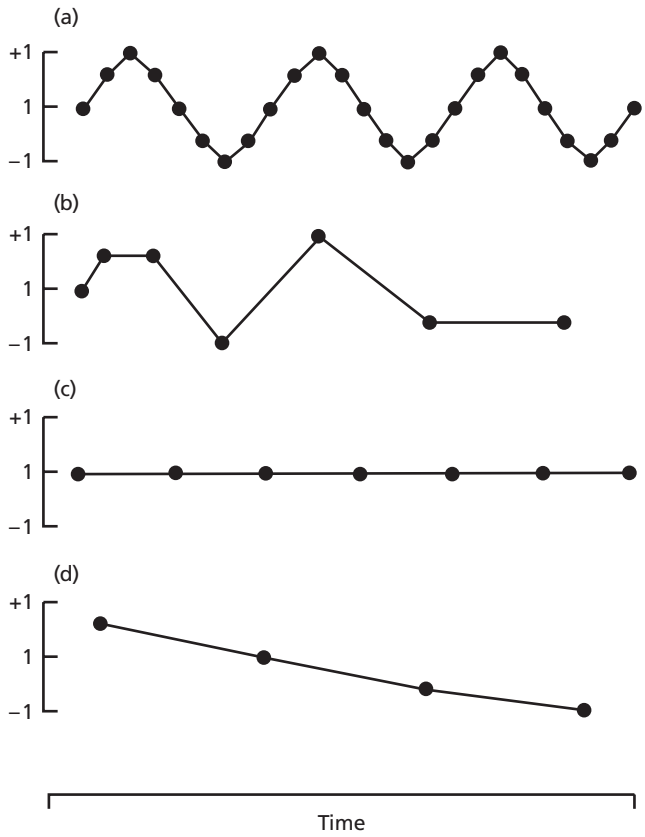

Fig. 3.9. For a real oscillation (a), an incorrect assessment of its period can be made using inappropriate sampling intervals (b, c, and d). From [20].

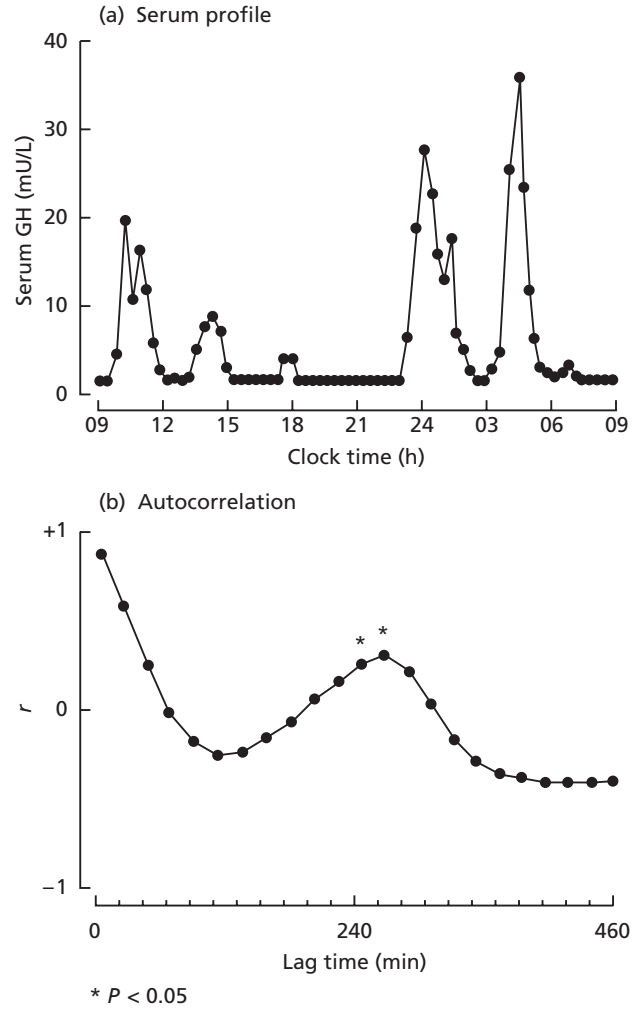

Fig. 3.10. Autocorrelation function (b) of serum growth hormone (GH) concentration profile (a) with a regular waveform (**) occurring at 240 min or 4-hourly. This can be cross-referenced with the actual data array. From [20].

Approximate entropy

Approximate entropy is a measure that attempts to quantitate regularity in data in conjunction with standard measures such as the mean and root mean squared [23,24]. Entropy is a concept that addresses system randomness and predictability. The greater the entropy, the more the randomness and the less the system order. The higher the value of approximate entropy, the more random the time series.

Deconvolution analysis

The concentration of a hormone measured at any point in time represents a balance between secretion from the gland of origin and clearance from the circulation. It is possible, from knowledge of hormone clearance or by making a priori assumptions about clearance, to work back from (deconvolute) the measured concentration [25]. The program that is used most is Deconv/Pulse [25]. This multiple-parameter deconvolution technique includes the calculation of a subject-specific hormone half-life, temporal positions of secretory bursts, as well as the mass of the hormone secreted.

Another deconvolution method was developed in which GH kinetics was calculated assuming a two-compartment model as a basis [26]. The results of deconvolution were similar to the area under the curve, so that this could serve equally to estimate the amount of the hormone secreted. Another algorithm, DETECT [27], is less used.

Autocorrelation and Fourier analysis

Another approach is the application of mathematical procedures, such as autocorrelation and Fourier transformation, to hormone profiles [20,28]. Autocorrelation (Fig. 3.10) is a technique for establishing whether there are regularly recurring waveforms (of any shape) within a data array. The method is independent of the shape of the wave and the start point of the profile. The end result is an estimate of period and an assessment of its significance.

If more than one frequency or rhythm is present in the time series, these underlying frequencies may be difficult to assess by inspection of the autocorrelation function because different frequencies can obscure each other. In this situation, as with any complex waveform, these can be deconvoluted into

a series of sinusoids. Amplitude and frequency components of the sinusoids can be dissected, and this assessment is called Fourier transformation. The method allows groups of data to be compared and information to be provided on frequency, frequency modulation, amplitude, and amplitude modulation [29].

Distribution methods

With distribution methods, the log-transformed hormone concentration values are sorted in ascending order regardless of their temporal attribute. The distribution can then be plotted and compared between patient groups [18]. This method is also suitable for assessing the trough concentration [18,30]. It determines the proportion of time of the whole profile that is occupied at certain concentrations. For example, in the case of GH, the concentration at or below which the profile spent 5% of its time might be considered as a marker of trough activity. Estimates of the trough values of hormone profiles may be needed to understand pathological situations, e.g. acromegaly and Cushing syndrome.

Co-pulsatility

If one wishes to evaluate synchronized activity of two or more hormonal systems objectively, two programs are available. Hypergeo [31] is based on the principle of hypergeometric distribution. Its input consists of the number of samples in a profile, the number of pulses observed for two hormones, and the number of coincident pulses. AnCoPuls [32] counts the number of coincidences in a given profile, allowing for phase shifts and increasing windows defining "coincidence."

Clinical implications

Sequential hormone measurements are mostly performed for GH. In theory, it should be the gold standard for endogenous GH secretion in a particular child, but it is assessed in only a minority of patients because it is time-consuming and burdensome both for the patient and for medical and nursing staff. Other problems are that the amount of blood drawn must be small in relation to the patient's blood volume, and that it may be difficult to establish a peripheral catheter large enough to draw blood from it and to remain patent in small children.

A further problem is that it is almost impossible to obtain control data. In addition, in the rare studies in which healthy children have undergone 24-h sampling, a remarkably large variation in 24-h GH secretion was observed [33]. Part of this large variation appears to result from day-to-day variability, which further decreases the value of the test [34]. Another part of the variation may be due to interindividual differences in GH sensitivity.

A 12- or 24-h GH profile may deserve a place in the diagnostic armamentarium in those patients in whom a clinical suspicion of GH deficiency, in combination with low plasma IGF-I and IGF binding protein (IGFBP)-3 values, is not confirmed by a low GH peak at a provocation test. In these cases, there are three possibilities: a discrepancy between a low spontaneous secretion and a normal GH peak after provocation (termed neurosecretory dysfunction [35]), an abnormal GH molecule, or GH insensitivity. Neurosecretory dysfunction may be the most frequent of the three options. The discovery of polymorphisms with different activity of the GH promoter sites lends further support to the possibility that neurosecretory dysfunction really does exist [36]. However, the alternative explanation, that neurosecretory dysfunction applies to the group of children with idiopathic short stature with a false-positive test result in the 24-h GH profile test, cannot be ruled out.

Another hormone with a strong pulsatile character is LH, particularly in the early phases of puberty, the first sign of which is augmented pulsatile secretory activity of LH during sleep [14,37]. As puberty progresses, the amplitude and frequency of LH pulses increase, initially only during the night but, when puberty progresses, also during daytime, leading ultimately to the adult pattern of circhoral release of LH bursts [15]. If a gonadotropin-releasing hormone (GnRH) test and GnRH analogue test still leave the clinician in doubt as to whether puberty has started, night-time sampling of LH and follicle-stimulating hormone (FSH) can be performed, with an interval of not more than 20 min.

Stimulation and suppression tests

Stimulation tests are used to assess the maximum secretion of a hormone. In principle, the tests measure the responsiveness to the stimulus and the hormonal reserve in the target cells, which is not necessarily identical to the endogenous secretion. Indeed, there is only a moderate correlation between 24-h profile and GH stimulation test results [38,39], which is probably due to considerable intraindividual variation in both tests. Still, for practical reasons, GH stimulation tests are generally used as a proxy parameter of endogenous secretion.

Theoretically, a stimulation test should be more sensitive to pick up subtle deficiencies that would not be picked up by assessing spontaneous hormone concentrations, as compared with baseline hormone concentrations. For example, a subclinical (compensated) hypothyroidism may become apparent only by elevated stimulated TSH values after a thyrotrophin-releasing hormone (TRH) bolus. Similarly, a partial deficiency or heterozygosity of one of the adrenal enzymes, or partial (compensated) primary or secondary hypocortisolism, can become apparent only after adrenocorticotrophic hormone (ACTH) stimulation.

A problem with every stimulation test is that standardization is poor. As with all tests in pediatrics, there are considerable difficulties obtaining age-matched controls. Usually, protocols are based on historical arguments, for example the first protocol that was reported.

Suppression tests are performed to study whether the hormone production is still under physiological control. Examples include the oral glucose tolerance test to check whether GH secretion can be suppressed and the dexamethasone suppression test to check whether cortisol or androgens can be suppressed.

Chromosomal analysis and molecular tests

Although chromosomal analysis has been used for five decades, a newer development is fluorescent *in situ* hybridization (FISH) analysis, which detects whether two copies of a gene are present (the normal situation) or one (haplodeficiency) or three (duplication), or detects translocations. Molecular tests (DNA testing) are playing an increasing role in endocrine diagnosis.

Chromosomal analysis is a crucial part of endocrine diagnosis of girls with short stature. Even without the classical stigmata, short girls may have Turner syndrome, which can be confirmed only by assessing the karyotype. When the karyotype of the leukocytes is reported as normal, Turner syndrome still cannot be completely ruled out as mosaicism may occur (although rarely), in which the chromosomal abnormality is absent in leukocytes, but present in other cell types, such as skin fibroblasts. Chromosomal analysis is also crucial in children with ambiguous genitalia and in boys suspected of Klinefelter syndrome (XXY) or an XYY karyotype, and is helpful in children with dysmorphic features. One should bear in mind that this technique detects only numerical abnormalities, gross deletions, or translocations.

FISH analysis is a valuable addition to chromosomal analysis. Its principle is that, if a certain disorder is suspected, the probe for its gene or critical region and one or two probes for control genes (with another color) on the same chromosome are added to the chromosomal smear. In normal circumstances, one dot for the critical region and one for the control gene are visible on each of the two sister chromosomes. In the case of a deletion of the gene on one chromosome, only one colored dot is detected in contrast to two dots of the control gene. Duplication of the gene on one of the chromosomes is detected by three dots (Fig. 3.11). In the case of a translocation, the dots for the gene of interest and the control gene are located on different chromosomes.

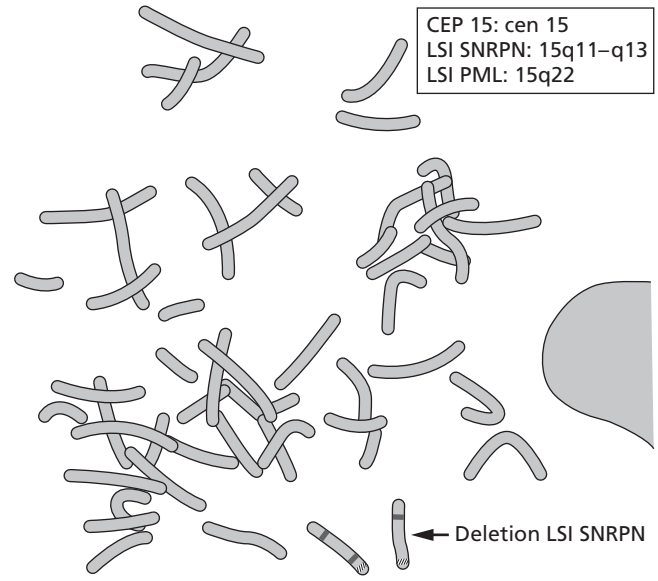

Fig. 3.11. FISH of a patient with a deletion of the critical region for the Prader–Willi–Labhart syndrome (PWS). The LSI SNRPN probe is specific for the critical region of PWS located within 15q11–q13. The CEP15 (15p11.2) and LSI PML (15q22) serve as control probes for chromosome 15. Courtesy of Drs K. Hansson and C. Ruivenkamp, Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

Mutations and polymorphisms

The purpose of DNA testing is to find a mutation in the DNA that is responsible for the disorder of the patient. A mutation is a change in the primary nucleotide sequence of DNA. Mutations can occur in the germline, during embryogenesis, or in somatic tissues. Mutations that occur during development lead to mosaicism. A mutation can influence the function of a gene and, thus, the protein it encodes, but whether this occurs is dependent on the location of the mutation and its nature. Polymorphism has been defined either in terms of prevalence (a mutation that occurs in more than 1% or 5% of the population) or in terms of functionality (a mutation that does not have any distinguishable effect).

With respect to the location, a mutation can occur in a coding or a non-coding region. In the coding region, various point mutations can be found, such as small insertions or deletions of one or a few nucleotides or single-nucleotide alterations, which can be divided into neutral, nonsense, and missense mutations. The addition or removal of one (or a number that cannot be divided by three) nucleotide(s) leads to a frameshift, i.e. a shift in the reading frame. Thereby, the code of all consequent codons changes, so that a strongly abnormal protein is formed. Usually, one of the codons changes into a stop codon, so that the formation of the protein stops prematurely. This is almost invariably associated with disease. The same usually applies to a nonsense mutation, as this leads to a premature stop codon. A neutral mutation indicates a nucleotide alteration that does not result in a change of amino acid in the translated protein. Usually, this has no functional relevance, except if the nucleotide alteration affects mRNA stability or protein translation, e.g. when the resulting codon is rarely used in humans.

If the mutated codon codes for another amino acid, it is not always clear whether the mutation causes disease. The protein may still retain its full function or part of it. The phenotype can thus vary from a complete deficiency to no signs at all. Additional functional tests may be needed in such cases

before the mutation is causally associated with the disease. If the mutation results in a protein with an amino acid with a different charge, particularly if it is located in a functional domain of the protein that is crucial for protein–protein interactions, folding, or other aspects of secondary and tertiary structure, one can be almost sure that the mutation is functional. If a mutation leads to less clear changes in the protein, one can check whether the mutation is found in control subjects. If it is not, the likelihood of a functional mutation increases, but no absolute certainty is obtained yet. A next step is to check whether the mutation is present in family members with or without the disease. If the mutation segregates with the disease, it provides additional evidence and, depending on the number of family members available for analysis, can even prove causality. Finally, biochemical tests in cellular models or knockout models may be necessary to clarify the functional importance of the mutation and its relation to disease.

The great majority of mutations in non-coding regions have no functional relevance, but there are three exceptions: a mutation in the promoter region or a response element of the promoter can cause diminished transcription and therefore less protein production [36]; a mutation at the boundary between an exon and an intron or in its close vicinity can disturb the action of the splicing machinery, leading to a defective RNA molecule due to aberrant splicing; a mutation in the untranslated tail or polyadenylation signal of the gene can diminish the stability of the mRNA, causing a lower protein production.

Approximately 50% of point mutations occur in CpG dinucleotides (a cytosine nucleotide linked via a 5′ phosphate group with a guanine nucleotide), so-called "hotspots" for mutation. A CpG can then change into a TpG or CpA. If the same CpG mutation occurs in different families, additional molecular tools are available (a haplotype or pedigree analysis) to distinguish a new mutation within a family from an inherited mutation that can be tracked to a unique mutation many generations before.

Most mutations in an important part of the gene cause loss-of-function. Most are rare and occur heterozygously. Inactivating mutations are usually recessive. Alternatively, deletion of a single allele can result in haplodeficiency, a situation in which one normal allele is not sufficient to maintain a normal phenotype. Other heterozygous mutations can result in loss-of-function due to a dominant-negative effect, in which the affected allele impairs the function of the second normal allele. In some instances, a mutation leads to a gain-of-function. These mutations are characterized by a complete or incomplete dominant inheritance. In some genes, for example the parathyroid hormone (PTH) receptor and the calcium-sensing receptor, both loss-of-function and gain-of-function mutations have been found.

For the proper expression of a gene, not only has the gene itself to be normal but also its regulation. The transcription of genes is controlled primarily by transcription factors that bind to DNA sequences in the regulatory regions of genes (promoter) usually located upstream of the transcription start site. Other proteins (co-activators and co-repressors) interact with the DNA-binding transcription factors to generate large regulatory complexes. Gene expression is also influenced by epigenetic events, such as X inactivation and imprinting.

Indications for molecular tests

Molecular tests are usually performed to confirm a clinical and/or biochemical diagnosis and to make the diagnosis more precise in terms of the molecular defect. In patients suspected of a disorder of which the genetic cause is known (e.g. achondroplasia, hypochondroplasia) or in a family where one index case is known with a genetic disorder, a direct investigation of the expected affected gene or mutation is performed. In some disorders, one particular mutation or just a few are observed in a disease, e.g. the different mutations in the FGFR-3 gene in achondroplasia and hypochondroplasia. In other disorders, an almost unlimited number of different mutations is found, e.g. in congenital adrenal hyperplasia due to 21-hydroxylase deficiency [40]. The additional information on the exact genetic defect is of value for the patient because it can provide certainty about the diagnosis and, in some cases, it provides more reliable information about the clinical course and prognosis of the disorder. Furthermore, it can serve as a basis for genetic counseling and prenatal diagnosis. It is also of value in terms of clinical research, because an analysis of the clinical phenotype and genetic defects of a group of patients will provide a better insight into the genotype–phenotype correlation. In turn, this will be of use for a firmer diagnosis of and information to future patients. Examples of this are the genotype–phenotype studies in patients with congenital adrenal hyperplasia due to a 21-hydroxylase defect, which have led to a better understanding of which mutations lead to a severe clinical phenotype and which to the non-classical presentation [40].

While this form of molecular testing is still in line with classical biochemical tests, and is in fact an extension of them, molecular tests can also be used to unravel the causes of a disorder that is different from ones described before. The clinician should always keep an open mind in such cases and try his/her best to find the genetic explanation in collaboration with clinical geneticists and molecular biologists. There could be a new mutation in a known gene or an abnormality in a gene that was not associated with disease before. This investigation is greatly facilitated by the Human Genome Project (HGP), which has generated genetic and physical maps of the majority of the human genome. Thus, an important advantage of techniques of molecular biology is that they enable the detection of disorders that do not lend themselves to conventional hormone measurements or have not been

defined in their pathophysiology [41]. In this way, the identification of defective genes can pinpoint cellular pathways involved in key physiological processes.

For the practicing pediatric endocrinologist, the family history remains an essential step in recognizing the possibility of a hereditary disorder. It is useful to draw a detailed pedigree of the first-degree relatives. In patients suspected of a genetic disorder with no clear positive family history (for example the vast majority of cases with Sotos syndrome), it might be useful to collect DNA not only from the index case but also from the parents to check whether a mutation has occurred *de novo* (i.e. is only present in the index case but not in both parents). In these cases, a *de novo* mutation provides convincing evidence for its pathogenicity. If no candidate gene is known, it is helpful to obtain material from as many as possible family members, affected and unaffected.

Sample preparation, DNA/RNA extraction, and isolation

Sample preparation must be performed according to the instructions of the laboratory where the DNA analysis will take place. In many cases, it is sufficient to take a blood sample (5–10 mL) in a tube treated with EDTA (ethylene diamine tetra-acetic acid) and mail it to the laboratory at room temperature. High-quality DNA can be isolated from these samples even when samples are stored for more than 2 weeks at room temperature.

In the laboratory, the first step is to isolate DNA from the whole blood sample. There are numerous protocols for doing so, and commercial DNA extraction kits are available. If isolated properly, DNA can be stored for prolonged periods of time at 4°C without quality reduction, until appropriate tests are available for a genetic disorder of as yet unknown origin.

In some situations, it may be advisable to obtain RNA instead of DNA. The handling of RNA is much more complicated than that of DNA. RNA degrades rapidly, so it must be extracted directly from fresh tissue under specific conditions or tissue must be stored immediately at –80°C for later processing. To ensure RNA for future testing, it is useful to perform a skin biopsy from which a dermal fibroblast culture is established. Similarly, patient lymphocytes can be Epstein–Barr virus (EBV) immortalized. Both fibroblasts and immortalized lymphocytes can be stored indefinitely in liquid nitrogen. In the case of possible somatic mutations, which are limited to a neoplastic tissue (for example in McCune–Albright syndrome), a sample of this lesion can be used for extraction of DNA or RNA.

Frequently used techniques for detection of a mutation

Advances in molecular biology have greatly facilitated clinical genetics. Two important milestones are the discovery of the polymerase chain reaction (PCR) [42] and the completion of the Human Genome Project (HGP).

PCR is an *in vitro* method for copying a given DNA sequence exponentially. PCR has simplified and accelerated the isolation and cloning of DNA fragments dramatically. It is at the basis of most procedures currently used for the detection of mutations and was fundamental to the completion of the HGP. The components of a PCR include:

1 a DNA template (usually genomic DNA, but also cDNA obtained after reverse transcription of mRNA; see below);
2 oligonucleotide primers: short (about 20 nucleotides long), biochemically synthesized, single-stranded DNA molecules complementary to the DNA sequences that bracket the target DNA sequence of the template, present in great excess;
3 the nucleotides dATP, dGTP, dTTP, and dCTP (dNTPs) as substrate for the DNA copies and energy donors for the polymerization process;
4 a heat-resistant DNA polymerase; and
5 buffers that create an optimal environment for both polymerase activity and primer annealing.

There are three stages in a PCR cycle. At high temperature, the double-stranded DNA template is denatured (made single-stranded). Then, the temperature is lowered to enable the primers to bind to their complementary DNA sequence (annealing). Thereafter, the temperature is raised again to create the optimal temperature for the action of DNA polymerase, whereby the new DNA strand is made (extension). Then a new cycle starts again, usually 20–30 times. The nucleotide sequence of the amplified PCR product is confirmed by sequencing.

PCR can also be used for the amplification of mRNA. To accomplish this, one has first to transcribe the mRNA into complementary DNA (cDNA) using the enzyme reverse transcriptase. This process is called reverse transcription. In short, mRNA is incubated with an oligonucleotide primer of Ts, complementary to the poly-A tail of the mRNA. After annealing of the primer sequence to the mRNA, the primer sequence is extended by the enzyme reverse transcriptase, for which the mRNA serves as a template. Alternatively, random priming can be performed using hexanucleotides, which facilitate the amplification of 5′ regions. The result of the activity of the reverse transcriptase is a double-stranded RNA–DNA duplex, the cDNA of which can be used as input in a PCR. This procedure is called reverse transcription (RT)-PCR and is useful as a qualitative or, with some modifications, a quantitative measure of gene expression.

For the detailed analysis of a piece of DNA, frequently obtained by PCR, the nucleotide sequence is determined with sequencing [43]. The present form of this technique is a unidirectional PCR (instead of two primers necessary for exponential amplification, only one primer is added to the reaction resulting in a linear amplification). On top of the natural nucleotides, abnormal nucleotides (dideoxynucleotide triphosphate or ddNTP) are added, labeled with different

fluorochromes. In each tube, a different one is used representing each of the four ddNTPs that stop the PCR randomly at a certain point. The resulting mixture consists of fluorescently labeled fragments of various lengths that are separated by gel electrophoresis. Subsequently, the sequence is read using the fluorochromes. This method can be automated, and sequences of various DNA templates can be determined simultaneously. Sequence analysis of PCR-amplified DNA fragments representing a candidate disease gene is the method of choice to search for pathogenic mutations. It is simple, cheap, reliable, and has a high sensitivity for the detection of mutations. Developments in this area, including the automation of the procedure and the use of PCR and sequencing robots, have reduced the use of various other more time-consuming and laborious screening techniques, such as single-strand conformation polymorphism (SSCP) [44], denaturing gradient gel electrophoresis (DGGE) [45], temperature gradient gel electrophoresis [TGGE], and heteroduplex detection by denaturing HPLC (dHPLC) [41,46].

Traditional cloning techniques, which were successful in identifying many hormone receptors, such as expression cloning and cloning by similarity, are used less frequently, partly because of the completion and spinoff of the HGP. Cloning refers to the creation of a recombinant DNA molecule that can be propagated indefinitely. This can be effected by inserting the gene or a cDNA into a vector that can replicate within a host cell, often the bacterium strain *Escherichia coli* K-12. If the DNA fragment is not too large, a plasmid can be used as a vector, which is a small, circular double-stranded piece of DNA that can be propagated in a bacterium. The plasmid usually also contains a gene for resistance to an antibiotic, often ampicillin. For cloning larger pieces of DNA, another vector, such as phage lambda or bacterial or yeast artificial chromosomes, can be used. Computational methods and bioinformatics can now be used to predict gene function of previously uncharacterized genes and to test directly hypotheses about gene function in experimental models. An example of a hormone receptor that is cloned by conventional strategies is the PTH receptor, the rat homolog of which was cloned by expression cloning, using radiolabeled PTH as bait. Subsequently, the rat cDNA was used for isolation of the mouse and human homologs [47,48].

Mutation detection in case of certainty about the gene involved

Knowledge of the identity of the disease-causing gene is fundamental for the detection of a pathogenic mutation. When the genetic defect of a particular disorder is known, mutation analysis is straightforward and can be performed in specialized centers for clinical genetics or in a standard laboratory setting equipped for molecular biology. If information on the gene involved is lacking, various approaches can be followed to localize and identify the genetic defect (Fig. 3.12).

In the flow chart in Figure 3.12, the steps in the identification of a pathogenic mutation in a disorder of which the gene defect is known are depicted schematically. After isolation of genomic DNA from the index case, overlapping fragments of the candidate gene covering all coding exons and approximately 50 bp of flanking intron sequences are amplified by PCR. The required oligonucleotide primers can either be derived from the literature or be designed using software programs freely available on the internet and the sequence information of the gene provided by the HGP. The nucleotide sequence of the PCR products is determined and compared with a reference sequence. In this way, one can detect nucleotide alterations, in either heterozygous or homozygous form, with high specificity. The alterations have subsequently to be classified as either disease-causing (pathogenic) or as a non-functional polymorphism using the strategies described above. Using this method, the majority of disease-causing mutations can be detected. A few exceptions are pathogenic mutations located in intronic sequences or in the promoter region of the gene, which are not amplified by the PCR. Microdeletions involving, for example, one of the two copies of an exon are not detected using this method. In this case, the normal allele is amplified, and a false-negative score is obtained. In these cases, the expression pattern of the mRNA or sequencing of the corresponding cDNA may be needed for the detection of the mutation. New PCR-based techniques have become available, such as multiplex amplifiable probe hybridization (MAPH) and multiplex ligation-dependent probe amplification (MLPA). Both rely on sequence-specific probe hybridization to genomic DNA, followed by amplification of the hybridized probe, and semi-quantitative analysis of the resulting PCR products. The relative peak heights or band intensities from each target indicate their initial concentration. These techniques are ideally suited for the detection of small deletions or amplifications [49].

Mutation detection in case of uncertainty about the gene involved

There are several ways to identify a gene responsible for a genetic endocrine disorder. One strategy is to search the literature for arguments that one or more genes can be considered candidate genes for the disorder. These candidate genes can subsequently be screened for the occurrence of pathogenic mutations by direct sequencing as outlined above. For example, the discovery of the etiology of the combined pituitary deficiency (GH, prolactin, and TSH) in the Snell mouse (a mutation in the Pit-1 gene) led to the discovery of similar mutations in the human equivalent gene POU1F1 [50]. Later, the discovery of another transcription factor in pituitary ontogenesis, Prop-1 in the mouse, was followed by the detection of mutations in the human homolog [51]. Indeed, in recent years, resemblance of phenotypes observed in transgenic mice with human disease has led to a rapid increase

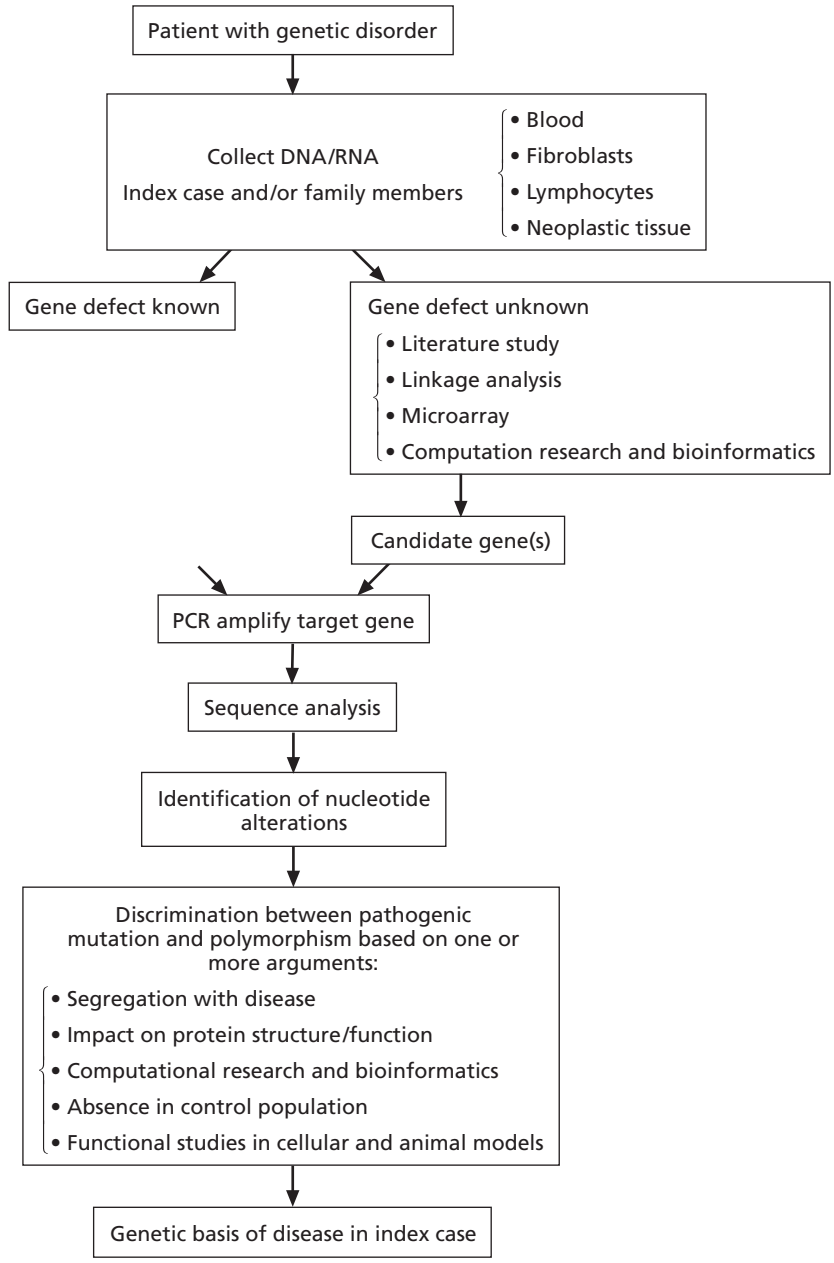

Fig. 3.12. Flow diagram for the identification of disease-causing mutations. See text for details.

in the elucidation of many genetic disorders of unknown etiology.

In other cases, the presence of one patient with an abnormal karyotype, caused for example by a translocation, can help in the identification of the gene involved. In such cases, it can be suspected that one of the breakpoints is located in the gene itself or in its close vicinity, which causes the disease. In such cases, the HGP will give information about the genes located in the neighborhood of the breakpoint. This strategy led, for example, to the discovery of the NSD-1 gene implicated in Sotos syndrome [52].

In the absence of more or less direct clues for a candidate gene, other techniques may be required to identify the responsible gene, such as "positional cloning," referring to the technique in which a gene is isolated on the basis of information about its chromosomal location. In the past, this was a time-consuming and laborious effort often taking several years to identify the genetic origin of a disease. Positional cloning has been simplified dramatically by the completion of the HGP. Information on the chromosomal region involved in the disease is derived from linkage analysis.

Linkage studies

Because most of the human genome does not code for protein, a large amount of sequence variation exists between indi-

viduals. If these variations in DNA sequence, referred to as DNA polymorphisms, can be followed from one generation to the next, they can serve as genetic markers for linkage studies. Linkage means that the gene for the disease and the DNA marker are co-inherited. Polymorphic means that several variations (alleles) of the DNA marker occur in the population. Thus, if there is no candidate gene and if one or several large families are known with a disease, a genome-wide linkage study can be performed. In such an analysis, the location on the chromosome where the gene of interest is located is spotted by a linkage between the disease and an inherited polymorphic DNA marker situated on a known spot on a chromosome. If the mutated gene lies close to a polymorphic marker, there is a strong likelihood that the mutated gene joins the marker during the process of recombination in meiosis. Linkage is expressed as a lod (logarithm of odds) score. High lod scores of +3 are generally accepted as supporting linkage, and a score of –2 is consistent with the absence of linkage. Using a polymorphic marker set evenly distributed over all human chromosomes, it is possible to pinpoint the genomic region that is involved in the disease.

Restriction fragment length polymorphisms (RFLPs) were the first type of molecular markers used in linkage studies, but single nucleotide polymorphism (SNP) databases generated by the HGP offer a more efficient approach. SNPs are DNA polymorphisms that can serve as markers in linkage studies. These polymorphisms are usually neutral. A fixed combination of alleles of several SNPs that inherit together is called a haplotype. SNPs will be of increasing importance for linkage analysis, as screening of these haplotypes can be relatively easily formatted in automated high-throughput screening protocols.

Another useful type of DNA polymorphism consists of variable number of tandem repeats (VNTRs), composed of a variable number of repetitions of a one-, two-, or three-base sequence. Such polymorphisms, also known as simple sequence repeats (SSRs) or microsatellites, for example dinucleotide repeats such as CACACA, occur in many places in the DNA. Polymorphic in this context means that the number of repetitive elements (repeats) in this marker, and thus its length, is highly variable within the population. The location of many dinucleotide repeats is known, and the length can easily be analyzed by PCR. The segregation in families of a polymorphic marker of specific length with disease can identify the chromosomal location of the disease-causing gene.

A phenomenon called linkage disequilibrium is the basis for another strategy, which can afford a higher degree of resolution in mapping studies in some cases. This technique is not necessarily confined to large families but can be performed if material is available on large numbers of (apparently) unrelated patients with the disease.

Microarray

Microarray technology is a rapidly evolving approach to identify genes that are involved in disease processes. Microarrays (DNA chips) can be used for various purposes, such as the study of gene expression patterns (at the RNA level) in various tissues or cells, the diagnosis of gene mutations, and SNP analysis. Microarrays are also used to develop genetic fingerprints of different types of malignancies. On a chip or glass plate, a large number of single-stranded DNA sequences, representations of the coding sequences of genes or SNPs, are spotted. From the tissue or cells that one wishes to study, RNA is isolated. It is first transformed to cDNA with reverse transcriptase. The cDNA is labeled with a fluorescent dye and then allowed to hybridize on the array, where it binds to the complementary DNA sequences. When two samples are compared, two different fluorescent labels (red and green) are used. The intensity of fluorescence is measured for each spot and is a measure of the quantity of hybridized DNA on each spot, and thus of the quantity of RNA in the original sample. One can also make a special array on which all possible mutations of one or more genes are represented. Hybridization with the patient sample shows which mutation is present.

Conclusion

For a proper use of hormone measurements in blood and urine, the pediatric endocrinologist needs knowledge of the principles of evidence-based medicine in order to decide about the optimal diagnostic strategy to use for a patient and to evaluate the results. Knowledge about biochemical principles and characteristics of the usual biochemical tests, particularly immunoassays, is necessary. Endocrine tests, plasma hormone profiles, or stimulation tests are a vital part of pediatric endocrinology, but DNA testing has become increasingly important, and the results of the Human Genome Project have led to further rapid developments. Continuous training is mandatory for the clinician who wants to use molecular tests for clinical and scientific purposes to optimal effect.

Acknowledgment

We are grateful for the technical assistance of Bart Ballieux.

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4 Congenital disorders of the hypothalamic–pituitary axis

Ameeta Mehta and Mehul T. Dattani

Introduction

The pituitary gland is the central regulator of growth, reproduction, and homeostasis. It functions through hormone-signaling pathways that co-ordinate signals from the brain and the hypothalamus to target organs, such as the adrenals, thyroid, and gonads. The pituitary lies within the sella turcica at the base of the brain, and the mature gland consists of the adenohypophysis (anterior and intermediate lobes) and the neurohypophysis (posterior lobe).

The anterior pituitary consists of somatotrophs [growth hormone (GH)], thyrotrophs [thyrotrophin or thyroid stimulating hormone (TSH)], corticotrophs [corticotrophin or adrenocorticotrophic hormone (ACTH)], gonadotrophs [follicle-stimulating hormone (FSH) and luteinizing hormone (LH)] and lactotrophs (prolactin). The intermediate lobe, which produces pro-opiomelanocortin (POMC), the precursor to melanocyte-stimulating hormone (MSH) and endorphins, involutes in the human adult gland.

The posterior lobe consists of axons of neurons, the cell bodies of which reside in the hypothalamus. The posterior pituitary stores and releases oxytocin, which is required during parturition and lactation, and arginine vasopressin (AVP), which regulates water balance.

The function of the pituitary gland is intricately linked with that of the hypothalamus. Stimulatory and inhibitory releasing hormones are secreted from the hypothalamus, which regulate hypothalamo-pituitary–target gland axes. These include corticotrophin-releasing hormone (CRH), thyrotrophin-releasing hormone (TRH), GH-releasing hormone (GHRH), gonadotropin-releasing hormone (GnRH), and the inhibitory hormones dopamine and somatostatin. The hormones secreted by the posterior lobe are synthesized in the magnocellular neurons of the paraventricular and supraoptic nuclei that lie within the hypothalamus (Fig. 4.1).

The hypothalamus lies superior to the mature pituitary gland and has neural projections into the cerebral cortex and the median eminence. The portal blood system carries its hormones to the anterior pituitary. The infundibulum or pituitary stalk carries both the portal blood supply and the neural tracts to the pituitary gland, so damage to the pituitary stalk results in anterior and posterior pituitary dysfunction.

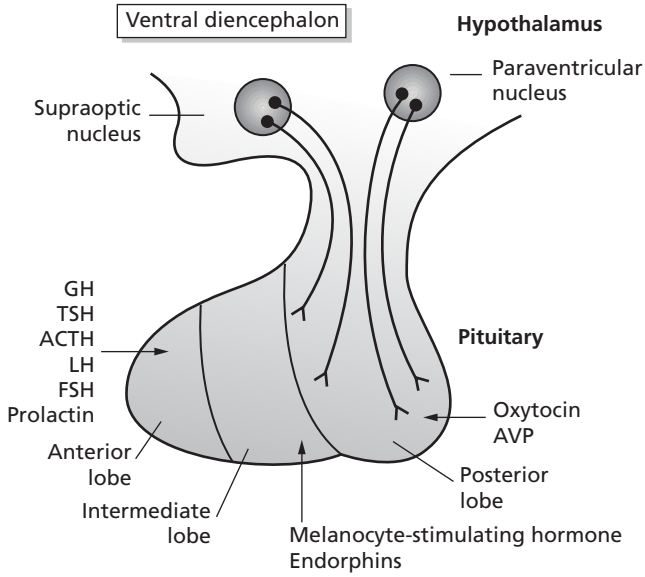

Fig. 4.1. Relationship between the hypothalamus and the pituitary gland.

Clinical features of hypopituitarism are variable in severity and in the number of hormone deficiencies. Clinical features may appear in the neonatal period, often with a stormy perinatal course, or later with growth failure. Isolated GH deficiency is the commonest endocrinopathy. In many patients with hypopituitarism, the problem lies within the hypothalamus rather than the pituitary. The evolution of additional hormone deficiencies with time is a well-recognized feature of hypopituitarism, and comprehensive evaluation of the hypothalamo-pituitary axis is indicated in any patient suspected of having one hormonal deficiency.

Hormonal deficits can be one part of a syndrome, with patients manifesting abnormalities in extrapituitary structures, usually in structures sharing a common embryological origin, such as the eye and forebrain.

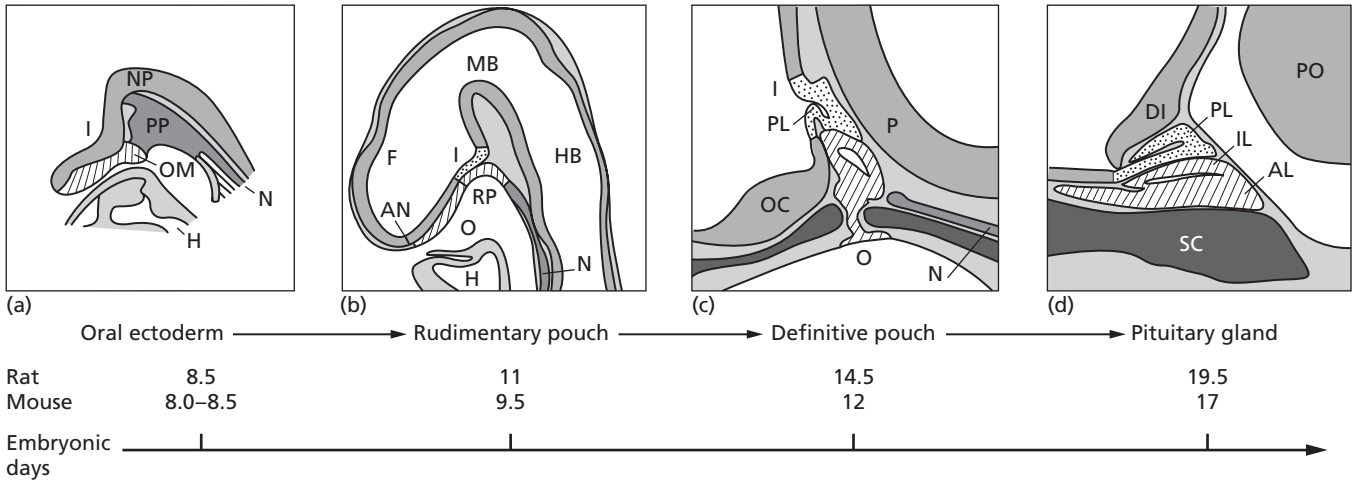

Fig. 4.2. Rodent pituitary development. Four stages of pituitary development: (a) pituitary placode; (b) rudimentary pouch; (c) definitive pouch; (d) adult pituitary gland. AL, anterior lobe; AN, anterior neural pore; DI, diencephalon; f, forebrain; H, heart; HB, hindbrain; I, infundibulum; IL, intermediate lobe; MB, midbrain; N, notochord; NP, neural plate; O, oral cavity; OC, optic chiasma; OM, oral membrane; P, pontine flexure; PL, posterior lobe; PO, pons; PP, prechordal plate; RP, Rathke's pouch; SC, sphenoid cartilage. Taken from Sheng HZ, Westphal H. *Trends Genet* 1999; 15: 236–40.

Hypothalamo-pituitary development

The development of the pituitary gland is similar in all vertebrates and has been extensively studied in the mouse (Fig. 4.2). The anterior and intermediate lobes are derived from the oral ectoderm, and the posterior pituitary from neural ectoderm [1]. The development of the anterior pituitary occurs in four distinct stages:

1 Formation of the pituitary placode derived from the ectoderm at the roof of the primitive oral cavity, which makes contact with the floor of the ventral diencephalon. This stage occurs on embryonic day (E) 8 in the mouse, corresponding to 4–6 weeks' gestation in humans. The apposition of the oral ectoderm forming Rathke's pouch and the neural ectoderm of the diencephalon is maintained throughout early organogenesis, and this relationship, together with various animal experimental manipulations, suggests that inductive tissue interactions are involved.

2 Invagination of the oral ectoderm and formation of a rudimentary Rathke's pouch with evagination of the ventral diencephalon to form the posterior pituitary.

3 Formation of the definitive Rathke's pouch.

4 Spatial and temporal differentiation of the various cell types within the mature anterior pituitary gland.

Figure 4.3 shows the cascade of signaling molecules and transcription factors that play a role in organ commitment, cell proliferation, cell patterning, and terminal differentiation (Fig. 4.4). Genetic interactions dictate normal development, and the final product is a culmination of a co-ordinated process whereby repression and activation of target genes allow normal development to continue. In comparison with the rodent, little is known about pituitary development in humans, but it appears that its embryological development mirrors that in the rodent. Spontaneous or artificially induced mutations in the mouse have led to significant insights into human pituitary disease, and identification of mutations associated with human pituitary disease have been invaluable in defining the genetic cascade responsible for the development of this embryological tissue. Mutations involved in human hypothalamo-pituitary disease are listed in Table 4.1.

Early developmental genes and transcription factors

A number of signaling molecules and transcription factors are implicated in early pituitary organogenesis and lineage differentiation. They are expressed sequentially at critical periods of pituitary development, and the expression of many is subsequently attenuated.

Morphogenetic signals (*BMP, FGF, SHH, Wnt*)

Close interaction between the oral and neural ectoderm is required for initial pituitary development. Extrinsic molecules within the ventral diencephalon and the surrounding structures, such as bone morphogenetic proteins 2 and 4 (*BMP 2, 4*), fibroblast growth factor 8 (*FGF8*), Sonic Hedgehog (*SHH*), Wingless (*Wnt4*), and thyroid transcription factor (*Ttf1*; also called *Nkx2.1*), play critical roles in early organogenesis. Rathke's pouch develops in a two-step process requiring at least two sequential inductive signals from the diencephalon. First, the induction and formation of the pouch rudiment is dependent upon *BMP4*, which is present only in the hypothalamus and not in Rathke's pouch. Secondly, *FGF8*, which is also present in the hypothalamus and not in Rathke's pouch,

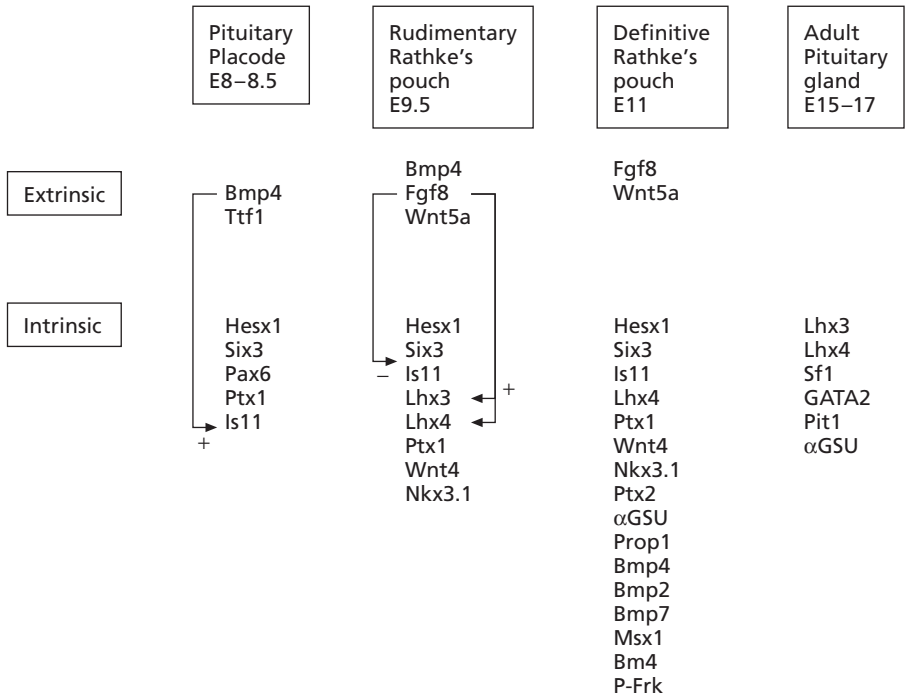

Fig. 4.3. Transcription factors and signaling molecules involved in anterior pituitary development. Revised from Watkins-Chow DE, Camper SA. *Trends Genet* 1998; 14: 284–90.

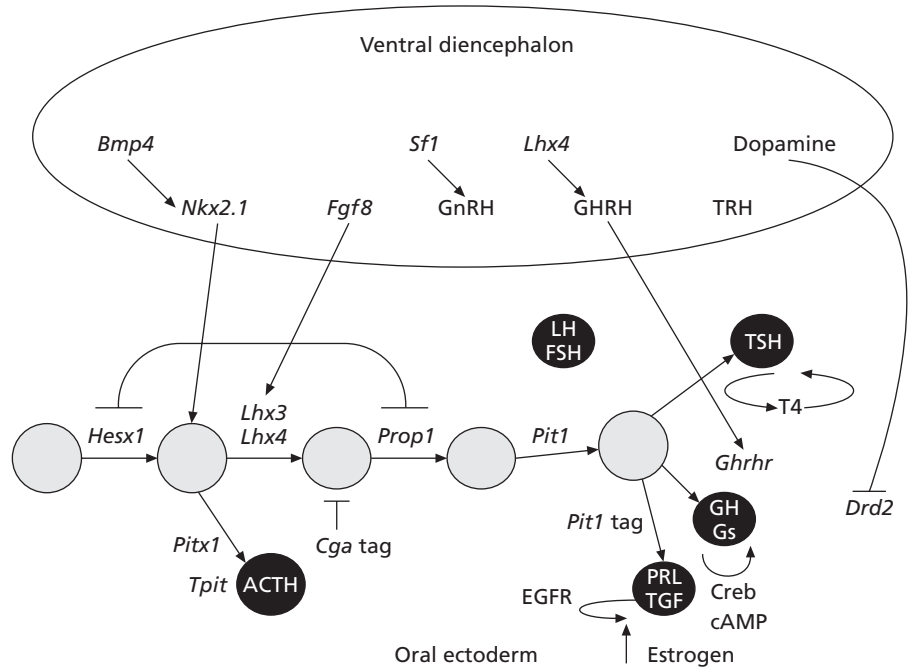

Fig. 4.4. Schematic representation of the developmental cascade of genes implicated in human pituitary development with particular reference to pituitary cell differentiation.

activates the key regulatory genes *Lhx3* and *Lhx4* essential for subsequent development of the pouch rudiment into a definitive pouch. Murine mutations within *Nkx2.1*, which is expressed only in the presumptive ventral diencephalon, can cause severe defects in the development not only of the diencephalon but also of the anterior pituitary gland.

Hesx1

Hesx1 is one of the earliest markers of the pituitary primordium, suggesting that it has a critical role in early determination and differentiation of the gland. It is also called *Rpx* (Rathke's pouch homeobox) and is a member of the paired-like class of homeobox genes. HESX1 is a transcriptional

Table 4.1. Human mutations causing abnormal hypothalamo-pituitary development and function.

Gene	Phenotype	Inheritance
Isolated hormone abnormalities		
GH1	Isolated growth hormone deficiency	R, D
GHRHR	Isolated growth hormone deficiency	R
TSH beta	Isolated TSH deficiency and secondary hypothyroidism	R
TRHR	Isolated TSH deficiency and secondary hypothyroidism	R
T-PIT	ACTH deficiency	R
PC 1	ACTH deficiency, hypoglycemia, impaired glucose tolerance, HH, obesity	R
POMC	ACTH deficiency, obesity, red hair	R
DAX1	Adrenal hypoplasia congenital and HH	XL
GnRHR	Isolated gonadotropin deficiency and HH	R
KAL-1	Kallman syndrome	XL
FSH beta	Primary amenorrhea; defective spermatogenesis	R
LH beta	Delayed puberty	R
AVP	Diabetes insipidus	R, D
Combined pituitary hormone deficiency (CPHD)		
PIT1	GH, TSH, prolactin deficiencies	R, D
PROP1	GH, TSH, LH, FSH, prolactin, evolving ACTH deficiency	R
Specific syndrome		
HESX1	Septo-optic dysplasia	R, D
LHX3	CPHD (GH, TSH, LH, FSH, prolactin), short neck, limited rotation	R
LHX4	CPHD (GH, TSH, ACTH) with cerebellar abnormalities	D
SOX3	IGHD and mental retardation	XL
GLI2	Holoprosencephaly and multiple midline defects	D
GLI3	Pallister–Hall syndrome	D
Pitx2	Rieger syndrome	D

HH, hypogonadotrophic hypogonadism; R, recessive; D, dominant; XL, X-linked.

repressor, although its downstream targets remain unknown. A highly conserved region in the N-terminus of HESX1, the engrailed homology domain (eh-1), is crucial for its repressor function and binds TLE1, a mammalian homolog of the *Drosophila* co-repressor Groucho. (Engrailed is a protein in *Drosophila* and contains a special domain, which is a repressor domain. This is highly conserved in many species and some repressor proteins such as Hesx1 through evolution. Hence, it is called an "engrailed homology domain.") The homeodomain also interacts with the nuclear co-repressor (NcoR). The N-terminal domain-binding TLE permits co-operative binding of NcoR, HDAC1, and Sin3A/B to the homeodomain, thereby making Hesx1 a strong repressor.

The gene is first expressed during mouse embryogenesis in a small patch of cells in the anterior midline visceral endoderm as gastrulation commences. Expression is then induced in the adjacent ectoderm in an area fated to give rise to the ventral prosencephalon, which in turn will form the forebrain. Subsequently, *Hesx1* is expressed at the anterior extreme of the rostral neural folds with resolution to the ventral diencephalon by E9. At this stage, *Hesx1* is also expressed in the thickened layer of oral ectoderm that will give rise to Rathke's pouch. *Hesx1* continues to be expressed in the developing anterior pituitary until E12, when its transcripts disappear in a spatiotemporal sequence that corresponds to progressive pituitary cell differentiation. Expression is undetectable by E13.5. The extinction of *Hesx1* is important for the activation of other downstream genes such as *Prop1*.

Prop1 is a pituitary-specific paired-like homeodomain activator. Both transcription factors are believed to bind to the same DNA response elements. It has been suggested that *Hesx1* and *Prop1* function as opposing transcription factors and that the careful temporal regulation of their expression is critical for normal pituitary development. *Hesx1* and *Prop1* exhibit temporally distinct but overlapping patterns of expression over the entire period of pituitary organ commitment, patterning, and cell type determination. Premature expression of *Prop1* can block pituitary organogenesis. Prolonged expression of *Hesx1* with the obligate co-repressor TLE1 can block Prop1-dependent activation, which normally results in the appearance of somatotrophs, lactotrophs, thyrotrophs, and gonadotrophs. Hence, the switch between binding of a paired homeodomain repressor Hesx1 for a paired homeodomain activator Prop1 is critical for normal organogenesis. There is also evidence to suggest that Prop1 activation is itself a prerequisite for the extinction of *Hesx1*. *Hesx1* expression is prolonged in the Ames dwarf mouse, the phenotype of which results from a point mutation within the

Prop1 gene. Lhx3 is also important for the maintenance of *Hesx1* expression.

Targeted disruption of *Hesx1* in the mouse revealed morphological defects in homozygote embryos from E9 and also in mutant neonates and adults. These consisted of a reduction in prospective forebrain tissue, absence of developing optic vesicles, markedly decreased head size, and severe microphthalmia. The phenotype was highly variable and often asymmetrical. Other abnormalities included absence of the optic cups, the olfactory placodes, and Rathke's pouch, reduced telencephalic vesicles, hypothalamic abnormalities, and aberrant morphogenesis of Rathke's pouch. In 5% of the null mutants, the phenotype was characterized by a complete lack of the pituitary gland. In the majority of the mutant mice, by E12.5, the mice had multiple oral ectoderm invaginations and, hence, multiple pituitary glands.

Between E13.5 and E15.5, *Hesx1* mutants were characterized by a dramatic cellular overproliferation. The formation of multiple pouch invaginations and pituitary overproliferation can occur independently. Deletion of the *Hesx1* gene causes a rostral extension of *FGF8* and *FGF10* expression in the ventral diencephalon, leading to ectopic Lhx3 induction and the formation of supernumerary pituitary glands, confirming that FGF8/FGF10 signaling is required and sufficient to signal pituitary commitment from oral ectoderm. These data suggest that the paired-like homeodomain repressor Hesx1 serves to establish boundaries of FGF8/10 gene expression in the ventral diencephalon and to restrict the spatial domains at which pituitary organogenesis can occur [4].

Pitx1 and *Pitx2*

Pitx1 and *Pitx2* are paired-like homeobox genes expressed in the fetal pituitary and in most cells of the adult gland. They play an important role in the development of Rathke's pouch and anterior pituitary gland.

Pitx1 is initially expressed in the first branchial arch mesenchyme at E9. On E9.5, it is expressed throughout the oral epithelium lining the roof of the buccal cavity and in Rathke's pouch ectoderm. Expression continues throughout development in all regions of the anterior pituitary. In adults, *Pitx1* is specifically expressed at higher levels in cells of the α-glycoprotein subunit (αGSU) lineage. Although a fraction of pro-opiomelanocortin (POMC)-expressing cells expresses high concentrations of Pitx1, most corticotrophs do not. *Pitx1* expression overlaps with that of *Lhx3* and appears to be required for the sustained expression of *Lhx3*. *Pitx1* is essential for sustained expression of αGSU and for the maintenance of cell-specific transcription in corticotrophs and gonadotrophs.

A T-box factor, Tpit, present only in POMC-expressing cells within the pituitary, is essential for initiating POMC cell differentiation and for activating POMC transcription synergistically with Pitx1. POMC transcription is also enhanced by other factors such as leukemia inhibitory factor (LIF) and the basic helix–loop–helix heterodimer NeuroD1/Pan1 [also known as corticotroph upstream transcription factor (CUTE)]. Pitx1 also appears to modulate steroidogenic factor 1 (sf1) activity in the gonadotrophs, activation of the GH promoter, and synergistic activation of the prolactin promoter with Pit1 [5].

Mice rendered deficient in Pitx1 demonstrate abnormalities within the hindlimb and the palate. The number of gonadotrophs and thyrotrophs is reduced with an increase in the concentrations of ACTH transcripts and peptides in corticotrophs. To date, no mutations have been described within Pitx1 in humans.

Pitx2 is first expressed in the mouse embryo in oral epithelium and oral ectoderm. At E9.5, *Pitx2* is expressed in the developing Rathke's pouch in addition to the mesenchyme near the optic eminence, the basal plate of the central nervous system (CNS), the forelimbs, and domains of the abdominal cavity. It appears to be required for pituitary development shortly after formation of the committed pouch. It may be required for one or more anterior pituitary cell types or may act in concert with other transcription factors. It is also expressed in lungs, kidney, testes, and tongue.

Pitx2 is implicated in left–right asymmetry, because it is expressed initially in the lateral plate mesoderm in the embryo. It is subsequently expressed in several organs that are asymmetric with respect to the left–right axis of the embryo and thereby plays a crucial role in determining left–right asymmetry. There are at least three isoforms of Pitx2: Pitx2a and Pitx2b are expressed in the adult pituitary gland in the thyrotrophs, gonadotrophs, somatotrophs, and lactotrophs but not in the corticotrophs, where Pitx1 is highly expressed. Pitx2c is expressed in all five cell lines. Mutations in *Pitx2* are associated with Rieger syndrome [6].

Lhx3 and Lhx4

Lhx3 and *Lhx4* belong to the LIM family of homeobox genes that are expressed early in Rathke's pouch. At least three different isoforms of *Lhx3* have been described in mammals, each with distinct expression patterns and transcriptional properties. *Lhx3* is detected in the developing nervous system, and the *Lhx3a* isoform is first expressed at E8.5 in the mouse embryo. *Lhx3b* is first expressed at E9.5. At E9.5, *Lhx3* is expressed in Rathke's pouch and the closing neural tube. Subsequently, *Lhx3* is expressed in the anterior and intermediate lobes of the gland, the ventral hindbrain, and the spinal cord. Maintenance of Lhx3 persists in the adult pituitary, suggesting a maintenance function for one or more of the anterior pituitary cell types.

Lhx3 activates the αGSU promoter and, together with Pit1, acts synergistically to activate TSH-β and prolactin promoters and the Pit-1 enhancer. *Lhx3* is one of the earliest markers for cells destined to form the anterior and intermediate lobes, and continued expression is essential for the formation of gonadotrophs, thyrotrophs, somatotrophs, and lactotrophs. In *Lhx3* null mutant mice, Rathke's pouch is formed but fails

to grow. *Hesx1* is expressed but the expression is switched off early (E12.5). There is failure of expression of *αGSU*, *TSH-β*, *GH* and *Pit1* transcripts, but specification of the corticotroph cell lineage does occur, although there is failure of POMC cells to proliferate.

Lhx4 is a closely related gene expressed in specific fields of the brain and spinal cord. Like *Lhx3*, *Lhx4* is expressed throughout the invaginating pouch at E9.5. Subsequent expression at E12.5 is restricted to the future anterior lobe. Its expression is reduced by E15.5. Null mutants of *Lhx4* show the formation of Rathke's pouch with expression of *αGSU*, *TSH-β*, *GH* and *Pit1* transcripts, demonstrating that the various anterior pituitary cell lineages are specified, although the numbers are reduced. *Lhx3*−/−, *Lhx4*−/− double mutant mice show a more severe phenotype than either single mutant with an early arrest of pituitary development, thereby suggesting that these two genes may act in a redundant manner during early pituitary development.

Human mutations in *LHX3* are associated with GH, TSH, prolactin, and gonadotropin deficiencies. *LHX4* mutations result in GH, TSH, and ACTH deficiencies.

Terminal cell differentiation

Terminal pituitary cell differentiation is a culmination of the interaction between extrinsic signaling molecules, such as FGF8, BMP2, BMP4, BMP7, and Wnt5A, and transcription factors, such as Lhx3, Lhx4, GATA2, Isl1, Prop1, and Pit1. *GATA2* encodes a transcription factor that is important in the differentiation of gonadotrophs and thyrotrophs. Other transcription factors involved in maturation of the gonadotroph lineage are steroidogenic factor 1 (sf1) and Dax1. *Pit1* and *Prop1* are the best characterized in terms of function in both humans and mice.

Prop1

Prop1 (Prophet of Pit1) is a pituitary-specific paired-like homeodomain transcription factor, first expressed in the dorsal portion of the murine Rathke's pouch at E10–10.5, followed by maximal expression at E12 in the caudomedial region and subsequent extinction by E15.5. It is believed to be required for the expression of *Pit1*, as there is a failure of determination of *Pit-1* lineages, lack of *Pit-1* gene activation, and absence of progression to mature cells in the Ames dwarf mouse, which harbors a homozygous missense mutation (S83P) in the *Prop1* gene. The size of the pituitary gland is considerably reduced, and the adult Ames dwarf mouse has less than 1% of the normal complement of somatotrophs, markedly reduced numbers of lactotrophs and thyrotrophs, and reduced expression of gonadotropins [7]. Humans with *PROP1* mutations have GH, TSH, prolactin, and gonadotropin deficiencies, suggesting a role for Prop1 in gonadotroph differentiation. Prop1 is also important in regulating the expression of Hesx1.

Pit1

Pit1 (now called *POU1F1*) is a pituitary-specific transcription factor belonging to the POU homeodomain family. It has also been called GH factor 1 (*GHF1*), because it was first identified as a regulator of *GH1* transcription. Apart from GH1, Pit1 binding sites have been identified in the promoters of the prolactin and TSH-β genes. Pit1 is expressed relatively late during pituitary development (E13.5 in the mouse), and expression persists throughout life. It is believed that the expression pattern would be similar in humans. Pit1 usually binds to multiple sites on target genes, and dimerization of Pit1 on DNA seems to be important for high-affinity DNA binding and consequent transcriptional activation. Although Pit1 is sufficient to activate the minimal elements in the *GH1* promoter necessary for cell-specific expression, it requires other factors, such as Zn-15, a zinc finger transcription factor, for synergistic activation of the *GH1* gene.

Pit1 is essential for the development of somatotrophs, lactotrophs, and thyrotrophs in the anterior pituitary. The transcripts first appear in cells within the caudomedial region of the anterior pituitary at E14.5, followed by detection of the protein within somatotrophs and lactotrophs and subsequent expression of the GH and prolactin genes on E16 and E17 respectively. Pit1-dependent thyrotroph population arises on E15.5. Pit1 is required for transactivation of the TSH-β promoter.

Two naturally occurring murine models have shed light on the role of Pit1 in normal pituitary development. In the Snell dwarf (dw) mouse, a recessive point mutation (W261C) results in the absence of somatotrophs, lactotrophs, and thyrotrophs [8]. A similar phenotype results in the Jackson dwarf mouse (dw^J), which harbors a recessive null mutation resulting from rearrangement of Pit1. Apart from its role in proliferation and maintenance of somatotrophs, lactotrophs, and thyrotrophs, Pit1 binding sites have also been found in the *GHRHR* and the *Pit1* gene itself. In the Snell dwarf mouse, Pit1 transcripts appear at the normal time in the expected region of the pituitary gland but, by E18.5, there is a significantly decreased level of Pit1 compared with the wild-type animals.

These data suggest that autoregulation of Pit1 is required to sustain *Pit1* gene expression once Pit1 protein has reached a critical threshold.

Anterior pituitary hormones and their deficiencies

Growth hormone

Somatotrophs account for 4–10% of the net weight of an adult pituitary gland. The human GH gene (*GH-N* or *GH1*) forms part of a cluster of five homologous genes [*GH1*, *hCS-L* (*CSHP1*), *hCS-A* (*CSH1*), *hGH-V* (*GH2*), *hCS-B* (*CSH2*)] located on the long arm of chromosome 17 (17q22–24),

spanning 66.5 kilobases (kb). Its expression is regulated not only by a proximal promoter but also by a locus control region (LCR) 15–32 kb upstream of the *hGH-1* gene. The LCR confers pituitary-specific, high-level expression of human growth hormone (hGH) [9]. The full-length transcript from the GH-N gene encodes a 191-amino-acid 22 kDa protein that contains two disulfide bridges and accounts for 85–90% of circulating GH. Alternative splicing of the mRNA transcript generates a 20 kDa form of GH that accounts for the remaining 10–15%. Within both the proximal promoter and the LCR are located binding sites for the pituitary-specific transcription factor Pit1.

GH binds in the circulation to two binding proteins, high- and low-affinity GHBP [10]. Little is known about low-affinity GHBP, which accounts for approximately 10–15% of GH binding with a preference for binding to 20 kDa hGH. High-affinity GHBP is a 61 kDa glycosylated protein that represents a soluble form of the extracellular domain of the GH receptor that can bind to both 20 and 22 kDa hGH and thereby prolong the half-life of GH. *In vivo* studies, in which GH and GHBP have been co-administered to hypophysectomized and GH-deficient rats, have demonstrated a potentiation of weight gain and bone growth, although similar studies have not been performed in man.

The half-life of hGH is less than 20 min. It binds to the GH receptor (GHR), which is present in a number of tissues. The hormone sequentially dimerizes its receptor, activating a receptor-associated tyrosine kinase JAK2 that in turn is autophosphorylated and also phosphorylates the GHR. This leads to signal transduction using the mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription (STAT) and phosphatidylinositol (PI) 3-kinase pathways. The endresult is activation of a number of genes that mediate the effects of GH. These include early response genes encoding transcription factors (c-jun, c-fos, and c-myc implicated in cell growth, proliferation, and differentiation) and insulin-like growth factor I (IGF-I), which mediates the growth-promoting effects of GH [11,12].

Actions

GH is secreted in a pulsatile fashion. Peak concentrations are achieved during sleep, but secretion is also increased during emotional stress, exercise, hypoglycemia, protein meals, and prolonged fasting. Pharmacological agents used to increase hGH secretion include insulin, glucagon, clonidine, L-dopa, and propranolol. Apart from its actions on linear growth, GH is anabolic, lipolytic, and diabetogenic. It increases calcium absorption and is believed to improve bone density. Administration of hGH results in a reduction in body fat and an increase in muscle mass.

GH acts indirectly on bone growth by stimulating the synthesis of IGF-1, the main GH-dependent growth factor. IGF-1 is a single-chain polypeptide containing 70 amino acids. It shares considerable homology with insulin. It is synthesized in the liver and circulates bound to several binding glycoproteins. The principal binding protein is IGFBP-3, the secretion of which is also regulated by GH. Measurement of IGF-1 correlates well with spontaneous GH secretion and is used in the diagnosis of GH deficiency, but its concentration is altered in a number of other disease states, such as hypothyroidism, malnutrition, poorly controlled diabetes, and chronic disease.

Regulation

The secretion of GH is pulsatile with a predominant nocturnal component. Pulsatility is regulated by GH-releasing hormone (GHRH), a 44-amino-acid protein that stimulates GH secretion, and somatostatin, an inhibitory hormone containing 14 amino acids. The secretion of these hypothalamic hormones is influenced by neurotransmitters and neuropeptides, such as dopamine, catecholamines, histamine, serotonin, gamma aminobutyric acid (GABA), and opiates. GH and growth factors such as IGF-I and IGF-II exert negative feedback on the hypothalamic regulators of GH secretion, whereas sex steroids such as testosterone and estrogen increase hGH secretion.

Use of synthetic GH-releasing peptides (GHRP) has led to the identification of a GH secretagogue (GHS) receptor (GHS-R type 1a), which is strongly expressed in the hypothalamus. Specific binding sites for GHRP have also been identified in other regions of the CNS and in peripheral endocrine and non-endocrine tissues. The endogenous ligand for the GHS receptor, ghrelin, has now been isolated from the stomach and is an octynylated peptide consisting of 28 amino acids [13]. Although it is expressed predominantly in the stomach, smaller amounts are produced within the bowel, pancreas, kidney, the immune system, placenta, pituitary, testis, ovary, and hypothalamus. Ghrelin leads not only to the secretion of GH but also stimulates prolactin and ACTH. It influences endocrine pancreatic function and glucose metabolism, gonadal function, appetite, and behavior. It controls gastric motility and acid secretion and has cardiovascular and anti-proliferative effects. Its role in normal growth during childhood remains unclear. Both Ghrelin and GHRPs release GH synergistically with GHRH, but the efficacy of these compounds as growth-promoting agents is poor.

Isolated GH deficiency (IGHD)

Etiology and clinical features

Congenital GH deficiency may occur in isolation or associated with other anterior and posterior pituitary hormone deficiencies with or without extrapituitary features such as optic nerve hypoplasia and midline forebrain defects. The condition may be sporadic or familial. The reported incidence is 1 in 3500 to 1 in 10 000 live births, with the majority of cases being idiopathic. Familial cases account for 5–30% of cases, and there are four well-described familial forms

Table 4.2. Isolated growth hormone deficiency.

Inheritance	Type	Phenotype	Gene	Nature of mutations
Autosomal recessive	IA	Severe short stature, anti-GH antibodies on treatment	GH	Deletions, amino acid substitutions
	IB	Less severe short stature. No anti-GH antibodies	GH/GHRHR	Splice site mutations, amino acid substitutions
Autosomal dominant	II	Less severe short stature. No antibodies	GH	Splice site mutations
X-linked recessive	III	Short stature (GHD) with agammaglobulinemia	?	?

(Table 4.2). Mutations within the homeobox gene *HESX1* can also cause isolated GH deficiency.

IGHD type IA

Patients with IGHD type IA have a complete absence of GH and lack tolerance to exogenous GH producing hGH antibodies [14]. They present with early and profound growth failure. Serum GH concentrations are undetectable or extremely low on provocation testing. The condition is characterized by an initial response to exogenous hGH treatment, followed by development of antibodies to GH, resulting in a markedly decreased final height as an adult.

The disorder is inherited in an autosomal-recessive manner. The majority of patients have large deletions within the GH1 gene; those identified to date range from 6.7 to 45 kb [15], but microdeletions, such as that of a single basepair at codon 10 leading to an altered reading frame with premature termination of translation and an ensuing truncated protein, have been described. Table 4.3 lists all the mutations identified to date in the GH1 gene.

The prevalence of this disorder is unclear. Sporadic cases may go unrecognized, resulting in the possible low incidence of this disorder. A prevalence of 9–38% for GH1 deletions in markedly short (height < −4 standard deviations) individuals has been suggested. All reported families to date are consanguineous.

There is marked heterogeneity in the phenotype of these patients, in addition to considerable variability in antibody formation and response to hGH treatment, even within families with the same deletions. Patients with larger deletions (> 7.6 kb) respond better to GH treatment compared with those with smaller deletions. Recombinant IGF-1 has been used, particularly in patients with a poor initial response to hGH treatment and formation of high antibody titers. With improvements in recombinant technology, purer forms of GH can be produced, which alleviate the problem of antibody formation to some extent.

Table 4.3. Mutations identified to date within the *GH1* gene.

Mutation	Anti-GH antibodies	Type
Gross deletions		
6.7 kb gross deletion	?	1A
7.0 kb gross deletion	✓	1A
7.6 kb gross deletion	✓/X	1A
45.0 kb gross deletion	✓	1A
Double (GH1, CSH1, GH2, CSH2)	✓	1A
Microdeletions		
C10del microdeletion	✓	1A
IVS3 18 bp del microdeletion		II
2 bp deletion S54del (compound heterozygote with 6.7 kb del)	X	1A
Point mutations		
W7X nonsense mutation	✓	1A
E4X nonsense mutation	X	1A
W20X nonsense mutation	X	1A
P89L missense mutation	X	II
R183H missense mutation	X	II
V110F missense mutation	X	II
R77C missense mutation	X	II
Splice site mutations		
E3+1 G>T	X	II
E3+5 A>G	X	II
IVS2–2 A>T	X	II
IVS3+1 G>A	X	II
IVS3+1 G>C	X	II
IVS3+2 T>C	X	II
IVS3+5 G>A	X	II
IVS3+5 G>C	X	II
IVS3+6 T>C	X	II
IVS3+6 T>G	X	II
IVS3+28 G>A	X	II
IVS4+1 G>C	X	IB
IVS4+1 G>T	X	IB
IVS4+5 G>C	X	II

IGHD type IB

Isolated GH deficiency type IB is also associated with a prenatal onset of GH deficiency but is milder than IGHD type IA, with detectable concentrations of GH after provocation testing. The condition is inherited as an autosomal-recessive trait. Children present with marked short stature and poor growth velocity. There is a good response to exogenous hGH with no formation of GH antibodies.

IGHD type IB results from homozygous splice site mutations within the *GH1* gene or mutations within the GHRH

Table 4.4. *GHRHR* mutations identified to date.

Mutation	*N*	Year	Country
E72X	22	1996–98	India/Pakistan/Sri Lanka
IVS1+1G→A	30	1999	Brazil
Del1121–1124	1	2000	Japan
L144H	1	2001	Spain
L144H/F242C	1	2001	USA
A222E	1	2001	Pakistan
H137L/del 1140–1144	2	2001	?
IVS7+1G→C	2	2001	Morocco
K329E/124 A>C	1	2002	?
Q43X/IVS3+1G→A	2	2002	?
A176V	2	2003	Pakistan

receptor (*GHRHR*). The human *GHRHR* gene consists of 13 exons spanning approximately 15 kb and has been mapped to chromosome 7p15. It encodes a protein containing 423 amino acids. The receptor is a G-protein-coupled receptor characterized by seven transmembrane domains with a high binding affinity for GHRH. Expression of *GHRHR* is upregulated by PIT1. GHRHR is required for proliferation of somatotrophs and therefore plays an important role in anterior pituitary development.

The first reported cases of *GHRHR* mutations were in two first cousins who were found to have a G→T substitution leading to a stop codon and a severely truncated protein lacking the membrane-spanning domains with a consequent inability to bind to GHRH [16]. Since then, approximately 65 patients with GHRHR mutations have been reported, including splice site mutations [17]. Table 4.4 lists the GHRHR mutations reported to date.

IGHD type 2

IGHD type 2 is inherited as an autosomal-dominant condition and is mostly the result of splice site mutations in intron III (IVSIII) within the GH1 gene. Patients present with marked short stature and respond well to exogenous hGH treatment with no formation of antibodies.

These splice site mutations lead to the production of two alternatively spliced GH molecules, 20-kDa and 17.5-kDa hGH. The 17.5-kDa form of hGH, generated as a result of the skipping of exon 3 and consequent loss of amino acids 32–71 (del 32–71GH), has a dominant-negative effect preventing the secretion of the normal wild-type 22-kDa hGH with a consequent deleterious effect on pituitary somatotrophs. In a murine model of this dominant-negative mutation, there is evolution of the phenotype with later failure of prolactin, TSH, and gonadotropin secretion [18]. More recently, mutations in an exon splice enhancer within exon 3 of the *GH1* gene have been associated with autosomal-dominant GHD [19]. This mutation is associated with the preferential use of stronger splice sites that lead to the production of 17.5 and 20 kDa forms of GH. Several different splice site mutations have been reported to date. In addition, four missense mutations (R77C, R183H, P89L, and V110F) have recently been implicated in IGHD type 2 (Table 4.3). These patients have a normal GH1 allele but are unable to secrete the normal form of GH in appropriate concentrations. The mutant protein therefore exerts a dominant-negative effect, although the mechanism is unclear.

IGHD type 3

This disorder is inherited in an X-linked recessive manner. In addition to GH deficiency, the patients may manifest agammaglobulinemia. No abnormalities have been documented within the GH1 gene, and the mechanism for the phenotype is unknown. Recently, a polyalanine expansion within SOX3, a transcription factor related to Sry and implicated in CNS development, has been described in a pedigree with X-linked mental retardation and GHD [20]. The mechanism by which this polyalanine expansion leads to a phenotype remains unknown.

Thyrotrophin or thyroid-stimulating hormone

Thyrotrophin is a glycoprotein consisting of two noncovalently bound chains of amino acids (α and β) that is synthesized and stored within the thyrotrophs of the pituitary gland. The α-chain consists of 92 amino acids and shares homology with other pituitary glycoproteins FSH and LH. The β-chain contains 110 amino acids and is TSH specific.

Actions

The primary function of TSH is to stimulate the thyroid gland to secrete triiodothyronine (T_3) and thyroxine (T_4). Its actions include stimulation of the iodide pump on the cell membrane transporting iodide into the cell, stimulation of the synthesis of the thyroidal storage protein thyroglobulin, and stimulation and synthesis of T_4 and T_3 and their release from their complexes with thyroglobulin. TSH binds to its cell membrane receptor, which consists of seven transmembrane domains, four intracellular domains, and a long extracellular sequence with six potential glycosylation sites. It is a G-protein-coupled receptor that stimulates adenyl cyclase activity, activation of protein kinase A, and subsequent phosphorylation.

Regulation

TSH secretion is pulsatile with peak concentrations at night. Its secretion is stimulated by hypothalamic TRH acting via its G-protein-coupled receptor and inhibited by somatostatin and dopamine. The thyroid hormones exert negative feedback at the pituitary level on TSH secretion and at the

hypothalamic level on TRH. Other factors impinging on TSH secretion include estrogen, which increases the number of TRH receptors on the thyrotrophs, and a decrease in the ambient temperature, which is a potent stimulator of TSH.

Isolated TSH deficiency

Clinical features

Central hypothyroidism has a reported prevalence of 1 in 50 000 live births. Neonates can present with non-specific symptoms such as lethargy, poor feeding with failure to thrive, prolonged hyperbilirubinemia, and cold intolerance. Babies with central hypothyroidism may be born with a normal or above average birthweight and a birth length that is below average. Central hypothyroidism is generally milder than primary hypothyroidism. Collu *et al*. reported a patient with a TRH receptor mutation who presented only with short stature and delayed bone maturation. Although he had a subnormal intelligence quotient (IQ), this was possibly related to the low socioeconomic status of the family as the IQ of unaffected sibs was similar [21].

Etiology

Isolated central hypothyroidism is very rare with only 60 reported cases until 1998. It may be sporadic, but familial cases have been reported. Dacou-Voutetakis *et al*. first reported a homozygous nonsense mutation in exon 2 of the TSH β-subunit gene in three children affected by congenital TSH-deficient hypothyroidism within two related Greek families [22]. Affected individuals showed symptoms of severe mental and growth retardation. This mutation gives rise to a truncated peptide including only the first 11 of 118 amino acids of the mature TSH β-subunit peptide.

Collu *et al*. were the first to report an inactivating mutation of the TRH receptor gene as a cause for isolated central hypothyroidism [21]. The patient had complete absence of TSH and prolactin responses to TRH. Mutational analysis revealed that the patient was a compound heterozygote for two different mutations, having inherited a different mutated allele from each of the parents. The mutation resulted in a failure of TRH to bind the mutated TRH receptor with a consequent failure of TSH secretion.

Adrenocorticotrophic hormone (ACTH)

ACTH is a 39-amino-acid polypeptide with a biological half-life of approximately 8 min. It is synthesized and stored within the corticotrophs, which account for about 10% of the adenohypophysis. The initial precursor prohormone is pro-opiomelanocortin (POMC). Post-translational processing of POMC is species specific. The POMC gene on chromosome 2 spans approximately 12 kb and consists of three exons. Cleavage of the POMC precursor into biologically active peptides is a critical process. The main enzymes involved are prohormone convertases, particularly PC1 within the anterior pituitary corticotrophs. PC1 cleaves POMC to generate N-POC and β-lipotrophin. N-POC is then cleaved to form pro-γ-melanocyte-stimulating hormone (pro-γ-MSH), a joining peptide, and ACTH. There is further evidence to suggest that another enzyme PC2 cleaves ACTH into αMSH and a corticotrophin-like intermediate lobe peptide within the intermediate lobe, and β-lipotrophin into β-endorphin and γ-lipotrophin. αMSH plays an important role as an agonist for the MC1 receptor in causing pigment deposition in the hair follicle and as an agonist for the MC4 receptor in the hypothalamus where it controls appetite.

Actions

The primary function of ACTH is to stimulate the zonae fasciculata and reticularis of the adrenal glands to produce glucocorticoids (mainly cortisol) and adrenal androgens. Like other peptide hormones, ACTH binds to its specific membrane receptor on the adrenocortical cells to increase the formation of cyclic AMP and activate various protein kinases.

Regulation

The secretion of ACTH follows a circadian rhythm with peak concentrations in the early hours of the morning and low concentrations in the late evening. As a result of this, cortisol secretion is circadian with peak concentrations at around 08.00 hours and a nadir at midnight. This rhythm can be disrupted by shifts in day–night patterns.

Hypothalamic CRH, a 41-amino-acid peptide, binds with high affinity to its specific cell membrane receptors on the corticotrophs to increase transcription of the POMC gene and ACTH synthesis. CRH neurones are also found in other areas of the brain including the hypothalamus, brainstem, and cerebral cortex. AVP acts synergistically with CRH to stimulate ACTH release from the corticotrophs. Other neurotransmitters (serotonin, noradrenaline, neuropeptide Y, interleukins 1 and 6, tumor necrosis factor, and leukemia inhibitory factor) are involved in the regulation of ACTH secretion. Stressors (e.g. surgical stress, infection, pain, acute illness, fever, hypoglycemia, and other pathological states) increase CRH secretion resulting in an increase in ACTH and cortisol. Exogenous glucocorticoids reverse this effect. The mechanism underlying the increased secretion of the steroid hormones is unclear, although it is thought to be mediated by interleukins.

Isolated ACTH deficiency

Clinical features

Isolated congenital ACTH deficiency is rare; ACTH deficiency is more commonly associated with multiple pituitary hormone deficiencies. The clinical features of isolated

congenital ACTH deficiency are poorly defined. Patients usually present in the neonatal period with non-specific symptoms such as poor feeding, failure to thrive, and hypoglycemia. More acute signs of adrenal insufficiency include vascular collapse, shock, and bradycardia. Serum aldosterone secretion is controlled by the renin–angiotensin system, and abnormalities in salt excretion are unusual in isolated ACTH deficiency. Females rely on adrenal androgens for the development of pubic and axillary hair, and women with isolated ACTH deficiency lack both.

Etiology

Only a few cases of isolated ACTH deficiency have been reported to date. No abnormalities have been detected in CRH or CRH receptor in these patients. Krude *et al*. first described two patients with mutations in the POMC gene [23]. The first patient was a compound heterozygote with one missense mutation leading to a frameshift at codon 144, and the other was a single nucleotide change leading to a premature stop codon. The second patient was homozygous for a point mutation in exon 2 leading to a start codon. Both patients presented with early-onset isolated ACTH deficiency and obesity with red hair resulting from the lack of αMSH production. Symptoms of hypoglycemia and cholestasis resolved with hydrocortisone supplementation. Both sets of heterozygous parents were asymptomatic. There have been reports of a further three patients with POMC gene mutations, all presenting with isolated ACTH deficiency, red hair, and obesity.

A compound heterozygous mutation in the PC1 gene in a female patient with extreme early-onset obesity and ACTH deficiency was described in 1997 [24]. She also had defective processing of other prohormones and presented with insulin-dependent diabetes mellitus and hypogonadotrophic hypogonadism [25]. More recently, a child with isolated ACTH deficiency, red hair, and a severe enteropathy was found to harbor mutations within PC1 [26].

Mutations in the transcription factor Tpit, which is important for the terminal differentiation of the pituitary corticotrophs, were first described in 2001 [27]. The authors reported a mutation frequency of 73% in patients with isolated ACTH deficiency of neonatal onset. Eight patients belonging to six unrelated families were found to harbor seven different Tpit mutations. All patients were homozygous for the mutations apart from one compound heterozygote. The heterozygote parents were asymptomatic, indicating an autosomal-recessive mode of inheritance. The patients manifested severe ACTH and cortisol deficiency resulting in episodes of sudden and severe hypoglycemia associated with seizures and prolonged neonatal cholestatic jaundice.

Gonadotropins

The reproductive system is unique as a result of the changes that occur in the concentrations of the reproductive hormones throughout life. FSH and LH are glycoproteins composed of two subunits, α and β. The α-subunit is identical to the α-subunit of TSH, and the specific biological activity of the hormone resides in the β-subunit. The LH β-subunit gene is located on chromosome 19. The human chorionic gonadotropin β-subunit has similar biological activity to the LH β-subunit. LH secretion is pulsatile in both sexes, but sexual dimorphism of secretory patterns becomes evident with maturity of the hypothalamo-pituitary–gonadal axis. Increased nocturnal LH release is the first sign of the onset of puberty. The FSH β-subunit gene is located on chromosome 11.

Actions

Both hormones bind to membrane receptors in ovary and testis, activating the G-protein-coupled complex and stimulating adenyl cyclase. FSH regulates gametogenesis in males and females, while LH is thought to be primarily responsible for gonadal steroid secretion.

Regulation

Pulsatile release of hypothalamic gonadotropin-releasing hormone (GnRH) regulates the secretion of the pituitary hormones LH and FSH, which stimulate the testis and ovary at puberty to increase gonadal steroid secretion and develop secondary sexual characteristics. There is a surge in gonadotropin and gonadal steroid secretion in the neonatal period with concentrations similar to those reached during puberty. Following the first few months of life, the gonadotrophin axis remains quiescent until puberty, when concentrations rise again. Dopamine has a dose-dependent regulatory effect on gonadotropin secretion.

Estrogens and progesterone have a negative effect on gonadotropin secretion but, if plasma estrogens are high for longer than about 36 h in the absence of plasma progesterone, positive feedback is exerted with a surge of LH.

FSH secretion is also regulated by inhibin, a protein molecule secreted by the follicular granulosa cells in the female and the Sertoli cells in the male.

Isolated gonadotropin deficiency: hypogonadotrophic hypogonadism

Hypogonadism may result from abnormalities within the hypothalamo-pituitary axis or within the gonad itself (hypergonadotrophic hypogonadism). Hypogonadotrophic hypogonadism (HH) is particularly heterogeneous with a phenotype ranging from undescended testes at birth with absent pubertal development to normal puberty with infertility at the other extreme. It is four times more common in males than females and has an incidence variably reported from 1 in 10 000 to 1 in 86 000 [28,29]. The commonest

presentation is at puberty with a lack of pubertal development, although the diagnosis is sometimes suspected at birth in patients who present with cryptorchidism. The prevalence of abnormalities at birth is low, suggesting that maternal HCG rather than fetal gonadotropins are responsible for testosterone secretion in the fetus. The diagnosis of isolated HH at puberty is made with low concentrations of LH and FSH and a poor rise to stimulation with exogenous gonadotropin-releasing hormone (GnRH test).

HH may be isolated or combined with other pituitary hormone deficiencies. Mutations in several transcription factors, such as PROP1, LHX3, and HESX1, are associated with deficiencies of gonadotrophic hormones [30–32]. The condition may be sporadic or familial inherited in an autosomal-dominant, autosomal-recessive, or X-linked manner.

X-linked inheritance

The association between isolated HH and anosmia (Kallman syndrome) was first reported by Maestre de San Juan. Kallman detailed the genetic basis to this disorder due to mutations in the *KAL1* gene resulting in an X-linked inheritance. The anosmia results from agenesis of the olfactory bulbs, the development of which is closely linked to that of GnRH-synthesizing neurons. Although these patients are capable of synthesizing and secreting a normal GnRH protein, the improper location of the GnRH neurones results in an inability of GnRH to reach the pituitary gland to stimulate the gonadotropins. Neuroimaging is often used in clinical practice to identify the abnormal olfactory bulbs in order to make the diagnosis. Approximately 75% of patients with Kallman syndrome have agenesis of the olfactory bulbs on neuroimaging [33]. Mutations in *KAL1* are responsible for the X-linked form of Kallman syndrome [34,35], and other features, such as mirror movements, renal aplasia, high-arched palate, deafness, and pes cavus, are associated with the X-linked form of the disorder [35].

DAX1 (dosage-sensitive sex reversal, adrenal hypoplasia congenita critical region on the X chromosome) mutations in humans cause HH and adrenal hypoplasia congenita that can result in severe neonatal adrenal crisis. Most often, the HH presents itself at puberty [36]. The condition is inherited as an X-linked disorder resulting from inactivating mutations of the DAX1 gene. DAX1 is a transcription factor expressed in several tissues and in the hypothalamus and pituitary. It interacts closely with SF1 (steroidogenic factor 1), another transcription factor that is critical for adrenal and gonadal development. Duplications of DAX1 result in persistent Müllerian structures and XY sex reversal, suggesting that the gene acts in a dosage-sensitive manner. Females are phenotypically normal.

Autosomal inheritance

Inactivating mutations of the gonadotropin-releasing hormone receptor (GnRHR) were first reported in 1997, and seven different pedigrees have been described since [37]. There is a wide range of phenotypes from hypogonadism with undescended testes and presentation at birth to mild pubertal delay. Although mutations in GnRHR are rare, patients with milder phenotypes harboring mutations may not yet be identified.

Recently, a homozygous missense mutation was identified within a novel gene, GPR54, in a highly consanguineous pedigree of patients with hypogonadotrophic hypogonadism [38]. A second patient with isolated hypogonadotrophic hypogonadism was found to be a compound heterozygote for two different mutations within the gene. Further pedigrees have been identified that harbor deletions within the gene. GPR54 is believed to be a regulator of GnRH secretion at the hypothalamic level.

Mutations in PC1 are associated with defective processing of prohormones, and a mutation in PC1 in a female patient has been reported with extreme early-onset obesity, ACTH deficiency, insulin-dependent diabetes, and HH [24].

Leptin is a product of the adipocyte and acts as a satiety factor. Apart from its role in regulating nutrition, it appears to play an important role in several neuroendocrine functions by acting at a hypothalamic level. Mutations in leptin and its receptor are associated with obesity, marked hyperphagia, metabolic abnormalities, and HH [39]. Treatment of a 12-year-old female patient with a frameshift mutation in the leptin gene with leptin has resulted in significant weight loss and normalization of nocturnal LH secretion [40].

Prolactin

Prolactin is a 199-amino-acid protein with its gene located on chromosome 6. Its principal functions are growth and development of the breasts and initiation and maintenance of lactation. It plays a role in gonadal function by stimulating the generation of LH receptors in the gonads in both sexes. The mechanism of action of prolactin is by stimulating the tyrosine kinase pathway and subsequent intracellular protein phosphorylation.

The release of prolactin is under the control of the hypothalamus with afferent impulses from sensory receptors, primarily around the nipples. The dominant hypothalamic influence is inhibitory, and the principal inhibitory hormone is dopamine. Other molecules exerting an inhibitory role are noradrenaline, histamine, and serotonin acting at either a hypothalamic or a pituitary level. TRH, in addition to stimulating the release of TSH, is the principal prolactin-stimulatory hormone. Thyroxine and estrogen can modulate the number of TRH receptors in the lactotrophs, thereby influencing prolactin release. Thyroxine decreases the number of TRH receptors, while estrogens increase their availability.

The maternal pituitary is the main source of serum prolactin during pregnancy, and the only known clinical effect of prolactin hyposecretion in adults is the failure of lactation

in puerperal women. Prolactin deficiency may be combined with other anterior pituitary hormone deficiencies, as seen in patients with *POU1FI* and *PROP1* mutations. It may occur as an isolated deficiency [41].

Posterior pituitary hormones

The neurohypophysis consists of the supraoptic and paraventricular nuclei, which contain the cell bodies of the magnocellular neurosecretory neurones that secrete vasopressin and oxytocin, the supraoptico-hypophyseal tract, which includes the axons of these neurones, and the posterior pituitary, where the axons terminate on capillaries of the inferior hypophyseal artery.

Arginine vasopressin (AVP)

Vasopressin is a basic nonapeptide with a disulfide bridge between cysteine residues at positions 1 and 6. Most mammals have the amino acid arginine at position 8. The vasopressin gene lies on chromosome 20, in tandem with the oxytocin gene and separated by 8 kb of DNA. The gene contains three exons and encodes a polypeptide precursor that consists of a 19-amino-acid amino-terminal signal peptide, 9-amino-acid vasopressin peptide, a diamino acid linker, the 93-amino-acid neurophysin peptide (NPII), a single amino acid linker, and a 39-amino-acid carboxyl-terminal glycopeptide copeptin.

The preprohormone is synthesized in the magnocellular neurone cell body, after which the signal peptide is cleaved away. The prohormone then folds and places AVP into a binding pocket of NPII, which protects it from proteolysis and promotes high-density packing in neurosecretory granules by oligomerization of AVP–NPII dimers. Following the formation of seven disulfide bonds within NPII and one within AVP and the glycosylation of copeptin, the prohormone is packaged into neurosecretory granules and cleaved into the product peptides during axonal transport to the posterior pituitary.

The mature hormone and NPII are stored as a complex in secretory granules within the nerve terminals of the posterior pituitary. Stimulation of vasopressinergic neurons results in the opening of voltage-gated calcium channels in the nerve terminals through a transient influx of calcium, which results in fusion of the neurosecretory granules with the nerve terminal membrane and release of their contents into the circulation. The half-life of vasopressin is 5–15 min.

Actions

Vasopressin acts via three G-protein-coupled receptors:

1 It achieves its pressor effects via V1 receptors.

2 Its main renal effects are achieved via V2 receptors (V2-R). Activation of the V2-R leads to a biphasic increase in the expression of the water channel protein Aquaporin 2. This allows reabsorption of water from the duct lumen along an osmotic gradient, with excretion of concentrated urine.

3 Vasopressin acts via V3 receptors on corticotrophs to secrete ACTH, in synergy with CRH.

Regulation

The main regulatory factors in determining vasopressin secretion are osmotic status, blood pressure, and circulating volume. Neurotransmitters, such as dopamine and norepinephrine, are also thought to play a role in vasopressin secretion, as does angiotensin II.

Central diabetes insipidus

Congenital causes of central diabetes insipidus (DI) are rare. Familial central DI is an autosomal-dominant disorder of AVP secretion. Affected patients present with polyuria and polydipsia, usually in the first 10 years of life. Overt hypertonic dehydration occurs only if the patient is unable to obtain water. Food consumption may be decreased leading to loss of weight and slow growth. During infancy, common clinical features include hyperthermia, vomiting, failure to thrive, and constipation. Neonatal manifestations are uncommon, suggesting that the pathophysiology of familial central DI involves progressive postnatal degeneration of AVP-producing magnocellular neurones.

A number of mutations have been described in children with central DI in the AVP neurophysin gene. These include signal peptide mutations that decrease the ability of the signal peptidase to initiate removal of the signal peptide from the preprohormone [42]. A second group of mutations occurs within the AVP or amino-terminal domain of the NPII coding sequence, and these interfere with the binding of AVP to NPII or in the folding of NPII [43]. A third group of mutations results in the synthesis of a truncated neurophysin molecule [44].

The mutations described to date may lead to abnormal folding and processing of the preprohormone. The mutant protein may then accumulate within the endoplasmic reticulum, where it may kill the cells by interfering with the orderly processing of other essential proteins. Hence, heterozygous mutations within the AVP NPII gene may result in the production of an abnormal preprohormone that cannot be processed properly and destroys AVP-processing neurones.

A pedigree with autosomal-recessive DI has also been described, with a homozygous mutation in exon 1 leading to partial loss-of-function.

Other causes include Wolfram syndrome, an autosomal-recessive condition that includes DI, diabetes mellitus, optic atrophy, and sensorineural deafness. WFS1 is a novel gene on chromosome 4 encoding an 890-amino-acid glycoprotein (wolframin), predominantly localized in the endoplasmic

reticulum. Mutations in WFS1 underlie autosomal-recessive Wolfram syndrome. Sixty-six mutations over the entire coding region have been reported to date and are typically inactivating, suggesting that a loss-of-function causes the disease phenotype [45].

DI may also be a feature of midline disorders such as septo-optic dysplasia and holoprosencephaly.

Oxytocin

The oxytocin gene lies on chromosome 20, consists of three exons, and, like vasopressin, encodes a polypeptide precursor with an amino-terminal signal peptide, the oxytocin peptide, neurophysin, and a carboxy-terminal peptide. The human oxytocin promoter contains estrogen response elements and interleukin-6 response elements. The significance of this is unclear. The half-life of oxytocin is short. Oxytocin binds to a G-protein-coupled cell surface receptor on target cells to regulate lactation, parturition, and reproductive behavior, but women lacking posterior pituitary function can breast-feed normally, so oxytocin is not essential for lactation in humans.

Combined pituitary hormone deficiency

Clinical features

Combined pituitary hormone deficiency (CPHD) is defined as a deficiency in two or more pituitary hormones. The condition varies considerably in severity, and the signs and symptoms are due to a combination of individual hormone abnormalities. The symptoms may be non-specific in the early neonatal period and become obvious with time. Occasionally, the condition may be life-threatening, especially in patients with ACTH deficiency, and an early diagnosis is mandatory.

Neonatal presentation

The presentation of hypopituitarism in the neonatal period may be non-specific with poor feeding, lethargy, apnea, jitteriness, and poor weight gain. Hypoglycemia is present in the majority of patients with CPHD, probably due to ACTH deficiency, although it has also been reported in patients with isolated GH deficiency. Measurement of capillary blood glucose is routine in sick neonates, and measurement of true blood glucose should be undertaken if the capillary glucose is less than 2.6 mmol/L. At the same time, blood should also be taken for measurement of random GH, cortisol, insulin, non-esterified fatty acids, and ketone bodies.

Low serum insulin in the presence of hypoglycemia will rule out hyperinsulinemia, but low serum GH and/or cortisol concentrations should point to a diagnosis of hypopituitarism. Infants with TSH deficiency may present with temperature instability and prolonged unconjugated hyperbilirubinemia. Conjugated hyperbilirubinemia is a marker of cortisol deficiency and, together with recurrent sepsis, apnea, and seizures, should prompt investigation for hypopituitarism.

Occasionally, neonates present with diabetes insipidus, although this is much more common in patients with associated midline defects. A history of breech delivery or other instrumental delivery is commoner in patients with hypopituitarism. Patients with gonadotropin deficiency, particularly LH, may present with undescended testes and a micropenis, as growth of the penis is dependent upon normal secretion of LH and testosterone in the second and third trimesters.

Growth failure

Birthweight and length have been reported to be normal in patients with congenital hypopituitarism. There has however been considerable controversy over the role of GH in the postnatal period and in early infancy. Recent studies have shown evidence for severe growth failure in infants with congenital hypopituitarism [46]. Short stature is generally the primary complaint in patients with hypopituitarism who present later in infancy and childhood.

Development

Patients with untreated hypothyroidism under the age of 2 years can develop severe brain damage and global developmental delay. Prolonged undetected hypoglycemia can also result in profound central nervous system damage. Diabetes insipidus associated with either severe hyponatremia (due to overtreatment) or hypernatremia (due to inadequate treatment or fluid deprivation) can also lead to brain damage.

Puberty

Male patients with HH may present with a micropenis and undescended testes. The diagnosis is less well recognized in females at birth. Patients with HH can have a wide spectrum of abnormalities ranging from absent, delayed, or arrested pubertal progress or infertility in later life. Additional GH deficiency will result in failure of the pubertal growth spurt.

Diabetes insipidus

Patients with panhypopituitarism can present with diabetes insipidus in an unusual fashion. The symptoms are polyuria and polydipsia with weight loss, but cortisol is essential for the excretion of a water load, and the diagnosis of diabetes insipidus may be masked in patients with ACTH deficiency. Treatment with hydrocortisone will unmask diabetes

insipidus, and caution should be exercised in patients with multiple pituitary hormone abnormalities when they are commenced on glucocorticoid replacement treatment, with careful monitoring for diabetes insipidus.

Visual problems

Patients with hypopituitarism and visual abnormalities should be reviewed by an ophthalmologist to rule out optic nerve hypoplasia.

Etiology

The majority of cases with hypopituitarism are idiopathic, with approximately 5–20% of cases being familial and therefore suggesting a genetic basis. Familial cases may be inherited in an autosomal-recessive or -dominant manner. The condition is highly heterogeneous with respect to the clinical presentation, hormonal phenotype, neuroanatomy, natural history of the disease, and family history. Mutations in a number of transcription factors involved in pituitary development have been identified in association with specific patterns of CPHD with (*HESX1*, *LHX3*, *LHX4*) or without (*POU1F1*, *PROP1*) extrapituitary manifestations. Table 4.5 highlights the phenotypic differences between patients with *POU1F1*, *PROP1*, and *LHX3* mutations.

POU1F1

The human *POUIF1* has been localized to chromosome 3p11 and consists of six exons spanning 17 kb. It encodes a 291-amino-acid protein with a molecular mass of 33 kDa. The protein has three functional domains, a transactivation domain, a POU-specific (POU-S) domain, and a POU homeodomain (POU-H). The POU-S and POU-H domains are both critical for high-affinity DNA binding on the GH and prolactin promoters.

The first mutation within *POUIFI* was identified by Tatsumi *et al*. in a child with GH, prolactin, and profound TSH deficiency [47]. The patient was homozygous for a nonsense mutation in *PIT1* resulting in a severely truncated protein of 171 amino acids, lacking half the POU-S domain and all the POU-H domain. Such a protein would be incapable of binding to GH and PRL promoters and thus unable to activate the transcription of these genes. The spectrum of hormone deficiency varies considerably in patients with *POU1F1* mutations. GH deficiency generally presents early in life, along with prolactin deficiency. However, TSH deficiency can be highly variable with presentation later in childhood [48,49].

Pfaffle *et al*. described mutations in two Dutch families with GH, PRL, and TSH deficiencies [48]. Two children in the first family had mild central hypothyroidism and were homozygous for an A158P substitution. In the second family, the children had more severe central hypothyroidism and had inherited the A158P allele from their father and a deletion of the entire *PIT1* gene from their mother. The A158P substitution retained promoter-binding activity but failed to activate transcription from GH or PRL promoters. Nevertheless, the two children in the first family had normal-sized anterior pituitary glands on magnetic resonance imaging (MRI) with normal serum thyroxine levels before beginning GH treatment. The compound heterozygotes in the second family had severe hypothyroidism and small anterior pituitaries. In addition to these recessive mutations, a heterozygous point mutation R271W has also been described. When co-transfected with wild-type *POU1F1*, this mutant protein prevented transcriptional activation by the wild-type protein and acted as a dominant negative.

MRI demonstrates a small or normal anterior pituitary with a normal posterior pituitary and infundibulum. Abnormalities within midline structures are not associated with *POU1F1* mutations. The inheritance is both autosomal-dominant and -recessive, the former associated with a dominant-negative effect whereby the mutant protein interferes with the function of the normal protein. Since the first report, several mutations have been identified in *POU1F1*, all of which have been associated with a broadly similar phenotype of GH, TSH, and prolactin deficiency [2]. The dominant R271W mutation is a "hotspot" for *POU1F1* mutations [50] and has been identified in several unrelated patients of different ethnic backgrounds.

More recently, a patient with GH deficiency, normal basal serum prolactin but lack of response to stimulation with TRH, and evolving secondary hypothyroidism has been identified with a novel mutation (K216E) that can bind to DNA and activate transcription, but does not support retinoic acid induction of the *POU1F1* gene distal enhancer either alone or in combination with wild-type *POU1F1* [51]. Hence, functional analysis of many of these mutations suggests that some mutations disrupt DNA binding, whereas others disrupt transcriptional activation or other properties

Table 4.5. Clinical features of hypopituitarism due to mutations in *POU1F1*, *PROP1*, and *LHX3* causing CPHD in humans.

Phenotype	*PROP1*	*POU1F1*	*LHX3*
Presentation	Delayed	Congenital	Congenital
GH	Deficient	Deficient	Deficient
TSH	Deficient	Deficient	Deficient
Prolactin	Deficient	Deficient	Deficient
LH, FSH	Deficient	Normal	Deficient
ACTH	May evolve	Normal	Normal
Pituitary size	S, N, E	S, N	S, N, E
Non-pituitary phenotype	Nil	Nil	Short cervical spine

S, small; N, normal; E, enlarged.

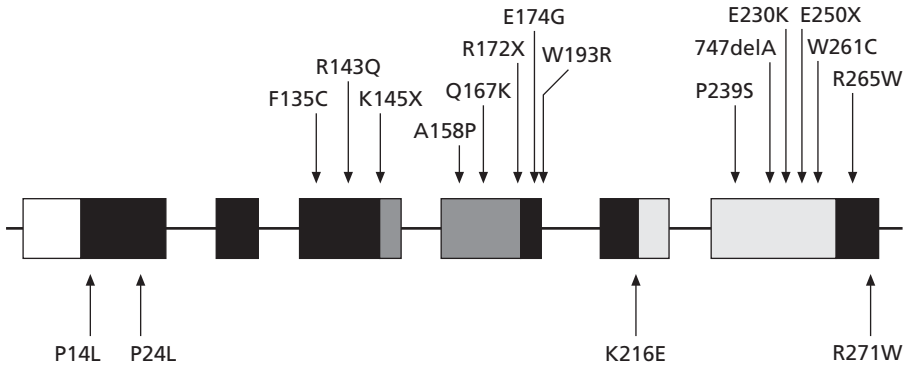

Fig. 4.5. Diagram illustrating the reported *POU1F1* mutations to date. Mutations illustrated at the bottom are inherited dominantly while those shown at the top are recessive mutations. The POU-specific domain and the POU homeodomain are shown by the dark and light gray bars respectively.

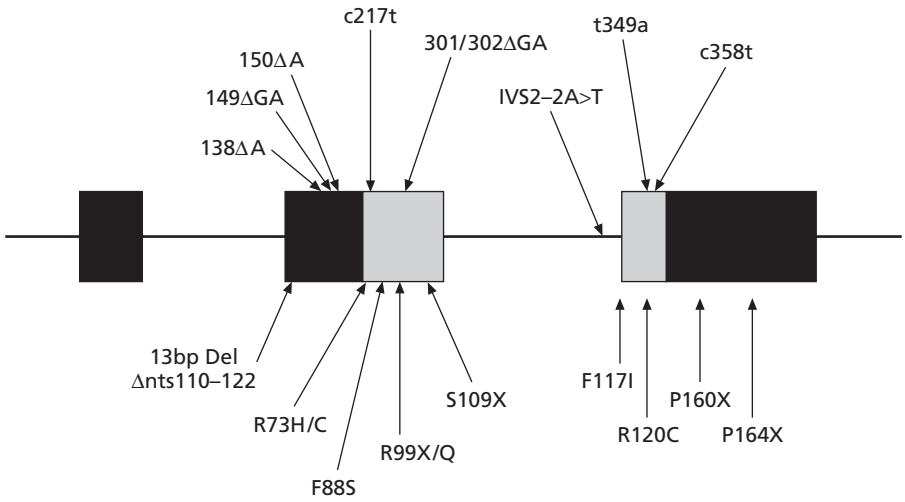

Fig. 4.6. Human mutations in *PROP1* causing combined pituitary hormone deficiency. All mutations are inherited recessively. Gray bar denotes the paired-like homeodomain.

such as autoregulation. Figure 4.5 illustrates the reported human mutations in *POU1F1* causing CPHD.

PROP1

In contrast to patients with mutations within *POU1F1*, mutations in *PROP1* are associated with GH, TSH, prolactin, and gonadotropin deficiencies and are commoner. The human *PROP1* gene has been mapped to chromosome 5q and is a member of the paired-like homeobox gene family. Expression of *Prop1* in the mouse precedes and appears to be required for the expression of *Pit1* [52]. The gene spans 3 kb and consists of three exons encoding a protein product of 226 amino acids. The DNA-binding homeodomain consists of three α-helical regions, and most mutations reported to date affect this region (Fig. 4.6).

Wu *et al*. first reported *PROP1* mutations in four unrelated pedigrees with an endocrine phenotype consistent with GH, TSH, prolactin, LH, and FSH deficiencies [30]. The first family harbored a homozygous mutation (R120C) in the paired-like homeodomain. The second and third pedigrees were found to have what is now believed to be a mutational "hotspot" within the PROP1 gene, a 2 bp deletion (delA301,G302, also known as 296del GA). This mutation involves a 2 bp GA or AG deletion among three tandem GA repeats (296-GAGA-GAG-302) within exon 2, resulting in a frameshift with a stop codon at codon 109, thereby leading to a truncated protein (S109X) that contains the N-terminus and only the first helix of the homeodomain, thus disrupting both DNA binding and transcriptional activation. The patient in the fourth pedigree was a compound heterozygote (delA301,G302/F117I). Since then, several mutations have been identified, all inherited in an autosomal-recessive manner (Fig. 4.6).

Deficiency of GH, prolactin, and TSH is more profound in patients with mutations within *POU1F1* than in patients with *PROP1* mutations. Although most patients present with early-onset GH deficiency, normal growth in early childhood and normal final height in an untreated patient with a *PROP1* mutation have been reported [53]. The normal final height was achieved at the expense of considerable weight gain at the time of puberty. As observed in patients with *POU1F1* mutations, the TSH deficiency in these patients is highly variable and may not be present from birth. Although *PROP1* is essential for the differentiation of gonadotrophs in fetal life, the spectrum of gonadotropin deficiency is again extremely variable ranging from hypogonadism and lack of puberty

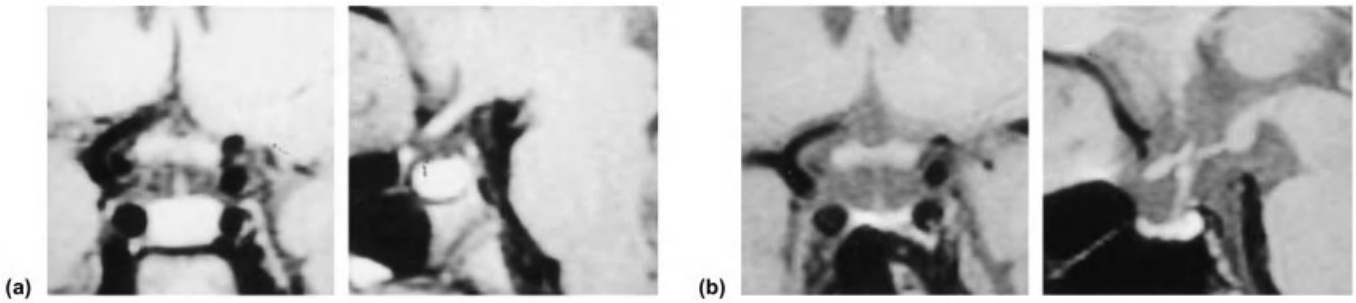

Fig. 4.7. MRI of a patient with A301, G302 deletion in *PROP1*. (a) At 8.8 years, neuroimaging revealed an enlarged anterior pituitary. (b) Repeat scan at 15 years demonstrated spontaneous involution with a pituitary gland of reduced size. Taken from Mendonca BB *et al*. *J Clin Endocrinol Metab* 1999; 84: 942–5.

to spontaneous pubertal development and infertility [52]. The exact mechanism underlying this "acquired" deficiency is unclear, although it suggests that Prop1 is not required for gonadotroph determination but is required for differentiation.

Patients with *PROP1* mutations also demonstrate an evolving cortisol deficiency with age, although the youngest patient described with cortisol deficiency is 7 years old. The underlying mechanism for the cortisol deficiency is unknown, especially as Prop1 is not expressed in corticotrophs. Various hypotheses have been postulated such as a gradual attrition of corticotrophs as Prop1 may be required for the maintenance of the corticotroph population, or an expanding pituitary mass, which then involutes with secondary loss of corticotrophs [54].

Although most patients with *PROP1* mutations have a small or normal anterior pituitary on MRI some patients have an enlarged anterior pituitary suggesting a tumor [55]. These masses can regress with time and lead to complete pituitary involution and an empty sella syndrome. Figure 4.7a illustrates the MRI of a patient with a 2 bp deletion (A301, G302) in PROP1 showing a massively enlarged anterior pituitary. A repeat scan 7 years later demonstrated spontaneous pituitary involution (Fig. 4.7b). Pituitary enlargement has not been reported in the Ames dwarf, but pituitary size has been shown not to be constant [56]. To date, the underlying mechanism for the mass remains unknown. Prolonged expression of HESX1 or LHX3, and hence of undifferentiated precursor cells that remain viable for a longer time, may be an explanation [7]. There has been only one report of a biopsy of the "tumor," and the histology was non-specific with the presence of amorphous material and no signs of apoptosis, nor were there any recognizable cell lines. These changes in pituitary size may therefore reflect a natural progression of the disease. There is no correlation between the finding of an enlarged pituitary or its subsequent involution and evolving cortisol deficiency.

X-linked hypopituitarism

Duplications of Xq26–27 have been described recently in association with hypopituitarism [57]. The phenotype has not been well-described to date, and the smallest duplication to date is approximately 3.9 Mb. It has been speculated that the hypopituitarism is due to overdosage of a gene that lies in the critical region of 3.9 Mb.

Dysmorphic syndromes

HESX1 and septo-optic dysplasia

Septo-optic dysplasia is a rare congenital anomaly with a relative prevalence of 6.3 per 100 000. It is a heterogeneous disorder characterized by a clinical triad of midline forebrain abnormalities, optic nerve hypoplasia (ONH), and hypopituitarism (Fig. 4.8). It is thought to be equally prevalent in males and females and, although the condition is generally sporadic, familial cases have been described. The first reported case of ONH associated with absence of the septum pellucidum was in a 7-month-old infant with congenital

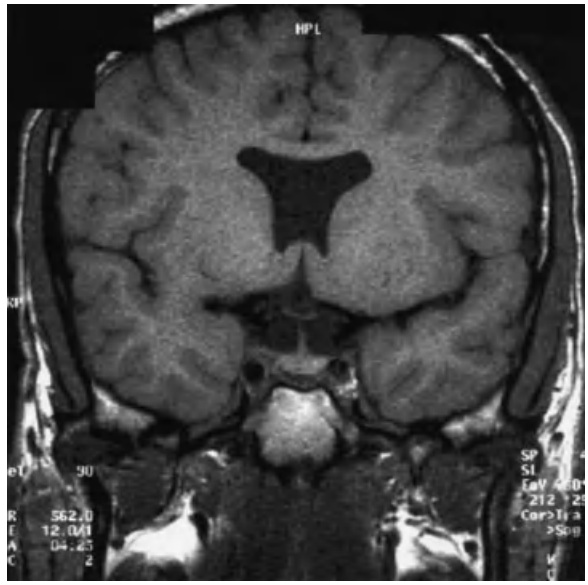

Fig. 4.8. MRI scan of patient with septo-optic dysplasia (SOD) illustrating an absent septum pellucidum.

blindness, absent pupillary reflexes, and normal development, more than 50 years ago. Before that, ONH had only been described as a rare and isolated anomaly for almost a century. De Morsier described the post-mortem findings of ONH and agenesis of the septum pellucidum and coined the term septo-optic dysplasia (SOD) in 1956. Pituitary abnormalities in association with the disorder were described subsequently in a patient with extremely short stature.

The phenotype is highly variable, and each of the three components can occur in isolation or in combination with at least one of the other components. Approximately 30% of SOD cases have the complete clinical triad; 62% of patients have some degree of hypopituitarism, and 60% have an absent septum pellucidum [58]. De Morsier, in his initial report, reported that optic nerve abnormalities were present in only nine of 36 cases with absent septum pellucidum. Acers studied 45 individuals with ONH and found that 12 of these patients had associated midline brain defects, with six of these individuals also demonstrating hypopituitarism [59].

Optic nerve hypoplasia may be unilateral or bilateral and may be the first presenting feature, with later onset of endocrine dysfunction. Bilateral ONH is commoner (88% compared with 12% unilateral cases). There appears to be little correlation between the size of the optic nerve and its visual function. Neuroradiological abnormalities are present in up to 75–80% of patients with ONH [60,61].

Endocrine deficits vary from isolated GH deficiency to panhypopituitarism. There has been some suggestion that abnormalities of the septum pellucidum and hypothalamo-pituitary axis on neuroimaging can predict the severity of endocrine dysfunction [62]. A decrease in growth rate resulting from GH deficiency is the commonest feature, with hypoglycemia, and polyuria and polydipsia being less common. Sexual precocity or failure to develop in puberty may occur. Abnormal hypothalamic neuroanatomy or function and diabetes insipidus may be a feature.

The endocrinopathy may evolve with progressive loss of endocrine function over time. The commonest endocrinopathy is GH deficiency followed by TSH and ACTH deficiency. Gonadotropin secretion may be retained in the face of other pituitary hormone deficiencies. Commencement of GH treatment in SOD children with GH deficiency may be associated with accelerated pubertal maturation.

Neurological deficit is common, but not invariably so and, in one study, was documented in 15 of 24 children with a severe degree of ONH. The deficit ranged from global retardation to focal deficits such as epilepsy or hemiparesis. Other neuroanatomical abnormalities include cavum septum pellucidum, cerebellar hypoplasia, schizencephaly, and aplasia of the fornix.

The etiology of the condition is unknown and likely to be multifactorial with a combination of genetic and environmental factors. The development of the forebrain is highly complex occurring at a very early stage of embryonic development, as early as E8.5 in the mouse, in which it has been studied extensively [63]. This corresponds to as early as 3–6 weeks' gestation in the human embryo. Non-neural structures such as the anterior pituitary, the olfactory placode, and the nasal cavity ectoderm and neural structures such as the hypothalamus, the posterior pituitary, the optic vesicles, the optic nerves, and the forebrain develop from the same region of the embryo, the anterior neural plate. Any insult at this critical stage of embryonic development could account for the features of SOD. There has been an increased understanding of the genetic basis of this condition led by the discovery of a transcription factor (Hesx1) that co-ordinates the sequential development of these structures in the mouse embryo.

HESX1

In the light of the phenotype demonstrated in *Hesx1* null mutant mice, the human homolog of the gene was screened for mutations in patients with SOD. *HESX1* maps to chromosome 3p21.1–3p21.2, and its coding region spans 1.7 kb with a highly conserved genomic organization consisting of four coding exons. The 185-amino-acid open reading frame is highly conserved compared with the mouse and Xenopus open reading frames, particularly across the homeodomains that share 95% and 80% homology respectively. An additional conserved domain is located near the amino-terminus, which is also present in other homeodomain classes including *engrailed* and *goosecoid* and has been shown to play a role in transcriptional repression.

A homozygous missense mutation (Arg160Cys) was found in the homeobox of *HESX1* in two siblings within a highly consanguineous family in which two affected siblings presented with ONH, absence of the corpus callosum, and hypoplasia of the pituitary gland with panhypopituitarism [32]. The parents were heterozygous for the mutation and phenotypically normal. Screening of extended members of the family revealed a further nine phenotypically normal heterozygotes within this highly consanguineous pedigree, consistent with an autosomal-recessive inheritance.

The mutation led to a complete loss of DNA binding and, unusually, was associated with an *in vitro* dominant-negative effect, even though heterozygotes for the mutation did not manifest a phenotype. Given the occasional heterozygous murine phenotype, the possibility that heterozygous mutations of *HESX1* might be associated with various pituitary phenotypes was considered.

Five additional heterozygous mutations of *HESX1* have been identified in children with various forms of pituitary disease and SOD. These included three non-conservative amino acid substitutions [64], a 2 bp insertion [65], and a deletion in nucleotide position 1684 [66] (Table 4.6). Haplotype analysis using markers that closely flanked *HESX1* revealed that such heterozygous *HESX1* mutations are associated with a dominant inheritance that is incompletely

Table 4.6. *HESX1* mutations with variable phenotype and neuroanatomy.

Mutation	Inheritance	*N*	Phenotype	MRI	Year
R160C	HM	2	SOD	SAP, EPP, ONH, absent SP, thin CC	1998
S170L	HT	2	IGHD	(one patient with ONH)	2001
T181A	HT	1	IGHD	SAP, absent PP	2001
Q6H	HT	1	CPHD	SAP, EPP	2001
306/307 ins AG	HT	1	CPHD	SAP, EPP, left ONH	2003
I26T	HM	1	CPHD	SAP, EPP	2003
1684delG	HT	1	SOD	Absent CC, ONH, SAP, absent PP	2003

HM, homozygous; HT, heterozygous; SAP, small anterior pituitary; EPP, ectopic posterior pituitary; ONH, optic nerve hypoplasia; SP, septum pellucidum; CC, corpus callosum.

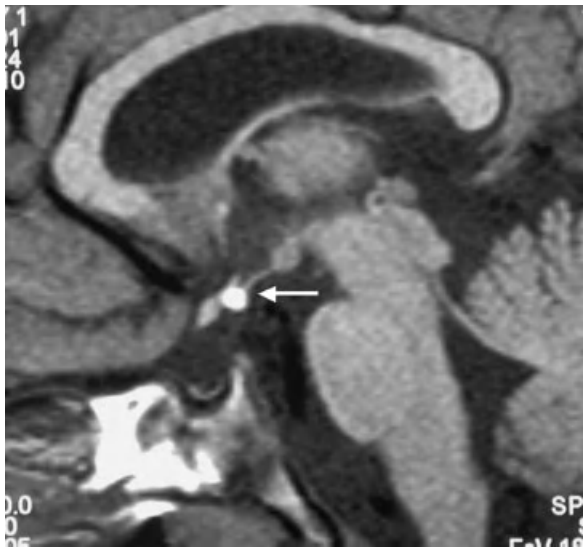

Fig. 4.9. MRI scan of patient with an undescended posterior pituitary as highlighted by the bright signal.

penetrant, although the insertion was a *de novo* mutation. These data are consistent with the finding that *Hesx1*$^{+/-}$ mice display low penetrance of a mild phenotype.

The phenotypes associated with heterozygous mutations are, on the whole, milder, classically characterized by isolated GHD with an ectopic/undescended posterior pituitary (Fig. 4.9). However, both ONH and midline forebrain abnormalities have also been associated with heterozygous mutations.

Recently, a second homozygous mutation (I26T) has been identified in a girl who initially presented with GHD and subsequently developed gonadotropin, TSH, and ACTH deficiencies during adolescence and early adulthood [67]. This was the first mutation to be described in the critical eh-1 domain of HESX1, and functional analysis revealed partial loss of repression and loss of interaction with the co-repressor TLE1. Intriguingly, the patient manifested no evidence of midline defects. The MRI scan revealed anterior pituitary hypoplasia with an ectopic/undescended pituitary. Hence, both homozygous and heterozygous mutations of *HESX1* are associated with highly variable pituitary/forebrain phenotypes, and no clear genotype–phenotype correlations have been identified.

The overall frequency of *HESX1* mutations is low, suggesting that mutations in other known or unknown genes contribute to this complex disorder. Environmental agents such as viral infections, vascular or degenerative changes could contribute to the phenotype. Young maternal age and antenatal exposure to alcohol or drugs have been postulated to play a role in the etiology, although this has been disputed. In some studies, there is a preponderance of primigravida mothers.

Neck abnormalities and *LHX3*

LHX3 maps to human chromosome 9q. Mutations within the gene have now been identified in four patients within two unrelated consanguineous families [31]. The patients presented with an endocrine phenotype similar to that observed in PROP1-deficient patients (GH, TSH, LH, FSH, prolactin deficiencies). They also had a characteristic phenotype with a rigid and short cervical spine and limited head rotation. MR images demonstrated small anterior pituitaries with a normal posterior pituitary and normal midline structures in the two patients within the first family. However, as observed with *PROP1* mutations, the fourth patient demonstrated a markedly enlarged anterior pituitary that was not evident in a MR scan performed 10 years earlier. Mutations within *LHX3* are rare and associated with a highly characteristic phenotype.

Cerebellar abnormalities and *LHX4*

There has been one report of a mutation within *LHX4* to date [68]. The patient presented with short stature and had GH, TSH, and ACTH deficiencies. Apart from CPHD, the patient had extrapituitary manifestations that included a poorly formed sella turcica and pointed cerebellar tonsils. MRI

scanning revealed a small anterior pituitary, an ectopic posterior pituitary, and an absent infundibulum. This suggests that *LHX4* may be a factor that tightly co-ordinates brain development and skull shaping.

Rieger syndrome and *PITX2*

Mutations within *PITX2* or RIEG are associated with Rieger syndrome in humans. Rieger syndrome is an autosomal-dominant condition with variable manifestations, including anomalies of the anterior chamber of the eye, dental hypoplasia, a protuberant umbilicus, mental retardation, and pituitary abnormalities. Eight heterozygous mutations in Pitx2 (five missense mutations, two splicing mutations, and one in-frame duplication of 21 bp affecting the homeodomain) have been reported to date [2]. Although affected mice have hypopituitarism and a hypoplastic gland, it remains unclear whether humans with *PITX2* mutations have pituitary hormone deficiencies.

Midline defects and *GLI2*

Recently, the Sonic Hedgehog (SHH) signaling pathway has been implicated in more complex disorders of pituitary development. Mutations within SHH are associated with holoprosencephaly. Three *Gli* genes have been implicated in the mediation of SHH signals. Heterozygous mutations within *GLI2* have been identified in seven out of 390 patients with holoprosencephaly [69]. Phenotypic penetrance was variable, with the parent carrying the mutation showing no obvious phenotype in some cases. In all affected patients, pituitary gland function was abnormal, accompanied by variable craniofacial abnormalities. Other features included post-axial polydactyly, single nares, single central incisor, and partial agenesis of the corpus callosum.

Holoprosencephaly

Holoprosencephaly (HPE) is a brain anomaly whereby the cleavage of the forebrain is abnormal. Three types have been identified – alobar, semilobar, and lobar. It is associated with a number of other anomalies, such as nasal and ocular abnormalities, in addition to abnormalities of the olfactory nerves and bulbs, corpus callosum, hypothalamus, and pituitary gland. The clinical phenotypes are variable, and the pituitary abnormality most commonly associated with HPE is diabetes insipidus, but other anterior pituitary hormone deficiencies may arise.

Major advances have been made in understanding the etiology of the condition. At least 12 chromosomal regions on 11 chromosomes may contain genes implicated in holoprosencephaly. Additionally, autosomal-recessive and -dominant forms of the condition have been described. Mutations within *SHH*, *ZIC2*, *TG1F*, and *SIX3* have been implicated.

Miscellaneous conditions

A number of conditions are associated with variable hypopituitarism. These include Pallister–Hall syndrome, Fanconi anemia, solitary single central incisor, cleft lip and palate, and ectrodactyly–ectodermal dysplasia-clefting syndrome.

Investigation and treatment of hypopituitarism

Establishing the diagnosis of CPHD

The diagnosis of CPHD may be evident in the newborn period when the infant presents with hypoglycemia or prolonged neonatal hyperbilirubinemia. Additionally, males may present with undescended testes and a microphallus. The diagnosis is often clear on assessing basal thyroid function, with a low concentration of free thyroxine (FT_4) and a concomitant low concentration of TSH. The random cortisol is low, and a 24-h plasma cortisol may confirm low concentrations of cortisol throughout the day. The concentration of the GH-dependent factors IGF-I and IGFBP-3 is also useful, although the sensitivity and specificity of the test is poor in isolation. Growth hormone provocation tests are contraindicated in children less than 1 year of age.

In the older child, the diagnosis of CPHD is based upon the documentation of a low growth velocity in conjunction with GHD on provocative testing. TSH deficiency is characterized by a low TSH in conjunction with a low thyroxine level. A routine TRH test is not mandatory to establish the diagnosis of central hypothyroidism in children with CPHD [70]. Prolactin deficiency is confirmed by a suboptimal response to TRH, and gonadotropin deficiency by a poor response to gonadotropin-releasing hormone, although the latter is dependent upon the age at which the test is performed. ACTH deficiency can be diagnosed as a poor cortisol response to provocation with either hypoglycemia or exogenous ACTH (synacthen). In males, stimulation of testes with human chorionic gonadotropin (HCG) can be used in the diagnosis of hypogonadotrophic hypogonadism, when the testes often show a very poor testosterone response to HCG. Magnetic resonance imaging of the brain is extremely useful in detecting abnormalities of forebrain and pituitary development and in assessing the size of the optic chiasm and optic nerves. The size of the anterior pituitary gland is highly variable. The posterior gland may be eutopic or ectopic. Midline defects of the forebrain may also be present.

Management of CPHD

The mainstay is replacement therapy with the appropriate hormones. Thyroxine should be commenced if the free or total thyroxine concentration is low. Growth should be

carefully monitored and, if the growth velocity is poor and GHD is confirmed on provocative testing, treatment with recombinant hGH should be commenced and continued until linear growth ceases. There is a case for the use of GH treatment in young adults with GHD given the possible metabolic effects on fat, lean body mass, and bone mineral density. Sex steroids in the form of estrogen or testosterone should be commenced at puberty if gonadotropin deficiency is confirmed. Hydrocortisone should be commenced if cortisol secretion is impaired. If a mutation within *PROP1* is documented and cortisol secretion is normal, it should be assessed at regular intervals as it may become impaired. If a pituitary mass is present, serial MRI scans are indicated in order to monitor the size of the mass. In patients with SOD, adequate ophthalmological, neurological, and social support should be offered to the patients and their families, given the visual disability that is part of the condition.

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5 Normal and disordered growth

Leena Patel and Peter E. Clayton

Normal growth

Introduction

Growth can be defined as a process of increase in size by accretion of tissue. It is observed in the whole organism, in body regions, in organ systems, and in the cellular environment. It is dependent on cell hyperplasia (an increase in cell number), cell hypertrophy (an increase in cell size), and apoptosis (programmed cell death). Hyperplasia and apoptosis are genetically regulated to limit the size of an organ or the body. The speed and success of growth depend on the relative rates of these three cellular events. Their coordination allows a fertilized egg to develop into a mature adult, while their disruption may generate variable degrees of whole body or regional growth failure.

The control of human growth is related to internal cues, such as genotype, external factors, such as nutrition and environment, and internal signaling systems, such as hormones and growth factors. Tissue growth may not end with the completion of whole body growth. Some cells, notably those in the liver and endocrine tissues, retain their ability to proliferate. Others, such as blood and epidermal cells, can be renewed but remain terminally differentiated. Yet others, such as those in the nervous system, have a limited capacity to regenerate.

Whole body growth is not a smooth symmetrical event: there is marked variation in organ and regional growth through childhood and adolescence (Fig. 5.1) [1]. Thus, the time at which an organ system is vulnerable to adverse events will vary.

Fig. 5.1. Growth in different parts of the body in boys [data derived from [1], head circumference (Tanner, 1978), and sitting height/subischial leg length (Tanner and Whitehouse, 1978) charts].

Stages of growth

The growth trajectory of each stage of growth [fetal, infant (I), childhood (C), and pubertal (P)] can be represented mathematically by the "ICP growth model" (Fig. 5.2) [2,3]. Growth during the first three years results from a combination of a rapidly decelerating infancy component and a slowly decelerating childhood component. The latter dominates the mid-childhood years but at adolescence is altered by the pubertal contribution, which is modeled on a sigmoid curve.

Throughout the growing years, it is useful to consider those attributes required for normal growth (Table 5.1). Chronic illness is a potent cause of growth failure, a result of the adverse effects of the disease on dividing cells and secondary effects on nutritional intake. It is for this reason that growth monitoring is such an important and essential component of any child health surveillance program and that auxological assessment should be undertaken whenever a child attends hospital. A secure and caring environment is a prerequisite for normal growth: emotional deprivation is a potent cause of dramatic growth failure. An adequate nutritional intake must be taken, although energy requirements for normal growth are modest.

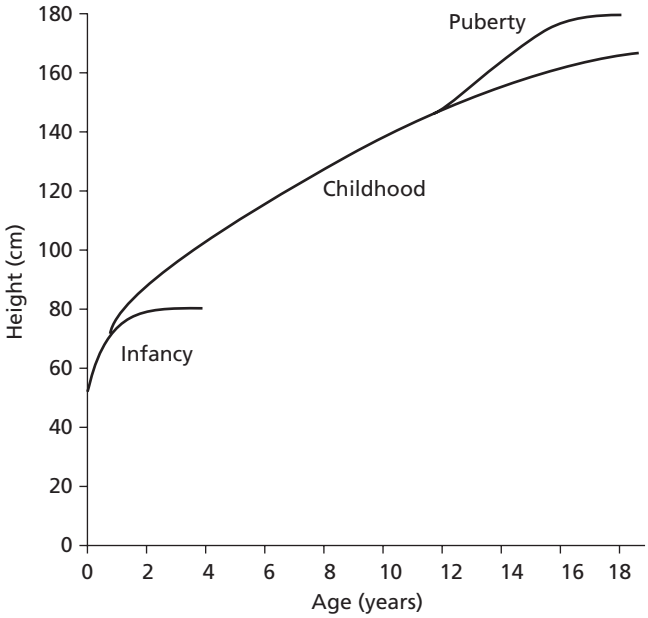

Fig. 5.2. The infancy–childhood–puberty (ICP) model of growth for boys. Data shown are the mean height values (cm) for age (from [2,3]).

Total daily energy expenditure (TEE) is based on four components: basal metabolic rate (accounting for 55–60% of TEE), thermic effect of food (accounting for 10%), energy expended in activity (highly variable but accounting for approximately 25%), and energy required for growth. The last of these, which varies with growth rate, makes the smallest contribution to TEE, so food/energy intake, which would normally match TEE, must be severely restricted to cause growth failure.

Finally, cells have to have the capacity to divide and respond to hormonal and growth factor influences. This is amply demonstrated in conditions where there is an abnormality in cell growth, such as a mutation in fibroblast growth factor-3 receptor resulting in achondroplasia, or in those infants with intrauterine growth retardation (IUGR) who fail to catch up postnatally (e.g. Russell–Silver syndrome).

Table 5.1. Requirements for normal human growth.

Absence of chronic disease
Emotional stability, secure family environment
Adequate nutrition
Normal hormone actions
Absence of defects impairing cellular/bone growth

The fetus

During the first trimester, the fetus establishes tissue patterns and organ systems. In the pre-embryonic period, from weeks 1 to 3, the ectoderm, mesoderm, and endoderm are formed within the embryonic disk. From weeks 4 to 8, there is rapid growth and differentiation to form all the major organ systems in the body. In the second trimester, the fetus undergoes major cellular hyperplasia and, in the third, organ systems mature in preparation for extrauterine life. Changes in crown–rump and crown–heel length and weight through gestation are illustrated in Figure 5.3 [4,5]. From weeks 4 to 12, crown–rump growth velocity is 33 cm/year, from 12 to 24 weeks 62 cm/year, and from 24 weeks to term 48 cm/year. Weight gain over the same intervals, extrapolated to kg/year, shows a different pattern with modest gains initially (0.1 kg/year from weeks 4 to 12) followed by 2.7 kg/year in weeks 12 to 24, escalating in the last 16 weeks to 8.7 kg/year. Growth velocity is therefore maximal in the second trimester, but maximal weight gain is achieved in the third trimester, although there is a declining weight velocity in the last weeks of pregnancy (Fig. 5.3).

The orchestration of this combination of rapid cell division and differentiation as well as morphogenesis is dependent in part on a class of developmental genes belonging to

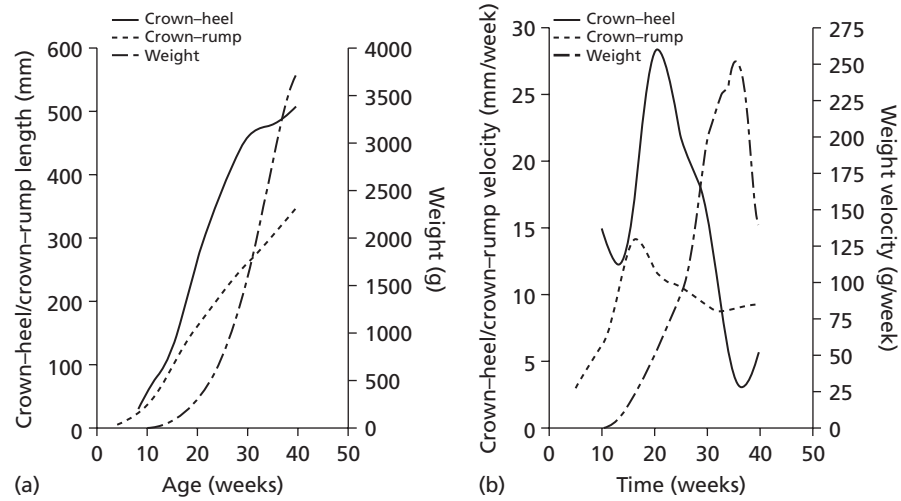

Fig. 5.3. Crown–heel length, crown–rump length, and weight (a) and velocity (b) of the fetus from 4 weeks' gestation (data taken from [4] and [5]).

Table 5.2. Examples of homeobox gene mutations causing human growth/developmental disorders [6–8].

PAX 6	Aniridia; Peter's anomaly (defect of anterior chamber of the eye); anophthalmia
MSX-1	Craniosynostosis
SHOX	Short stature in Turner syndrome; rare cause of idiopathic short stature; Leri–Weill dyschondrosteosis
HESX-1	Familial septo-optic dysplasia
PIT-1	Combined GH, TSH, and prolactin deficiencies
PROP-1	Congenital hypopituitarism

the homeobox family [6]. The homeobox (a 180-bp DNA sequence, encoding a 60-amino-acid homeodomain) was originally discovered in the genome of the fruit fly *Drosophila*, but is present in all multicellular organisms. Homeobox-containing genes encode for proteins that include a homeodomain and bind DNA, thereby controlling gene expression and hence cell differentiation and organ development. Class I *Hox* homeobox genes are involved in patterning embryonic structures such as the axial skeleton, the limbs, digestive and genital tracts, and in craniofacial and nervous system development. Abnormalities in human homeobox genes usually give rise to specific organ malformation, but there are also genes that have a wider impact on whole body growth (Table 5.2) [6–8].

Throughout gestation, fetal growth is constrained by maternal factors and placental function but is co-ordinated by growth factors [9]. These can act locally in a paracrine manner [e.g. insulin-like growth factor (IGF)-I and IGF-II, fibroblast growth factor, epidermal growth factor, transforming growth factors α and β] or as endocrine hormones (e.g. insulin). Nutrition from the mother plays a rate-limiting role. Placental transport of nutrients and metabolites can occur by simple diffusion, where transfer is limited by blood flow through the placenta and placental surface area (e.g. oxygen, carbon dioxide, urea), carrier-mediated facilitated diffusion down a concentration gradient not requiring energy (e.g. glucose and lactate), and active transport using carrier proteins and energy (e.g. amino acids). Factors that control nutrient transport are not fully characterized, but growth hormone and IGF-I acting on the maternal and/or fetal sides of the placenta have been shown to alter diffusion capacity and lactate uptake in sheep [10].

Understanding factors that can control fetal growth has assumed increasing importance since epidemiological studies have established links between intrauterine and early extrauterine growth retardation (I/EUGR) and the risk of developing a host of health problems in later life [11]. These include hypertension, cardio- and cerebrovascular disease, insulin resistance, and non-insulin-dependent diabetes mellitus. The link between early growth and later disease is postulated to occur through programming, where an insult (e.g. maternal undernutrition) at a critical period in fetal development leads to a permanent deleterious metabolic alteration that renders that individual prone to specific disease later in life. Hormones and growth factors are potential targets for programming. Evidence is accumulating that insulin and the growth hormone (GH)–IGF axis are modified in those born with IUGR. Exactly how these changes translate into disease is not yet defined.

The infant

During the first year, infants grow rapidly but at a sharply decelerating rate (Fig. 5.4) [1,12]. A similar pattern is observed for weight gain. It has been postulated that nutritional input is the principal regulator of growth over this period with minimal contribution from growth hormone. Data from humans and transgenic animal models suggest that the hormones and receptors within the GH–IGF axis also play their part in this early phase of growth. Nevertheless, it is during this period that alterations in dietary intake are likely to have the greatest impact on growth. Early onset of obesity in an otherwise normal infant is more likely to lead to tall stature than obesity developing later in childhood.

The correlation between length and weight at birth and mean parental size is poor (*r*-value for length is approximately 0.2), reflecting the dominant influence of the intrauterine environment over the genotype [4]. Once this effect is removed, a period of "catch-up" or "catch-down" growth commonly occurs during the first 2 years, while the infant establishes its own growth channel. Catch-up growth starts soon after birth and is completed over 6–18 months, while catch-down growth commences between 3 and 6 months and is completed by 9–20 months. By 2 years of age, this process has increased the correlation between length and one parent's height to an *r*-value of 0.5, and between length and mid-parental height to an *r*-value of 0.7–0.8. Likewise, the correlation between an individual's birth length and their adult height only has an *r*-value of 0.3, but the correlation between height and final height has increased to an *r*-value of 0.8 by 3 years of age.

Although growth charts give the impression that growth is linear, most studies of short-term growth (day to day, week to week) find it to be non-linear [13]. There is dispute over its exact form: one model proposes that all infant growth occurs in short intense bursts over 24 h, separated by long periods of growth *stasis* [14]. The question of how day-to-day activity within the GH–IGF axis might generate such an infant growth trajectory has not been addressed.

The child

By 4 years of age, average growth velocity has declined to 7 cm/year and remains relatively steady until adolescence, the prepubertal nadir in average velocity being 5 to 5.5 cm/year (Fig. 5.4). On an individual basis, there is a

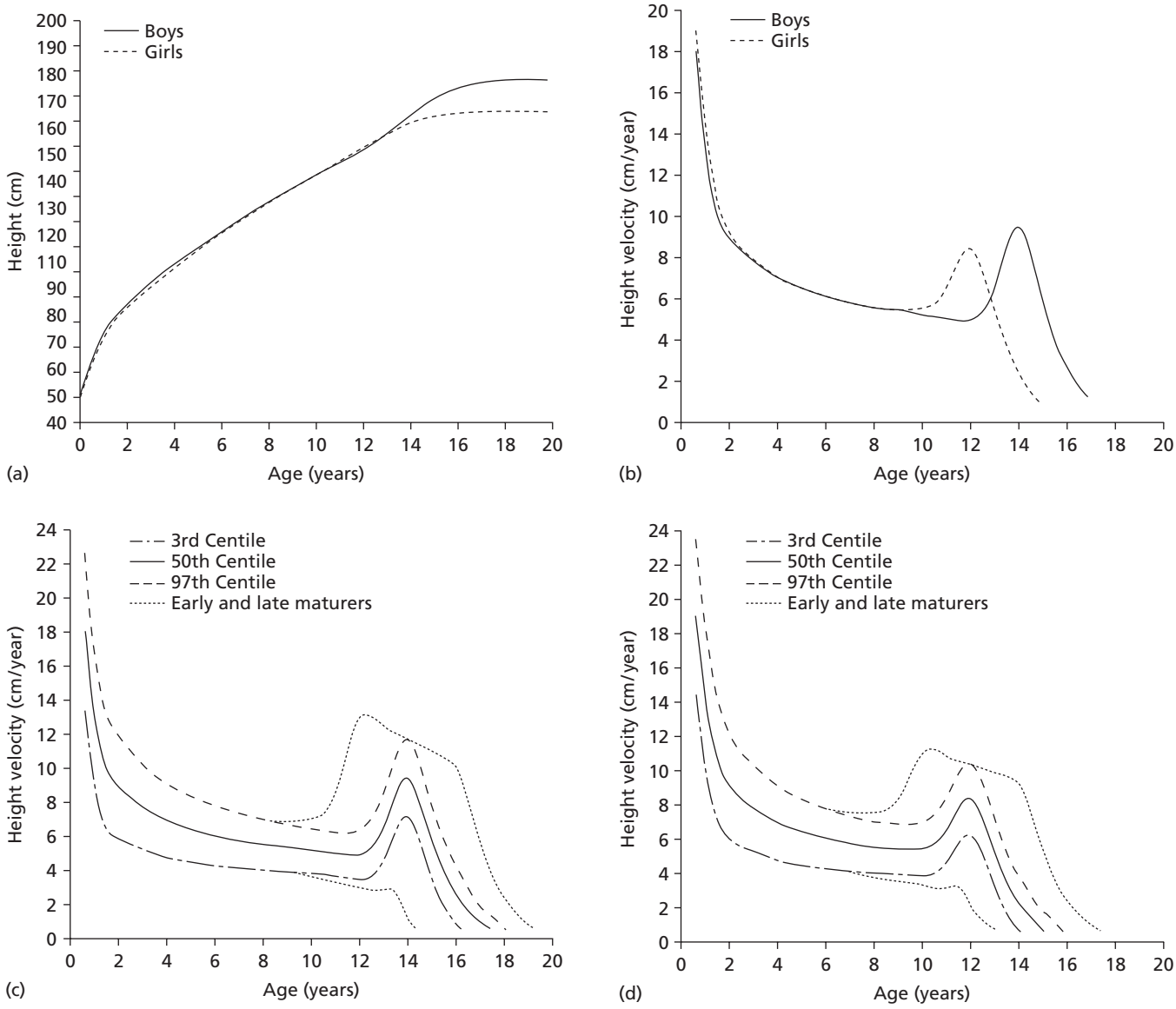

Fig. 5.4. Height (a) and mean height velocity (b) for boys and girls according to age (from [1] and [12]). Timing of the pubertal growth spurt for early and late maturers in boys (c) and girls (d) (from Tanner–Whitehouse growth velocity charts [18]).

well-recognized mid-childhood growth spurt. Additionally, if an individual is measured throughout childhood, oscillations in growth velocity of variable amplitude are observed with a periodicity of approximately 2 years (Fig. 5.5) [15]. Seasonal variation in growth rate with a velocity nadir in the winter months is well described [13]. Thus, the whole process requires variability controlled by inherent, presumably genetic, mechanisms as well as external influences.

During childhood, GH, in addition to thyroid hormone, is the major determinant of growth. It is therefore the time when dysfunction in the GH axis may be recognized. In the ICP model, the childhood component is first recognized at 6 months of age, but becomes predominant over the waning infancy component by 3 years of age. In the Swedish study, in which this model was devised, it was possible to detect an abrupt change in growth velocity in 76% of the subjects as the childhood component was initiated [3]. This was thought to represent the time at which GH became active, but there is abundant evidence that the GH axis is active in the fetus, and the increasing influence of GH in childhood growth is likely to be gradual rather than an "on–off" phenomenon.

There is relatively little difference in height between boys and girls (Figs 5.4 and 5.6) [16]. Body composition, as measured by dual-energy X-ray absorptiometry (DEXA) scanning, shows similar amounts of fat and fat-free mass in the two sexes [17]. Nevertheless, girls do have some subtle differences in maturation, demonstrating skeletal maturity that is 3–6 weeks ahead of that seen in boys at birth.

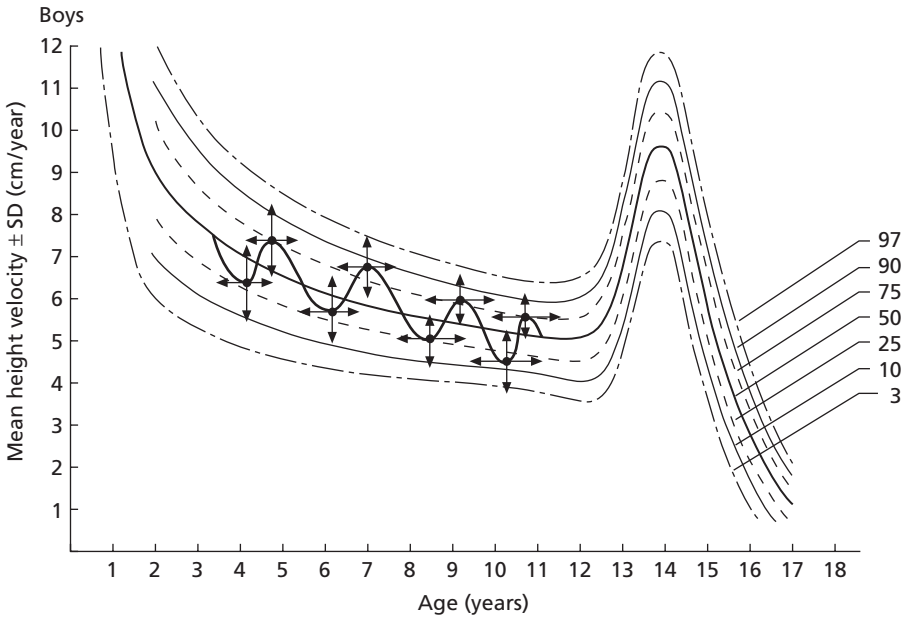

Fig. 5.5. Oscillations of growth velocity in the Edinburgh Growth Study (from [15]). The horizontal and vertical arrows represent the range of timing and amplitude, respectively, for each growth spurt.

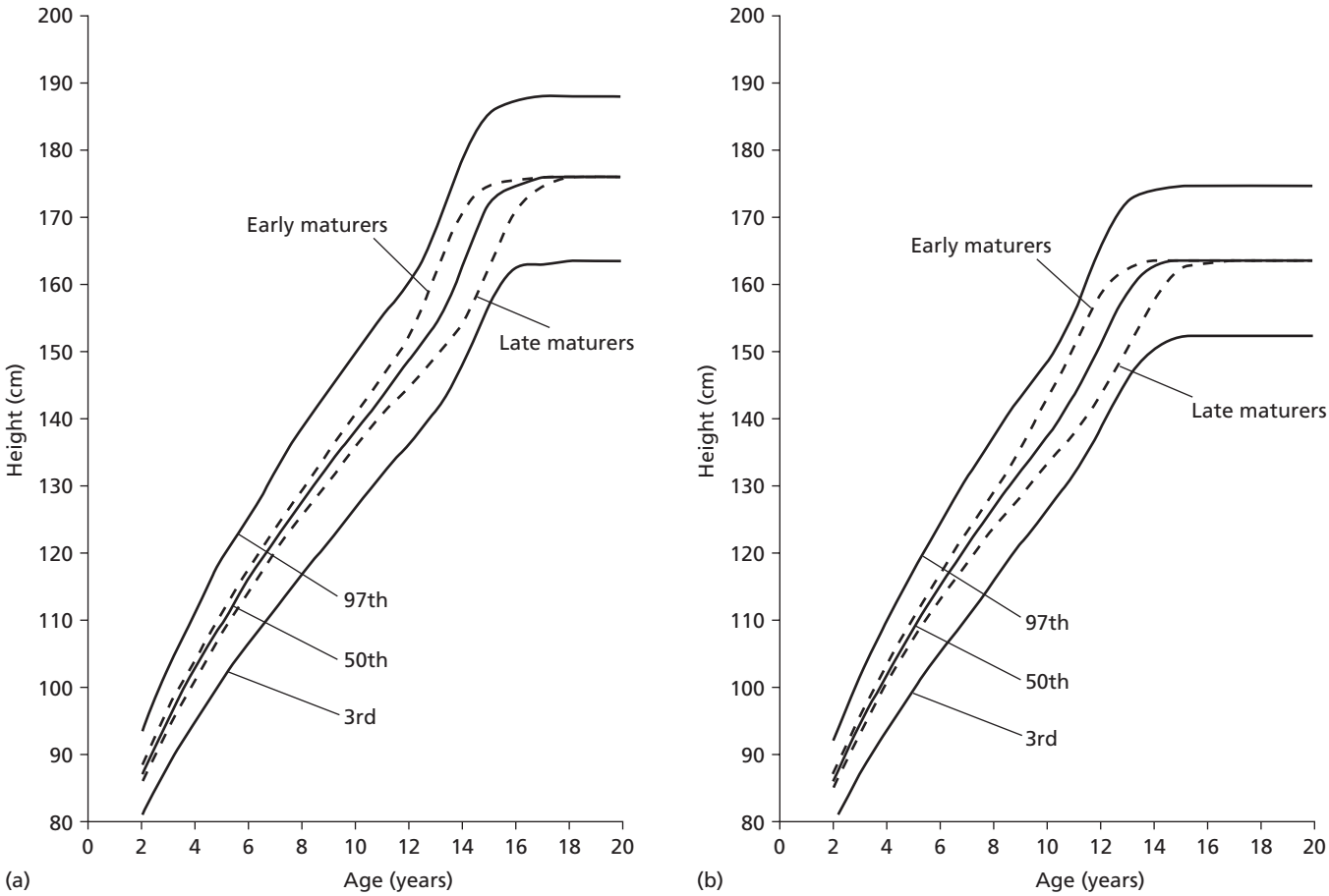

Fig. 5.6. Male (a) and female (b) growth charts with the 3rd, 50th, and 97th centiles for height indicated by solid lines. The dashed lines represent the 10th and 90th centiles for tempo (i.e. 10th, late maturers; 90th, early maturers) (from [16]).

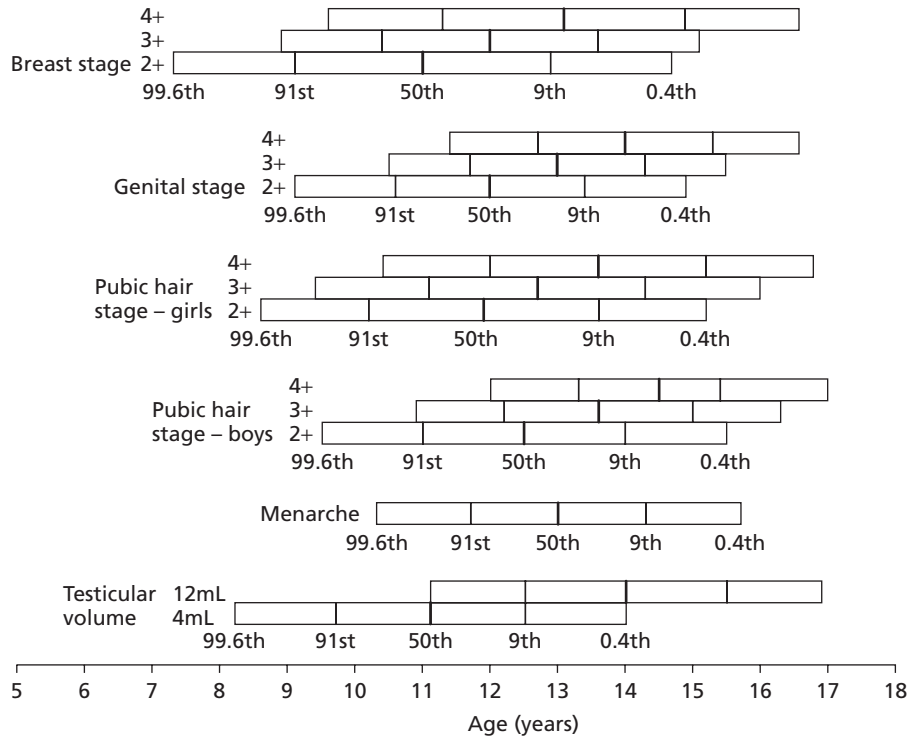

Fig. 5.7. Comparison of the timing of stages of puberty in boys and girls (data taken from Child Growth Foundation 1996 growth charts).

Puberty

In physical terms, puberty may be defined as the transition from the prepubertal state through the development of secondary sexual characteristics to the achievement of adult stature. There is marked sexual dimorphism in the timing of these events (Fig. 5.7) [18]. There is also wide variation in pubertal timing within each sex. The later onset of puberty in boys gives them two additional years of prepubertal growth compared with girls. The height gained in this time (8–10 cm), in addition to the greater amplitude of pubertal growth in boys (3–5 cm more than the female growth spurt), gives rise to the 12.5 cm difference in adult height between the sexes.

It is during this phase that the issue of growth tempo becomes obvious. Constitutional delay of growth and puberty is common and can be considered a variant of normal. The condition can be associated with chronic disease, for example atopy, but more often occurs in isolation. Pubertal development commences late and the growth spurt is blunted. Although constitutional delay in growth and puberty (CDGP) may actually present in the pubertal years, some children may have shown slow growth much earlier in childhood. The corollary is constitutional tall stature and early puberty, which is associated with more intense pubertal growth than normal. The net result is that both early and late maturers should achieve a comparable height (Fig. 5.6). The genes that contribute to this variation in physical development have not been characterized.

Marked changes in body composition occur during puberty. Using skinfold thickness to derive fat mass (FM), fat-free mass (FFM), and percentage body fat (%BF), boys gain approximately 30 kg in weight from 12 to 18 years, 82% being accounted for by FFM. Girls gain approximately 18.5 kg, 68% being FFM. There are relatively small changes in %BF, 1.7% in boys and 3% in girls [19]. If data on FM and FFM are derived from DEXA scanning, a similar change in FFM is reported over male puberty, but only 50% of weight gained was due to FFM in girls [17].

Clinical recognition of normal growth

The ability to quantitate growth over time against well-constructed population standards is the cornerstone of auxological assessment. The variations in normal growth mean that observation needs to be undertaken over a year or longer in order to define a problem. The tools required are simple. They include growth charts (for height, weight, sitting height and subischial leg length, body mass index, height velocity, and head circumference), standards for pubertal timing (usually included on growth charts), radiographs of non-dominant wrists for the assessment of bone age, and an orchidometer to measure of testicular volume.

Height and weight

Height or length measurements should be undertaken in a standard manner, ideally by trained personnel using

calibrated well-maintained equipment. Height should be plotted on a growth chart, on which biological parental heights have also been charted [1]. Ideally, these should be actual rather than reported heights. The point at which parental height is plotted is sex dependent. If the index case is male, father's height and (mother's height + 12.5 cm) will be plotted. The reverse holds for a girl. The mid-parental height is defined as the midpoint between these (corrected) heights, while the target range is 10 cm (for boys) or 8.5 cm (for girls) either side of this point. If it is considered that a major secular trend in height has occurred between the child's generation and that of the parents, then up to 3 cm can be added to the mid-parental height. If the child's height falls outwith the target range, a growth disorder may be present. There may of course still be an abnormality of growth when the height is within the target range, if the mid-parental height or one parent's height falls outside the lower centile. In these cases, an autosomal-dominant growth disorder may be present.

One further way to express height (and any other auxological data), which controls for age and sex, is to derive a standard deviation score (SDS). This requires knowledge of the mean and standard deviation of height at all ages in each sex, and is calculated as:

SDS = (observed height–mean height for age and sex) ÷ standard deviation for that age and sex.

95% of normal values would be expected to fall between ± 2 SDS.

Weight is considered an important parameter for the assessment of infant well-being, in the phase of life where nutrition has such an important impact on growth. In childhood, weight measurements (ideally taken on calibrated electronic scales) are usually taken coincidentally with height. They should be plotted on a chart, and their relation to height assessed. Generating a weight-for-height can do this. The age at which a child's height falls on the 50th centile is defined ("height age"). The mean weight for a child of this age is read off the chart and data expressed as (observed weight ÷ mean weight for the height age) × 100%, which gives the percentage of expected weight-for-height. A value > 120% would be considered obese, while a value < 85% would indicate poor nutritional status. Alternatively, and better, body mass index [defined as weight (kg) ÷ height (metres)^2] can be plotted on a centile chart [20].

Growth velocity

A single height measurement plotted in relation to parental heights gives useful information, but does not reflect the dynamic nature of growth. For this, serial height measurements should be taken. Height velocity (cm/year) is then calculated as [the amount grown (cm) ÷ the time interval between measurements (years)] [12]. In order to maintain a position close to a given height centile, velocity should not fall below the 25th or above the 75th centile in successive years. Even in experienced hands, the technical error of measurement of height is of the order of 0.15 cm. If velocity is based on measurements taken at short intervals, such as 3 months, measurement error will be magnified four times when extrapolating to an annual growth rate. If this is combined with the non-linearity of growth, an accurate perspective of growth is difficult to obtain. Even if growth is monitored over 12 months, the cycles of growth through the prepubertal years could lead to a false impression about growth performance, and measurement over longer periods may be required. The probability that growth velocity over successive years will be inadequate becomes increasingly small. The theoretical chance of annual growth velocities in a normal child being below the 25th centile over 2 years (assuming that the velocity in each year is independent) would be 0.25 × 0.25 = 6.25%, and below the 10th centile would be 1%.

The degree of physical development and the timing of the pubertal growth spurt complicate assessment of growth velocity around puberty. It is this variation that gives rise to the wide variation in peak height velocity on growth charts (Fig. 5.4).

Bone age

Bone maturation can be observed directly by visualization of epiphyseal growth plates on X-ray. In normal children, there is an orderly development of the epiphyseal centers in growing bones. It has therefore been possible to generate standards for bone maturation in each sex throughout childhood and adolescence. Bone age has evolved as a measure that quantifies physical maturation. The epiphyseal centers in the non-dominant hand and wrist X-ray can be compared with those found in age-matched representative X-rays from normal boys and girls, as shown in the atlas of Greulich and Pyle (G&P) [21]. It is likely that the maturity of the epiphyseal centers in any one child will vary and, hence, comparison with a number of standard X-rays may be required to define an average bone age. Alternatively, the maturity of each epiphyseal center can be scored individually by the Tanner–Whitehouse standards (TW-II) to generate an overall bone age. These techniques are in part observer dependent.

Diagnostic information is not obtained from bone age estimation, but marked bone age retardation (> 3 years) can be associated with hypothyroidism, growth hormone (GH) deficiency, and hypopituitarism. A diagnosis of constitutional delay in growth and puberty would be supported by bone age delay > 2 years in the absence of an endocrine deficit.

Accurate bone age assessment may be difficult or impossible in certain circumstances, such as some skeletal dysplasias or in children exposed to long-term immunosuppressive steroid treatment. The main use of bone age estimations is to

monitor growth potential over time, particularly if a treatment to modify growth or puberty or both is introduced. Bone age estimates can also be used as a means of predicting adult height. For instance, adult height can be predicted using the tables of Bayley and Pinneau [22] and a G&P bone age, or using an equation incorporating current age, height, and TW-II bone age, each multiplied by a constant defined by age and sex.

Puberty

There is marked variation in the timing of events in puberty (Fig. 5.7) [18]. It is therefore important to compare an individual's development with population standards in order to define normality. Once puberty is initiated, the sequence of development should be ordered and completed within the appropriate timeframe. Failure to progress as well as failure to enter puberty could indicate abnormality. The pubertal growth spurt starts early in puberty in girls but later in boys, but the magnitude of the spurt is inversely related to the age at peak height velocity in both sexes.

Secular trends

This phenomenon describes the changes in growth and development (either positive or negative) that occur in a population from one generation to the next. Such changes have mostly been positive for anthropometric measurements and have been linked to improvements in nutrition and economic conditions [23]. The secular trend can therefore be considered as a barometer of a nation's health. Considerable changes in height have been documented in many countries over the last 200 years. One dramatic example of this was the increase in height of Japanese boys between 1950 and 1960, which peaked at an increment of 8 cm in height per decade at age 14 years. More recently, there is evidence that the secular trend in adult height is diminishing, particularly for example in Norway and Sweden. Rates throughout the world vary from 0.3 cm to 3.0 cm per decade. As there is no apparent secular trend in birth size, increases in stature develop soon after birth. For Europe and the USA between 1880 and 1980, stature has altered by 1.5 cm/decade in children, 2.5 cm/decade in adolescence, and 1 cm/decade in adulthood.

A secular trend has also been noted in the tempo of growth, such that the initiation of the pubertal growth spurt, age at peak height velocity, and age at menarche are occurring earlier. As height has already increased in childhood, reducing the time available for growth does not impact on final height. Again, there is some evidence that these trends are slowing: the age at menarche in Norway and in parts of the USA has stabilized. There are data showing that age at menarche is increasing in some European populations.

These trends can be more prominent in certain sections of a population. The secular trend in growth and maturation tends to be more pronounced in those from low socioeconomic groups, those with poorly educated parents, and those from rural areas. The effect of this is to raise the lower height centiles and to reduce the variability in the timing of puberty. This can obviously have implications for the clinician whose task is to assess whether a child is showing normal development. It is important therefore for growth standards to be kept updated.

The endocrine control of growth

The principal hormones influencing growth are GH and the IGFs, but there are many other hormones that contribute, including thyroid hormones, adrenal androgens, sex steroids, glucocorticoids, vitamin D, leptin, and insulin. Often, this contribution is channeled through interaction with the GH–IGF axis.

Hypothalamic control of GH secretion

GH is secreted from the anterior pituitary in discrete pulses every 3–4 h, with very low concentrations of GH present between pulses (Fig. 5.8). This pattern is determined by the interaction between growth hormone-releasing hormone (GHRH), ghrelin and somatostatin (SS) [24]. The amplitude of the GH peak is determined by GHRH, which stimulates the pituitary somatotrophs to increase both the secretion of stored GH and GH gene transcription. Ghrelin, a 28-amino-acid octanoylated peptide, acts in synergy with GHRH to promote GH release. Ghrelin also has a potent orexigenic action, indicating a link between nutritional and growth control. SS tone determines the trough levels of GH by inhibiting GHRH release from the hypothalamus and GH secretion from the pituitary (Fig. 5.9). Withdrawal of SS is the most important factor in determining the time of a pulse as GH pulsatility is maintained under constant infusion of GHRH.

Pituitary control of GH secretion

The human GH gene forms part of a cluster of five similar genes found on the long arm of chromosome 17 [24]. Two genes for GH isohormones (GH-N and GH-V), two genes for placental lactogen, and a single gene encoding a placental lactogen-like protein are found within this gene cluster. The main form of GH in the circulation comes from the GH-N (or normal) gene expressed primarily in the pituitary. The full-length transcript from the GH-N gene encodes a 191-amino-acid, 22 kDa protein, which constitutes 80–90% of pituitary GH. Alternative splicing of the mRNA transcripts generates a 20 kDa species that lacks amino acids 32–46 and accounts for the remaining 10–20%. Deletion of the GH-N gene in humans leads to severe postnatal growth failure and, in these individuals, treatment with GH generates a short-

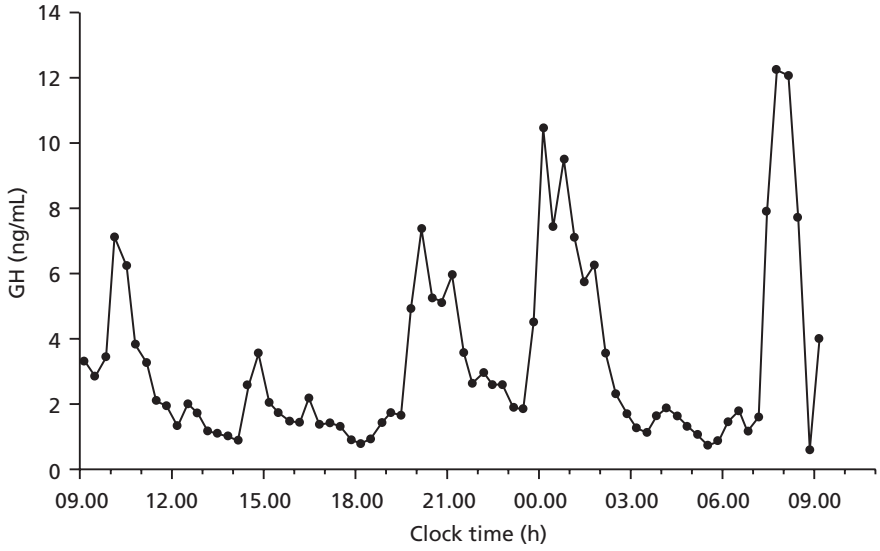

Fig. 5.8. Changes in the serum concentration of GH (ng/mL) over 24 h with samples measured at 20-min intervals.

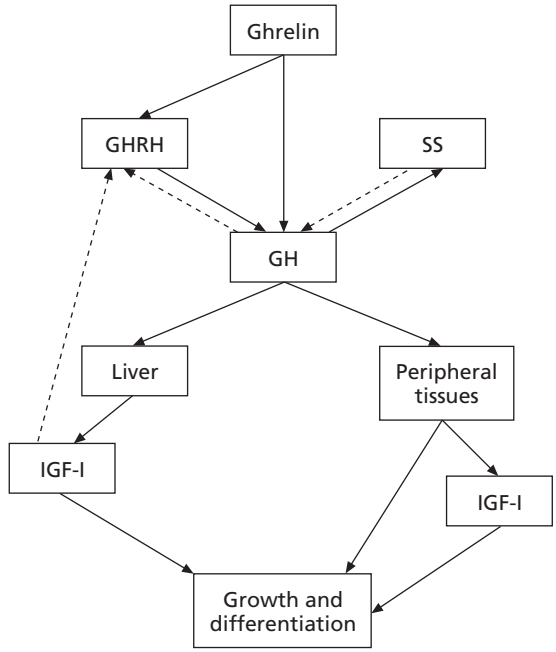

Fig. 5.9. Schematic representation of the GH–IGF axis. GH is released from the pituitary under the regulation of growth hormone-releasing hormone (GHRH) and somatostatin (SS) with additional control coming from the influence of ghrelin. GH then acts on either the liver or the peripheral tissues to stimulate the production of IGF-I, which along with GH itself mediates growth and differentiation.

lived growth response resulting from the development of anti-GH antibodies.

Within the promoter of the GH-N gene are binding sites for the homeobox transcription factor Pit-1, which determine pituitary-specific expression of GH. Pit-1 is also essential for normal pituitary development. It is one of a number of homeodomain gene products that are required for pituitary organogenesis [25].

Other factors influencing GH secretion

There are a considerable number of other factors that influence GH secretion acting directly on the pituitary by stimulation of GHRH, inhibition of SS, or a combination of both. These include neuropeptides (e.g. galanin, opioids), metabolites (e.g. glucose, free fatty acids), hormones (e.g. estrogen, testosterone), physical exercise, and sleep [24]. GH itself and its downstream effector, IGF-I, are both capable of regulating secretion via negative feedback mediated by somatostatin.

A negative relationship exists between body mass index, a marker of body fatness, and GH secretion in normal prepubertal children as well as in obese children. The fact that GH secretion increases after weight loss or fasting suggests that diminished GH is a result of obesity rather than a cause. The signal by which adiposity effects the reduction in GH secretion is not known. Hyperinsulinemia in obese subjects has also been proposed as an alternative mechanism for the reduction in GH secretion. In obese children, insulin and IGF-I concentrations are either normal or elevated despite the reduction in GH levels. Such subjects exhibit normal or increased growth. The hyperinsulinemia may stimulate increased IGF-I, which stimulates growth but also suppresses GH secretion by negative feedback.

GH in the circulation

Growth hormone can be detected in the fetal pituitary from around 8 weeks of gestation and in the serum from 10 weeks

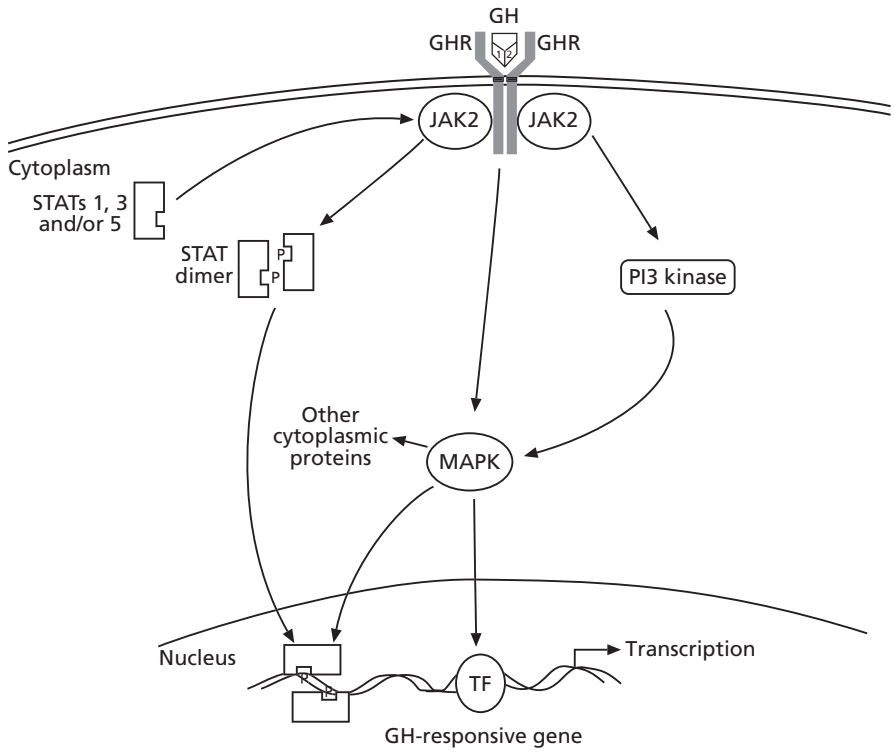

Fig. 5.10. Schematic representation of the action of GH at the cellular level. A single molecule of GH binds to two GH receptors (GHR) followed by the recruitment and activation of a receptor-associated tyrosine kinase (JAK2). The GH signal can be transmitted to the nucleus through the STAT pathway or via the MAPK pathway either directly or via PI3-K with the net result of activating transcription factors (TF), which act on GH-responsive genes.

before the maturation of the hypothalamic–pituitary axis is complete [26]. Serum levels of GH rise to high levels by 24 weeks, decline towards birth and fall further after the first 2 weeks of postnatal life. The high concentrations of GH throughout gestation are a reflection of the time taken for the neuroendocrine control of GH secretion to develop. In early gestation, GH release from the pituitary is uncontrolled. The decrease in GH after 24 weeks is then associated with the development of the inhibitory mechanisms governing GH release.

Few data exist on the longitudinal changes in GH secretion with age in prepubertal children, but cross-sectional data suggest that GH pulse amplitude increases with age [27]. This has been confirmed by studies using urinary GH measurement as a surrogate for pituitary GH, which show a gradual increase in GH through the prepubertal years [28]. The most profound changes in GH secretion occur through the pubertal years with a marked increase in the amplitude of GH pulses [27]. Androgens and estrogens both increase GH secretion during puberty, although the androgen effect may be mediated through the estrogen receptor by aromatization of testosterone to estrogen. Maximal levels of GH secretion coincide with the timing of peak height velocity in both sexes, and secretion declines thereafter into adulthood.

In prepubertal and pubertal children, episodic GH release generates large peaks of GH lasting 1–2 h separated by periods of low basal secretion (Fig. 5.8). The pattern of GH secretion is important in generating the diversity of actions mediated by GH. In rodents, the sexual dimorphism in GH secretion parallels differences in growth rates [29]. Male rats secrete GH in discrete pulses separated by low trough levels and grow at a faster rate than females who exhibit high basal GH levels and less pulsatility. In addition, pulsatile infusion of GH in GH-deficient rats stimulates growth while continuous infusion is associated with its metabolic actions.

This sexual dimorphism in GH secretion is also evident in humans [30]. Average daily GH output is greater in women compared with men, a difference that disappears when corrected for estrogen concentrations. There are also differences in the profile of 24-h GH secretion between the sexes: in men, there are small pulses in daylight hours with large nocturnal pulses, but there is less diurnal variation with more frequent pulses in women. There are associations between the peak and trough attributes of 24-h GH secretion and different endpoints of GH action: trough concentrations of GH are correlated with body composition and metabolic parameters, while peak concentrations correlate with IGF-I production [31].

Cellular actions of GH

Growth hormone exerts its effects on target tissues by binding to a specific GH receptor (GHR), which is expressed in a variety of tissues, particularly the liver [32]. Figure 5.10

shows a simplified scheme for the transduction of GH signals at the cellular level. A single molecule of GH binds sequentially to two GHRs, the process of dimerization being critical to the activation of intracellular signal transduction. GH contains two different regions, sites 1 and 2, which interact with the extracellular domains of the two GHRs. Binding of GH at site 1 to a single GHR is followed by recruitment of a second GHR, which interacts at site 2 to form the GHR–dimer complex. The GHR contains no intrinsic tyrosine kinase activity but, upon activation by GH, is phosphorylated by a receptor-associated tyrosine kinase, JAK2, a member of a family of tyrosine kinases that is involved in signal transduction by cytokine receptors. Activation of JAK2 by GH receptor dimerization results in JAK2 autophosphorylation, GHR phosphorylation, and the initiation of phosphorylation cascades. GH signal transduction can proceed through a number of pathways, of which the mitogen-activated protein kinase (MAPK), signal transducers and activators of transcription (STAT), and the phosphatidylinositol 3-OH kinase (PI3-K) pathways are the best characterized.

The net result of GH signal transduction is the activation of genes involved in mediating the effects of GH. An important target of GH is the IGF-I gene, the product of which mediates many, if not all, its growth-promoting actions *in vivo*. The STAT pathway in particular has been implicated in the generation of IGF-I in response to GH. A patient has been described with marked short stature, high circulating levels of GH, but very low levels of IGF-I and IGF binding protein (IGFBP)-3 (consistent with GH insensitivity), who has a homozygous mutation in STAT5b. This patient also has immune abnormalities, illustrating the importance of STAT5 to cytokine signaling in general.

Insulin-like growth factors and their binding proteins

IGF-I and IGF-II are single-chain polypeptide hormones with structural homology to proinsulin that are expressed in multiple organs and tissues under both endocrine and tissue-specific autocrine and/or paracrine regulation. Both are important in fetal growth and development, but only IGF-I appears to be critical for postnatal growth. This may be due to the fact that IGF-II does not appear to be regulated by GH, which is the primary determinant of circulating IGF-I concentrations in postnatal life. IGFs in the circulation bind to the IGFBPs, a family of distinct but structurally homologous proteins that share the ability to bind both IGFs with high affinity. The IGFs are present in the circulation at concentrations approximately 1000 times that of insulin, and one major role of the IGFBPs is to prevent the potential insulin-like activity of IGFs [33]. Nearly all IGF in the circulation will be associated with a binding protein. The IGFBPs extend the half-life of the IGFs within the vascular space creating a circulating reservoir of IGF activity and provide a transport mechanism regulating movement of IGFs across the capillary walls. They also play a role in controlling IGF distribution to specific cell types or receptor subtypes, thus modulating the paracrine effects of IGFs.

IGFBP-3 is the major IGFBP in the circulation accounting for most of the IGF-I binding capacity and, like IGF-I, is strongly GH dependent. The main site of IGFBP-3 production is in the liver under GH control, although it is also expressed in most peripheral tissues. When bound to IGF-I, IGFBP-3 associates with another GH-dependent protein, the acid-labile subunit (ALS), to form a ternary complex of 150 kDa molecular weight. In general, the molar concentration of IGFBP-3 in the circulation is equal to the sum of both IGF-I and IGF-II, reflecting the role of IGFBP-3 as the major carrier of IGF. ALS is present in a molar excess, indicating that virtually all IGFBP-3 and IGF exist in the form of the ternary complex.

Two distinct receptors for the IGFs have been identified, the type I or IGF-I receptor, which is homologous to the insulin receptor, and the type II or IGF-II receptor, also known as the mannose-6-phosphate receptor [33]. The mitogenic actions of IGF-I and IGF-II are mediated almost entirely through the IGF-I receptor using a signal transduction cascade similar to that used by insulin. In comparison with IGF-I, little is known about the interactions and consequences of IGF-II binding to the mannose-6-phosphate receptor. IGF-II does not appear to mediate any of its biological effects through this receptor, acting instead through the IGF-I receptor.

Physiological actions of GH and IGF-I on bone growth

The major role of GH during growth and development is to promote longitudinal bone growth. Two hypotheses have been generated to explain the mode of action of GH in generating a growth response. The somatomedin hypothesis proposes that GH mediates its effects on its target tissues via stimulation of hepatic IGF-I production, which in turn acts as a classical endocrine hormone (Fig. 5.11a). The alternative hypothesis, the dual effector theory, is based on the premise that growth is a result of the differentiation of precursor cells, followed by clonal expansion. According to this hypothesis, GH directly promotes the differentiation of cells and the development of IGF-I responsiveness. Clonal expansion of these differentiated cells is then mediated by local production of IGF-I in response to GH (Fig. 5.11b) [34]. A mouse model has been developed in which hepatic IGF-I expression alone has been "knocked out." This mouse exhibits normal pre- and postnatal growth, indicating that, in the absence of endocrine IGF-I, autocrine and/or paracrine production of IGF-I is sufficient to sustain normal growth.

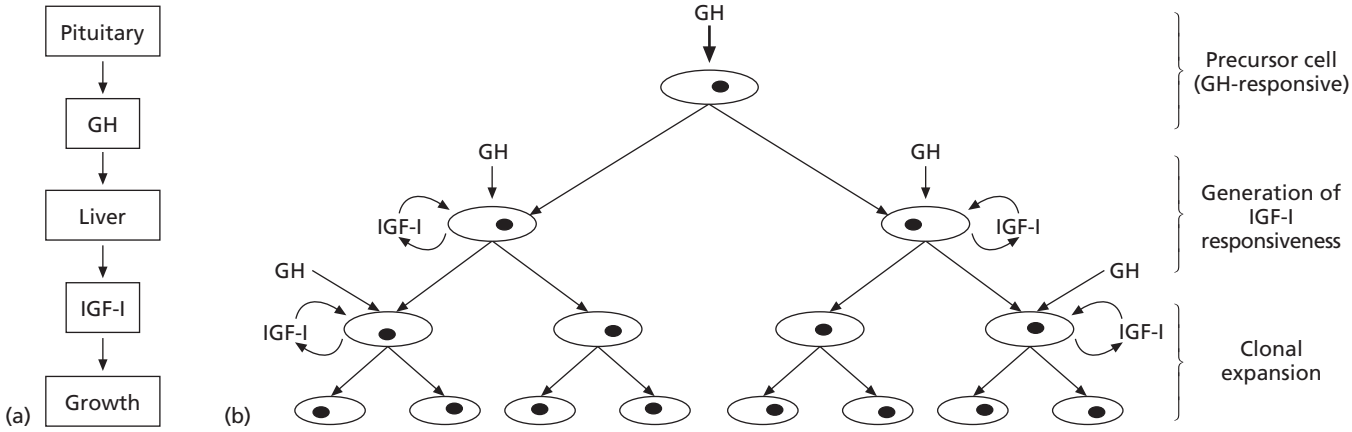

Fig. 5.11. The somatomedin hypothesis (a) and dual effector theory (b) of GH action.

Integration of endocrine signals into whole body growth

Human growth occurs over 16 years in girls and 18 years in boys and is a non-linear process with week-to-week, seasonal and year-to-year variation. Most studies of GH–IGF hormone output have been undertaken in subjects who have a growth disorder on one day of that child's growing life and then related to a short period of their current growth. In these circumstances, relationships between growth rate, stature, and hormone output have been found. It has been less easy to relate growth rate and GH output in normal children. In studies that have collected longitudinal measurements of the GH–IGF axis, it has become clear that there is considerable variation in hormone levels from day to day, week to week, and month to month: coefficients of variation for serum IGF-I are 40%, for urinary GH 55%, and for urinary IGF-I 37%. In normal children, significant rhythms in urinary GH excretion used as a surrogate of integrated serum GH concentrations have been identified over these intervals [35]. These data suggest that, just as the pulsatile pattern of serum GH within 24 h impacts on the actions of GH, so the pattern in which GH is released over time is likely to be an important factor in generating normal growth.

Disorders of growth

Introduction

Growth is a sensitive indicator of a child's health, nutritional state, and genetic background. Disturbances are a frequent cause of referral to an endocrine clinic. Accurate anthropometry is critical for clinical evaluation. In the initial assessment, it is essential to establish the parental pattern of growth and puberty so that the child's growth can be compared with the parents'. Estimation of skeletal maturity from a wrist radiograph allows prediction of the child's growth potential and likely final height. Serial measurements at a minimum of 6-monthly intervals reveal the child's pattern of growth and allow growth velocity to be calculated. Changes in growth rate serve as pointers to growth disorders and health problems.

Short stature and growth failure

Definition

Short stature is relative and needs to be defined according to the growth performance of a given population and reference charts relevant to it. Arbitrary cutoffs, for example below the second (approximately –2.05 SDS) or third centile (–1.88 SDS), are useful when a single measurement is available, but do not take into consideration the genetic background of the child or the growth rate. In addition, children of tall parents may be short but not necessarily have height below the defined cutoff. Short stature may be normal for some children but a feature of disease in others. The probability of organic disease is greater the further below the second or third centile that a child's height is.

Growth failure implies a poor height velocity for age and stage of puberty, irrespective of whether a child is "short," "normal," or "tall" from a height measurement and parents' heights, and is a sensitive indicator of pathology (Fig. 5.12). After a transient period of growth inhibition, the phenomenon of accelerated linear growth (owing to supranormal height velocity), which allows a child to return to his/her original preretardation growth curve, is called catch-up growth [36].

Evaluation is recommended for a child with

- height below the 0.4th centile,
- a significant discrepancy between their centile and mid-parental target centile,

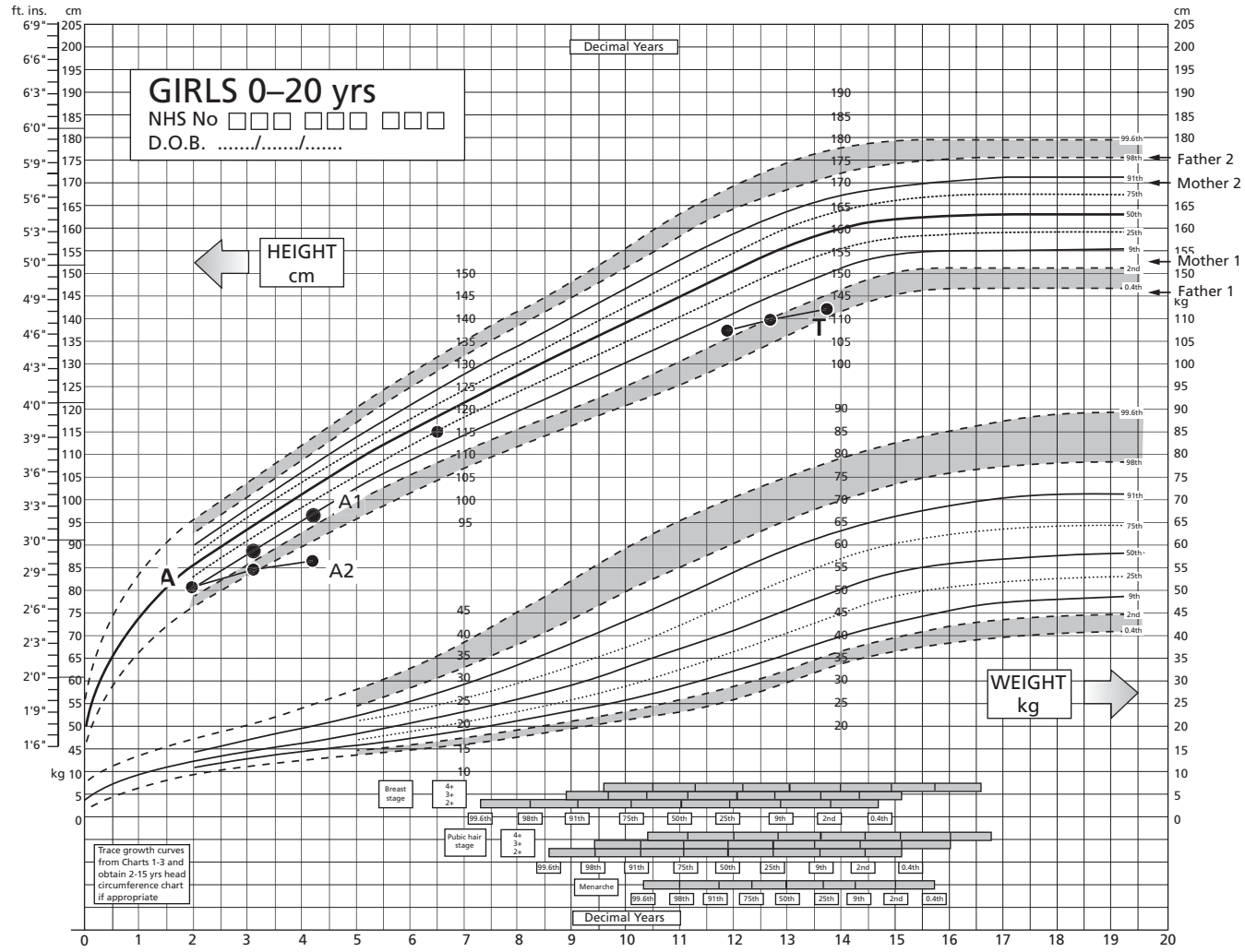

Fig. 5.12. Growth chart illustrating the importance of taking into consideration heights of parents' and stage of puberty when interpreting growth data and the value of serial over single height measurements. The height of child A is appropriate for Mother 1/Father 1 but not for Mother 2/Father 2. Subsequent measurements reveal whether child A has normal (A1) or abnormal (A2) growth. Serial measurements of teenager T do not necessarily imply growth failure if she has delayed puberty.

- height curve crossing two centile lines, even if the measurements are not below the 0.4th centile or
- in whom there are parental or professional concerns.

Children with height between the 0.4th and second centiles or height curve crossing one centile line should have their height velocity monitored carefully.

Epidemiology

The major causes of short stature in children are shown in Table 5.3. A prospective prevalence study of 114 881 school-aged children from randomly selected schools in Utah examined the frequencies of growth abnormalities outside growth clinics [37]. Initially, from single height measurements, 1.2% of the children were found have height < –2 SDS. These "short" children were remeasured in the second year, and less than 50% had height < third centile and growth rate < 5 cm/year. Non-endocrine causes accounted for the growth failure in the majority (familial short stature in 37%, constitutional delay in growth in 27%, familial short stature/constitutional delay in growth in 17%, systemic disease in 9%). Only 5% of the children had an endocrine problem (GH deficiency in 3%, hypothyroidism in < 1%, and Turner syndrome in 3% of the girls) [37].

Psychosocial impact

Short stature does not pose significant psychosocial problems

Table 5.3. The major causes of short stature.

No disproportion between trunk and limbs: Looks normal			Dysmorphic features and/or disproportionate short stature: Looks abnormal	
Normal height velocity	**Low height velocity**		**Dysmorphic features, recognizable syndrome**	**Disproportion between limbs and trunk**
	Underweight	**Weight appropriate for height or relatively overweight**		
Appropriate for parents: familial short stature Short for parents: constitutional delay	Psychosocial: child abuse/neglect anorexia nervosa Systemic disease: Respiratory, e.g. cystic fibrosis, asthma Cardiovascular, e.g. congenital heart disease Gastrointestinal, e.g. celiac disease, Crohn's disease Nutritional, e.g. rickets, protein calorie malnutrition Renal, e.g. chronic renal failure, renal tubular acidosis Infections, e.g. HIV, tuberculosis Musculoskeletal, e.g. juvenile arthritis Neurological, e.g. tumor	Endocrine disorder: hypothyroidism growth hormone deficiency hypopituitarism pseudohypoparathyroidism Cushing syndrome	Low birthweight: Russell–Silver syndrome Three M slender-boned dwarfism Seckel syndrome Chromosome abnormality: Turner syndrome Prader–Willi syndrome Autosomal dominant: Noonan syndrome Autosomal recessive: Bloom syndrome Fanconi pancytopenia Mulibrey Nanism X-linked dominant: Aarskog syndrome Rare syndromes: Floating Harbor Kabuki makeup	Short limbs: achondroplasia hypochondroplasia dyschondrosteosis metaphyseal chondrodysplasia multiple epiphyseal dysplasia Short limbs and trunk: metatropic dysplasia Short trunk: mucopolysaccharidosis spondyloepiphyseal dysplasia

for the majority of children or adults. However, and especially for those presenting to a specialist, it can have a negative influence on the affected child and parents [38]. Some short children and adolescents feel insecure, have low self-esteem, are introverted, withdrawn, depressed, socially isolated, anxious, aggressive, and immature. They may be bullied and have difficulty adjusting at school. These problems need to be recognized so that the child and family can receive appropriate psychological support.

Clinical assessment

The aims of assessment of a short child are to determine whether the child is otherwise normal or has pathology and to determine the psychosocial impact of appearance on the child. Few children with short stature have an endocrine disorder. Careful clinical assessment (Table 5.4), a plot of serial height measurements, and determination of height velocity are needed to separate those who are small but growing normally from those who have an abnormal pattern of growth. A radiograph of the non-dominant hand and wrist allows determination of the bone age and the growth potential. The mid-parental height can be calculated to estimate height expectation. Stature that is out of keeping with the family background is likely to be significant (e.g. a child whose height is on the second centile but whose mid-parental height falls on the 75th centile). Physical examination may reveal abnormal body proportions, dysmorphic features, and the underlying problem. The clinical impression usually dictates the investigation pathway and, while pathology must not be missed, overly zealous evaluation should be avoided.

Endocrine causes of short stature

Growth hormone deficiency

GH deficiency may be congenital (e.g. associated with septo-optic dysplasia) or acquired (e.g. secondary to cranial irradiation or, rarely, a tumor in the area of the hypothalamus or pituitary) (Table 5.5).

Table 5.4. Important aspects of the history and examination in a child with short stature.

History

Reason for referral

- History of growth problem
  - Time of first concerns about stature, change in stature over time
  - Birth size in relation to gestation: weight, length, head circumference
  - Pubertal development: age of onset and progression of secondary sexual characteristics
- Pregnancy and perinatal events
  - Clues to growth retardation *in utero* from infections, drugs, smoking, alcohol
  - Gestation, vertex or breech presentation, mode of delivery, condition at birth
  - Postnatal problems such as hypoglycemia (congenital hypopituitarism), jaundice (congenital hypothyroidism or hypopituitarism), floppiness and feeding difficulty (Prader–Willi syndrome), puffy hands and feet (Turner syndrome)
- Medical history
  - Problems associated with specific syndromes such as Turner syndrome
  - Symptoms of an endocrine disorder such as hypothyroidism
  - Symptoms of tumor around the pituitary gland
  - Systemic illness
  - Treatments that can impair growth, e.g. corticosteroids, radiotherapy, methylphenidate
  - Developmental problems in specific areas such as speech, hearing, learning, vision
- Psychosocial history to determine the impact of short stature on the child
  - Self-image and parents' perceptions
  - Teasing/bullying at school
  - School adjustment
  - Personality, emotional, and behavioral problems
- Family history
  - Heights of parents and siblings
  - Age of onset of puberty in parents
  - Consanguinity, affected family member, known inherited conditions

Examination

Measurements: weight, standing height, sitting height, head circumference

Height in relation to previous heights (height velocity), parents' heights, stage of puberty, weight

Genitalia and pubertal development

Body composition: subcutaneous fat and muscle bulk

Unusual or dysmorphic features in face, eyes, nose, ears, mouth, hairline, neck, upper limbs, hands, palms, fingers, nails, feet, or skin

Signs of specific syndromes such as Turner or Noonan syndrome

Signs of specific endocrine disorders such as hypothyroidism, growth hormone deficiency, or corticosteroid excess

Signs of a congenital (e.g. septo-optic dysplasia) or acquired (e.g. craniopharyngioma) lesion affecting the hypothalamus, pituitary (and growth hormone secretion), and the optic chiasm: visual fields, fundi, pupils, squint, nystagmus, acuity

Signs of chronic systemic disease

Table 5.5. Causes of growth hormone deficiency (GHD).

GHD can be on a hypothalamic or a pituitary basis

I GHD resulting from congenital malformations of the hypothalamus/pituitary
- A Septo-optic dysplasia and optic nerve hypoplasia
- B Other midline abnormalities, e.g. holoprosencephaly

II GHD resulting from irradiation of the hypothalamus/pituitary

III Trauma to the hypothalamus/pituitary

IV Mutations or gene deletions of transcription factors necessary to pituitary development:
- A HESX1
- B PROP1
- C POUF1 (Pit1)

V Mutations within the GH gene

VI Mutation of the GHRH receptor

VII GHD of undefined etiology (idiopathic) (including those with abnormal pituitary morphology on MRI – pituitary hypoplasia, interrupted or hypoplastic stalk, ectopically placed posterior pituitary lobe)

VIII Bioinactive GH

A congenital deficiency may arise through deletion of the GH gene, in which case no GH molecule can be produced, or by mutations within the gene, often at exon–intron splice sites, that result in an abnormal dysfunctional GH molecule. The difference between complete absence of the GH gene and relative deficiency is important, as children with the former have no immunological tolerance to the GH molecule and thus develop antibodies when exposed to therapy.

Isolated GH deficiency presents in early childhood without a family history. It is usually associated with pituitary hypoplasia secondary to a deficiency in the secretion of GH-releasing hormone from the hypothalamus. Thin-slice magnetic resonance imaging (MRI) through the hypothalamic region may also reveal an absent or attenuated pituitary stalk and/or ectopically positioned pituitary bright spot. These features can indicate that isolated GH deficiency may evolve with the development of other anterior pituitary hormone deficits. MRI scans can, however, be normal. The etiology of this form of GH deficiency is unclear. There may be a history of birth trauma or prematurity, and abnormalities in pituitary development genes may contribute. GH deficiency may be associated with midline congenital abnormalities such as optic nerve hypoplasia/septo-optic dysplasia. GH deficiency may be isolated or associated with hypopituitarism.

If GH deficiency is an isolated pituitary hormone problem, birthweight is characteristically normal, and the early postnatal course is uneventful. A diminished growth velocity becomes obvious towards the end of the first year as the hormone-dependent childhood phase of growth fails to take over from the nutrition-dependent infant phase. However, despite the congenital nature of this disorder, GH deficiency may not present until later childhood. The lack of sufficient GH causes a characteristic appearance with truncal obesity, immature cherubic facies, and central crowding of the facial features from maxillary hypoplasia. Head circumference is normal for age. Boys may have small genitalia, but a very small penis is suggestive of gonadotropin deficiency. Body proportions are normal before puberty, but patients tend to have poorly developed musculature. Puberty, if it occurs spontaneously, is usually slightly, but not significantly, delayed. Problems in the newborn period with hypoglycemia or conjugated hyperbilirubinemia [secondary to adrenocorticotrophic hormone (ACTH) deficiency] are likely when there are multiple pituitary hormone deficiencies.

Investigations to assess growth failure should comprise testing the whole hypothalamic–pituitary axis and should be undertaken in a center properly staffed and equipped for the safe investigation of children with endocrine problems. Methods of testing the hypothalamic–pituitary GH axis include provocation with a variety of stimuli (insulin hypoglycemia, arginine, glucagon, L-dopa, clonidine, pyridostigmine). Peak GH responses are more reproducible with agents that stimulate GHRH secretion and control endogenous somatostatin tone (e.g. arginine, pyridostigmine). The results need to be interpreted in the context of the circumstances, particularly in patients with delayed puberty or obesity. In those with delayed puberty, GH response can be amplified by the administration of 100 mg of testosterone given intramuscularly 3 days before the test (to a boy) or of 20 Bg of ethinylestradiol for 3 days (to a girl).

Many assays for GH, using both polyclonal and monoclonal antibodies, are available. The reported values for GH vary widely between assays done on identical samples. Each endocrine unit should therefore be acquainted with the performance of its GH assay. GH-dependent peptide IGF-I should also be measured because a level below an age- and sex-matched normal range, in association with a low peak GH level during a provocation test, would confirm a diagnosis of GH deficiency.

Because of the recognized fallibility of GH tests (suggesting GH deficiency when it is not present), the diagnosis of moderate/partial GH deficiency is difficult. This should be based on a multifaceted process that takes into consideration clinical and auxological assessment, combined with biochemical tests of the GH–IGF axis and MRI evaluation of the hypothalamic–pituitary axis [39]. In addition, it is crucial for those with a diagnosis of GH deficiency through childhood and adolescence to have pituitary function, in particular GH reserve, retested at the completion of growth to determine whether they should continue GH replacement throughout life. Some patients who have isolated GH deficiency in childhood are able to generate normal levels of GH when retested in adolescence and do not seem to require GH replacement in adulthood.

GH deficiency is treated with daily injections of recombinant GH (dose 25–50 μg/kg/day or 0.7–1.4 $\mu g/m^2$/day). The response to treatment is predictable from the pretreatment growth rate and the dose of GH administered.

Growth hormone-insensitive states

The phenotype of GH insensitivity is very similar to GH deficiency, but the biochemical hallmark is raised basal and stimulated GH levels coupled with low IGF-I and IGF-binding protein-3 (IGFBP-3) levels. GH insensitivity may be congenital or acquired. The latter occurs with fasting, poor nutrition, and catabolic states. Congenital GH insensitivity (Laron syndrome) is very rare. In the majority, it is a recessive condition caused by mutations of the GH receptor gene, most commonly in the extracellular domain of the receptor. The mutations usually result in a truncated nonsense protein, and therefore circulating GH-binding protein (GHBP), produced normally by cleavage of the extracellular domain from the cell surface, is absent. However, GH insensitivity with a normal or raised GHBP has been described, caused by mutations within the transmembrane or intracellular domains of the GH receptor. The latter can be inherited as a dominant trait, thus demonstrating, in a way similar to autosomal-dominant

GH deficiency, that genetic short stature may have an endocrine origin.

Hypothyroidism

Congenital hypothyroidism is invariably detected on a screening program in the first few weeks of life. However, some inborn errors, such as abnormalities in the Pendrin gene, may present later. Outside infancy, hypothyroidism is usually acquired and caused by Hashimoto thyroiditis or iodine deficiency. Short stature, growth failure, and poor school performance are characteristic. Bone age tends to be very retarded, and children maintain infantile proportions because of poor linear bone growth.

Pseudohypoparathyroidism (Albright's hereditary osteodystrophy)

Pseudohypoparathyroidism is inherited as an autosomal-dominant trait and characterized by peripheral resistance to the actions of parathyroid hormone (PTH) and often TSH. The typical phenotypic features include short stocky build, advanced bone age, round face, short thick neck, obesity, short metacarpals (especially the fourth and fifth), short distal phalanx of the thumb, and decreased intelligence in 50–70% of cases. Calcification in the subcutaneous tissues, kidneys, and brain is common. The diagnosis may be confirmed by low or normal levels of serum calcium, hyperphosphatemia, and raised PTH level. Following PTH infusion, urinary cyclic adenosine monophosphate (cAMP) fails to rise in type I. In type Ia, cAMP response occurs, but there is no phosphate diuresis. Type I cases are subdivided into those with (type Ia) and without (type Ib) a defective G-protein. The condition is treated by administering high doses of vitamin D.

Cushing syndrome

Cushing syndrome due to excess endogenous cortisol secretion (e.g. an adrenal adenoma in early childhood and an ACTH-secreting pituitary adenoma in later childhood) is unusual in childhood. It is most frequently associated with corticosteroid treatment for atopy, inflammatory disorders, or immune suppression. The growth suppression is less with alternate-day oral regimens compared with daily treatment. Short stature results from decreased linear growth (corticosteroid-suppressed GH secretion and direct inhibitory effects of corticosteroids on the growth plate). However, skeletal maturation is generally retarded, and the long-term effects of treatment are largely on the timing of onset of puberty rather than actual final height.

Idiopathic or "normal variant" short stature

Idiopathic or "normal variant" short stature (NVSS) refers to children with familial/genetic short stature (where mid-parental height falls below the population 10th centile) or constitutional delay in growth and puberty (CDGP; a disorder of the tempo of growth), who are otherwise healthy. These are diagnoses of exclusion. Subtle abnormalities in GH secretion and/or action have been suggested in children with idiopathic short stature because they have lower mean secretion of GH and lower mean concentrations of circulating IGF-I compared with control subjects [40]. Defects in the *SHOX* (short stature homeobox-containing) gene have been described in some individuals with idiopathic short stature, as well as Lange mesomelic dwarfism and Leri–Weill dyschondrosteosis [41,42]. GH has been used to treat children with normal variant short stature to final height [40]. The mean gain in height (the difference between predicted and achieved adult height among treated children compared with those not treated) was 9.2 cm for boys and 5.7 cm for girls.

Familial short stature

A dominant mechanism is proposed to be responsible for familial short stature, and autosomal-dominant disorders such as some skeletal dysplasias need to be excluded. Characteristically, the child is not inappropriately short compared with parents, has a normal growth rate, bone age is not delayed, and projected adult height falls within the parental target.

Constitutional delay in growth and puberty

Constitutional delay in growth and puberty is one of the most common causes of short stature, delayed puberty, or both. Boys are more likely to present than girls because of greater concerns about short stature and immature physique. The short stature is temporary and associated with delayed skeletal (bone age delayed > 2 years with height appropriate for bone age) and pubertal development. A normal growth spurt in puberty can be predicted, and eventual height will fall within the target predicted from parents' heights. The history often reveals delayed puberty in one or both parents. Unidentified genetic factors and nutrition may play a role. The children tend to be lean or have weight appropriate for their height. The most important aspects of management are explanation and reassurance that the delay in growth and puberty is simply part of the normal variation in maturation. For those with major psychosocial problems, treatment with low doses of sex steroids for a brief period of 3–6 months can be offered to activate the pubertal timing mechanism without accelerating bone age. The treatment does not enhance adult height but is safe, in that it does not compromise final height.

Small for gestational age

While intrauterine growth retardation (IUGR) implies impaired fetal growth and can be considered a prenatal diagnosis, small for gestational age (SGA) describes birth length and/or weight in relation to gestational age and is, therefore, a post-partum diagnosis. IUGR does not always result in infants being SGA, and SGA is not necessarily a consequence

of IUGR [43]. SGA is most widely defined as birth length and/or weight below –2 SDS (or below the third centile) for the reference population mean for gestational age. Low birth length is a stronger predictor of subsequent short stature than weight. In one cohort, the majority of otherwise healthy full-term SGA infants (birth length < –2 SDS) showed catch-up growth within the first year, and approximately 8% remained short as adults [44,45]. Those who failed to catch-up by 2 years were at high risk of remaining short in later life.

While multiple factors must influence growth in this heterogeneous group of short children born SGA, a relative resistance to GH and IGF-I is also likely to contribute. Thus, high doses of GH (dose 35–67 Bg/kg/day) may help some children to normalize height during the growing phase, maintain normal growth, and attain adult height within the normal range and within parental target [46]. However, the long-term safety of high doses of GH during childhood and adolescence on glucose metabolism and the consequences of relatively high IGF-I levels during this period remain uncertain.

Systemic causes of short stature

A systemic disease should be suspected in short thin children. History and examination generally provide useful clues to the diagnosis. Exceptionally, children who grow slowly for no obvious reason may have an occult condition (e.g. celiac disease, inflammatory bowel disease, renal tubular acidosis). Skeletal maturation is often delayed, and there is potential for catch-up growth if the underlying condition can be treated successfully. It is likely that the major factors that impair growth in children with systemic disease, such as chronic inflammation, undernutrition, and corticosteroid treatment, do so by attenuating the functional integrity of the GH–IGF-I axis through complex interactions. Evidence from an animal model and from children with juvenile chronic arthritis suggests that an interleukin-6-mediated decrease in IGF-I may be an important mechanism by which growth is impaired in chronic inflammatory conditions, independent of nutrition [47].

Psychosocial and emotional deprivation

Psychosocial and emotional deprivation are uncommon causes of growth failure in infancy and early childhood. These children display behavioral abnormalities, such as apathy, watchfulness, and autoerotic activity, as well as delayed developmental behavior. The history often reveals rejection or neglect, non-accidental injury, or lack of physical handling. The children have poor growth at home but catch-up in hospital or when fostered. They may have high levels of fasting GH and also cortisol non-responsiveness with the insulin tolerance test. GH deficiency that is reversible when the caring environment is improved has also been described.

Syndromes with short stature

Children with dysmorphic features may have a recognizable syndrome associated with a chromosomal abnormality (e.g. Turner syndrome, Prader–Willi syndrome), low birthweight (e.g. Russell–Silver syndrome, Three M slender-boned dwarfism, Seckel syndrome), autosomal-dominant (e.g. Noonan syndrome), autosomal-recessive (Bloom syndrome, Fanconi pancytopenia, Mulibrey Nanism), X-linked dominant (Aarskog syndrome), or rare conditions (e.g. Floating Harbor, Kabuki makeup).

Disproportionate short stature

A skeletal dysplasia should be suspected in patients who are short, are growing normally but have a reduced height prediction, have abnormal body proportions, abnormalities of the limbs and trunk, and who fail to have a normal growth spurt despite appropriate pubertal development. Abnormal body proportions may not be clinically apparent but can be discerned from plots of sitting height and subischial leg length. A full skeletal radiographic survey and interpretation by an experienced radiologist are needed to make a diagnosis.

Hypochondroplasia

Hypochondroplasia is an autosomal-dominant condition with variable penetrance and a wide spectrum of disease severity. Mutations in the tyrosine kinase domain of the fibroblast growth factor receptor 3 (FGFR3) gene, mapped to chromosome 4p, have been described in some cases [48,49]. Patients with a severe phenotype have disproportionate short stature and rhizomelic limb shortening in early childhood akin to achondroplasia. In others, the short stature may not become manifest until adolescence, when a reduced pubertal growth spurt results in significant reduction in final height. The characteristic radiological finding is decreased interpedicular distance between lumbar vertebrae L1 and L5 with short pedicles, which may not be evident until the second or third year of life. The results of GH treatment on final height have not been fully evaluated but do not appear to be impressive. Surgical limb lengthening procedures based on the principle of distraction osteogenesis offer significant gain in length of both lower and upper limbs and allow normalization of body proportions.

Tall stature

Definition

Children whose height is above the 99.6th centile or who have a significant discrepancy between the child's centile and mid-parental target centile should be evaluated for tall stature.

Table 5.6. The major causes of tall stature.

Looks normal			Looks abnormal	
Normal height velocity	**Increased height velocity**		**Abnormal features**	**Disproportion between limbs and trunk**
	Signs of puberty	**No precocious puberty**		
Familial tall stature Obesity	Precocious puberty: Central Gonadotropin independent	Endocrine disorder: Hyperthyroidism Growth hormone excess Familial glucocorticoid deficiency	Recognizable syndrome: Cerebral gigantism Beckwith–Wiedemann syndrome Weaver syndrome	Long limbs: Marfan syndrome Homocystinuria XYY boys Long limbs and hypogonadism: Gonadotropin deficiency Klinefelter syndrome

Causes

There are few pathological causes of tall stature (Table 5.6). Most tall children represent the upper end of the normal distribution of height and have a family history of tallness in one or both parents, i.e. constitutional tall stature. Obese children, especially if the onset of obesity occurred in infancy, are often taller than average for their age and, if skeletal maturity is also advanced, puberty may occur earlier.

Assessment

The assessment of a child with tall stature requires a detailed history, family history of stature and age of onset of puberty, measurements to include sitting height and arm span, determination of height velocity and pubertal status. Tall stature may represent a considerable handicap, and parents may be especially concerned if they experienced psychosocial problems during childhood. Adult height prediction using bone age and available equations is essential in the management of tall children [22].

Management

Treatment with sex steroids (girls 10–50 Bg/day ethinylestradiol; boys 50–250 mg of testosterone ester depot at two- to four-weekly intervals) to induce puberty early and accelerate its progress has been used to limit growth. Doses higher than these have not been found to be more effective but are associated with significant side-effects. The reduction in height is greater when treatment is started at a lower bone age (when the remaining growth potential is greater) and is continued until complete fusion of the epiphyses at the knees. Nausea, headaches, weight gain, striae, breast discomfort, abdominal pain, calf cramps, and irregular periods are the most commonly observed side-effects of high doses of estrogen. Thromboembolism and hypertension are rare but significant risks of treatment. Potentially serious effects on lipids, glucose tolerance, liver function, and development of carcinoma have not been established. The side-effects of high doses of testosterone are acne, fluid retention, hypertension, and temporary decrease in testicular size. Surgical damage to knee epiphyses by epiphysiodesis, generally performed to correct limb length inequality, is an alternative treatment option to limit height, but the results of this procedure have not been systematically compared with medical treatment.

Endocrine causes of tall stature

The most common endocrine causes of tall stature in childhood are precocious puberty and hypogonadism. The former is suggested by tall stature associated with increased growth velocity and advanced bone maturation in the preadolescent age group. Final height in children with precocious puberty is likely to be reduced by premature epiphyseal fusion. Hypogonadism (central or due to gonadal failure) may give rise to tall stature, continuing growth into late adolescence, and a eunuchoid habitus.

Hyperthyroidism

An increase in growth rate associated with advanced bone age is seen in hyperthyroidism and also in hypothyroidism overtreated with thyroxine. Measuring thyroxine, triiodothyronine, and TSH levels makes the diagnosis. A thyroid-releasing hormone test should be carried out where doubt still exists.

Growth hormone excess (hyperpituitary gigantism)

Growth hormone excess, from a GH-secreting tumor, in childhood or adolescence is extremely rare. It is characterized by extremely rapid linear growth, but bone age is not advanced,

and overgrowth of soft tissues and metabolic changes are similar to those observed in patients with acromegaly. Levels of GH and IGF-I are raised, contrasting with levels in constitutional tall stature. The tumor can be diagnosed using cranial MRI. Treatment is aimed at reducing the excessive GH secretion. As for acromegaly, treatment modalities include trans-sphenoidal removal of the tumor, GH antagonist, somatostatin analogs, and/or radiotherapy.

Familial glucocorticoid deficiency
Familial glucocorticoid deficiency results from mutations of the ACTH receptor (MC2-R) [50]. Inheritance is probably autosomal recessive. Patients are excessively tall for their parents, but linear growth is normalized with glucocorticoid replacement. They have hyperpigmentation associated with high ACTH levels, increased head circumference, and characteristic facial appearance including hypertelorism, epicanthic folds, and frontal bossing.

Tall stature associated with an abnormal appearance

Sotos syndrome (cerebral gigantism)
Sotos syndrome is a pre- and postnatal overgrowth syndrome caused by deletions or mutations in the nuclear receptor-binding SET domain 1 (NSD1) gene on chromosome 5q [51]. The children have increased birth length, weight, and head circumference. They have rapid growth in early childhood with advanced bone age, but do not attain excessively tall adult height. Characteristic features include a large elongated head, prominent forehead, down-slanting palpebral fissures, mild hypertelorism, high-arched palate, small pointed chin, and acromegalic appearance to hands and feet. Most have subnormal intelligence. They have normal GH secretion and no evidence of thyroid, adrenal, or gonadal dysfunction.

Marfan syndrome
Marfan syndrome is an autosomal-dominant condition resulting from a mutation in the fibrillin-1 (FBN-1) gene on chromosome 15q. It affects approximately 1 in 5000 people. Patients with Marfan syndrome have long limbs with narrow hands and long slender fingers. The metacarpal index (the ratio of the length to the width of the largest metacarpal on a hand radiograph) is usually greater than 8.4. Arm span is greater than height, and the lower segment is much greater than the upper segment. Other features include mild joint laxity, skin striae, kyphoscoliosis, deformities of the rib cage, high arch palate, and lens dislocation. Aortic root dilatation, aortic aneurysms, and mitral valve prolapse are important cardiac features and associated with increased mortality in early adult life. Echocardiographic and ophthalmic assessment should be undertaken in a child with marfanoid features.

Homocystinuria
Homocystinuria is a rare (≈ 1:250 000) autosomal-recessive disorder caused by defects in cystathionine synthase. Phenotypically, patients resemble those with Marfan syndrome, but they usually have subnormal intelligence, osteopenia, and a tendency to fatal thromboembolism. Lenticular dislocation also occurs, usually in a downward direction.

Klinefelter syndrome
Klinefelter syndrome has a frequency of around 1 in 1000 liveborn males, and the incidence increases with maternal age. Most have the XXY karyotype, and about 10% are mosaics (XY/XXY). In childhood, presentation is usually with tall stature or poorly developed secondary sexual characteristics, and adults may present with infertility. Patients tend to be tall with disproportionately long limbs, feminine distribution of body fat, gynecomastia, and mild learning difficulties. Onset of puberty is not delayed, but testicular volumes do not increase more than 8–10 mL. Testicular histology shows seminiferous tubule dysgenesis, increase in Leydig cells, and interstitial fibrosis.

XYY syndrome
The incidence of XYY syndrome is 1 in 1000 males, but the majority are not recognized. Features in some include increased linear growth in mid-childhood, tall stature with disproportionately long limbs, mild delay in the onset of puberty, above average peak height velocity, and a tendency to compulsive and socially deviant behavior.

Underweight

Of the various anthropometric methods available (Table 5.7), weight interpreted using available centile charts is the simplest and most reasonable measure of a child's nutritional

Table 5.7. Anthropometric measures of a child's nutritional state.

Anthropometric measure	Definitions	
	At risk of malnutrition	Malnutrition
Weight for age	0.4th–second centile	< 0.4th centile
BMI	0.4th–second centile	< 0.4th centile
BMI as % of population median BMI for age	80–90%	< 80%
Actual weight divided by 50th centile weight for age as % of the actual height divided by 50th centile height for age	80–90%	< 80%

Table 5.8. Causes of weight loss, poor weight gain, and failure to thrive.

Inadequate calorie intake
Breast-feeding failure
Behavioral problems resulting in poor feeding and excessive consumption of fluids
Psychosocial deprivation
Poverty
Anorexia nervosa, bulimia
Difficulty swallowing due to congenital abnormality (e.g. cleft palate), neurodevelopmental delay, or breathing difficulty (heart failure or respiratory disease)
Increased losses
Recurrent vomiting (e.g. gastroesophageal reflux)
Diarrhea from malabsorption or other gastrointestinal disorder (e.g. cystic fibrosis, celiac disease, inflammatory bowel disease, giardiasis)
Endocrine disorder
Diabetes mellitus
Diabetes insipidus
Thyrotoxicosis
Congenital adrenal hyperplasia (salt wasting)
Adrenal insufficiency
Pheochromocytoma
Idiopathic hypercalcemia of infancy
Congenital lipodystrophy
Other organic causes
Metabolic: galactosemia, urea cycle disorders, organic aciduria, hereditary tyrosinemia
Renal: renal tubular acidosis, cystinosis
Cardiac: large left-to-right shunt
Respiratory: chronic lung disease, bronchiectasis
Chronic inflammation: polyarteritis nodosa, chronic infection (e.g. tuberculosis)
Immunodeficiency: severe combined immunodeficiency, AIDS
Hematological: anemia
Malignancies: renal, brain

state. Underweight means weight loss or failure to gain weight at an appropriate rate so that weight falls to lower centiles. Acute transient weight loss can occur with common childhood illnesses associated with fever, diarrhea, and vomiting. Acute weight loss is also seen in children with endocrine and systemic diseases of recent onset, the most common being diabetes mellitus (Table 5.8). A prolonged period of poor weight gain in children under 2 years of age is referred to as failure to thrive. Healthy children whose initial weight is between the ninth and 91st centiles often cross one centile space during the first year [52]. A sustained fall through two centile spaces is described as mild to moderate, and three centile spaces (= 2 SD scores) as severe failure to thrive [52]. Linear growth and head growth are not affected with transient periods of being underweight, but linear growth may be impaired with long-term failure to thrive.

The causes of failure to thrive or being chronically underweight are predominantly nutritional (Table 5.8). Major organic disease accounts for 5% or less and is generally diagnosed from overt clinical features [52]. Non-organic failure to thrive refers to children with psychosocial deprivation, abuse, or neglect, and who do not have an organic cause. Abuse or neglect has been reported in 5–10% of children with failure to thrive [52]. Undernutrition owing to poor feeding, inadequate dietary intake, and excessive consumption of juice or milk is the commonest cause of failure to thrive in otherwise healthy children. Assessment of a child who is failing to thrive or chronically underweight should include feeding and dietary history (variety and quantity of foods offered and eaten, meal time routines and behavior, drinking pattern, parent's interest in food and cooking), bowel habits, and family history. Specific features of endocrine disorders and other organic diseases need to be explored and identified.

Overweight and obesity

Overweight and obesity are defined as body mass index above the 85th and 95th centiles respectively. The aims of assessment of an overweight child are to determine the cause (Table 5.9) and identify problems associated with it, as well as risk factors for complications. The important aspects of history and examination are presented in Table 5.10.

Table 5.9. The major causes of obesity.

Height above average and/or normal height velocity	Height low compared with weight and/or low height velocity	
Simple obesity	Endocrine disorder:	Hypogonadism, dysmorphic features:
Polycystic ovary syndrome	Hypothyroidism	Prader–Willi syndrome
	Growth hormone deficiency	Laurence–Moon–Biedl syndrome
	Cushing syndrome	Carpenter syndrome
	Pseudohypoparathyroidism	Cohen syndrome
	Hypothalamic lesion:	Alstrom syndrome
	Craniopharyngioma	

Table 5.10. The important aspects of assessment of an overweight/obese child.

History
Birthweight
Age of onset of overweight
Diet, quality, and quantity of food and drink consumed
Leisure activities and lifestyle, frequency and nature of physical activity
Psychological consequences, bullying
Physical consequences: tiredness, reduced exercise tolerance, joint pain
Family history
Parents' weights and heights
Obesity
Associated problems
Examination
Weight in relation to height
Pubertal status
Distribution of fat
Striae
Hirsutism, acne
Acanthosis nigricans
Blood pressure
Features of endocrine disorder or dysmorphic syndrome

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6 Genetic syndromes and dysmorphology

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Genetic and dysmorphic syndromes can be associated with either short or tall stature. The effect on stature is dependent not only on the genetic makeup but results from a combination of environmental and social conditions and the impact of associated illness. Most genetic disorders involving short stature are rare, but the number of diseases and syndromes that include short stature is large. A search of the London Dysmorphology Database under the heading "short stature, general abnormalities" identified 1072 syndromes. Increased stature is less common, with generally less social perception that the achieved stature is unacceptable. In contrast to the situation with short stature, a search of the London Dysmorphology Database under the heading "tall stature, general abnormalities" identified only 74 syndromes.

Genetic syndromes are recognized by their effect on children's growth rates and pubertal development and by the presence of stigmata associated with certain conditions. The impact of genes on growth has been demonstrated in twin and parent–child studies, with the mean difference in height at age 4 years being 1.1 cm in monozygotic and 3.2 cm in dizygotic twins [1].

The study of growth is fundamental to the practice of pediatrics and pediatric endocrinology and an essential part of the diagnostic process to determine the etiology of either short or tall stature. Centile growth charts permit assessment of normal and abnormal growth over time, highlighting the need for further investigation where a child's height deviates from the pattern predicted by the relevant growth chart. Growth charts also allow monitoring of a response to treatment such as growth hormone or recovery of growth when an adverse influence (e.g. chronic disease) is ameliorated.

Disease-specific growth charts facilitate monitoring of growth in a range of conditions in which growth deviates from the norm as a result of underlying genetic, chromosomal, or dysmorphic syndromes. Use of disease-specific growth charts may also allow more accurate prediction of final height and thus assist in individual and family counseling.

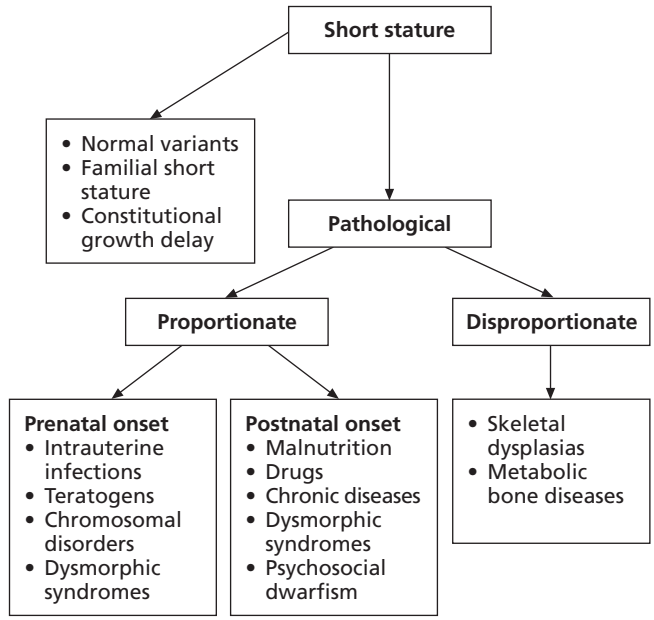

Fig. 6.1. Classification of short stature.

Genetic and dysmorphic syndromes with short stature

The classification of short stature can be approached in a number of ways. Figure 6.1 demonstrates a classification based on a differentiation of normal variant and pathological growth and whether the short stature is proportionate or disproportionate. Proportionate growth is divided into growth with a predominantly prenatal or postnatal onset. The normal variants of growth (familial short stature and constitutional delay) account for the majority of children presenting for evaluation of short stature. Intrauterine infections and teratogens (e.g. alcohol, warfarin) may cause significant prenatal-onset growth retardation.

Chromosomal abnormality	Frequency	Features
Trisomy 21	1:600–1:700	Typical facies (see text), congenital heart disease (50%),
Trisomy 18	1:3000–1:7000	macroglossia and tongue protrusion, small hands and feet Peculiar face, clenched hand, congenital heart disease (80%)
Trisomy 13	1:4000 – 1:10 000	Polydactyly, cleft palate, hypertelorism, scalp defects Dysplastic calvarium, syndactyly 3–4, cystic placenta
Triploidy	Uncertain	
Turner syndrome (45,X syndrome)	1:2500	Webbed neck, short fourth metacarpal, congenital heart disease, broad chest, wide-set nipples

Table 6.1. Common numerical chromosome abnormalities associated with short stature.

Short stature syndromes of prenatal onset include those involving chromosomal disorders and those dysmorphic syndromes caused by a single gene defect. Most chromosomal disorders, with the exception of the multiple X and Y syndromes, result in intrauterine growth restriction. The incidence of chromosomal anomalies is widely different in embryos, fetuses, abortuses, and still or live births. Unbalanced chromosome aberrations are found in 50–60% of abortions. Chromosomal problems may include abnormalities of number, structure, and imprinting. The common numerical chromosomal abnormalities causing short stature are summarized in Table 6.1.

Turner syndrome

Turner syndrome (TS) combines characteristic physical features with complete or partial absence of one of the X chromosomes, frequently accompanied by cell mosaicism [2]. TS was first described by Ullrich in 1930 [3] and Turner in 1938 [4] and is the most common sex chromosome abnormality of females, affecting an estimated 3% of all females conceived. Over 99% of fetuses with the 45,X karyotype are spontaneously aborted, especially in the first trimester, giving a prevalence rate of 1 in 2–2500 live female births. It is thought that only fetuses with the least severe manifestations of the chromosomal abnormality survive.

In 50% of cases, one entire X chromosome (45,X karyotype) is missing. Other females possess a range of karyotypic abnormalities, including partial absence of the second X chromosome and mosaicism. The presence of Y chromosomal material may be associated with the development of gonadoblastoma. The risk for this has been quoted at 30% but is more probably in the range of 7–10%. Gonadectomy is recommended to prevent malignancy.

The short stature of TS may be explained partly by haploinsufficiency of the short stature homeobox (SHOX) gene on the pseudoautosomal region of the X and Y chromosome at Xp22.33 [5], which escapes X inactivation. This gene has also been implicated in idiopathic short stature [5] and the short stature and skeletal abnormalities of the Leri–Weill dyschondrosteosis [6]. SHOX gene haploinsufficiency may account for the skeletal defects of TS, including mesomelia (forearm shortening), cubitus valgus, high arched palate, short metacarpals, and Madelung deformity.

The most common features of TS are short stature and ovarian failure (Fig. 6.2), and the more common phenotypic features are summarized in Table 6.2. These may not be obvious, and any girl with unexplained short stature or ovarian dysfunction should have a karyotype to exclude TS.

Lymphedema in a neonate is a key diagnostic pointer to TS, but it may be a lifelong problem and may be exacerbated by the initiation of growth hormone or estrogen treatment. Conservative treatments such as diuretics and support stockings may be required. Plastic surgery for the neck webbing associated with intrauterine cystic hygroma may be considered, but girls with TS are particularly prone to the formation of keloid scars.

A range of skeletal abnormalities may be found in TS, and there may be overlap in the skeletal phenotypes of the two SHOX haploinsufficiency syndromes, Leri–Weill syndrome (LWS) and TS. Features in common include Madelung deformity (present in 74% of LWS and 7% of TS), high arched palate, skeletal disproportion, increased carrying angle, short fourth metacarpals, and scoliosis. In addition, infants with TS have an increased risk of congenital dislocation of the hip.

Low-set ears and mild malformations of the outer ear occur in 30–50% of TS girls [7]. Conductive and sensorineural hearing loss is common, with the sensorineural dip occurring as early as 6 years of age. Hearing loss may be progressive into adult life. The outer, middle, and inner ear are all affected, and hearing problems and ear malformations correlate with the karyotype. Otitis media is extremely common in girls with TS and may progress to mastoiditis and/or cholesteatoma, particularly in very young children. Speech and language problems may occur. Myopia, squint, and ptosis may all be more common, and ophthalmological assessment should be performed in childhood.

Congenital cardiac disease occurs in approximately 30% of girls with TS. Left-sided cardiac abnormalities are most common, with bicuspid aortic valve in 30–50%, coarctation of

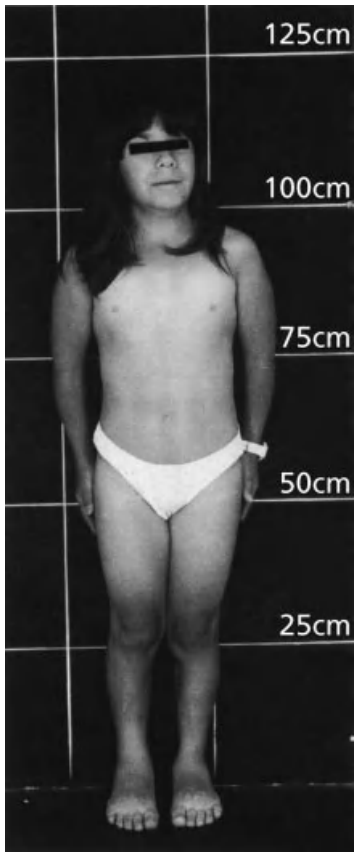

Fig. 6.2. An 11-year-old with Turner syndrome. Her birthweight was 2 kg at 37 weeks, and she presented to the pediatrician at the age of 10 years because of concerns about short stature and slow growth velocity. She had a past history of recurrent ear infections. Her height SDS at presentation was –3.3 and weight SDS was –1.5. Her karyotype was 45XO. Note the rather solid appearance with a broad chest. Mild webbing of the neck was present, as was an increased carrying angle of the elbows and hyperconvex nails. Lymphedema of the fourth and fifth toes was present in the first few months of life.

Table 6.2. Phenotypic features of Turner syndrome in childhood and adolescence.

Feature	Frequency (%)
Short stature	88–100
Ovarian failure	87–96
Lymphatic abnormalities	
Neck webbing	23–65
Low posterior hairline	40–80
Lymphedema	21–47
Nail convexity/dysplasia	43–83
Skeletal abnormalities	
Micrognathia	60
High arch palate	35–84
Short fourth/fifth metacarpals	35–77
Increased carrying angles	27–82
Madelung deformity	7
Kyphoscoliosis	12–16
Broad chest	33–75
Abnormal upper–lower segment	90
Recurrent middle ear infections	60
Hearing problems	50 (?)
Congenital cardiac problems	30
Bicuspid aortic valve	30–50
Coarctation of aorta	30
Aortic root dilation	5 approx.
Renal abnormalities	30
Nevi	22–78
Endocrine	
Thyroiditis	10–30
Glucose intolerance	34

Information summarized from [7] and [80].

the aorta in 30%, and aortic dilation in 5%. The last is an uncommon but serious complication, particularly if leakage or rupture occurs. It is usually associated with another congenital abnormality such as bicuspid aortic valve, coarctation, or hypertension. Because cardiac disease is so common in TS, all patients should have a cardiological assessment, including echocardiography, at diagnosis, and annual blood pressure assessment is advised [7]. Identification of a cardiac lesion requires regular follow-up and prophylaxis for bacterial endocarditis. Monitoring for aortic root dilation should be performed, particularly if a cardiac lesion (bicuspid aortic valve, coarctation, or hypertension), known to be associated with this problem, is present. At adolescence, even in the absence of previous cardiac pathology, repeat assessment of the aortic root [possibly including magnetic resonance imaging (MRI)] should be performed as aortic dilation may sometimes occur in the absence of other cardiac pathology. Aortic root dilation and rupture is a potentially life-threatening complication of TS, particularly during pregnancy.

Renal tract malformations are present in up to 30% of TS girls. Horseshoe kidney and duplex collecting systems are the most frequent pathology. The presence of renal disease may compound the likelihood of hypertension, and urinary tract infections should be treated promptly. All girls with TS should have renal ultrasound at diagnosis, and ultrasound and urine cultures should be performed routinely every 3–5 years [7].

Primary hypothyroidism due to Hashimoto thyroiditis occurs in up to 30% of girls with TS. Diabetes mellitus occurs uncommonly in TS, but insulin resistance and glucose intolerance may be present and may rarely be exacerbated by treatment with oxandrolone or growth hormone. Healthy weight maintenance may be a problem for girls with TS, and short stature and statural disproportion may exacerbate the tendency to obesity.

A range of other problems has been reported in TS [7]. Specific learning difficulties, particularly in relation to nonverbal and visuospatial processing tasks, are common. Liver

Table 6.3. Common syndromes with specific growth charts.

Syndrome	Growth charts	Chromosomal/genetic abnormality
Turner	Lyon *et al*. (1985) [8] Ranke *et al*. (1988) [11] Lippe *et al*. (1993) [12] Rongen-Westerlaken *et al*. (1997) [13] Suwa (1992) [14] Bernasconi *et al*. (1994) [15]	Complete/partial absence of X chromosome
Trisomy 21	Cronk *et al*. (1988) [20] Styles *et al*. (2002) [21] Cremers *et al*. (1993) [22] Piro *et al*. (1990) [23] Myrelid *et al*. (2002) [24]	Trisomy 21
Noonan	Witt *et al*. (1986) [28] Ranke *et al*. (1988) [29]	Mutation in PTPN11 gene, 12q24.1
Prader–Willi	Butler *et al*. (1991) [34]	Loss of paternal alleles 15q11–13
Silver–Russell	Wollman *et al*. (1995) [39]	Maternal uniparental disomy, chromosome 7 (7–10%)
Achondroplasia	Horton *et al*. (1978) [48]	Mutation of FGF-3 receptor gene (transmembrane portion)
Hypochondroplasia	Appan *et al*. (1990) [49]	Mutation of FGF-3 receptor gene (tyrosine kinase domain)
Spondyloepiphyseal dysplasia	Horton (1962) [51]	Mutations of collagen II gene
Marfan syndrome	Pyeritz (1983) [68] Pyeritz (1985) [67]	Mutation of fibrillin gene, 15q21.1

abnormalities have been reported, and TS girls may have a higher risk of developing celiac disease than the general population. Girls with TS may have more dental crowding and malocclusion problems than other girls because of retrognathia.

The diagnosis should be made by obtaining a karyotype counting a sufficient number of cells to exclude the possibility of low-level mosaicism. If TS is strongly suspected on clinical grounds and the blood karyotype is normal, a karyotype should be obtained on a peripheral skin biopsy. If a patient with TS has evidence of virilization or if a marker chromosome suggestive of Y-containing material is located, cytogenetic probing for Y chromosome material should be performed by DNA hybridization or fluorescent *in situ* hybridization (FISH) using a Y-centromeric or short-arm probe.

The growth of girls with TS should be plotted on Turner-specific growth charts. The short stature of TS is characterized by mild intrauterine growth retardation [8], slow growth during infancy [8], delayed onset of the childhood component of growth [9], and growth failure during childhood and adolescence without a pubertal growth spurt. This growth failure leads to an adult height approximately 20 cm below the female average for the ethnic-specific population. Mean final heights of women with TS range between 136.7 cm (Japan) and 146.9 cm (Germany) [10]. A number of growth curves based on analysis of national Turner syndrome growth data have been constructed (Table 6.3 [8,11–15]). Ullrich's first reported case showed a final height of 144.6 cm [16].

Because of the almost uniform problem of short stature, many treatments have been tried over the years to improve the final height of TS girls, including estrogen, oxandrolone, and growth hormone (GH). The Recommendations for the diagnosis and management of Turner syndrome [7] state that "initiation of GH therapy should be considered as soon as a patient with TS has dropped below the fifth percentile of the normal female growth curve. Therapy may be started as early as 2 years of age, although there is only limited experience of treating TS girls of this age. For girls below 9–12 years of age, therapy can be started with GH alone. In older girls (> 9–12 years of age), or in girls above 8 years in whom therapy is started when the individual is already below the fifth percentile of the normal growth curve, consideration should be given to the concomitant administration of oxandrolone." The appropriate use and timing of estrogen for feminization has been the subject of many studies [2].

The diagnosis of TS at any age may be associated with psychological distress, grief, and loss for the family and girl/adolescent. Psychological support should be made available, and families and adolescents with TS should be encouraged to contact their local or national TS support group. The peer and family support provided by such groups is an invaluable educational and emotional assistance for a TS girl and her family. Women with TS need lifelong medical surveillance [17].

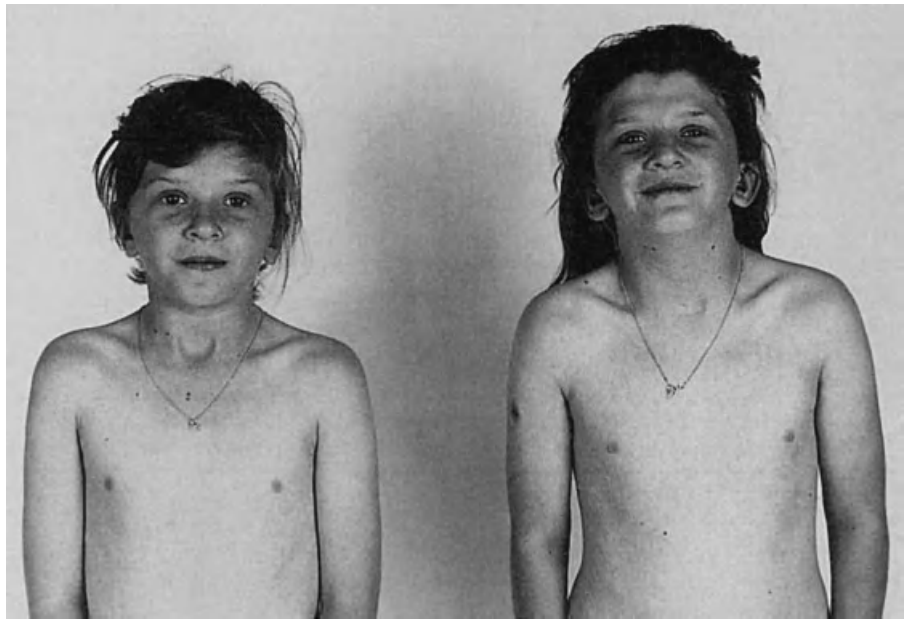

Fig. 6.3. Sisters aged 10 and 12 years with Noonan syndrome are shown. Both have short stature, –2.4 SDS, and no evidence of puberty. Phenotypic features include increased carrying angle (not shown in photograph), low-set posteriorly rotated ears, and broad chest. The younger girl had mild pulmonary stenosis.

Trisomy 21

Trisomy 21 (Down syndrome) occurs in 1 in 600–700 live births and is the most common single cause of mental retardation. It is associated with malformation of several organs and characterized by short stature. Growth velocity is most reduced between the ages of 6 months and 3 years. Puberty tends to be early, but the pubertal growth spurt is impaired. The mean final height for an individual with Down syndrome (DS) is approximately 20 cm below the target height [18]. Men with DS are generally infertile, while women with DS are fertile. GH deficiency is not usually present in DS, although suboptimal GH secretion due to hypothalamic dysfunction has been suggested [19]. Subjects with DS often have thyroid disease: the incidence of congenital hypothyroidism is increased, and acquired thyroid dysfunction resulting from autoimmune thyroiditis is common.

GH treatment has been used to treat the short stature of DS. Results vary with some studies showing normalization of growth velocity, but the effect of GH on other parameters including metabolic effects, effects on psychomotor and motor function, and predisposition to malignancy requires further study. GH treatment for non-GH-deficient individuals with DS is currently not generally recommended.

The growth of children with DS should ideally be plotted on Down-specific growth charts, which have been constructed for American [20], British [21], Dutch [22], Sicilian [23], and Swedish DS children [24] (Table 6.3).

Noonan syndrome

Noonan syndrome (NS) occurs in 1:1000–2500 live births [25]. The clinical features include typical facial features, short stature, congenital heart disease, pectus chest deformity, webbing of the neck, coagulation defects, visual disturbance, hearing impairment, undescended testes, and delayed puberty. The facial features include hypertelorism, ptosis, downslanting palpebral fissures, and low-set posteriorly rotated ears (Fig. 6.3). The typical cardiac defects (in decreasing frequency of occurrence) are pulmonary valve stenosis, left ventricular hypertrophic cardiomyopathy, and secundum atrial septal defect. Not all clinical features may be present, and clinical scoring systems have been developed [26].

Noonan syndrome is caused by a mutation in the PTPN11 gene, which encodes the protein tyrosine phosphatase SHP-2 (a protein that controls cardiac semilunar valvulogenesis and has other diverse effects on cell proliferation, differentiation, and migration) on the long arm of chromosome 12 (12q24.1) [27].

The mean final height of adults with NS is 162.5 cm in males and 153 cm in females, with growth charts for NS available [28,29] (Table 6.3). These demonstrate a 2-year delay in the pubertal growth spurt. GH has been used in Noonan syndrome and has shown short and longer term benefit, although the results of final height analyses are not yet available [30,31]. NS patients with echocardiographic features of hypertrophic cardiomyopathy may be at risk from GH therapy because of the known effect of GH on cardiac muscle. Such patients should be identified by cardiological assessment before GH therapy and should be reviewed annually with this issue in mind while on GH [30].

Prader–Willi syndrome

Prader–Willi syndrome (PWS) was first described in 1956 by Prader, Willi and Labhart [32]. It occurs in approximately 1 in

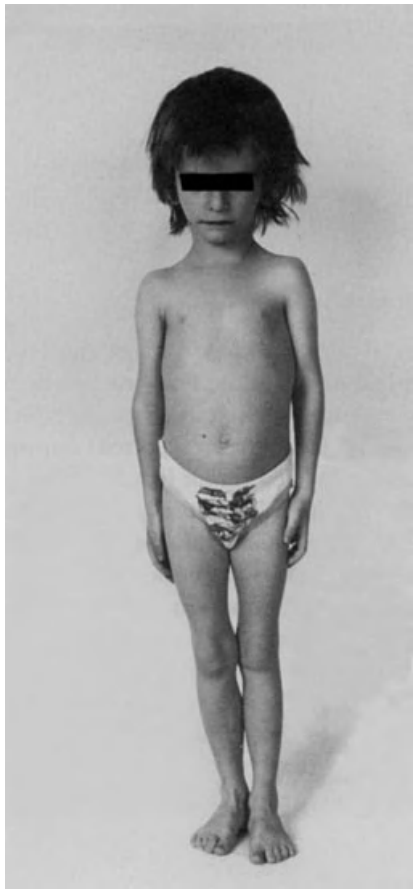

Fig. 6.4. Four-year-old male with Silver–Russell syndrome. His birthweight was 1.68 kg at term and, at age 4 years, his height SDS is –5.3 and weight SDS is –4.5. A triangular face is present with a broad forehead and narrow point to the mandible. There is obvious body asymmetry with the right arm and right leg greater than the left side.

15 000 live births and affects males and females equally in all ethnic groups. Typical clinical features include short stature, muscular hypotonia, hypogonadism, mild to moderate mental retardation, and hyperphagia leading to extreme obesity if not controlled. The symptoms vary with age. In neonates and during infancy, muscular hypotonia, feeding problems, and underweight are the most prominent symptoms. With increasing age, short stature, hyperphagia, and behavioral problems become more predominant. Many of the symptoms are non-specific, and clinical diagnosis may be difficult. Diagnostic clinical criteria were developed in 1993 [33], and growth charts were published in 1991 [34] (Table 6.3).

The majority of individuals with clinical PWS have a chromosomal abnormality, with loss of paternal alleles on the long arm of chromosome 15 in the region 15q11–13. Approximately 70% are caused by paternal alteration, 25% by maternal disomy, and 5% by translocation or other structural abnormalities [35].

The endocrine and GH/insulin-like growth factor (IGF) axis in individuals with PWS has been studied extensively, with low spontaneous and stimulated GH secretion and IGF-I levels. Evaluation of the true GH status of PWS individuals may be complicated by obesity. GH treatment has been used in children and adults, with benefits demonstrated in growth and body composition [36]. Sudden death in GH-treated children with PWS has been recently reported [37].

Silver–Russell syndrome

The Silver–Russell syndrome (SRS) is characterized by intrauterine and postnatal growth restriction with dysmorphic features including a small triangular facies, skeletal asymmetry, and fifth finger clinodactyly (Fig. 6.4). The genetic etiology of SRS is heterogeneous [38]. Maternal uniparental disomy for chromosome 7 [mUPD (7)] occurs in 7–10% of patients, with strong evidence that disruption of imprinted gene expression, as opposed to mutation of a recessive gene, underlies the SRS phenotype in these cases. The pattern of postnatal growth is well described, and the final adult height is generally 3–4 SDs below normal [39] (Table 6.3).

Children with SRS are usually included in clinical trials of GH treatment on intrauterine growth retardation, the etiology of which is often not known. In these trials, initial growth response is usual, with some concern regarding excess bone age advancement. Predicted adult height has tended to increase, but final height has shown little benefit [40]. It is concerning that the catch-up growth seen by some small for gestational age children may be associated with adverse metabolic sequelae such as insulin resistance [41].

The Aarskog–Scott syndrome

The Aarskog–Scott syndrome is a genetically heterogeneous developmental disorder [42,43]. It is characterized by craniofacial dysmorphism (hypertelorism, downslanting palpebral fissures), brachydactyly, urogenital abnormalities, and disproportionate short stature. A broad range of mild developmental delay or learning difficulty has occasionally been reported. Because of the clinical overlap with other syndromes (e.g. Noonan syndrome, Short syndrome, pseudohypoparathyroidism, and Robinow syndrome), overdiagnosis has probably occurred. The gene responsible for the X-linked form, FDG1, was identified and characterized by positional cloning in a family in which the phenotype was associated with a balanced X-autosome translocation [44]. It is thought that this gene is important in the signaling pathway of skeletal formation and morphogenesis.

Birthweight and length are usually in the lower half of the normal range, indicating that growth failure may start prenatally. Growth becomes impaired during the first year of life in about one-third of patients, and between 1 and 3 years of age in the remainder. Nearly all children fall below the third centile for height by about 3 years of age. Growth is slow during childhood, puberty is usually delayed, and the

normal puberty growth spurt is suppressed. Final height has been reported to be between –2 and –3 SDS [45].

GH deficiency is uncommon; a positive effect of GH treatment for up to 3 years of treatment has been reported [45].

The DiGeorge/velocardiofacial syndrome

The DiGeorge/velocardiofacial (DG/VCF) syndrome is due to a microdeletion of chromosome 22q11.2 (del 22q11) and is characterized clinically by facial dysmorphism, conotruncal heart defects, palatal anomalies, immunological defects with thymic hypoplasia, and neonatal hypocalcemia. Auxological parameters are characterized by weight deficiency in the first years of life due to feeding difficulties, normalization of weight in the following years, and development of obesity in adolescence with normal final height. A small group of patients has shown constitutional delay in growth with slight delay in bone age in infancy and head circumference corresponding to the lower centiles [46].

Skeletal dysplasias

Skeletal dysplasias comprise a large and variable group of uncommon disorders characterized by disproportionate short stature in childhood and adult life [47]. Estimates vary but, overall, these disorders occur with an incidence of < 1:10 000 live births.

Achondroplasia is caused by a mutation in the transmembrane portion of the fibroblast growth factor receptor-3 (FGF-3) gene. In 90% of cases, the paternal allele is affected. Most cases are sporadic (90%), and inheritance is autosomal dominant. The typical appearance of patients includes a large head, frontal bossing and mid-face hypoplasia, severe shortening (rhizomelic) of the extremities but normal trunk length. Mental function is normal. Adult height is severely impaired, generally between 100 and 140 cm. Growth curves have been published for this disorder [48] (Table 6.3).

Hypochondroplasia may result from a mutation of the tyrosine kinase domain of the FGFR-3 gene, but often no genetic abnormality can be identified. The clinical picture has variable severity and involves mild disproportion and short stature, with final height in males of 145–165 cm and in females of 130–150 cm [49]. Growth curves for this disorder have been published [49] (Table 6.3).

Spondyloepiphyseal dysplasia is the term used for a group of skeletal dysplasias that includes spondyloepiphyseal dysplasia congenita, spondylometaphyseal dysplasia, and Kneist dysplasia. The disorder is caused by autosomal-dominant mutations of the collagen II gene (COL2A1). The skeletal deformities are severe and include kyphoscoliosis. Adult height is severely impaired (84–132 cm) [50]. Growth curves for this condition have been established [51] (Table 6.3).

Leri–Weill dyschondrosteosis is a form of mesomelic (shortening of forearms and lower legs) short stature with Madelung's deformity [52]. It is caused by haploinsufficiency of the SHOX gene (short stature homeobox containing gene) located within the pseudoautosomal region of the X and Y chromosomes [6]. There is heterogeneity in the clinical expression of this condition in terms of short stature and other skeletal manifestations (e.g. short fourth metacarpals, scoliosis, exostoses). Adult height of males is 156–171 cm and of females is 135–164 cm [53].

There are limited data available on GH treatment of bone dysplasias. Recent reports suggest that the response may be better in hypochondroplasia and Leri–Weill dyschondrosteosis [54] than in achondroplasia [55].

Growth disorders in the chromosome 18 syndromes

The 18q syndrome is caused by a deletion of a portion of the long arm of chromosome 18 [56]. It is characterized by dysmyelination, speech failure, hypotonia, mental retardation, and short stature [57]. Some children have normal growth, as defined by normal height and growth velocity, normal growth factors, and normal responses to GH stimulation testing. Others have classical growth hormone deficiency (GHD). There have also been several reports of patients with 18p syndrome who have a deletion of the short arm of chromosome 18 and who also have GHD. Thus, children with chromosome 18 abnormalities have a high frequency of growth failure and GHD and merit thorough clinical and biochemical evaluation if growth failure is present.

A range of other dysmorphic syndromes can be associated with short stature, including Charge syndrome [58], Fanconi anemia [59], Williams syndrome [60], and the Bardet–Biedl syndrome [61].

Genetic and dysmorphic syndromes with tall stature

Tall stature and excessive growth are relatively rare concerns in pediatric practice. Genetic and dysmorphic syndromes can cause both prenatal and postnatal overgrowth and resultant tall stature. Syndromes associated with prenatal overgrowth include the Beckwith–Wiedemann syndrome, Simpson–Golabi–Behmel syndrome, Perlman syndrome, Sotos syndrome, Weaver syndrome, Marshall–Smith syndrome, and Elejalde syndrome [62].

Klinefelter syndrome

Klinefelter syndrome (KS) is associated with an additional X chromosome in males due to non-disjunction of the sex chromosomes during the first meiotic division in one of the parents. The most frequent karyotype is 47XXY (93%), but other karyotypes also occur (e.g. 46XY, 47XXY, 48XXYY,

49XXXX7) and are associated with a similar phenotype. KS occurs in 1 in 500–1000 live male births. The syndrome is characterized by primary hypogonadism with small testes and hyalinization and fibrosis of the seminiferous tubules resulting in infertility, tall stature with long legs, behavioral and psychological problems, and gynecomastia at puberty.

Learning and behavioral problems may occur in KS, although IQ is usually in the normal range. Other problems include mitral valve prolapse, Berry aneurysm, venous ulcers, deep vein thrombosis, and pulmonary embolism. There is also an increased risk of carcinoma of the breast, autoimmune disorders, such as systemic lupus erythematosus, diabetes, hypothyroidism, and osteoporosis due to androgen deficiency.

Birthweight and head circumference have been reported to be smaller than those in normal control subjects, although still in the normal range. Height velocity is increased during childhood, and an increase in leg length relative to height (low upper to lower segment ratio) is evident from the age of 3 years. Upper limb span is not increased. Tall stature is evident before puberty and related to extra leg length. Tall stature in association with extra copies of the short stature homeobox (SHOX) gene has been reported recently [63]. As individuals with KS have at least three copies of the SHOX gene, the tall stature may be due to SHOX gene effects rather than androgen deficiency and delayed growth plate fusion. Skeletal maturation is generally appropriate for chronological age throughout childhood.

At puberty, testicular size may increase to 10 mL, but progressive hyalinization and fibrosis of the seminiferous tubules results in small adult testes. Despite relatively normal early pubertal androgen levels in some males with KS, hypergonadotrophic hypogonadism is evident by mid-puberty in virtually all males with KS, and androgen replacement is essential to prevent gynecomastia. Final mean height is reported as approximately 10 cm taller than in XY males [64].

Treatment in KS needs to address the issues of possible learning and behavioral problems, puberty, stature and infertility, gynecomastia, and the need for long-term androgen replacement. Testosterone therapy may be introduced from the age of 12 years to induce puberty and prevent physical and psychological effects of hypogonadism. A minority of boys enter puberty spontaneously and do not require testosterone replacement until later puberty or early adult life. Treatment can commence with oral testosterone (e.g. testosterone undecanoate) or intramuscular testosterone esters with doses increased over time to induce a normal pubertal progression and physical development. Long-term replacement may be facilitated using testosterone implants, patches, or gels.

Marfan syndrome

Marfan syndrome (MS) is an autosomal-dominant syndrome of connective tissue [65]. It occurs in 1 in 10 000 births with equal sex prevalence. The underlying defect is caused by a mutation in the fibrillin-I gene located at 15q21.1. Fibrillin is the main component of 1–12 nm extracellular microfibrils that are important for elastogenesis, elasticity, and homeostasis of elastic fibers. Patients may arise as a fresh mutation, so a family history is not a prerequisite for diagnosis.

The syndrome consists of the clinical triad of excessively long limbs, ocular abnormalities, and cardiovascular problems. Skeletal manifestations include tall stature, long limbs, arachnodactyly, abnormal joint mobility, scoliosis, and chest wall deformity. Ocular manifestations can include dislocated lens (upward dislocation), severe myopia, flat cornea, elongated globe, and risk of retinal detachment. Cardiovascular manifestations can include dilation of the aortic root, aortic dissection, abdominal aortic aneurysm, and mitral valve prolapse or regurgitation. Diagnostic criteria have been suggested [66]. Tall stature develops early in infancy, and height remains elevated throughout childhood and adult life. Growth curves have been developed, with mean adult height of 177 cm in females and 187 cm in males [67,68] (Table 6.3). Cardiovascular manifestations are potentially the most severe and contribute to decreased lifespan. Life expectancy has been reported to have increased from 32 ± 16 years in 1972 to 41 ± 18 years in 1995.

Homocystinuria

Homocystinuria is an autosomal-recessive disorder of amino acid metabolism due to cystathione beta-synthase deficiency. The gene is located at 21q22.3, with many mutations found in different kindreds. The physical appearance is similar to Marfan syndrome with tall stature, Marfinoid body habitus, mental retardation, and downward lens dislocation. Thromboembolic disease and osteoporosis may complicate the condition. Homocystinuria occurs in 1 in 200 000 live births. Children with homocystinuria appear normal at birth, with the clinical features becoming manifest in the first few years of life.

The diagnosis may be made by newborn screening or as the result of diagnostic testing. Treatment consists of restriction of dietary methionine and supplementation of dietary cysteine. The etiology of the tall stature remains obscure. Good metabolic control is said to ameliorate the excess growth velocity seen in this situation [69].

Sotos syndrome

Sotos syndrome (SoS) is a childhood overgrowth syndrome characterized by excessive growth, distinctive craniofacial features, developmental delay, and advanced bone age. It occurs in 1 in 10 000–50 000 births [70]. A diagnosis is unlikely if one or more of four criteria (height > 97th centile, head circumference > 97th centile, bone age > 90th centile, and developmental delay) are not fulfilled [71].

Haploinsufficiency of the NSD1 gene has been identified as the major cause of SoS, with intragenic mutations or submicroscopic microdeletions being found in 60–75% of clinically diagnosed patients [72]. Weaver syndrome may resemble SoS, with recent reports of NSD1 mutations in both Sotos and Weaver syndromes [73].

Overgrowth is frequently evident at birth, and the growth velocity is excessive in the first few years of life and then parallels the charts in the high centiles or above the centiles. Bone age is usually advanced by 2–4 years during childhood, and puberty may occur relatively early, although within the normal range. The mean adult height attained is usually in the upper part of the normal range, with reports of mean final height of 172.9 ± 5.7 cm in females and 184.3 ± 6.0 cm in males [74]. Excessive final height is generally not a problem.

Beckwith–Wiedemann syndrome

Beckwith–Wiedemann Syndrome (BWS) is a prenatal and postnatal overgrowth syndrome that is characterized by omphalocele and macroglossia. Up to 50% of neonates have persistent neonatal hypoglycemia associated with hyperinsulinism [75]. Other common features are hemihyperplasia, ear anomalies including anterior lobe creases and posterior helical pits, umbilical hernia, visceromegaly, adrenocortical cytomegaly, renal abnormalities, and an increased risk of embryonal tumors. The prevalence is reported to be approximately 1 in 14 000, with no gender predilection.

The molecular genetics of BWS are complex and involve a number of growth regulatory genes in an imprinted gene cluster in the chromosome 11p15.5 region. A number of imprinted genes are known to be involved in the pathogenesis of BWS, including the paternally expressed IGF-2 and KvQTI-AS and the maternally expressed H19, p57 KIP2, and KvLQTI [76].

The overgrowth in BWS can be uniform or regional affecting any part of the body or organs. Infants are born with birthweight and length approximately 2 SD above the mean for gestational age; however, overgrowth may not manifest until the first year of life [77]. Growth velocity is usually above the 90th centile until 4–6 years of age, with skeletal age mildly advanced, and then growth velocity returns to normal through late childhood and puberty. Tall final height is common, but stature is not excessive [78].

Simpson–Golabi–Behmel syndrome

Simpson–Golabi–Behmel syndrome is an X-linked overgrowth syndrome with prenatal and postnatal overgrowth, craniofacial abnormalities, digital abnormalities (polydactyly, nail hypoplasia), and a wide variety of less common features. There is overlap between the BWS and the Simpson–Golabi–Behmel syndrome, with both syndromes including macroglossia, organomegaly, earlobe creases, hyperinsulinism and hypoglycemia, and risk of embryonal tumors. The syndrome is due to loss of function abnormalities of the Glypican 3 (GPC3) gene on Xq26. The GPC3 and IGF-2 receptors may be functionally related and may be co-dependent, possibly explaining the relationship with BWS [79].

Growth charts relating to many of the syndromes discussed in this chapter and elsewhere can be found in the appendix (see p. 565).

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7 Acquired abnormalities of the hypothalamic–pituitary axis leading to growth impairment

Dennis J. Brenner and Edward O. Reiter

Growth disorders may be subdivided into *primary growth abnormalities*, where the presumed gene-directed defect(s) appear(s) to be intrinsic to the growth plate, *secondary growth disorders* such as growth failure resulting from chronic disease or endocrine disorders, and *idiopathic short stature*, including variants of normal (constitutional delay of growth and puberty and genetic short stature), heterozygous mutations of the growth hormone (GH) receptor gene (a variant of GH insensitivity), post-GH or insulin-like growth factor (IGF) receptor dysfunction, and as yet unidentified mutations throughout the growth-related genome. The focus of this chapter is on the second.

Endocrine regulation of growth

Growth hormone

Human GH is produced as a single-chain, 191-amino-acid, 22-kDa protein with a pulsatile secretory pattern reflecting the interplay of two hypothalamic regulatory peptides, growth hormone-releasing hormone (GHRH) and somatostatin [somatotrophin release-inhibiting factor (SRIF)], with presumed modulation by other GH-releasing factors. The pulsatile secretion of GH *in vivo* is the apparent summation of a simultaneous reduction in hypothalamic somatostatin release and increase in GHRH release. Conversely, a trough of GH secretion occurs when somatostatin is released in the face of diminished GHRH activity.

Many external factors influence GH secretion including stress, sleep, hemorrhage, fasting, hypoglycemia, and exercise, and form the basis for a number of GH-stimulatory tests used in the evaluation of GH secretory capacity/reserve. GH secretion is also influenced by a variety of non-peptide hormones, including androgens, estrogens, thyroxine, and glucocorticoids, as well as the IGF peptides. The mechanisms by which these hormones regulate GH secretion may involve actions at both hypothalamic and pituitary sites. Hypothyroidism and glucocorticoid excess may each blunt spontaneous and provocative GH secretion, while gonadal steroids are responsible for the pubertal rise in GH secretion.

The episodic release of GH by the somatotropes results in intermittent increases in serum concentrations of GH separated by periods of low or undetectable concentrations, during which time GH secretion is minimal [1]. It is, consequently, impractical to assess GH secretion by random serum sampling. Mean concentrations of GH decrease from values of 25–35 μg/L in the neonatal period to approximately 5–7 μg/L through childhood and early puberty. Twenty-four hour GH secretion peaks during adolescence, contributing to the high serum concentrations of IGF-I characteristic of puberty. This pubertal increment in GH production is due to both enhanced pulse amplitude and increased mass of GH per secretory burst, rather than to a change in pulse frequency [1,2] (Figs 7.1 and 7.2).

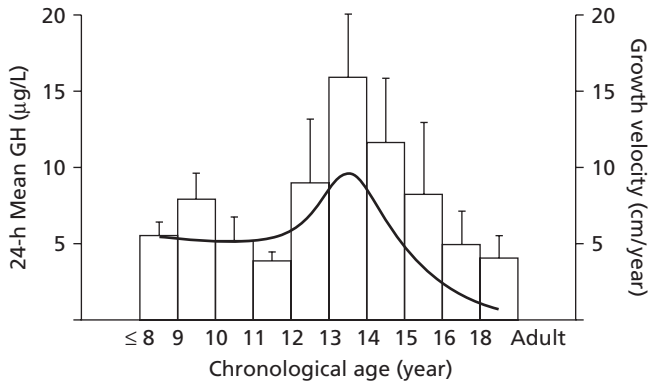

Fig. 7.1. Relation between 24-h mean growth hormone (GH) levels and age in boys and men. Bars represent values for the 24-h mean (± SE) levels of GH (left axis) from 60 24-h GH profiles of healthy boys and men subdivided according to chronological age. An idealized growth velocity curve reproduced from the 50th percentile values for whole-year height velocity of North American boys [9] is superimposed (from Martha PM Jr *et al. J Clin Endocrinol Metab* 1989; 69: 563–50).

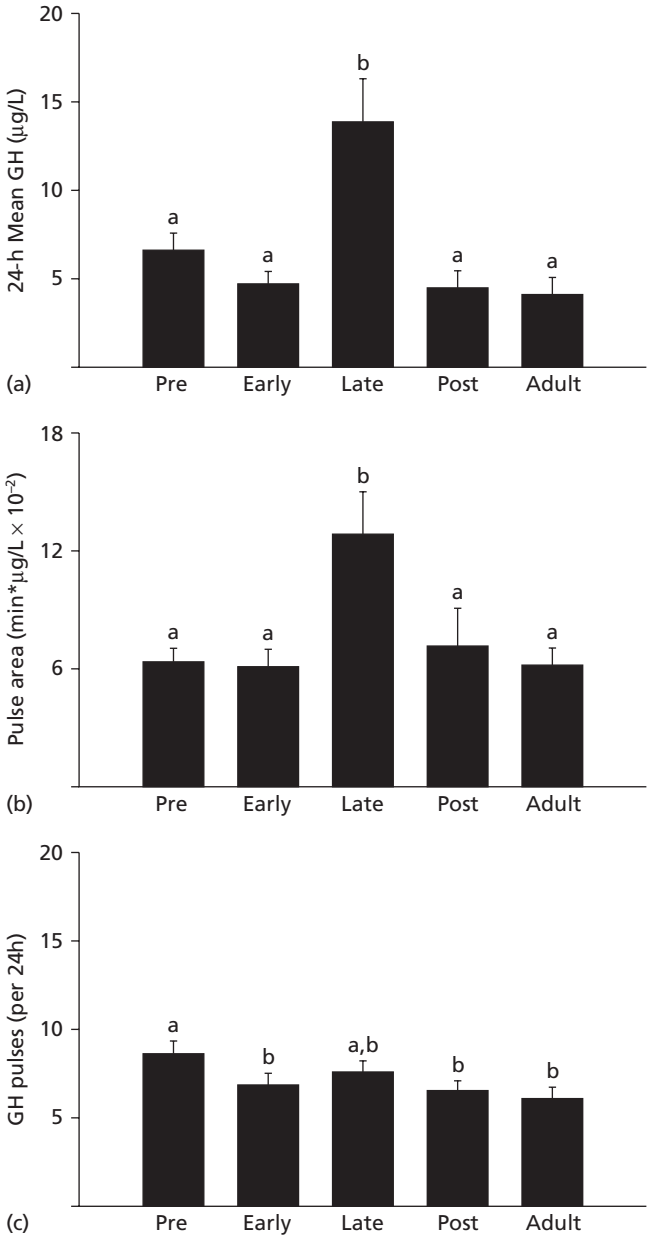

Fig. 7.2. (a) The mean (± SE) 24-h levels of growth hormone (GH) for groups of normal boys at varied stages of pubertal maturation. (b) The mean (± SE) area under the GH concentration versus time curve for individual GH pulses, as identified by the Cluster pulse detection algorithm. (c) The number of GH pulses (± SE), as detected by the Cluster algorithm, in the 24-h GH concentration profiles for boys in each of the pubertal study groups. Note: the mean 24-h GH concentration changes are largely mediated by changes in the amount of GH secreted per pulse rather than the frequency of pulses. In each panel, bars bearing the same letter are statistically indistinguishable (from Martha PM Jr *et al*. *J Clin Endocrinol Metab* 1989; 69: 563–70).

Physiological states that impact GH secretion, in addition to puberty and aging, include sleep, nutritional status, fasting, exercise, stress, and gonadal steroids. A circadian rhythm of somatostatin secretion, upon which is superimposed episodic bursts of GHRH release, could result in the nocturnal augmentation of GH production. Obesity in childhood and adolescence is characterized by low GH production but normal IGF and increased GHBP concentrations, and often increased linear growth [3]. Body mass also influences GH production in normal prepubertal and pubertal children and adults [2,4].

GH receptor (GH-R)/GH binding protein (GHBP)

In humans, the most important circulating GH binding protein appears to be derived from proteolytic cleavage of the extracellular domain of the GH receptor. The GH-R also contains transmembrane and intracellular domains. GHBP binds GH with high specificity and affinity but with relatively low capacity, as about 45% of circulating GH is bound [5]. An additional GHBP, not related to the GH receptor, binds approximately 5–10% of circulating GH with lower affinity [5]. Concentrations of GHBP are low in early life, rise through childhood, and plateau during the pubertal years and adulthood [6,7]. Impaired nutrition, diabetes mellitus, hypothyroidism, chronic liver disease, and a spectrum of inherited abnormalities of the GH receptor are associated with low concentrations of GHBP, while obesity, refeeding, early pregnancy, and estrogen treatment can cause elevated concentrations of GHBP. A direct correlation exists between GHBP concentrations and body mass index.

GH actions

A presumed sequence of steps in GH action is shown in Plate 6, facing p. 148. Binding of GH to the membrane-associated GH receptor leads to sequential dimerization of the GH receptor through binding to each of two specific sites on GH. Interaction of the GH receptor with JAK2, tyrosine phosphorylation of JAK2 and the GH receptor, changes in cytoplasmic and nuclear protein phosphorylation and dephosphorylation cause target gene transcription. GH- and JAK2-dependent phosphorylation and activation have been demonstrated for many cytoplasmic signaling molecules, which, after forming homodimers or heterodimers, translocate into the nucleus, bind DNA, and activate transcription [8]. The clarification of these events will presumably lead to the definition of some of the causes of growth impairment.

The anabolic actions of GH are mediated through the IGF peptides, previously called somatomedins. Although this is largely true, GH is also capable of inducing effects that are independent of IGF activity. Indeed, the actions of GH and IGF are, on occasion, contradictory, as evident in the "diabetogenic" actions of GH and the glucose-lowering activity of IGFs. There are multiple sites of GH action and, frequently, it is not entirely clear which of these actions is mediated through the IGF system and which might represent IGF-independent effects of GH [9].

The sites of action include stimulation of epiphyseal growth. In bone, there is stimulation of osteoclast differentiation and activity, stimulation of osteoblast activity, and increase in bone mass by endochondral bone formation. In adipose tissue, acute insulin-like effects are followed by increased lipolysis, inhibition of lipoprotein lipase, stimulation of hormone-sensitive lipase, decreased glucose transport, and decreased lipogenesis. In muscle, there is an increase in amino acid transport, nitrogen retention, lean tissue, and energy expenditure. A series of organ-specific murine knockout experiments has also demonstrated that the elimination of circulating concentrations of IGF-I, presumably those largely generated through GH-mediated hepatic production, was still associated with growth. These studies suggested that the somatomedin hypothesis also had to be modified to include local IGF-I production, whether GH dependent or not [10].

Insulin-like growth factors

The insulin-like growth factors (or somatomedins) are a family of peptides that are, in part, GH dependent and mediate many of the anabolic and mitogenic actions of GH. IGF-I (formerly called somatomedin C) is a basic peptide of 70 amino acids, and IGF-II is a slightly acidic peptide of 67 amino acids. The two peptides share 45 of 73 possible amino acid positions and have approximately 50% amino acid homology to insulin. This structural similarity explains the ability of both IGFs to bind to the insulin receptor (and have approximately 8% of insulin's potency) and of insulin to bind to the type I IGF receptor. On the other hand, structural differences probably also explain the failure of insulin to bind with high affinity to the IGF-binding proteins.

Serum concentrations of IGF peptides

In human fetal serum, IGF-I concentrations are relatively low and are positively correlated with gestational age [11]. There is generally a good correlation between fetal cord serum IGF-I concentrations and birthweight. IGF-I concentrations in human newborn serum are generally 30–50% of adult concentrations. Serum concentrations rise during childhood and attain adult concentrations at the onset of puberty (Fig. 7.3). During puberty, IGF-I concentrations rise to two or three times the adult range [12]. Concentrations during adolescence correlate better with Tanner stage (or bone age) than with chronological age. Girls with gonadal dysgenesis show no adolescent increase in serum IGF-I, supporting the association of the pubertal rise in IGF-I with the production of gonadal steroids. The pubertal rise in gonadal steroids may stimulate IGF-I production indirectly through promoting a rise in GH secretion, but patients with GH insensitivity due to GH receptor mutations show a pubertal rise in serum IGF-I, thereby suggesting a direct effect of gonadal steroids upon IGF-I.

After adolescence, serum IGF-I concentrations demonstrate a gradual and progressive age-associated decline that is possibly responsible for the negative nitrogen balance, decrease in muscle mass, and osteoporosis of aging. This hypothesis is unproven but has generated interest in the potential use of GH and/or IGF-I therapy in normal aging.

The GH dependency of the IGFs was established in the initial report from Salmon and Daughaday [13] and further clarified with the development of sensitive and specific immunoassays. IGF-I concentrations are more GH dependent than are IGF-II concentrations and are more likely to reflect subtle differences in GH secretory patterns. However, serum IGF-I concentrations are influenced by age, degree of sexual puberty, and nutritional status. As a result, age-defined normative values may be misleading. IGF-I concentrations in normal children less than 5 years of age are low, and there is overlap between the normal range and values in GH-deficient children. Assessment of serum IGF-II concentrations is less age dependent, especially after 1 year of age, but IGF-II is less GH dependent than IGF-I.

There are a number of studies that demonstrate discordance between assessments of GH secretion and IGF concentrations. There are numerous reasons for this problem including other factors, such as nutrition or inflammatory cytokines, that regulate IGF production and intrinsic problems with the tests used to characterize GH production. The observation that many "normal short" children have low serum concentrations of IGF-I, IGF-II, or both calls into question the criteria by which the diagnosis of GH deficiency is made. Given that provocative GH testing is both arbitrary and non-physiological and the inherent variability in GH assays, it is not surprising that the correlation between IGF-I concentrations and provocative GH concentrations is imperfect.

IGF receptors

At least two classes of IGF receptors exist. High concentrations of insulin compete for occupancy of one form of IGF receptor (type I), but insulin has effectively no affinity for the second form of receptor. The demonstration that IGFs bound to the insulin receptor provided an early explanation for their insulin-like activity [14].

IGF binding proteins (IGFBP-1–6)

In contrast to insulin, the IGFs circulate in plasma complexed to a family of six binding proteins that extend the serum half-life of the IGF peptides, transport the IGFs to target cells, and modulate the interaction of the IGFs with surface membrane receptors. The identification and characterization of IGFBPs in body fluids and in conditioned media from cultured cells have been facilitated by the development of a number of biochemical and assay techniques. Many of the roles of the binding proteins are redundant, suggesting varied evolutionary

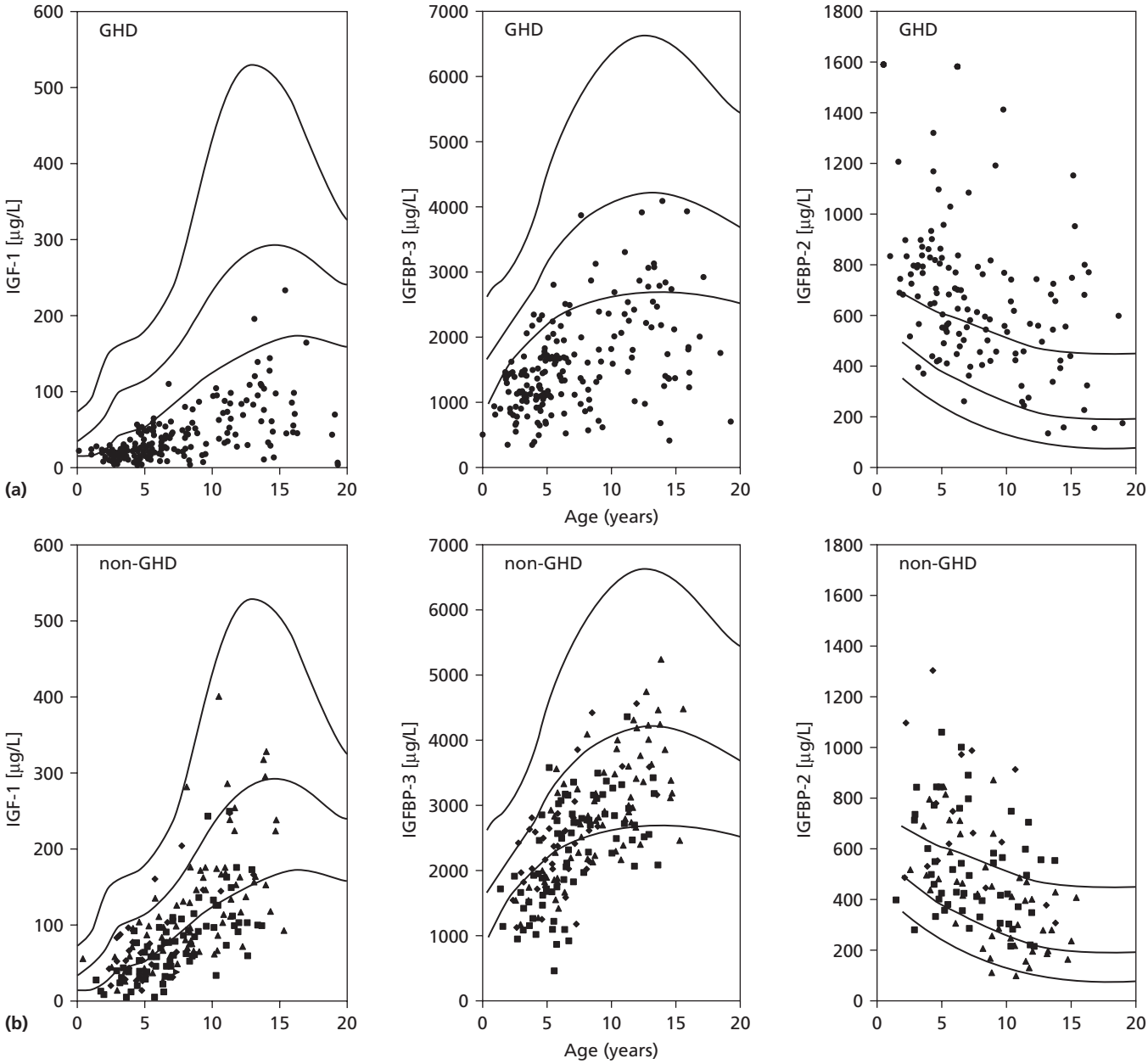

Fig. 7.3. Basal levels of IGF-I, IGFBP-3, and IGFBP-2 in children with (a) GHD (*N*=187) and (b) non-GHD (*n*=205) in comparison with normal ranges (lines mark 5th–50th and 95th centiles of normal) (from Ranke MB *et al*. *Horm Res* 54: 60–8).

changes so that the absence of any one might not have deleterious effects. These binding proteins are discussed in detail elsewhere [15].

IGFBP-3 is GH dependent, either because of a direct GH effect or, less likely, regulation by IGF. This binding protein has been studied extensively. It is the major serum carrier of IGF-I in association with an acid-labile subunit to generate the so-called ternary complex. Its measurement in the assessment of growth disorders is discussed later as it is an important component of such evaluations. Concentrations of IGFBP-2 and IGFBP-3 are illustrated in Figure 7.3.

Gonadal steroids

While androgens and estrogens do not contribute substantially to normal growth before puberty, the adolescent rise in serum gonadal steroid concentrations is an important part of the pubertal growth spurt. States of androgen or estrogen excess before epiphyseal fusion cause rapid linear growth and skeletal maturation. Thus, just as growth deceleration requires evaluation, growth acceleration can be as abnormal and may be a sign of precocious puberty or virilizing congenital adrenal hyperplasia.

A GH-replete state is obligatory for a normal growth response to gonadal steroids, and children with GH deficiency do not have a normal growth response to either endogenous or exogenous androgens. Gonadal steroids work, in part, by enhancing GH secretion and also stimulate IGF-I production directly, as evidenced by the rise in serum IGF-I concentrations and pubertal growth spurt in children with mutations of the GH receptor [16].

Both androgens and estrogens increase skeletal maturation. It is likely that androgens primarily act in this regard after conversion to estrogens by aromatase in extraglandular tissues, but they may also have independent action. Indeed, mutation of the estrogen receptor in a man was associated with tall stature and open epiphyses [17], and similar findings occur in patients with mutations of the gene encoding the aromatase enzyme [18].

Skeletal development, in terms of bone mass accretion, is an important pubertal phenomenon and is largely mediated by estrogen action. A longitudinal analysis of female pubertal calcium accretion found that approximately 26% of adult calcium is laid down during the two adolescent years of maximal growth [19]. The vast majority of skeletal mass is present by 18 years of age with estrogens appearing to regulate the timing of the growth spurt, stabilization of bone modeling, and endosteal mineral apposition [20]. Late puberty is characterized by epiphyseal closure and increased volumetric density (true bone mineral density that is not size based), which are apparently mediated more clearly by estrogen. Late menarche and delayed puberty may be risk factors for later osteopenia. Independent and synergistic effects of gonadal steroids, GH, and IGF-I contribute to the attainment of peak bone mass in adults.

Thyroid hormone

Thyroid hormone is a major contributor to postnatal growth, although, like GH, it is of relatively little importance to growth of the fetus. Hypothyroidism postnatally can cause profound growth failure and virtual arrest of skeletal maturation. In addition to a direct effect on epiphyseal cartilage, thyroid hormones appear to have a permissive effect on GH secretion. Patients with hypothyroidism have decreased spontaneous GH secretion and blunted responses to GH provocative tests. Treatment with thyroid hormone results in rapid "catch-up" growth, which is typically accompanied by marked skeletal maturation, potentially causing overly rapid epiphyseal fusion and compromise of adult height.

IGF-I deficiency syndrome

As IGF-I is a major mediator of skeletal growth, its deficiency can result in severe growth failure. Causes of IGF-I deficiency include central hypothalamic–pituitary dysfunction with failure of pituitary GH production and primary or secondary GH insensitivity (GHI). We use the term IGF-I deficiency syndrome to describe the generic condition, whether caused by GH deficiency, dysfunction, or insensitivity, to illustrate this unifying concept. Secondary GHI is associated with a wide range of chronic conditions, including malnutrition, renal failure, pulmonary disease, inflammatory bowel disease, hepatic dysfunction, systemic immunological diseases (e.g. rheumatoid arthritis), and infectious disorders. Table 7.1 puts the IGF deficiency syndromes into the larger perspective of growth impairment.

Table 7.1. Classification of growth retardation.

I. Primary growth abnormalities
A. Skeletal dysplasias
B. Chromosomal abnormalities
C. Intrauterine growth retardation
D. Genetic errors of bone growth (e.g. SHOX)

II. Secondary growth disorders
A. Malnutrition
B. Chronic disease (nutrition, cytokines, energy imbalance)
C. Endocrine disorders
  1. Hypothyroidism
  2. Cushing syndrome
  3. Pseudohypoparathyroidism
  4. Rickets
    a. Vitamin D-deficient or -resistant rickets
  5. IGF deficiency
    a. GHD due to hypothalamic dysfunction
    b. GHD due to pituitary GH deficiency
    c. GH resistance (expanded in Table 7.7)
      (1) Primary GH insensitivity
        i. GH receptor gene mutations
        ii. Post-receptor cascade errors
      (2) Secondary GH insensitivity (in chronic illness)
    d. Primary defects of IGF synthesis
    e. Primary defects of IGF transport and clearance
    f. IGF resistance
      (1) Primary IGF insensitivity
        i. Defects of the type 1 IGF receptor
        ii. Post-receptor cascade errors
      (2) Secondary IGF insensitivity (?in chronic illness)

III. Idiopathic short stature
A. Genetic short stature
B. Constitutional delay of growth and puberty
C. Heterozygous defects of the GH or IGF receptors
D. Genetic errors of post-receptor processes

Hypothalamic–pituitary disorders

It is not always possible to distinguish hypothalamic from pituitary dysfunction, as both organs may be involved in the same pathological process. In addition, embryonic development of the hypothalamus and pituitary appears to be co-dependent. A number of factors produced in the developing

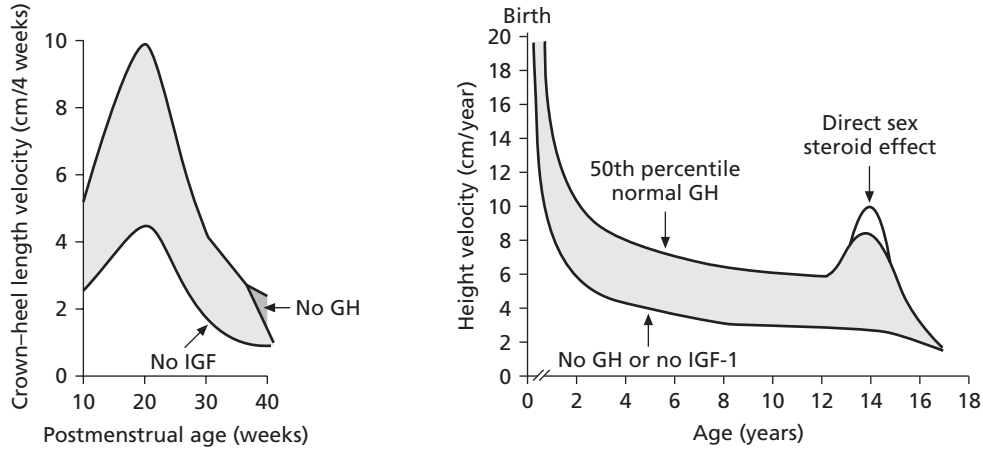

Fig. 7.4. Roles of insulin-like growth factor (IGF) and growth hormone (GH) in prenatal and postnatal growth. The upper (gray) line of the height velocity curve in both panels approximates the 50th percentile in boys. Different scales are used for prenatal growth (expressed as crown–heel length velocity in centimeters per 4 weeks) and postnatal height velocity (expressed as centimeters per year). The lower (red) line in the postnatal height velocity curves indicates the expected growth rate in children totally lacking GH. Clinical evidence suggests that a similar line can be drawn for children with severe IGF deficiency. The shaded region thus reflects IGF-dependent growth. A small, direct sex steroid effect that is independent of IGF is seen during puberty. Prenatally, the red line reflects the best estimate of intrauterine growth in the absence of IGFs (derived from limited case reports and murine models). IGF-dependent growth in prenatal life is largely independent of GH, except for a small effect in the last few weeks before birth (from Rosenfeld RG. *N Engl J Med* 2003; 349: 2184–6).

ventral diencephalon function as molecular signals for the initial formation and development of Rathke's pouch. Subsequent differentiation of the various anterior pituitary cell types appears to be regulated by a strict temporal and spatial pattern of pituitary transcription factors.

Clinical features of IGF deficiency

IGF-I deficiency resulting from hypothalamic dysfunction with abnormalities of GHRH, endogenous GHS or SRIF synthesis or secretion, primary or secondary decreased pituitary GH production, or GHI shares a common phenotype. The similarity emphasizes the role of IGF-I in mediating most of the anabolic and growth-promoting actions of GH. This is further supported by the ability of IGF-I therapy to correct growth in children with mutations of the GH receptor gene, and the clinical features of severe IGF deficiency are shared by all the conditions.

If GH or IGF deficiency is acquired, clinical signs and symptoms will appear at a later age. The importance of the GH/IGF axis in affecting growth through the life cycle is shown in Figure 7.4. Birth size is normal or near normal in most children with IGF-I deficiency but low in severe congenital GH deficiency, GHI, and in the single case of a deletion of the IGF-I gene [21]. Severe intrauterine growth retardation (IUGR) is not part of typical IGF deficiency but is present in infants with profound IGF deficiency, confirming the role of IGF-I in intrauterine growth. Infants with early-onset GHD may have birth lengths around –2 SD [22–25]. Half of infants diagnosed with isolated GHD or multiple pituitary hormone deficiencies before 2 years of age have birth lengths < –2 SD but birthweight is around –1 SD, lending an appearance of relative adiposity, even in the neonatal period [23].

These data support an intrauterine role for the GH–IGF system in growth regulation. Studies [15] performed in mice to determine the role of GH and the IGFs in fetal growth yield several conclusions: (1) IGF-I is important for both fetal and postnatal growth; (2) IGF-I is more important than GH for postnatal growth; (3) IGF-II is a major fetal growth factor; (4) the type 1 IGF receptor mediates anabolic actions of both IGF-I and IGF-II; (5) the type 2 IGF receptor is bifunctional, serving to both target lysosomal enzymes and enhance IGF-II turnover; (6) IGF-I production is involved in normal fertility; and (7) placental growth is impaired only with IGF-II knockouts. Whether these studies in mice are applicable to humans is not known.

A high frequency of abnormalities of the hypothalamic–pituitary area including dysgenesis of the pituitary stalk, ectopic placement of the posterior pituitary inferior to the median eminence, and diminished volume of the anterior pituitary has been defined by magnetic resonance imaging (MRI) [26]. Neonatal morbidity can include breech deliveries and perinatal asphyxia, hypoglycemia, and prolonged jaundice with direct hyperbilirubinemia due to cholestasis and giant cell hepatitis. When GHD is combined with deficiency of adrenocorticotrophic hormone (ACTH) and thyroid-stimulating hormone (TSH), hypoglycemia may be severe. The combination of GHD with gonadotropin deficiency can cause microphallus, cryptorchidism, and hypoplasia of the scrotum. GHD (or GHI) should, therefore, be considered in the differential diagnosis of neonatal hypoglycemia and of microphallus/cryptorchidism.

Postnatal growth is abnormal in severe congenital IGF deficiency. Most surveys of GHD and GHI indicate that growth failure can occur during the first months of life [22–24]. By 6–12 months of age, the growth rate is definitely slow and deviates from the normal growth curve, with lengths 3 to 4 SD below the mean. This stresses the importance of normal IGF-I production and action in the neonatal period and early childhood. The single most important clinical manifestation of IGF deficiency of all etiologies is growth failure, and careful documentation of growth rates is essential. Deviation from the normal growth curve should always be a cause of concern; between 2 years of age and the onset of puberty, growth deceleration (or acceleration) must always be considered pathological.

Skeletal proportions tend to be relatively normal but correlate better with bone age than with chronological age. Skeletal age may be substantially delayed below chronological age but, in the absence of hypothyroidism, is similar to "height age." In acquired GHD, as from a central nervous system (CNS) tumor that causes increased intracranial pressure, bone age may approximate chronological age; delayed skeletal maturation should not, therefore, be required for the diagnosis of GHD.

Assessment of volumetric bone mineral density reveals decreased mineralization beyond that dependent upon small size. Weight/height ratios tend to be increased, and fat distribution is often infantile or doll-like in pattern. Musculature is poor, especially in infancy, and can cause delay in gross motor development, and lead to the erroneous impression of mental retardation in an immature-appearing child. Facial bone growth may be particularly retarded with an underdeveloped nasal bridge and frontal bossing. Fontanelle closure is often delayed, but the overall growth of the skull is normal, leading to cephalofacial disproportion and the appearance of hydrocephalus. The voice is infantile because of hypoplasia of the larynx. Hair growth is sparse and thin, especially during early life; nail growth is also slow. Even with normal gonadotropin production, the penis is small. Puberty is usually delayed.

Final height data in patients with untreated GH deficiency are few. Wit *et al*. [27] summarized data from 36 untreated patients (22 men and 14 women) with severe isolated GH deficiency who had a mean final height SDS of −4.7. In 19 patients with multiple pituitary hormone deficiencies, thus lacking in gonadal steroids, mean final height was –3.1 SDS [28].

Causes of IGF-I deficiency

Two surveys of nearly 63 000 GH-treated patients, in databases managed by Pfizer (KIGS) and Genentech (NCGS), cared for by pediatric endocrinologists throughout the world, include more than half of the internationally treated patients [29,30]. The registry patients are diverse and include subjects with GHD and with Turner syndrome and miscellaneous other disorders. About 59% of the total group, or approximately 37 000 patients, had GHD (as defined by a stimulated GH level of <10 μg/L), of whom 78% had "idiopathic" GHD and 22% had "acquired" or "organic" (neoplasms, trauma, inflammation, miscellaneous) causes of GHD. The latter group includes patients with congenital (developmental) GHD-associated syndromes. The organic/acquired group is probably underestimated because many of the patients classified as idiopathic had not had definitive imaging assessments of the hypothalamic–pituitary region and possibly have congenital structural abnormalities.

With the availability of synthetic IGF-I for treatment of patients with inherited abnormalities of the GH receptor, around 200 patients with primary GHI have been identified. This is an exceedingly small number of subjects, even with the addition of the potentially larger group of individuals with heterozygous abnormalities of the GH receptor. In contrast, patients with secondary GHI, including those with malnutrition or chronic systemic disease, must be considered potentially a huge number on a worldwide basis.

An incidence of GHD of 1:60 000 live births has been reported from the United Kingdom [31], and a survey of Scottish school children indicated prevalence as high as 1:4000 [32]. The best estimate in the United States population is approximately 1:3480 [33]. It is likely, however, that childhood GHD is overdiagnosed. In particular, the diagnosis of acquired, idiopathic, isolated GHD should always be suspect. It is true that destructive or inflammatory lesions of the hypothalamus or pituitary may affect only GH secretion and that isolated GHD resulting from a mild mutation/deletion of the GHRH receptor gene or GH gene may appear late. Also, combined pituitary hormone deficiencies (CPHD) may present first with what appears to be isolated GHD, but such circumstances are unusual. In the absence of anatomical abnormalities seen on imaging studies and/or biochemical evidence of CPHD, the diagnosis of acquired, isolated, idiopathic GHD demands careful and thorough documentation, with greater skepticism as children approach puberty. Partial, transient GH insufficiency related to sex steroid deficiency in delayed puberty is particularly confounding.

Many of the disorders that affect hypothalamic regulation of GH synthesis and secretion impact directly upon pituitary function. Consequently, it is not always possible to establish definitively the primacy of hypothalamic or pituitary dysfunction, hence the term "idiopathic." Nevertheless, congenital (developmental) or functional abnormalities of the hypothalamus may account for most cases of "idiopathic" isolated GHD, and many such cases of GHD will yet prove to have a molecular basis. Acquired structural damage to this area (neoplastic, traumatic, etc.) causes a quarter of GHD cases.

Specific conditions

Inflammation of the brain and/or hypothalamus

Bacterial, viral, or fungal infections may result in hypothalamic/pituitary insufficiency. The hypothalamus and/or pituitary may also be involved in sarcoidosis.

Tumors of the brain and/or hypothalamus

Brain tumors are a major cause of hypothalamic insufficiency, especially midline brain tumors, such as germinomas, meningiomas, gliomas, ependymomas, and gliomas of the optic nerve. Although short stature and GHD are most often associated with suprasellar lesions in neurofibromatosis, they may also exist without such lesions; whether growth impairment antedates the pathological findings is not clear. Metastases from extracranial carcinomas are rare in children, but hypothalamic insufficiency can result from local extension of craniopharyngeal carcinoma or Hodgkin disease of the nasopharynx. The laboratory diagnosis of GHD in children with brain tumors may be difficult because concentrations of both IGF-I and IGFBP-3 are poor predictors, especially in pubertal patients [34]. Craniopharyngiomas and histiocytosis can cause hypothalamic dysfunction.

Radiotherapy

Cranial irradiation appears to be an increasing cause of hypothalamic–pituitary dysfunction. Taken in aggregate, there may be as many as 4000 pediatric cancer survivors who have GHD resulting from the broad range of cancer treatments [35]. Irradiation may impair both hypothalamic and pituitary function, and it is often not easy to discriminate between damage at the two levels. The hypothalamus is more radiosensitive than the pituitary and more often the site of damage, especially in the dose range usually given to children with malignancy. The degree of pituitary dysfunction is relative to the dose of irradiation received. Low doses typically cause isolated GHD, and higher doses cause multiple pituitary deficiencies.

The majority of long-term survivors develop GHD with the adverse effect of radiotherapy directly related to the biologically effective dose to the hypothalamus. Within 5 years of irradiation, nearly 100% of children receiving ≥ 30 Gy over 3 weeks to the hypothalamic–pituitary axis have subnormal GH responses to provocative tests, whereas GHD may not become apparent for a decade or more after doses of 18–24 Gy [35]. The degree of pituitary deficiency is also a function of the length of time after irradiation; children who test normally soon after therapy may develop pituitary deficiencies years later. Before GH secretory deficiency develops, GH insensitivity with low concentrations of IGF-I, IGFBP-3, and GHBP (presumably caused by the malignancy and the intensive chemo- and radiotherapy regimens) may decrease growth velocity.

Chemotherapy regimens by themselves may impair final adult height, although not nearly to the extent seen after irradiation. When such therapy is stopped, prepubertal children will have some degree of catch-up growth but also persistent abnormalities of the IGF/IGFBP system [36]. Even when serum GH responses to provocative testing are normal, spontaneous GH secretion may be blunted at X-ray doses as low as 18 to 24 Gy [37], probably yielding the best example of the syndrome of neurosecretory dysfunction of GH production.

Poor linear growth from decreased GH secretion may be exacerbated by the impact of irradiation in diminishing the pubertal acceleration of spinal growth. Surprisingly, cranial irradiation can result in precocious puberty, especially in children irradiated at young ages [38], causing early epiphyseal fusion. Sexual precocity appears to occur more frequently with low doses of irradiation, and gonadotropin deficiency is likely at high doses. Treatment with gonadotropin-releasing hormone (GnRH) analogs may be necessary to suppress the hypothalamic–pituitary gonadal axis in an attempt to attain normal final height.

Children with documented GHD and growth failure are candidates for exogenous GH treatment. No evidence demonstrates enhanced relapses of the primary neoplasm in patients treated with GH [39,40], but there appears to be a variable growth response to GH; spinal growth impairment, inadequate or delayed treatment, and sexual precocity may limit linear growth.

Bone marrow transplantation (BMT)

BMT for patients with inborn errors of metabolism, aplastic anemia, and malignancies requires preparative regimens that include total lymphoid or total body irradiation (TBI), often with chemotherapy and sometimes including cranial irradiation. Children in whom the clinical condition requires modest treatment programs before BMT have minimal loss of growth after BMT [41]. In children who had cranial irradiation followed by high-dose chemotherapy and TBI, especially in a single dose, as preparative regimens, growth failure is almost inevitable 2–5 years after BMT [41]. Pubertal growth is most affected. If the TBI is fractionated and if cranial irradiation was not previously needed, growth velocity and height 3 years after BMT are not compromised [42]. In the absence of cranial irradiation, there is a poor correlation between GH production and concentrations of IGF-1 or IGFBP-3 and growth in children after BMT, suggesting the importance of factors such as nutrition and irradiation-induced vertebral dysplasia or hypothyroidism. Final heights in long-term survivors of BMT are lower than at the time of the transplantation but generally within the normal adult range; such data suggest being conservative with regard to exogenous hormonal treatment.

Psychosocial dwarfism

An extreme form of "failure to thrive" is termed "psychosocial dwarfism" or "emotional deprivation dwarfism." Most cases of failure to thrive can be traced back to a poor home environment and inadequate parenting, with improved weight gain and growth upon removal of the infant from the dysfunctional home. Some children have dramatic behavioral manifestations beyond those in the typical failure to thrive infant, namely bizarre eating and drinking habits, such as drinking from toilets, social withdrawal, and primitive speech. Hyperphagia and abnormalities of GH production are associated. GH secretion is low in response to pharmacological stimuli but returns to normal upon removal from the home. Concomitantly, eating and behavioral habits return to normal, and a period of catch-up growth ensues.

Careful assessment of endogenous GH secretion will show reversal of the GH insufficiency within several weeks, including enhancement of GH pulse amplitude and frequency. The reversibility of GH secretion and the later growth increment in the context of the clinical findings described above confirm the diagnosis of psychosocial dwarfism.

The neuroendocrine mechanisms involved in psychosocial dwarfism remain to be elucidated. GH secretion is abnormal, and ACTH and TSH concentrations may also be low, although some patients have high plasma cortisol concentrations. Even when GH secretion is reduced, treatment with GH is not usually of benefit until the psychosocial situation is improved. Management of the environmental causes of the growth failure is imperative and often associated with substantial growth. In our experience, although psychosocial dysfunction is a common cause of failure to thrive in infancy, the constellation of bizarre behaviors described in psychosocial dwarfism is rare.

GH neurosecretory dysfunction

Because tests of GH secretion following pharmacological provocation may not accurately reflect normal GH secretion, a subset of children with "GH neurosecretory dysfunction" has been identified by frequent or continuous serum sampling over a 12- to 24-h period. This condition is characterized by short stature and poor growth, normal serum GH responses to provocative testing, but reduced IGF-I and 24-h serum GH concentrations. Previous cranial irradiation may be the most common cause of these findings. Patients with idiopathic short stature do not appear to have diminished 24-h GH production rates, especially when the very broad range of data in normal and short normal children is considered. There appears to be little doubt that some children with "GH neurosecretory dysfunction" secrete insufficient amounts of GH, even if they pass provocative GH testing; whether they should be identified by 24-h GH sampling or by determination of the GH-dependent peptides is unclear.

Prader–Willi syndrome (PWS)

PWS is a genetically determined syndrome complex, with a frequency of 1 in 10 000 to 25 000 live births, that includes profound neonatal hypotonia and subsequent diminished muscle mass and strength [43]. Growth failure may be evident at birth and is more impressive postnatally, with mean adult heights more than 2 SD below the mean and almost always below the mid-parental height target range. Cryptorchidism and microphallus are present neonatally, and hypogonadotropic hypogonadism persists into adult life. With advancing age, hyperphagia and obesity become prominent. The genetic defect in PWS is a functional deletion of the paternal allele within chromosome 15q11–13. Most patients with PWS have deletions of the long arm of the paternally derived chromosome 15; in some, both copies of 15q may be maternally derived, (uniparental disomy), while rarely there may be mutations of the imprinting center of chromosome 15q [43].

The probable cause of the short stature in PWS is deficient GH production due to as yet undefined hypothalamic dysfunction. MR assessment of the hypothalamic–pituitary area does not yield evidence for congenital structural abnormalities. The body habitus and composition are similar to classical GHD including small hands and feet, increased fat mass, and low muscle mass. Low mean serum GH concentrations or inadequate responses after provocative testing may reflect the impact of obesity, but serum concentrations of GH-dependent peptides are low in PWS, in contrast to the findings in obesity where these factors are produced normally despite diminished GH production. Thus, PWS is an IGF deficiency condition due to inadequate GH production, although the possibility of failure of upregulation of GH action, as seen in obesity, may yet be found to play a role. GH treatment of growth failure in PWS is now a Food and Drug Administration (FDA)-approved indication for GH use. Treatment results in improved growth velocity, normalization of final height potential, increased muscle mass and strength, and decreased fat mass. In view of the risk of developing obesity-related insulin resistance and diabetes, glycemic status must be monitored closely during GH therapy. Recent cases of upper airway obstruction and apnea-induced deaths have been reported during GH therapy [44].

Acquired, idiopathic, isolated GHD

In most pediatric endocrine centers, many children receiving GH are diagnosed as having acquired idiopathic, isolated GHD. As noted earlier, this diagnosis should always be considered somewhat suspect, especially peripubertally, although some patients may actually have undiagnosed gene defects in GH production or secretion or be experiencing the first manifestation of combined pituitary hormone

deficiency (CPHD). Many studies have reported data on retesting patients with GH-treated GHD during or after cessation of therapy. All the vagaries of different GH assays and GH provocative tests, varied "cutoff" concentrations of GH normalcy, diagnostic categorization of patients, and radiological interpretation of MRI findings certainly affect these evaluations. Nonetheless, several clear conclusions emerge. In 464 patients with isolated GHD, 207 (44%) had normal GH concentrations during provocative retesting [15]. Over 70% of subjects initially diagnosed as having "partial" GHD had normalization on retesting. In contrast, approximately 96% of 148 patients with CPHD, with or without structural abnormalities of the hypothalamic–pituitary area, had sustained GHD. Whether these results simply cast doubt upon the validity of the initial GH tests (or GH provocative testing in general) or whether children with earlier GHD may truly normalize is not clear. The entity of partial, transient GHD associated with delayed puberty may certainly be an example of this latter situation.

Tumors involving the pituitary

Many tumors that impair hypothalamic function also impair pituitary secretion of GH. In addition, craniopharyngiomas are a major cause of pituitary insufficiency [45]. These tumors arise from remnants of Rathke's pouch, the diverticulum of the roof of the embryonic oral cavity that normally gives rise to the anterior pituitary. Genetic defects in this condition, although certainly reasonable to suspect, have not yet been identified. This tumor is a congenital malformation present at birth that gradually grows over the ensuing years. The tumor arises from rests of squamous cells at the junction of the adenohypophysis and neurohypophysis, forming an enlarging cyst that contains degenerated cells and may calcify but does not undergo malignant degeneration. The cyst fluid ranges from a "machinery oil" to a shimmering cholesterol-laden liquid, and the calcifications may be microscopic or gross. About 75% of craniopharyngiomas arise in the suprasellar region, with the remainder resembling pituitary adenomas.

Craniopharyngiomas may appear clinically at any age from infancy to adulthood but usually in mid-childhood. The most common presentation is due to increased intracranial pressure, including headaches, vomiting, and oculomotor abnormalities. Visual field defects result from compression of the optic chiasm, and papilledema or optic atrophy may be present. Visual and olfactory hallucinations as well as seizures and dementia may exist. Most children with craniopharyngiomas have evidence of growth failure at the time of presentation. GH and the gonadotropins are the most commonly affected pituitary hormones in children and adults, but deficiency of TSH and/or ACTH may also occur; diabetes insipidus is present in 25–50% of patients [46]. Some 50–80% of patients have abnormalities of at least one anterior pituitary hormone at diagnosis [46].

Although lateral skull films may demonstrate enlargement or distortion of the sella turcica, frequently accompanied by suprasellar calcification(s), some children have normal plain films. MRI is the most sensitive diagnostic technique, allowing identification of cystic and solid components and delineation of anatomic relationships necessary for a rational operative approach. Operative intervention via either craniotomy or transphenoidal resection may result in partial or almost complete removal of the lesion. Post-operative irradiation, especially when tumor resection is incomplete, is commonly used.

In some patients, especially those who become obese, a syndrome of normal linear growth without GH may occur. The circulating growth-promoting substance(s) in this condition may include insulin and other poorly characterized mitogens. The long-term childhood and adolescent consequences of craniopharyngioma are substantial, with many quality of life issues exacerbating the hypopituitarism. Features of the adult dysmetabolic syndrome frequently appear [47].

Pituitary adenomas are infrequent during childhood and adolescence, accounting for less than 5% of operated patients at large centers [48]. Nearly two-thirds of tumors stain immunochemically for prolactin, and a small number stain for GH. There is variable experience as to the invasive nature of pituitary adenomas, although the prevailing opinion is that they are less aggressive in children than in adults. In 56 patients at the Mayo Clinic with non-ACTH-secreting adenomas removed transphenoidally, macroadenomas were about one-third more frequent than microadenomas, with girls outnumbering boys 3.3 to 1 [49]. The macroadenoma patients had about 50% incidence of hypopituitarism, compared with none in patients with microadenomas; long-term cure rates were 55–65% for both tumor sizes.

The localized or generalized proliferation of mononuclear macrophages (histiocytes) characterizes Langerhans cell histiocytosis, a diverse disorder occurring at all ages, with peak incidence at ages 1 to 4 years. This condition is characterized by an infiltration and accumulation of Langerhans cells in involved areas, such as skull, hypothalamo-pituitary stalk, CNS, and viscera. Although this disorder is classically associated with diabetes insipidus, approximately 50–75% of patients in selected series have growth failure and GHD at the time of presentation [50].

Diagnosis of IGF deficiency syndrome

As the way of diagnosing GHD is controversial, the concept of the IGF deficiency syndrome becomes more relevant. With availability of highly specific assays for the IGF peptides and binding proteins and with increasing understanding of the GH–IGF axis, evaluation of patients with growth failure should include a combination of auxological assessment and measures of the GH–IGF system. Documenting a deficiency

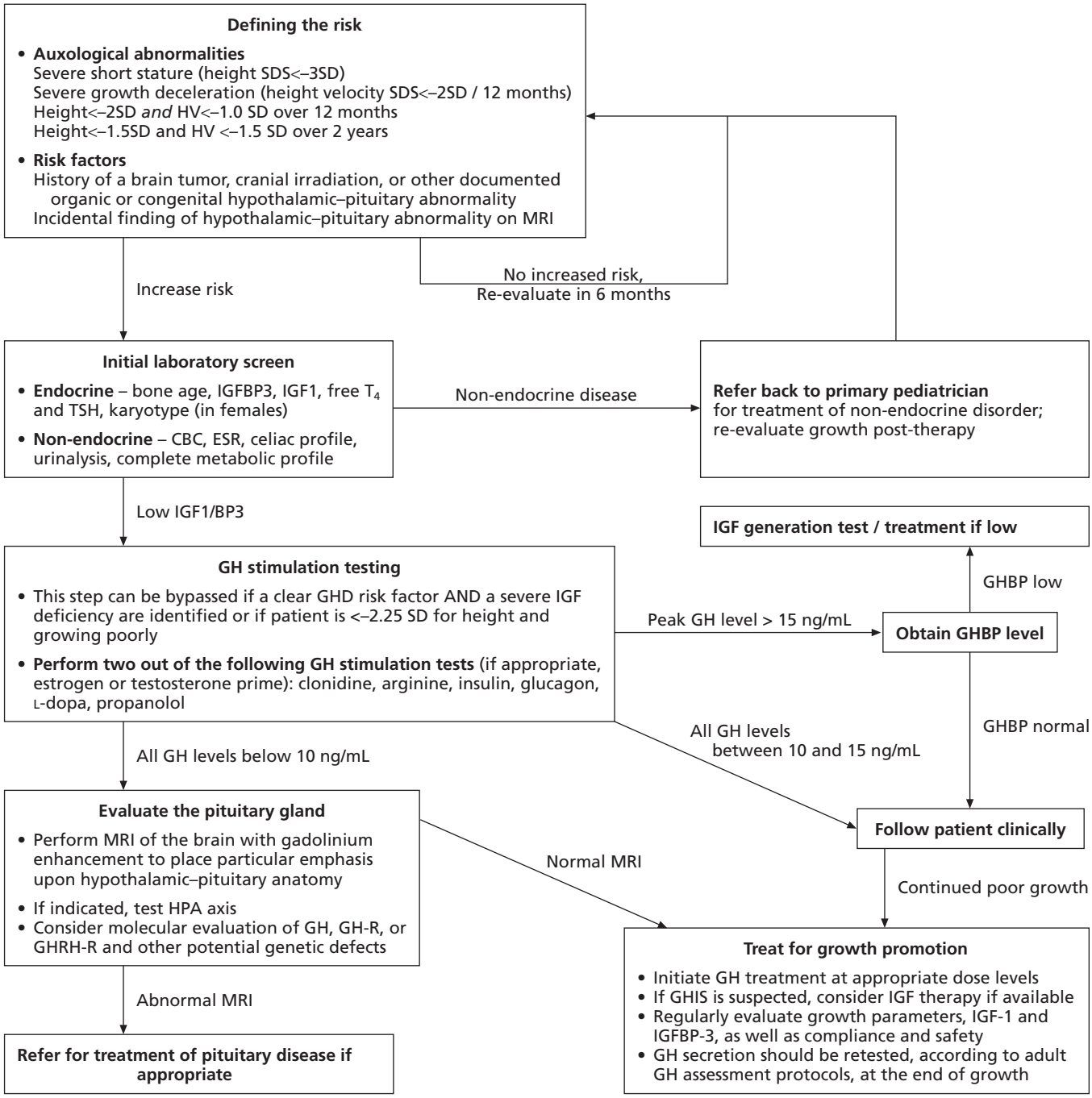

Fig. 7.5. Algorithm for the evaluation of the patient with short stature.

of IGF concentrations and concomitant alterations in serum concentrations of IGFBPs suggests an abnormality of GH secretion or activity and necessitates a thorough evaluation of hypothalamic–pituitary–IGF function.

The basis for diagnosis of IGF deficiency is the calculation of height velocity. In the absence of other evidence of pituitary GH secretory dysfunction, it is usually unnecessary to perform tests of GH secretion. Thus, the documentation of a normal height velocity in children below the fifth centile in height makes the diagnosis of IGF deficiency (and GHD) highly unlikely.

Figure 7.5 provides an algorithm for the evaluation of the child with growth failure. There are many illness-related causes of diminished growth. The possibility of hypothalamic–pituitary dysfunction should always be considered in children with documented growth deceleration, particularly

Table 7.2. Key history and physical examination findings that may indicate that GHD could be present (the GRS 2000 criteria [51]).

In the neonate, hypoglycemia, prolonged jaundice, microphallus, or traumatic delivery
Cranial irradiation
Head trauma or central nervous system infection
Consanguinity and/or an affected family member
Craniofacial midline abnormalities
Severe short stature (< –3 SD)
Height < –2 SD and a height velocity over 1 year < –1 SD
A decrease in height SD of more than 0.5 over 1 year in children over 2 years of age
A height velocity below –2 SD over 1 year
A height velocity more than 1.5 SD below the mean sustained over 2 years
Signs indicative of an intracranial lesion
Signs of multiple pituitary hormone deficiency (MPHD)
Neonatal symptoms and signs of GHD

in the face of known or suspected CNS pathology (tumors, irradiation, malformations, infection, trauma, blindness, nystagmus, etc.). Similarly, the neonate with hypoglycemia and/or microphallus warrants evaluation of pituitary function (including MRI). Children with documented TSH, ACTH, antidiuretic hormone (ADH), or gonadotropin deficiency are candidates for GHD. For children with proportionate short stature and documented growth deceleration, serum IGF-I and IGFBP-3 should be measured and, based upon the results, the possibilities of hypothalamic dysfunction and/or pituitary insufficiency and GHI can be explored.

Recent recommendations by the GH Research Society (GRS) [51] for defining GHD as the cause of IGF deficiency recognize no gold standard for that diagnosis. They propose that, in a child with slow growth, whose history and auxology suggest GHD (Table 7.2), testing for GH/IGF-I deficiency requires the measurement of IGF-I and IGFBP-3 concentrations as well as GH provocation tests (after hypothyroidism has been excluded). When isolated GHD is suspected, two GH provocation tests (sequential or on separate days) are required but, in those with defined CNS pathology, history of irradiation, CPHD, or a genetic defect, one test should be adequate. In patients who have had cranial irradiation or malformations of the hypothalamic–pituitary unit, GHD may evolve over years, and its diagnosis requires serial testing.

Some patients with auxology suggestive of GHD may have IGF-I and/or IGFBP-3 concentrations below the normal range on repeated tests, but GH responses in provocation tests above the "cutoff" level. Such children do not have classical GHD, but may have an abnormality of the GH/IGF axis that could, after the exclusion of systemic disorders affecting the synthesis or action of IGF-I, be considered for GH treatment. Cranial MRI with particular attention to the hypothalamic–pituitary region is needed in any child with GHD. These recommendations stress rational clinical judgment rather than specific tests in the diagnosis of childhood GHD.

Ultimately, GHD (or IGF deficiency) should be characterized on the basis of combined clinical and laboratory criteria. Short children who have well-documented normal height velocities do not require evaluation of GH secretion, and the finding of normal serum concentrations of IGF-I and/or IGFBP-3 is confirmatory. Children with Turner syndrome and short stature do not need GH provocative testing to qualify for GH therapy, as such treatment is not predicated on abnormal GH secretion. The child with documented growth deceleration must, however, have further evaluation, even if tests of GH secretion appear normal. Documentation of decreased serum IGF-I and IGFBP-3 concentrations affirm the diagnosis of IGF deficiency, and the differential diagnoses of GHD and GHI would need to be considered. The child with a history of cranial irradiation, decreased height velocity, and reduced serum concentrations of IGF-I and IGFBP-3 should be considered to have GHD (or GHR), even in the face of normal provocative tests.

Such patients may have normal IGFBP-3 concentrations with low GH concentrations in pharmacological tests. This approach still leaves a place for measurements of GH secretion. These determinations are critical for distinguishing between GHD and GHI as causes of IGF deficiency. Documentation of abnormal pituitary GH secretion raises the possibility of intracranial tumors and the potential for deficiency of other pituitary hormones. Evaluation for GHD permits concomitant assessment of ACTH/cortisol secretion during insulin-induced hypoglycemia.

The diagnosis of GHD in a newborn may be difficult. The presence of micropenis in a male newborn should always lead to an evaluation of the GH/IGF axis. A GH level must be measured in the presence of neonatal hypoglycemia occurring in the absence of metabolic disorders such as hyperammonemia or carnitine deficiency syndromes. A level of less than 20 mg/L in a polyclonal radioimmunoassay suggests GHD. The use of standard GH stimulation tests is not recommended in neonates, with the possible exception of the glucagon test. Normative data are not available for stimulated serum GH concentrations, but a cutoff of 25 ng/mL is probably appropriate, and stimulated values under 20 ng/mL should certainly raise suspicion. MRI is essential when the diagnosis is suspected and could yield useful information about developmental abnormalities of the hypothalamopituitary area. An IGFBP-3 level is of value for the diagnosis of neonatal GHD, but IGF-I concentrations are rarely helpful because normal values are so low.

In short, a child should be considered a candidate for GH therapy if he/she meets auxological criteria that are supported by biochemical evidence of GH deficiency based on sex steroid-primed provocative tests and/or evidence of IGF deficiency based on the measurement of IGF-I and IGFBP-3 concentrations. Such cases also need to have MRI imaging of the hypothalamus–pituitary and assessment of other pituitary hormone deficiencies. This approach will result in GH

Table 7.3. Tests to provoke growth hormone secretion (modified from [15]).

Stimulus	Dosage	Times samples are taken (min)	Comments
Exercise (screening)	Step climbing; exercise cycle for 10 min	0, 10, 20	Observe child closely when on the steps
Levodopa	< 15 kg: 125 mg 10–30 kg: 250 mg > 30 kg: 500 mg	0, 60, 90	Nausea, rarely vomiting
Clonidine (?screening)	0.15 mg/m^2	0, 30, 60, 90	Tiredness, postural hypotension
Arginine HCl (IV)	0.5 g/kg (max. 30 g) 10% arginine HCl in 0.9% NaCl over 30 min	0, 15, 30, 45, 60	
Insulin (IV)*	0.05–0.1 unit/kg	0, 15, 30, 60, 75, 90, 120	Hypoglycemia, must observe closely
Glucagon (IM)	0.03 mg/kg (max. 1 mg)	0, 30, 60, 90, 120, 150, 180	Nausea, occasional vomiting
GHRH (IV) (often with arginine)	1 μg/kg	0, 15, 30, 45, 60, 90, 120	Flushing, metallic taste

Tests should be performed after an overnight fast. Many investigators suggest that prepubertal children should be "primed" with gonadal steroids, e.g. 5 mg of Premarin orally the night before and the morning of the test, or with 50–100 μg/day ethinylestradiol for three consecutive days before testing, or 100 mg of depot testosterone 3 days before testing. Patients must be euthyroid at the time of testing.

*Insulin-induced hypoglycemia is a potential risk of this procedure, which is designed to lower the blood glucose by at least 50%. Documentation of appropriate lowering of blood glucose is recommended. If GHD is suspected, the lower dosage of insulin is usually administered, especially in infants. $D_{10}W$ and glucagon should be available.

treatment of some children with "idiopathic, isolated" GH deficiency or IGF deficiency, and such cases require careful monitoring of both pituitary status and responsiveness to GH treatment. The latter can be assessed relative to recently developed predictive models [52], and the diagnosis of GHD needs to be reconsidered in the child with idiopathic, isolated GHD, a normal MRI, and a subnormal clinical response to GH.

Table 7.4. Critical problems with standard GH provocative testing*.

Provocative GH testing is non-physiological
Arbitrary definitions of "subnormal" response to provocative tests
Age dependency and variable use of "priming" gonadal steroids
Variability of GH assay potency estimates limits discriminatory power
Expense, discomfort, and risks of provocative GH testing
Poor reproducibility of provocative tests

*Modified from [15,53].

GH deficiency

Assessment of pituitary GH production is difficult because GH secretion is pulsatile and varies with gender, age, pubertal stage, and nutritional status. Between normal pulses of GH secretion, serum GH concentrations are low (< 0.1 μg/L), often below the limits of sensitivity of most conventional assays (usually < 0.2 μg/L). Accordingly, measurement of a random serum GH concentration is virtually useless in diagnosing GHD, but may be useful in the diagnosis of GHI and GH excess.

Measurement of GH "secretory reserve" relies on the use of physiological or pharmacological stimuli, and such provocative tests (Table 7.3) have been the basis for the diagnosis of GHD for over 30 years. Physiological stimuli include fasting, sleep, and exercise; pharmacological stimuli include levodopa, clonidine, glucagon, propranalol, arginine, and insulin. Stimulation tests have often been divided into screening tests (exercise, fasting, levodopa, clonidine), which are characterized by ease of administration, low toxicity, low risk, and low specificity, and definitive tests (arginine, insulin, glucagon). To improve specificity, provocative tests are customarily combined or given sequentially, such as fasting + oral clonidine as a screening test to be followed by fasting + intravenous sequential arginine, oral clonidine, levodopa, or insulin as a definitive test. It is generally accepted that a child must fail provocative tests with at least two separate stimuli to be considered GHD.

Although provocative GH testing has been the foundation for the diagnosis of GHD since GH assays first became available, the appropriateness of such studies has been severely criticized for a number of reasons (Table 7.4 [53]). Another diagnostic approach involves measurement of spontaneous GH secretion by multiple sampling over 12–24 h. This approach is subject to many of the same limitations as provocative GH testing. The expense and discomfort of such testing are obvious and, although initially thought to be more reproducible than provocative GH tests, variability is a problem. The ability of such tests to discriminate between GHD and normal short children is also an issue. Rose *et al*. [54] reported that measurement of spontaneous GH secretion identified only 57% of children with GHD as defined by provocative testing. A longitudinal study of normal boys through puberty demonstrated a wide intersubject variance,

Table 7.5. Potential limitations of IGF-I determinations [15].

IGFBPs may interfere with assays so must be removed effectively
IGF-I concentrations are age dependent, being especially low in very young children in whom discrimination from normalcy may be difficult
IGF-I concentrations may also be very low in cases of primary or secondary GHI
IGF-I (and IGFBP-3) may be normal in adult-onset GHD or in patients with CNS tumors
Interlaboratory variation may be substantial

Table 7.6. Potential advantages of IGFBP-3 assay determinations (modified from [15]).

Technically simple, not requiring dissociation of a binding protein from the IGF peptide
Normal values are in the 1–5 mg/L range, obviating assay sensitivity issues
Less age variation than with IGF-I, allowing separation from "low" values in infancy
Less affected by other factors such as nutrition
IGFBP-3 is highly GH dependent

including many low 24-h GH production rates, despite fully normal growth [2].

Given the problems with GH testing, it is not surprising that provocative tests and 24-h GH profiles do not always correlate. Most children with GHD can be identified by 12–24-h GH profiles, which are superior, in both sensitivity and specificity, to provocative GH testing. Neurosecretory dysfunction probably does exist in children after cranial irradiation and characterizes a subgroup of children with GHD and IGF deficiency. The expense and discomfort of such GH profiles and the problems in GH determinations preclude them from being the test of choice in establishing the diagnosis of GHD.

An alternative means of diagnosing GHD is the assessment of the GH-dependent peptides IGF-I and -II and their binding proteins, especially IGFBP-3. GHD then becomes part of the differential diagnosis of IGF deficiency, which includes hypothalamic dysfunction, pituitary insufficiency, and GHI. With the development of sensitive and specific assays for IGF-I, IGF-II, and the IGFBPs, it is clear that these peptides accurately reflect integrated GH status. Furthermore, IGF-I and -II normally circulate in serum in sufficiently high concentrations such that assay sensitivity is not an issue. Serum concentrations are relatively constant during the day, so that provocative testing or multiple sampling is not necessary. However, IGF-I assays do have potential limitations, as listed in Table 7.5.

Even when these caveats are considered, the correlation between serum IGF-I concentrations and provocative or spontaneous GH measurements is imperfect. In a group of short children, IGF-I concentrations were below –2 SD in only 20/31 children with a diagnosis of GHD based upon provocative testing (64.5% sensitivity), and were normal in 65/73 children with a normal GH response (89% specificity) [55,56]. This imperfect correlation probably reflects limitations of GH testing rather than inadequacies of IGF measurements. When serum concentrations of both IGF-I and -II are determined, the correlation with GH testing improves, because serum IGF-II concentrations are low in GHD and normally do not increase with age after 1 year [57]. In one study, 18% of patients with low provocative GH concentrations had IGF-I concentrations in the normal range, but only 4% of GHD patients had normal serum concentrations of both IGF-I and -II [57]. Serum concentrations of both IGF-I and -II were reduced in only 0.5% of normal children and in 11% of normal short children.

The assay of GH-dependent IGFBP-3, normally the major serum carrier of IGF peptides, is an additional means of diagnosing IGF deficiency resulting from GHD because the concentrations of IGFBP-3 correlate with the sum of the concentrations of IGF-I and -II. The potential advantages of this peptide determination are listed in Table 7.6.

The use of IGFBP-3 assays in the diagnosis of GHD was evaluated by Blum and colleagues [58], who found that serum IGFBP-3 concentrations were below the fifth centile for age in 128/132 children (97%) diagnosed as GHD by conventional criteria. At the same time, 124/130 (95%) non-GHD, short children had normal IGFBP-3 concentrations. The former group consisted largely of children with severe GHD, as such a clear correlation between provocative GH testing and serum IGFBP-3 concentrations has not been observed consistently. For example, in one study, the sensitivity of the IGFBP-3 assay in complete GHD was 93% but only 43% in partial GHD [59]. Juul and Skakkebaek found that 47/48 prepubertal but only 74/94 pubertal children with normal GH provocative test results had normal IGFBP-3 concentrations [56]. IGFBP-3 concentrations may be falsely normal in patients with GHD as a result of intracranial lesions. The correlation between IGF and IGFBP-3 concentrations and assessments of spontaneous GH secretion is also imperfect. Even in normal children, the correlation between 24-h GH secretion and serum IGF-I and IGFBP-3 concentrations is modest. The measurement of the GH-dependent peptides may however be of value in recognizing that concentrations higher than approximately –1 SD for either could obviate the necessity for formal GH provocative testing.

It is not possible fully to resolve conflicts between assays of the IGF axis and measurements of GH secretion, as there is no definitive way to diagnose GHD. Studies of patients with GH receptor deficiency support the use of IGF-related determinations. Although such patients may have normal or elevated serum GH concentrations, mutations or deletions of the GH receptor gene render them unresponsive to GH, making them "functionally GH deficient." In approximately 70

Table 7.7. Proposed classification of growth hormone insensitivity syndromes [61].

Primary GH insensitivity (hereditary defects)
GH receptor defect (may be positive or negative for GH-binding protein)
Extracellular mutation
Cytoplasmic mutation
Intracellular mutation
GH signal transduction defect (distal to cytoplasmic domain of GH receptor)
Insulin-like growth factor-I (IGF-I) synthetic defect (IGF-I gene deletion)
IGF-I transport defect
IGF-I receptor defect
Bioinactive GH molecule
Secondary GH insensitivity (acquired defects)
Circulating antibodies to GH that inhibit GH action
Antibodies to the GH receptor
GH insensitivity caused by malnutrition, liver disease, catabolic states, etc.
Other conditions that cause GH insensitivity

In order to supplement this revised classification, the following definitions are proposed:
GH insensitivity: clinical and biochemical features of IGF-I deficiency and resistance to exogenous GH, associated with GH secretion that would not be considered abnormally low.
GH insensitivity syndrome: GH insensitivity associated with the recognizable dysmorphic features described by Laron *et al*. [109].
Partial GH insensitivity: GH insensitivity in the absence of dysmorphic features described by Laron *et al*. [109].

instances of GH receptor gene mutations, all had markedly reduced serum concentrations of both IGF-I and IGFBP-3 [60], with both IGF-I and IGFBP-3 correlating significantly with height.

GH insensitivity (GHI) (Table 7.7)

The combination of decreased serum concentrations of IGF-I, IGF-II, and IGFBP-3 with increased serum concentrations of GH is highly suggestive of a diagnosis of GHI [16]. The possibility of GH receptor deficiency (GHRD) is supported by a family history consistent with autosomal-recessive transmission. Much more commonly, GHI is secondary to chronic illness.

Savage and Rosenfeld [61] devised a scoring system for evaluating short children for the diagnosis of GH receptor deficiency, based upon five parameters: (1) basal serum GH > 10 mU/L (approximately 5 Bg/L); (2) serum IGF-I ≤ 50 Bg/L; (3) height SDS < –3; (4) serum GHBP < 10% (based upon binding of [^{125}I]GH); and (5) a rise in serum IGF-I concentrations after GH administration of more than twofold the intra-assay variation (approximately 10%). Blum *et al*. [62] proposed that these criteria could be strengthened by: (1) evaluating GH secretory profiles, rather than isolated basal concentrations; (2) employing an age-dependent range and the 0.1 centile as the cutoff level for evaluation of serum IGF-I concentrations; (3) employing highly sensitive IGF-I immunoassays and defining a failed GH response as the inability to increase serum IGF-I concentrations by at least 15 Bg/L; and (4) measuring both basal and GH-stimulated IGFBP-3 concentrations.

These criteria fit well with the population of GHRD patients in Ecuador, but that is a homogeneous population with severe GHI [16]. The applicability of these criteria elsewhere remains to be evaluated.

An important biochemical marker is the response of IGF-I and IGFBP-3 to GH stimulation. Normal ranges and age-defined responses to GH of serum IGF-I and IGFBP-3 concentrations have been established in this Ecuadorian population and enabled separation of patients with GHD and GHI from normal subjects [63,64].

Idiopathic short stature

Many children and early adolescents are short (< third centile) with slowed linear growth velocity (< 25th centile), delayed skeletal maturation, and an impaired or attenuated pubertal growth spurt. There may or may not be a family history. Not all children have all these clinical features, but they have no chronic illness or apparent endocrinopathy. They usually have normal GH secretory dynamics, although provocative tests may be blunted under some circumstances. GH-dependent peptides are lower than expected on a chronological, although usually not skeletal, age basis. Treatment with exogenous GH usually augments linear growth.

Such children are usually (and possibly inappropriately) considered to be variants of normal and achieve a final adult height within the lower part (or often below) of the family target range. The etiology of the slowed childhood growth and frequently delayed pubertal spurt has not been established in most such children. As this is the largest group of short children, efforts are continuing to develop a rational classification and a means of separating these children from those with an abnormality of the GH/IGF axis.

Constitutional delay in growth and puberty (CDGP)

The term constitutional delay describes children with a normal variant of pubertal tempo characterized by short stature but relatively normal growth rates during childhood, delayed puberty with a late and attenuated pubertal growth spurt, and attainment of normal adult height. Most children with constitutional delay begin to deviate from the normal growth curve during the early years of life and, by age 2 years, are at or slightly below the fifth centile for height. During mid-childhood years, height SDS may gradually drift lower, but this does not appear to affect adult height

Table 7.8. Criteria for presumptive diagnosis of constitutional delay of growth and puberty*.

No history of systemic or chronic illness
Normal nutrition
Normal physical examination, including body proportions
Normal thyroid function and GH/IGF axis screening tests
Normal complete blood count (CBC), sedimentation rate, electrolytes, blood ureanitrogen (BUN), creatinine
Height at or below the third centile but with annual growth rate < fifth centile for age
Delayed puberty:
Males: failure to achieve Tanner G2 stage by age 13.8 years or P2 by 15.6 years
Females: failure to achieve Tanner B2 stage by age 13.3 years
Delayed bone age
Normal predicted adult height:
Males: > 163 cm (64 in)
Females: > 150 cm (59 in)

*Modified from [15].

outcome. Final height, although usually within the normal population range, is often in the lower part of the parental height target zone. The predicted final height, especially when the skeletal age is extremely delayed, exceeds the true adult stature. The delayed growth spurt may adversely affect spinal and vertebral mineralization, which may not be overcome when pubertal growth finally accelerates. The consequences of this altered tempo of bone calcium accretion could be a risk factor for osteoporosis in later adult life.

GH secretion may be decreased, with transient partial GHD at the time of the delayed pubertal growth spurt apparently the consequence of inadequate production of gonadal steroids. Such children have delayed skeletal ages, normal or slightly low serum IGF-I, but usually normal IGFBP-3 concentrations for skeletal age and normal GH provocative tests (if pretreated with gonadal steroids). By definition, children with pure CDGP should have bone ages sufficiently delayed to result in normal predicted adult heights (> 163 cm in males and > 150 cm in females) (Table 7.8), although the correlation between predicted and final height is imperfect and must be viewed with caution. When CDGP occurs in the context of familial short stature, children may experience both a delayed adolescent growth spurt and a short final height.

Some have attributed the diminished growth in the peripubertal period in CDGP to a transient GH deficiency or to a lazy pituitary, a concept that is probably due to the inadequacies of GH testing, especially to the failure to pretreat patients with a brief course of gonadal steroids. Low serum concentrations of IGF-I and IGFBP-3 and/or a poor GH response to provocative testing (after priming with gonadal steroids) should mandate an investigation for underlying pathology, such as intracranial tumors.

Genetic (familial) short stature

The control of growth in childhood and the final height attained are polygenic in nature. For this reason, familial height impacts upon the growth of an individual, and evaluation of a specific growth pattern must be placed in the context of familial growth and stature. Formulae have been developed to determine parental target height, and growth curves that relate a child's height to parental height are available [15]. As a general rule, a child who is growing at a rate that is inconsistent with that of siblings or parents warrants further evaluation.

Many organic diseases characterized by growth retardation are transmitted genetically. This includes GH insensitivity due to mutations of the GH receptor gene, GH gene deletions, mutations of the PROP-1 or POUF-1 gene, pseudohypoparathyroidism, diabetes mellitus, and some forms of hypothyroidism. Inherited non-endocrine diseases characterized by short stature include skeletal dysplasias, dysmorphic syndromes associated with IUGR, inborn errors of metabolism, renal disease, and thalassemia. Identifying short stature as inherited thus does not, by itself, relieve the physician of responsibility for determining the underlying cause of growth failure.

Nonetheless, a constellation of clinical findings describes a condition referred to as genetic short stature (GSS) (or familial short stature) that differs from the syndrome of CDGP discussed above. In GSS, childhood growth is at or below the fifth centile, but the velocity is generally normal. The onset and progression of puberty is normal or even slightly early and more rapid than normal, so that skeletal age is concordant with chronological age. Parental height is short (both parents are often below the 10th centile), and puberty is normal. Final heights in these individuals are short and in the target zone for the family. The GH/IGF system is apparently normal, but exogenous GH therapy during the middle childhood years may increase linear growth velocity substantially without disproportionate augmentation of skeletal maturation.

Heterozygous mutations of the GH receptor

The concentration of the GH receptor may be genetically determined, although modulated by such factors as nutritional status; GH production appears to be inversely related to GH receptor/GHBP concentrations. Accordingly, GHBP concentrations have been assessed in subjects with idiopathic short stature [65]. Serum concentrations of GHBP in 90% of these children are lower than the normal mean, 20% being below the normal range, especially a subgroup with low IGF-I and higher mean 12-h concentrations of GH [65]. Such data raise the possibility that an abnormality of GH receptor content or structure could impair GH action. The inverse

relationship of GHBP concentrations to GH production is consistent with this hypothesis. In a small group of patients with growth failure, low concentrations of IGF-I and poor response to exogenous GH, heterozygous GH receptor mutations were present in 28% [66].

In contrast to the rarity of homozygous GH receptor mutations in GHR, heterozygosity is more common and may be a frequent cause of short stature. In heterozygotes, protein from the mutant allele may disrupt the normal dimerization that occurs when GH interacts with its receptor, leading to diminished GH action and growth impairment. The IGF-I/IGFBP-3 generation test following 4 days of GH administration may reveal individual patients with findings of low basal and provoked peptides and modestly elevated GH concentrations that might represent partial GHI. Detailed biochemical and genetic confirmation of abnormalities in the post-GH cascade would appear reasonable in these children. It is likely that new diagnoses, especially in errors of post-receptor genes, will continue to emerge.

Treatment of growth disorders

When growth failure is the result of a chronic underlying disease, such as renal failure, cystic fibrosis, or malabsorption, therapy must be directed at treatment of the underlying condition. Although growth acceleration may occur in such children with GH or IGF-I therapy, complete catch-up requires correction of the primary medical problem. If treatment of the underlying condition involves glucocorticoids, growth failure may be profound and is unlikely to be correctable until steroids are reduced or discontinued.

Correction of growth failure associated with chronic hypothyroidism requires appropriate thyroid replacement. As discussed earlier, thyroid therapy causes dramatic catch-up growth but also markedly accelerates skeletal maturation, potentially limiting adult height. More gradual thyroid replacement and/or the use of gonadotropin inhibitors to delay puberty may be necessary to obtain maximal final height.

Constitutional delay in growth and puberty (CDGP)

This normal variant can be managed by careful evaluation to rule out other causes of abnormal growth and/or delayed puberty combined with appropriate explanation and counseling. If short stature and delayed puberty are psychologically disabling the preadolescent or teenager with poor self-image and diminished socialization, there is a place for the short-term use of gonadal steroids, usually depot testosterone. Both short stature and delayed puberty are addressed by androgen treatment. It is important to emphasize to the patient that he is normal, that the treatment is short term and designed to provide some pubertal development earlier than he would on his own, but that it will not increase adult height. In view of the important role of estrogen in the process of skeletal maturation, aromatase inhibitors could be used in conjunction with androgen therapy to prevent an acceleration of bone age and further enhance final adult height. The use of non-aromatizable androgens (e.g. oxandrolone) has been another approach to treat the short stature component without advancing bone age.

One must be assured that such patients do enter into true puberty. In the year after testosterone treatment, boys should achieve testicular enlargement and a serum testosterone concentration in the pubertal range. The diagnosis of hypothalamo-pituitary insufficiency or hypogonadotrophic hypogonadism should be considered if such progression does not occur. Although the diagnosis of constitutional growth delay remains most likely in such patients, some eventually do prove to be gonadotropin deficient especially if still prepubertal late in teenage. Referrals for constitutional delay are more common in boys than in girls, probably reflecting cultural values. When constitutional delay is a problem in girls, short-term estrogen therapy can be used, but the advancement of bone is a greater hazard when doses that enhance growth velocity and sexual puberty are used.

Treatment of growth hormone deficiency

The names for the various biosynthetic GH preparations reflect the source and the chemical composition of the product. Somatrophin refers to GH of the same amino acid sequence as that in naturally occurring human GH. Somatrophin from human pituitary glands is abbreviated GH or pit-GH; recombinant origin somatrophin is termed recombinant GH or rGH (we use the generic term GH). Somatrem refers to the methionine derivative of recombinant GH and is abbreviated met-rGH; it is no longer used.

Historical perspective [15]

As untreated patients with GH deficiency have profound short stature (averaging nearly –5 SDS), there was clinical urgency to use GH therapy as soon as it was available. The action of GH is highly species specific, and humans do not respond to animal-derived GH. Human cadaver pituitary glands were for many years the only practical source of primate GH for treatment of GHD, and more than 27 000 children with GHD worldwide were treated with pit-GH [67]. The limited supplies of pit-GH, low doses, and interrupted treatment regimens resulted in incomplete growth increments. Therapy was usually discontinued in boys who reached 5 feet 5 inches or 165 cm and in girls who reached 5 feet or 150 cm. This treatment did increase linear growth and enhanced final adult height. The dose–response relationship and the relation of age to GH response were recognized during this period.

Distribution of pit-GH was halted in the United States and most of Europe in 1985 because of concern about a causal

Table 7.9. New modalities for treatment of GHD.

Liquid formulations
Pen-type delivery devices
Needleless devices
Oral secretagogues
Long-acting GH formulations
GHRH
Inhaled GH delivery systems (theoretical)
GH transdermal patches (theoretical)

relationship with Creutzfeldt–Jakob Disease (CJD), a rare and fatal spongiform encephalopathy that had been reported previously to be capable of iatrogenic transmission through human tissue (reviewed in April 2003 at www.niddk.nih.gov/health/endo/pubs/creutz/update.htm). In North America and Europe, this disorder has an incidence of approximately one case per million in the general population; it is exceedingly rare before the age of 50 years. To date, over 160 young adults who had received human cadaver pituitary products have been identified as cases of CJD, with the sad likelihood that all will die from the disease. In patients in the United States, the onset of CJD was 14–33 years after starting treatment, while the large cohort of French patients had a median incubation period approximately 5 years shorter [68]. Vigilant surveillance for this dreadful complication continues. Fortunately, by the time the risks of pituitary-derived GH were discovered, rGH was being tested for safety and efficacy.

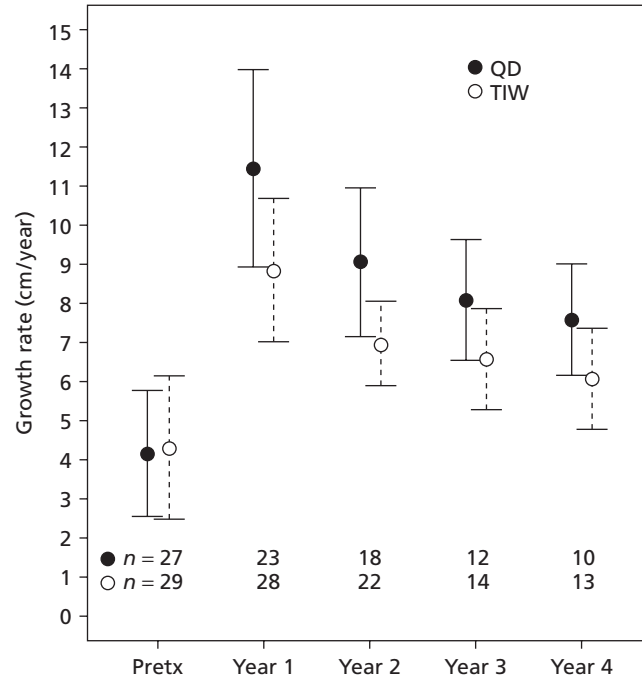

Fig. 7.6. Annual growth velocity (mean ± SD) for prepubertal patients with growth hormone (GH) deficiency prior to and during 4 years of GH treatment, contrasting results with daily (QD) and thrice-weekly (TIW) injections. The mean annual growth velocity in the QD group was significantly greater than that of the thrice-weekly group during each year, although significance diminished from year 1 to year 4 (from [71]).

Treatment regimens

The recommended starting dose in GHD is 0.18–0.35 mg/kg body weight per week, administered in seven daily doses, with the mean American dose being 0.3 mg/kg/week. Alternative regimens include a 6 day/week or 3 day/week schedule, with the same weekly dosage. In general, the growth response to GH is a function of the log dose given, so that increasing dosage further enhances the growth rate [52,67], but daily dosing may be the more important treatment parameter. Either subcutaneous or intramuscular administration has equivalent growth-promoting activity; the former is now used almost exclusively.

GH is available in several vehicles, and multiple systems are now available for administering GH (Table 7.9). The standard preparation is lyophilized GH, which is highly water soluble so may be brought into solution with a small volume of diluent. An aqueous solution is "ready to use" and has 28-day stability. A sustained release preparation of GH with protein integrity in a poly (lactide–coglycolide) polymer that is biocompatible and biodegradable permits once- or twice-monthly treatments [69]. Either reconstituted or liquid GH is administered in insulin syringes with ultrafine needles that are almost pain free in skilled hands. Pen devices with internal reconstitution of GH are frequently used because of ease, accuracy, and "hidden needles." Needle-free, jet injector systems are available and yield a normal serum immunoreactive and bioactive GH profile [70]. The sustained release GH is administered through a short, larger bored needle, but the injection pain is balanced against the low frequency of treatments. At this time, all GH preparations yield comparable short-term growth outcomes, except long-acting GH, in which mean first year growth rates are around 2 cm lower per year.

Growth responses to exogenous GH vary, depending on the frequency of administration, dosage, age (greater absolute gain in a younger child, although not necessarily of growth velocity SDS), weight, and GH receptor amount, as assessed by serum GHBP concentrations. On this general regimen, nonetheless, the typical GHD child accelerates growth from a pretreatment rate of 3–4 cm/year to 10–12 cm/year in year 1 of therapy and 7–9 cm/year in years 2 and 3. Progressive waning of GH efficacy occurs and is poorly understood. The importance of dosage frequency is illustrated in Figures 7.6 and 7.7 by growth responses of prepubertal naive GHD children randomly assigned to receive thrice-weekly or daily GH at the same total weekly dose (0.30 mg/kg/week) [71]. The mean total height gain was 9.7 cm greater in the daily treated patients (38.4 vs. 28.7 cm, $P < 0.0002$) with comparable bone

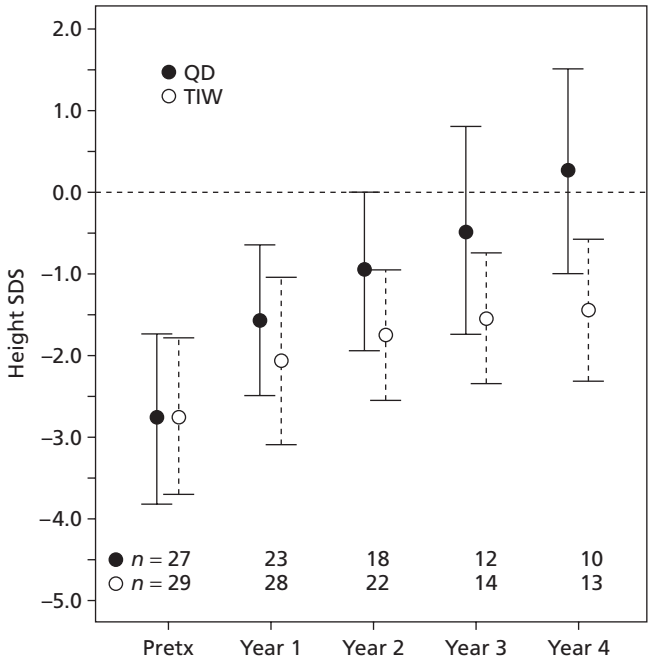

Fig. 7.7. Height standard deviation score (SDS) (mean ± SD) for prepubertal patients with growth hormone deficiency (GHD) before and during 4 years of growth hormone (GH) treatment, contrasting results with daily (QD) and thrice-weekly injections (TIW). The mean SDS in the QD group was significantly greater throughout the treatment period. Younger patients had the greatest increase in height SDS, and the effect of age was more marked in the QD group (from [71]).

age advancement and no acceleration of pubertal onset. At a dosage of 0.30 mg/kg/week, the approximate current cost of GH therapy for a 20-kg child is $12–15 000/year.

Sophisticated mathematical models [52] have examined many laboratory and auxological parameters that influence response to GH therapy. As age at onset of treatment is inversely correlated with growth responses and the smaller, lighter child requires less GH (with marked economic benefit), it is important to assess growth data in early treated children. In short-term studies of 134 patients [15], treated before age 3 years, marked early catch-up occurred with a mean height gain of around 3 SDS by 4 years of therapy allowing most children to reach the normal height range by mid-childhood. Mean height in one study [25] reached −0.4 SDS after 8 years of treatment. Near-adult height data in 13 patients treated before 5 years [72] did not differ from the mid-parental target height (−0.9 vs. −0.7 SDS).

Longer term/final height results

Patients treated largely with biosynthetic GH [15] have improved actual or near-final adult height SDS, with average final height in more than 1400 patients approximating −1.3 SD below the mean. Data from the two largest databases [29,30], representing the North American and European experiences as reported by pediatric endocrinologists, are shown in Table 7.10.

Despite this availability of GH therapy, long-term studies still show that most patients fail to achieve their genetic target heights. Evaluation of adult heights in 121 patients with childhood GHD treated in Genentech GH research trials indicates a mean adult height in both male and female patients of −0.7 SDS, with 106 (87%) being within 2 SDS for normal adult Americans [73]. Even in these closely followed patients, however, a −0.4 to −0.6 SDS difference from mid-parental targeted height occurred. The achievement of the genetic target is possible, however, as a Swedish subgroup of consistently treated patients reached a median final height SDS of −0.32, which was equivalent to the mid-parental target height [29]. By multiple regression analysis, factors found to correlate with enhanced adult height were baseline height, younger age at onset of treatment, longer treatment duration, and a greater growth velocity during the first year of treatment.

While the development of recombinant GH has solved the problem of supply experienced in the pituitary GH era, delays in diagnosis and initiation of therapy have still compromised adult height. The extreme importance of the amount of growth achieved in GH-treated prepubertal children in predicting their total growth has been amply demonstrated.

Table 7.10. Adult height in children with GHD treated with biosynthetic GH.

Study	Sex	*n*	Dose	Duration (years)	Age (years)	Ht SDS	Δ Ht SDS	Ht vs. MPH
KIGS [110,111]	M	154	0.16	8.3	18.9	−0.9	+1.8	−0.3
	F	115	0.18	8.0	17.1	−1.2	+1.6	−0.7
NCGS [75,112]	M	2095	0.28	5.2	18.2	−1.1	+1.4	−0.7
	F	1116	0.29	5.0	16.7	−1.3	+1.6	−0.9

GH dose is mg/kg/week; MPH is the mid-parental target height. KIGS is the Pharmacia (now Pfizer) international growth database. NCGS is the Genentech National Cooperative Growth Study.

Despite the recognition that the prepubertal growth achievement is critical, attempts to increase total pubertal growth have also been made. In an effort to increase final height of GHD patients, the use of high-dose GH during puberty has been studied. This was based on the rationale that GH secretion normally rises two- to fourfold during the pubertal growth spurt with dramatic concomitant increases in serum IGF-I concentrations, and that the pubertal growth spurt normally accounts for approximately 17% of adult male height and 12% of adult female height. Mauras *et al*. [74] evaluated higher pubertal GH doses (0.1 vs. 0.043 mg/kg/day) and found that the higher dosage resulted in a 4.6-cm increase in near-final height. Mean height SDS achieved in the 0.043 mg/kg/day group (as in an earlier report [73]) was −0.7 ± 0.9 but 0.0 ± 1.2 in the 0.1 mg/kg/day group. The higher GH dosage did not result in more rapid acceleration of bone age, but was often associated with concentrations of IGF-I exceeding upper limits of normal.

Another attempt at enhancing GH-induced growth during puberty was the addition of gonadotropin-releasing hormone agonists to prolong the process of skeletal maturation; this treatment regimen has had mixed results. Blocking estrogen production with aromatase inhibition or estrogen receptor binding with estrogen receptor antagonists are likely to diminish the rate of skeletal maturation.

The use of higher doses of GH, the ability to treat until growth cessation, early initiation of treatment, progressive weight-related dose increments, attention to compliance with daily administration, and appropriate thyroid hormone and glucocorticoid replacement therapy are important factors in improved adult height outcomes. As final height correlates with height at the onset of puberty in GHD patients, every effort must be made to enhance growth velocity during prepuberty. In data from NCGS and KIGS, the height gained during puberty in patients with GHD was generally comparable to that in healthy children with delayed bone ages [29,75]. The total pubertal height gain is negatively correlated with the age of pubertal onset. When normal or precocious puberty limits the response to GH, it may be appropriate to delay puberty by the use of a GnRH analog. Use of this strategy in pubertal GHD patient groups, however suggestive, is not yet clearly documented to enhance final height [15]. Nevertheless, the earlier the age of pubertal onset, the lower the final height outcome, and GHD patients with delayed puberty or hypogonadotropic hypogonadism have a taller adult height.

GH treatment of Prader–Willi syndrome

GH treatment of growth failure in PWS is now an FDA-approved indication with the recommended dose being approximately 1 mg/m^2/day or 0.24 mg/kg/week. Many clinical trials document the efficacy of GH and confirm that the characteristic IGF deficiency state is due to GH deficiency. After 5 years of GH therapy, Lindgren and Ritzen [76] (at a dose of 0.23 mg/kg/week) showed that mean height SDS approached 0.5 with a gain of nearly 2 SDS. Other shorter (6 to 24 months) treatment programs have found increased growth rates but also provide important information on the changes in abnormal metabolic parameters during GH treatment. Such data demonstrate reduction in body fat mass and percentage, increased fat-free mass, improved muscle strength and agility, and increased fat oxidation [77–80]. Respiratory muscle weakness, which is found in PWS, was improved after GH treatment [78]. In view of the association of insulin resistance and type 2 diabetes with obesity, glycemic status should be monitored in GH-treated patients with PWS [43]. Recent data also suggest a risk of death related to upper airway obstruction in these GH-treated children [44].

Combined pituitary hormone deficiencies

If GHD is part of a multiple pituitary insufficiency, it is necessary to address each endocrine deficiency both for general medical reasons and to ensure maximal effect of GH therapy. TSH deficiency is often unmasked during the initial phase of therapy, and thyroid function should be assessed both before the onset of therapy and during the first 3 months of GH treatment, and annually thereafter. The pituitary–adrenal axis is evaluated during the insulin stimulation test in the workup for GHD. If ACTH secretion is impaired, patients should be placed on the lowest safe maintenance dose of glucocorticoids, certainly no more than 8–10 mg/m^2/day hydrocortisone and less if possible. Higher doses may impair the growth response to GH therapy but are necessary during times of stress.

Gonadotropin deficiency may be evident in the infant with microphallus. This can usually be treated with 3- to 4-monthly injections of 25 mg of testosterone enanthate. Management at puberty is more complicated, as physical and psychological benefits of promoting sexual puberty are juxtaposed with the effects upon epiphyseal fusion. When GH therapy is initiated in childhood and growth is normal before puberty, it is appropriate to begin gonadal steroid replacement at a normal age (e.g. 11–12 years in girls and 12–13 years in boys). In boys, this can be done by beginning with monthly injections of 50–100 mg of testosterone enanthate, gradually increasing to 200 mg/month, and eventually moving to the appropriate adult replacement regimen as determined by the monitoring of plasma testosterone concentrations. In girls, therapy involves the use of very low doses of conjugated estrogens or ethinylestradiol and eventual cycling with estrogen and progesterone.

Monitoring GH therapy (Table 7. 11)

While most pediatric endocrinologists simply document changes in growth velocity as the signal parameter of

Table 7.11. Elements of monitoring GH therapy.

Close follow-up with a pediatric endocrinologist every 3–4 months
Determination of growth response (change in height *z*-score)
Annual measurement of serum IGF-I and IGFBP-3 concentrations and fasting glucose/insulin ratio
Screening for potential adverse effects
Evaluation of compliance
Consideration of dose adjustment based on IGF values, growth response, and comparison with growth prediction models

therapeutic efficacy, this may not be sufficient. Treatment models that predict growth rate with quite narrow confidence limits provide quantitative estimates of whether the individual patient is responding appropriately to GH. A model [52] explaining 61% of growth response variability for the first year of therapy includes inverse relationships with maximum GH response during provocative testing, age and height SDS minus mid-parental height SDS, and positive correlation with body weight SDS, GH dose, and birthweight SDS. The most important predictive factor for years 2 through 4 is the first-year height velocity. Indeed, unpublished data from KIGS show that the amount of growth during the first year is also predictive of total growth achieved during GH treatment. Clearly, after age at diagnosis, GH dose management is the variable most affected by the physician.

Changes in the GH-dependent peptides, IGF-I, IGFBP-3, and the acid-labile subunit (ALS), along with the aggregate ternary complex correlate with growth responses. Measurement of IGF-I and possibly of IGFBP-3 may give added information on the growth-promoting and fat-mobilizing actions of GH, as well as of the spectrum of childhood responsivity to exogenous GH. Use of auxological parameters in conjunction with scrutiny of the concentrations of the GH-dependent peptides will enhance our ability to optimize and individualize the treatment program. Safety monitoring should include yearly assessment of IGF-I, IGFBP-3, and fasting glucose/insulin ratios.

Poor growth responses

The growth response to GH diminishes after several years but should continue to be equal to or greater than the normal height velocity for age throughout treatment. A suboptimal response to GH can result from several causes: (1) poor compliance; (2) improper preparation of GH for administration or incorrect injection techniques; (3) subclinical hypothyroidism; (4) co-existing systemic disease; (5) excessive glucocorticoid therapy; (6) prior irradiation of the spine; (7) epiphyseal fusion; (8) anti-GH antibodies; (9) incorrect diagnosis of GHD as an explanation for growth retardation; or (10) the presence of true GH resistance. Although 10–20% of recipients of recombinant GH develop anti-GH antibodies, growth failure is rarely, if ever, due to such antibodies. Maximal growth response to GH should follow early diagnosis and initiation of therapy and careful attention to compliance with psychological support. Although in earlier studies, many boys and, especially, girls with idiopathic GHD did not achieve normal adult heights, normal height (i.e. reaching the family-specific target height) should be attained in most cases.

GH treatment during the transition to adulthood and in adulthood

The clinical consequences of GHD in adults and the potential benefits of GH therapy in such patients are becoming apparent [81,82]. Signs and symptoms of adult GHD have included reduced lean body mass and musculature, increased body fat, reduced bone mineral density, reduced exercise performance, and increased plasma cholesterol. Adults with GHD have had significantly increased risk of death from cardiovascular causes, a finding potentially linked to the increased adiposity and serum cholesterol [83]. GHD adults may suffer depression, anxiety, reduced energy and vitality, and social isolation. GH therapy of adult GHD patients results in marked alterations in body composition, fat distribution, bone density, and sense of well-being. Whether these effects of GH therapy will be sustained and, if so, what the optimal GH regimen will be remain to be determined.

Based on adult data, which suggest profound metabolic derangements associated with untreated GHD, continuation of GH treatment in the late adolescent patient who shows GHD on retesting is reasonable. In nearly 500 patients with isolated GHD, 207 (44%) had normal GH concentrations during provocative retesting [15]. In contrast, approximately 96% of patients with CPHD, with or without structural abnormalities of the hypothalamic–pituitary area, had sustained GHD. The presence of multiple anterior pituitary hormone deficiencies or structural disease should obviate the absolute need for subsequent retesting.

One unresolved issue is the strict pharmacological definition of GHD in young adults: there are no convincing normative data, and it does not seem reasonable to use the much lower GH responses to testing that characterize older adults. Many patients do not wish to continue daily injections, but data support their necessity. Loss of energy and strength is frequent. Total body and abdominal fat in untreated patients increases significantly, while lean body mass is lost relative to control subjects or comparable GH-treated patients or upon reinstitution of therapy.

As bone mass accrual is not completed until the third decade, late adolescence is an important time for GH sufficiency to prevent later osteopenia. In young adults (mean age 24 years) in whom GH therapy had been discontinued since late teenage, 57% had mean spine bone mineral

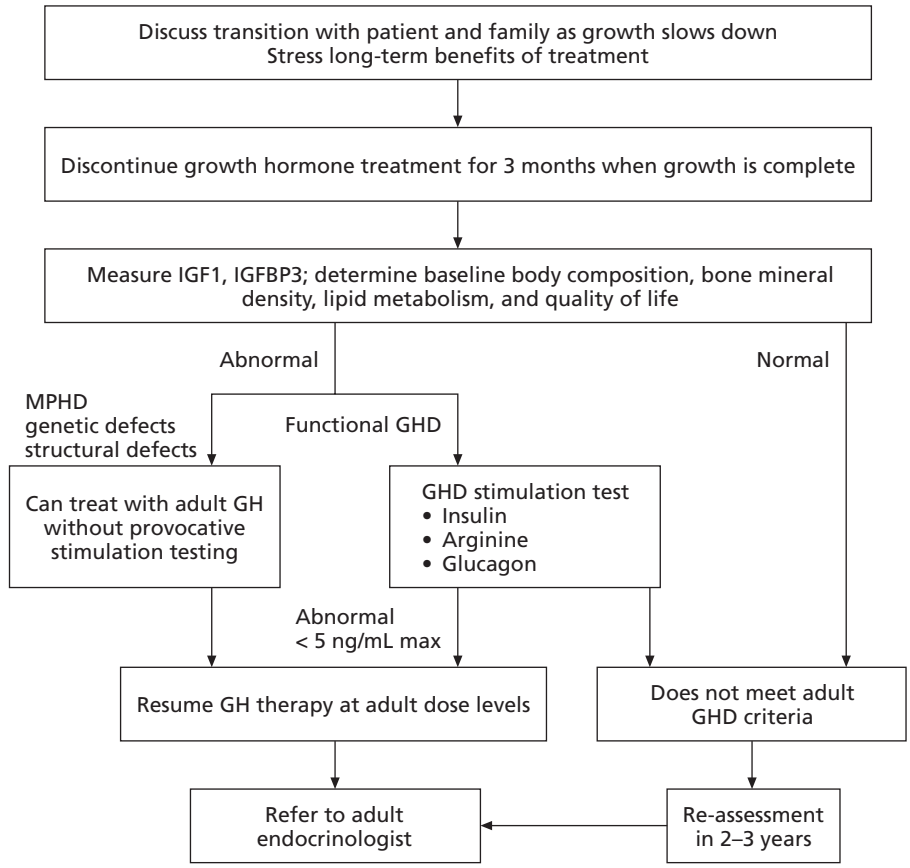

Fig. 7.8. Algorithm for the transition of pediatric growth hormone patients to adult growth hormone therapy.

density (BMD) more than –1 SD below the mean, while this figure would have been expected to be 37% in a normal population. Two years of GH treatment resulted in a 5.2% increase after a transient decrement at 6 months of therapy [84]. After several years off GH therapy, concentrations of IGF-I and IGFBP-3 decrease appreciably; resumption of GH permits normalization of the values. Such data affirm the necessity of continuing GH treatment in late adolescence, albeit at non-childhood growth-promoting doses, to prevent the development of adverse cardiovascular risk, diminished bone mineralization, and an overall lowering of energy level.

Our method of assessing the late adolescent patient is based on the retesting data discussed earlier and the recommendations of the GRS [51]. An algorithm to guide this transition is shown in Figure 7.8. Upon completion of skeletal growth, GH therapy should be halted for approximately 3 months, with retesting if necessary. In places where an insulin tolerance test is mandatory for the patient to qualify for further GH therapy, this test should be performed. Other pituitary hormones and serum IGF-I and IGFBP-3 concentrations are also measured.

The likelihood of GHD persisting into adult life in patients with CPHD, structural abnormalities of the hypothalamus–pituitary, or documented hypothalamic–pituitary molecular defects far exceeds that in patients with idiopathic, isolated GHD. The gradual and progressive development of hypothalamic dysfunction after cranial irradiation demands individualization of this process. Assessment of body composition, BMD, fasting lipids, insulin, and quality of life, before and after discontinuation of GH therapy establishes baseline data before long-term GH treatment and for longitudinal assessment of untreated patients.

The most conservative approach would suggest that all children diagnosed as GHD should be retested by insulin-induced hypoglycemia upon completion of skeletal growth and before a commitment is made for long-term adult treatment. A strong argument can be made, however, that patients with a very high likelihood of persistent GHD do not necessarily require retesting or, at most, have IGF-I and IGFBP-3 determinations. It is our recommendation that such adolescents should not stop treatment and continue on appropriate doses of GH.

On the other hand, the child who has carried a diagnosis of idiopathic, isolated GHD should always be retested. When the diagnosis of adult GHD is established, we continue GH therapy at 12.5 to 25 μg/kg/day with monitoring of IGF-I concentrations to assure appropriate dosing. Caution should be exercised when considering the decision of continuing GH

therapy in conditions where there is a known risk of diabetes or malignancy. The transition to adult GH replacement should ideally be arranged with close collaboration between pediatric and adult endocrinologists who should discuss the reinitiation of treatment with the patient.

Use of GH in non-GH-deficient short stature

Most normal short children treated with GH have growth acceleration ("catch-up"), which is sustained over the initial years of therapy (although attenuation of the response occurs as in all other instances of GH treatment). Slower pretreatment growth velocity and higher weight–height ratio, i.e. more approximating GHD, and a lesser degree of bone age retardation are associated with better early growth responses [87]. Longer term data are now available to begin to determine the impact of therapy on adult height.

Nearly 3000 children were classified as idiopathic short stature (ISS) in KIGS [88] with 153 having reached final height in 1999. GH treatment (0.2–0.25 mg/kg/week) resulted in the achievement of target height in familial (FSS, genetic) short stature patients, although at a short stature (–1.7 SDS in males and –2.2 SDS in females) with a mean gain during therapy of 0.6–0.9 SDS. In non-FSS children, the mean final height was greater in males (–1.4 SDS) but not in females (–2.3 SDS), with mean gains having been 1.3 and 0.9 SDS respectively. These heights are nonetheless distant from the mid-parental target heights that were near 0 SDS.

Hintz *et al*. [89] assessed adult height in 80 North American children with ISS treated for up to 10 years at a GH dose of 0.3 mg/kg/week. Mean height SDS at conclusion was –1.4 with a gain of 1.3 SDS, quite similar to the broader KIGS experience. McCaughey *et al*. [90] showed that GH treatment (about 0.34 mg/kg/week) of eight prepubertal girls led to a mean height SDS gain of 1.28 after 6.2 years of treatment, a 7.6 cm greater gain than in a control group whose mean height SDS did not change.

These data suggested that GH treatment of prepubertal children with ISS does increase growth velocity and final height, but the range of results reflects the patient heterogeneity. Contrasting data, which challenge the value of GH treatment in such patients, showed final heights of –1.5 SDS in boys and –1.6 SDS in girls in a collaborative study of 229 untreated ISS children from nine European countries [91]. Concerns that GH treatment might accelerate pubertal onset and progression resulting in failure to improve long-term height SDS have not been substantiated by the studies described above. The value of delaying puberty with a gonadotropin-releasing hormone agonist in GH-treated short children is controversial with contradictory data [92]. At this time, the added cost along with the potential negative impact of stopping puberty in a child already shorter and less mature than his/her peers diminishes our enthusiasm for such a delaying regimen.

The picture has recently been clouded further by the United States FDA's July 2003 approval of GH for the treatment of non-GHD children more than 2.25 SD below the mean for height [93]. Two randomized, multicenter trials, one placebo-controlled and one dose–response, were conducted. In the double-blind trial, patients received either GH at a dose of 0.22 mg/kg/week (so quite low-dose treatment) or placebo, with final height measurements after a mean treatment duration of 4.4 years. The treatment group achieved a mean final SDS of –1.8 vs. –2.3 for the placebo-treated group ($P = 0.017$). More patients had final heights above the fifth centile in the treatment group than in the placebo group (41% vs. 0%, $P < 0.05$), and more gained at least 1 SDS in the treatment group as well (50% vs. 0%, $P < 0.05$). The dose–response study randomized patients into several treatment groups, ranging from 0.24 mg/kg/week to 0.37 mg/kg/week. The higher dose group had a greater increase in mean height velocity after 2 years of treatment, with a greater mean final height achievement over the predicted heights (7.2 cm vs. 5.4 cm). None of the patients had heights above the fifth centile at baseline, but 82% of those receiving 0.37 mg/kg/week and 47% of those at 0.24 mg/kg/week reached a final height above the fifth centile. It should be noted that details of the above two studies come from the package insert of the commercially available GH product and have not been published in a peer-reviewed form as of the writing of this text (March 2004) [94].

The financial, ethical, and psychosocial impact of GH therapy of short children (whether at the bottom 5%, 3%, 1%, or 0.1%) should be considered. Five percent of the population will be below the fifth centile, whether we treat with GH or not, and perhaps focusing upon short stature potentially handicaps an otherwise normal child, psychologically or socially. No convincing data have been presented to date that GH treatment of these short children improves psychological, social, or educational function. Furthermore, the final adult height in children with constitutional delay of growth and puberty (probably the most frequent diagnosis) will be adequate without any treatment. Finally, the treatment risks of GH therapy, both known and unknown, must be considered when treatment of otherwise normal children is an issue.

Based on these accumulated data, we recommend the following approach:

1 Controlled therapeutic trials of "non-GHD short stature" should continue to be carried out to adult height.

2 Appropriate evaluation should include analysis of the GH–IGF axis, especially IGF-1 and IGFBP-3 measurements (before and after GH administration) before labeling a short child as "normal." GH stimulation testing in short patients with normal IGF-1 and IGFBP-3 seems unnecessary but may be demanded by payers. The need to characterize the full spectrum of potential genetic abnormalities in the GH–IGF system is apparent.

3 Proper assessment of pretreatment growth velocity should be over a minimum of a 6-month period and preferably for 12 months.
4 In the otherwise normal child with severe short stature (at least 2.25 SD below the mean for age) and a poor height velocity (e.g. < 25th centile for age: < 6 cm/year before age 4 years; < 5 cm/year at age 4–8 years; < 4 cm/year at any time before puberty) that would lead to an adult height outcome below the normal range, the possibility of GH treatment should be discussed with patient and family. This includes an assessment of normal and familial growth patterns along with the predicted pubertal and statural development. The inconveniences, discomforts, and potential risks of GH treatment should be fully described. It is the physician's responsibility to ensure that the expectations of the child and the parents are realistic in regard to short-term growth and ultimate height. Where appropriate, counseling and psychological support should be provided.
5 If a trial of GH therapy is desired, treatment should be for a minimum of 6 months, at the approved dosage of up to 0.37 mg/kg/week.
6 Therapy with GH should be continued beyond 6 months only if growth is accelerated (defined as an increase in the height velocity of at least 2 cm/year). Efficacy of treatment requires continuous monitoring especially in partial GHR patients where the possibility of IGF-I therapy is a future alternative. The use of multivariate models to assess growth response should be considered.
7 Growth acceleration with GH treatment does not relieve the need to try to ascertain the cause of the growth failure. Appropriate studies should be repeated, when indicated and as newer molecular techniques evolve.
8 Treatment must be monitored carefully for side-effects of GH treatment.
9 Continued psychological support should be provided for the child and family. This includes guiding the patient through puberty and providing post-treatment follow-up.

Side-effects of growth hormone

Pituitary-derived human GH had an enviable safety record for a quarter of a century but proved to be the agent for transmission of the fatal spongiform encephalopathy, CJD. Although pit-GH was removed from use in the United States in 1985 and, later, throughout the world, over 160 more patients with GH-derived CJD have been identified and cases are likely to continue to be found over the next several decades. Although this risk does not exist with recombinant DNA-derived GH, the experience with pituitary GH serves as a grim reminder of the potential toxicity that can reside in "normal" products and "physiological replacement."

Extensive experience with recombinant GH over more than 20 years has been encouraging. Every attempt has been made to seek physiological replacement rather than pharmacological therapy, but this is often not possible. Concerns have been raised about a number of potential complications, which clearly require continued follow-up and assessment. This evaluation has been greatly facilitated by the extensive databases established by GH manufacturers, in particular Genentech (National Collaborative Growth Study, NCGS) and Pfizer (Pfizer International Growth Database, KIGS).

Development of leukemia

Leukemia as a complication of GH therapy was first reported in five cases from Japan in 1988 [95], and more than 50 cases of leukemia have been reported in GH-treated patients. A confounder in the assessment of a potential role of GH treatment in this disorder is that many GHD children have conditions that may predispose the development of leukemia, such as histories of prior malignancies, irradiation, or syndromes associated with the development of leukemia (Bloom syndrome, Down syndrome, Fanconi anemia). GH-treated patients who develop leukemia do so at a later age than the normal population. Patients have included recipients of both pit-GH and rGH, and leukemia has occurred both during treatment and following termination of therapy. Calculations of relative risk are imprecise but vary from sevenfold in Japan to two- to fourfold in the United States. Leukemia has been reported in GHD individuals without any history of GH therapy, raising the possibility that the GHD state, by itself, might be a predisposing factor [39,40].

If GH is a causative agent in the development of leukemia, the increased risk appears modest and may arise from the underlying state rather than from GH therapy. The number of cases worldwide of new leukemia in children treated with GH but in whom there are no known risk factors is approximately what would be expected on a patient–year basis. Although this issue should be discussed with all potential recipients of GH, the concern appears to be limited to those children with high risk factors. Particular care should be used in prescribing GH therapy for children with past histories of leukemia or lymphoma or other disorders conveying an increased risk of leukemia. In a study of over 600 children with prior leukemia who were treated with GH, the relapse rate was within the expected range, consistent with no effect of GH replacement therapy on the recurrence of leukemia [15]. In addition, data from nearly 60 000 GH-treated patients with over 193 000 at-risk patient–years did not reveal an increased risk for non-leukemic extracranial neoplasms [39,40].

Recurrence of CNS tumors

As many recipients of GH have acquired GHD from CNS tumors or their treatment, the possibility of tumor recurrence with therapy is of obvious importance. Estimates of CNS tumor recurrence rates in non-GH-treated children and

adolescents are difficult to obtain, bearing in mind the vast array of treatment programs used in the past three decades. In a total of 1083 patients compiled in 11 reports, not treated with GH, 209 or 19.3% had recurrences [15]. Such data in a heterogeneous group, including craniopharyngiomas, gliomas, ependymomas, medulloblastomas, and germ cell tumors, provide a background for assessing recurrence rates in GH-treated youth. Reports from nine centers, encompassing 390 patients, indicate recurrence in 64 or 16.4%, or quite similar to the much larger number of untreated patients [15].

In a particularly well done comparative study at three pediatric neuro-oncology centers having data on 1071 brain tumor patients (180 treated with GH for a mean treatment period of 6.4 years, with 31 followed for more than 10 years), relative risk of recurrence or death was similar in both groups [96]. Extensive analysis of 4410 patients with brain tumor or craniopharyngioma histories before GH therapy in the NCGS and KIGS databases [39,40] showed a similar lack of increased tumor recurrence. In the NCGS series, recurrence rates of the most common CNS neoplasms, craniopharyngioma (6.4%), primary neuroectodermal tumors (medulloblastoma, ependymoma) (7.2%), and low-grade glioma (18.1%) were lower or similar to those reported in non-GH-treated children. Despite such reassuring data, the relatively short follow-up times, even in huge international databases, tempers willingness to eliminate any relationship of GH therapy to recurrence of CNS tumors.

Pseudotumor cerebri

Pseudotumor cerebri (idiopathic intracranial hypertension or IIH) occurs rarely in GH-treated patients. The disorder may develop within months of starting treatment or as long as 5 years into the course; it appears to be more frequent in patients with renal failure than in those with GHD. The mechanism for the effect is unclear but may reflect changes in fluid dynamics within the CNS. Pseudotumor has also been described following thyroid hormone replacement in hypothyroidism. In any case, physicians should be alert to complaints of headache, nausea, dizziness, ataxia, or visual changes. Because of the possible association of pseudopapilledema with GHD, perhaps representing a variant of optic nerve hypoplasia, careful ophthalmological evaluation should be undertaken in patients with suspected GH therapy-associated pseudotumor cerebri to avoid overdiagnosis and invasive treatments.

Slipped capital femoral epiphysis (SCFE)

SCFE is associated with both hypothyroidism and GHD. Whether GH therapy plays a role has been difficult to determine, in part because the incidence of SCFE varies with age, sex, race, and geographic locale, being reported at between 2–142 cases per 100 000. The data in the KIGS and NCGS studies are in this range [39,40]. Accordingly, while SCFE cannot be attributed to GH therapy per se, complaints of hip and knee pain and/or limp should be evaluated carefully.

Diabetes mellitus

The association of GH treatment with insulin resistance has long been recognized. A retrospective analysis of the KIGS database found 43/23 333 children with abnormalities of glucose regulation including 11 with type 1 and 18 with type 2 diabetes mellitus [97]. The heterogeneity of this patient group, and the failure to corroborate these findings with a similar retrospective analysis of NCGS data (type 2, 6.2/100 000) put the report into question. Nonetheless, the reduction of insulin sensitivity by GH is a concern that demands close assessment of high-risk patients such as those with Prader–Willi or Turner syndromes and a history of intrauterine growth retardation. At present, it would seem most likely that the relationship of the development of diabetes in childhood/adolescent GH recipients is due to a common genetic linkage rather than a GH side-effect.

Miscellaneous side-effects [39,40]

Other potential side-effects of GH therapy include prepubertal gynecomastia, pancreatitis, growth but not malignant degeneration of nevi, behavior changes, scoliosis and kyphosis, worsening of neurofibromatosis, hypertrophy of tonsils and adenoids, and sleep apnea. This list is obviously only partial. Clinicians must remember that GH and the IGFs that it regulates are potent mitogens with diverse metabolic and anabolic actions. All patients receiving GH treatment, even as replacement therapy, will continue to be carefully monitored.

For the most part, the side-effects of GH are minimal and rare. When they occur, careful history and physical examination are adequate to identify their presence. Management of these side-effects may include either transient reduction of dosage or temporary discontinuation of GH. In the absence of other risk factors, there is no evidence that the risk of leukemia, brain tumor recurrence, slipped capital femoral epiphysis, or diabetes is increased in recipients of long-term GH treatment. Any patient receiving GH who has a second major medical condition, such as being a tumor survivor, should be followed in conjunction with an appropriate specialist such as an oncologist and a neurosurgeon. While GH has been shown to increase the mortality of critically ill patients in the intensive care unit (ICU) [98], there is no evidence that GH replacement therapy needs to be discontinued during intercurrent illness in GHD children.

The question of long-term cancer risk

Several epidemiological studies suggest an association between high serum IGF-I concentrations and incidence of

malignancies [99,100]. The calculated risk of cancer in those studies was also increased for patients with low IGFBP-3 concentrations. While additional studies are being conducted to verify or disprove these associations, the role of GH should also be carefully examined. Although IGF-I concentrations were not statistically associated with cancer risk, the combination of high IGF-I and low IGFBP-3 was related to a heightened risk [101]. As GH positively influences the production of both peptides, this casts doubt on its role as a driving force in the IGF–cancer relationship. Epidemiological studies assessing the risk of malignancy in patients with acromegaly found differing results, with some [102,103] but not others identifying significant associations between acromegaly and colon cancer risk [15,104]. Acromegaly is associated with a marked increase in the incidence of benign hyperplasia of several organs, including colonic polyps [105]. Such findings suggest that the GH–IGF axis may lead to symptomatic benign proliferative disease, which could be associated with symptoms, such as rectal bleeding, that would then lead to a potential detection (or ascertainment) bias.

Children receiving GH do not have a greater risk of *de novo* or recurrent tumors [39,40]. No increased incidence of cancer was found in GH recipients among adults who were treated for GH deficiency [106,107]. These reports represent imperfect, uncontrolled studies, but the experience gained at least suggests that GH therapy will not be associated with later development of neoplasms in the absence of other risk factors. The use of IGF-I and IGFBP-3 in the monitoring of GH recipients, both adult and pediatric, has been recommended and endorsed by international bodies such as the GH Research Society [51]. Until the issue of cancer risk in GH therapy is fully resolved, regular monitoring of both IGF-I and IGFBP-3 and altering the GH dose to diminish the theoretical risk profile is suggested; it is quite unusual to have a GH-treated patient with high IGF-I and low IGFBP-3 concentrations. As lifelong treatment of GH-deficient patients becomes routine, the importance of long-term, regular monitoring of the GH-dependent peptides seems prudent, along with the maintenance of the databases to monitor safety data [108]. Although decades more remain to assess the question of untoward consequences, current data support the safety of present indications for use of GH in children and adults.

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8 Development of the reproductive systems

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Reproductive development is a complex and highly integrated process that starts in early gestation (5 weeks) and is complete some 14 years later when fertility is achieved during puberty. Abnormalities can present with diverse signs or symptoms at different ages, including genital ambiguity or undescended testes in the newborn period, early or late puberty in adolescence, or infertility in adult life (Table 8.1). Making a diagnosis and management plan for patients with disorders of reproduction is important for successful long-

Table 8.1. Disorders of reproductive development.

Disorders of chromosomal sex
Klinefelter syndrome (47,XXY)
Turner syndrome (45,X)
Mixed gonadal dysgenesis (45,X/46,XY)
True hermaphroditism (46,XY/46,XX) (rare)

Disorders of gonadal and phenotypic sex
Undervirilized karyotypic male (46,XY)
Disorders of testis development
  True hermaphroditism (46,XY)
  Gonadal dysgenesis (including mutations in *WT1*, *SF1*, *SRY*, *SOX9*, *DHH*, and *ATRX*)
  Absent testis syndrome

Disorders of androgen synthesis
LH receptor mutations
Smith–Lemli–Opitz syndrome
Steroidogenic acute regulatory protein mutations
Cholesterol side-chain cleavage (*CYP11A1*) deficiency
3β-Hydroxysteroid dehydrogenase 2 (*HSD3B2*) deficiency
17α-Hydroxylase/17,20-lyase (*CYP17*) deficiency
Oxidoreductase (POR) deficiency
17β-Hydroxysteroid dehydrogenase 3 (*HSD17B3*) deficiency
5α-Reductase 2 (*SRD5A2*) deficiency

Disorders of androgen action
Androgen insensitivity syndrome
Androgen receptor cofactor defects
Anti-androgenic drugs
Environmental modulators

Syndromic associations of male genital development (include)
Robinow, Aarskog, hand–foot–genital, popliteal pterygium (*IRF6*)

Other disorders affecting males (46,XY)
Persistent Müllerian duct syndrome
Vanishing testis syndrome

Table 8.1. (*continued*)

Isolated hypospadias
Cryptorchidism (*INSL3*, *GREAT*)

Virilized karyotypic female (46,XX)
Ovarian transdifferentiation
  True hermaphroditism (46,XX)
  XX male (usually *SRY* positive)
Increased androgen synthesis
  3β-hydroxysteroid dehydrogenase 2 (*HSD3B2*) deficiency
  21-hydroxylase (*CYP21A2*) deficiency
  11β-hydroxylase (*CYP11B1*) deficiency
  Oxidoreductase (*POR*) deficiency
  Aromatase (*CYP19*) deficiency
  Glucocorticoid receptor mutations
Increased androgen exposure
  Maternal virilizing tumors (e.g. luteomas of pregnancy)
  Androgenic drugs

Syndromic associations (include)
Fraser syndrome, cloacal anomalies, neurofibromatosis

Other disorders affecting females (46,XX)
Ovarian dysgenesis
Müllerian agenesis/hypoplasia (e.g. MURCS, cat eye syndrome)
Uterine abnormalities (e.g. Fryns syndrome, MODY5)
Vaginal atresis (e.g. McKusick–Kaufman syndrome)

Disorders of gonadotropin release and action
Disorders of neuronal migration (Kallmann syndrome)
X-linked Kallmann syndrome (*KAL1*)
Autosomal-dominant Kallmann syndrome (*FGFR1*, *NELF*)

Disorders of GnRH release and action
Processing defects (PC-1, *PCSK1*)
Regulatory defects (*leptin*, *leptin receptor*, *SF1*, *DAX1*, *GPR54/KiSS1*)
GnRH resistance (*GnRH receptor*)

Disorders of pituitary development
Multiple pituitary hormone defects (*HESX1*, *LHX3*, *PROP1*)
Gonadotrope (*SF1*, *DAX1*)

Disorders of gonadotropin action
Gonadotropin mutations (*FSHβ*, *LHβ*)
Congenital disorders of glycosylation
Gonadotropin resistance (*FSH receptor*, *LH receptor*, PHPIa/*GNAS*)

Syndromic associations (include)
Hypogonadotropic hypogonadism: Prader–Willi, Bardet–Biedl (Laurence–Moon), Alström, CHARGE, Gordon–Holmes spinocerebellar ataxia
Ovarian failure: Perrault, Maximilian, Quayle and Copeland, Pober, Malouf, ataxia telangiectasia, Nijmegen, Cockayne, Rothmund–Thompson, Werner, bletharophimosis–ptosis–epicanthus syndrome (BPES, *FOXL2*)

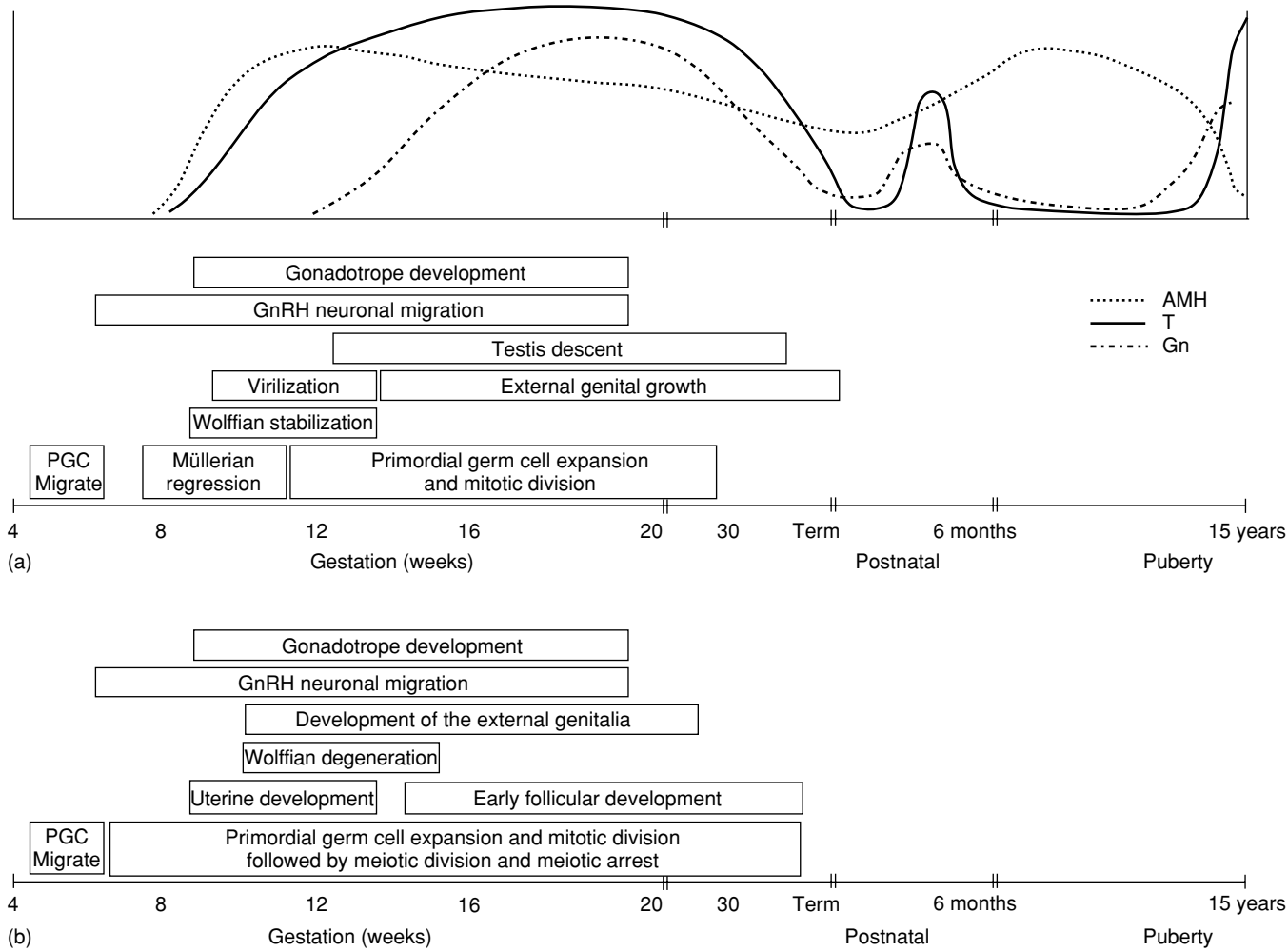

Fig. 8.1. Key events and endocrine changes throughout reproductive development in the male (a) and female (b). Modified with permission from ref. 6. PGC, primordial germ cells; GnRH, gonadotropin-releasing hormone; AMH, anti-Müllerian hormone; T, testosterone; Gn, gonadotropins.

term endocrine, reproductive, and psychosexual outcome. Beyond the more obvious presentations, approximately one in six couples are infertile, and some of these patients have milder abnormalities in reproductive development, so understanding the genetic and endocrine mechanisms underlying these processes is essential for the adult endocrinologist and gynecologist [1–5].

Development of the reproductive systems

Development of the reproductive systems can be divided into four stages (Fig. 8.1a and b): (1) sex determination and sexual differentiation from 5 weeks' gestation in humans; (2) development of the fetal hypothalamic–gonadotrope axis from 6 weeks' gestation; (3) postnatal reproductive endocrine events from birth to 6 months; and (4) puberty.

Many of the factors involved in reproductive development maintain reproductive function and fertility throughout adult life and influence the hypothalamo–pituitary–gonadal (HPG) axis at many levels. For example, the orphan nuclear receptor DAX-1 (*NR0B1*) is expressed above Rathke's pouch in early fetal life, is involved in cellular organization and differentiation in the testis, and plays a role in regulating puberty in the hypothalamus and pituitary. Peptide hormones such as anti-Müllerian Hormone [AMH, *MIS* (Müllerian inhibiting substance)] not only stimulate regression of the primitive uterus and Fallopian tubes from around 7 weeks' gestation but also regulate pituitary gonadotropin secretion and ovarian primordial follicle recruitment in later life. In contrast, events such as the "wave" of SRY expression seen in the developing testis occur only at critical times during development. Thus, dividing reproductive development into discrete stages can help clinical investigation and management appropriately.

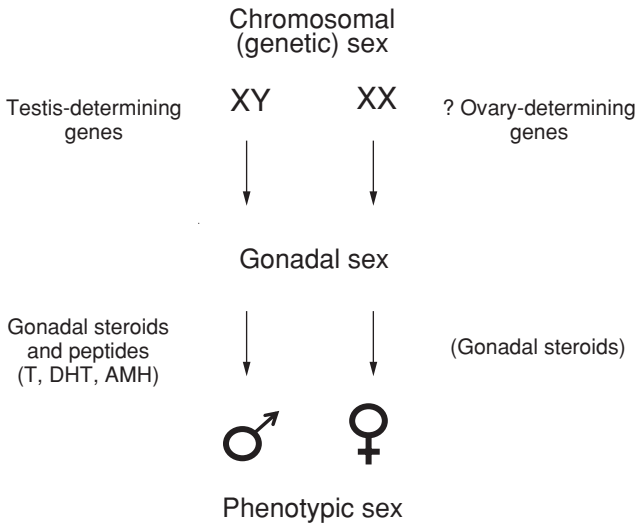

Fig. 8.2. The principle mechanisms involved in sex determination and sexual differentiation. Reproduced with permission from Achermann JC, Jameson JL. Disorders of sexual differentiation. In: Kasper *et al*., eds. *Harrison's Principles of Internal Medicine*, 16th edn. New York: McGraw-Hill, 2004.

Sex determination and sexual differentiation

Sex determination involves development of a bipotential gonad into a testis or ovary. *Sexual differentiation* requires the gonad to develop and function appropriately to produce peptide hormones and steroids. In the developing male, the process of sexual differentiation results in regression of Müllerian structures (uterus and Fallopian tubes), stabilization of Wolffian structures (which develop into the seminal vesicles, vasa deferentia, and epididymes), virilization of the external genitalia (penis and scrotum), and descent of the testes. The consequences of ovarian differentiation are less obvious in the developing female until the time of puberty when estrogen synthesis stimulates breast development. Ovarian development was formerly considered a constitutive (default) pathway, but it appears to require many active processes.

Sex determination and sexual differentiation are divided into three major components: (1) chromosomal sex; (2) gonadal sex (the result of sex determination); and (3) phenotypic sex (the result of sexual differentiation) (Fig. 8.2).

Chromosomal sex

The Y chromosome

Chromosomal sex describes the complement of sex chromosomes present in an individual (46,XY male; 46,XX female). The presence of a single, normal Y chromosome is usually sufficient for testis determination to occur, even when multiple X chromosomes are present. Thus, individuals with 47,XXY, 48,XXXY, or variants usually develop as phenotypic males, although fertility can be compromised.

The X chromosome

Loss of an X chromosome impairs gonadal development. Therefore, women with Turner syndrome have streak gonads or ovarian dysgenesis, although some of them enter puberty spontaneously, and fertility has been reported in a small proportion of women, usually with Turner mosaicism (46,XX/45,X). Patients with deletions of Xq13–25, Xq26–28, and/or the short arm distal to Xp11 usually have streak gonads and complete failure of pubertal development [7]. Reproductive phenotypes associated with other variants of Turner syndrome can be unpredictable, and key X-specific genes necessary for ovarian development have not been demonstrated. Fetuses with no X material (45,Y) are not viable.

Mixed gonadal dysgenesis

Mixed gonadal dysgenesis (45,X/46,XY) causes a wide spectrum of phenotypes ranging from mild virilization to normal male appearance [8]. This chromosome complement can be found in males investigated for infertility or detected incidentally during antenatal amniocentesis screening for unrelated issues, suggesting that a 45,X/46,XY karyotype is underdiagnosed in the general population. Most patients with 45,X/46,XY mixed gonadal dysgenesis present with genital ambiguity, and issues of sex of rearing, growth, gonadal tumorigenesis, and fertility potential need to be addressed. Other features include renal and cardiac abnormalities similar to Turner syndrome, probably reflecting the effects of sex chromosome haploinsufficiency. The proportion of 45,X/46,XY cell mosaicism can vary between tissues and, rarely, the mosaic cell line may be detected only in the gonad. The milder phenotypes associated with 45,X/46,XX and 45,X/46,XY mosaic genotypes highlight the importance of gene dosage effects in reproductive development. These gene dosage phenomena are also seen following chromosomal rearrangements (deletions or duplications) or through functional gene dosage effects of single gene disorders.

True hermaphroditism

True hermaphroditism refers to the presence of ovarian and testicular tissue in the same individual, either as ovotestes or as distinct ovarian or testicular structures [9]. For unknown reasons, testicular development is often more pronounced on the right side. Occasionally, true hermaphroditism occurs as a result of true sex chromosomal chimerism (46,XY/46,XX), but most true hermaphrodites have a single chromosomal complement. Most frequently, this is 46,XX, especially in patients from the African subcontinent. The genetic basis of the condition is not known.

Chromosomal rearrangements

True hermaphroditism and other abnormalities in gonadal development can occur following sex chromosomal or autosomal rearrangements. In many cases, these changes have helped to identify some of the critical genes involved in

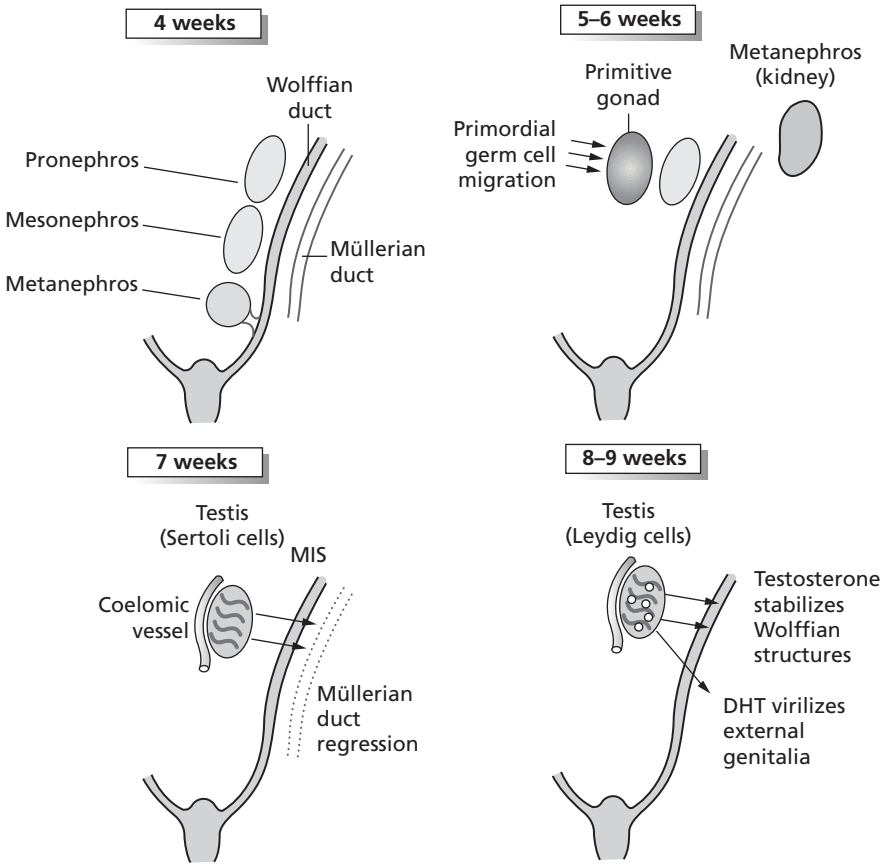

Fig. 8.3. The principal morphological and functional stages of early gonad/testis development in humans. Reproduced with permission from Achermann JC, Jameson JL. Testis determination. *Topical Endocrinol* 2003; 22: 10–14 [13].

sex determination and sexual differentiation. For example, deletion of the Y chromosome region (Yp11.3) containing SRY (sex determination region on the Y chromosome) impairs sexual development in karyotypic 46,XY males, as do deletions of 17q24 (containing *SOX9*, campomelic dysplasia), 9p (*DMRT1/2*), 10q (gene unknown), Xq13.3 (*ATRX*, X-linked α-thalassemia, mental retardation syndrome), and 16p (*SOX8*, ATR-16 syndrome with α-thalassemia, mental retardation). Duplications of certain genomic regions are also associated with impaired development in 46,XY males, suggesting that genes in these regions may "oppose" testis development (e.g. Xp21 containing *DAX1*; 1p35 containing *WNT4*).

Approximately 80% of "46,XX males" have a translocation of Y-chromosomal material containing *SRY*. These observations were paramount in localizing this key testis-determining factor [10]. Seminal experiments showed that *XX Sry*-positive transgenic mice developed testes and had a male phenotype, confirming the role of *Sry* as a primary testis-determining gene in mammals [10]. Reports of mosaic duplication of chromosome 17q23–24 (containing *SOX9*) in a virilized 46,XX female, coupled with studies of *Sox9* overexpression in the mouse (*Odsex*), have demonstrated that other (autosomal) factors downstream from SRY may be sufficient to promote testis development in a dosage-sensitive manner [11,12].

Gonadal sex

Gonadal sex refers to the development of the gonadal tissue as testis or ovary. The principal embryological and morphological changes involved in gonad development are shown in Fig. 8.3.

The bipotential gonad

The primitive gonad arises from a condensation of the mesonephric region of the urogenital ridge at approximately 4–5 weeks' gestation in humans (Fig. 8.3). This region contains cells derived from the coelomic cavity as well as the mesonephros. This primitive gonad remains bipotential ("indifferent") until about 40 days' gestation in humans, so testes and ovaries are morphologically indistinguishable.

Several important genes that facilitate development of the bipotential gonad are expressed in the developing urogenital ridge (Fig. 8.4, Table 8.2). The transcription factors Lim1, Emx2, and Lhx9/Lim9 are all expressed during this developmental stage, and deletion of these genes causes severe abnormalities of gonadal development in mice. Although heterozygous mutations in *EMX2* have been found in patients with schizencephaly (without gonadal dysgenesis), mutations in these early factors have not been found in humans, but more

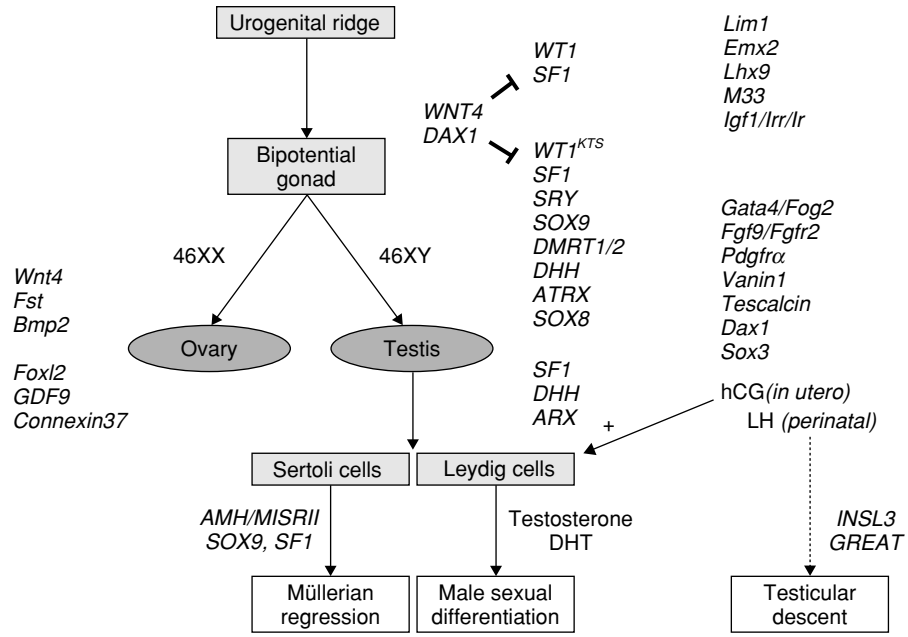

Fig. 8.4. Genetic events involved in gonad determination and differentiation. Mutations or deletions in the genes shown in upper case letters have been found in patients with disorders of gonadal (testis) development. The genes shown in lower case letters have been identified as important components of sex determination and sexual differentiation pathways from studies of mice. Adapted with permission from ref. 1. Copyright 2004, The McGraw Hill Companies.

Table 8.2. Genetic disorders of testis development (46,XY).

Gene	Protein	OMIM	Locus	Inherited	Gonad	Uterus	External genitalia	Associated features
WT1	TF	607102	11p13	AD	Dysgenetic testis	+/–	Female or ambiguous	Wilms' tumor, renal abnormalities, gonadal tumors (WAGR, Denys–Drash and Frasier syndromes)
SF1 (NR5A1)	Nuclear receptor TF	184757	9q33	AR/AD	Dysgenetic testis	+/–	Female or ambiguous	Primary adrenal failure
SRY	TF	480000	Yp11.3	Y	Dysgenetic testis or ovary	+/–	Female or ambiguous	
SOX9	TF	608160	17q24–25	AR	Dysgenetic testis or ovary	+/–	Female or ambiguous	Campomelic dysplasia
DMRT1	TF	602424	9p24.3	?AD	Dysgenetic testis	–	Female or ambiguous	
ATRX	TF	300032	Xq13.3	X	Dysgenetic testis	–	Female or ambiguous	α-Thalassemia, mental retardation
SOX8	TF	605923	16p13.3	?AD	ND	–	Ambiguous	ATR-16 syndrome, α-thalassemia, mental retardation
ARX	TF	300382	Xp22.13	X	Dysgenetic testis	–	Ambiguous	X-linked lissencephaly
DHH	Signaling molecule	605423	12q13.1	AR	Testis/streak	+	Female	Minifascicular neuropathy
DAX1 (NR0B1)	Nuclear receptor TF	300018	Xp21.3	dupXp21	Dysgenetic testis or ovary	+/–	Female or ambiguous	
WNT4	Signaling molecule	603490	1p35	dup1p35	Dysgenetic testis	+	Ambiguous	

AR, autosomal recessive; AD, autosomal dominant; Y, Y-chromosomal; X, X-chromosomal; TF, transcription factor; ND, not determined; WT1, Wilms' tumor-related gene 1; SF1, steroidogenic factor 1; DHH, desert hedgehog. Modified with permission from [4]. Copyright 2002, The Endocrine Society. Chromosomal rearrangements likely to include key genes are included.

complex phenotypes, including cranial and/or renal abnormalities, might be expected to be seen (*LIM1*, *EMX2*).

Wilms' tumor-related gene 1 (WT1) is a four-zinc-finger transcription factor expressed in the developing genital ridge, kidney, gonads, and mesothelium. Homozygous (complete) deletion of the gene encoding *Wt1* in mice prevents gonad and kidney development. The WT1 protein is subject to complex post-translational modification and splicing processes, so that multiple WT1 isoforms exist. Deletions or mutations of WT1 cause well-defined syndromes in humans (Table 8.2).

Haploinsufficiency of WT1 due to deletion of the chromosomal locus containing *WT1* and *PAX6* (11p13) causes WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities and mental retardation). Dominant-negative point mutations in WT1 cause Denys–Drash syndrome (gonadal dysgenesis, genital ambiguity, nephropathy, and predisposition to Wilms' tumor), whereas mutations in the exon 9 splice site of *WT1*, causing an altered ratio of +KTS to –KTS isoforms of WT1, result in Frasier syndrome (gonadal dysgenesis, late-onset nephropathy, and predisposition to gonadoblastoma) [14]. Although these isoforms may play different roles in regulating various stages of renal and gonad development, it is likely that significant phenotypic overlap exists in the last two conditions [15].

Another key transcription factor expressed in the urogenital ridge is steroidogenic factor 1 (SF1, *NR5A1*). SF1 is a member of the orphan nuclear receptor superfamily that regulates the transcription of an array of genes involved in gonadal development, adrenal development, steroidogenesis, and reproduction. Complete deletion of the gene encoding Sf1 in mice results in apoptosis of the developing gonad and apparent gonadal agenesis. Other features of these homozygous deleted animals include persistent Müllerian structures, adrenal agenesis, hypogonadotropic hypogonadism, abnormalities of the ventromedial hypothalamus, and late-onset obesity in adult animals rescued by adrenal transplantation.

Heterozygous animals have reduced gonadal size and impaired adrenal stress responses. A similar phenotype (primary adrenal failure, severe 46,XY gonadal dysgenesis, and persistent Müllerian structures) has been reported in two patients. One child had a *de novo* heterozygous point mutation in the "P"-box primary DNA-binding region of SF1, which causes severe loss-of-function [16]. The other child had a homozygous point mutation in a secondary DNA-binding region, the "A"-box, which causes partial loss-of-function (see Plate 7a, facing p. 148). The variable modes of inheritance again highlight the exquisite sensitivity of reproductive development to gene dosage effects [17].

It is becoming apparent that other heterozygous loss-of-function mutations in SF1 can be associated with partial gonadal dysgenesis and normal adrenal function [18]. The role of SF1 in human ovarian development remains unclear. One 46,XX girl with primary adrenal failure resulting from a point mutation in SF1 has apparently normal ovarian development but has not yet reached the age of puberty when ovarian function can be assessed.

Duplication of a region of the X chromosome (Xp21, *dosage-sensitive sex reversal*) containing the gene *DAX1* has been reported in a small number of 46,XY patients with impaired testicular development or ovotestes. These reports suggested that the orphan nuclear receptor DAX1 [and a related signaling molecule, WNT4 (dup1p35)] could act to antagonize testis development as "anti-testis" genes (Fig. 8.4) [19]. This concept has been supported by *in vitro* studies showing that DAX1 can repress SF1 transactivation, and by studies in mice in which overexpression of *Dax1* causes impaired male development in the presence of a "weakened" *Sry* locus. Targeted deletion of *Dax1* in a similar mouse strain also causes impaired testis development or ovotestis, and patients with X-linked adrenal hypoplasia congenita (due to mutations in *DAX1*) have abnormal testis architecture and infertility, suggesting that critical doses of these factors have important roles at different stages of development. Both under- or overactivity could have deleterious effects at different stages of gonadal development (Fig. 8.4) [20].

In addition to the role of these transcription factors, early gonadal development requires a host of paracrine and autocrine interactions. For example, complete lack of testis development and downstream Sry expression occurs following compound deletion of three members of the insulin signaling receptor family (insulin receptor, insulin-like growth factor 1 receptor, and insulin-related receptor) [21]. Significant functional redundancy between these systems must exist, as deletion of single factors had no obvious effect. This study also highlights the importance of the wave of SRY expression, one of the first events to commit the bipotential gonad to testis formation at around 40 days of age.

Primordial germ cell migration

Primordial germ cells (PGCs) are the embryonic precursors of gametes (spermatocytes or ova). Surprisingly, PGCs in all species arise some distance from the developing gonad and undergo a process of migration during the early stages of embryogenesis. In humans, PGCs arise from a region around the yolk sac/allantois and migrate into the primitive gonad between 5 and 6 weeks' gestation, under the influence of signaling molecules, receptors, and extracellular matrix proteins such as c-KIT, Steel, β1-integrin, and E-cadherin (Fig. 8.3) [22].

In the first few months of gestation, PGCs undergo multiple cycles of mitotic division. In the testis, a "self-renewable" population of germ cells exists. These undifferentiated PGCs are maintained by factors such as OCT4, whereas Plzf is required in adult male germ cells for stem cell self-renewal [23]. Most PGCs commit to differentiation following the expression of signaling molecules and transcription factors such as Pog. After several cycles of mitotic division, these cells enter mitotic arrest. Subsequent testicular development can occur in the absence of this germ cell population.

In the developing ovary, primordial ova (oogonia) undergo mitotic expansion in the first few months of gestation (5–24 weeks) followed by meiotic division (8–36 weeks) and a process of meiotic arrest. This meiotic arrest occurs in the first prophase when the chromatids of homologous pairs have begun to separate but are fixed by chiasmata (diplotene stage). The presence of these PGCs is believed to be necessary to sustain ovarian development.

More than six million PGCs exist in the developing ovary around 16 weeks of gestation. At this stage, somatic pregran-

ulosa cells associate with these primordial ova to form primitive or primordial follicles. Approximately 80% of oogonia fail to form follicles and undergo apoptosis, so that only one million germ cells are present in the ovary at the time of birth. These "resting" primordial follicles can remain in this stage of development throughout the woman's reproductive life, and meiosis progresses only in response to ovulation of the Graafian follicle (approximately 400 in a woman's reproductive lifetime). It was widely held that the population of germ cells present at birth represented a fixed pool that gradually reduced with time through apoptosis (and ovulation), but this view has been challenged by the discovery of a self-renewable population of germline stem cells in the mouse ovary that are active into adult life [24]. Whether such a mechanism exists in primates remains to be seen.

Testis determination

Testis determination is an active process that begins around 6 weeks' gestation in humans and consists of several distinct genetic and morphological events. The first noticeable event is a transient wave of SRY expression through the undifferentiated gonad, initially centrally, then in cells located at the cranial and caudal poles [25]. *SRY* is a single-exon gene that encodes a 203-amino-acid, high-mobility group (HMG)-box transcription factor that binds to specific DNA response elements (AACAAT/A) [9]. SRY is believed to regulate target gene expression through inducing a structural bend in DNA (see Plate 7b, facing p. 148). Mutations and deletions in *SRY* tend to cluster within the region encoding the HMG-box and have been reported in approximately 20% of patients with sporadic or familial 46,XY gonadal dysgenesis [10]. This SRY expression is believed to "switch" the fate of the progenitor cells into pre-Sertoli cells and is associated with subsequent expression of SOX9 (see Plate 8, facing p. 148) [11,26].

SOX9 is an SRY-related HMG-box factor that is proving to be a testis-determining factor in its own right (Fig. 8.4). In addition to its role in the testis, SOX9 is expressed in developing cartilage under the regulation of PTHRP/Indian hedgehog signaling pathways. Heterozygous loss-of-function mutations or deletions in *SOX9* result in campomelic dysplasia (Table 8.2). Approximately 75% of patients with campomelic dysplasia have some degree of gonadal dysgenesis. Many children with this condition do not survive childhood because of severe thoracic dysplasia, recurrent chest infections, and respiratory compromise, but disruption of the extensive upstream regulatory region of *SOX9* as a result of rearrangements in chromosome 17q23–24 can cause less marked effects on SOX9 function and a less marked phenotype.

Around the time of SRY and SOX9 expression (and nuclear localization), the developing testis undergoes a series of distinct cellular and morphological changes. The understanding of these processes has resulted from studies in mice (Plate 8, facing p. 148) [26].

The first stage of testis development involves a proliferation of Sf1-positive somatic cells, resulting in an increase in Sertoli cell precursors and Sertoli cell differentiation. This process is influenced by growth factors such as Fgf9 and Fgfr2. These primitive Sertoli cells coalesce with peritubular myoid cells to form primary sex cords, which then condense to form primitive seminiferous cords at around 7 weeks' gestation in humans. Sex cord development is supported by a striking reorganization of the gonadal vasculature in the developing testis but not the ovary (Fig. 8.3 and Plate 8, facing p. 148). These changes include the development of a discrete coelomic vessel, restriction of endothelial cells to the interstitial space between the sex cords, and increasing branching of blood vessels. The development of these vascular systems is influenced by growth factors such as Pdgfrα and can be repressed by the Wnt4/follistatin system. The changes in vascular architecture play an important role in determining cellular patterning and organization in the developing testis, in supporting paracrine interactions, and in the export of androgens from the developing Leydig cells to the perineal and systemic circulation (Plate 8, facing p. 148).

Although the expression of SRY plays a crucial role as a testis-determining factor, it is becoming clear that many other factors are necessary for normal development (Fig. 8.4, Table 8.2). Some of them may be expressed exclusively within the developing testis, whereas others may play a facilitative role in supporting gonad development and are expressed in other developing tissues, such as brain, kidney, and heart (Fig. 8.4, Table 8.2). Mutations in *ARX* cause X-linked lissencephaly and ambiguous genitalia (XLAG), whereas mutations in the gene encoding desert hedgehog (*DHH*) have been found in patients with severely impaired testicular development with or without minifascicular neuropathy [27,28].

Studies of targeted mutagenesis and ENU-induced mutagenesis in mice together with differential gene expression studies are starting to identify some of the many genes involved in gonad development (Fig. 8.4). A single gene disorder is found in only a small proportion of patients with 46,XY testicular dysgenesis. It is possible that mutations in other testis-specific genes involved in gonadal development will be identified in these patients in the future or that combinations of genetic events will prove to be responsible for some of the developmental phenotypes.

Ovarian development

Ovarian development occurs in the absence of testis-determing genes. It is not known whether specific ovarian-determining genes exist, but genes involved in maintaining or supporting ovarian development certainly do, and this process is much more active than previously thought [29].

Unlike testis development, ovarian development is dependent on the presence of PGCs. As outlined above, these primordial oocytes undergo several cycles of division before

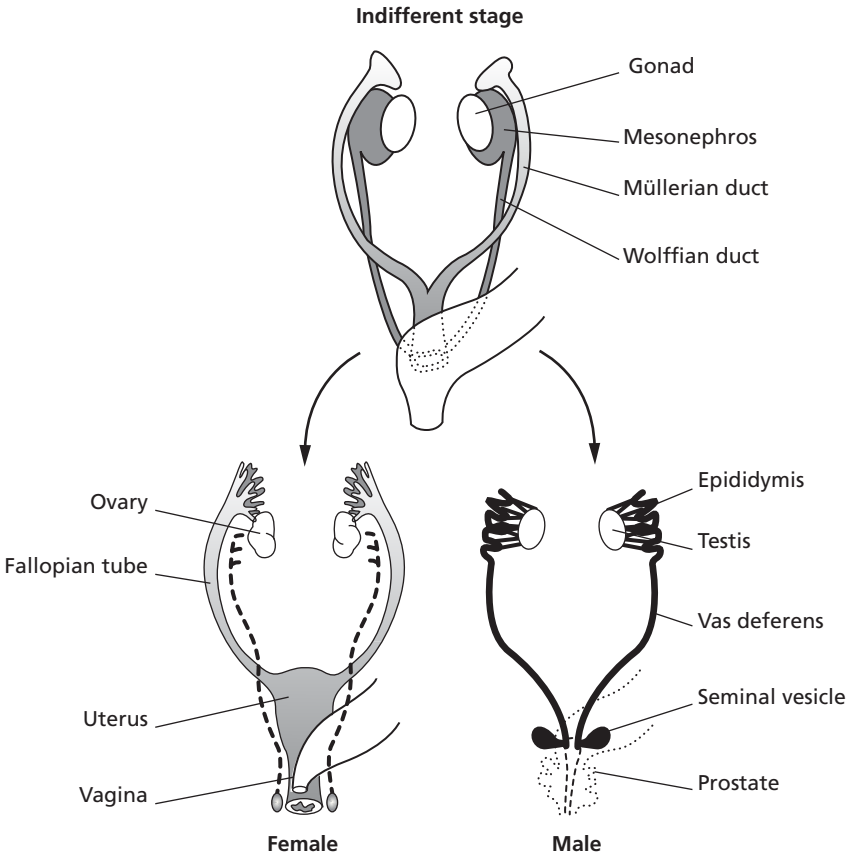

Fig. 8.5. Changes in Müllerian and Wolffian structures in the developing female and male fetus.

entering meiosis and undergoing meiotic arrest. In fact, meiotic germ cells may play an active role in preventing testis development [30]. Similarly, factors such as Wnt4, follistatin, and estrogen receptors α/β may also oppose testis development, as deletion of these factors in mice can result in pre- or postnatal transdifferentiation of the ovary to testicular tissue [31,32]. Although human gonadal tissue may be more resistant to such changes, it is clear that many factors are involved in maintaining ovarian development (e.g. FIGα, Dazla, Bmp8b, Smad5, connexin 37, Foxl2). The basis of most forms of ovarian agenesis or ovarian failure is not known.

Phenotypic sex

The developing gonad produces several steroid and peptide hormones that cause sexual differentiation and result in the phenotypic sex seen at birth. The critical role of testicular androgens in this process was first shown by surgical removal of the gonads during embryonic development of the rabbit. Jost's classic experiment resulted in the development of female reproductive characteristics, regardless of chromosomal sex of the embryo [33].

Male sexual differentiation

Sertoli cells and Müllerian regression

Sertoli cells play an important role in supporting germ cell survival and produce two important peptide hormones, AMH (MIS) and inhibin B. AMH is a member of the transforming growth factor (TGF)-β superfamily and is first secreted from around 7 weeks' gestation. AMH causes regression of Müllerian structures (Fallopian tubes, uterus, upper two-thirds of the vagina) by its paracrine action on the MIS type II receptor (Figs 8.3 and 8.5) [34]. Müllerian structures appear to be maximally sensitive to AMH between 9 and 12 weeks' gestation, a time when the developing testis produces peak concentrations of AMH but before the onset of significant AMH production by the developing ovary. Boys with mutations in either *AMH* or the MIS receptor gene can present with persistent Müllerian duct syndrome and undescended testes but otherwise normal virilization. In contrast, karyotypic 46,XY males who have severe early gonadal dysgenesis can have persistent Müllerian structures due to impaired Sertoli cell development and AMH release. In most cases, Leydig cell development is compromised, so marked undervirilization of external genitalia is also present.

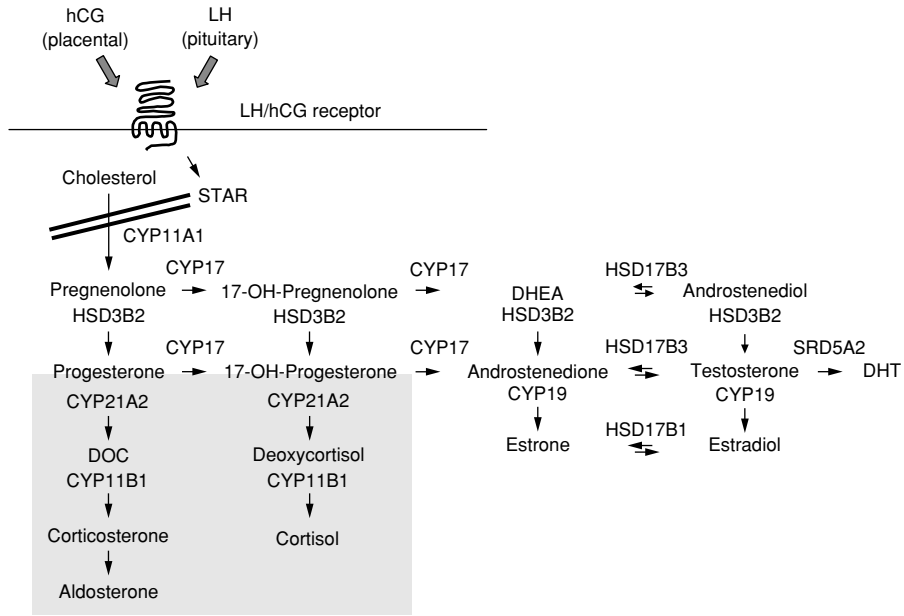

Fig. 8.6. Simplified pathways of steroidogenesis. In the developing gonad, stimulation by hCG or LH (from 18 weeks) results in androgen synthesis in the fetal Leydig cell. Blocks in the mitochondrial transport of cholesterol (steroidogenic acute regulatory protein, *STAR*) and early steroidogenesis (side-chain cleavage, *CYP11A*; 3β-hydroxysteroid dehydrogenase type II, *HSD3B2*) result in defects in virilization as well as impaired mineralocorticoid and glucocorticoid production by the adrenal gland (inset). Abnormalities in the dual-function enzyme 17α-hydroxylase/17,20-lyase (*CYP17*) result in impaired virilization but with salt retention and hypertension with age. In humans, the predominant steroid pathway occurs through conversion of Δ5 steroids (17-hydroxypregnenolone to dehydroepiandrosterone) by 17,20-lyase, rather than through the conversion of Δ4 steroids (17-hydroxyprogesterone to androstenedione) that occurs in rodents. Abnormalities in the final enzymes involved in androgen production (17β-hydroxysteroid dehydrogenase type III, *HSD17B3*; 5α-reductase, *SRD5A2*), as well as the androgen receptor, will result in undervirilization of the developing male fetus. In contrast, overproduction of adrenal androgens due to mutations in 21-hydroxylase (*CYP21A2*), 11β-hydroxylase (*CYP11B1*), or the P450 electron transfer enzyme oxidoreductase (*POR*) will cause virilization of the developing female fetus, as will increased testosterone and androgen exposure following mutations in aromatase (*CYP19*).

Inhibin B suppresses pituitary follicle-stimulating hormone (FSH) activity, and it seems likely that both these peptide hormones have more extensive autocrine, paracrine, and endocrine effects on reproductive development and function at multiple levels of the HPG axis than originally thought [35].

Fetal Leydig cells and steroidogenesis

Fetal Leydig cells develop within the interstitium of the developing testis and secrete androgens by 8–9 weeks' gestation (Fig. 8.3). The pathways of testicular steroidogenesis are shown in Fig. 8.6. A massive expansion in fetal Leydig cells occurs between 14 and 18 weeks' gestation, resulting in peak testosterone secretion at around 16 weeks. Fetal Leydig cell steroidogenesis is stimulated by placental human chorionic gonadotropin (hCG) during the first two trimesters of pregnancy, but the developing hypothalamic–gonadotrope system produces significant amounts of luteinizing hormone (LH) from around 20 weeks' gestation (Fig. 8.1). Local production of testosterone is necessary for stabilization of Wolffian structures, such as the epididymides, vasa deferentia, and seminal vesicles, whereas the potent metabolite dihydrotestosterone induces virilization of the external genitalia (Figs 8.5 and 8.7). In the male, the urogenital sinus develops into the prostate and prostatic urethra, the genital tubercle develops into the glans penis, the urogenital fold fuses to form the shaft of the penis, and the urogenital swellings form the scrotum (Fig. 8.7).

Several disorders of androgen synthesis and action result in undervirilization of the developing male fetus. These present with a spectrum of phenotypes ranging from complete lack of penile development to genital ambiguity or even micropenis (Table 8.3). The influence of environmental agents with estrogenic or anti-androgen effects on male sexual development *in utero* is the subject of much interest at present given the increase in incidence of disorders such as hypospadias. Drugs such as inhibitors of 5α-reductase (used in the treatment of male pattern baldness) and other environmental chemical disruptors could potentially impair virilization of the developing male fetus if exposure occurs during the early stages of pregnancy.

Testicular descent

Testicular descent starts at around 12 weeks' gestation and is usually complete by the middle of the third trimester, although 3–5% of term male infants will have an undescended testis at birth. Factors that influence transabdominal testicular descent through contraction of the gubernacular

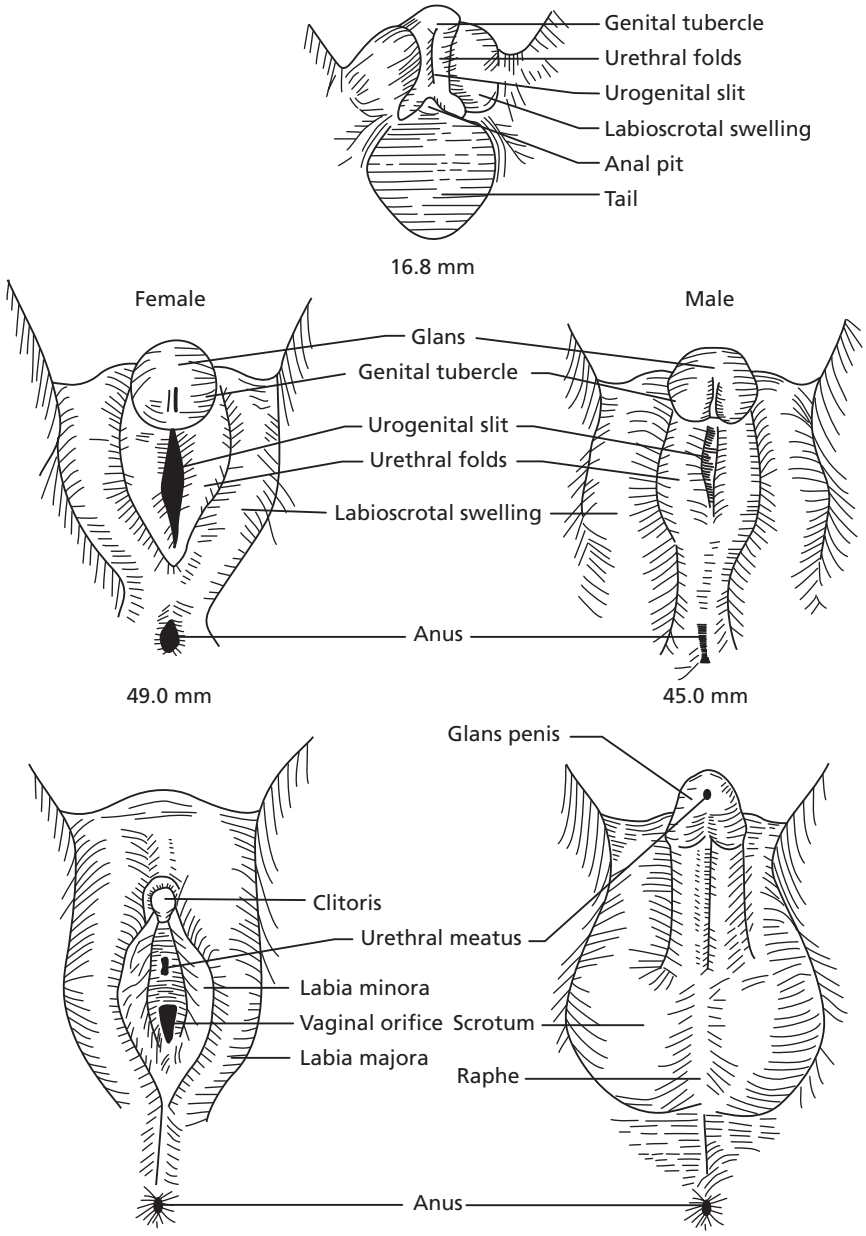

Fig. 8.7. Physical processes involved in the development of the external genitalia. Reproduced with permission from ref. 6.

ligament include insulin-like 3 (*INSL3*, relaxin-like factor) and its G-protein-coupled receptor, GREAT. Mutations in this ligand–receptor system have been described in a small proportion of patients with cryptorchidism [36,37]. Other testicular factors are likely to be involved in testicular descent, as most dysgenetic testes are intra-abdominal. In contrast, patients with disorders of androgen synthesis and action usually have testes palpable in the inguinal region, but the scrotum is poorly formed. Pituitary LH continues to stimulate Leydig cell steroidogenesis in the latter stages of pregnancy and has a direct influence on the inguinal phase of testicular descent (Fig. 8.4). Thus, boys with congenital gonadotropin deficiency have micropenis and cryptorchidism, rather than genital ambiguity.

Subsequent testicular development

During the second and third trimesters, the testes show several distinct morphological changes, including a reduction in fetal Leydig cell mass and elongation and coiling of seminiferous cords. There is no further significant development of germ cells during this time, and seminiferous cords do not canalize until later in childhood. Nevertheless, certain developmental insults can affect the testis at this stage. For example, vanishing (absent) testis syndrome probably represents a late fetal event because boys with this condition have adequate virilization and no Müllerian structures. The etiology of this disorder is not known but may reflect a vascular event such as fetal testicular torsion.

Table 8.3. Genetic disorders of androgen synthesis and action (46,XY).

Gene	Protein	OMIM	Locus	Inherited	Gonad	Uterus	External genitalia	Associated features
LHGCR	G-protein receptor	152790	2p21	AR	Testis	–	Female, ambiguous or micropenis	Leydig cell hypoplasia
DHCR7	Enzyme	602858	11q12–13	AR	Testis	–	Variable	Smith–Lemli–Opitz syndrome: coarse facies, second–third toe syndactyly, failure to thrive, developmental delay, cardiac and visceral abnormalities
STAR	Mitochondrial membrane transporter	600617	8p11.2	AR	Testis	–	Female	Congenital lipoid adrenal hyperplasia (primary adrenal failure), pubertal failure
CYP11A1	Enzyme	118485	15q23–24	AR	Testis	–	Female or ambiguous	Congenital adrenal hyperplasia (primary adrenal failure), pubertal failure
HSD3B2	Enzyme	201810	1p13.1	AR	Testis	–	Ambiguous	CAH, primary adrenal failure, partial virilization due to ↑ DHEA
CYP17*	Enzyme	202110	10q24.3	AR	Testis	–	Female, ambiguous or micropenis	CAH, hypertension due to ↑ corticosterone and 11-deoxycorticosterone (except in isolated 17,20-lyase deficiency)
HSD17B3	Enzyme	605573	9q22	AR	Testis	–	Female or ambiguous	Partial virilization at puberty, ↑ androstenedione:testosterone ratio
SRD5A2	Enzyme	607306	2p23	AR	Testis	–	Ambiguous or micropenis	Partial virilization at puberty, ↑ testosterone–dihydrotestosterone ratio
Androgen receptor	Nuclear receptor TF	313700	Xq11–12	X	Testis	–	Female, ambiguous, micropenis or normal male	Phenotypic spectrum from complete androgen insensitivity syndrome (female external genitalia) and partial androgen insensitivity (ambiguous) to normal male genitalia and infertility

AR, autosomal recessive; X, X-chromosomal; TF, transcription factor; LHGCR, luteinizing hormone/choriogonadotrophin receptor; DHCR7, 7α-dehydrocholesterol reductase; STAR, steroidogenic acute regulatory protein; CYP11A1, P450 side-chain cleavage; HSD3B2, 3β-hydroxysteroid dehydrogenase type II; CYP17, 17α-hydroxylase/17,20-lyase; HS017B3, 3β-hydroxysteroid dehydrogenase type III; SRD5A2, 5α-reductase type II.
*Mutations in the co-enzyme oxidoreductase may also cause impaired virilization. Modified with permission from ref. 4. Copyright 2002, The Endocrine Society.

Female sexual differentiation

The processes of female sexual differentiation are less obvious than in the male and do not involve significant changes in the external genitalia. Müllerian structures persist to form the Fallopian tubes, uterus, and upper portion of the vagina (Fig. 8.5). Normal uterine development is not a passive process and requires a host of factors to support development (e.g. Pax2, Lim1, Emx2, Wnt4/Lp, Hoxa13) and differentiation (e.g. Wnt7a, Hoxa10, Hoxa11, Hoxa13, progesterone, and estrogen receptors) [38]. The lack of local testosterone production leads to degeneration of Wolffian structures (Fig. 8.5). The urogenital sinus develops into the urethra and lower portion of the vagina, the genital tubercle develops into the clitoris, the urogenital (urethral) folds form the labia minora, and the urogenital labioscrotal swellings form the labia majora (Fig. 8.7).

In contrast to the testis, the developing ovary does not express FSH and hCG/LH receptors until after 16 weeks' gestation. At around 20 weeks' gestation, plasma concentrations of FSH reach a peak, and the first primary follicles are formed. By 25 weeks' gestation, the ovary has developed definitive morphological characteristics (see Plate 9, facing p. 148) [39]. Folliculogenesis can proceed, and a few Graafian follicles will have developed by the third trimester. However, the amount of estrogen secreted by the developing ovary is likely to be insignificant compared with placental estrogen synthesis, and the ovary remains generally quiescent until activation at the time of puberty. Abnormalities in several factors (e.g. connexin 37, GDF9, FSH receptors, estrogen receptor β, progesterone receptor) can interfere with this early folliculogenesis [40].

Several conditions can affect female sexual development *in utero* (Table 8.4). Exposure of the fetus to androgens results in virilization of the external genitalia. A uterus will be present, but the local testosterone concentration is not usually sufficient to stabilize Wolffian structures. Virilization of

Table 8.4. Genetic disorders causing virilization of karyotypic females (46,XX).

Gene	Protein	OMIM	Locus	Inheritance	Gonad	Uterus	External genitalia	Associated features
SRY	TF	480000	Yp11.3	Translocation	Testis or ovotestis	–	Male or ambiguous	
SOX9	TF	608160	17q24	dup17q24	ND	–	Male or ambiguous	
HSD3B2	Enzyme	201810	1p13	AR	Ovary	+	Ambiguous	CAH, primary adrenal failure, partial virilization due to ↑ DHEA
CYP21A2	Enzyme	201910	6p21–23	AR	Ovary	+	Ambiguous	CAH, phenotypic spectrum from severe salt-losing forms associated with adrenal failure to simple virilizing forms with compensated adrenal function, ↑ 17-hydroxyprogesterone
CYP11B1	Enzyme	202010	8q21–22	AR	Ovary	+	Ambiguous	CAH, hypertension due to ↑ 11-deoxycortisol and 11-deoxycorticosterone
POR (oxidoreductase)	CYP enzyme electron donor	124015	7q11.2	AR	Ovary	+	Ambiguous	Mixed features of 21-hydroxylase deficiency, 17α-hydroxylase/17,20-lyase deficiency, and aromatase deficiency; associated with Antley Bixler craniosynostosis
CYP19	Enzyme	107910	15q21	AR	Ovary	+	Ambiguous	Maternal virilization during pregnancy, absent breast development at puberty
Glucocorticoid receptor	Nuclear receptor TF	138040	5q31	AR	Ovary	+	Ambiguous	↑ ACTH, 17-hydroxyprogesterone and cortisol; failure of dexamethasone suppression

AR, autosomal recessive; TF, transcription factor; ND, not determined; CAH, congenital adrenal hyperplasia; ACTH, adrenocorticotrophic hormone; HSD3B2, 3β-hydroxysteroid dehydrogenase type II; CYP21A2, 21-hydroxylase; CYP11B1, 11β-hydroxylase; CYP19, aromatase.

the 46,XX fetus is most often due to disorders of adrenal steroidogenesis (21-hydroxylase deficiency, 11β-hydroxylase deficiency, oxidoreductase deficiency) or to the mild androgenic effects of dehydroepiandrosterone (DHEA) in 3β-hydroxysteroid dehydrogenase deficiency type II (Table 8.4). Rare causes of virilization include aromatase deficiency, glucocorticoid resistance, and maternal virilizing tumors (e.g. luteoma of pregnancy). Exposure to certain chemical agents in pregnancy has also been proposed as a cause of fetal virilization.

Abnormalities in uterine development can result in bicornate uterus (Fryns syndrome), uterine hemiagenesis or hypoplasia, or uterine agenesis. These conditions can be associated with renal and cervical spinal abnormalities as part of Mayer–Rokitansky–Kuster–Hauser syndrome or MURCS (Müllerian, renal, cervical spine) [38]. The etiology of these conditions is not known, although a WNT4 mutation has been described [41]. Uterine abnormalities have been associated with maturity-onset diabetes of the young type 5 (MODY5, *HNF1β*) and vaginal abnormalities with hand–foot–genital syndrome (*HOXA13*) and McKusick–Kaufman syndrome (*BBS6*).

Development of the hypothalamic–gonadotrope axis in the fetus

Fetal hypothalamic–gonadotrope development occurs from 6 weeks' gestation in parallel with the processes of sex determination and sexual differentiation. Pituitary gonadotrophin release probably does not influence the gonad until around 20 weeks' gestation (Fig. 8.1), but development of the hypothalamic–gonadotrope axis has some unique features, and abnormalities can cause congenital forms of hypogonadotropic hypogonadism (HH) (Table 8.5).

Development and migration of gonadotropin-releasing hormone (GnRH)-synthesizing neurons

Embryonic development of the hypothalamic GnRH neurosecretory system is intimately related to development of the extra- and intracranial olfactory apparatus, and the GnRH-synthesizing neurons originate extracranially before migrating to their final position in the fetal hypothalamus in all species studied.

In humans, GnRH-synthesizing neurons first appear in

Table 8.5. Genetic causes of hypogonadotropic hypogonadism.

Gene	Protein	OMIM	Locus	Inherited	Associated features
KAL1	Extracellular matrix protein	308700	Xp22	X	Anosmia, renal agenesis, synkinesia, cleft lip/palate, oculomotor and visuospatial defects, gut malrotations
FGFR1	Receptor	136350	8p11	AD/AR	Anosmia/hyposmia, cleft lip/palate, facial dysmorphia
NELF	Guidance molecule	608137	9q34.3	AD	Anosmia
Leptin	Cytokine	164160	7q31	AR	Obesity
Leptin receptor	Receptor	601007	1p31	AR	Obesity
PC1 (PCSK1)	Enzyme	162150	5q15–21	AR	Obesity, hypocortisolemia, hypoglycemia, diarrhea
GPR54	G-protein receptor	604161	19p13.3	AR	
GnRHR	G-protein receptor	138850	4q21	AR	
HESX1	TF	601802	3p21	AD/AR	Septo-optic dysplasia, MPHD (or isolated GHD)
LHX3	TF	600577	9q34	AR	MPHD (ACTH usually spared), cervical spine rigidity
PROP1	TF	601538	5q35	AR	MPHD (ACTH usually spared)
SF1 (NR5A1)	Nuclear receptor TF	184757	9q33	AD/AR	Primary adrenal failure, XY sex reversal, uterus, obesity
DAX1 (NR0B1)	Nuclear receptor TF	300200	Xp21	X	Primary adrenal failure, impaired spermatogenesis

AR, autosomal recessive; AD, autosomal dominant; X, X-chromosomal; TF, transcription factor; MPHD, multiple pituitary hormone deficiency; FGFR1, fibroblast growth factor receptor 1; NELF, nasal embryonic LHRH factor; PC1, prohormone convertase 1; GnRHR; gonadotropin-releasing hormone receptor; SF1, steroidogenic factor 1. Modified with permission from ref. 4. Copyright 2002, The Endocrine Society.

the embryonic medial olfactory placode at around 6 weeks' gestation and begin to migrate along axons of the terminal vomeronasal nerve complex on a neural cell adhesion molecule (N-CAM)-rich scaffold (Fig. 8.8a). At around 6.5 weeks' gestation, these migrating neurons pass through the primitive cribriform plate and penetrate the forebrain, medial and caudal to the developing olfactory bulbs. GnRH neurons then migrate posteriorly in the submeningeal space by the interhemispheric fissure before proceeding laterally to reach their final position in the fetal mediobasal hypothalamus from around 14 weeks' gestation [42]. Fetal GnRH neuron migration is complete by around 19 weeks' gestation, by which stage pulsatile GnRH release is established.

A number of cell adhesion molecules and signaling factors have been implicated in this migratory process (e.g. anosmin-1/*KAL*, FGFR1, NELF, AXL/GAS6), and GnRH neurons express different transcription factors at different stages of maturation. Furthermore, other neuroendocrine cells co-migrate with GnRH-synthesizing neurons. Some of these cells synthesize neurotransmitters and hormones such as neuropeptide Y, leptin, corticotrophin-releasing factor (CRF), and glutamate, factors that may interact with GnRH neurons along the migratory path or in their final position in the hypothalamus.

Kallmann syndrome (KS) is the association of HH with anosmia (lack of a sense of smell) and can occur in X-linked, autosomal-dominant, and autosomal-recessive forms. Molecular advances in the past decade have identified two factors responsible for these conditions. X-linked Kallmann syndrome is due to mutations or deletions in the gene *KAL1*. *KAL1* encodes the protein, anosmin-1, which is expressed in the human forebrain from 5 weeks' gestation and appears to stimulate afferent projections to the olfactory bulb. Anosmin-1 contains a whey acidic protein (WAP) domain and four fibronectin domains, suggesting a putative role as an extracellular matrix protein. Studies of a 19-week-old fetus with X-linked KS have shown arrest of GnRH neuronal migration during passage through the cribriform plate but before entering the meninges. Patients with X-linked KS have hypoplasia or aplasia of olfactory bulbs and olfactory tracts (Fig. 8.8b). Ansomin-1 is also expressed in several other developing structures accounting for associated features such as synkinesia (mirror-image movements), unilateral renal agenesis, and oculomotor abnormalities (Table 8.5) [43]. However, the penetrance of these features is highly variable in families with *KAL1* mutations, suggesting that other modifier genes or epigenetic phenomena influence phenotypic expression.

Loss-of-function mutations in *FGFR1* have been identified as a cause of autosomal-dominant (and rarely recessive) forms of KS [44]. Associated features can include cleft lip or palate and facial dysmorphia in addition to anosmia or hyposmia. Although data are limited, it is emerging that the degree of hypogonadism associated with *FGFR1* changes may be milder than that associated with mutations in *KAL1*, and some patients may present with adult-onset HH or a "fertile eunuch" phenotype. Furthermore, a heterozygous

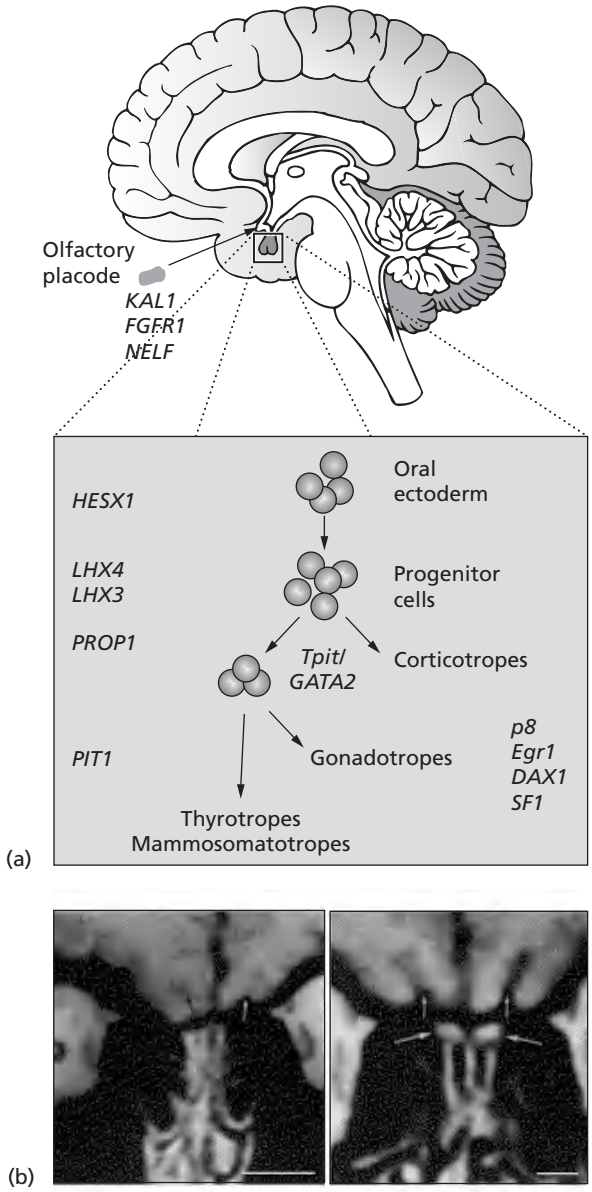

Fig. 8.8. Development of the hypothalamic–gonadotrope axis. (a) Migration of GnRH neurons from the olfactory placode to the fetal hypothalamus is facilitated by proteins such as anosmin-1 (*KAL1*), FGFR1, and NELF. Inset. Differentiation of the anterior pituitary gland. The gradient of factors such as GATA2 and Tpit may provide a "switch" for gonadotrope differentiation. Mutations in several transcription factors are associated with multiple pituitary hormone deficiency in humans. Mutations affecting isolated gonadotrope factors have not yet been described, except as part of complex phenotypes associated with mutations in SF1 or DAX1. (b) Coronal magnetic resonance image (MRI) scan showing absent olfactory bulbs and shallow sulci in a patient with X-linked Kallman syndrome (arrows, left) compared with a normal control (right). Reproduced with permission from refs. 42,55. Copyright 2003, Nature Publishing Group [41]; 2002, Elsevier [55].

splice-site mutation in the human homolog of *NELF* (nasal embryonic LHRH factor) has been proposed as the cause of an autosomal-dominant form of KS in a Japanese patient [45].

GnRH synthesis and action

GnRH-synthesizing neurons are localized throughout the hypothalamus with a concentration in the arcuate nucleus. GnRH is synthesized as a precursor polypeptide, which undergoes cleavage and enzymatic processing to form a mature 10-amino-acid hormone that is stored in secretory granules. GnRH is released from neuronal projections at the medial eminence into the portal blood system in a pulsatile manner from the second trimester. No mutations in GnRH have been identified in humans, although targeted deletion of this gene causes hypogonadism in the *hyp*/*hyp* mouse, and deletion of a region of chromosome 8 that contains the *GNRH* gene has been described in a patient with HH.

GnRH synthesis and release can be influenced by a host of neuroendocrine factors and other regulators, and several single gene disorders have congenital or adolescent HH as part of the condition (Table 8.5). These conditions include mutations in prohormone convertase-1 (*PCSK1*), leptin, and the leptin receptor. Mutations in the G-protein-coupled receptor, GPR54, and its natural ligand, kisspeptin-1 (metastin, *KISS1*), have been reported in patients with autosomal-recessive forms of HH, including micropenis and undescended testes [46,47]. Although these factors are expressed in pituitary gonadotropes, initial data suggest that the GPR54/kisspeptin-1 system regulates GnRH synthesis and release primarily at the level of the hypothalamus. Related galanin-like receptors and peptides are also emerging as potential regulators of GnRH.

Finally, pulsatile GnRH exerts its action on the gonadotrope by signaling through the G-protein-coupled GnRH receptor (*GNRHR*). *GNRHR* mutations are found in approximately 40% of patients with autosomal-recessive familial HH and in approximately 10% of patients with idiopathic isolated gonadotropin deficiency [48]. These changes affect receptor signaling and membrane localization.

Pituitary gonadotrope development and function

During development, cells destined to form the anterior pituitary arise within the oral ectoderm and Rathke's pouch, and undergo a process of differentiation into corticotropes, thyrotropes, mammosomatotropes, and gonadotropes. Developmental events that affect differentiation of early progenitor cells can cause congenital gonadotropin insufficiency as part of a multiple pituitary hormone deficiency. For example, mutations or variations in transcription factors such as HESX1, LHX3 and PROP1 have all been associated with HH, although the onset of this is often delayed until adulthood (e.g. PROP1) (Table 8.5, Fig. 8.8a) [49].

Table 8.6. Selected genetic causes of gonadotropin resistance, gonadal failure, or abnormal spermatogenesis in males.

Gene	Protein	OMIM	Locus	Inherited	Associated features
FSHB	Glycoprotein hormone	136530	11p13	AR	Increased LH, azoospermia
LHB	Glycoprotein hormone	152780	19q13	AR	Increased LH (bioinactive), increased FSH, pubertal delay, arrested spermatogenesis
FSHR	G-protein receptor	136435	2p16–21	AR	Variable spermatogenic defects
LHCGR	G-protein receptor	152790	2p21	AR	Phenotype ranges from complete undervirilization to micropenis and abnormal puberty
GNAS	G-protein subunit	139320	20q13.1	AD (imprinted)	Pseudohypoparathyroidism, hormone resistance
DAX1 (NR0B1)	Nuclear receptor TF	300200	Xp21.3	X	X-linked adrenal hypoplasia congenita, abnormal puberty, impaired spermatogenesis
ERα	Nuclear receptor TF	133430	6q25.1	AR	Tall stature, delayed epiphyseal fusion
CYP19	Enzyme	107910	15q21	AR	Maternal virilization, delayed epiphyseal fusion
AR	Nuclear receptor TF	313700	Xq11–12	X/CAG	Phenotype ranges from complete or partial androgen insensitivity to micropenis or impaired spermatogenesis
AIRE	TF	607358	21q22.3	AR	Autoimmune polyendocrinopathy syndrome type 1
DAZ	RNA-binding protein	400003	Yq11	Y	
RBMY	RNA-binding protein	400006	Yq11	Y	
USP9Y	Protease	400005	Yq11.2	Y	
DNAH5	Microtubule motor protein	603335	5p14–15	AR	Primary ciliary dyskinesia, left–right asymmetry
POLG	DNA polymerase	174763	15q25	CAG	
CFTR	Membrane channel	602421	7q31.2	AR	Cystic fibrosis

AR, autosomal recessive; AD, autosomal dominant; X, X-chromosomal; Y, Y-chromosomal; TF, transcription factor; CAG, CAG repeat variability.

Differentiation of the gonadotropin cell line from other pituitary lineages may be controlled by gradient-dependent "switches" such as GATA2 and Tpit (TBX19) [50,51]. A limited number of gonadotrope-specific factors have been identified, including P8, Egr1, SF1, and DAX1 (Fig. 8.8a) [52]. Patients with mutations in *SF1* may have partial deficits in gonadotropin synthesis, but the phenotype is more complex as it also involves gonadal dysgenesis. Mutations in *DAX1* cause X-linked adrenal hypoplasia congenita. Approximately 10% of boys with this condition have evidence of congenital gonadotropin insufficiency, but the early postnatal period of HPG axis activation is often present or even prolonged, and early puberty can occur in some cases. Nevertheless, most patients show evidence of disordered gonadotropin release/HH by the time of adolescence. No mutations in other gonadotrope-specific factors have been described, although these genes remain candidates for the significant number of patients with congenital or adolescent-onset HH where no cause is found.

Gonadotropins

Fetal pituitary gonadotropin synthesis and release begins from around 14 weeks' gestation and peaks around 20–22 weeks' gestation (Fig. 8.1). Synthesis of these hormones is regulated by hypothalamic GnRH, downstream signaling mechanisms such as PACAP, and transcription factors such as SF-1, DAX-1, Pitx1, Egr-1 (LHβ), and p8 (LHβ). The β-subunits of these hormones undergo post-translational modification and heterodimerize with a common α-subunit to form the mature glycoprotein hormones, FSH and LH [53].

The gonadotropin hormones are also influenced by inhibin, activin, and follistatin (see Plate 10a, facing p. 148) [54]. Inhibin is a heterodimeric hormone consisting of an α-subunit bound to one of two distinct β-subunits, βA or βB. Inhibin A and inhibin B are produced by the gonads (predominantly by ovarian granulosa cells and testicular Sertoli cells respectively) and placenta and suppress FSH. Activins stimulate FSH and consist of homo- or heterodimers of inhibin β-subunits (activin A, activin B, or activin AB). Follistatin binds activin to attenuate its action and reduce FSH release. These hormones are likely to function at a local (paracrine) level in the pituitary as well as having a systemic influence on gonadotropin release and signal through TGFβ/Smad pathways.

Mutations in genes encoding LH and FSH have been described in a small number of patients with abnormalities of

Table 8.7. Selected genetic causes of gonadotropin resistance and ovarian failure in females.

Gene	Protein	OMIM	Locus	Inherited	Associated features
FSHB	Glycoprotein hormone	136530	11p13	AR	Delayed puberty, absent breast development, primary amenorrhea
LHB	Glycoprotein hormone	152780	19q13	AR	Possible infertility
FSHR	G-protein receptor	136435	2p16–21	AR	Variable pubertal failure and primary or secondary amenorrhea
LHCGR	G-protein receptor	152790	2p21	AR	Normal pubertal development, oligo- or amenorrhea
GNAS	G-protein subunit	139320	20q13.1	AD (imprinted)	Pseudohypoparathyroidism, hormone resistance
AIRE	TF	607358	21q22.3	AR	Autoimmune polyendocrinopathy syndrome type 1
FMR1	Possible methylation locus	309550	Xq27.3	X/CAG	Premature ovarian failure
Inhibin-α	Glycoprotein hormone subunit	147380	2q33–36	AR	Premature ovarian failure
FOXL2	TF	605597	3q23	AD	Bletharophimosis–ptosis–epicanthus inversus syndrome

AR, autosomal recessive; AD, autosomal dominant; X, X-chromosomal; TF, transcription factor; CAG, CAG repeat variability.
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puberty or spermatogenesis (Tables 8.6 and 8.7, Plate 10b, facing p. 148), but severe congenital abnormalities associated with these conditions have not been described [53,55,56]. Mutations in the G-protein-coupled FSH receptor are associated with variable spermatogenic defects in males and variable pubertal development and menstrual disturbance in females (Tables 8.6 and 8.7). In contrast, LH receptor loss-of-function affects virilization of the developing male fetus as it signals both hCG and LH activity, whereas women with LH receptor changes have normal pubertal development but oligo- or amenorrhea [53].

Variations in the α-subunit of inhibin (INHA) have been linked to premature ovarian failure and adrenocortical tumors in children. Targeted deletion of this gene causes stromal tumors in mice. Deletion of the βB-subunit of activin suppresses reproduction in female mice, whereas deletion of the βA-subunit is lethal. Deletion of the activin receptor (*Acvr2*) delays or impairs fertility in mice and is associated with micrognathia and other dysmorphic features. Finally, overexpression of follistatin can affect reproductive function in mice too. The potential role of changes in the inhibin/activin/follistatin system in human reproductive disorders remains poorly understood.

Early postnatal changes in the hypothalamus–pituitary (gonadotrope)–gonadal axis

At birth, the baby is removed from the influence of maternal and placental hormone and undergoes a series of distinct endocrine changes.

In the male, low concentrations of testosterone can be detected by standard assays at birth, but these fall in the first few days of life. However, a reactivation of the HPG axis occurs from around 6 weeks of age, which results in peaks of testosterone nearing mid-pubertal levels at between 2 and 3 months after birth (Fig. 8.1) [57]. The HPG axis then becomes relatively quiescent by 6 months of age until the onset of puberty. Inhibin B concentrations are high at birth and fall in the first 2 years of life before once again rising with the onset of puberty between 11 and 15 years (58). In contrast, AMH concentrations remain high from birth through childhood and decline to low concentrations with the onset of puberty. Thus, AMH and inhibin B can be useful markers of active testicular tissue in boys with cryptorchidism [59].

The early postnatal endocrine events in girls are less well understood. Placental estrogen exposure can result in breast development before birth, and a small episode of menstrual bleeding can occur several days after birth following withdrawal of estrogen and progesterone. It remains unclear whether girls have a discrete activation of the HPG axis in infancy, but concentrations of estradiol (20–80 pmol/L) and inhibin B (50–200 pg/mL) can be measured in the first few months of life, and surprisingly high concentrations of FSH with marked interindividual variability can be found during infancy and early childhood [median 3.8 IU/L (1.2–18.8 IU/L, 2.5–97.5%) at 3 months of age in healthy term girls] [59]. Inhibin A has been proposed as a test of ovarian tissue in the newborn period in children with possible true hermaphroditism, but this hormone is below the limits of detection in many normal term newborn girls [60].

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9 Ambiguous genitalia

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In the vast majority of infants at birth, sex assignment is instantaneous. Parents faced with a situation in which that may not be the case find this unbelievable. The neonatologist and, in turn, the pediatric endocrinologist must manage the problem in an organized fashion centered on a logical series of investigations to try to establish a cause for the abnormal genital development. It should be recognized from the outset that a cause may not be found, especially in the XY infant with ambiguous genitalia. Nevertheless, there should not be undue delay in sex assignment.

An understanding of the genetic and hormonal control of fetal sex development is fundamental to the investigation and management of ambiguous genitalia. Allied to this must be knowledge about postnatal psychosexual development and an appreciation of the sociocultural influences on gender [1] as well as the psychosexual aspects of the subject [2,3].

Genital ambiguity is compounded by the use of terminology confusing to physicians, let alone for families with affected children. The terms relevant for the assessment of ambiguous genitalia need clarification based on the functional classification of causes. These dictate a practical approach to investigation and early management.

Terminology

The phrase ambiguous genitalia is generally used synonymously with intersex and refers to abnormal genital development at birth. Strictly defined, the terms describe an infant in whom immediate sex assignment is not possible. However, external genital phenotypes such as severe perineoscrotal hypospadias with micropenis would also be included under the umbrella term even though sex assignment is not generally an issue. Geneticists use the term sex reversal, prefaced by XX or XY, but the use of the terminology XY sex reversal, subdivided as complete or partial, is descriptive and is applied generally to disorders of sex determination manifest as gonadal dysgenesis.

True hermaphroditism, defined as the presence of both ovarian and testicular tissue in the one individual, may be in the form of separate gonads or combined as an ovotestis. The diagnosis can be confirmed only by histology. The terminology pseudohermaphroditism prefaced by female and male to describe causes of masculinization in females and undermasculinization in males, respectively, has long been used, but these terms are confusing not only to professionals but also to families of children with intersex disorders. The descriptive model is used in this chapter. Postnatal psychosexual development and the sociocultural influences on gender are not the brief of this chapter. Nevertheless, the pediatric endocrinologist must be familiar with current concepts in this field and the polarization of views espoused on the subject [4,5]. The following definitions are germane to understanding the management of ambiguous genitalia:

- gender (sex) assignment – the decisive allocation of male or female at birth, which is usually instantaneous;
- gender identity – the sense of self as being male or female;
- gender role – denotes aspects of behavior and preferences in which males and females differ;
- sexual orientation – refers to the target of sexual arousal;
- gender attribution – assigning as male or female on first encounter with a person;
- gender dysphoria – a transsexual state associated with a gender identity disorder. There is a dichotomy between the body habitus and gender identity. The process of fetal sex determination and sex differentiation appears to be normal, and transsexualism is not generally considered within the ambit of ambiguous genitalia.

In general, gender identity and gender role, together with the symbols that attribute to gender manifestations, are congruent. Furthermore, the subject of erotic desires is generally toward the opposite sex. It is against this background that the complex assessment of adults who were born with ambiguous genitalia and may have been sex reassigned must take place.

Table 9.1. Causes of ambiguous genitalia: a functional classification.

Type/cause	Illustrative examples
Masculinized female	
Fetal androgens	CAH, placental aromatase deficiency
Maternal androgens	Ovarian and adrenal tumors
Undermasculinized male	
Abnormal testis determination	Partial (XY) and mixed (XO/XY) gonadal dysgenesis
Androgen biosynthetic defects	LH receptor-inactivating mutations 17βOH-dehydrogenase deficiency 5α-reductase deficiency
Resistance to androgens	Androgen insensitivity syndrome variants
True hermaphroditism	
Presence of testicular and ovarian tissue	Karyotypes XX, XY, XX/XY
Syndromal	Denys–Drash, Frasier Smith–Lemli–Opitz

Causes of ambiguous genitalia – a simple classification

A classification system based on descriptive criteria rather than an exhaustive list of causes generally found in textbooks is shown in Table 9.1. An understanding of the embryology of the reproductive system and the genetic and hormonal control of fetal sex development is essential to manage ambiguous genitalia appropriately (see Chapter 8). This is particularly the case for abnormal genital development in the male.

The masculinized female

The placenta contains an aromatase enzyme system that is generally extremely efficient in protecting a female fetus from the effects of androgens in the maternal circulation. For example, women with congenital adrenal hyperplasia (CAH) who may become pregnant and have elevated testosterone levels throughout gestation do not have female offspring that are virilized [6,7]. Androgen-secreting tumors of the adrenals and ovaries can masculinize the mother and a female fetus, presumably because the androgenic substrates overwhelm the capacity of the placental aromatase system. Luteoma of pregnancy and hyperreactio luteinalis are benign tumors but produce large ovarian masses. Luteomas predominate in multiparous Afro-Caribbean women who may have a pre-existing polycystic ovarian syndrome. These tumors regress post partum but can recur in subsequent pregnancies [8]. Other virilizing ovarian tumors include arrhenoblastoma, hilar cell tumor, and Krukenberg tumor. Use of progestational agents with some intrinsic androgenic activity to prevent recurrent miscarriage is obsolete, but Danazol, a derivative of 17β-ethinyltestosterone, has a place in the medical adjunctive treatment of endometriosis. It readily crosses the placenta, and cases of masculinized female infants are recorded [9].

Placental aromatase deficiency is a recognized cause of ambiguous genitalia in a female infant whose mother is also virilized during pregnancy [10,11]. A single *CYP19* gene is expressed in several tissues, including the gonads, placenta, and adipocytes, through the action of tissue-specific promoters. The aromatase enzyme is a key regulator of production of estrogens from androgens via the fetal–placental–maternal unit (Fig. 9.1).

The fetal adrenals produce large quantities of dehydroepiandrosterone sulfate (DHEAS), which is 16β-hydroxylated

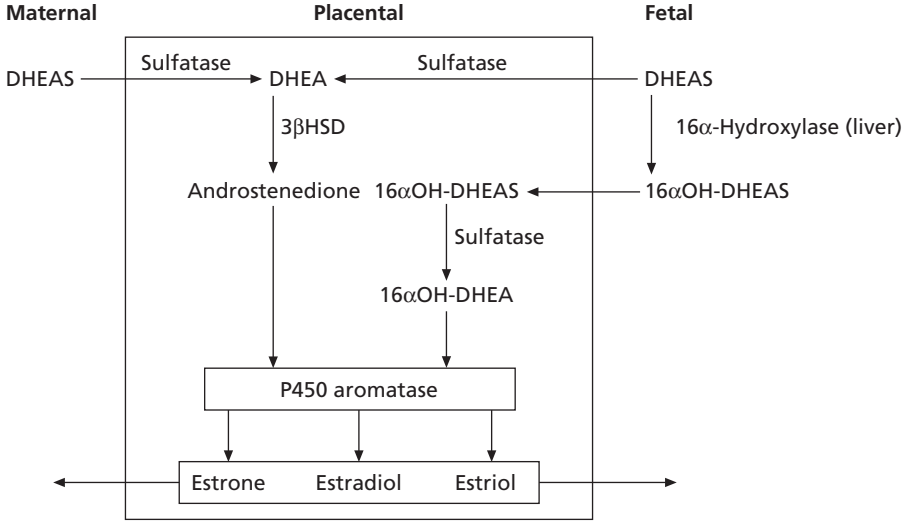

Fig. 9.1. The fetal–placental–maternal steroid unit. The androgen substrate DHEAS is synthesized in both the maternal and the fetal adrenals and cleaved to DHEA by placental sulfatase. The fetal liver also hydroxylates DHEAS prior to sulfatase cleavage by the placenta. Androgen substrates are aromatized to estrogens, particularly estriol. DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; 3βHSD, 3β-hydroxysteroid dehydrogenase.

in both the adrenal and the fetal liver. After transfer to the placenta, the sulfate moiety of 160H-DHEAS is removed by placental sulfatase. Deficiency of this enzyme causes the X-linked ichthyosis syndrome [12]. The substrates DHEA and 160H-DHEA are converted to more potent androgens, such as androstenedione and testosterone. These are aromatized to estrone and estradiol respectively. A large amount of estriol is also produced by aromatization of androgen substrates. Maternal urinary estriol measurement is no longer routinely used as a test of placental function, but levels would be low in the last trimester of a normal pregnancy as a result of either placental sulfatase or placental aromatase deficiency. Serial measurements of urinary estriol do still have a useful role in monitoring prenatal treatment of CAH with dexamethasone [13].

The degree of maternal and fetal masculinization can be quite profound in placental aromatase deficiency. The mother, however, may escape signs of virilization when as little as 1–2% activity of mutant enzyme is present. This illustrates the capacity of this enzyme to convert androgens to estrogens. The internal genitalia of affected female infants are normal, but ovarian cysts may develop in later childhood. There is failure of breast development at puberty, onset of virilization, and polycystic changes in the ovaries. A spectrum of mutations is distributed throughout the *CYP19* gene, including some affecting the critical heme binding site [14]. Aromatase deficiency should be considered when CAH has been excluded in a female newborn with ambiguous genitalia.

Apparent combined deficiency of the P450 17α-hydroxylase and 21-hydroxylase enzymes can also cause mild degrees of maternal and fetal masculinization which is self-limiting after birth. Mutations are found not in the *CYP17* or *CYP21* genes but in the gene encoding for cytochrome P450 oxidoreductase [15]. This enzyme functions as an electron donor to microsomal cytochrome P450s, including the aromatase enzyme. This may partly explain the masculinization from accumulation of fetal adrenal androgens as a result of partial placental aromatase deficiency. However, there is also impairment of androgen biosynthesis in oxidoreductase deficiency such that affected males are undermasculinized. The explanation for this paradox may lie with observations of steroid biosynthetic pathways in the tammar wallaby [16]. Here, there is evidence that the potent androgen DHT can be produced by a "backdoor" pathway involving the precursor steroid, androstanediol and avoiding testosterone as an intermediary substrate. Such a pathway may exist in the human fetus only to switch to the more classical pathway of androgen biosynthesis after birth. Further studies of masculinized newborn females and undermasculinized males of unknown cause using specific urinary steroid analyses will indicate whether oxidoreductase deficiency is a significant cause of ambiguous genitalia.

In the context of ambiguous genitalia, CAH is the commonest cause and is usually the most straightforward diagnosis to establish. This must be undertaken promptly in view of the potential life-threatening consequences to the infant of glucocorticoid and mineralocorticoid deficiency. Giving dexamethasone to the mother from early in pregnancy can successfully prevent any masculinization of the external genitalia in an affected female infant [17]. This is a unique example of preventing a major congenital malformation by medical intervention.

The undermasculinized male

The list of causes in this category is huge, not least because of inclusion of disorders such as simple hypospadias and isolated micropenis, which are not true examples of ambiguous genitalia. The choice of using the following broad categories related to testis determination, androgen biosynthesis, and androgen action stems directly from an understanding of the normal processes of male fetal sex development.

Defects in testis determination

Normal development and function of Sertoli cells and Leydig cells is essential for hormone-mediated sex differentiation of the internal and external genitalia in the male. Failure of these specialized cells to develop gives rise to a dysgenetic gonad and the clinical disorder, gonadal dysgenesis. Gonadal histology is quite variable and is the determinant of the sex phenotype. Thus, streak gonads are completely undifferentiated and are composed mainly of fibrous tissue with no germ cells, Sertoli cells, interstitial steroid-secreting cells, tubules, or follicles. When both gonads are streaks, the phenotype is female, whatever the karyotype. Consequently, XY complete gonadal dysgenesis (Swyer syndrome) leads to complete sex reversal and no ambiguity in sex development. Approximately 15–20% of patients have a mutation of the *SRY* gene [18]. There are familial cases of XY complete gonadal dysgenesis in which the genetic cause is unknown and the pattern of inheritance can be X-linked or autosomal recessive [19]. There is a high risk of gonadal tumors such as gonadoblastoma and germinoma.

The partial form of gonadal dysgenesis gives rise to ambiguity of the genitalia because of the preservation of some Leydig cell function. There are generally Müllerian duct remnants, reflecting inadequate Sertoli cell production of anti-Müllerian hormone (AMH). Histology shows a thin and loosely organized tunica albuginea, underdeveloped seminiferous tubules with wide intertubular spaces, abundant infantile Sertoli cells, scanty germ cells, and a dense stroma containing calcified psammoma bodies [20]. These appearances are not dissimilar from those of an early developing testis so dysgenetic gonads in partial gonadal dysgenesis syndromes represent a failure in gonad maturation.

Partial gonadal dysgenesis is the term generally applied in association with an XY karyotype, whereas the term mixed gonadal dysgenesis is used when there is associated 45,XO/46,XY chromosomal mosaicism. In this syndrome, gonadal morphology is typically a testis on one side and a streak gonad on the contralateral side. The condition is not due to mutations in the *SRY* gene [21,22]. A wide spectrum of phenotypes can result from XO/XY sex chromosome mosaicism with varying degrees of sex reversal [23]. Abnormalities of the external genitalia may be in the form of a severe hypospadias with cryptorchidism but a penis of normal size. Alternatively, the degree of undermasculinization can be manifest solely as a hypertrophied clitoris. There is no correlation between the phenotype and the proportion of XO vs. XY cell lines, whether this is determined in blood or fibroblasts. In later childhood, stigmata of Turner syndrome may appear. The pediatric endocrinologist and pediatric surgeon see a skewed population of XO/XY infants that present at birth with genital anomalies. More than 90% of fetuses with 45,XO/46,XY on prenatal cytogenetic studies have normal male genitalia [24,25]. There is no information about the longer term follow-up of these cases in relation to growth, puberty, fertility, and risk of gonadal tumors.

A number of eponymous syndromes are associated with gonadal dysgenesis and should be considered in the assessment of an infant with ambiguous genitalia. Important examples are two related disorders, the Denys–Drash and Frasier syndromes [26–28]. Both are caused by mutations in *WT1*, a gene essential for gonadogenesis and nephrogenesis. In the Denys–Drash syndrome, there are usually genital anomalies at birth in XY cases, a characteristic nephropathy resulting from diffuse mesangial sclerosis, and a predisposition to Wilms' tumor with a median age of onset by 12 months [29]. There is a relative "hotspot" within exon 9 of the gene where most of the heterozygous mutations occur. A *WT1* mutation can occur in isolated hypospadias without evidence of a nephropathy or a Wilms' tumor [30]. The rarity of this occurrence does not merit screening for WT1 mutations in all infants with hypospadias, although there may be a case for prospective screening for nephropathy (proteinuria) and Wilms' tumor (renal ultrasound) in XY infants with ambiguous genitalia.

Frasier syndrome differs with respect to a more severe gonadal dysgenesis generally resulting in XY complete sex reversal, a nephropathy characterized by focal segmental glomerulosclerosis, and a predisposition to a gonadoblastoma rather than a Wilms' tumor. The characteristic WT1 abnormality in Frasier syndrome is caused by a donor splice site mutation in intron 9, which leads to an alteration in the normal ratio of WT1 protein isoforms. The WAGR syndrome (Wilms' tumor, aniridia, genital anomalies, mental retardation) is a contiguous gene deletion syndrome involving a chromosome 11p locus that includes the *WT1* and *PAX6* genes.

SOX9, an SRY-related protein, is a transcription factor involved in both chondrogenesis and early testis determination. Heterozygous mutations in the *SOX9* gene can cause campomelic dysplasia, a multiskeletal disorder, together with sex reversal in the majority of affected males [31]. Not all affected patients have both gonadal dysgenesis and genital anomalies. This appears to be related to the function of the SOX9 protein binding to DNA either as a dimer or as a monomer [32]. Dimerization is a mandatory requirement for chondrogenesis. Mutations in *SOX9* do not lead solely to genital anomalies [33]; hence, analysis of this gene is indicated only in the investigation of XY gonadal dysgenesis associated with skeletal abnormalities.

Another key gene in development of the gonads is *SF1* (steroidogenic factor 1). The gene is also required for development of the adrenals and the hypothalamus [34]. Disruption of this gene causes combined XY sex reversal and adrenal insufficiency, although a patient without adrenal insufficiency has also been reported [35,36]. Screening for *SF1* mutations in patients with gonadal dysgenesis of unknown cause is probably worthwhile.

ATRX syndrome is another multisystem disorder associated with XY gonadal dysgenesis [37]. The syndrome comprises β-thalassemia, mental retardation, and multiple congenital anomalies. The clinical spectrum extends to eponymous syndromes such as Juberg–Marsidi and Smith–Fineman–Myers. The ATR-X gene is located on Xq13.3 and encodes for a protein that belongs to a family of DNA helicases. Their precise function is unknown, but they appear to regulate gene expression via chromatic remodeling [38].

Defects in androgen biosynthesis

The principal components of the pathway that are key to the production of androgens by the fetal testis are shown in Figure 9.2. Fetal serum concentrations of testosterone rise to within the normal adult male range toward the end of the first trimester, a period when Wolffian duct stabilization occurs, followed later by growth of the external genitalia (Fig. 9.3). It is evident that the timing and magnitude of the rise in both androgen and AMH concentrations are critical determinants for normal male sex differentiation. Abnormalities in a number of biosynthetic steps can result in inadequate androgen production and hence an undermasculinized male infant. Some of these abnormalities also affect steroidogenesis.

Fetal Leydig cell androgen synthesis is initially placental human chorionic gonadotropin (hCG) dependent but is dependent later in pregnancy on luteinizing hormone (LH) stimulation from the fetal pituitary glands. Both ligands bind to a common LH/hCG receptor, a member of the family of G-protein-coupled receptors comprising seven transmembrane regions. Inactivating mutations in the *LHR* gene in XY individuals cause a wide range in severity of undermasculinization, including complete sex reversal, severe hypospadias

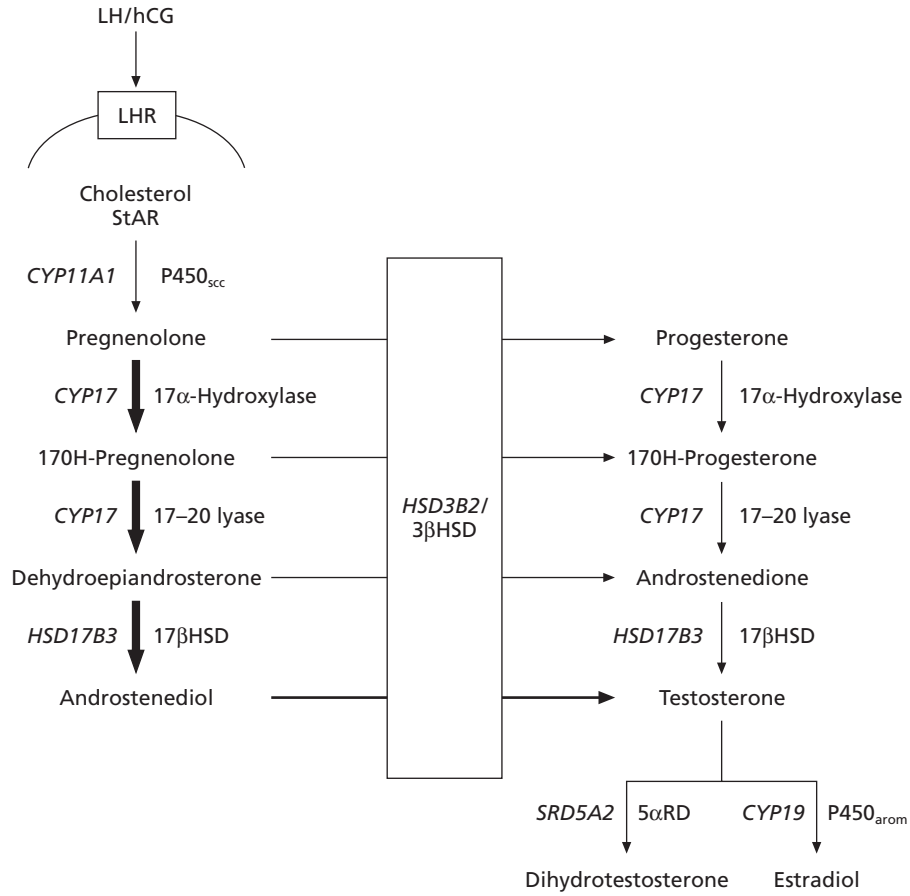

Fig. 9.2. Pathways of testosterone synthesis in the human testis. The predominant pathway is indicated by the bold arrows. The enzymes encoded by their respective genes (italicized) are shown. LHR, LH receptor; $P450_{scc}$, cytochrome P450 side-chain cleavage; 17βHSD, 17β-hydroxysteroid dehydrogenase; 3βHSD, 3β-hydroxysteroid dehydrogenase.

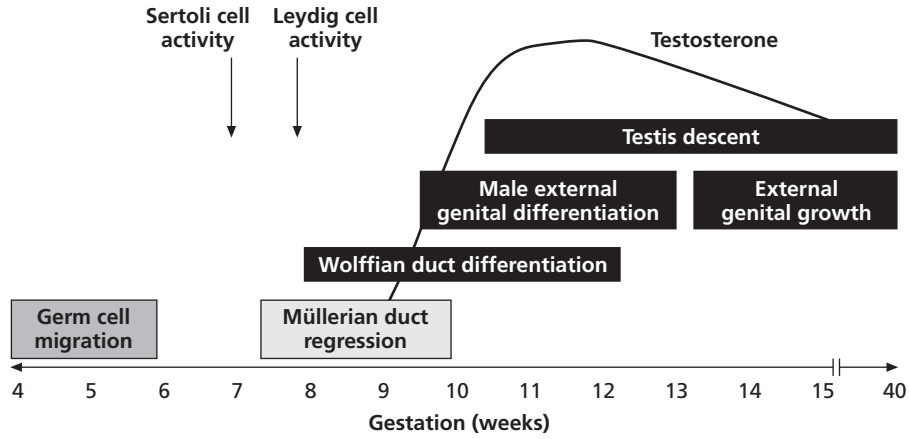

Fig. 9.3. Key events in fetal male development. The timing of the rise in fetal serum testosterone is shown, peak levels approximating the lower end of the normal adult range (10–30 nmol/L).

with ambiguous genitalia, or just isolated micropenis [39]. The expected endocrine profile is low testosterone and elevated LH levels and no testosterone response following hCG stimulation. Testis histology shows the appearance of Sertoli cells only and no Leydig cells in the interstitium. These features are difficult to confirm in the prepubertal child who may have an *LHR* mutation causing abnormal genital development. It is an intriguing observation that Wolffian ducts are stabilized despite the apparent lack of normal fetal testosterone production.

A number of enzymes are needed for testosterone and dihydrotestosterone (DHT) biosynthesis, but those relevant to testis-specific defects are 17β-hydroxysteroid dehydrogenase and 5α-reductase enzyme deficiencies. A unique feature of both these enzyme defects is their presentation at birth with severe undermasculinization, often to the extent of

complete sex reversal, yet a remarkable degree of virilization of the external genitalia with the onset of puberty. This phenomenon of "double sex reversal" is not fully explained, other than the suggestion that peripheral production of testosterone and DHT at puberty occurs through the utilization of alternative isoenzymes of the mutant enzyme. The penultimate step in testosterone synthesis is catalyzed by the 17β-hydroxysteroid dehydrogenase type 3 enzyme using androstenedione as substrate. A spectrum of mutations in the *HSD17B3* gene generally results in complete XY sex reversal [40,41]. The disorder may be mistaken for complete androgen insensitivity syndrome. Some affected infants are more masculinized and can be raised male. Gonadectomy must be performed before puberty when sex has been assigned as female. The uterus is absent as a result of normal testicular AMH action, but Wolffian ducts are stabilized, perhaps as a result of sufficient androgenic effect from locally acting high concentrations of androstenedione. Females affected with this enzyme deficiency are asymptomatic.

DHT is more potent than testosterone as an androgen because of binding more avidly to the androgen receptor. The type 2 5α-reductase enzyme is expressed in the genital anlagen so that growth of the genital tubercle and fusion of the labioscrotal folds is preferentially a DHT-dependent process. The type 2 isoenzyme in adulthood is expressed in the prostate, epididymis, seminal vesicles, and liver. In contrast, the type 1 isoenzyme is expressed only in skin and liver. Mutations in the *SRD5A2* gene have been reported worldwide, often in pockets within ethnic populations. These include the Dominican Republic (where the disorder was first fully characterized), New Guinea, Turkey, and Egypt [42]. Affected infants are rarely completely female at birth and may be sufficiently masculinized to be sex assigned as male. Sometimes, just isolated micropenis or hypospadias is the genital anomaly [43]. This suggests that testosterone may also have some direct effect on development of the genital anlagen.

Defects in androgen action

Failure of development of the external genitalia in a male with a normal 46,XY karyotype and no defect in determination of testes, which produce age-appropriate circulating concentrations of androgens, defines a form of tissue-specific resistance to the action of androgens. This is the commonest cause of male undermasculinization. Total resistance to androgens leads to complete XY sex reversal and no ambiguity of the external genitalia. This is the complete androgen insensitivity syndrome (CAIS), also known as the testicular feminization syndrome [44,45]. Some tissue response to androgens results in the partial androgen insensitivity syndrome (PAIS). The degree of response may be manifest as mild clitoromegaly, true ambiguity of the genitalia, hypospadias alone, or impaired fertility in an otherwise normal male [46].

Hormone levels in CAIS and PAIS are consistent with the definition of target resistance; typically, testosterone is elevated and LH levels are unsuppressed. Androgens are aromatized to estrogens resulting in breast development in XY males unopposed by any androgen action. Thus, the patient with CAIS has a normal female phenotype at puberty, except for absent or scanty growth of pubic and axillary hair. Clinical presentation occurs typically in adolescence for assessment of primary amenorrhea, but the condition may present in infancy with inguinal herniae, which are found to contain testes at the time of surgical repair. The patient with PAIS and a more male phenotype develops gynecomastia at puberty, and breast cancer has been reported [47]. Breast cancer does not seem to occur in CAIS, although there is one report of a juvenile fibroadenoma of the breast developing after estrogen replacement was started after gonadectomy in a young adult with CAIS [48].

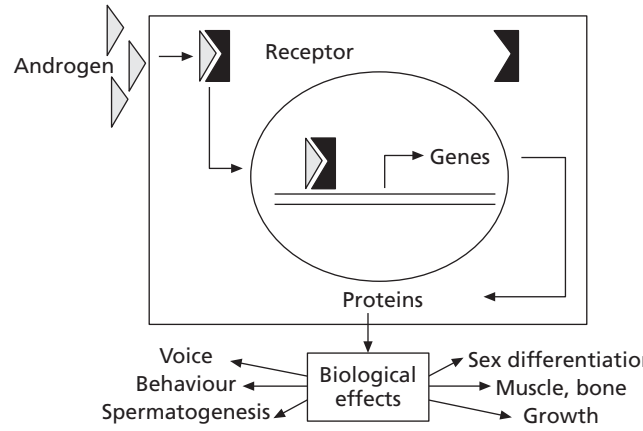

Fig. 9.4. A simplified diagram of the intracellular mode of action of androgens. Some of the biological effects mediated by the androgen–receptor complex are indicated.

The pathophysiology of CAIS and PAIS is related to a defect in the intracellular action of androgens (Fig. 9.4). The androgen receptor (AR) is located in the cytoplasm of androgen target cells until bound to testosterone or DHT, when the hormone–receptor complex translocates to the nucleus. Acting as a transcription factor, this complex together with co-regulator proteins promotes the expression of androgen-responsive genes. It is possible to postulate a number of steps in this pathway that may result in resistance to androgens. The best characterized involves the AR itself, where mutations either affect androgen binding or disrupt interaction of the hormone–receptor complex with DNA [49]. The AR is a member of a large family of nuclear hormone receptors that are ligand activated by steroid and thyroid hormones, retinoic acids, oxysterols, fatty acids, and xenobiotics [50]. There is also a large class of nuclear receptors for which no ligand has been identified, the so-called orphan receptors. One example is the DAX1 protein.

All nuclear receptors comprise four general functional domains, an N-terminal transactivation domain, a central

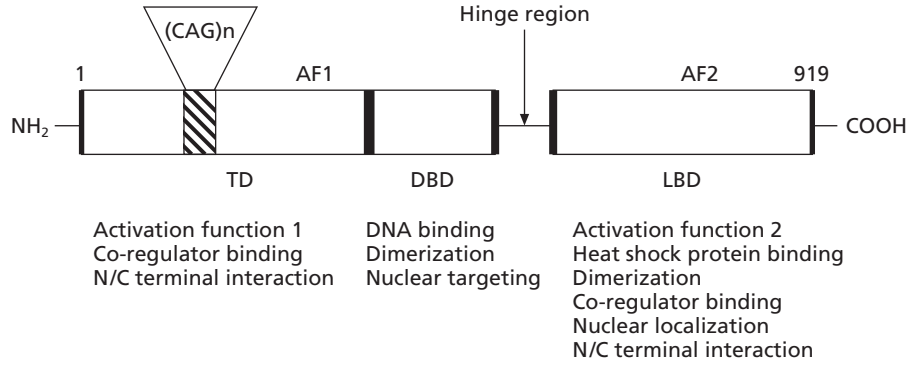

Fig. 9.5. Functional domains of the androgen receptor. There are three primary domains together with the hinge region. AF1 and AF2 are subdomains, and the multiple functions of the different domains are shown. (CAG)n indicates a polyglutamine tract in the N-terminal domain, which varies in length in the normal UK population within the range 11–31. Hyperexpansion of this glutamine tract causes spinobulbar muscular atrophy (Kennedy's disease).

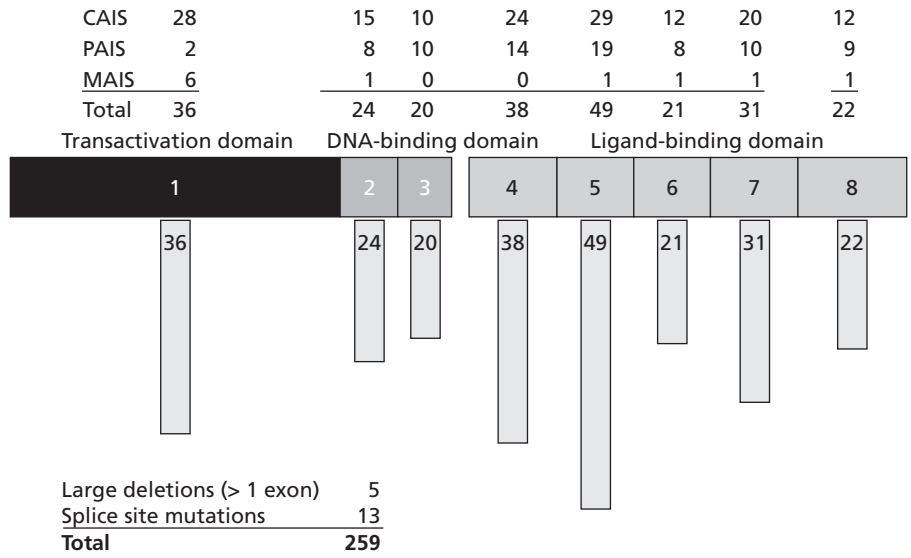

Fig. 9.6. Spectrum of *AR* gene mutations identified in patients with androgen insensitivity syndrome. The frequency of mutations according to the functional domains is shown. The pattern is similar to that recorded on the international mutation database. CAIS, complete androgen insensitivity syndrome; PAIS, partial androgen insensitivity syndrome; MAIS, minimal androgen insensitivity syndrome (e.g. male factor infertility alone).

DNA-binding domain, a hinge region, and a C-terminal domain to which the ligand binds (Fig. 9.5). Subdomains are involved in dimerization, nuclear localization, and transcriptional regulation. The *AR* gene is located on chromosome Xq11–12. Numerous mutations have been identified throughout this 90 kb gene that cause either CAIS or PAIS. They are recorded on an international database (http://www.mcgill.ca/androgendb). More than 300 mutations are described, and a selection of some of the mutations identified in my laboratory is shown in Figure 9.6. Severe mutations such as deletions and premature stop codons predictably result in no AR function and a CAIS phenotype. However, most mutations are missense and generally located in the ligand-binding domain. The same mutation may cause CAIS in one family but be manifest as PAIS in another affected family. The factors that modulate receptor activity and androgen responsiveness in such examples remain unknown.

A mutation is identified in the *AR* gene in about 80% of XY sex-reversed females who have clinical, biochemical, and histological evidence of CAIS, but only about 15–20% of patients with PAIS have an AR mutation. It is possible that resistance to androgens is not the explanation of the genital abnormality in these patients and that this proportion is derived following thorough evaluation and exclusion of other known causes that can cause a similar phenotype.

The ligand activation of the AR is but one of several molecular components to androgen action. Little is known about the identity of androgen-responsive genes expressed in the developing male reproductive tract. The AR contains a polymorphic trinucleotide CAG repeat, which encodes a polyglutamine tract in the N-terminal domain. The range of repeats in the normal population is about 11–31. The tract is hyperexpanded in spinomuscular bulbar atrophy or Kennedy's disease [51]. Affected males display signs of mild androgen insensitivity. Transcriptional efficiency of the AR *in vitro* is inversely proportional to the numbers of CAG repeats. This appears to be biologically relevant as variations in the number of CAG repeats within the normal range show associations with androgen-related conditions such as hypospadias [52], prostate cancer [53], male infertility [54], and polycystic ovarian disease [55]. A practical application of quantifying the number of CAG repeats on X chromosome alleles is to

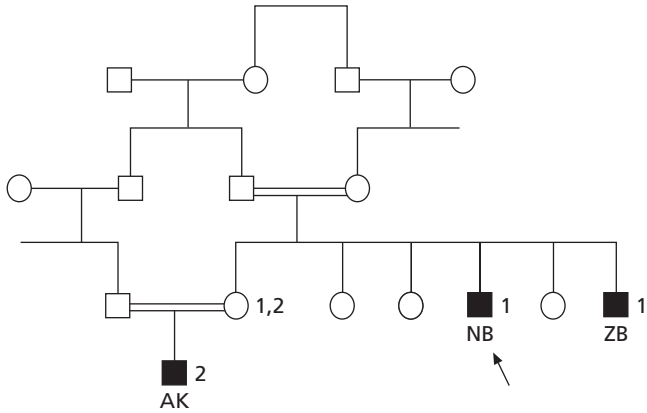

Fig. 9.7. Pattern of CAG triplet repeat alleles in a consanguineous family with familial hypospadias. Affected siblings NB and ZB have different alleles from their affected nephew, AK. Thus, X linkage for the *AR* gene is excluded and, hence, PAIS as the cause of the familial hypospadias.

exclude X-linked androgen insensitivity in familial cases of idiopathic hypospadias (see Fig. 9.7) and in carrier detection for CAIS [56].

There is a risk of tumors developing in the gonads of patients with CAIS or PAIS. Tumor types include gonadoblastomas, seminomas, and germ cell tumors. The risk is probably less than the 20–30% reported previously and certainly much lower before puberty [46].

True hermaphroditism and other sex chromosome anomalies

The term hermaphroditism should only be applied to individuals possessing both testicular and ovarian tissue that is well differentiated. The ovaries must contain follicles. The most frequent karyotype is 46,XX followed by a third of cases with 46,XX/XY mosaicism and less than 10% having a 46,XY karyotype [57]. The prevalence of this intersex disorder is particularly high in South African black people where true hermaphroditism accounts for half the number of cases of intersex seen in a single pediatric surgical unit [58]. Most patients have ambiguous genitalia with perineal hypospadias, bifid scrotum, and usually a normal-sized phallus. Ovotestis is the most common gonad. The *SRY* gene is present in only 10% of cases with a 46,XX karyotype, but there is *SRY* mosaicism confined to the gonads in a minority of the *SRY*-negative cases [59].

The XX male generally has normal differentiation of the external genitalia, although hypospadias may occur infrequently [60]. The condition affects 1 in 20 000 male births. The testes are small and firm, height is below the average for normal males (unlike Klinefelter syndrome), and gynecomastia is usual. There is an increased risk of carcinoma of the breast. Affected males are infertile. The majority are *SRY* positive as a result of X–Y chromosomal interchange during paternal meiosis. *SRY*-negative XX males are more likely to have associated genital anomalies. The development of testes in these individuals may be the result of *SRY* expression confined only to the gonads or a mutation in a testis repressor gene that is autosomal or X-linked. Klinefelter syndrome (47,XXY) affects around 1 in 600 males and is not usually associated with ambiguous genitalia. However, there are single case reports of genital anomalies comprising hypospadias, penoscrotal transposition, and even a few cases with complete sex reversal consistent with androgen insensitivity [61].

Clinical assessment

Newborn infants with the following features should be investigated:

- infants with ambiguous genitalia;
- severe hypospadias with or without undescended testes, micropenis, or bifid scrotum or shawl scrotum;
- a male infant with non-palpable testes;
- female infant with inguinal herniae;
- isolated clitoromegaly and/or labial fusion (examine for maternal virilization);
- genital anomalies associated with syndromes.

It has been estimated that the frequency of deviation of the genital anatomy from the "ideal" male or female newborn is as high as 2% of live births based on a literature survey [62]. The "idealized" male infant was defined as one with a penile size between 2.5 to 4.5 cm in length, normal position of the urethral meatus, testes in the scrotum, and an XY karyotype. The "idealized" female infant had a clitoris ranging in size from 0.2 to 0.85 cm, a normal female reproductive tract, and an XX karyotype. Included in the "deviations" from normal were conditions such as sex chromosome aneuploidies, simple hypospadias, and undescended testes. Clearly, frequency calculated in this study is an overestimate for the number of newborns who require a detailed genetic and endocrine investigation. Nevertheless, a large number of newborn infants need to be investigated. A logical protocol of tests supported by appropriate facilities for reliable hormone measurements, imaging techniques, and genetic analyses must be followed.

Examination

The clinical assessment of an infant with ambiguous genitalia needs a full history and physical examination, just as in any other area of clinical practice. Family history and exposure to potential reproductive tract teratogens are particularly relevant. Examination of the external genitalia should record the following details:

- phallus – size and presence of chordee, is it a micropenis or clitoromegaly?

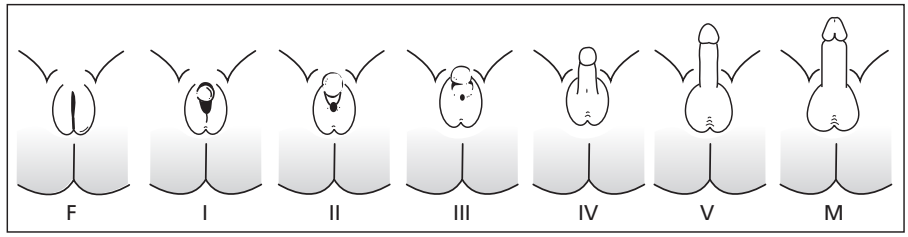

Fig. 9.8. A recognized scoring system for the degree of virilization of the external genitalia in a female infant with congenital adrenal hyperplasia (Prader score I–V). F, normal female; M, normal male. The Quigley score for androgen insensitivity to quantify the degree of undermasculinization has a similar basis [42].

- site of urethral opening – has a urine stream been observed?
- one or two external orifices on the perineum?
- development of labioscrotal folds – whether there is a bifid scrotum, fused labia, rugosity, and pigmentation of skin;
- whether gonads are palpable and their position.

A number of grading systems have been devised to assess the degree of undermasculinization as applied to the androgen insensitivity syndrome [40] or the degree of masculinization by the Prader score as applied to female infants with CAH (Fig. 9.8). An alternative method of assessing the degree of undermasculinization is to assign a score in relation to the presence or absence of micropenis, the position of the urethral opening (normal, glanular, penile shaft, perineal), scrotal sac fused or not, and the position of the gonads (scrotal, inguinal, abdominal, absent) [49,63]. Micropenis has been defined as a stretched penile length which is more than 2.5 SD below the mean for age. This equates to a lower limit of 1.9 cm for an infant up to 5 months of age [64]. A value of 2.5 cm or less for stretched penile length is often used to define micropenis, although it may be necessary to consider ethnic variations in penile size [65]. A normal range for penile length in preterm infants between 24 to 36 weeks of gestation is available [66]. Applying some quantitative system to characterize the degree of undermasculinization in XY infants with ambiguous genitalia seems desirable, particularly if treatment with androgens is to be tried. The remainder of the examination should include looking for signs of adrenal insufficiency and any congenital malformation syndromes associated with genital anomalies.

Investigations

Many schedules have been suggested for the investigation of a newborn infant with ambiguous genitalia, but each pediatric endocrine unit must formulate a protocol that is determined by local practice and facilities [67]. Many centers are equipped to perform initial screening investigation, but more detailed investigations to establish a definitive diagnosis may have to be undertaken at another center. Table 9.2 lists a range of investigations that should lead to a functional diagnosis in most newborns with ambiguous genitalia and allow early sex assignment.

Table 9.2. Investigating an infant with ambiguous genitalia.

Genetics
FISH (X centromeric and SRY probes)
Karyotype (high resolution; abundant mitoses)
Save DNA
Endocrine
170H-progesterone, 11-deoxycortisol (plus routine biochemistry; save serum) renin,
ACTH 24-h urinary steroids (also check proteinuria) testosterone, androstenedione, DHT
LH, FSH, AMH, inhibin B
hCG stimulation test (define dose, timing)
Imaging
Pelvic, adrenal, renal ultrasound
MRI
Cystourethroscopy and sinogram
Surgical
Laparoscopy
Gonadal biopsies
Genital skin biopsy (AR studies, extract DNA and RNA)

The commonest cause of newborn ambiguous genitalia is CAH due to 21-hydroxylase deficiency. A provisional indication of the sex chromosomes can be obtained rapidly by fluorescent *in situ* hybridization (FISH) analysis using X-chromosome centromeric probes and a probe for the *SRY* gene. A full karyotype is required to confirm the FISH result and a sufficient number of mitoses analyzed to exclude mosaicism. Measurement of serum 170H-progesterone is a reliable test for 21-hydroxylase deficiency. In a 46,XX infant with ambiguous genitalia, elevated 170H-progesterone (generally greater than 300 nmol/L), and a uterus visualized on pelvic ultrasound, the diagnosis is CAH. Ancillary biochemical tests should establish whether the infant is also a salt loser. It may be necessary to perform a synacthen stimulation test, especially if CAH is suspected in a preterm infant or there is a possibility of one of the rarer enzyme defects. Measurement of urinary steroid metabolites by gas chromatography and

mass spectrometry is the definitive test to define the type of enzyme defect [68]. Male infants with non-palpable testes must have a karyotype; it is unacceptable to miss this opportunity to establish a diagnosis of CAH in a female masculinized to a Prader score V degree.

In the XY or XO/XY infant with ambiguous genitalia, investigations are aimed at establishing location and function if testes are present. The hCG stimulation test is pivotal in this situation, coupled with imaging and laparoscopy to identify gonad site and histology. There is no uniform practice for an hCG stimulation test. I have most experience with using 1500 units daily for 3 days, with a post-hCG blood sample collected 24 h after the last injection. Occasionally, a longer test is needed using a twice-weekly injection regimen for 2 weeks. Pre- and post-hCG blood samples should be analyzed for androstenedione, testosterone, and DHT. Concomitant 24-h urine collections can be performed for urinary steroid analysis, which is reliable in the newborn for the diagnosis of 5α-reductase deficiency but not for 17β-hydroxysteroid dehydrogenase deficiency. Expressing the ratio of testosterone to androstenedione following hCG stimulation is a useful screen for 17β-hydroxysteroid dehydrogenase deficiency in the differential diagnosis of XY undermasculinization [69]. A ratio less than 0.8 is consistent with this enzyme deficiency; in contrast, this is seldom the case with androgen insensitivity. Making such distinctions on biochemical criteria is an important prelude to molecular studies of the appropriate gene.

Sertoli cell function can be assessed by measurement of AMH and inhibin B. Both proteins are elevated in serum during infancy. Circulating levels of AMH remain high until puberty when levels fall in response to the effect of testosterone. Hence, AMH levels are elevated in intersex states associated with androgen insensitivity but low in disorders of gonadal dysgenesis [70]. An undetectable value suggests anorchia, so this is a useful test in the investigation of an XY infant with ambiguous genitalia with no palpable gonads [71]. Inhibin B is also undetectable in anorchia, and the levels correlate with the increment in testosterone following an hCG stimulation test in boys with gonadal dysgenesis and androgen insensitivity [72]. Basal measurements of AMH and inhibin B alone may be sufficient for confirming anorchia, but baseline LH and follicle-stimulating hormone (FSH) measurements together with a suitably performed hCG stimulation test should not be omitted from the protocol of investigations required in the XY infant with ambiguous genitalia.

Imaging with ultrasound and magnetic resonance imaging (MRI) is used to delineate the internal genital anatomy, including localizing the site and, possibly, the morphological nature of the gonads. Only histology will provide precise details of the gonads, and many infants with ambiguous genitalia require a laparoscopy to obtain as much detail to reach a diagnosis.

Management

This is a complex area involving a multidisciplinary practice that needs to be provided to the patient from birth to adulthood. Early management is focused on establishing a diagnosis, particularly for the infant who may have a life-threatening disorder with adrenal insufficiency. If this is the case, treatment with appropriate replacement of glucocorticoids, mineralocorticoids, glucose, and fluid is required.

The XY infant with ambiguous genitalia poses more difficulties in management. It is possible that a definitive diagnosis may not be reached, even after extensive investigation. Sometimes, a trial of testosterone treatment can give an indication about androgen responsiveness and future treatment options. A decision about sex assignment may be delayed until such a trial is complete. Infants with disorders of inadequate fetal androgen production are likely to respond to androgens with an increase in phallic growth. However, this can also happen in PAIS due to some missense mutations, so a positive response to androgens should not assume that androgen insensitivity is excluded as a diagnosis.

Despite the hubris about how to manage intersex disorders, there is consensus that the newborn infant with ambiguous genitalia should be sex assigned as soon as is practicable after birth. When surgery should be undertaken and the nature of the procedure are the issues that remain to be resolved. The results of some adult outcome studies of intersex patients are beginning to alter early surgical practice. Thus, surgery is not performed so readily for the enlarged clitoris, creating a vagina is not necessary before puberty, and an XY infant with a micropenis should not necessarily be sex assigned female [73–75]. The early management of ambiguous genitalia is undertaken by engaging the family fully in a climate of openness with the truth. This must include explaining the current limitations in knowledge about diagnosis and longer term outcome studies. Above all, professional staff have a responsibility to support the family in reaching a decision on sex assignment and to continue that support as appropriate through childhood and adolescence.

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10 Normal and abnormal puberty

Mehul T. Dattani and Peter C. Hindmarsh

Introduction

The changes of puberty occur because of orderly, sequential changes in endocrine activity. They commence toward the end of the first decade of life, but the axis has already been active *in utero* and during the first year of life [1]. There is then reduced gonadotropin secretion (a restraint) until reactivation, or rather increased amplitude, of gonadotropin pulsatility takes place.

Normal puberty

Secular trends in puberty

The age of puberty in girls is earlier than in past centuries. The age of menarche in industrialized European countries and in the United States has fallen 2–3 months per decade over the past 100–150 years [2]. This trend has generally ceased in Western Europe, at least when puberty is defined by the age of menarche. If pubertal onset is defined by the development of breast buds (breast stage 2), the secular trend is probably continuing. As age at menarche has remained constant, the duration of puberty has lengthened [3]. These observations in the western world contrast with cultures in which the standard of living has changed little, where no trend toward earlier menarche has been documented [4] or where the standard of living has improved and events are accelerated [5].

The interaction between nutritional status and puberty is important when food supply is limited. Chronic disease and malnutrition are common causes of delayed puberty. Strenuous physical activity in girls can delay puberty, especially when associated with thin habitus [6]. Moderate obesity is associated with earlier menarche and advanced physical development; in contrast, pathological obesity is associated with delayed menarche [7]. This may explain the current trend to earlier puberty, in that a rapid early increase in body size above that expected for parental size and secular trend does not lead to an increase in final height due to an earlier puberty. Figure 10.1 illustrates this in Japanese children [8]. The most interesting aspect of Figure 10.1 is at birth, where mean length has not changed in 40 years. There has been an appreciable trend of 10 mm/decade at age 2 years and in adulthood, which means that Japanese children aged 2 years were 4 cm taller in 1990 than in 1950 and the same was true of young adults.

Fig. 10.1. The mean secular increase in height (cm) of Japanese children by age and sex, 1950–1990. Note the lack of a trend at birth, a trend of 1 cm/decade at 2 and 17 years, and a trend of 3–4 cm/decade at 12–14 years.

The age of onset and completion of puberty in boys is less well defined and documented than the age of menarche in girls. Overall, there has been little change with respect to the timing of onset of puberty in boys [9,10].

Genetic factors play an important role in the onset of puberty. African-American girls achieve menarche at a mean age of 12.2 years, whereas Caucasians do so at an average age of 12.9 years [5]. The ethnic differences in the age of

secondary sexual development remain, even when the effects of social or economic factors are eliminated. Further evidence for the genetic influence is provided by the concordance of the age of menarche between mother–daughter pairs.

Physical changes of puberty

The physical changes of puberty in individuals are defined by the Tanner stages [11,12]. Figure 10.2 describes the components and consonance of the process for girls and boys.

Breast formation is primarily controlled by estrogen secreted by the ovaries. Breast development may be unilateral for several months, which may cause unfounded concern in girls or their parents. Even though the growth of pubic and axillary hair is mainly under the influence of adrenal androgens, the stage of breast development usually correlates well with the stage of pubic hair development in normal girls. However, as different endocrine organs control these two processes, the stages of each phenomenon should be classified separately. Peak height velocity is attained 6–9 months after the appearance of breast stage 2 development.

In boys, an increase in testicular volume to 4 mL is usually the first sign and can easily be assessed using the Prader orchidometer. Most of the increase is due to enlargement of the Sertoli, rather than the Leydig, cells. In gonadotropin-independent precocious puberty (testotoxicosis), the testes remain small in relation to the growth of the phallus and pubic hair, as the condition is due to constitutive activation of the luteinizing hormone (LH) receptor with consequent hyperplasia of Leydig rather than Sertoli cells. The growth of the penis and genitalia in the male usually correlates well with pubic hair development, as both features are regulated by androgen secretion. However, stages for pubic hair and genital development should be determined independently as valuable clinical information can be accrued. For example, pubic hair growth without testicular enlargement suggests an adrenal rather than a gonadal source of androgens.

Peak height velocity occurs relatively late in puberty in boys compared with girls, and usually coincides with a testicular volume of 10–12 mL. Voice changes in boys can be noted at 8 mL testicular volume and become obvious by 12 mL volume.

Ovarian development in puberty

Oogonia arise from the primordial germ cells in the wall of the yolk sac near the caudal end of the embryo [13]. By the sixth month of fetal life, the cells have migrated to the genital ridge and progressed through sufficient mitoses to reach a complement of 6–7 million oogonia, which represents the maximal number of primordial follicles the individual will have throughout life. Meiosis begins but is not completed as the nucleus and chromosomes persist in prophase to mark the conversion of the oogonia to primary oocytes. Primordial follicles are composed of the primary oocyte surrounded by a single layer of spindle-shaped cells that will develop into granulosa cells and a basal lamina which will be the boundary of the theca cells later in development. Owing to apoptosis, 2–4 million primordial follicles are present at birth but only 400 000 remain at the onset of menarche [13].

At the time of the first ovulation, the first meiotic metaphase converts the primary oocyte into the secondary oocyte, which is extruded into the Fallopian tubes [14]. The ovum does not form until the time of sperm penetration, when the second polar body is eliminated. While some follicles in the fetus and child progress to the large antral stage, all developing follicles undergo atresia prior to puberty, and few large follicles develop in the child. However, the presence of more than six follicles with a diameter of more than 4 mm indicates the presence of pulsatile gonadotropin secretion and may be seen in normal prepubertal girls, in pubertal girls prior to menarche, and in patients recovering from anorexia nervosa. This "multicystic" appearance is considered to be characteristic of a phase of mainly nocturnal pulsatile gonadotropin secretion prior to positive feedback [15].

Standards for ovarian and uterine size and shape are available for normal girls and those with Turner syndrome [16–18]. The uterus lies in a craniocaudal direction in childhood without the adult flexion. The myometrium enlarges during early puberty, thereby enlarging the corpus leading to the adult corpus-to-cervix ratio. The cervix develops its adult shape and size just before menarche, and the cervical canal enlarges.

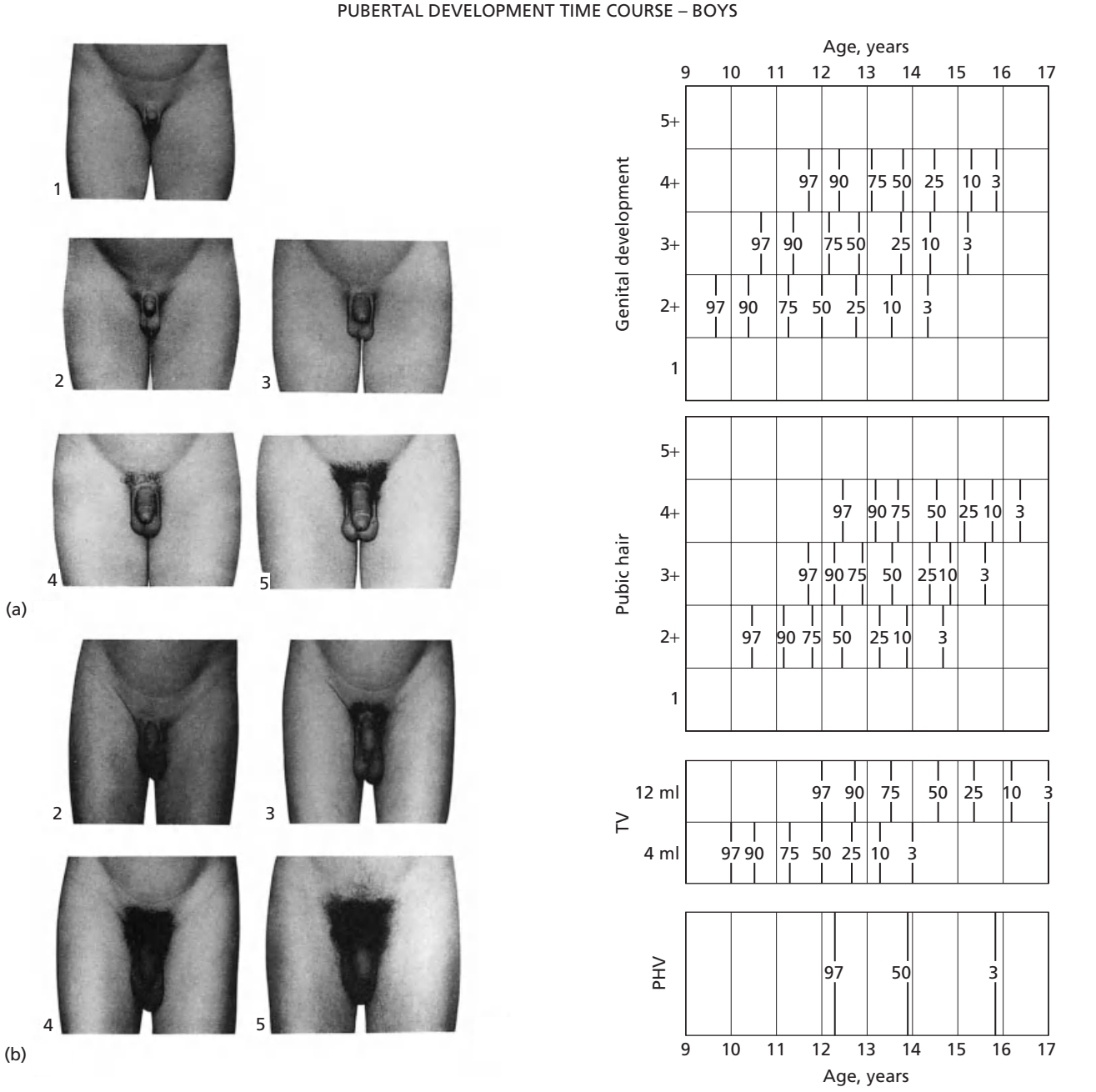
Testicular development in puberty

The prepubertal testes consist mainly of Sertoli cells, but adult testes are mostly composed of germ cells in the seminiferous tubules. The seminiferous tubules enlarge during puberty and form tight occlusive junctions leading to the development of a blood testicular junction [19]. Leydig cells are present in small numbers in prepuberty, although the interstitial tissue is mainly composed of mesenchymal tissue. At puberty, the Leydig cells become more apparent.

Spermatogenesis can be detected histologically between ages 11 and 15 years, and sperm is found in early morning urine samples by 13.3 years of age (spermarche) [20]. Ejaculation occurs by a mean age of 13.5 years without consistent relationship to testicular volume, pubic hair development, or phallic enlargement. While adult morphology, motility, and concentration of sperm is not found until the bone age advances to 17 years [21], immature-appearing boys can be fertile.

Gynecomastia

Breast enlargement occurs to some degree in 39–75% of boys, usually during the first stages of puberty [22] on account of



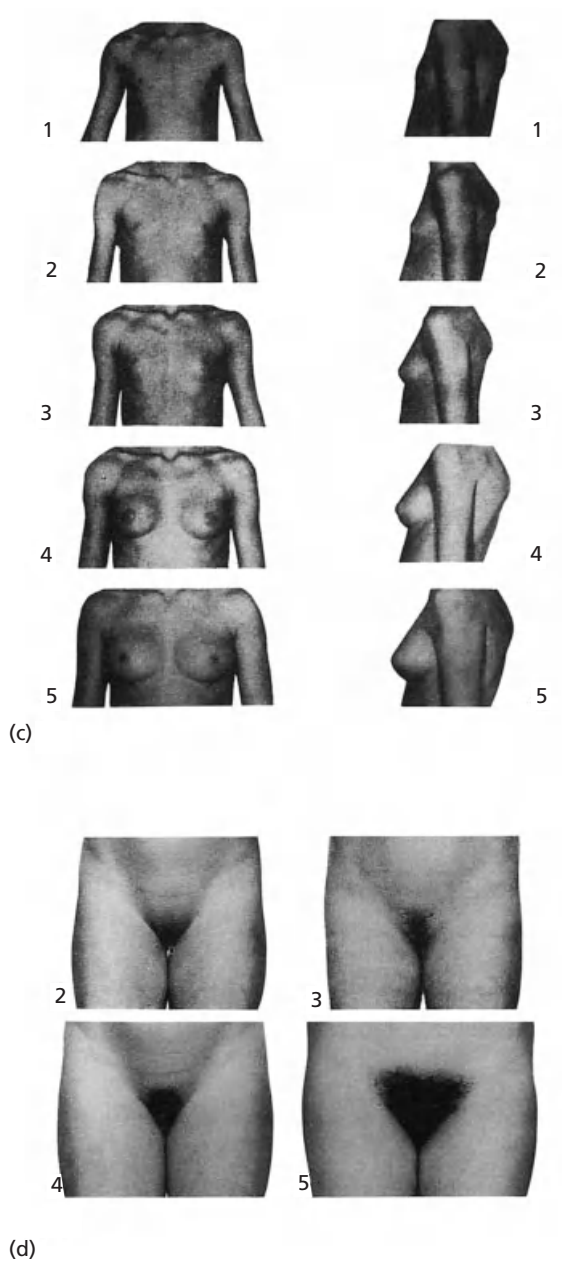
Boys: genital development

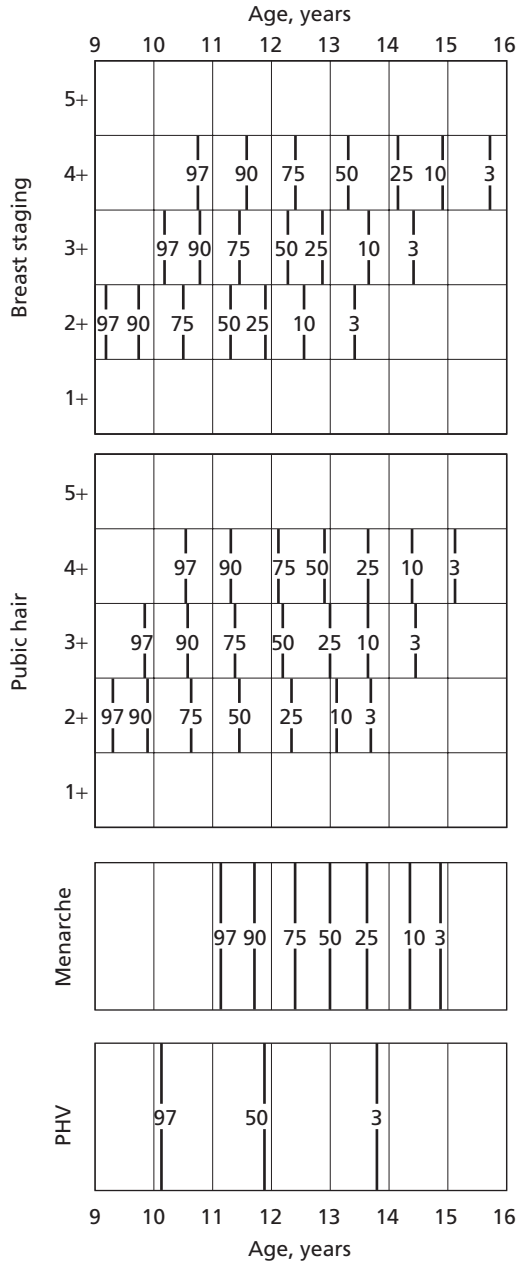
Stage 1: Preadolescent. The testes, scrotum and penis are of about the same size and proportions as in early childhood.
Stage 2: Enlargement of the scrotum and testes. The skin of the scrotum reddens and changes in texture. Little or no enlargement of the penis.
Stage 3: Lengthening of the penis. Further growth of the testes and scrotum.
Stage 4: Increase in breadth of the penis and development of the glans. The testes and scrotum are larger; the scrotum darkens.
Stage 5: Adult.

Boys: pubic hair

Stage 1: Preadolescent. No pubic hair.
Stage 2: Sparse growth of slightly pigmented downy hair chiefly at the base of the penis.
Stage 3: Hair darker, coarser and more curled, spreading sparsely over the junction of the pubes.
Stage 4: Hair adult in type, but covering a considerably smaller area than in the adult. No spread to the medial surface of the thighs.
Stage 5: Adult quantity and type with distribution of a horizontal pattern and spread to the medial surface of the thighs. Spread up linea alba occurs late, in about 80% of men, after adolescence is complete, and is rated Stage 6.

Fig. 10.2. Pubertal assessment is an important component of the assessment of gonadal function. Staging of each of the components is separate and should be recorded as such to allow discordance in development to be identified. The figures show the relationship in time of each of the components, and each should be related to other parts of the puberty process. Note that the peak height velocity in girls takes place some 2 years before that in boys.





Girls: breast development

Stage 1: Preadolescent. Elevation of the papilla only.
Stage 2: Breast bud stage. Elevation of the breast and papilla as a small mound. Enlargement of the areola diameter.
Stage 3: Further enlargement and elevation of the breast and areola, with no separation of their contours.
Stage 4: Projection of the areola and papilla above the level of the breast.
Stage 5: Mature stage, projection of the papilla alone due to recession of the areola.

Girls: pubic hair

Stage 1: Preadolescent. No pubic hair.
Stage 2: Sparse growth of slightly pigmented downy hair chiefly along the labia.
Stage 3: Hair darker, coarser and more curled, spreading sparsely over the junction of the pubes.
Stage 4: Hair adult in type, but covering a considerably smaller area than in the adult. No spread to the medial surface of the thighs.
Stage 5: Adult quantity and type with distribution of a horizontal pattern and spread to the medial surface of the thighs. In about 10% of women, after adolescence is complete pubic hair spreads up the linea alba and is rated Stage 6.

Fig. 10.2. (*continued*)

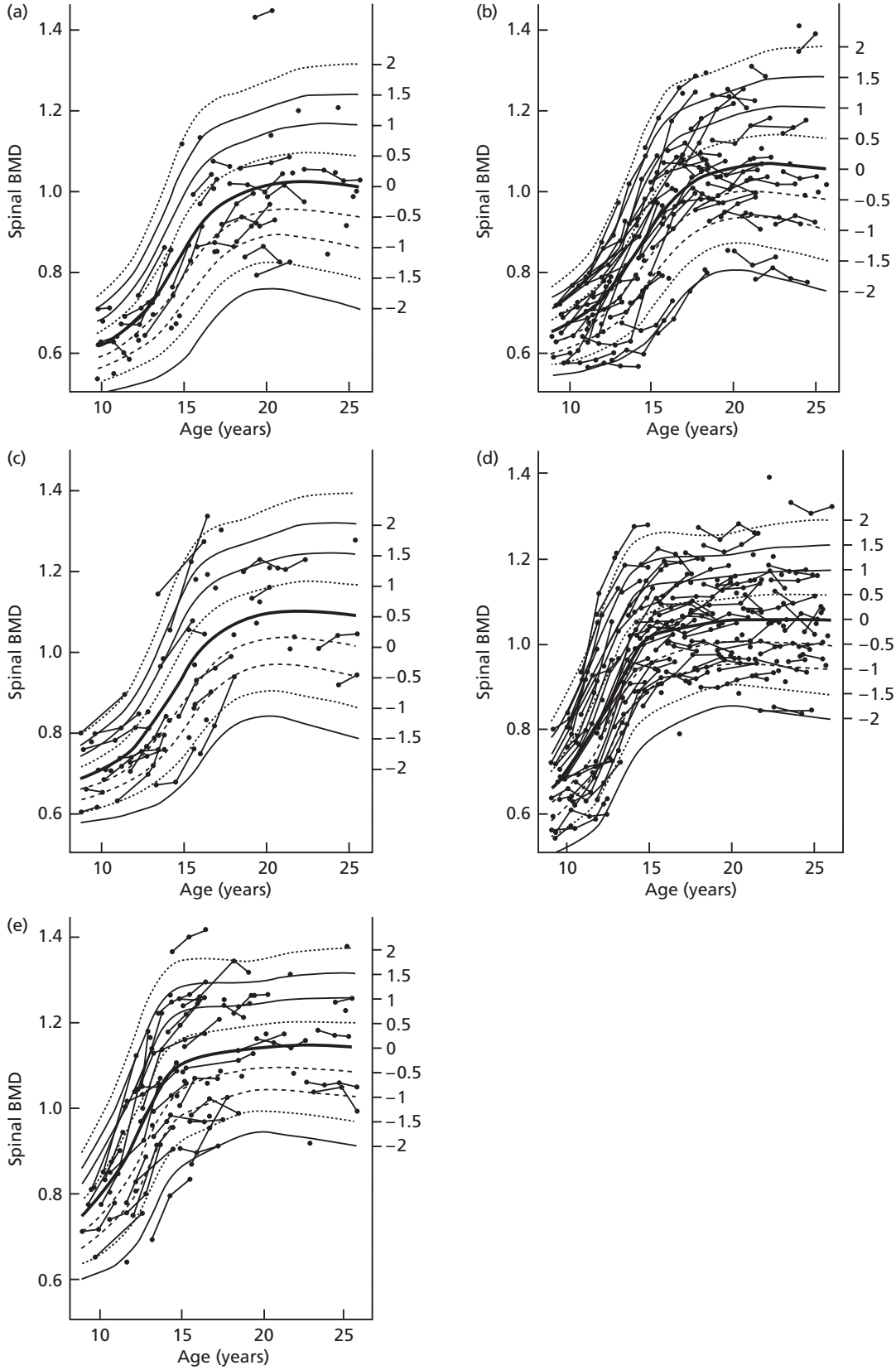


Fig. 10.3. Spinal bone mineral density determined in 423 males and females showing the effect of puberty in (a) Hispanic males, (b) Asian/white males, (c) black males, (d) non-black females, and (e) black females.

an increase in estrogen production (by aromatization of testosterone) before testosterone secretion achieves concentrations that can oppose the estrogen. In most cases, the tissue regresses within 2 years but, occasionally, in normal, often obese, boys, and frequently in pathological conditions such as Klinefelter syndrome or partial androgen resistance, in which the effective amount of bioactive testosterone is reduced, gynecomastia remains permanent. Surgery, usually through a peri-areolar incision, is the only effective mode of therapy at present, although non-aromatizable androgens or aromatase inhibitors are under study as potential treatments.

Bone mineral density (BMD)

The most important phases of bone accretion occur during infancy and puberty. In the teenage years, girls reach peak mineralization between 14 and 16 years while boys reach a later peak at 17.5 years [23] (Fig. 10.3). Both peaks are attained after peak height velocity has been achieved in either sex. BMD is influenced not only by sex steroids but also by genetics [24], exercise [25], and growth hormone (GH) secretion.

There is a poor correlation between calcium intake and BMD during puberty or young adulthood, suggesting that the normal age of puberty is the most significant factor in achieving peak bone mineralization [26]. It seems prudent nonetheless to insure adequate calcium intake in patients with delayed or absent puberty or those treated with gonadotropin-releasing hormone (GnRH) analogs to hold up puberty, until more is learned about the biology of the control of bone accretion in puberty.

Body composition

Percentages of lean body mass, skeletal mass, and body fat are equal between prepubertal boys and girls but, as boys go through puberty, total body bone mass and fat-free mass continue to increase whereas, in girls, only body fat and fat-free mass increase [27]. The increase in lean body mass starts at 6 years in girls and at 9.5 years in boys and is the earliest change in body composition in puberty [28]. At maturity, men have 1.5 times the lean body mass and almost 1.5 times the skeletal mass of women, while women have twice as much body fat as men.

Growth in puberty

The pubertal growth spurt encompasses the most rapid phase of postnatal growth after the neonatal period and follows the decreasing growth rate of the late childhood phase. It can be detected in girls prior to the onset of secondary sexual characteristics. In boys, the growth spurt starts on average 2 years after that in girls. Peak height velocity occurs at a mean of 13.5 years in boys and 11.5 years in girls [29], corresponding to genitalia stage 3–4 in boys and breast stage 2–3 in girls.

The mean difference in adult height between men and women of 12.5 cm is due mainly to the taller stature of boys at the onset of the pubertal growth spurt and also to the increased height gained during the pubertal growth spurt in boys compared with girls [30]. A girl who has experienced menarche usually has no more than 2–3% of her growth remaining as menarche closely accords to a bone age of 13 years, the only event of puberty more closely related to skeletal than chronological age. A post-menarcheal girl has 5–7.5 cm of growth remaining before adult height is reached, although the range of post-menarcheal growth extends to 11 cm.

The pubertal growth spurt is mediated by many endocrine influences. Sex steroids exert a direct effect upon the growing cartilage as well as an indirect effect mediated by increasing GH secretion. Increasing sex steroid production at puberty stimulates increased amplitude (but not frequency) of spontaneous GH secretion [31] as well as peak stimulated GH, and this in turn stimulates increased production of insulin-like growth factor (IGF)-I.

Estrogen, either from the ovary or aromatized from testicular testosterone, is the factor that mediates the increased GH response during puberty [32]. A prepubertal child given an androgen that can be aromatized to estrogen, such as testosterone, will have augmented GH secretion, whereas non-aromatizable dihydrotestosterone will not increase GH secretion. An estrogen-blocking agent such as tamoxifen will reduce GH secretion [33].

Thyroid hormone is necessary to allow the pubertal growth spurt to proceed. The rapid growth rate is accompanied by an increase in markers of bone turnover such as serum alkaline phosphatase, serum bone alkaline phosphatase, osteocalcin, Gla protein, and the amino-terminal propeptide of type III procollagen; thus, normal adult values of these proteins are lower than concentrations found in puberty [34].

Estrogen has a biphasic effect on growth; low concentrations stimulate growth while higher concentrations lead to cessation of growth (35). Estrogen plays a major role in the final stages of epiphyseal fusion. Patients with either estrogen receptor deficiency or aromatase deficiency have tall stature, they continue growth into the third decade as a result of lack of fusion of the epiphyses of the long bones, and have increased bone turnover, reduced bone mineral density, osteoporosis, and absence of a pubertal growth spurt [36]. Estrogen is the main factor that fuses the epiphyses of the long bones and causes cessation of statural growth. These observations have raised the possibility of the use of aromatase inhibitors for the further management of short stature associated with a variety of conditions, with the rationale that this might allow more time for growth before epiphyseal fusion [37].

Endocrine changes in puberty

Hypothalamic GnRH

Gonadotropin-releasing hormone (GnRH) is a 10-amino-acid peptide generated from a larger 69-amino-acid prohormone precursor. The gene encoding GnRH is located on chromosome 8 [38]. Neurons producing GnRH originate in the primitive olfactory placode early in the development of mammals and then migrate to the medial basal hypothalamus [39]. The control of this migration is related to the *KAL* gene located at Xp22.3. The absence of the *KAL* gene, or rather the gene product ANOSMIN-1, causes Kallman syndrome, a decrease in or lack of gonadotropin secretion with hyposmia due to disordered development of the olfactory bulb [40].

GnRH is released in episodic bursts into the hypothalamo-pituitary portal system. The frequency of release varies with development, sex, and the stage of the menstrual period. Variation in the frequency of secretion of GnRH changes the relative concentrations of LH and follicle-stimulating hormone (FSH) released and, because of their differing half-lives, the serum concentrations of each will also be altered.

GnRH is localized mainly in the hypothalamus and, to a degree, in the hippocampus, cingulate cortex, and the olfactory bulb. There is no discrete nucleus that contains all the GnRH neurons, although the arcuate nucleus plays a key role. Gonadotropins are normally released into the bloodstream in a pulsatile manner as a result of the pulsatile nature of GnRH secretion (see Plate 11, facing p. 148). Episodic secretion appears to be an intrinsic property of the hypothalamic neurons that produce and secrete GnRH [41]. This GnRH pulse generator, which is the basis of the central nervous system (CNS) control of puberty and reproductive function, is affected by biogenic amine neurotransmitters, peptidergic neuromodulators, neuroexcitatory amino acids, and neural pathways; for example, adrenaline and noradrenaline increase GnRH release whereas dopamine, serotonin, and opioids decrease GnRH release.

Testosterone and progesterone inhibit GnRH pulse frequency. However, the decrease in gonadotropin secretion during childhood before the onset of puberty appears to be mediated by the CNS. Gamma amino butyric acid (GABA) is probably the major cause of the suppression of GnRH secretion that occurs physiologically during mid-childhood [42]. Damage to the CNS from increased intracranial pressure or tumor may release the inhibition and bring about premature pubertal development.

GnRH stimulates the production and secretion of LH and FSH from the gonadotrophs by binding to a cell surface receptor [43,44], which triggers increased intracellular calcium concentration and phosphorylation of protein kinase C in a manner similar to other peptide–receptor mechanisms. There appear to be readily releasable pools of LH, which lead to a rise in serum LH within minutes after a bolus of GnRH, as well as other pools of LH that take longer to mobilize. While episodic stimulation by GnRH increases gonadotrophin secretion, continuous infusion of GnRH decreases LH and FSH secretion and downregulates the pituitary receptors for GnRH. This phenomenon is used in the treatment of central precocious puberty. Estrogens increase and androgens decrease GnRH receptors. These alterations in the GnRH receptor have an important role in regulating gonadotroph function.

Pituitary gonadotropins (Fig. 10.4)

FSH and LH are glycoproteins composed of two subunits, an α-subunit that is identical for all the pituitary glycoproteins and distinct β-subunits that confer specificity. The β-subunits are 115 amino acids long with two carbohydrate side-chains. Human chorionic gonadotropin (hCG) produced by the placenta is almost identical in structure to LH except for an additional 32 amino acids and additional carbohydrate groups. The LH β-subunit gene is on chromosome 19q13.32, close to the gene for β-hCG, while the FSH gene is located at 11p13. There are rare cases of mutations in the β-subunit of gonadotropin molecules that cause pathological effects. An inactivating mutation of β-LH caused absence of Leydig cells and lack of puberty in one male. Inactivating mutations of β-FSH led to lack of follicular maturation and amenorrhea in two females and to azoospermia in two males [45,46].

The same gonadotroph cell produces both LH and FSH. The gonadotrophs are distributed throughout the anterior pituitary gland and abut the capillary basement membranes to allow access to the systemic circulation. Inactive gonadotroph cells that are not stimulated, e.g. as a result of disease affecting GnRH secretion, are small in diameter, while the gonadotroph cells of castrate individuals or those with absence of gonads such as in Turner syndrome, which are stimulated by large amounts of GnRH, are large and demonstrate prominent rough endoplasmic reticulum.

Serum gonadotropin concentrations change during the progress of puberty (Table 10.1). Because of the episodic nature of gonadotropin secretion, a single gonadotropin determination will not reveal the secretory dynamics of the hormones. However, newer third-generation assays are sufficiently sensitive to indicate the onset of puberty in single basal unstimulated samples. The role of this form of clinical assessment remains unclear.

GnRH must stimulate gonadotropin release before any other factors can affect gonadotropin secretion. However, in the presence of GnRH stimulation, sex steroids and gonadal peptides can change gonadotropin secretion. Negative feedback inhibition is manifest when sex steroids decrease pituitary LH and FSH secretion at the hypothalamic and pituitary levels and is exemplified in individuals with gonadal dysgenesis, who have very high concentrations of LH and FSH

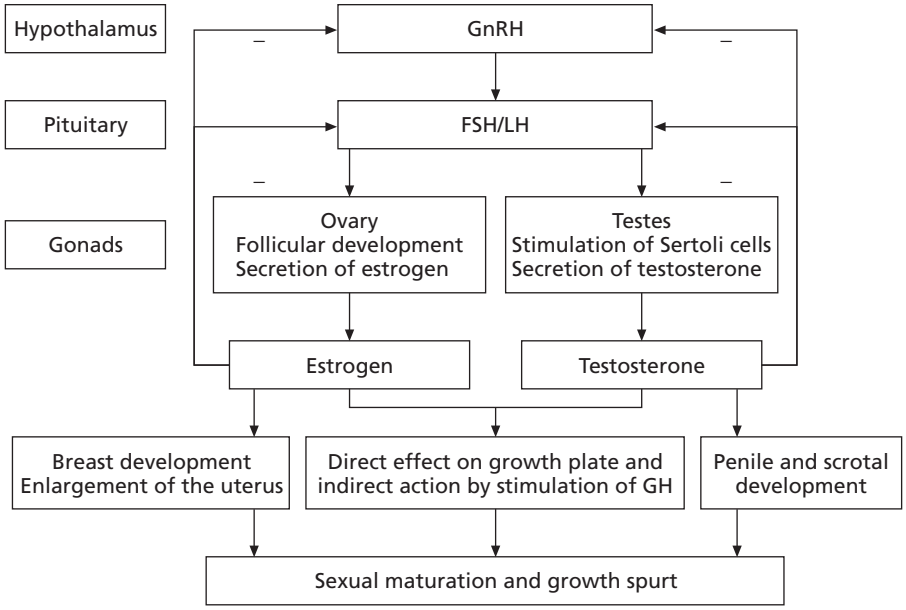


Fig. 10.4. Overall feedforward and feedback loops in the hypothalamo-pituitary–gonadal axis.

Table 10.1. Serum concentrations of luteinizing (LH), follicle-stimulating (FSH) hormones, and the sex steroids at different pubertal stages.

Females Tanner stages	LH (U/L)	FSH (U/L)	Estradiol (pg/mL)
I	0.01–0.21	0.50–2.41	5–10
II	0.27–4.21	1.73–4.68	5–115
III	0.17–4.12	2.53–7.04	5–180
IV	0.72–15.01	1.26–7.37	25–345
V	0.30–29.38	1.02–9.24	25–410

Males Tanner stages	LH (U/L)	FSH (U/L)	Testosterone (ng/dL)
I	0.02–0.42	0.22–1.92	2–23
II	0.26–4.84	0.72–4.60	5–70
III	0.64–3.74	1.24–10.37	15–280
IV	0.55–7.15	1.70–10.35	105–545
V	1.54–7.00	1.54–7.00	265–800

during infancy and puberty. The proteins inhibin, a product of both ovary and testis, and follistatin, an ovarian product, exert direct inhibitory effects upon FSH secretion at the pituitary level. Progesterone slows LH pulse frequency.

Estradiol decreases gonadotropin secretion at low concentrations but causes positive feedback at higher values. This developmental stage predominates in females at approximately mid-puberty [47]. By this process, a rising concentration of estradiol (> 200–300 pg/mL) persisting for more than 48 h [48] triggers the release of a burst of LH from the pituitary gonadotrophs, which stimulates ovulation about 12 h later. Several steps must prepare the hypothalamo–pituitary–gonadal axis for positive feedback, including a pool of LH adequate to release and prime the ovary to produce adequate estrogen. Estradiol increases pituitary gland sensitivity to GnRH which, in addition to an increase in GnRH pulse frequency, increases LH secretion. Thus, a follicle must be of adequate size to produce adequate estrogen to exert the positive feedback effect, the pituitary gland must have sufficient readily releasable LH to effect a surge of LH release, and the hypothalamus must be able to secrete adequate GnRH to cause the stimulation of pituitary release. The increase in estrogen also suppresses FSH to allow, in the presence of LH, luteinization of the follicle.

Sex steroids

The Leydig cells of the testes synthesize testosterone through a series of enzymatic conversions for which cholesterol is the precursor (Fig. 10.5). When LH binds to Leydig cell membrane receptors, the ligand–receptor complex stimulates membrane-bound adenyl cyclase to increase cyclic adenosine monophosphate (cAMP), which then stimulates protein kinase. This causes the conversion of cholesterol to pregnenolone by P450scc (side-chain cleavage enzyme), the first step in the production of testosterone. After exposure to LH, the number of receptors for LH and the post-receptor pathway decrease their responsiveness to LH for at least 24 h. This explains the clinical finding of insensitivity to LH after daily injections of LH compared with every-other-day injections. When assessing the response of testes to LH, hCG or LH must

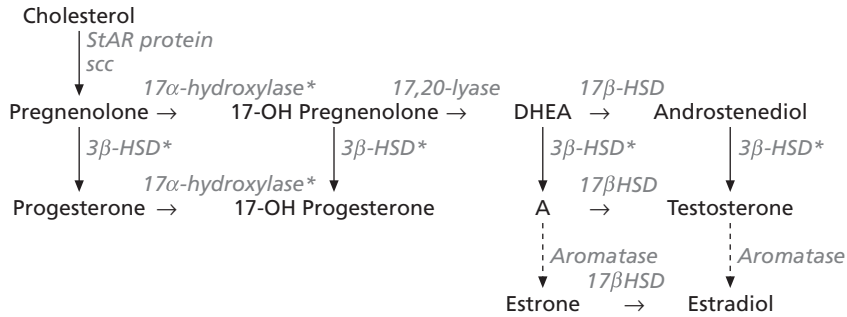


Fig. 10.5. Steroid biosynthetic pathway in the gonad.

be administered at 2- to 3-day intervals to eliminate such downregulation.

When testosterone is secreted into the circulation, it is bound to sex hormone-binding globulin. The remaining free testosterone is conventionally considered to be the active moiety. At the target cell, testosterone dissociates from the binding protein, diffuses into the cell, and may be converted by 5α-reductase type 2 (a surface enzyme located on the genital skin and elsewhere and encoded by a gene on chromosome 2 [49]) to dihydrotestosterone or by aromatase (CPY19) to estrogen [50]. Testosterone or dihydrotestosterone binds to an androgen receptor that is encoded by a gene on the X chromosome (Xq11–q12) [51]. The testosterone/dihydrotestosterone–receptor complex then attaches to the steroid-responsive region of genomic DNA to initiate transcription and translation.

The effects of testosterone are different from those of dihydrotestosterone because a fetus without dihydrotestosterone will not virilize fully. The androgen receptor has a greater affinity for dihydrotestosterone than for testosterone. Testosterone will suppress LH secretion, maintain Wolffian ducts, and produce the male body habitus while dihydrotestosterone is mostly responsible for the virilization of the external genitalia and for much of the secondary sexual characteristics of puberty, including phallic growth, prostate enlargement, androgen-induced hair loss, and beard growth. Androgens exert other effects in the body: testosterone promotes muscle development, stimulates enzymatic activity in the liver, and stimulates hemoglobin synthesis. Androgens must be converted to estrogen to stimulate bone maturation at the epiphyseal plate [36].

FSH binds to specific receptors on the cell surface of Sertoli cells and causes a sequence of events that culminates in increased protein kinase in a manner similar to the stimulatory effect of LH on Leydig cells. However, FSH causes an increase in the mass of seminiferous tubules and, in an undefined way, supports the development of sperm.

Estrogen is produced mainly by the follicle cells of the ovary utilizing the same initial steps as testosterone production with a final aromatization process. In the female, LH binds to membrane receptors of ovarian cells and stimulates the activity of adenyl cyclase to produce cAMP, which stimulates the production of the low-density lipoprotein (LDL) receptor to increase binding and uptake of LDL cholesterol and the formation of cholesterol esters. LH stimulates the rate-limiting enzyme P450scc, which converts cholesterol to pregnenolone, initiating steroidogenesis. After the onset of ovulation, LH exerts major effects upon the theca of the ovary. FSH binds to its own cell-surface receptors on the glomerulosa cells and stimulates the conversion of testosterone to estrogen.

The main active estrogen in humans is estradiol. Estrogens circulate bound to sex hormone-binding globulin (SHBG) and follow the same general pattern of action at the cell level as described for testosterone. Estradiol affects breast and uterine development, the distribution of adipose tissue, and bone mineral accretion. Low concentrations of estradiol are difficult to measure in standard assays. New experimental bioassays for estrogen are sensitive enough to differentiate between boys and girls in prepuberty, between prepuberty and puberty in girls, and between normal girls and those with premature thelarche, who secrete only a small amount of estrogen above age-matched controls [52].

Activin and inhibin

Inhibin is a heterodimeric glycoprotein member of the transforming growth factor (TGF)-βfamily produced by the Sertoli cells in the male and by the ovarian granuloma cells and the placenta in the female [53]. Inhibin suppresses FSH secretion from the pituitary gland and provides another explanation for different serum concentrations of LH and FSH with only one hypothalamic peptide (GnRH) stimulating them. Activin is a subunit of inhibin and has the opposite effect, stimulating the secretion of FSH from the pituitary gland. Inhibin B secretion rises in early puberty in both boys and girls and then plateaus [54]. The infant male has values of inhibin B higher than those achieved in adult males for the first 1–1.5 years after birth, indicating the activity of the testes during this early period. Absence of inhibin due to gonadal failure causes a greater rise in serum FSH than LH in pubertal and adult subjects.

Anti-Müllerian hormone

Anti-Müllerian hormone (AMH) belongs to the same TGF-β family as inhibin and is produced from the Sertoli cells of the fetal testes and the granulosa cells of the fetal ovary [55]. In normal males, AMH is high in the fetus and newborn but decreases thereafter, with a further drop at puberty. Patients with dysgenetic testes have decreased serum AMH. Values are elevated in males with Sertoli cell tumors or females with granulosa cell tumors. AMH assays might be used to differentiate a child with congenital anorchia who has no testicular tissue from one with undescended testes who has testicular tissue that can produce AMH. Girls have low concentrations of AMH in the newborn period.

Ontogeny of endocrine pubertal development

Fetal testosterone secretion early in pregnancy is caused by placental hCG stimulation. The fetal hypothalamus contains GnRH-containing neurons by 14 weeks of gestation, and the fetal pituitary gland contains LH and FSH by 20 weeks [56]. The hypothalamo-pituitary portal system develops by 20 weeks of gestation, allowing hypothalamic GnRH to reach the pituitary gonadotrophs. Stimulation by GnRH causes gonadotropin secretion to rise to extremely high concentrations at mid-gestation with a decrease in responsivity thereafter. Initially unrestrained GnRH secretion by the hypothalamus comes under restraint from the CNS by mid-gestation, and probably also to some degree from increased circulating sex steroid concentrations, which exert a restraining effect upon gonadotropin secretion until after birth.

At term, gonadotropin concentrations are lower than at mid-gestation but still relatively high. Gonadotropin values rise once again in an intermittent pattern after birth with episodic peaks noted up to 2–4 years after birth [57]. Estrogen and testosterone from the infantile gonads also rise episodically during this period, but mean serum values of gonadotropins and sex steroids during infancy remain much lower than those found in the fetus and the pubertal subject but higher than those found during mid-childhood. Because sex steroids suppress gonadotropin secretion to a significant degree during the first years after birth, agonadal patients, such as those with Turner syndrome, exhibit high (castrate) serum gonadotropin concentrations while maintaining the same pattern of pulsatile gonadotropin secretion as normal girls but with higher pulse amplitudes [58,59].

During the quiescent childhood phase, which reaches a nadir around 7 years of age [60], gonadotropin pulsatility and gonadal activity remain at a low level, restrained by the CNS [61]. Even in children without gonadal function, such as those with Turner syndrome, serum gonadotropin concentrations are low, demonstrating that the presence of the gonads is not necessary to suppress gonadotropin secretion during this period. Changes in gonadotropin secretion arise as a result of alterations in pulse amplitude with pulse frequency unchanged [62]. Likewise, testosterone and estrogen are measurable in the circulation using sensitive assays, demonstrating low but definite activity of the prepubertal gonads.

During the peripubertal period, prior to physical changes, gonadotropin secretion increases first at night (Plate 11, facing p. 148). Sequential sampling demonstrates a rise in sex steroid secretion during the late night/early morning, which follows the pulses of the gonadotropins by hours. Girls also have circadian variation in testosterone production with higher values once puberty starts compared with prepubertal girls; the levels in prepuberty correlate with dehydroepiandrosterone sulfate (DHEAS) secretion, suggesting an adrenal origin of the testosterone whereas, in puberty itself, the testosterone appears to originate from the ovaries [63]. As puberty progresses, the secretion of gonadotropins and sex steroids increases during the day until little circadian rhythm remains.

During the peripubertal period, there is also a change in the response of pituitary gonadotrophs to exogenous GnRH administration. The pattern of LH release increases, so that the adult pattern of response to GnRH is achieved during puberty. The release of FSH shows no such change with development, although female subjects have more FSH release than male subjects at all developmental stages.

Leptin is a hormone produced in the adipose cells that suppresses appetite by attaching to its receptor in the hypothalamus. Leptin plays a major role in puberty in mice and rats as the leptin-deficient mouse (ob/ob) will not commence puberty until leptin is replaced, and leptin administration will induce puberty in an immature but normal mouse. The leptin-deficient human also has pubertal delay, and the introduction of leptin treatment was associated with the appearance of gonadotropin peaks [64]. These data suggest that leptin might trigger the onset of puberty, but clinical studies show that leptin increases in girls during puberty in synchrony with the increase in fat mass, while leptin decreases in puberty in boys with a decrease in fat mass and an increase in fat-free mass; leptin varies with body composition only, and no sex differences are noted [65,66]. Thus, in otherwise normal adolescents, there is no convincing evidence that leptin triggers pubertal development. Leptin appears to be permissive of puberty, but not the cause of its onset or progression.

Adrenarche

The adrenal androgens, dehydroepiandrosterone (DHEA) and androstenedione, produced by the zona reticularis, increase in concentration two or more years before the increase in the secretion of gonadotropins and sex steroids [67]. This process (adrenarche) begins by 6–8 years of age in normal subjects and continues until late puberty. Adrenarche occurs as a result of increased adrenal 17, 20-lyase, and 17α-hydroxylase activities [68]. These androgens cause an

Table 10.2. Age at stage of puberty in girls.

Stage	British girls		Swiss girls		US girls	
	Mean (years)	SD	Mean (years)	SD	Mean (years)	SD
Breast stage 2	11.50	1.10	10.9	1.2	11.2	0.7
Pubic hair stage 2	11.64	1.21	10.4	1.2	11.0	0.5
Breast stage 3	12.15	1.09	12.2	1.2	12.0	1.0
Pubic hair stage 3	12.36	1.10	12.2	1.2	11.8	1.0
Breast stage 4	13.11	1.15	13.2	0.9	12.4	0.8
Pubic hair stage 4	12.95	1.06	13.0	1.1	12.4	0.9
Menarche	13.47	1.12	13.4	1.1		
Breast stage 5	15.33	1.74	14.0	1.2		
Pubic hair stage 5	14.41	1.21	14.0	1.3	13.1	

Table 10.3. Age at stage of puberty in boys.

Stage	British boys		Swiss boys		US boys	
	Mean (years)	SD	Mean (years)	SD	Mean (years)	SD
Genitalia stage 2	11.64	1.07	11.2	1.5	11.2	0.7
Pubic hair stage 2	13.44	1.09	12.2	1.5	11.2	0.8
Genitalia stage 3	12.85	1.04	12.9	1.2	12.1	0.8
Pubic hair stage 3	13.90	1.04	13.5	1.2	12.1	1.0
Genitalia stage 4	13.77	1.02	13.8	1.1	13.5	0.7
Pubic hair stage 4	14.36	1.08	14.2	1.1	13.4	0.9
Genitalia stage 5	14.92	1.10	14.7	1.1	14.3	1.1
Pubic hair stage 5	15.18	1.07	14.9	1.0	14.3	0.8

increase in height velocity (the mid-childhood growth spurt), excessive secretion of apocrine sweat, the development of pubic and axillary hair, and an advance in bone age.

The presence or absence of adrenarche does not seem to influence the onset of puberty. Patients with Addison disease who have no adrenal function experience puberty at an appropriate, albeit delayed, age, and children with premature adrenarche also enter gonadarche at a normal age. Thus, adrenarche is usually temporally co-ordinated with gonadarche during pubertal development in normal individuals, but appears not to play an important role in the progression of gonadarche.

Abnormal puberty

Limits of normal pubertal development

Given the discussion on secular trends, care is needed in defining the limits of the normal timing of puberty. European data (Tables 10.2 and 10.3) suggest that precocious puberty should be considered when secondary sexual characteristics appear under 8 years of age (breast stage 2) in girls and 9 years (genitalia stage 2) in boys [69,70]. Data from the USA suggest that sexual precocity should be defined by the onset of secondary sexual development before 6 years in black girls and before 7 years in white girls. Given the fact that age at menarche remains effectively unchanged, a slightly wider definition as suggested by the American data seems reasonable. However, care does need to be exercised with these lower limits, and clinical evaluation outwith the European time limits probably remains the best option at present [71] until sensitivity/specificity analysis has been undertaken using different age limits.

The definitions for pubertal delay have not changed. Boys should enter the early stages of puberty by 13.5 years (14 years is usually used for convenience) and girls by 13 years to avoid the label of delayed puberty. Puberty does not often occur spontaneously after 18 years of age. English girls complete secondary sexual development in a mean of 4.2 years (range 1.5–6 years) and boys in 3.5 years (range 2–4.5 years) [11,12].

Precocious puberty

The consequences of precocious puberty may be lifelong, and a careful diagnostic and therapeutic approach is indicated. The premature secretion of gonadal steroids in children

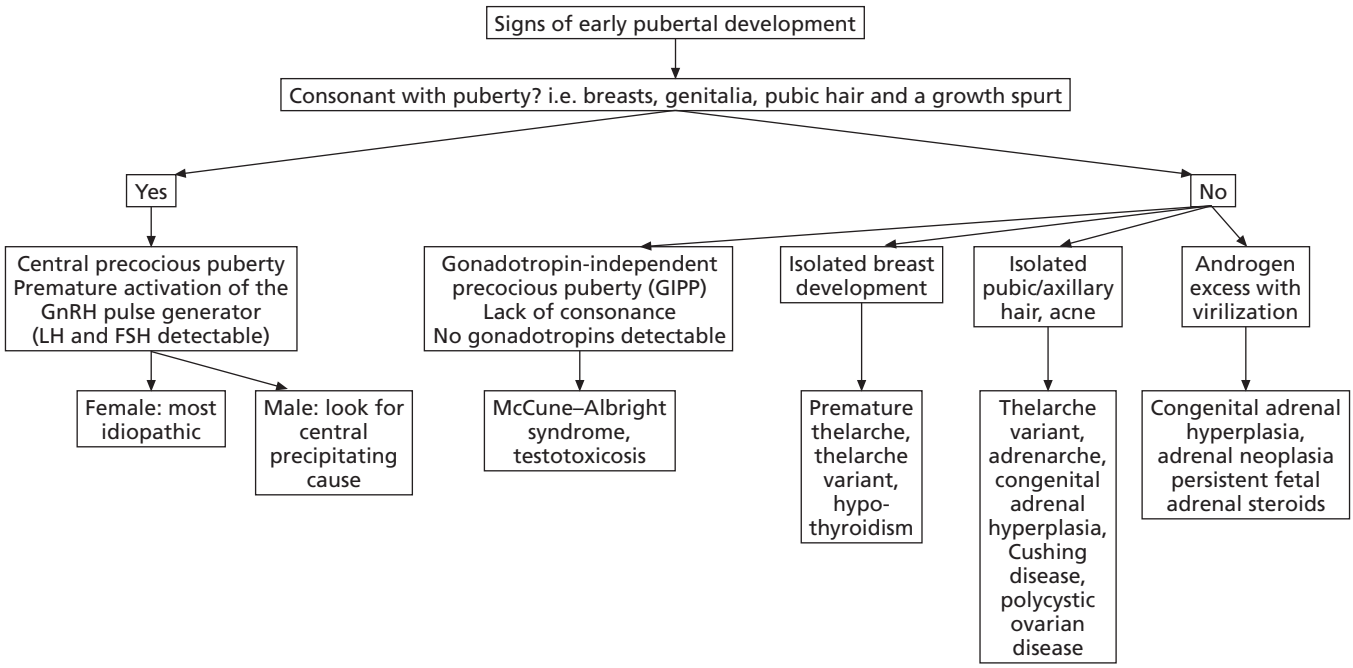


Fig. 10.6. Algorithm for evaluating early puberty.

results in the paradox of tall stature during childhood due to an accelerated rate of linear growth but with eventual short stature as an adult due to early fusion of the epiphyseal growth plates. The psychological consequences of early puberty are considerable.

Etiology

The causes of early puberty can be divided into those with consonance of puberty and those in which there is a loss of consonance (Fig. 10.6). The former include central or gonadotropin-dependent precocious puberty, in which there is premature activation of the hypothalamo-pituitary–gonadal axis, whereas the latter includes isolated early development of breast tissue (premature thelarche/thelarche variant) or pubic/axillary hair (premature adrenarche, late-onset congenital adrenal hyperplasia, adrenal tumors). Additionally, there may be activation of the ovaries or testes independently of gonadotropin secretion, so-called gonadotropin-independent precocious puberty. The causes are listed in Table 10.4.

Precocious puberty is much commoner in girls than in boys [69–72]. This may be because activation of the hypothalamo-pituitary–gonadal axis requires a lower dose of GnRH in girls than in boys (73), and suppression of sexual precocity with a GnRH analog is more difficult in girls than in boys (74).

Gonadotropin-dependent precocious puberty

In central or gonadotropin-dependent precocious puberty (GDPP), the hypothalamo-pituitary–gonadal axis is prematurely activated, the pattern of endocrine change is the

Table 10.4. Causes of premature sexual development.

I.	Gonadotrophin-dependent precocious puberty
	Idiopathic central precocious puberty – commonest cause in females
	Secondary central precocious puberty
	congenital anomalies, e.g. septo-optic dysplasia
	brain neoplasms, e.g. optic nerve gliomas, hamartomas, etc.
	cysts
	hydrocephalus
	post infection
	post trauma
	post cranial radiotherapy
	neurofibromatosis
	adoption
	HCG-producing neoplasms, e.g. choriocarcinoma, hepatoblastoma, germ cell tumors of CNS or mediastinum
II.	Gonadotrophin-independent precocious puberty
	Ovarian cysts
	Defects of LH receptor function: McCune–Albright syndrome, testotoxicosis
III.	Abnormal patterns of gonadotrophin secretion
	Premature thelarche (isolated breast development)
	Thelarche variant and slowly progressing variants of central precocious puberty
	Hypothyroidism
IV.	Sexual precocity due to adrenal androgens
	Steroid secretion by the normal adrenal gland – adrenarche
	Adrenal enzyme defects – congenital adrenal hyperplasia
	Adrenal tumors – Cushing syndrome and virilizing tumor
V.	Gonadal tumors secreting sex steroids
VI.	Exogenous sex steroids

same as in normal puberty, and the pubertal development is consonant. Idiopathic precocious puberty accounts for the majority (> 90%) of cases in girls, but only 10% of cases in boys and is a diagnosis of exclusion. In males with GDPP, neuroradiological imaging [either computed tomography (CT) or magnetic resonance imaging (MRI)] is mandatory. In girls, in the absence of neurological signs, the diagnostic return is only 15% at all ages with the majority of findings not requiring intervention [69,72,75]. Interventional returns are increased in patients under 4 years of age.

Secondary GDPP results from CNS lesions that can provoke premature activation of the hypothalamo-pituitary–gonadal axis, even if not in the region of the hypothalamus. These include tumors such as optic and hypothalamic gliomas (Fig. 10.7), astrocytomas, ependymomas, and pineal tumors, hydrocephalus, trauma, radiotherapy, post-CNS infection, and neurofibromatosis [76]. Hamartomas of the tuber cinereum are congenital tumors composed of a heterotopic mass of GnRH neurosecretory neurones, fiber bundles, and glial cells, which are frequently associated with GDPP, often before 3 years of age. Gelastic epilepsy and developmental delay may be associated, and the characteristic appearance on neuroradiological imaging is that of a sessile or pedunculated mass usually attached to the posterior hypothalamus between the tuber cinereum and the mamillary bodies [77]. The tumor is thought to secrete GnRH, rather than stimulating secretion from a normal hypothalamus, and is associated with very high serum LH concentrations in response to GnRH administration.

The prevalence of GDPP is increased after cranial irradiation for local tumors or leukemia. Low-dose cranial irradiation (18–24 Gy) employed in the CNS prophylactic treatment of acute lymphoblastic leukemia is associated with a downward shift in the distribution of ages at pubertal onset and menarche in girls [78,79]. Moderate radiation doses (25–47.5 Gy) used for the treatment of brain tumors in children are associated with precocious puberty with a direct relationship between ages at pubertal onset and therapy [79]. Higher doses are usually associated with gonadotropin deficiency.

The scenario may be complicated by co-existing growth hormone deficiency (GHD) in those children who have received cranial radiotherapy, as well as those children with sexual precocity secondary to congenital anomalies, trauma, or CNS infection. Careful evaluation of these children reveals that GH-deficient children with GDPP grow at a rate that is somewhere between that of children who are GH sufficient with GDPP and that of children who have GHD without sexual precocity. As children who have received cranial irradiation are often obese and as obesity is associated with a reduction in GH secretion, tests of GH secretion can be misleading in this group of patients and need careful interpretation. Treatment of those children with GDPP and true GHD entails the administration of GH and a GnRH agonist.

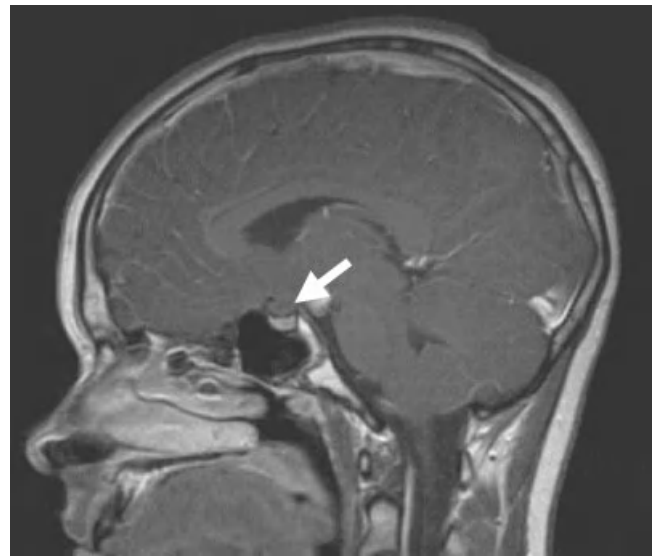


Fig. 10.7. Sagittal magnetic resonance image from an 8-year-old boy with clinical features of gonadotrophin-dependent precocious puberty showing a hypothalamic mass with the growth characteristics of a glioma.

Table 10.5. Conditions in which earlier sex steroid exposure is associated with gonadotrophin-dependent precocious puberty.

Condition	Exposure
Congenital adrenal hyperplasia	Late diagnosis or poor treatment
Gonadotrophin-independent precocious puberty	Autonomous sex steroid secretion
Exposure to xenoestrogens	Environment
?? Premature adrenarche	Adrenal androgen secretion

Children adopted from developing countries and moved to a more affluent environment have an increased incidence of early and precocious puberty [3,5]. Sexual abuse has been reported as a precipitating cause of GDPP and, in these cases, the development can regress with a change in environment [80]. Sex steroid exposure has a direct maturational effect on the hypothalamus and can accelerate the onset of centrally mediated puberty (Table 10.5) [81–83].

Rarely, gonadotropin-releasing tumors (usually hCG) lead to sexual precocity. These tumors are mostly intracranial, such as pineal germ cell tumors and teratomas, or hepatoblastomas and teratomas. Tumor markers such as α-fetoprotein and pregnancy-specific β1-glycoprotein are often present. Pure gonadotropin-secreting tumors of the pituitary occur but are rare.

Gonadotropin-independent precocious puberty (GIPP)

In these conditions, the secretion of sex steroids is autonomous and independent of the hypothalamic GnRH pulse

generator. There is loss of normal feedback regulation, and sex steroid concentrations can be very high with low gonadotropin secretion. The disorders are associated with an abnormally functioning LH receptor. The LH receptor belongs to the G-protein coupled receptor superfamily and is characterized by the presence of seven transmembrane α-helices. The LH receptor is linked to an associated G-protein, which is vital for signal transduction and the intracellular actions of the hormone. LH binds to its receptor, and this then activates the G-protein, which leads to the conversion of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). This causes an increase in intracellular cAMP, which sets off a chain of events culminating in the synthesis and secretion of sex steroids. Phosphorylase activity of the G-protein converts GTP to GDP, which terminates the action of LH.

In boys, familial testotoxicosis or male-limited GIPP is associated with premature Leydig cell and germ cell maturation. It is inherited as an autosomal-dominant condition, which only manifests in males. Virilization occurs with very high concentrations of testosterone and enlargement of the testes to the early or mid-pubertal range, although they seem smaller than expected in relation to the stage of penile growth. Premature Leydig and Sertoli cell maturation and spermatogenesis occur [84]. Unstimulated gonadotropin concentrations are prepubertal with a minimal prepubertal response to GnRH stimulation. There is a lack of the usual pubertal pattern of LH pulsatility. In adulthood, fertility is achieved, and an adult pattern of LH secretion and response to GnRH is demonstrable.

Testotoxicosis is associated with a number of constitutively activated mutations of the LH receptor [85,86]. The majority of these activating mutations are in the transmembrane domain of the receptor. Two boys have been described who manifest the combination of testotoxicosis and pseudohypoparathyroidism [87]. Both had a mutation in the gene encoding the Gs α-subunit of G-proteins. The mutation resulted in the substitution of an alanine residue at position 366 with serine (Ala366Ser), a mutation that led to constitutive activation in the adenyl cyclase of the LH receptor. This mutation is stable at the lower temperature of the testes but is degraded at 37°C, the temperature of the cAMP-dependent receptor for parathyroid hormone, which leads to parathyroid hormone resistance.

McCune–Albright syndrome (MAS) is a multisystem disorder that occurs in both boys and girls. It is characterized by the classical triad of irregularly edged hyperpigmented macules or café-au-lait spots, a slowly progressive bone disorder (polyostotic fibrous dysplasia), which can involve any bone, with frequent facial asymmetry and hyperostosis of the base of the skull and, most commonly in girls, GIPP [88]. There is often a lack of consonance in pubertal development, and menses may be observed with minimal breast development. Autonomous hyperfunction most commonly involves the ovary, but other endocrine involvement includes the thyroid (nodular hyperplasia with thyrotoxicosis), the adrenals (multiple hyperplastic nodules with Cushing syndrome), the pituitary (adenoma with gigantism, acromegaly, or hyperprolactinemia), and the parathyroids (adenoma or hyperplasia with hyperparathyroidism). At least two of these features should be present for the diagnosis to be made. The condition is sporadic and is due to a somatic activating missense mutation in the gene encoding the α-subunit of the G-protein (Gsα), which stimulates cAMP production (see above). The mutation results in a failure of phosphorylation of GTP to GDP and therefore constitutive activation [89]. The mutation is somatic, and individuals are chimeric for the condition, hence the variability of the phenotype.

The sexual precocity in girls with MAS is caused by autonomously functioning luteinized follicular cysts of the ovaries. Multiple follicular cysts with an occasional large solitary cyst may be present. Estrogen production is associated with a prepubertal pattern of LH secretion with an absent LH response to GnRH. Later, GnRH-dependent puberty ensues with ovulatory cycles. Sexual precocity is rare in boys with MAS. When it does occur, it is associated with asymmetric enlargement of the testes in addition to signs of sexual precocity. The seminiferous tubules are enlarged and exhibit spermatogenesis. Leydig cells may be hyperplastic.

Abnormal patterns of gonadotropin secretion

Premature thelarche

Thelarche describes the phenomenon of isolated breast development, which may be unilateral or bilateral, often with a fluctuating degree of development. It is not accompanied by other signs of puberty. Growth velocity is normal, and bone age is not advanced. Premature thelarche is often present from infancy, usually occurs by the age of 2 years, and onset is rare after the age of 4 years. Incidence is 20 per 100 000 patient–years. Up to 60% of cases occur between 6 months and 2 years of age, and most regress 6 months to 6 years after diagnosis. Significant nipple development is usually absent, and estrogen-induced thickening and dulling of the vaginal mucosa or enlargement of the uterus on ultrasonography is uncommon. Growth in stature is normal. It is usually a benign self-limiting disorder, although some girls progress into early or precocious puberty.

Premature thelarche is typically associated with FSH secretion, antral follicular development, and ovarian function that is greater than that of prepubertal control subjects. Unstimulated and GnRH-stimulated plasma levels of FSH are increased, whereas those of LH are prepubertal [90].

Thelarche variant

Variations of premature thelarche occur on a spectrum towards precocious puberty. Most cases of premature thelarche present in the first two years of life and regress

before puberty. Children who present later may demonstrate continuing breast development, which may advance with accompanying growth acceleration and bone age advancement. The name given to this condition is thelarche variant or "a slowly progressive variant of precocious puberty in girls" [91]. The condition may result from a disorder of ovarian follicular maturation as mean ovarian volume exceeds the normal prepubertal size. Cyclic breast growth may be seen which does not resolve spontaneously. The condition demonstrates considerable heterogeneity in terms of gonadotropin secretion: pulsatile FSH-predominant gonadotropin secretion is intermediate between premature thelarche and GDPP [91], LH secretory profiles are more like those observed in normal puberty [92], and, in some girls with a rapid but transient onset of estrogenization, suppress responsiveness to GnRH.

Primary hypothyroidism

In some patients with primary hypothyroidism, in addition to elevated TSH concentration, FSH concentrations are also increased. *In vitro* studies have demonstrated that TSH has weak agonist properties at the human FSH receptor [93]. Ovarian stimulation results, with isolated breast development in girls. In boys, testicular enlargement without any other secondary sexual characteristics results [94]. There is no pubertal progression in the majority of cases, bone age is delayed, and growth velocity poor. Nevertheless, in certain cases, normal gonadotropin-dependent puberty occurs at an inappropriately early age upon instigation of thyroxine treatment. Overall, the prognosis is excellent with reversal of puberty once treatment is commenced, but final height may be affected if diagnosis is delayed or if normal puberty occurs at an early age.

Sexual precocity due to adrenal androgens

Adrenarche

The fetal adrenal gland secretes DHEAS, and this can manifest as pubic hair or clitoromegaly in infancy, especially in premature babies [95]. Congenital adrenal hyperplasia (CAH) and virilizing adrenal tumors need to be excluded. Adrenal androgen concentrations diminish as the fetal adrenal zone regresses, and appearances return to normal.

When adrenarche occurs before the age of 8 years in girls and 9 years in boys, it is called premature adrenarche or pubarche and is commoner in children from an Asian, Mediterranean, or Afro-Caribbean background. An association with low birthweight has been described [96]. In some studies, there appears to be an increased prevalence of minor defects of adrenal steroidogenesis in these children [97], particularly when genital enlargement is present. In spite of the increase in height velocity and advance in bone age, final height is unaffected, although there may be an increased prevalence of functional ovarian hyperandrogenism in the mid-teenage years [98].

Congenital adrenal hyperplasia

The classical form of CYP21 deficiency may present with salt loss and clitoromegaly in girls in the neonatal period. In boys who do not have the salt-losing form and, in some very virilized girls who are not diagnosed as they are raised as boys, presentation may be with tall stature, increased height velocity, advanced bone age, clitoromegaly in girls, genital maturation in the absence of testicular enlargement in boys, and the development of pubic and axillary hair in both sexes. CYP11A deficiency presents in a similar way but with the additional complication of hypertension.

The non-classical or late-onset form of CAH may present in childhood or adolescence with early pubic hair and acne or in early adulthood with menstrual irregularities, hirsutism, or infertility. GDPP and the polycystic ovary syndrome are common sequelae. Final height is usually compromised. Virilization also occurs in undertreated children with CAH.

Approximately 5–10% of children with premature adrenarche are estimated to have late-onset CAH, although this estimate varies depending on the ethnicity of the population sampled [97]. The adrenocorticotrophic hormone (ACTH) stimulation test can differentiate between children with late-onset CAH and precocious pubarche/premature adrenarche. Unstimulated and stimulated concentrations of 17α-hydroxypregnenolone (17PGN) and 17PGN:17-OHP, DHEA, and androstenedione concentrations are higher in children with premature adrenarche than in control subjects or those with non-classical 21-hydroxylase deficiency.

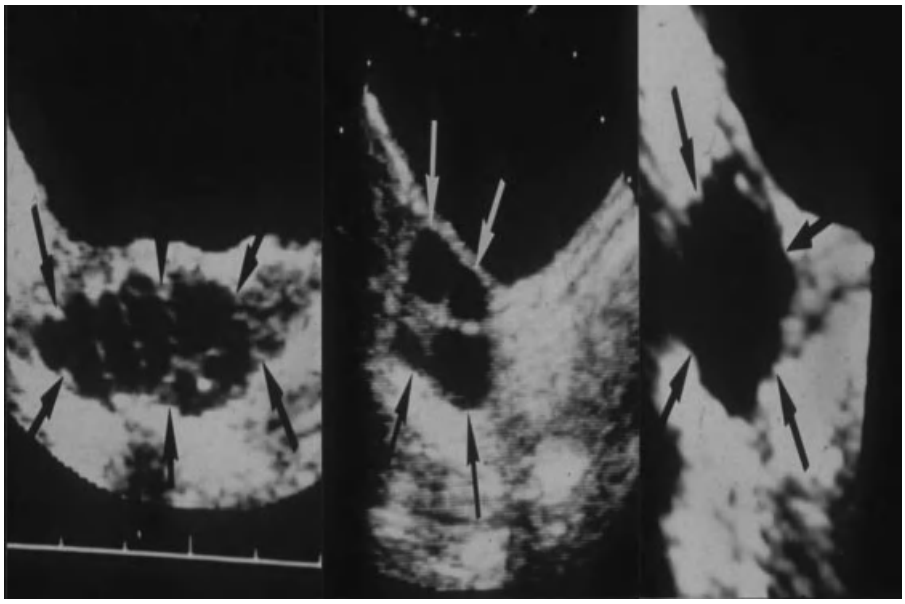
Adrenal tumors

The characteristic picture is a short history of virilization, accelerated growth rate, and advanced bone age. Cushing syndrome may be present if there is hypersecretion of cortisol. The diagnosis can be revealed only by urinary steroid profile analysis. Imaging of the adrenal glands should be performed. Treatment involves surgical resection of the tumor, with the option of adjuvant chemotherapy. The prognosis is usually guarded, and neither operative findings nor histology are of much help. The only factor influencing whether the lesion is likely to behave in a malignant manner or not is tumor size. Tumors less than 5 cm diameter are nearly always benign. An immediate fall in serum or urinary androgen markers of tumor secretion is encouraging, and adjuvant chemotherapy or radiotherapy has not been demonstrated to improve long-term prognosis. Adrenal tumors may be associated with syndromes of increased cancer risk (e.g. Li–Fraumeni syndrome).

Gonadal sex steroids

Gonadal tumors

These are rare tumors leading to sexual precocity. Pubertal development is not consonant, and sex steroid concentrations are high, above the normal adult range. Leydig cell tumors of the testis are associated with virilization, but conversion of testosterone to estradiol leads to gynecomastia. Granulosa



(a) (b) (c)

Fig. 10.8. Pelvic ultrasound examinations of girls with (a) GDPP showing multicystic ovary characteristic of the effects of pulsatile gonadotrophin secretion, (b) the ovary in thelarche variant with several large cysts, and (c) the large single cyst present in McCune–Albright syndrome. Arrows outline ovarian structure. Marker bar depicts 1-cm intervals.

cell and germ cell tumors of the ovary can secrete both androgens and estradiol.

Exogenous sex steroids

Exogenous sex steroids can occasionally be the cause of sexual precocity. Hormones used in chicken-rearing are occasionally implicated as a cause of "epidemics" of premature thelarche, although the relationship is unproven.

Problems associated with sexual precocity

Growth

As puberty occurs abnormally early, the growth spurt will also take place early. GH concentrations and the increase in growth velocity are similar to those observed in normally timed puberty. However, as the growth spurt has occurred abnormally early, insufficient childhood growth will have taken place, so adding the relatively fixed increment of approximately 30 cm that comes with puberty will lead to a restricted final height. Sex steroid exposure will result in rapid bone age advance, diminishing adult height potential in turn.

Psychological problems

These problems are often the major issue for children with sexual precocity and their families as sex steroid exposure in young children results in disruptive behavior and the child looks older than their chronological age [99]. Most children experience problems at school, which are compounded by the difficulties that teachers and fellow pupils have in understanding them. The child and his/her family may later have problems dealing with normally timed pubertal development and are frequently apprehensive about stopping suppressive treatment. In girls, menstruation at an early age presents practical difficulties.

Additionally, girls may be subject to sexual advances with which they are unable to deal, while boys may have embarrassing erections. Children with special educational needs as a result of a cerebral lesion or hydrocephalus are particularly vulnerable to these problems. Early maturity within the normal range correlates to some extent with an earlier onset of sexual behavior [99].

Clinical and diagnostic approach to sexual precocity

A careful history and clinical examination should be performed in the first instance, height measured, and Tanner pubertal stage recorded. Bone age estimation should be performed. Follow-up of height velocity and pubertal progress is important for the differentiation of potentially benign, non-progressing conditions, such as premature thelarche and adrenarche, from progressive conditions such as GDPP, GIPP, CAH, and gonadal tumors. Most children presenting with sexual precocity do not require extensive investigation, but sinister underlying causes for sexual precocity such as tumors should always be considered.

Imaging

All girls with early development of secondary sexual characteristics warrant a transabdominal pelvic ultrasound examination [100]. In GDPP, the ovaries are active with multiple (> 6) cysts that are greater than 4 mm in diameter (Fig. 10.8a). Larger cysts are sometimes seen in premature thelarche (< 3 cysts) [101], thelarche variant (3 – 6 cysts) (Fig. 10.8b) [91], and MAS (Fig. 10.8c) [102]. In practice, ovarian appearances

may overlap between GDPP and premature thelarche, although children with premature adrenarche have appearances that are similar to control subjects.

The first signs of estrogenization of the uterus is a change in shape from a tubular structure, where the diameter of the fundus and the cervix are similar, to a pear-shaped structure, where the fundus expands so that its diameter exceeds that of the cervix. In GDPP, the changes resemble those of normal puberty whereas, in premature thelarche or thelarche variant, the uterus remains prepubertal in shape. Endometrial thickening suggests that pubertal concentrations of estrogen have been attained, and an endometrium of around 6–8 mm implies imminent menarche.

When GDPP is treated, the uterus does not return to its prepubertal state, although the endometrium should remain thin. The ovaries may remain large for the child's age, but the continuing development of large follicles indicates inadequate suppression. In children in whom an ovarian tumor is suspected, the pelvic ultrasound scan may be highly informative.

Neuroradiological imaging in the form of an intracranial MRI scan is mandatory if there are any neurological signs. Boys with GDPP must have neuroradiological imaging because idiopathic GDPP is extremely uncommon in boys. In girls, the need for neuroradiology is more contentious but is indicated in girls presenting under the age of 4 years.

If an adrenal tumor is suspected in patients with virilization, a CT or MRI scan of the adrenal glands is indicated. Ultrasound scan of the adrenal glands is of limited use. In cases where MAS is suspected, a bone scan and skeletal survey are indicated.

Biochemistry

The combination of a GnRH stimulation test and measurement of serum concentrations of sex steroids is a useful start point. This entails the administration of a single intravenous bolus of GnRH (2.5 μg/kg to a maximum of 100 μg) with measurement of plasma LH and FSH concentrations at 0, 20, and 60 min. Normal prepubertal children have an increment of 3–4 IU/L LH and 2–3 IU/L FSH. Regardless of age, the increment is greater in puberty, although the cutoffs vary depending upon the assay. In GDPP, a pubertal LH-dominant response is observed whereas, in GIPP and sexual precocity secondary to gonadal tumors or ovarian cyst formation, gonadotropin concentrations are suppressed by the autonomous sex steroid secretion. The response to GnRH in precocious adrenarche is prepubertal. In premature thelarche, FSH tends to be dominant while, in thelarche variant, response is intermediate between thelarche and GDPP with FSH predominating.

In children with virilization, unstimulated plasma 17-OHP concentrations that are elevated suggest a diagnosis of CAH. The response to synacthen with measurement of plasma 17-OHP and/or urinary steroids will confirm the diagnosis. Rapid virilization suggests the presence of an endocrine-secreting neoplasm. Testosterone, dihydrotestosterone, DHEAS, and androstenedione are all elevated in adrenal virilizing tumors. Plasma cortisol may also be elevated with a loss of the normal circadian rhythm if Cushing syndrome is a feature. There is a failure of suppression in response to dexamethasone if an adrenal tumor is present, whereas in premature adrenarche and CAH, dexamethasone administration will lead to suppression of adrenal steroids. A urinary steroid profile is also of considerable diagnostic value if an adrenal tumor is suspected [103].

A raised serum hCG level suggests an hCG-secreting neoplasm. The response to GnRH will be prepubertal. Thyroid function tests should be undertaken in a girl with premature thelarche or a boy with enlargement of the testes in the face of a lack of virilization combined with short stature, a poor growth velocity, and delayed bone age to exclude primary hypothyroidism.

Treatment

General

Adrenarche, thelarche, and thelarche variant have an excellent prognosis and do not require treatment. Hypothyroidism is treated with a cautious introduction of thyroxine with subsequent increase to standard maintenance doses. Non-classical CAH is treated with hydrocortisone and fludrocortisone if indicated by a raised plasma renin concentration. Although some groups suggest that the condition should not be treated, lack of treatment may compromise final height and fertility.

An abnormality underlying GDPP or GIPP needs treatment. The decision to treat precocious puberty per se is based upon several factors such as the age of onset, the rate of progression of puberty, the emotional impact of experiencing early puberty, and effects on final height. Not all children with precocious puberty require treatment to suppress gonadotropin secretion. Indications for therapy include the avoidance or amelioration of the psychological consequences of early puberty, with particular emphasis on menarche in young girls, initial tall stature with its attendant problem of unrealistic expectations by adults, a projected severe restriction in final adult height, and an increased risk of sexual abuse [104]. In general, the impact on final height and the psychological problems are usually worst in the youngest children, and most of these will need treatment. In girls with untreated idiopathic GDPP, a mean final height of 151–155 cm is not uncommon [105–107], whereas in boys, limited data suggest a greater restriction in final height. Slowly progressive forms of precocious puberty have been described which impact little on final height as the majority of these patients achieve their genetic target heights without intervention [108].

Treatment will halt pubertal progress and the pubertal growth spurt, but significant regression of the signs of sexual precocity does not usually occur, although there may be a

reduction of breast size and cessation of menses in girls and of testicular volume in boys. Aggressive behavior is reduced, as is the number of erections in boys. The shape of the uterus does not revert to the prepubertal form.

Gonadotropin-dependent precocious puberty

In the past, progestational agents were used for the treatment of patients with precocious puberty. Cyproterone acetate is a peripherally acting anti-androgen with some progestogenic and glucocorticoid actions, suppressing both gonadotropin and gonadal steroid secretion. It was effective in halting the progress of the physical features of puberty and useful in suppressing menstruation, but had no effect on final height [109].

More complete suppression of the hypothalmo-pituitary–gonadal axis results from the action of continuous exposure of the GnRH receptor to its ligand. GnRH agonists (GnRHa) are synthetic analogs with a D-tryptophan inserted instead of the naturally occurring L-form to increase the duration of receptor occupancy. This results initially in stimulation followed by receptor downregulation and cessation of gonadotropin secretion [110]. Later in the course of treatment, receptor levels return to normal, but desensitization persists because of an uncoupling of the receptor from the intracellular signaling effector pathway [111]. Synthetic depot preparations of GnRHa are available with enhanced activity and a longer half-life than natural GnRH. These are administered subcutaneously on a once-monthly or quarterly basis.

Gonadal suppression is observed in most children treated with GnRHa. It has been suggested that the response to treatment is best monitored by measuring basal sex steroid concentrations and peak plasma LH concentrations following a GnRH test [112]. However, in clinical practice, because the suppression is so complete, assessment of pubertal stage, height velocity, skeletal maturation, and pelvic ultrasound scan is often sufficient for monitoring the response. The depot preparations are more effective in suppressing height velocity and bone maturation than the intranasal preparations, which need to be administered thrice-daily. Initial stimulatory effects can be prevented by the use of cyproterone acetate in conjunction with the GnRHa over the first 4–6 weeks at a dose of 50 mg/m^2/day. If the endometrium is thickened, vaginal bleeding may occur at the start of treatment as a result of estrogen withdrawal and can usually (but not always) be prevented by the administration of cyproterone.

Although it has been suggested that treatment with GnRHa improves final adult height, the evidence points more to the age at the onset of treatment as the crucial factor [113–115], with a more clearcut effect in younger children. Data on final height suggest that treatment with GnRHa analogs and cyproterone acetate cannot recover lost height potential, and children cannot attain their target mid-parental height [116,117].

Children with precocious puberty have higher circulating concentrations of GH and IGF-1 than controls of a similar chronological age. Treatment with GnRHa may lead to a fall in circulating GH and IGF-1 concentrations with a consequent reduction in growth velocity. It has been suggested that a combination of GH and GnRHa may result in an increase in final height. Although the combination treatment leads to an increase in height velocity and height standard deviation score for bone age and in predicted adult height in girls with precocious puberty, there are limited data to support a similar increase in final height [118].

There are a number of published studies examining the effects of treatment with cyproterone acetate or GnRHa with or without hGH on final height in children with GDPP. Ideally, the comparison of the final height of a group of treated children with GDPP with that of a well-matched untreated control group in a randomized double-blind study would be the most meaningful method for determining the efficacy of treatment on final height. Most studies are small in size and use final height-predicted height as the arbiter of efficacy. Using predicted height creates its own problems.

Werder *et al*. demonstrated that there was an increase in height prediction with time in a group of untreated girls with GDPP [119]. Zachmann *et al*. demonstrated that the accuracy of final height prediction was poor in children with abnormal growth patterns, including untreated precocious puberty [120]. Bayley–Pinneau predictions are more accurate on the whole than other methods in precocious puberty [121]. However, the Bayley–Pinneau method for height prediction can overestimate the final height, particularly in those children who have a greatly advanced bone age for chronological age or who have a bone age greater than 7 years [114,116]. They are not applicable to the younger cohort where any likely effect will be maximal but in whom height predictions are the most inaccurate.

GnRHa treatment is safe and well-tolerated. However, withdrawal of sex steroids due to GnRHa results in a reduction in bone mineral density [122,123], although there are no long-term data on how this translates into long-term bone health [115]. Sex steroid withdrawal in children can result in "menopausal" symptoms, such as hot flushes and mood swings, occasionally necessitating low-dose estrogen therapy. Cyproterone is used less frequently in the treatment of GDPP and is associated with tiredness and lethargy with profound ACTH and adrenal suppression. Additionally, there is a risk of hepatocellular carcinoma.

Gonadotrophic-independent precocious puberty

Affected individuals do not exhibit a pubertal LH response to GnRH administration or a pubertal pattern of pulsatile LH secretion. Hence, they do not respond to the chronic administration of GnRHa with a reduction in gonadal steroid production. The treatment of GIPP is difficult. Agents such as testolactone, spironolactone, ketoconazole, flutamide, cyproterone, and medroxyprogesterone acetate have been used. Testolactone inhibits those functions of testosterone that are dependent upon its conversion to estrogen by acting as

an inhibitor of aromatase [124]. Spironolactone acts as an anti-androgen [124,125]. However, escape from the effects of treatment may occur after 1–3 years. The antifungal ketoconazole acts as an inhibitor of steroid synthesis by inhibiting cytochrome P450c17, which regulates 17-hydroxylation. It therefore suppresses both gonadal and adrenal steroid biosynthesis [126] and is effective in halting pubertal progress, albeit in doses much higher than those used for antifungal action. It can lead to adrenal insufficiency and, occasionally, severe hepatic dysfunction. The use of the anti-androgen flutamide is, at best, anecdotal. Cyproterone remains the drug of choice in the majority of cases of GIPP.

Stopping treatment and long-term follow-up

For psychological reasons, treatment should be stopped once the child has reached an age where puberty is acceptable. There is no evidence that longer treatment improves final height. As hypothalamic maturity is not affected by treatment, pubertal growth and development is then recommenced at an advanced stage approximately 3 months later. Gonadotropin secretion recommences approximately 4 months after cessation of depot GnRHa, and most girls menstruate within 1 year of stopping treatment [116]. Normal fertility has been documented in girls with both treated and untreated GDPP [127–129].

Children with GIPP have been reported to experience centrally mediated puberty at an appropriate time, although in MAS, the abnormal gonadal activation continues into adult life and may result in irregular menses and fertility problems [129].

Delayed puberty

Assessment

A temporary delay in sexual maturation is not uncommon and resolves with time leading to normal development, optimum final height, and fertility. By temporary is meant not much longer than 6 months in any one stage of puberty because, in patients with an underlying organic pathology, early diagnosis and treatment is essential to insure normal pubertal progress and adequate final height. A useful algorithm is depicted in Figure 10.9.

Assessment should include a history of symptoms of chronic illness, medications, symptoms suggestive of other hormone deficit or excess, previous treatment or surgery, abnormal eating patterns, and a history of the family, including parental heights and ages at onset of puberty. Chronic illness is often associated with delayed puberty, particularly asthma (and/or its treatment) [130], eczema [131], cystic fibrosis [132], and inflammatory bowel disease [133].

The majority of those that present with idiopathic delayed puberty are boys, more often because of their short stature than the delay in sexual development. In girls, delay is unusual and should prompt a systematic search especially to include eating issues and intense exercise programs.

A thorough examination should include details of present and past heights and weights with pubertal staging. Testicular size should be measured using the Prader orchidometer. Careful documentation for body disproportion with

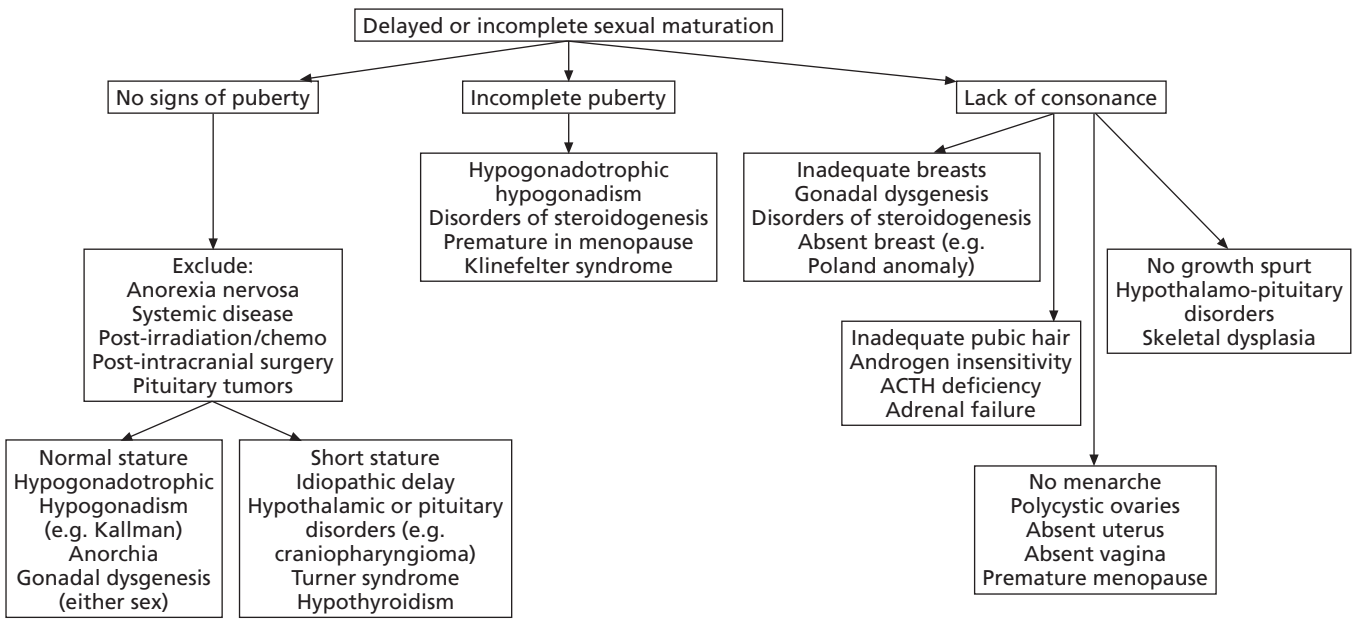


Fig. 10.9. Algorithm for evaluating late puberty.

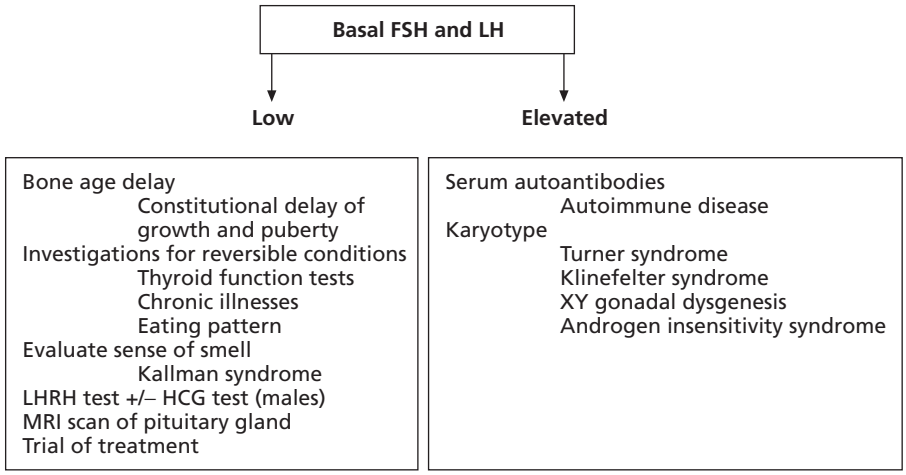


Fig. 10.10. Conditions of pubertal delay associated with high and low gonadotrophin concentrations.

estimation of the upper and lower body segments may suggest Klinefelter syndrome. Patients with Turner syndrome are short, have a low hairline, webbing of the neck, prominent ears, broad chest, renal and cardiac abnormalities with streak gonads, but presenting features may be more subtle. Presence of other dysmorphic features may reveal multisystem syndromes, such as CHARGE, Prader–Willi or septo-optic dysplasia. A careful neurological examination should be performed to include visual field deficits, sense of smell, and fundoscopy. Anosmia is suggestive of Kallman syndrome.

Initial investigations should include a radiograph of the wrist to estimate skeletal age. Based on the skeletal age, it is possible to calculate a predicted adult height range and its relation to the genetic potential (mid-parental height). Further investigations and management should depend on the skeletal age, stature, symptoms, and extent of delay. Laboratory measurement of serum FSH and LH concentrations will help to differentiate patients with hypergonadotrophic hypogonadism (Fig. 10.10). Serum gonadotropin concentrations are low in all normal children before puberty, and caution must be exercised in the interpretation of low serum gonadotropin concentrations especially below the age of 12 years. GnRH testing has been studied extensively in pubertal delay but rarely clarifies whether an individual will progress in puberty or has a permanent defect [134,135]. Overnight sampling may demonstrate gonadotropin pulsatility but is unhelpful for prognosis. Pelvic ultrasound examination is helpful in girls, where it may reveal the multicystic pattern classical of early puberty.

Differential diagnosis

Constitutional delay in puberty

This is the commonest cause of delayed sexual maturation, especially in males. In addition to delayed puberty, it is characterized by short stature that is appropriate when skeletal age is taken into account. Growth velocity is normal for a prepubertal individual. As a rule, mean height velocity is 5 cm/year at 12 years of age and declines at a rate of 1 cm/year for every year thereafter that puberty is not entered. The variance on this is ± 1 cm/year. Constitutional delay is often familial and has an excellent long-term prognosis. Patients have low serum gonadotropins, as do patients with gonadotropin deficiency.

The rules on growth rate are important and can help to prevent unnecessary investigation of the GH axis. Unless "priming" of the system is undertaken with sex steroids, low GH secretion may be documented and GH therapy instigated.

Individuals with pubertal delay can experience social difficulties [136,137]. In these patients, treatment with oxandrolone, a weak non-aromatizable androgen, in doses of 2.5 mg once daily orally for 3–6 months, improves growth velocity [138] without advancing skeletal maturation or pubertal progress. Sex steroids in small doses for about 6 months will induce puberty that will progress spontaneously, leading to normal sexual development and final height [139] (Fig. 10.11).

Hypogonadotrophic hypogonadism (Table 10.6)

This is defined as a permanent absence of spontaneous pubertal development due to a lack of serum gonadotropin production or action. The deficiency may be isolated or associated with combined pituitary hormone deficiencies, congenital or acquired [140]. Isolated gonadotropin deficiency can be idiopathic or part of X-linked Kallman syndrome associated with anosmia [141], in association with X-linked adrenal hypoplasia [142] or X-linked ichthyosis [143]. Increasingly other genes will be implicated such as GPR54 [144].

Acquired gonadotropin deficiency may be due to intracranial trauma, tumors, surgery, or radiotherapy. Hemochro-

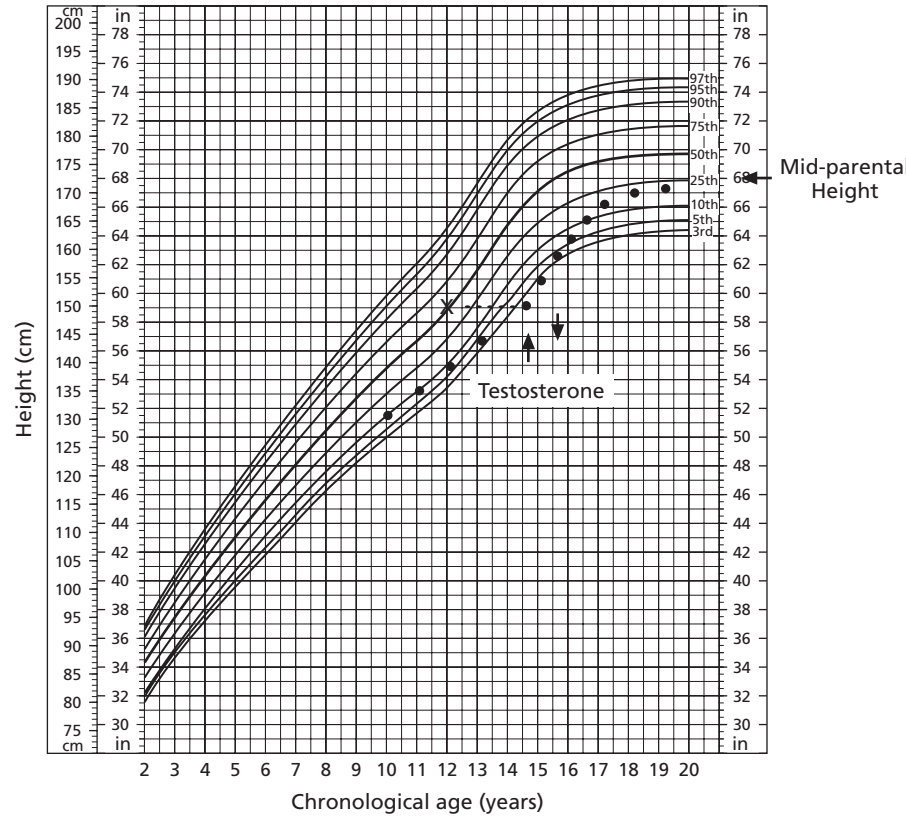


Fig. 10.11. Effect of low-dose testosterone in the form of Sustanon 50 given intramuscularly on a 4-weekly basis on stature in a boy with constitutional delay of growth and puberty. Note centile crossing from age 10–15 years but with a normal prepubertal growth rate. x represents bone age, which is delayed. Testosterone treatment points between the upright and inverted arrows. Final height appropriate for parents.

matosis associated with transfusion can result in permanent gonadotropin deficiency [145].

The condition in boys is best diagnosed, or rather excluded [146], with a GnRH test in combination with a hCG test to stimulate the testicular release of testosterone [147]. In girls, consideration needs to be given to the use of FSH to test ovarian function; further work is needed in this area [148]. More definitive studies include the use of repeated pulses of GnRH to test more fully gonadotroph secretory potential [149]. In many cases, it is necessary to rule out other pituitary hormone deficiencies and perform neuroimaging of the hypothalamus and pituitary gland. In some cases, the interpretation of the GnRH test is not straightforward, and endocrine reassessment may be necessary at a later time after completion of growth and puberty to ascertain the need for long-term replacement.

Hypergonadotrophic hypogonadism (Table 10.7)

Elevated serum gonadotropin concentrations in the absence of pubertal signs may suggest gonadal insufficiency. Radiotherapy, chemotherapy, and surgery, particularly orchidopexy for very high placed testes, can all result in gonadal failure [150].

Turner syndrome (1 in 2500 live female births) should be considered in all short girls, even in the presence of pubertal signs. Patients with Turner syndrome may show markedly elevated serum gonadotropin concentrations from as early as 8–9 years of age [58] due to lack of negative feedback. Pure XX and XY gonadal dysgenesis both present with delayed puberty, raised serum gonadotropins, and low sex steroid concentrations. The XY gonadal dysgenesis group reared as females have a high risk of gonadal tumors and need surgery for the removal of the gonads. Gonadal failure in females is also associated with autoimmune ovarian failure, which may be associated with autoimmune polyendocrinopathy syndrome [151] or galactosemia [152].

In boys, tall stature, pubertal delay, and learning difficulties are the classic features associated with Klinefelter syndrome (47XXY). The learning difficulties may be mild and the stature not excessive [153]. Many patients enter puberty but rarely progress beyond 8 mL testicular volumes. The pubertal appearance is discordance between the genital and pubic hair stages and the small testes [154]. The early introduction of testosterone prevents gynecomastia. Other causes in boys include anorchia, torsion, or infection.

Treatment

Treatment should be instituted at an appropriate age. There is no advantage in delaying puberty in terms of adult stature, and psychosocial problems are exacerbated by delay.

Table 10.6. Causes of hypogonadotrophic hypogonadism.

Developmental and genetic causes
Kallmann syndrome (HH and anosmia): X-linked (*KAL*), AD, AR
Impaired GnRH release and action (e.g. *leptin*, *leptin R*, *PC1*, *GnRHR*)
Multiple pituitary hormone deficiency (e.g. *HESX1*, *LHX3*, *PROP1*)
Isolated gonadotrophin deficiency
Isolated LH deficiency/mutation
Congenital glycosylation disorders
Effects on multiple levels of the HPG axis (e.g. *DAX1*, *SF1*)
Midline defects

Chromosomal abnormalities
Deletions and rearrangements

Syndromic associations
Prader–Willi syndrome
Laurence–Moon syndrome
Gordon–Holmes spinocerebellar ataxia
CHARGE
Others

Physical causes
CNS tumors
  Craniopharyngioma, germinoma, hypothalamic glioma, optic nerve glioma
  Pituitary tumors
Langerhans cell histiocytosis
Post infection
Granulomatous disorders
Vascular malformations
Trauma/pituitary stalk transection
Cranial irradiation

Functional causes
Chronic renal disease
Chronic gastrointestinal disease/malnutrition
Sickle cell disease/iron overload
Chronic lung disease/cystic fibrosis/asthma
Acquired immune deficiency syndrome
Poorly controlled diabetes mellitus
Hypothyroidism
Cushing disease
Hyperprolactinemia
Metabolic conditions (e.g. Gaucher disease)
Anorexia nervosa
Bulimia nervosa
Psychogenic/stress
Extreme exercise
Drugs

Table 10.7. Causes of hypergonadotrophic hypogonadism.

Girls
Chromosomal abnormalities
Turner syndrome and variants (e.g. 45,X; 46,XX/45,X; X chromosome abnormalities)
Mixed gonadal dysgenesis (e.g. 46,XY/45,X)
Deletions and rearrangements (e.g. Xq22, Xq26–28)

Abnormalities in gonadal development
Ovarian dysgenesis

Syndromic associations
Perrault, Maximilian, Quayle and Copeland, Pober, Malouf syndromes
Ataxia telangiectasia, Nijmegen, Cockayne, Rothmund–Thompson, Werner syndromes
Bletharophimosis–ptosis–epicanthus syndrome (BPES, *FOXL2*)

Disorders of steroid synthesis and action
LH resistance
FSH resistance
Pseudohypoparathyroidism 1a
SF1, StAR, CYP11a, HSD3B2, Cyp17, aromatase (CYP19) (46,XX karyotype)
HSD17B2, AIS, SRD5A2 (46,XY karyotype)

Other causes of primary ovarian failure
Autoimmune (e.g. AIRE)
Metabolic (e.g. galactosemia, storage disorders)
Hyperandrogenism/polycystic ovarian syndrome
Pelvic/spinal irradiation
Chemotherapy

Boys
Chromosomal causes
Klinefelter syndrome and variants (e.g. 47,XXY; 46,XY/47,XXY)
Mixed gonadal dysgenesis (e.g. 46,XY/45,X)
Deletions and rearrangements

Abnormalities in gonadal development
Testicular dysgenesis (e.g. loss of functional Sry, Sox9, SF1, WT1, DMRT)

Syndromic associations
Noonan syndrome
Robinow syndrome
Others

Disorders of steroid synthesis and action
LH resistance (e.g. *LHR*, *GNAS*)
SF1, StAR, CYP11a, HSD3B2, HSD17B2, PAIS

Other causes of primary testicular (Leydig cell) failure
Anorchia
Cryptorchidism
Sertoli cell only syndrome
Testicular irradiation
Chemotherapy
Infection (e.g. mumps)

Patients with constitutional delay, chronic illnesses, or eating disorders need small amounts of either testosterone or ethinylestradiol for not more than 4–6 months. This results in triggering endogenous sex steroid production that will sus-

Table 10.8. Suggested treatment regimens for pubertal induction.

Males	Females
Induction	*Induction*
Depot testosterone (intramuscularly)	Ethinylestradiol (orally)
25–50 mg every 4–6 weeks	2 μg daily for 6 months
100 mg every 4 weeks	5 μg daily for 6 months
200–250 mg every 4 weeks	10 μg daily for 6 months
	15 μg daily for 6 months
Adult replacement	Progesterone
Adult dosage (250 mg every 2–4 weeks)	Started with the onset of breakthrough bleeding or when ethinylestrogen dosage is 15–20 μg
or	
Transdermal scrotal patches	*Adult replacement*
	Ethinylestradiol 20–30 μg daily with cyclic progesterone treatment
	Oral contraceptive pill
	Transdermal patches

tain further development. Patients with organic causes need lifelong sex steroid replacement, but treatment should allow the patient to progress at a normal rate. Suggested dosage schedules are shown in Table 10.8. Patients with Turner syndrome may require GH and/or oxandrolone treatment in addition to achieve adequate final height and bone mass.

Anorchic males and those with hypogonadotrophic states need to be informed about testicular prostheses for cosmetic and psychological reasons. This is best undertaken with silastic implants as a one-stage procedure in the middle to later stages of puberty, when the scrotum is large enough to accommodate the prostheses. Fertility options in both sexes include sperm or ova donation or adoption. Pregnancy rates of *in vitro* fertilization with ovum donation in girls with Turner syndrome have been reported at 20–25%, but adequate uterine growth during a slow induction of puberty plays an important role in success [155].

Pulsatile subcutaneous GnRH has been used successfully to induce puberty, with a pattern of administration to mimic normal puberty [156]. Recombinant FSH allows for testicular growth to be undertaken and possibly spermatogenesis, although the lack of the postnatal FSH surge may be a key factor in determining success in hypogonadotrophic hypogonadism [157].

As delay in puberty is often associated with short stature, it can lead to bullying in school. Although early treatment does not alter long-term effects on height or puberty, a delay in treatment can lead to difficulties in interpersonal relationships and disturbed psychosocial adjustment, especially during the period of delay. The outcome in untreated individuals is not different from those with a normal timing of puberty, but most volunteer that they would have preferred earlier intervention [158]. Poor bone mineralization as a result of suboptimal therapy may result in bone fractures and osteoporosis. There are some inconclusive data that delayed puberty can result in permanent loss of bone mass [159–161].

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11 Gynecology

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Introduction

Gynecological problems are not uncommon among children and adolescents. However, serious pathology is rare, and many conditions often need only explanation and reassurance. Careful and sensitive investigation will determine etiology and management. It is important not to neglect future issues such as sexual health and fertility even in the youngest child, as these will eventually assume great importance.

Gynecological input is becoming more important in children with other complex congenital anomalies. Improved neonatal and pediatric care and surgery mean that children who may not previously have survived into adult life now do so. They have the same sexual and reproductive expectations as their peers and deserve optimal gynecological input to achieve these goals.

Common gynecological problems prior to puberty

Vulvovaginitis

Persistent vulval irritation or vaginal discharge is the commonest reason for a gynecological referral in a prepubertal girl [1]. The peak age for presentation is between 3 and 7 years. Symptoms may have been present for several months or years and may be intermittent. Most children will be found to have non-specific bacterial infection, although a small number may have symptoms associated with a systemic illness. Sexual abuse can cause vaginal symptoms and must be considered.

Presentation is usually with a yellow-green offensive discharge and/or vaginal soreness and itching. Vaginal bleeding is not a typical feature of vulvovaginitis, although it can occur. If bleeding is present, careful evaluation is essential before assuming that vulvovaginitis is the main pathology. On inspection of the genital area in vulvovaginitis, the vulva typically has a red "flush" spreading toward the anus. The skin may be excoriated, and there may be a pool of discharge at the posterior fourchette. A low vaginal swab can be taken. Examination under anesthetic is not indicated unless a foreign body is suspected.

Table 11.1. Conservative treatment measures for vulvovaginitis.

Improved hygiene
Wipe from front to back
Avoid soaps/bubblebaths
Urinating with knees apart
Barrier creams, e.g. nappy cream
Cotton underwear
Bioyogurts/probiotics

Non-specific vulvovaginitis is thought to be due to poor perineal hygiene combined with a lack of estrogen. The anatomical appearance of the prepubertal vulva also contributes, with small, flattened labia and close proximity of the vagina and anus. Chemical irritation due to bubblebaths and detergents exacerbates the condition. Vaginal swabs may be negative but may grow coliforms and group A streptococcus [2]. Candida is rarely found in otherwise healthy children.

The mainstay of treatment is conservative with improved vulval hygiene and avoidance of soaps (Table 11.1). If the swab is positive, broad-spectrum antibiotics may help temporarily, but recurrence is common. Symptoms resolve as the child approaches puberty.

The presence of a foreign body is not as common as is widely assumed, occurring in only 4% of girls with genital symptoms without vaginal bleeding [3]. Vaginal bleeding or a persistent foul-smelling discharge refractory to treatment should raise suspicions of a foreign body. The child may also admit insertion of a foreign body and, in this situation, an examination under anesthetic is necessary. Imaging is not usually diagnostic, although it may be suggestive (Fig 11.1).

Other systemic infections can cause vaginal discharge and soreness (Table 11.2). Symptoms usually clear with the

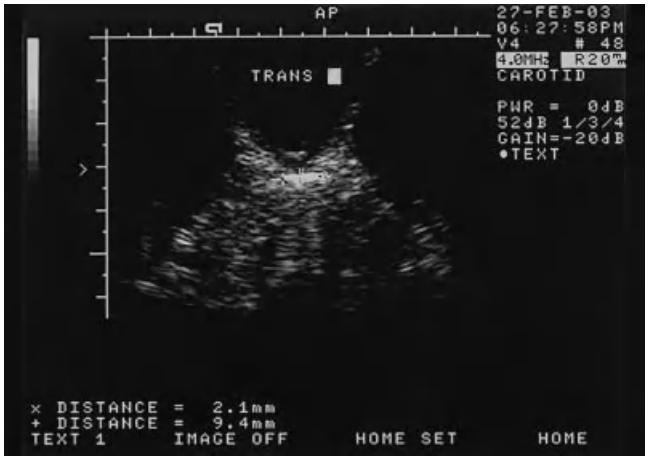

Fig. 11.1. Ultrasound of foreign body in the vagina in a 5-year-old girl. At vaginoscopy, a small charm from a bracelet was found and removed.

Table 11.2. Systemic causes of vulvovaginitis.

Respiratory pathogens, e.g. group A β-hemolytic streptococcus, *Streptococcus pneumoniae*
Gastrointestinal pathogens, e.g. *Shigella* spp., *Yersinia enterocolitica*
Varicella
Measles (Koplik's spots)
Rubella
Diphtheria
Leukemia
Crohn's disease
Behçet syndrome

resolution of the acute systemic illness. Threadworms cause anal and vaginal itching. Another less common cause of persistent watery discharge is an ectopic ureter, and a renal ultrasound may show a duplex kidney. Persistent symptoms must raise the possibility of sexual abuse. The detection of organisms usually transmitted by sexual intercourse, such as *Neisseria gonorrheae*, *Chlamydia*, or herpesvirus, or genital warts, means appropriate referral is mandatory. Other organisms such as *Gardnerella* have been associated with sexual abuse but can also occur in its absence [4].

Labial adhesion

Labial adhesion or fusion is estimated to occur in 3% of prepubertal girls [5]. The peak incidence is in the first year of life, but it is never present at birth. The appearance is typical, with fusion of the labial skin extending from the posterior fourchette toward the urethral opening. A thin membranous line in the midline where the tissues fuse is clearly visible. The urethra may be a pinhole opening in extensive fusion. The etiology is unknown, but lack of estrogen is probably contributory. Labial fusion has been seen in association with premature thelarche, which would not support this theory [6]. Mild vulvitis may make fusion more likely.

Most children are asymptomatic. If symptoms do occur, they are usually urinary. Urine may pool behind the vaginal adhesions and cause post-micturition dribble, soreness, and vulval irritation. Parents may be anxious about the possibility of an absent vagina, but the appearance is typical, and the diagnosis can be made on examination. No investigations are indicated, although the ultrasound demonstration of a uterus may be reassuring. Spontaneous resolution is common, and treatment is not necessary in the asymptomatic child. If the child is symptomatic, treatment is with estrogen cream applied topically to the midline fusion for no longer than 6 weeks. The labia will buttonhole, then separate. Recurrence after discontinuing estrogen is common. A small amount of estrogen is absorbed systemically through the vagina, and breast swelling, breast tenderness, and vaginal spotting have been reported. Surgical separation is rarely needed unless urinary symptoms are persistent and estrogen therapy has failed. Recurrence is common even after surgery and can lead to children having multiple surgical procedures (see Plate 12, facing p. 148).

Adolescent gynecology

Common problems

Menstrual dysfunction

Menstrual disorders in adolescent girls are thought to be common, but the incidence is unknown as teenagers are loath to present due to embarrassment as well as lack of knowledge of what is normal and of potential treatment options. Presentation with a menstrual disorder may disguise other worries such as contraception, sexually transmitted infection, or pregnancy. Vaginal examination should be performed only in consenting adolescents who are sexually active and only when it is likely to add value to the assessment.

Menorrhagia

Troublesome periods may be too frequent, irregular, and/or heavy. A menstrual diary may be helpful for reassurance and explanation of the extent of normal variation. In most girls in the early months following menarche, the commonest cause is anovulatory cycles. The menstrual cycle then becomes irregular, and late periods may be prolonged. Cystic glandular hyperplasia of the endometrium has been reported, but the incidence is rare, perhaps because early intervention now is more common [7]. These symptoms can be regarded as normal for the first 2 years while the hypothalamic–pituitary–ovarian axis matures to establish regular cycles. A very small number of girls require hospital admission with severe and profuse bleeding causing cardiovascular compromise and severe anemia.

Acquired and congenital bleeding disorders are relatively common causes of menorrhagia and may account for 10–15%

Table 11.3. Contraindications to the use of oral contraceptives.

Pregnancy
Prior or current arterial or venous thrombosis
Ischemic heart disease
Migraine
Diabetes mellitus (relative contraindication)
Liver disease
Hypertension
Collagen vascular diseases, e.g. systemic lupus erythematosus

of cases [8]. Conditions such as Von Willebrand disease and immune thrombocytopenic purpura should be excluded in any girl with severe menorrhagia refractory to simple treatments [9]. An ultrasound scan is usually requested, although it rarely shows pathology.

Treatments include the use of antifibrinolytic drugs such as tranexamic acid, which is effective in reducing blood loss but does not make cycles more regular [10]. Cyclical progesterones are widely used, although their efficacy is poorly established. In order to be effective, progesterone needs to be given for 21 days each month rather than just during the luteal phase, as has been traditional. Disadvantages can include acne and hirsutism with norethisterone, which may interfere with compliance. The mainstay of treatment is the combined oral contraceptive pill, which reduces blood loss and regulates the cycle length. The contraindications to their use are shown in Table 11.3.

Dysmenorrhea

Pain during menstruation may have a significant impact on schooling and examination performance. Early periods may often be pain free, and the advent of pain usually coincides with the establishment of regular ovulatory cycles. Pain is attributed to higher levels of prostaglandins, and so anti-prostaglandin drugs such as mefenamic acid can be helpful. Suppression of ovulation with the combined oral contraceptive pill is very effective.

Girls who fail to respond to these measures need further evaluation. Dysmenorrhea can be associated with obstruction of the lower genital tract. A double uterus may have one uterine horn that is non-communicating and becomes obstructed causing worsening pain each month. The girl is usually found to have a large pelvic mass clinically and on ultrasound. The anatomy may be complex, and pelvic magnetic resonance imaging (MRI) is usually required before planning treatment [11]. Treatment is surgical, but removal of an obstructed horn can usually be accomplished laparoscopically (see Plate 13, facing p. 148).

Endometriosis is a recognized cause of dysmenorrhea and is not a condition restricted to adult women. Some 38% of adolescents presenting with chronic pelvic pain have endometriosis [12]. If pelvic pain is refractory to non-steroidal anti-inflammatory drugs and the oral contraceptive, a diagnostic laparoscopy is indicated. Treatment options are currently as for adult women with a combination of surgical and drug treatment. Psychological support and contact with similarly affected adolescents is though to be of benefit [13].

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is common and is diagnosed with increasing frequency in adolescent girls. Polycystic ovaries are found in about 25% of normal women and are usually asymptomatic but PCOS, which includes hyperandrogenicity, can be responsible for irregular cycles and episodes of secondary (or rarely primary) amenorrhea [14]. Sometimes, but not always, there will be accompanying weight gain, acne, and hirsutism. Classically, the luteinizing hormone (LH) will be higher than follicle-stimulating hormone (FSH), with a 3:1 ratio. Testosterone may be at the higher end of normal and, in such a situation, it is worth checking the concentration of 17-hydroxyprogesterone to exclude congenital adrenal hyperplasia. Estrogen values are normal. Ultrasound shows a densely thickened ovarian stroma, with a "chain" of follicles around the edge of the ovary.

Treatment of PCOS is not simple but, at least in adolescence, fertility is not the prime object. In overweight teenagers, weight loss and exercise must be addressed. A change in lifestyle may be all that is needed for symptom improvement. Long-term associations such as diabetes are more common in the obese, and acanthosis nigricans may be a pointer to this. Other treatments depend upon the most troublesome symptoms. Periods may be regulated with the oral contraceptive pill. All combined pills help with skin problems to some extent, but the addition of an anti-androgen is particularly helpful with hirsutism. Yasmin, a newer combined contraceptive pill containing ethinylestradiol and drospirenone, is also useful. Metformin is used in PCOS to increase insulin sensitivity. Several studies have confirmed its efficacy in menstrual regulation and fertility. Metformin is most effective in obese women, but there is little information on its use in adolescent girls. It is appropriate for those unable or unwilling to take the oral contraceptive pill.

Contraception

Young women are starting sexual activity at a younger age. Early sexual activity and frequent partner changes mean a high risk of pregnancy and sexually transmitted infections. One in four adolescents under 16 years of age claim to be sexually active but, in the UK, only 50% of adolescents aged under 16 years use contraception at first intercourse.

The legal age of consent for sex in England is 16 years, but it is legal to prescribe contraception for a child under 16 years if they are deemed "Gillick competent," that is they are judged

able to give informed consent. Adequate and appropriate sex education has been identified as a key factor in reducing early sexual activity, but sex education in schools is often patchy, and teenagers fail to access contraceptive services due to ignorance, confusion, and embarrassment.

All young women requesting contraception need full information on the range of options. This must be done in easily understandable terminology with written information where possible. Routine vaginal examinations and cervical smears are unnecessary in the early stages of contraceptive use and may deter teenagers from attending a clinic.

Condoms are often chosen as they are easy to obtain, but failures are common, which may be due to the unplanned nature of teenage sexual activity, to the use of drugs and/or alcohol, and to lack of instruction given to young men on the use of condoms.

The combined oral contraceptive pill is the most commonly used method as it is effective and simple to use. The beneficial effects on periods and skin make it additionally appealing to young girls. The main disadvantage is the lack of protection against sexually transmitted infections such as chlamydia. The "double Dutch" approach of condoms with a hormonal method should be recommended to all adolescents.

In those teenagers who find it impossible to remember to take their pill, longer acting methods, such as injectables and implants, are appropriate (Depo-Provera, Implanon). The longer duration of action means less reliance on a daily routine. Cycle irregularities are common during the first few months of use, although amenorrhea is common with prolonged use. Depo-Provera has been associated with a reduction in bone mineral density, although there is no evidence as yet that it increases the risk of osteoporosis in later life. Depo-Provera is still appropriate if other methods are unsuitable but use should be reviewed after 2 years.

Contraceptive services for adolescents should be easily accessible and user friendly. As well as providing contraception, they should have a major role in health education in an attempt to reduce teenage pregnancy and sexually transmitted infections such as chlamydia, which may damage future reproductive health.

Congenital anomalies of the lower genital tract

Primary amenorrhea in the presence of normal secondary sexual characteristics (including pubic hair) is most commonly caused by anatomical anomalies such as an absent uterus or vaginal obstruction.

Imperforate vagina

Obstruction to the vagina can prevent the escape of menstrual flow. This causes worsening cyclical pain and a pelvic mass due to hematocolpos. The obstruction most commonly occurs at the junction of the lower third of the vagina, at the level of the hymen. It may be possible to visualize a bulging hymenal membrane, once the labia are gently parted. Ultrasound can confirm the diagnosis. Resection of the obstructing membrane will release the blockage and allow further normal menstruation.

In very rare cases, the obstruction may be due to a transverse vaginal septum. This may be an isolated anomaly, although it has been described as part of McKusick–Kaufman syndrome [15]. If the septum is thin and low, it may be possible to resect from below, but care must be take to insure the whole septum is removed to prevent contracture. If the septum is thicker and higher in the vagina, a combined abdominal and perineal approach is needed. It may be possible to remove the septum and anastamose the proximal and distal vagina, but sometimes the distance is too great and must be bridged by a skin graft or section of bowel.

Rokitansky syndrome

Approximately 1 in 5000 girls are born with congenital absence of the uterus and some or all of the vagina [16], the Rokitansky or Mayer–Rokitansky–Kuster–Hauser (MRKH) syndrome. The clinical spectrum is poorly documented, but patients may frequently have other developmental disorders outside the genital tract. Associated congenital anomalies of the upper urinary tract, which include ectopic kidney, renal agenesis, horseshoe kidney, and abnormal collecting ducts, occur in 30–40% of all cases. Some 12% of cases have skeletal anomalies, mostly involving the spine [17]. Some fit into other known syndromes such as Müllerian–renal–cervicothoracic somite (MURCS) association, McCusick–Kaufman syndrome, Bardet–Biedl syndrome, Fraser syndrome, and Klippel–Feil syndrome.

There is little agreement about the etiology, clinical syndromes, and natural history of this syndrome. Its pathogenesis is unknown, and it is unclear whether normal vaginal and Müllerian structures failed to develop, whether development was arrested at some point, or whether destruction of developed structures occurred, e.g. through inappropriate apoptosis. The embryological development of the vagina and Müllerian structures is poorly understood, especially with regard to the proportion of the vagina derived from the urogenital sinus or the Müllerian duct. This leads to difficulty predicting etiological factors in the abnormal development of these structures. At present, there are no well-recognized etiologies for Rokitansky syndrome, although possible culprits include environmental, genetic, hormonal, or receptor factors.

The most common presentation is with primary amenorrhea in the presence of otherwise normal pubertal development. The function of the hypothalamo-pituitary–ovarian axis is normal: FSH, LH and estrogen levels are normal, and the karyotype is 46XX. On examination, the vagina will be blind

ending and is likely to be short. Ultrasound will confirm the presence of ovaries, but no uterus will be demonstrated. Pelvic ultrasound is not easy in children, and it is not uncommon for a scan to report vestigial uterine tissue with no endometrium when the uterus is actually absent. MRI may add more information. There is no indication for routine laparoscopy or ovarian biopsy in this group of patients.

Treatment options focus on psychology and on the creation of a vagina comfortable for penetrative intercourse. There is currently no treatment available to transplant or create a uterus. Uterine transplants have been carried out successfully in mice [18], but occasional attempts in humans have ended in disaster [19]. Tissue engineering may be an answer in the future with growth of bladder and penis already reported [20]. Women with MRKH syndrome may have their own genetic children, using retrieval of ova, assisted conception techniques, and a surrogate mother. Such a pathway is set with difficulties and may prove to be too costly in financial and emotional terms for many women.

The discovery of an absent vagina or uterus is a devastating event for a teenage girl and her family. There are few data available looking at the psychological impact of a diagnosis of Rokitansky syndrome. Input from a clinical psychologist experienced in the area is essential. The treatment of choice in creating a vagina sufficient for intercourse consists of using plastic dilator moulds to stretch the vaginal area (see Plate 14, facing p. 148). This is thought to be successful in about 85% of women, although there is little information on compliance or subsequent sexual function. Ideally, dilation should be practiced at least once daily for 3–6 months in order to optimize success. At present, there have been no prospective studies of the impact of vaginal hypoplasia interventions on sexual function. In cases where dilators are unsuccessful or where compliance is difficult, newer techniques such as the laparoscopic Vecchietti [21] or Davidov [22] procedures may be more acceptable and equally as effective as the traditional vaginoplasty with skin grafting or intestinal replacement.

Complete androgen insensitivity syndrome (CAIS)

Intersex conditions such as CAIS may also present with primary amenorrhea in the presence of normal breast development.

The gynecological management of CAIS focuses on psychological support and treatments for vaginal hypoplasia. The psychological aspects of living with CAIS are paramount to successful management. There are few long-term data, but we know that the initial strong reactions of shock, anger, grief, and shame can persist for many years in affected individuals and their families [23].

Once a diagnosis of androgen insensitivity is confirmed, gonadectomy is often performed. The rationale behind this is the small (2–5%) risk of malignancy in the testes [24]. However, delaying gonadectomy until later in life will allow spontaneous puberty without the need for exogenous estrogen administration. Delaying gonadectomy until after puberty may also lead to improved adult bone density, although there are few data to support this. If the testes are retained, assiduous follow-up is necessary although monitoring is difficult. Clinical palpation is possible only if the gonads are in the groin or inguinal canal. Ultrasound is not accurate enough reliably to detect early malignant changes.

Vaginal development in CAIS is variable, but vaginal hypoplasia is common. The vagina is composed of a lower urogenital portion and an upper portion derived from Müllerian structures. Owing to the effects of anti-Müllerian hormone, the Müllerian portion of the vagina will not develop. Normal vaginal length in the general population has been reported as a mean of 11.1 cm [25], whereas vaginal lengths within a CAIS population have been reported to range from 2 to 11 cm [26]. There is a high prevalence of sexual dysfunction within women with CAIS [26]. Clearly, psychological factors are important, but physical factors such as a short vagina also contribute. Treatments – as for Rokitansky syndrome – concentrate mainly on vaginal self-dilation to increase vaginal length. Small retrospective studies have shown this to be a beneficial approach [27], but there are no prospective studies on this treatment and its impact on sexual function. The advantages of this method are that risks and side-effects of treatment are low, and the patient is in charge of her own treatment. However, the time taken to create a vagina can be several months, and some women find the dilators difficult and unpleasant to use. As described above for Rokitansky syndrome, there are several surgical options available. Outcome studies are scarce, and there is little information about the comparative risks and benefits of the various techniques available.

Congenital adrenal hyperplasia (CAH)

CAH causes virilization of the external genitalia. The degree of virilization varies from mild clitoromegaly to complete fusion of the labial folds with a prominent phallus. Standard practice is to perform corrective clitoral and vaginal surgery during the first year of life. A feminizing genitoplasty consists of clitoral, vaginal, and labial surgery, with the aim of reducing the size of the clitoris, opening the vaginal introitus, and achieving a feminine appearance.

Clitoral reduction is the procedure that is currently practiced by pediatric surgeons. It involves removal of part of the erectile tissue with preservation of the glans and dorsal neurovascular bundle. Clitorectomy, the complete removal of the paired corpora and the glans, is no longer performed in the UK, although it may have been carried out as recently as 10 years ago and is still standard practice at some European centers [28]. Many adult women will have had clitorectomy performed as children.

Vaginal surgery may be performed in different ways depending upon the degree of virilization. In mild cases where mainly labial fusion is present, a simple inverted U-shaped incision is made over the perineal body, and the underlying tissue is divided. The skin may then be laid down to refashion the posterior fourchette. Flap vaginoplasties may be performed where the vagina joins the urethra in an intermediate position. Perineal skin is used to reconstruct the posterior fourchette, but the anterior vagina is not repositioned. In more severe cases, where the vagina joins the urethra in a higher position, a "pull-through" procedure is performed. The upper vagina is mobilized and brought down to meet the newly created introitus.

Vaginal stenosis can occur with the formation of scar tissue, following the original procedure (see Plate 15, facing p. 148). It is important to examine the vagina around the time of puberty to insure there is a passage for menstrual flow. Further surgery is required in up to 80% of cases [29] to allow menstrual flow, the use of tampons, and subsequent sexual intercourse.

Recent challenges

Although the above management has been accepted for 50 years, it has become increasingly apparent that some adult women are unhappy with the outcome of their surgical treatment and particularly with its effects on sexual function. Some also bitterly resent that their parents were asked to make decisions without being clear of the potential risks. Active peer support groups have contributed to the medical debate, and several groups have called for a moratorium on all childhood cosmetic genital surgery [30].

This has posed a major challenge for pediatricians and pediatric surgeons, and has prompted a major rethink on the role and timing of feminizing genitoplasty surgery.

The rationale behind surgery was based on an "optimal gender policy" [31]. This involved establishing the endocrinological diagnosis and commencing treatment as soon as possible. An important part of the management involved surgical correction of the genitalia in order to fit the sex of rearing. It was argued that this should be performed as soon as possible in order to minimize the period of gender uncertainty and to allow the parents to establish and reinforce the gender identity. There is however little in the literature to confirm the success or failure of this approach.

Long-term outcomes

Long-term outcomes following feminizing genitoplasty include cosmetic appearance, ability to allow menstrual flow, and sexual function. Although the majority of corrective surgery is performed by pediatric surgeons, these outcomes may not be fully assessed until adulthood is reached. Available studies report disappointing results with high incidences of vaginal stenosis requiring revision surgery [32,33]. The effects of surgery on future sexual function are unclear. Little objective data are available in the literature. Dyspareunia, vaginismus, and anorgasmia are rarely used as outcome measures when assessing long-term surgical outcomes, although all are prevalent in the normal female population. The clitoris is the most densely innervated structure in the human body, and surgery to this area may disrupt the neurovascular network. Recent work has indicated a higher prevalence of anorgasmia following clitoral surgery compared with controls [34]. In addition, clitoral surgery causes significant impairment of genital sensation [35]. If steroid suppression therapy is poor, the clitoris may regrow, despite surgical reduction. In one study, regrowth occurred in 39% of patients [29] with many of these children undergoing multiple further operations to the clitoris. It seems likely that repeated surgery may be more damaging to sexual function than one procedure.

Decision making

The decision of the parents will understandably be influenced by the advice given them by the medical team. Parents must be clear that the surgery is cosmetic and not life-saving, that if procedures are carried out in infancy further operative procedures may be needed in adolescence, and that the damage to future sexual function remains unquantified. There should always be the option to defer surgery until the child is old enough to be part of the decision-making process. These decisions about surgery are difficult and require early assessment by a multidisciplinary intersex team comprising pediatric surgeons or urologists, endocrinologists, gynecologists, and psychologists who are experienced in caring for babies and children with CAH. In most cases, vaginoplasty can be deferred until after puberty, which allows the patient input into the decision-making process. The implications of clitoral surgery must be carefully discussed, and parents should be clear that there is an option of not having surgery, especially in cases of mild or moderate clitoromegaly. More data are needed to evaluate sexual function in the long-term following surgical procedures.

Conclusion

A careful and sensitive approach is needed when evaluating children with gynecological symptoms. It must always be borne in mind that treatment options – particularly surgery – may have a significant impact on future sexual function and fertility. Parents must be given all available information, and the child must be involved in the decision-making process as soon as she is old enough.

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12 The thyroid and its disorders

Rosalind S. Brown and Stephen Huang

Introduction

Thyroid dysfunction in infancy and childhood results in the metabolic abnormalities found in the adult and also affects growth and development. Because these thyroid hormone-dependent effects on tissue maturation are developmentally regulated and organ or tissue specific, the clinical consequences of thyroid dysfunction depend on the age of the infant or child.

Untreated hypothyroidism in the fetus or newborn infant results in permanent abnormalities in intellectual and/or neurological function, reflecting the pivotal role of thyroid hormone on brain development at this time of life. After the age of 3 years, when most thyroid hormone-dependent brain development is complete, hypothyroidism results in slow growth and delayed skeletal maturation, but there usually is no permanent influence on cognitive or neurological development.

Thyroid hormonogenesis

The thyroid is composed of follicles that secrete thyroid hormone. They are composed of two types of cells that surround a central core of colloid. Thyroid hormone-secreting follicular cells, the major cellular constituent of the follicle, are interspersed with calcitonin-secreting parafollicular C cells, which are of neurogenic origin. A basal membrane surrounds the follicle and separates it from surrounding blood and lymphatic vessels as well as nerve terminals. The major constituent of the colloid is thyroglobulin (Tg), a very large iodinated, dimeric glycoprotein that functions as a thyroid hormone precursor and permits storage of iodine and of iodinated tyrosyl residues covalently bound within its protein structure.

The synthesis and secretion of thyroid hormone includes a complex series of events, each proceeding simultaneously in the same cell (Fig. 12.1) [1]. Dietary iodine, I_2, is converted to iodide in the gut and concentrated 20–40 times in the thyroid by an active transport mechanism involving the Na^+/I^- symporter (NIS), located within the basal plasma membrane. At the apical border, iodide transport into the lumen is facilitated by a newly recognized anion transporter encoded by pendrin (PDS), a gene on chromosome 7q22–31 with sequence homology to several sulfate transporters [2].

At the same time, Tg, synthesized within the follicular cell, undergoes a number of post-translational steps to attain the proper tertiary and quaternary structure. These steps include glycosylation and folding, the latter with the aid of chaperone molecules. Tg is transported by exocytosis into the follicular lumen (colloid). Here, at the colloid–apical cell membrane interface, Tg forms the backbone for a series of reactions that result in the oxidation of I_2 to an active intermediate and the iodination of tyrosyl residues ("organification") to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). Iodide oxidation and organification are both catalyzed by thyroid peroxidase (TPO), a key membrane-bound, glycosylated hemoprotein enzyme. TPO also catalyzes the coupling of iodotyrosines within the Tg molecule to form the thyroid hormones, triiodothyronine (T_3) and tetraiodothyronine or thyroxine (T_4). T_3 is formed by the coupling of one DIT and one MIT molecule; the coupling of two molecules of DIT results in T_4. Iodination also requires hydrogen peroxide, the generation of which is regulated in part by two recently identified enzymes, thyroid oxidase (THOX)1 and THOX2, which are inserted in the apical membrane of the thyroid follicular cell [3].

Thyroid hormones, stored in the colloid, are released into the circulation by a series of steps that result initially in their incorporation into the apical surface of the follicular cell by a process known as endocytosis. The ingested colloid droplets fuse with apically streaming proteolytic enzyme-containing lysosomes to form phagolysosomes, in which Tg hydrolysis occurs. The free MIT, DIT, T_3, and T_4 within the phagolysosomes are then released into the follicular cells. T_3 and T_4 released in this way diffuse from the thyroid follicular cell into the thyroid capillary blood. The released MIT and DIT

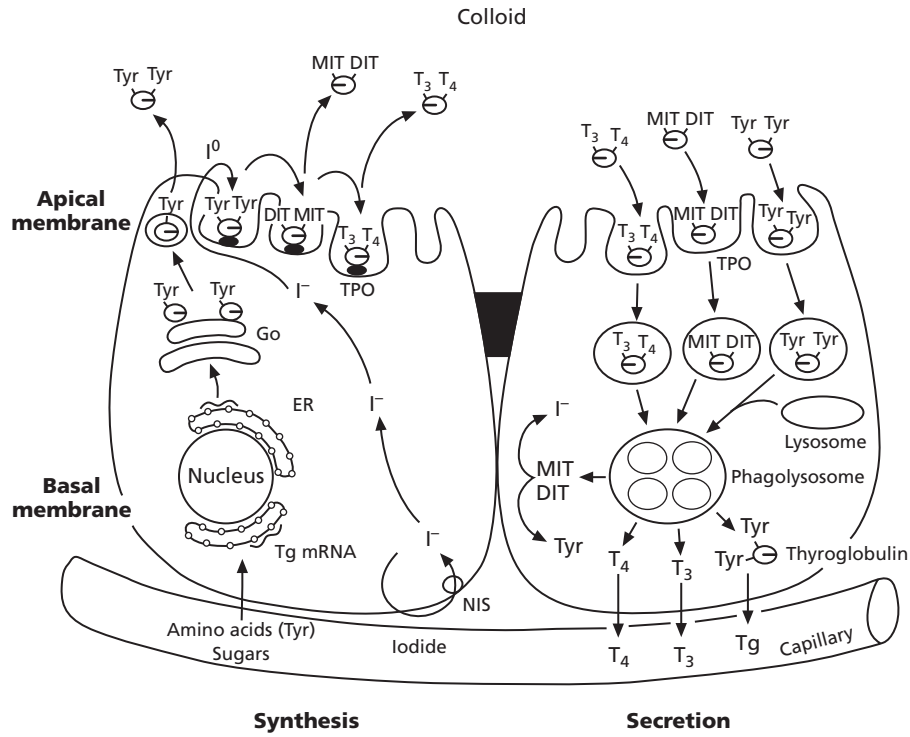


Fig. 12.1. Synthesis (left) and secretion (right) of thyroid hormones in thyroid follicular cells. These processes, which proceed simultaneously in the same cell, are diagrammed separately for clarity. Iodide (I^-), amino acids (tyrosine, Tyr, and others), and sugars, concentrated by follicular cells, are assembled into thyroglobulin (Tg), packaged into apical vesicles, and released into the lumen. At the apical membrane, Tyr residues on the Tg backbone (⊖) interact with reactive iodine (I^-) species to form the mono- and di-iodotyrosines MIT and DIT, a reaction catalyzed by thyroid peroxidase (TPO). MIT and DIT couple to form triiodothyronine (T_3) and thyroxine (T_4). These products are stored in extracellular colloid. Secretion involves invagination and the formation of intracellular colloid droplets, which fuse with enzyme-laden lysosomes to form phagolysosomes. Here, Tg is hydrolyzed to release MIT, DIT, T_3, and T_4. MIT and DIT are deiodinated and the iodide reutilized; T_3 and T_4 are released into the circulation. Not pictured are the pendrin gene, PDS, an apical I^- transporter, and THOX1 and THOX2, apical membrane-associated enzymes important in peroxidase generation. NIS, Na^+/I^- symporter; ER, endoplasmic reticulum; Go, Golgi apparatus (reprinted from [1]; see text for details).

are largely deiodinated by a deiodinase, the iodide re-entering the intracellular iodide pool to be reutilized for new hormone synthesis. Deiodination of T_4 to generate T_3 is a second source of T_3 within the thyroid.

Cloning of the genes for Tg, TPO, NIS, PDS, and, most recently, THOX1 and THOX2 has permitted a greater understanding of the specific events involved in thyroid hormonogenesis and of their regulation at both a molecular and a cell biological level. In addition, cloning of these genes has elucidated the molecular basis for many of the inborn errors of thyroid hormonogenesis discussed later in this chapter. Tg, TPO, and NIS also serve as targets of immune attack in patients with autoimmune thyroid disease. In view of the location of TPO and Tg in the interior of the cell, these proteins are unlikely to be the primary trigger of immune attack but are accessible to the immune system only after the cell has been injured.

Regulation of thyroid function

Thyrotrophin (TSH)

The major regulator of thyroid function is thyrotrophin (TSH), a glycoprotein hormone secreted by the pituitary gland. Like other pituitary glycoprotein hormones with which TSH shares structural homology, the TSH molecule is composed of a common α-subunit and a TSH-specific β-subunit. TSH stimulates both thyroid gland function and growth by binding to a specific receptor located on the basal plasma membrane [4]. The TSH receptor, which is a member of the subgroup 2, G-protein-coupled receptor superfamily, is composed of a large, extracellular domain, seven hydrophobic transmembrane-spanning regions, and a short intracytoplasmic tail (Fig. 12.2). The N-terminal extracellular domain appears to be sufficient for binding of hormone, whereas the cytoplasmic loops and C-terminal tail are important in signal transduction.

Through effects mediated primarily by the cyclic adenosine monophosphate (cAMP) signal transduction pathway, TSH exhibits transcriptional control of the genes for Tg, TPO, and NIS, and stimulates an array of cellular events, including iodine uptake and organification, as well as thyroid hormone synthesis and secretion. TSH also stimulates follicular cell proliferation and growth. Although the effects of TSH are mediated primarily through the adenyl cyclase–protein kinase A signal transduction pathway, at higher concentrations, TSH also stimulates the phosphoinositol–protein kinase C pathway.

In view of the pivotal importance of the TSH receptor in regulating thyroid function, it is not surprising that both germline and somatic mutations can lead to abnormalities

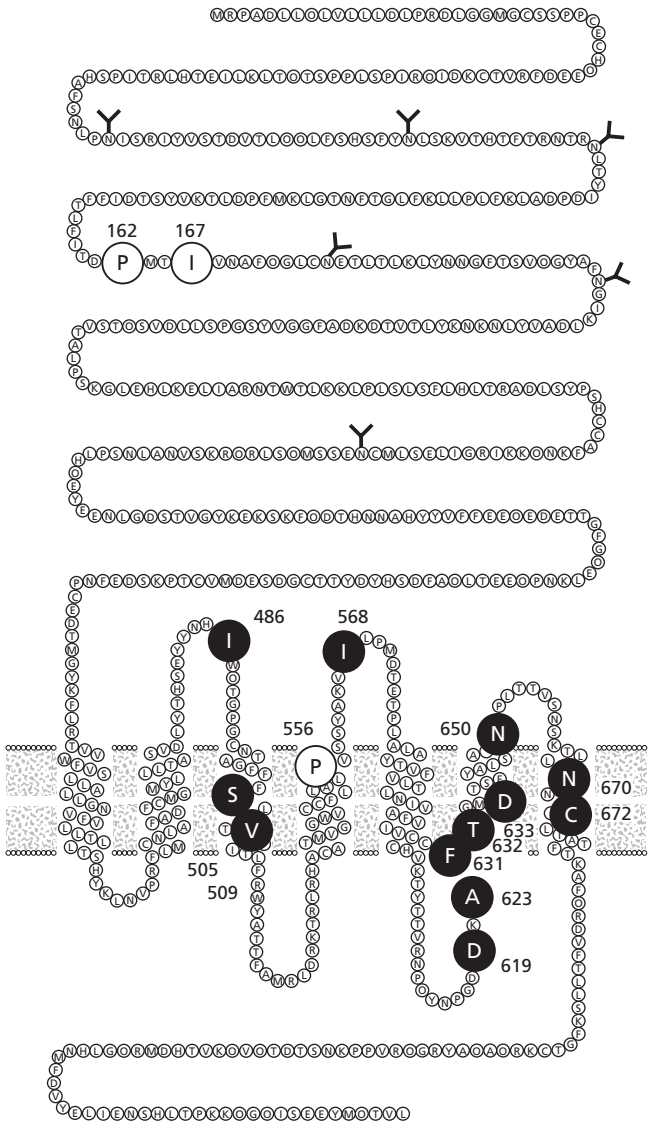


Fig. 12.2. Schematic representation of the human TSH receptor and the site of some disease-causing mutations. Like other members of the subgroup 2, G-protein-coupled receptor superfamily, the TSH receptor is composed of a large, extracellular domain, seven hydrophobic transmembrane-spanning regions, and a short intracytoplasmic tail. The white symbols refer to loss-of-function mutations while the black symbols refer to gain-of-function mutations. Note the transmembrane location of the loss-of-function Pro556Leu mutation in the hyt/hyt mouse (from [5] with permission).

of thyroid growth and function in patients (Fig. 12.2) [5]. In addition, unlike Tg and TPO, the TSH receptor is accessible to immune attack as it is located not in the interior of the cell but at the basal plasma membrane adjacent to both the blood and lymphatic vessels. Thus, both stimulatory and blocking TSH receptor antibodies (Abs) may occur in patients and result in stimulation and/or inhibition of TSH-induced thyroid cell growth and function.

The secretion of TSH by the pituitary gland is under positive feedback control by hypothalamic TSH-releasing hormone (TRH), a small tripeptide synthesized in the hypothalamus and transported to the pituitary via the pituitary portal vascular system. TSH secretion is under negative feedback control by thyroid hormone, the latter acting at the level of both the hypothalamus and the pituitary gland. Dopamine, somatostatin, and high doses of corticosteroids also inhibit pituitary release of TSH. Decreasing environmental and/or body temperature increases TRH release.

Iodide

Adequacy of dietary iodine is a critical regulator of thyroid gland function through adaptive mechanisms that respond to both its deficiency and its excess. This is understandable because the major thyroid hormones T_4 and T_3 are 65% and 59% iodine by weight respectively. The normal daily requirement of iodine is 150 μg for adults, 90 μg for infants and children, 40 μg for premature infants, and 200 μg for pregnant women.

In iodine deficiency, there is increased trapping of iodide by the thyroid gland as a result of both TSH-independent and TSH-dependent mechanisms. In addition, increased TSH secretion results in a stimulation of thyrocyte proliferation and hormonogenesis. Tg secretion is increased but, because of the reduced iodine content, there is preferential synthesis and secretion of the less iodinated compounds MIT and T_3 compared with DIT and T_4. Iodine deficiency also results in an increased peripheral conversion of T_4 to T_3. The reverse is true in the presence of iodine excess.

Excess iodine inhibits a number of different steps in thyroid hormonogenesis, including organification of iodide and subsequent hormone synthesis (the Wolff–Chaikoff effect), Tg synthesis, hormone release, and thyroid growth. Fortunately, under normal circumstances, the iodide-induced inhibition is transient, and normal hormone synthesis resumes (adaptation to or escape from the Wolff–Chaikoff effect). The escape from the Wolff–Chaikoff effect appears to be due, at least in part, to a decrease in NIS mRNA and protein expression, with a resultant decreased iodide transport into the thyroid [6]. This adaptation lowers the intrathyroidal iodine content below a critical inhibitory threshold, allowing organification of iodide to resume.

Other

A wide variety of other extracellular stimulatory signals have also been shown to bind to thyroid membranes and affect thyroid function and/or growth *in vitro*, but their importance *in vivo* is not yet known. These include adrenergic agents, growth factors such as insulin-like growth factor (IGF)-1 and epidermal growth factor (EGF), and purinergic agents. It is of particular interest that thyroid cells contain thyroid hormone

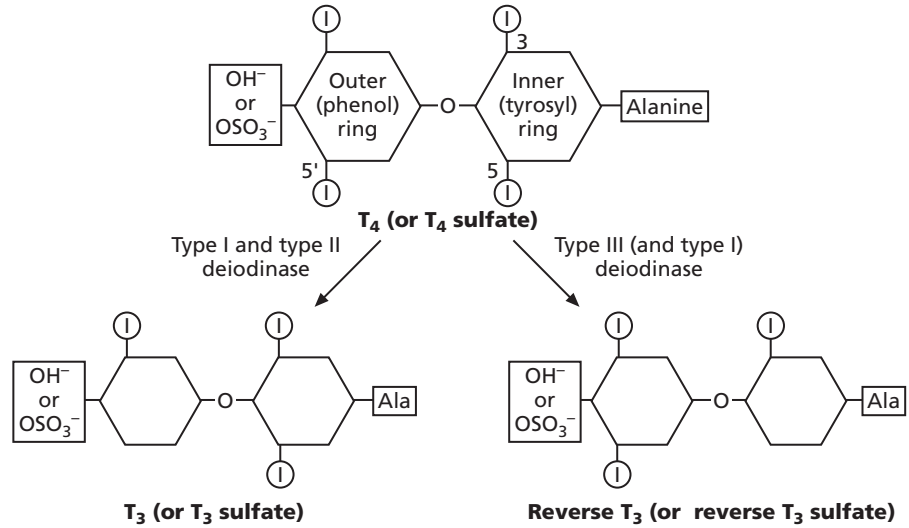


Fig. 12.3. Structure of the major thyroid hormones and the action of the monoiodothyronine deiodinase enzymes. The type I and type II deiodinases deiodinate the outer (phenol) ring, while the type III (and type I) deiodinases deiodinate the inner (tyrosyl) ring. See text for details.

receptors so that thyroid hormone itself could function as a regulator of thyroid function by a short-loop feedback mechanism. In addition, cytokines, produced by infiltrating lymphocytes in patients with autoimmune thyroid disease, and even by the thyroid follicular cells themselves can directly modulate both thyroid function and growth.

Thyroid hormone transport

T_4 and T_3 released into the circulation are transported to their target cells in non-covalent linkage with carrier proteins [7]. These binding proteins, produced in the liver, include thyroxine-binding globulin (TBG), transthyretin, and the secondary carrier protein, albumin. TBG, although the least abundant, is the most important carrier protein for T_4. Transthyretin binds T_4 but not T_3 and appears to play a role in T_4 transport into the brain. In the euthyroid steady state, almost all circulating thyroid hormone is bound to protein. This is especially true for T_4, 99.97% of which is bound, compared with 99.7% of T_3. Transport proteins function as an extrathyroidal storage pool of thyroid hormone that enables the release of free hormone on demand while at the same time protecting tissues from excessive hormone. However, they are not essential for normal thyroid function. Thus, the importance of thyroid hormone-binding proteins clinically lies in an appreciation of how abnormalities, whether secondary to genetic defects, drugs, or illness, may impact on the assessment of thyroid function.

Thyroid hormone metabolism

Thyroid hormone synthesized and secreted by the thyroid gland is both activated and inactivated primarily by a series of monodeiodination steps in target tissues. Sulfation is an additional method of thyroid hormone metabolism of particular importance in the fetus. In contrast to T_4, the sole source of which is the thyroid gland, only 20% of T_3 is derived by coupling of tyrosyl residues within the thyroid gland itself. The remainder (approximately 80%) of T_3 is derived from the peripheral conversion of T_4 to T_3 in the peripheral tissues, primarily the liver, kidney, brain, and pituitary gland.

T_4 and T_3 are thyronine molecules that consist of an inner (tyrosyl or α) ring and outer (phenolic or β) ring (Fig. 12.3). Monodeiodination of the outer ring of T_4 results in T_3, which is three or four times more metabolically active than T_4 *in vivo*. Monodeiodination of the inner ring produces reverse T_3 (rT_3), a metabolically inactive metabolite. Nearly all rT_3 (almost 98%) is derived from peripheral conversion and only 2% from the thyroid gland. Progressive tissue monodeiodination results in a series of diiodinated, monoiodinated, and non-iodinated forms of thyronine, all of which are metabolically inactive.

Three selenoprotein iodothyronine monodeiodinase enzymes have been described. Two of these enzymes, deiodinase (D)l and D2, are activating enzymes because they deiodinate the outer ring; there is one inactivating deiodinase, D3, which deiodinates the inner ring (Fig. 12.3) [8]. Dl is also capable of inner ring monodeiodination, particularly of sulfated iodothyronines. These deiodinases are developmentally regulated and differ in their tissue distribution and properties. In the cerebral cortex, for example, > 50% of the intracellular T_3 is derived from the intracellular conversion of T_4 to T_3. In contrast, in liver, only 25% of the intracellular T_3 is generated from T_4, the remainder being derived from plasma. As a consequence of these variations in deiodinase activity, the relative amounts of T_4 and T_3 in the serum do not necessarily correspond to their intracellular proportions.

D1, responsible for most of the circulating T_3, is expressed predominantly in liver and kidney. In contrast, the highest concentration of D2 is in brain, pituitary, placenta, and brown

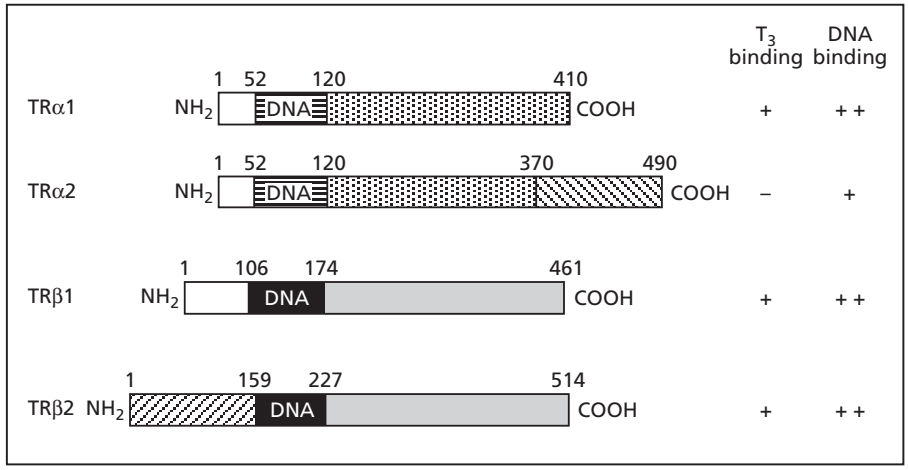


Fig. 12.4. The deduced amino acid structure and functional domains of the known thyroid hormone receptor (TR) subtypes (α and β) and isoforms (1 and 2). Note that, unlike the other TRs, Trα2 does not bind thyroid hormone (from [10] with permission).

adipose tissue. D3 is present predominantly in fetal tissues and the utero-placental unit, underlying the importance of protecting the fetus from the effects of thyroid hormone excess. Adaptive mechanisms in the activity of these deiodinases at a cellular level are an important prereceptor level of control that results in the preferential shunting of thyroid hormone to areas of need. For example, increased conversion of T_4 to T_3 by the fetal brain in the presence of hypothyroidism is a critical protective mechanism that accounts, in part, for the normal or near-normal cognitive outcome of babies with congenital hypothyroidism as long as postnatal therapy is early and adequate.

Thyroid hormone action

Thyroid hormone has multiple effects in cells, including stimulation of thermogenesis, water and ion transport, acceleration of substrate turnover, and amino acid and lipid metabolism. Thyroid hormone also potentiates the action of catecholamines, an effect that is responsible for many of the clinical manifestations seen in patients with thyroid overactivity. Unique to infants and children is the stimulation of growth and development of various tissues including the brain and skeleton.

Thyroid hormone is transported into the cell in association with a number of recently identified families of solute carriers, including organic anion-transporting polypeptides, amino acid transporters, and monocarboxylate transporters (MCTs) [9]. Within the cell, thyroid hormone initiates its action by binding to specific receptors located in the cell nucleus. The binding of T_3 to the thyroid hormone receptor (TR) is 10 times higher than that of T_4 [10]. In addition to the four classical TRs, TRα1, TRα2, TRβ1, and TRβ2, multiple other transcripts that encode protein products have been identified [11]. The gene encoding the TRα subtype is located on chromosome 17, while the gene encoding the TRβ subtype is on chromosome 3; the respective isoforms (1 and 2) result from alternative splicing of the initial mRNA transcripts.

The TRs are composed of a carboxy-terminal portion that is important for ligand binding and interactions between receptors, a DNA-binding domain with two loop structures known as "zinc fingers," and an amino-terminal domain with no known function (Fig. 12.4). TRα2, and other splice variants, such as TRvα2 and TRvα3, do not bind T_3 and may inhibit the binding of the other TRs to DNA (dominant-negative inhibition) [11]. The TRα gene also produces an orphan receptor, Rev-erbAα, that plays a role in cerebellar development [12]. TRs exist as monomers, homodimers, or heterodimers with other nuclear proteins such as the retinoid X receptors. The heterodimeric structure is the active form of the receptor. TRα1, TRβ1, and TRβ2 act to stimulate or suppress responsive genes. This activity requires the interaction of numerous co-activators and co-repressors. In the unliganded state, TRs repress gene function. Tissue specificity of thyroid hormone action derives from multiple factors, including the predominant TR isoform expressed, the cofactor(s) involved, and the type of receptor with which the TR partners. As a result, different genes are stimulated or inhibited in different tissues.

Like the iodothyronine deiodinases, the various TRs are expressed differentially in tissues and are developmentally regulated. TRα1 and TRα2 are widely distributed among tissues. The highest concentration of TRβ1 mRNA is found in brain, developing ear, liver, kidney, and heart, whereas TRβ2 mRNA expression is restricted to pituitary and brain tissues.

Ontogenesis of thyroid function and regulation in humans

The ontogeny of thyroid function involves both hypothalamic–pituitary and thyroid gland organogenesis and maturation,

as well as the development of each of the component systems required for mature activity: thyroid hormone transport, metabolism, and action. In addition, the placenta plays a pivotal role not only by regulating the transport of essential factors and hormones, particularly T_4 and iodide, but by synthesizing and metabolizing hormones as well.

Hypothalamic–pituitary development

Hypothalamic development, described in Chapter 4, involves a cascade of transcription factors, including sonic hedgehog (SHH) and ZIC-2 (a homolog of the *Drosophila* odd-paired gene). SF-1 and the LIM class homeodomain factors LHX-3 and LHX-4 also play a role. Transcription factors involved in pituitary development include the pituitary homeobox gene (PTX-1), thyroid transcription factor (TTF)-1 (also called TITF1, T/EBP, and NKX2.1), and the LIM class homeodomain transcription factors LHX-3 and LHX-4. TTF-1 is also involved in thyroid gland and lung development (see below). The terminal factors in the cascade are Prop-1 and POU1F1 (formerly called Pit-1). POU1F1 is essential for the differentiation of thyrotrophs, lactotrophs, and somatotrophs, whereas PROP-1, a homeodomain protein that is expressed briefly in the embryonic pituitary, is necessary for POU1F1 expression.

Thyroid gland development

The thyroid gland is derived from the fusion of a medial outpouching from the floor of the primitive pharynx, the precursor of the T_4-producing follicular cells, and bilateral evaginations of the fourth pharyngeal pouch, which give rise to the parafollicular or calcitonin (C)-secreting cells. Commitment toward a thyroid-specific phenotype as well as the growth and descent of the thyroid anlage into the neck results from the co-ordinate action of a number of transcription factors, including TTF-1 and TTF-2 (also called TITF2 and FKHL15), and PAX-8 [11–13]. TTF-1 is important for the development of both follicular cells and C-cells, whereas PAX-8 is involved only in thyroid follicular cell development. In addition, other homeodomain-containing or Hox genes (Hoxa-3 and the paralogous gene Hoxb-3) appear to regulate the expression of PAX-8 and TTF-1 respectively [1]. As each of these transcription factors is also expressed in a limited number of other cell types, it appears to be the specific combination of transcription factors, and possibly non-DNA-binding cofactors, acting co-ordinately that determines the specific phenotype of a cell. TTF-1, TTF-2, and PAX-8 also regulate thyroid-specific gene expression.

Recent studies of cadherin expression suggest that the caudal translocation of the thyroid anlage may also arise indirectly, as a result of the growth and expansion of adjacent tissues, including the major blood vessels [14]. In late organogenesis, the sonic hedgehog (SHH) gene plays an important role in the symmetric bilobation of the thyroid; SHH also suppresses the ectopic expression of thyroid follicular cells [15].

In the rat, at fetal day 15, despite early evidence of Tg, TPO, and TSH receptor gene expression, the thyroid gland is difficult to distinguish from the surrounding structures, and iodine organification, thyroid hormonogenesis, or evidence of a follicular structure is not present. This suggests that TTF-1 and Pax-8 are necessary but not sufficient for the expression of the fully differentiated thyroid phenotype. On fetal day 17, TSH receptor gene expression is significantly upregulated, and this is accompanied by significant growth and by rapid development in both structural and functional characteristics [16].

Expression of Tg and TPO mRNA is increased at this time, thyroid follicles first appear on morphological examination, TPO function can be demonstrated, and there is evidence of thyroid hormonogenesis. These findings suggest that the TSH receptor plays an important role only at this later stage of development but is not involved earlier in gestation. In support of this interpretation, hyt/hyt mice that have a loss-of-function (Pro556Leu) mutation in the transmembrane domain of the TSH receptor have severe hypothyroidism and hypoplastic but normally located thyroid glands with a poorly developed follicular structure [17]. Similar findings are detected in babies born to mothers with potent TSH receptor-blocking Abs as well as in babies with severe loss-of-function mutations of the TSH receptor.

Developmental events in humans parallel those in rodent species, but the timing of maturation differs (Fig. 12.5) [1]. Embryogenesis is largely complete by 10–12 weeks' gestation, equivalent to fetal day 15–17 in the rat. At this stage, tiny follicle precursors are first seen, Tg can be detected in follicular spaces, and evidence of iodine uptake and organification is first obtained. Low concentrations of T_4 and T_3 are detectable in fetal serum at 10–12 weeks, although it is likely that a fraction of the thyroid hormone measurable at this early stage of the development is maternal in origin.

Tg, first identified in the follicular spaces by 10–11 weeks, can be identified in the human fetal circulation at gestational age 27–28 weeks, but when Tg can first be detected in serum is not known. The secretion of a poorly iodinated thyroid hormone precursor and impaired clearance of this glycoprotein from the circulation by the immature liver result in a higher serum concentration of Tg in the premature fetus than at term.

Despite the fact that iodide uptake by the thyroid can be demonstrated at 10–11 weeks' gestation, the capacity of the fetal thyroid to reduce iodide trapping in response to excess iodide (the Wolff–Chaikoff effect) does not appear until 36–40 weeks' gestation (Fig. 12.5). Thus, premature infants are much more likely to develop hypothyroidism when exposed to excess iodine than are full-term babies.

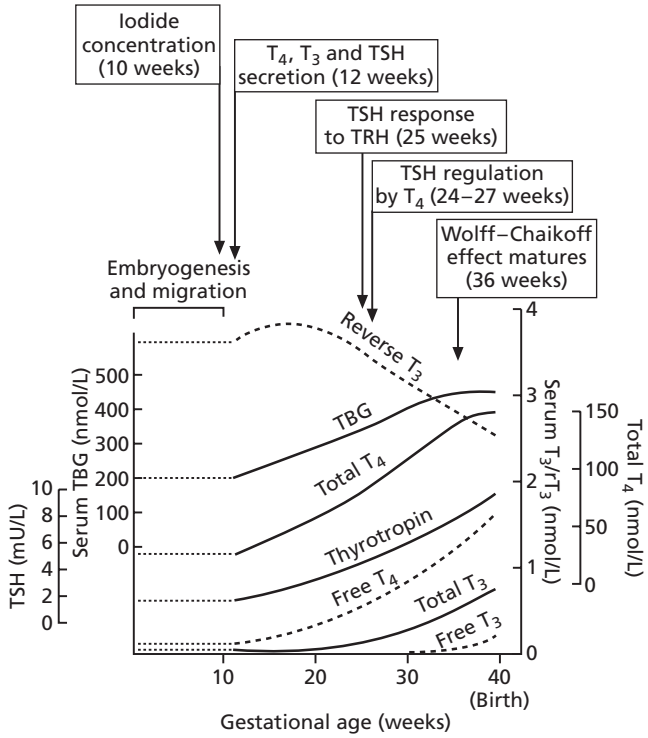


Fig. 12.5. Maturation of thyroid gland development and function during gestation. See text for details (from [76] with permission).

Maturation of the hypothalamic–pituitary–thyroid axis

TSH is detectable in fetal serum at levels of 3–4 mIU/L at 12 weeks' gestation and increases from 18 weeks to levels of 10 mU/L at term. This is accompanied by a parallel increase in fetal thyroid radioiodine uptake and by a progressive increase in the serum concentrations of both total T_4 and free T_4. The serum concentration of TBG also increases during gestation as a consequence of placental estrogen effects on the fetal liver. However, there is a progressive increase in the ratio of free T_4 to TSH concentration during the second half of gestation, suggesting changes in both the sensitivity of the pituitary thyrotroph to the negative feedback effect of thyroid hormones and the thyroid follicular cell sensitivity to TSH.

Maturation of hypothalamic–pituitary–thyroid feedback control is first observed early in the third trimester as indicated by an elevated fetal serum TSH in response to hypothyroxinemia and a suppressed TSH in fetuses with hyperthyroidism due to maternal Graves' disease. Similarly, a fetal TSH response to exogenously administered TRH has been demonstrated as early as 25 weeks' gestation.

Serum levels of TRH are higher in the fetal circulation than in maternal blood, the result both of extrahypothalamic TRH production (placenta and pancreas) and decreased TRH degrading-activity in fetal serum. The physiological significance of these increased levels of TRH in the fetal circulation is not known.

Maturation of thyroid hormone metabolism

Activity of D1, the major activating deiodinase, is low throughout gestation. In contrast, D3, the major inactivating deiodinase, is highly expressed in fetal tissues and in the placenta. As a result, circulating T_3 concentrations in the fetus are quite low and, at birth, are of the order of 50–60 ng/dL (≈ 1 nmol/L). In addition, the concentrations of the specific substrates metabolized by D1, rT_3 and the sulfate conjugates of T_4, are markedly elevated in the fetal circulation as well as in amniotic fluid. The physiological rationale for the maintenance of reduced circulating T_3 concentrations throughout fetal life is still unknown, but it has been suggested that its function may be to avoid tissue thermogenesis and potentiate the anabolic state of the rapidly growing fetus.

In contrast to D1, D2, highly expressed in brain and pituitary, is detectable by mid-gestation. As a consequence, fetal brain T_3 levels are 60–80% those of the adult by fetal age 20–26 weeks, despite the low levels of circulating T_3. In the presence of fetal hypothyroidism, D2 increases while D3 decreases. These co-ordinate adjustments are of critical importance and serve to preserve near-normal brain T_3 levels providing that maternal T_4 levels are maintained at normal concentrations (see below).

The ontogeny of thyroid hormone metabolism is closely associated with maturation of thyroid hormone action both temporally and spatially. This is best illustrated with the example of the cochlea, where D2 activity in the mouse rises dramatically to reach a peak level at postnatal day 6, a few days prior to the onset of hearing [18]. D2 expression is localized to connective tissue immediately adjacent to the sensory epithelium and spiral ganglion where thyroid hormone receptors (TRs) are found. This suggests that D2-containing cells in the connective tissue take up T_4 from the circulation, convert T_4 to T_3, and then release T_3 to the adjacent responsive cells. A similar paracrine relationship is found in the cerebral cortex where D2 is expressed predominantly in glial cells, whereas TRs are found in the adjacent neurons and oligodendrocytes [19]. In other areas of the brain, such as the pituitary gland, hippocampus, and caudate nucleus, D2 and TRs are co-expressed. Unlike D2, D3 is co-expressed with TRs in neurons, perhaps underlying the importance of protecting affected tissues from the effects of excess thyroid hormone.

Maturation of thyroid hormone action

Like thyroid hormone metabolism, the ontogenesis of thyroid hormone-mediated responsiveness is tissue specific

and developmentally regulated. Whereas thyroid hormone-mediated effects in the pituitary, brain, and bone can be detected prenatally, thyroid hormone-dependent action in brown adipose tissue, liver, heart, skin, and carcass is apparent only postnatally. Three examples illustrate the complexity and specificity of thyroid hormone action in different target organs or tissues.

Thyroid hormone and brain development

In the brain, the action of thyroid hormone and its developmental regulation are complex and only beginning to be understood [12,19,20]. At a functional level, thyroid hormone provides the induction signal for the differentiation and maturation of a diverse array of processes that lead to the establishment of neural circuits during a critical window of brain development. These processes include neurogenesis and neural cell migration (occurring predominantly between 5 weeks and 24 weeks), neuronal differentiation, dendritic and axonal growth, synaptogenesis, gliogenesis (late fetal to 6 months post partum), myelination (second trimester to 24 months post partum), and neurotransmitter enzyme synthesis. The absence of thyroid hormone appears to delay rather than eliminate the timing of critical morphological events or gene products, resulting in a disorganization of intercellular communication.

TRs are found in highest concentration in developing neurons and in multiple areas of the fetal brain, including the cerebrum, cerebellum, auditory and visual cortex. Consistent with a nuclear receptor-mediated mode of action, thyroid hormone stimulates numerous developmentally regulated genes, including genes for myelin, neurotrophins and their receptors, cytoskeletal components, transcription factors, extracellular matrix proteins and adhesion molecules, intracellular signaling molecules, as well as mitochondrial and cerebellar genes. In some cases, these genes appear to be direct targets of thyroid hormone action as thyroid hormone response elements can be detected in the DNA regulatory region and/or the genes are stimulated in cell culture. In other cases, thyroid hormone control may occur secondarily as a consequence of effects on terminal differentiation. In addition, thyroid hormones regulate some genes at the level of mRNA stability or mRNA splicing.

Until recently, one of the unexplained paradoxes has been the surprising lack of developmental abnormalities seen in mutant mice lacking TRβ1, TRα, or both, in contrast to the severe abnormalities observed in hypothyroid animals. Emerging evidence suggests that the reason for the abnormal brain development observed after thyroid hormone deficiency but not TR deficiency is transcriptional repression by the unliganded TR. For example, when mutant mice lacking the TRα1 receptor were made hypothyroid, no effects on cerebellar development were seen, contrary to findings in wild-type animals [19].

It is likely that the complexity of maturational control of thyroid hormone action involves developmental regulation of a myriad of factors that affect TR activity. These factors include co-repressors and co-activators as well as transcription factors that compete with TRs for thyroid hormone response elements (TREs) on target genes, providing further levels of modulation [20].

There is also some evidence that the action of T_4 on the developing central nervous system (CNS) may involve, in part, a non-nuclear mechanism. The work of Farwell *et al*. has shown that T_4-regulated actin polymerization plays an integral role in the regulation of deiodinase activity, and he and his colleagues have proposed that the action of T_4 on the actin cytoskeleton might be important in cellular migration, neurite outgrowth, and dendritic spine formation [21]. It is of interest that deafness, found in the TRβ1 knockout mouse, is also a frequent finding in patients with severe endemic cretinism and in some patients with thyroid hormone resistance due to a deletion in the TRβ1 gene.

Thyroid hormone and bone

A second important thyroid hormone target in the perinatal period is bone, as evidenced by the striking growth retardation, decreased growth velocity, and delayed ossification of the epiphyseal growth plate characteristic of longstanding, untreated hypothyroidism in infancy and childhood. Thyroid hormone-mediated bone maturation involves both a direct and an indirect action, the latter mediated by regulation of growth hormone gene expression and the IGF system [22,23]. At a direct level, T_3 regulates endochondral ossification and controls chondrocyte differentiation in the growth plate both *in vitro* and *in vivo* [22,24]. Both osteoblasts and growth plate chondrocytes express TRs, and several T_3-specific target genes have been identified in bone [25]. T_3 also stimulates closure of the skull sutures *in vivo*, the basis for the enlarged anterior and posterior fontanelle characteristic of infants with congenital hypothyroidism [26]. Analogous to findings in the brain, the growth retardation observed in hypothyroid mice is more severe than that seen in $TR\alpha^{0/0}\beta^{-/-}$ double knockout mice. This is consistent with the effect of the unliganded aporeceptor in mediating the deleterious effects of thyroid hormone deficiency [11].

Thyroid hormone and brown adipose tissue

During the perinatal period, brown adipose tissue is essential for non-shivering thermogenesis. In this tissue, thyroid hormone stimulates transcription of thermogenin (also called uncoupling protein, UCP 1), a unique protein that uncouples nucleotide phosphorylation and the storage of energy as adenosine triphosphate (ATP). As the child matures, shivering thermogenesis assumes greater importance, and brown adipose tissue disappears.

The role of the placenta

The placenta plays an important role in fetal thyroid development and function by regulating the passage of certain maternal hormones, substrates, and drugs, and by serving as an important site of thyroid hormone metabolism. Although the placenta also synthesizes some hormones that can affect the fetal thyroid (e.g. human chorionic gonadotropin, TRH), these appear to have little influence on the fetus.

Thyroid hormone

Under normal circumstances, the placenta has only limited permeability to thyroid hormone, and the fetal hypothalamic–pituitary–thyroid system develops relatively independently of maternal influence [27]. This relative barrier to thyroid hormone transport results primarily from the high placental content of D3, which serves to inactivate most of the thyroid hormone presented from the maternal circulation. The iodide released in this way can then be used for fetal thyroid hormone synthesis.

When a significant T_4 gradient between the maternal and fetal compartment exists, however, there is an increased net flux of maternal thyroid hormone to the fetus. Such a situation occurs when the fetus is hypothyroid, as illustrated by infants with the complete inability to synthesize T_4 on account of an inherited absence of the TPO enzyme. These infants nonetheless have cord T_4 concentrations between 25% and 50% of normal. Similar results are obtained in retrospective studies of cord serum in infants with sporadic congenital athyreosis.

There is also accumulating evidence that maternal–fetal T_4 transfer occurs in the first half of pregnancy, when fetal thyroid hormone levels are low [28]. Low concentrations of T_4, presumably of maternal origin, have been detected in human embryonic coelomic fluid as early as 6 weeks' gestation and in fetal brain as early 10 weeks' gestation prior to the onset of fetal thyroid function. Furthermore, both D2 and D3 activity as well as TR isoforms are present in human fetal brain from the mid-first trimester, indicating that the machinery to convert T_4 to T_3 and to respond to T_3 is present.

The transplacental passage of maternal T_4 (coupled with the co-ordinate adjustments in brain deiodinase activity discussed above) plays a critical role in minimizing the adverse effects of fetal hypothyroidism. Not only may it help to explain the normal or near-normal cognitive outcome of hypothyroid fetuses as long as postnatal treatment is early and adequate, it may also provide a partial explanation for the relatively normal clinical appearance at birth of over 90% of infants with congenital hypothyroidism. In contrast, when both maternal and fetal hypothyroidism occurs, whether this is due to severe iodine deficiency, potent TSH receptor blocking Abs, or maternal–fetal Pit-1 deficiency, there is a significant impairment in neuro-intellectual development despite the initiation of early and adequate postnatal thyroid replacement [29–31]. Even maternal hypothyroidism and hypothyroxinemia alone have been reported to cause significant cognitive and/or motor delay in the offspring, although the magnitude of the deficit is not as great as when both fetal and maternal hypothyroidism are present [32,33]. Unlike fetal hypothyroidism, the effects of maternal hypothyroidism are not reversible by early postnatal therapy.

Other hormones and factors

In contrast to thyroid hormone, the placenta is freely permeable to TRH and to iodide, the latter being essential for fetal thyroid hormone synthesis. The placenta is also permeable to certain drugs and to immunoglobulins of the immunoglobulin (Ig)G class. Thus, the administration to the mother of excess iodide, drugs (especially propylthiouracil or methimazole), or the transplacental passage of TSH receptor Abs from mothers with severe Graves' disease or primary myxedema may have significant effects on fetal and neonatal thyroid function.

Maternal TSH does not cross the placenta. Similarly, Tg is undetectable in the serum of athyreotic infants, indicating the absence of any transplacental passage of this large protein.

Thyroid function in the full-term and premature neonate, the infant, and during childhood

The neonate

Marked changes occur in thyroid physiology at the time of birth in the full-term newborn (Fig. 12.6). One of the most dramatic is an abrupt rise in the serum TSH that occurs within 30 min of delivery, reaching concentrations of 60–70 mU/L. This causes a marked stimulation of the thyroid, resulting in an approximate 50% increase in the serum T_4 and an increase of three- to fourfold in the concentration of serum T_3 within 24 h. Studies in experimental animals suggest that the increase in TSH is a consequence of the relative hypothermia of the ambient extrauterine environment. The marked increase in T_3 is due not only to the increase in TSH but also to maturation of D1 activity and the loss of placental D3 at the time of delivery. In contrast, the elevated concentrations of the other substrates of D1, rT_3, and T_3 sulfate, decrease relatively rapidly during the newborn period. Increased activity of D2 in brown adipose tissue at birth leads to an increase in T_3, which is required for optimal uncoupling protein synthesis and thermogenesis.

The premature infant

Thyroid function in the premature infant reflects the relative immaturity of the hypothalamic–pituitary–thyroid axis found

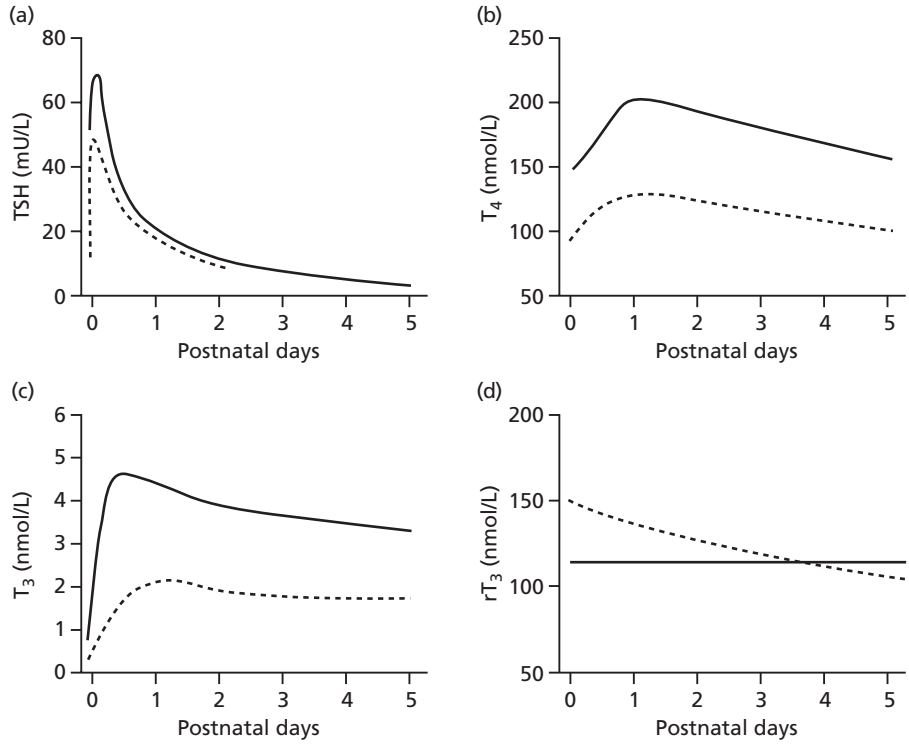


Fig. 12.6. Postnatal changes in the serum concentration of TSH, T_4, T_3, and rT_3 in term babies, (continuous line) compared with premature infants (discontinuous line) in the first week of life. Note that the postnatal surge in TSH is followed by a transient increase in the T_4 and T_3 concentration in the first few days of life. Changes in premature infants are similar to those seen in term babies, but are much less marked (from [77] as modified by [76] with permission). See text for details.

in comparable gestational age infants *in utero*. Thus, in cord blood samples obtained by umbilical cord sampling (cordocentesis), there is a progressive increase in the TSH, TBG, T_4, and T_3 concentration in fetuses with increasing degrees of maturity (Fig. 12.7) [34]. Following delivery, there is a surge in T_4 and TSH analogous to that observed in term infants, but the magnitude of the increase is less in premature neonates, and there is a more dramatic fall in the T_4 concentration over the subsequent 1–2 weeks (Fig. 12.8) [35]. This decrease in the T_4 concentration is particularly significant in very low birthweight infants (< 1.5 kg, approximately equivalent to < 30 weeks' gestation), in whom the serum T_4 may occasionally be undetectable. In most cases, the total T_4 is more affected than the free T_4, a consequence of abnormal protein binding and/or the decreased TBG in these babies with immature liver function. In addition to the aforementioned changes in T_4 and TSH concentrations, the serum rT_3 tends to stay higher, and serum T_3 is reduced for a longer period in the premature newborn, reflecting the greater immaturity of the type 1 deiodinase system.

The causes of the decrease in T_4 observed postnatally in premature infants are complex. In addition to the clearance of maternal T_4 from the neonatal circulation, preterm babies have decreased thyroidal iodide stores and are less able to regulate iodide balance [36]. This is a particular problem in borderline iodine-deficient areas of the world. Preterm infants are frequently sicker than their more mature counterparts and may be treated by drugs that affect neonatal thyroid function. In addition, as the capacity of the immature thyroid to adapt to exogenous iodide is reduced, there is an increase in sensitivity to the thyroid-suppressive effects of excess iodide found in certain skin antiseptics and drugs to which these babies are frequently exposed (see below).

Despite the reduced total T_4 observed in some preterm babies, the TSH concentration is not significantly elevated in most of them. Transient elevations in TSH are seen in some, the finding of a TSH concentration > 40 mU/L being more frequent the greater the degree of prematurity. In one study, for example, the prevalence of a TSH concentration > 40 mU/L in very low birthweight (< 1.5 kg) premature infants was eightfold higher and in low birthweight (1.5–2.5 kg) neonates twofold higher than the prevalence in term babies [37].

Although an elevated TSH concentration may reflect true primary hypothyroidism, the increase in TSH seen in the preterm infants at several weeks of age may reflect the elevated TSH observed in adults who are recovering from severe illness. Such individuals may develop transient TSH elevations, which are associated with still reduced serum T_4 and T_3 concentrations. These have been interpreted as reflecting a "reawakening" of the illness-induced suppression of the hypothalamic–pituitary axis. As the infant recovers from prematurity-associated illnesses such as respiratory distress syndrome (RDS), a recovery of the illness-induced suppression of the hypothalamic–pituitary–thyroid axis would also occur.

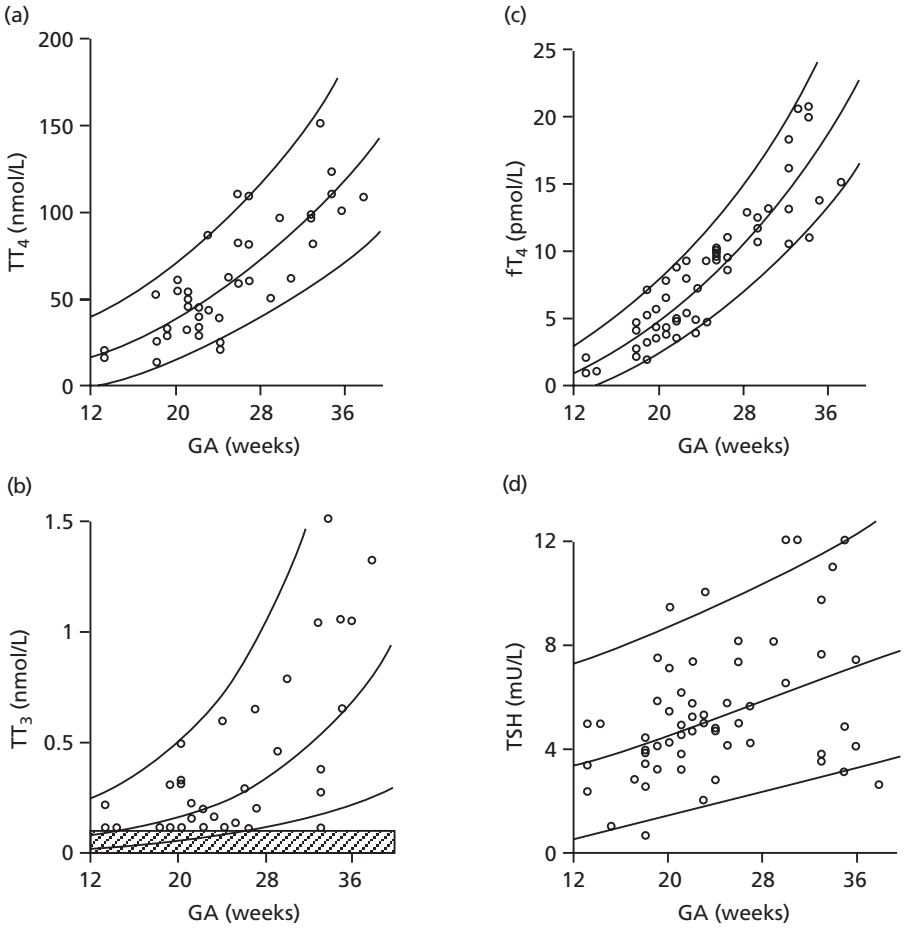


Fig. 12.7. Cord blood concentration of total T_4 (TT_4), total T_3 (TT_3), free T_4 (fT_4), and TSH in normal pregnancy (from [34] as modified by [78] with permission).

Infants and children

After the acute perturbations of the neonatal period, there is a slow and progressive decrease in the concentrations of T_4, free T_4, T_3, and TSH during infancy and childhood. Age- and gender-specific normative values in a large population of children have been published [38]. The higher concentrations of TSH and T_3 in children than in adults are particularly noteworthy. Adult normative data are frequently provided clinically, and apparent abnormalities in these values are not infrequent reasons for referral to a pediatric endocrinologist. The serum concentration of rT_3 remains unchanged or increases slightly. In addition, there is a higher T_4 turnover than in adults for whom reference values are often provided. In infants, T_4 production rates are estimated to be of the order of 5–6 μg/kg/day decreasing slowly over the first few years of life to about 2–3 μg/kg/day at ages 3–9 years. This is to be contrasted with the production rate of T_4 in the adult, which is about 1.5 μg/kg/day. Serum Tg levels also fall over the first year of life reaching concentrations typical of adults by about 6 months of age. The size of the thyroid gland increases slowly by about 1 g/year from approximately 1 g in the newborn to about 15–20 g at age 15 years when it has achieved its adult size. The thyroid lobe is comparable to the terminal phalanx of the infant or child's thumb.

Thyroid disease in infancy

Congenital hypothyroidism

Congenital hypothyroidism (CH) is the commonest treatable cause of mental retardation. Worldwide, the most common cause of CH is iodine deficiency, a problem that continues to affect almost 1 billion people despite international efforts aimed at its eradication. In areas where iodine deficiency is severe, CH is endemic ("endemic cretinism") and is characterized clinically by mental retardation, short stature, deaf mutism, and specific neurological abnormalities. Both endemic cretinism and iodine deficiency have been the subject of several excellent reviews [39,40].

Screening for congenital hypothyroidism

In iodine-sufficient areas and in areas of borderline iodine deficiency, CH is usually sporadic and occurs in 1 in

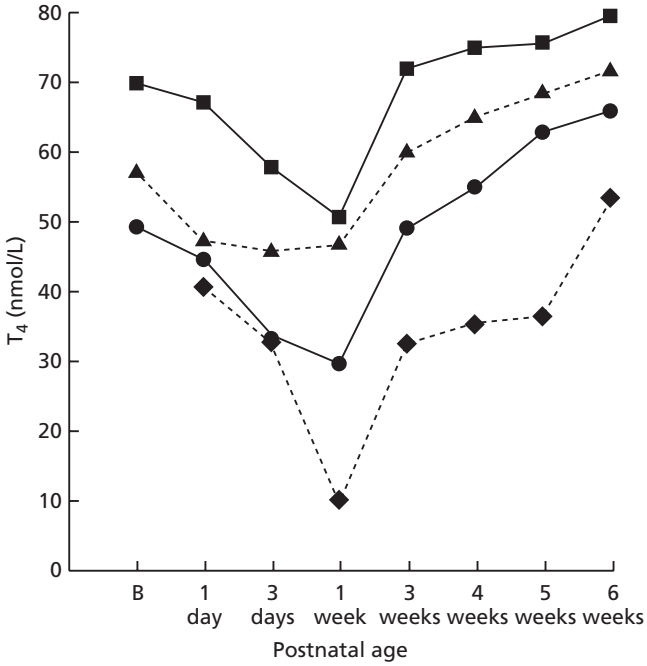


Fig. 12.8. Postnatal changes in the serum T_4 concentration in premature babies in the first 6 weeks of life. Note that, in very premature infants, no postnatal increase in the T_4 concentration in the first few days of life is observed. Instead, the T_4 concentration decreases with a nadir at 1 week of life. Values subsequently normalize by 3–6 weeks (from [35] with permission).

3000–4000 infants. In order to achieve optimal neurological outcome, treatment must be initiated soon after birth before affected infants are recognizable clinically. Neonatal screening programs have therefore been introduced in most industrialized areas of the world. Elsewhere, such as eastern Europe, South America, Asia, Oceania, and Africa, neonatal screening programs are under development.

By 1992, some 50 million infants had been screened for CH worldwide with 6000 cases detected annually. There continues to be disagreement as to whether minor neurointellectual sequelae remain in the most severely affected infants and what constitutes the best treatment strategy. Nonetheless, accumulating evidence suggests that a normal outcome is possible even in the latter group of babies as long as treatment is started sufficiently early and is adequate. Certainly, the main objective of screening, the eradication of mental retardation, has been achieved. In addition to the profound clinical benefit, it has been estimated that the financial benefit–cost ratio of neonatal screening programs is approximately 10:1, a ratio that does not include the loss of tax income that would result from impaired intellectual capacity in the untreated, but non-institutionalized, person. Newborn screening has also permitted an elucidation of the prevalence of the various causes of CH, including a series of transient disorders found predominantly in premature infants. Of note is the fact that the incidence of CH has been found to be four to five times more common than phenylketonuria for which screening programs were developed first.

Screening strategies for congenital hypothyroidism

Measurement of T_4 and/or TSH is performed on an eluate of dried whole blood collected on filter paper by skin puncture on day 1–4 of life. Two screening strategies for the detection of CH have evolved. In both, a two-tiered approach is used. In much of North America, primary T_4 screening is performed with TSH reserved for those specimens with a low T_4 (usually the lowest 10th–20th percentile). In most parts of Europe and Japan, a primary TSH/backup T_4 is employed. Recently, with the development of more sensitive, non-radioisotopic TSH assays, Canada and some states in the United States have switched to a primary TSH program. In practice, the choice of strategy utilized depends on the preference of the individual screening program.

Whichever method is used, babies whose initial TSH is > 50 mU/L are most likely to have permanent CH, whereas a TSH between 20 and 49 mU/L may be a false positive or may represent transient hypothyroidism. Transient CH is particularly common in premature infants in borderline iodine-deficient areas of Europe.

Each screening strategy has its advantages and disadvantages, but the two approaches appear to be equivalent in the detection of babies with permanent forms of CH. A primary T_4/backup TSH program will detect overt primary hypothyroidism, secondary or tertiary hypothyroidism (1 in 50 000 to 1 in 100 000 live births), babies with a low T_4 but delayed rise in the TSH, TBG deficiency, and hyperthyroxinemia; this approach may, however, miss compensated hypothyroidism. A primary TSH strategy, on the other hand, will detect both overt and compensated hypothyroidism, but will miss secondary or tertiary hypothyroidism, a delayed TSH rise, TBG deficiency, and hyperthyroxinemia. There are fewer false positives with a primary TSH strategy.

Both strategies will miss the rare infant whose T_4 and TSH levels on initial screening are normal but who later develops low T_4 and elevated TSH concentrations (< 0.5% of infants). This pattern has been termed "atypical" CH or "delayed TSH rise" and is observed most commonly in premature babies with transient hypothyroidism or in infants with less severe forms of permanent disease. Some programs have responded by performing a second screen on all infants at the time of their return visit to their pediatrician at 2–6 weeks of age. In addition, some of these programs request follow-up serum on any baby with a very low T_4 value (< third percentile) on two occasions or a very low filter paper T_4 below a critical value (< 3 μg/dL) on one occasion. Programs that perform a second screen report the detection of an additional 10% of CH cases, but this practice greatly increases the cost of screening [41]. Other screening programs routinely perform a second

screen only on patients at high risk of delayed TSH elevation, such as very low birthweight infants and babies in the neonatal intensive care unit. The latter programs report a 14-fold increased incidence of this problem in very low birthweight infants [42]. As noted previously, in some of these cases, it may not be certain as to whether the elevated TSH level is pathological or represents an appropriate compensatory response following hypothyroxinemia secondary to sick euthyroid syndrome. Other groups at high risk of delayed TSH rise are babies with cardiovascular anomalies, patients with Down syndrome, and monozygotic twins [43]. In the last group of infants, fetal cord mixing may occur and initially mask the presence of CH.

In both strategies, there is the very real possibility of human error in failing to identify affected infants. This can occur as a result of poor communication, lack of receipt of requested specimens, or the failure to test an infant who is transferred between hospitals during the neonatal period.

Newborn screening was performed initially at between 3 and 4 days of life, and the normal values that were derived reflected this postnatal age. The practice of early discharge from the hospital of otherwise healthy full-term infants has resulted in a greater proportion of babies being tested before this time. For example, it has been estimated that, in North America at the present time, 25% or more of newborns are discharged within 24 h of delivery and 40% in the second 24 h of life. Because of the neonatal TSH surge and the dynamic changes in T_4 and T_3 concentrations that occur within the first few days of life, early discharge increases the number of false-positive results. In California, the ratio of false-positive to confirmed CH has doubled from 2.5:1 to approximately 5:1. Some programs have responded by increasing their threshold value for TSH within the first day of life, but this increases the possibility of missing infants with a slowly rising TSH.

Another complicating factor is the dramatically increased survival of very premature infants. They greatly increase the cost of screening programs because blood T_4 concentrations are lower, and the incidence of transient hypothyroidism is much higher in them compared with full-term babies. It has been estimated that very low birthweight infants constitute only 0.8% of the population but increase the number of T_4 assays in a primary TSH program by 9%. Very low birthweight infants account for 8% of all TSH assays performed in a primary T_4 program.

In the last decade, normal values according to gestational age (and/or birthweight) for cord blood [34], filter paper at the time of screening [37], and serum in the first week of life [44], the last using newer, more sensitive assay techniques, have been published.

Thyroid dysgenesis

The causes of non-endemic CH and their relative frequencies are listed in Table 12.1. The most common cause, accounting for 85–90% of cases, is thyroid dysgenesis, almost always a sporadic disease. Thyroid dysgenesis may result in the complete absence of thyroid tissue (agenesis), or it may be partial (hypoplasia); the latter is often accompanied by a failure to descend into the neck (ectopy). Unilateral agenesis or hypoplasia may also occur, but this usually does not affect thyroid function in the newborn period. Females are affected twice as often as males. Thyroid dysgenesis is less frequent among African-Americans (1 in 32 000) and more common among Hispanics (1 in 2000) in the USA. Although a slightly higher incidence was originally reported in western Europe (1 in 3300) and a slightly lower figure was reported in Japan (1 in 5700) than in North America (1 in 4500), these differences

Table 12.1. Differential diagnosis of permanent congenital hypothyroidism.

Thyroid dysgenesis (1 in 4500)
Isolated thyroid aplasia, hemiagenesis, or hypoplasia ± ectopy)
Transcription factor defect (PAX-8)
Unknown*
Associated with other developmental abnormalities
Transcription factor defect (TTF-2, SHH, Tbx1)
Inborn errors of thyroid hormonogenesis (1 in 35 000)
Failure to concentrate iodide
Abnormal organification of iodine
Abnormal TPO enzyme
Abnormal H_2O_2 generation (THOX)
Pendred syndrome
Defective Tg synthesis or transport
Abnormal iodotyrosine deiodinase
Secondary and/or tertiary hypothyroidism (1 in 50 000 to 100 000)
Hypothalamic abnormality
Isolated TSH deficiency
TRH deficiency
Multiple hypothalamic hormone deficiencies
Isolated hypothalamic defect
Associated with other midline facial/brain dysmorphic features (e.g. septo-optic dysplasia, cleft lip/palate)
Pituitary abnormality
Isolated TSH deficiency
TRH resistance
Abnormal TSH β molecule
Multiple pituitary hormone deficiencies
Posterior pituitary eutopic (transcription factor defect, e.g. POU1F-1, PROP-1, LHX)
Posterior pituitary ectopic
TSH resistance
TSH receptor gene mutation
Post-receptor defect?
$G_{s\alpha}$ gene mutation
Thyroid hormone resistance (1 in 100 000)

*Most common.

Table 12.2. Genetic defects in thyroid development, hormonogenesis, and action.

Abnormality	Chromosome	Characteristic picture	Inheritance
Transcription factor		Thyroid gland usually dysgenetic	
TTF1 (NKX2-1)	14q13	Choreoathetosis, RDS, mild ↑TSH	?
TTF2(FKHL15)	9q22	Thyroid agenesis, cleft palate, spiky hair, choanal atresia, bifid epiglottis	?
PAX8	2q12–q14	Thyroid hypoplasia	AD
Tbx1?		Thyroid hemiagenesis, DiGeorge syndrome	
Sonic hedgehog?	7q11	Thyroid hemiagenesis, Williams syndrome	
↓TSH synthesis		Thyroid gland eutopic	
PROP1	5q	CPHD	AR
POU1FI (PIT1)	3p11	CPHD	AR, AD
LHX3	9q34.3	CPHD	AR
HESX1	3p22,1–p21.1	Septo-optic hypoplasia, CPHD	AR, AD
TRH	3p	↓TSH	AR
TRH receptor	8p23	↓TSH, ↓PRL	AR
TSH β-subunit	1p13	↓TSH	AR
↓TSH response		Thyroid gland eutopic	
TSH receptor	14q31	±Thyroid hypoplasia	AR
Gsα	20q13.2	±Thyroid hypoplasia, pseudoparathyroidism 1a (Albright hereditary osteodystrophy)	AD
↓T_4 synthesis		Thyroid gland eutopic, size may be ↑	
NIS	19p12–13.2	↓I_2 transport	AR
TPO	2p25	Abnormal I_2 organification	AR
THOX2	15q15.3	Abnormal H_2O_2 generation	AR
Thyroglobulin	8q24	Abnormal Tg synthesis	AR, AD
Dehalogenase	Unknown	Defect in dehalogenase enzymes	AR?
PDS	7q31	Congenital deafness, partial organification defect	AR
↓T_4 cellular transport		Thyroid gland eutopic	
MCT8	Xq13.2	Neurological abnormalities, deafness, mild hypothyroidism, TSH normal or ↑	

have decreased more recently as the original screening protocols have been modified.

Both genetic and environmental factors have been implicated in the etiology of thyroid dysgenesis, but the cause is unknown in most patients. The 2% familial occurrence, the reported gender and ethnic differences, as well as the increased incidence in babies with Down syndrome all suggest that genetic factors might play a role in some cases. The transcription factors TTF-1, TTF-2, and PAX-8 would appear to be obvious candidate genes in the etiology of thyroid dysgenesis in view of their important role in thyroid organogenesis and in thyroid-specific gene expression. To date, however, abnormalities in these genes have been found in only a small proportion of patients with thyroid dysgenesis unassociated with other problems [45]. For example, no germline mutations in the TTF-1 gene were found in a total of 76 CH patients studied by two different groups of investigators in Italy. Similarly, germline mutations of the PAX-8 gene were found in only three of 145 Italian and German CH patients with thyroid dysgenesis, in one of whom the abnormality was familial with an autosomal-dominant mode of inheritance. In these and other reported cases, a heterozygous loss-of-function mutation of the PAX-8 gene has been identified, and affected patients have had thyroid hypoplasia with or without ectopy. Table 12.2 summarizes known molecular defects in transcription factors and other causes of CH.

In contrast to the rarity of germline mutations of TTF-1, TTF-2, and PAX-8 in patients with isolated thyroid dysgenesis, emerging evidence suggests that heterozygous mutations in these genes may be a much more important cause of abnormal thyroid gland development when thyroid dysgenesis or abnormal thyroid function is associated with other dysmorphic findings. This is consistent with the important role of these transcription factors not only in the thyroid but in non-thyroid tissues as well during embryonic development. Heterozygous deletions of TTF-1 have been reported in a number of patients with CH, unexplained neonatal respiratory distress, and neurological manifestations, reminiscent of the findings of abnormal thyroid, lung pituitary, and fore-

brain development in mice with a targeted disruption of this gene. Similarly, a homozygous missense mutation in the TTF-2 gene has been associated with the syndrome of thyroid agenesis, bifid epiglottis, cleft palate, kinky hair, and choanal atresia. Most recently, it has been suggested that the thyroid hemiagenesis sometimes observed in patients with DiGeorge syndrome and Williams syndrome might be related to defects in the genes for Tbx1 and sonic hedgehog (or sonic hedgehog signaling) respectively [15]. Mice with a targeted disruption in both the sonic hedgehog and the Tbx1 genes, an important regulator of sonic hedgehog, also have thyroid hemiagenesis [15].

Inborn errors of thyroid hormonogenesis

Decreased T_4 synthesis due to an inborn error of thyroid hormonogenesis is responsible for most of the remaining cases (10–15%) of CH. A number of different defects have been characterized and include: (1) failure to concentrate iodide; (2) defective organification of iodide due to an abnormality in the TPO enzyme or in the H_2O_2 generating system; (3) defective Tg synthesis or transport; and (4) abnormal iodotyrosine deiodinase activity. The association of a partial organification defect with sensorineural deafness is known as Pendred syndrome. All the inborn errors of thyroid hormonogenesis are associated with a normally placed ("eutopic") thyroid gland of normal or increased size, and this feature forms the basis for the clinical distinction from thyroid dysgenesis.

Unlike thyroid dysgenesis, a sporadic condition, the inborn errors of thyroid hormonogenesis tend to have an autosomal-recessive form of inheritance consistent with a single gene mutation. It is not surprising, therefore, that a molecular basis for many of these abnormalities has now been identified [46]. These include mutations in the genes for NIS, TPO, and Tg respectively. Pendred syndrome has been shown recently to result from a defect in the pendrin (PDS) gene [2], and mutations in THOX2, important in hydrogen peroxide generation, have been shown to underlie many cases of apparent organification defect [3]. The gene for the iodotyrosine deiodinase enzyme has not been cloned to date.

TSH resistance

Decreased T_4 synthesis resulting from resistance to the action of TSH is a less common cause of congenital hypothyroidism. Babies with TSH resistance have a normal or hypoplastic gland; in rare cases, no thyroid gland at all is discernible on thyroid imaging, a picture indistinguishable from thyroid agenesis. Because the TSH gene is only expressed after the thyroid gland has migrated into the neck, loss-of-function mutations could only explain the finding of hypoplasia or apparent aplasia but not ectopy. Similar to the variability observed in thyroid gland size in this condition, the clinical findings in TSH resistance have varied from compensated to overt hypothyroidism depending on the severity of the functional defect. Some of these patients have been found to have a loss-of-function mutation of the TSH receptor, usually involving the extracellular domain [5]. Rarely, a loss-of-function mutation involves the transmembrane domain, analogous to the hyt/hyt mouse (Fig. 12.2). In a few affected infants, a discrepancy between presumed "athyreosis" on thyroid scintigraphy and the detection of either a "normal" serum Tg concentration or glandular tissue on ultrasound examination has been noted, but this has not been a consistent finding.

The relative frequency of TSH receptor gene mutations as a cause of TSH resistance is not known. In one study, inactivating mutations of the TSH receptor gene were found in only one of 100 patients with CH, indicating that abnormalities in this gene are not a common cause of thyroid hypoplasia or aplasia. A similar conclusion may be drawn from the failure to demonstrate linkage to the TSH receptor gene in 23 families, in a majority of which there were two or more children affected by CH and in whom there was appreciable consanguinity of the parents. Most familial cases of TSH resistance due to a loss-of-function mutation of the TSH receptor have an autosomal-recessive form of inheritance. Some of the remaining patients with TSH resistance are likely to have a post-receptor defect, possibly involving a signal transduction pathway.

Rarely TSH resistance may result from an inactivating mutation of the stimulatory guanine nucleotide-binding protein (Gs_α) gene. This syndrome, known as pseudohypoparathyroidism type la or Albright's hereditary osteodystrophy, is characterized by a variable resistance to G-protein-coupled receptors, most commonly the parathyroid hormone receptor. Unlike loss-of-function mutations of the TSH receptor, Albright's hereditary osteodystrophy has an autosomal-dominant inheritance with variable expression. The hypothyroidism at birth is usually mild.

Decreased TSH synthesis or secretion

CH resulting from TSH deficiency is only detected by newborn screening programs that utilize a primary T_4 strategy; the reported incidence is 1 in 50 000 to 1 in 100 000, < 5% of cases. TSH deficiency may be isolated, or it may be associated with other pituitary hormone deficiencies. Familial cases of both TSH deficiency and TRH deficiency have been described. TRH resistance due to a mutation in the TRH receptor gene has also been described in a child in whom secondary hypothyroidism was missed on newborn screening. In this patient, the diagnosis was suspected because of an absent TSH and prolactin response to TRH despite a normal pituitary gland on imaging.

TSH deficiency in association with other pituitary hormone deficiencies may be associated with abnormal midline facial and brain structures (particularly cleft lip and palate,

and absent septum pellucidum and/or corpus callosum) and should be suspected in any male infant with microphallus and prolonged hypoglycemia. Recently, one of the more common of these syndromes, septo-optic dysplasia, was shown to be due to a mutation in the HESX-1 homeobox gene in some cases. Non-dysmorphic causes of CH include pituitary hypoplasia, a disorder that is often associated with an ectopic posterior pituitary gland, and molecular defects in the genes for the transcription factors LHX, POU1F1, or PROP-1.

Decreased T_4 cellular transport

Decreased T_4 transport into target cells is a newly recognized congenital abnormality of thyroid hormone action [47]. In this syndrome, mutations in the monocarboxylate transporter 8 (MCT8) gene, located on the X chromosome, have been associated with male-limited hypothyroidism and severe neurological abnormalities, including global developmental delay, dystonia, central hypotonia, spastic quadriplegia, rotary nystagmus, and impaired gaze and hearing. Heterozygous females had a milder thyroid phenotype and no neurological defects.

Thyroid hormone resistance

Resistance to the action of thyroid hormone, although usually diagnosed later in life, may be identified in the newborn period by neonatal screening programs that primarily determine TSH. Affected babies are usually not symptomatic. Most cases of thyroid hormone resistance result from a mutation in the TRβ gene and follow an autosomal-dominant pattern of inheritance. The incidence has been estimated to be 1 in 50 000. Thyroid hormone resistance is discussed in further detail below.

Transient congenital hypothyroidism

Estimates of the frequency of transient CH vary greatly depending on how this condition is defined, i.e. whether transient hypothyroidism is considered to be all infants with a single elevated blood TSH concentration or only those babies in whom a low T_4 and elevated TSH are found in both the screening and the confirmatory serum sample, associated with disappearance of the condition within a few weeks with or without replacement therapy. In North America, a frequently quoted estimate for transient hypothyroidism is 10% of CH babies identified on newborn screening or 1 in 40 000 neonates. As noted earlier, transient hypothyroidism is most common in premature infants, the frequency increasing the greater the degree of prematurity. Causes of transient neonatal hypothyroidism are listed in Table 12.3. While iodine deficiency, iodine excess, and drugs are common causes of transient hypothyroidism in these babies, in some cases the cause is unknown. As noted above, a transient TSH elevation may represent a compensatory response in infants recovering from sick euthyroid syndrome.

Table 12.3. Differential diagnosis of transient congenital hypothyroidism.

Primary hypothyroidism
Prenatal or postnatal iodine deficiency or excess
Maternal antithyroid medication
Maternal TSH receptor-blocking antibodies
Secondary or tertiary hypothyroidism
Prenatal exposure to maternal hyperthyroidism
Prematurity (particularly < 27 weeks' gestation)
Drugs
Steroids
Dopamine
Miscellaneous
Isolated TSH elevation
Low T_4 with normal TSH
Prematurity
Illness
Undernutrition
Low T_3 syndrome

Iodine deficiency and iodine excess

Transient hypothyroidism due to both iodine deficiency and iodine excess is more common in relatively iodine-deficient areas of Europe than in North America, an iodine-sufficient region. In Belgium, for example, transient hypothyroidism was reported in 20% of premature infants, an eightfold higher prevalence than in North America. Administration of potassium iodide was successful in preventing this disorder. Because newborn infants are so susceptible to the adverse effects of iodine deficiency, the serum TSH on newborn screening has been shown to reflect the prevalence of iodine deficiency in a population. Premature infants are particularly at risk not only because of decreased thyroidal iodine stores accumulated *in utero*, but because of immaturity in the capacity for thyroid hormonogenesis, the hypothalamic–pituitary–thyroid axis, and in the ability to convert T_4 to the more metabolically active T_3. Furthermore, premature infants are in negative iodine balance for the first 1 or 2 weeks of postnatal life.

In addition to iodine deficiency, both the fetus and the newborn infant are sensitive to the thyroid-suppressive effects of excess iodine, whether administered to the mother during pregnancy or directly to the baby. This occurs, in part, because, as noted earlier, the fetus is unable to decrease thyroidal iodine uptake in response to an iodine load before 36 weeks' gestation. However, other factors, including increased skin absorption and decreased renal clearance of iodine in premature infants, are also likely to play a role.

Reported sources of iodine have included drugs (e.g. potassium iodide, amiodarone), radiocontrast agents (e.g. for intravenous pyelogram, oral cholecystogram, or amniofetography), and antiseptic solutions (e.g. povidone-iodine) used for skin cleansing or vaginal douches. In contrast to Europe, iodine-induced transient hypothyroidism has not been frequently documented in North America.

Maternal antithyroid medication

Transient neonatal hypothyroidism may develop in babies whose mothers are being treated with antithyroid medication [propylthiouracil (PTU), methimazole (MMI), or carbimazole] for the treatment of Graves' disease. The fetus appears to be particularly sensitive to the effects of antithyroid drugs even when the dosage used in the mother is within currently recommended guidelines. Babies with antithyroid drug-induced hypothyroidism characteristically develop an enlarged thyroid gland. At times, this goiter may be sufficiently large to cause respiratory embarrassment, especially with higher dosages. Both the hypothyroidism and the goiter resolve spontaneously with clearance of the drug from the baby's circulation. Replacement therapy is not usually required.

Maternal thyrotrophin receptor antibodies

Maternal TSH receptor blocking Abs, a population of Abs closely related to the TSH receptor stimulating Abs in Graves' disease, may be transmitted to the fetus in sufficient titer to cause transient CH. The incidence of this disorder has been estimated to be 1 in 180 000 in North America, equivalent to 20% of transient cases [48]. TSH receptor blocking Abs are found most often in mothers who have previously been treated for Graves' disease or who have the nongoitrous form of chronic lymphocytic thyroiditis ("primary myxedema"). Occasionally these mothers are not aware that they are hypothyroid, and the diagnosis is made in them only after CH has been recognized in their infants. Unlike TSH receptor-stimulating Abs that mimic the action of TSH, TSH receptor-blocking Abs inhibit both the binding and the action of TSH (see below). Because TSH-induced growth is blocked, these babies do not have a goiter; if the blocking Ab activity is sufficiently potent, rarely no thyroid tissue at all can be identified. More often, affected babies are misdiagnosed with thyroid agenesis because TSH-stimulated radioactive iodine uptake is inhibited. In contrast to findings on scintiscan, a normally placed thyroid gland can usually be visualized on ultrasound. The hypothyroidism generally resolves in 3 or 4 months when Ab is cleared from the neonatal circulation.

Babies with TSH receptor-blocking Ab-induced hypothyroidism are difficult to distinguish at birth from the more common thyroid dysgenesis, but they differ from the latter in a number of important ways (Table 12.4). They do not require lifelong therapy, and there is a high recurrence rate in subsequent offspring because of the tendency of these Abs to persist for many years in the maternal circulation. Unlike babies with thyroid dysgenesis in whom a normal cognitive outcome is found if postnatal therapy is early and adequate, babies with maternal blocking Ab-induced hypothyroidism may have a permanent deficit in intellectual development if feto-maternal hypothyroidism was present *in utero* [31].

Table 12.4. Comparison of clinical features of thyroid dysgenesis and TSH receptor-blocking Ab-induced congenital hypothyroidism.

Clinical feature	Thyroid dysgenesis	Blocking Ab-induced CH
Severity of CH	+ to ++++	+ to ++++
Palpable thyroid	No	No
^{123}I uptake	None to low	None to normal
Clinical course	Permanent	Transient
Familial risk	No	Yes
TPO Abs	Variable	Variable
TSH receptor Abs	Absent	Potent
Prognosis	Normal	May be delayed

Transient secondary and/or tertiary hypothyroidism

Occasionally, babies born to mothers who were hyperthyroid during pregnancy develop transient hypothalamic–pituitary suppression. This hypothyroxinemia is usually self-limited but, in some cases, it may last for years and require replacement therapy. In general, the titer of TSH receptor-stimulating Abs in this population of infants is lower than that in those who develop transient neonatal hyperthyroidism (see below). Other causes of transient secondary and tertiary hypothyroidism include prematurity (particularly infants < 27 weeks' gestation) and drugs frequently used in the neonatal intensive care unit (steroids, dopamine).

Other abnormalities of thyroid function discovered on newborn screening

Isolated hyperthyrotrophinemia

Isolated hyperthyrotrophinemia has been described primarily in screening programs that utilize a primary TSH method and is most common in premature infants. The etiology appears to be diverse. As a group, babies diagnosed with hyperthyrotrophinemia in infancy have a higher serum TSH compared with control children when re-examined in early childhood. Also, these infants have a higher prevalence of both thyroid morphological abnormalities, antithyroid antibodies, and mutations in thyroperoxidase and TSH receptor genes than do control infants. In babies whose blood specimen is obtained within the first day or two of life because

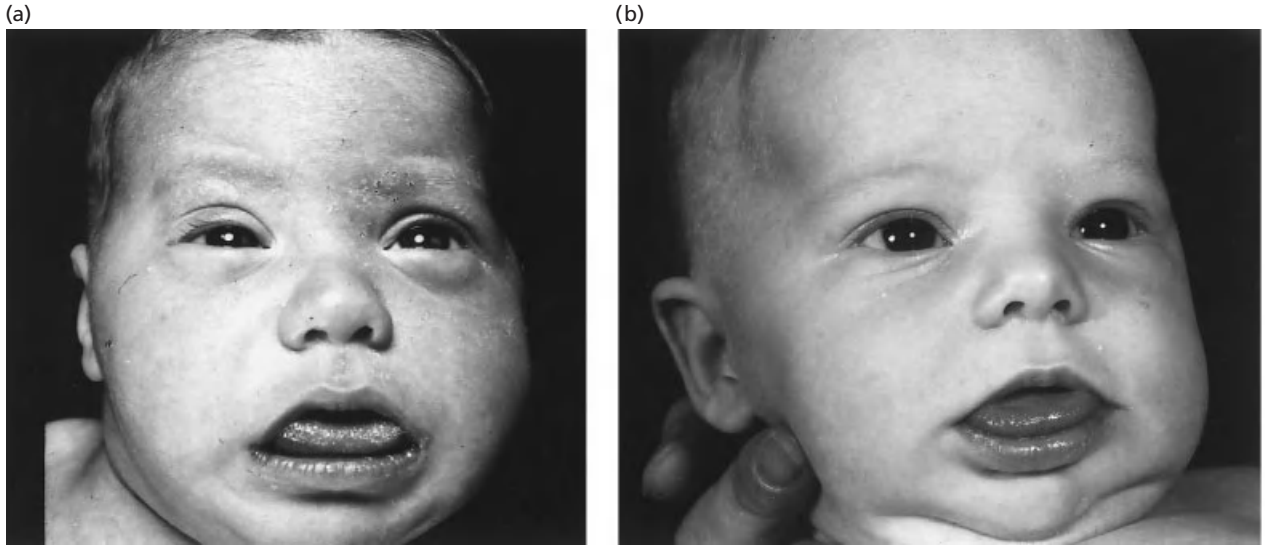


Fig. 12.9. Infant with severe, untreated congenital hypothyroidism diagnosed clinically prior to the advent of newborn screening (left), compared with an infant with congenital hypothyroidism identified through newborn screening (right). Note the striking difference in the severity of the clinical features.

of early discharge, isolated hyperthyrotrophinemia may be due to the cold-induced TSH surge observed postnatally. Maternal heterophile Abs that cross-react in the TSH radioimmunoassay have been implicated. Isolated hyperthyrotrophinemia of unknown etiology has been reported in babies in Japan. As some affected babies had a normal TSH and T_3 response to TRH and the TSH normalized without treatment, the hyperthyrotrophinemia was thought to have represented immaturity of the hypothalamic–pituitary–thyroid axis.

Hypothyroxinemia

Hypothyroxinemia in the presence of a "normal" TSH occurs most commonly in premature infants in whom it is found in 50% of babies of less than 30 weeks' gestation. Often, the free T_4, when measured by equilibrium dialysis, is less affected than the total T_4. In addition to hypothalamic–pituitary immaturity mentioned earlier, premature infants frequently have TBG deficiency due to both immature liver function and undernutrition, and they may have "sick euthyroid syndrome."

Abnormalities in thyroid-binding proteins, particularly TBG, may also cause hypothyroxinemia without associated hyperthyrotrophinemia. The incidence of TBG deficiency is 1 in 5000 to 1 in 12 000.

Low T_3 syndrome

The T_3 concentration in premature infants is lower than in full-term infants because of immaturity in the type 1 iodothyronine deiodinase enzyme. In addition, premature infants are frequently undernourished and suffer from a variety of illnesses, including respiratory distress syndrome, which aggravate the ability to convert T_4 to T_3. Serum T_3 values usually normalize within 2 months.

Clinical manifestations

Clinical evidence of hypothyroidism is usually difficult to appreciate in the newborn period. Many of the classic features (large tongue, hoarse cry, facial puffiness, umbilical hernia, hypotonia, mottling, cold hands and feet, and lethargy) are subtle and develop only with the passage of time. Figure 12.9 shows a baby with untreated CH diagnosed clinically compared with an infant in whom the diagnosis was made at 3 weeks of age in the early days of newborn screening. Non-specific signs that suggest the diagnosis of CH include prolonged, unconjugated hyperbilirubinemia, gestation longer than 42 weeks, feeding difficulties, delayed passage of stools, hypothermia, or respiratory distress in an infant weighing over 2.5 kg. A large anterior fontanelle and/or a posterior fontanelle > 0.5 cm is frequently present in affected infants but may not be appreciated.

In general, the extent of the clinical findings depends on the cause, severity, and duration of the hypothyroidism. Babies in whom severe feto-maternal hypothyroidism was present *in utero* tend to be the most symptomatic at birth. Similarly, babies with athyreosis or a complete block in thyroid hormonogenesis tend to have more signs and symptoms at birth than infants with an ectopic thyroid, the most common cause of CH.

Babies with CH are of normal size at birth. However, if diagnosis is delayed, subsequent linear growth is impaired. The finding of palpable thyroid tissue suggests that the hypothyroidism is due to an abnormality in thyroid hor-

monogenesis or in thyroid hormone action, or suggests that it will be transient.

Laboratory evaluation

Infants detected by newborn screening should be evaluated without delay, preferably within 24 h. The diagnosis of primary CH is confirmed by the demonstration of a decreased concentration of free T_4 and an elevated TSH level in serum. Most infants with permanent abnormalities of thyroid function have a serum TSH concentration > 50 mU/L, but even infants with less severe CH at birth have a higher incidence of permanent thyroid abnormalities than do babies with normal thyroid function at birth. Physicians should be aware that the serum T_4 concentration is much higher in full-term infants in the first 2 months of life (6.5–16.3 Bg/dL; 84–210 nmol/L) than in adults for whom reference values are given in many laboratories. Similarly, normal TSH values depend on gestational age and day of life. Normal values for thyroid function in the neonatal period have been published. Measurement of T_3 is of little value in the diagnosis of CH.

A bone age X-ray often is performed as a reflection of the duration and severity of the hypothyroidism *in utero*. Thyroid imaging provides information about the location and size of the thyroid gland. A radionuclide scan (either ^{123}I or ^{99m}pertechnetate) has long been the standard approach but, recently, color Doppler ultrasonography has been shown to be almost as sensitive as ^{123}I in identifying ectopic thyroid tissue, the most common cause of permanent CH [49]. Ectopic thyroid glands may be located anywhere along the pathway of thyroid descent from the foramen cecum to the anterior mediastinum. Thyroid imaging is helpful in verifying whether a permanent abnormality is present and aids in genetic counseling as thyroid dysgenesis is almost always a sporadic condition, whereas abnormalities in thyroid hormonogenesis are autosomal recessive. If scintigraphy is performed, ^{123}I, if available, is the preferred isotope because of the greater sensitivity and because ^{123}I, unlike technetium, is organified. Imaging with this isotope allows quantitative uptake measurements and tests for both iodine transport defects and abnormalities in thyroid oxidation. The lowest possible dose of ^{123}I, usually 25 μCi, should be used. Pertechnetate is cheaper and more widely available. There has been disagreement as to whether thyroid imaging by scintiscan should be performed in all babies because of the unknown risk of radiation exposure, particularly in centers where only ^{131}I is used in large doses. As color Doppler ultrasonography does not involve irradiation, this procedure offers promise as an alternative tool for initial thyroid imaging.

Apparent thyroid agenesis on scintiscan can be due to the presence of maternal TSH receptor-blocking Abs that completely inhibit TSH-induced thyroidal uptake of radioisotope, if present in a sufficiently high titer. Ultrasonography will usually document the presence of thyroid tissue in these cases. Autoimmune thyroid disease in the mother or a history of a previously affected sibling should alert the physician to this diagnosis, but such information is not always known. A binding assay (discussed further under Graves' disease) is appropriate for screening; bioassay can be done later if desired to demonstrate the biological action of the Abs.

In cases of TSH receptor Ab-induced CH, the blocking activity is extremely potent, half-maximal TSH binding-inhibition being reported with as little as a 1/20th to 1/50th dilution of serum; a weak or borderline result should cause a reconsideration of this diagnosis. TPO Abs, although frequently detectable in babies with blocking Ab-induced CH, are neither sensitive nor specific in predicting the presence of transient CH.

Other disorders that may mimic thyroid agenesis on thyroid scintigraphy include loss-of-function mutations of the TSH receptor, iodine excess, or an iodide-concentrating abnormality. Potential clues to the diagnosis of a loss-of-function mutation of the TSH receptor include a normal Tg and/or evidence of a thyroid gland on ultrasound examination despite the failure to visualize thyroid tissue on imaging studies. Verification of the diagnosis resides in the demonstration of a genetic abnormality in the TSH receptor gene.

Measurement of urinary iodine is helpful if a diagnosis of iodine-induced hypothyroidism is suspected. An iodide-concentrating defect should be suspected in patients with a family history of CH, particularly if an enlarged thyroid gland is present. The diagnosis is confirmed by the demonstration of decreased ^{123}I uptake on scan and by a salivary/blood ^{123}I ratio approaching unity. The detailed evaluation of infants suspected of having this and other abnormalities in thyroid hormonogenesis has been described elsewhere [50].

Measurement of Tg is most helpful in distinguishing a defect in Tg synthesis or secretion from other causes of thyroid dyshormonogenesis (iodide-trapping defect, organification defect). In the former condition, the serum Tg concentration is low or undetectable despite the presence of an enlarged, eutopic thyroid gland, but it is high in the latter condition. Serum Tg concentration also reflects the amount of thyroid tissue present and the degree of stimulation and is helpful, in association with ultrasound, in identifying patients with thyroid agenesis. For example, Tg is undetectable in most patients with thyroid agenesis and is intermediate in babies with an ectopic thyroid gland.

In babies in whom hypothyroxinemia unaccompanied by TSH elevation is found, free T_4 should be measured, preferably by a direct dialysis method, and the TBG concentration should be evaluated as well. The finding of a low free T_4 in the presence of a normal TBG may suggest the diagnosis of secondary or tertiary hypothyroidism, particularly if the patient has microphallus or a midline facial abnormality. In these cases, TRH testing has often been used to distinguish between a pituitary and a hypothalamic defect, but the utility

of this test has been questioned recently [51]. Pituitary function testing and brain imaging should also be performed in these infants.

In premature, low birthweight or sick babies in whom a low T_4 and "normal" TSH are found, the free T_4 when measured by a direct dialysis method is frequently not as low as the total T_4. In these infants, T_4 (and/or free T_4) and TSH should be repeated every 1–2 weeks until the T_4 normalizes because of the rare occurrence of delayed TSH rise. Thyroid function should also be monitored in other infants at risk of delayed TSH rise, such as severely ill babies in an intensive care setting and monozygotic twins in whom fetal blood mixing may initially mask the presence of CH. Even though many such babies will have transient hypothyroidism, treatment should be considered if values do not normalize within 1–2 weeks because any prolonged period of neonatal hypothyroidism, even if transient, could have adverse effects on cognitive development. In any infant, if signs or symptoms suggestive of hypothyroidism are present, thyroid function testing should be repeated because of the possibility of delayed onset of hypothyroidism and because of rare errors in the screening program.

An approach to the investigation of infants with abnormal results on newborn thyroid screening is presented in Figure 12.10.

Therapy

Replacement therapy with L-T_4 should begin as soon as the diagnosis of CH is confirmed. Parents should be counseled regarding the causes of CH, the importance of compliance, and the excellent prognosis in most babies if therapy is initiated early. Educational materials should be provided. Treatment need not be delayed in anticipation of performing a thyroid scan as long as this is done within 5–7 days of initiating treatment (before suppression of the serum TSH). An initial dosage of 10–15 Bg/kg is recommended to normalize the T_4 as soon as possible. Babies with compensated hypothyroidism may be started on the lower dosage, while those with severe CH [e.g. $T_4 < 5$ μg/dL (64 nmol/L)], such as those with thyroid agenesis, should be started on the higher dosage. Thyroid hormone may be crushed and administered with juice or formula, but care should be taken that all the medicine has been swallowed. Thyroid hormone should not be given with substances that interfere with its absorption, such as iron, soy, or fiber. Many babies will swallow the pills whole or chew the tablets with their gums even before they have teeth. Liquid preparations are unstable and should not be used.

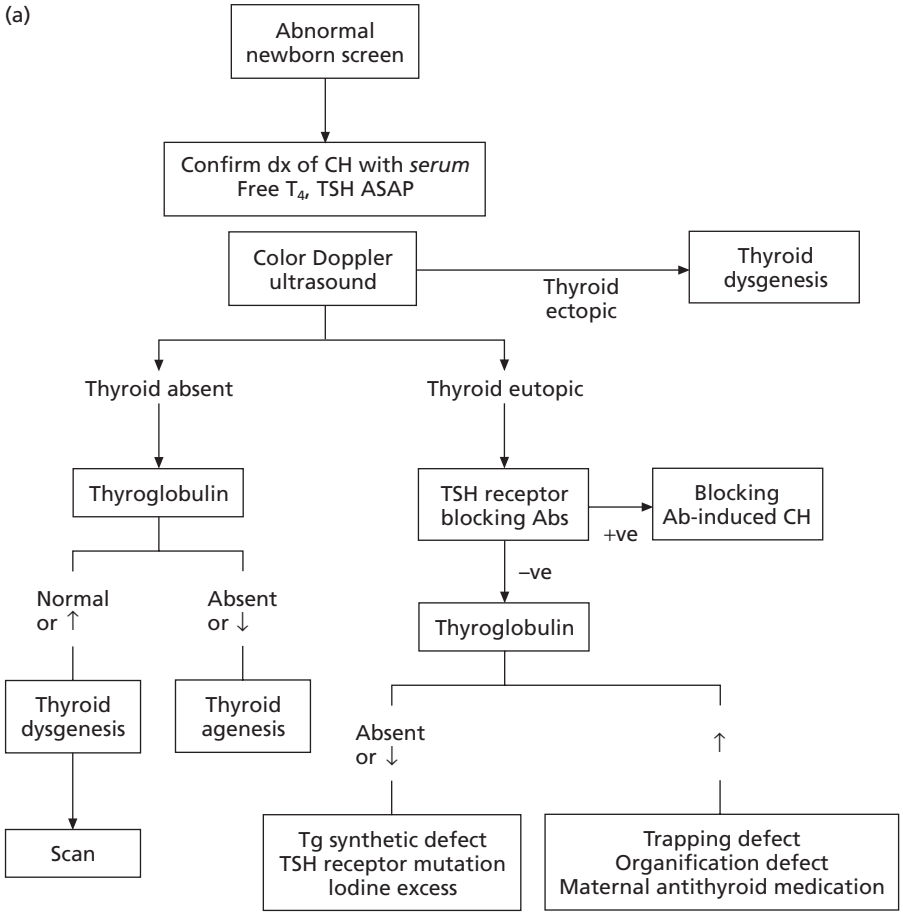
The aims of therapy are to normalize the serum T_4 concentration as soon as possible, to avoid hyperthyroidism, and to promote normal growth and development. On the above dose, serum T_4 concentrations normalize in most infants within 1 week and the TSH within 1 month. Whether or not more rapid normalization would improve outcome in the most severely affected infants is not known. Subsequent adjustments in the dosage of medication are made according to the results of thyroid function tests and the clinical picture. Some infants develop supraphysiological serum T_4 values, but the serum T_3 concentration usually remains normal, most affected infants are not symptomatic, and these short-term T_4 elevations have not been reported to be associated with adverse effects on growth, bony maturation, or cognitive development.

Normalization of the TSH concentration may sometimes be delayed because of relative pituitary resistance. In such cases, characterized by a normal or increased serum T_4 and an inappropriately high TSH level, the T_4 value is used to titrate the dosage of medication, but non-compliance is the most common cause and should be excluded.

Current recommendations are to repeat T_4 and TSH at 2 and 4 weeks after the initiation of L-thyroxine treatment, every 1–2 months during the first year of life, every 2–3 months between 1 and 3 years of age, and every 3–12 months thereafter until growth is complete. In hypothyroid babies in whom an organic basis was not established at birth and in whom transient disease is suspected, a trial of replacement therapy can be initiated after the age of 3 years when most thyroid hormone-dependent brain maturation has occurred.

Whether or not premature infants with hypothyroxinemia should be treated remains controversial. Early retrospective investigations failed to document a difference in cognitive outcome in premature infants with hypothyroxinemia compared with control subjects, but small numbers were studied. A relationship has been shown between severe hypothyroxinemia and both developmental delay and disabling cerebral palsy in preterm infants < 32 weeks' gestation. Whether or not the poorer prognosis in these infants is causal or coincidental cannot be determined, however, as the serum T_4 in premature infants, as in adults, has been shown to reflect the severity of illness and risk of death. There are conflicting results on the effect of therapeutic intervention with T_4 or T_3 on neurocognitive outcome, mortality rate, and respiratory function.

In the most thorough study to date, a placebo-controlled, double-blind trial of T_4 treatment, 8 μg/kg/day for 6 weeks was carried out in 200 infants less than 30 weeks' gestation [52]. Although overall no difference in cognitive outcome was found, there was an 18-point increase in the Bayley Mental Development Index score in the subgroup of T_4-treated infants < 27 weeks' gestation. Of some concern was the additional finding that treatment with T_4 was associated with a 10-point decrease in mental score ($P = 0.03$) in infants > 27 weeks' gestation. While further studies are needed, it would seem reasonable at the present time to treat any premature infant with a low T_4 and elevated TSH and to consider treatment of any infant < 27 weeks' with a low T_4 whether or not the TSH is elevated. A dosage of 8 μg/kg/day for these infants has been recommended. Whether or not to treat older



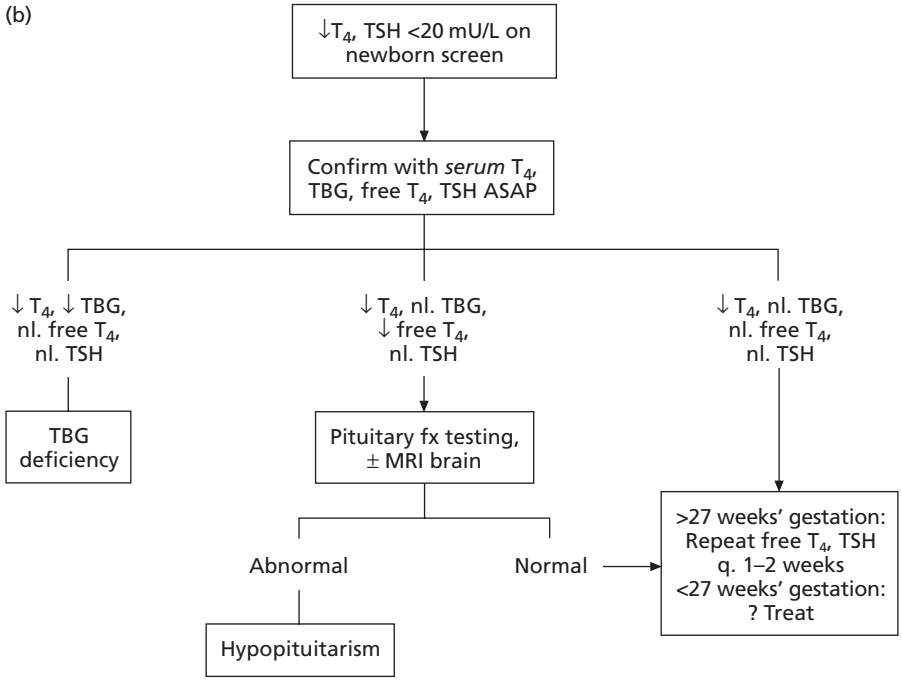


Fig. 12.10. Suggested initial approach to the investigation of an infant with congenital hypothyroidism (a) if TSH is elevated and (b) if TSH is normal. See text for details.

premature infants with hypothyroxinemia and what dosage to use remain uncertain.

Prognosis

Numerous studies have been performed to evaluate the cognitive outcome of babies with CH detected on newborn screening. In the initial reports, despite the eradication of severe mental retardation, the intellectual quotient (IQ) of affected infants was 6–19 points lower than that of control babies. Although this IQ deficit was small, it was nonetheless significant as judged by a fourfold increase in the need for special education in affected children. In addition, sensorineural hearing loss, sustained attention problems, and various neuropsychological variables were noted, although the frequency and severity of these abnormalities were much less than in the prescreening era. Those babies most likely to have permanent intellectual sequelae were infants with the most severe *in utero* hypothyroidism as determined by initial T_4 level [< 5 μg/dL (64 nmol/L)] and skeletal maturation at birth. These findings led to the widely held conclusion at the time that some cognitive deficits in the most severely affected babies might not be reversible by postnatal therapy.

In the initial programs, an L-T_4 dosage of 5–8 μg/kg was used and treatment was not initiated until 4–5 weeks of age. In contrast, accumulating data from a number of different studies have demonstrated that, when a higher initial treatment dose is used (10–15 μg/kg) and treatment is initiated earlier (before 2 weeks), this "developmental gap" can be closed, irrespective of the severity of the CH at birth. Dosage and timing of onset of therapy are independent variables [53]. Whether or not the higher starting dose is associated with increased temperamental difficulties and attention problems, particularly in less severely affected infants, remains controversial [54]. Combined therapy with T_4 and T_3 offers no advantage over T_4 alone [55].

Neonatal hyperthyroidism

Transient neonatal hyperthyroidism (neonatal Graves' disease)

Unlike CH which is usually permanent, neonatal hyperthyroidism is almost always transient. It results from the transplacental passage of maternal TSH receptor-stimulating Abs. Hyperthyroidism develops only in babies born to mothers with the most potent stimulatory activity in serum, corresponding to 2–3% of mothers with Graves' disease, or 1 in 50 000 newborns. The incidence is approximately four times higher than that for transient neonatal hypothyroidism due to maternal TSH receptor-blocking Abs. Both TSH receptor Ab potency, severity, and duration of *in utero* hyperthyroidism and maternal antithyroid medication are important determinants of neonatal thyroid status. Most babies born to mothers with Graves' disease have normal thyroid function. These outcomes are summarized in Table 12.5 [56].

Table 12.5. Thyroid function of neonates born to mothers with Graves' disease ($n = 230$)*.

	n	%
Thyrotoxicosis		
Clinical	6	2.6
Chemical	7	3.0
Hypothyroidism		
T_4 TSH	5	2.2
Normal T_4, TSH	18	7.8
Central hypothyroidism	2	0.9
Euthyroid	192	83.5

*From [56].

Some mothers have mixtures of stimulating and blocking Abs in their circulation, the relative proportion of which may change over time. Not surprisingly, the clinical picture in the fetus and neonate of these mothers is complex and depends not only on the relative proportion of each activity in the maternal circulation at any one time, but also on the rate of their clearance from the neonatal circulation after birth. Thus, one affected mother gave birth in turn to a normal infant, a baby with transient hyperthyroidism, and one with transient hypothyroidism. In another neonate, the onset of hyperthyroidism did not become apparent until 1–2 months post partum when the higher affinity blocking Abs had been cleared from the neonatal circulation. In the last case, multiple monoclonal TSH receptor-stimulating and -blocking Abs were cloned from peripheral lymphocytes in the mother's blood. Each monoclonal Ab recognized different antigenic determinants ("epitopes") on the receptor and had different functional properties. Neonatal hyperthyroidism may occur in infants born to hypothyroid mothers, the maternal thyroid having been destroyed by prior radioablation, surgery, or destructive autoimmune processes so that potent thyroid-stimulating Abs, present in the maternal circulation, are silent in contrast to the neonate whose thyroid gland is normal.

Clinical manifestations

Although maternal TSH receptor Ab-mediated hyperthyroidism may present *in utero*, the onset is usually toward the end of the first week of life. This is due both to the clearance of maternally administered antithyroid drugs from the infant's circulation and to the increased conversion of T_4 to T_3 after birth. The onset of neonatal hyperthyroidism may be delayed if higher affinity blocking Abs are also present.

Fetal hyperthyroidism is suspected in the presence of fetal tachycardia (pulse greater than 160/min) especially if there is

evidence of failure to thrive. In the newborn infant, characteristic signs and symptoms include tachycardia, irritability, poor weight gain, and prominent eyes. Goiter may be related to maternal antithyroid drug treatment as well as to the neonatal Graves' disease itself. Rarely, infants with neonatal Graves' disease present with thrombocytopenia, hepatosplenomegaly, jaundice, and hypoprothrombinemia, a picture that may be confused with congenital infections. Dysrhythmias and cardiac failure may develop and may cause death, particularly if treatment is delayed or inadequate. In addition to a significant mortality rate that approximates 20% in some older series, untreated fetal and neonatal hyperthyroidism is associated with deleterious long-term consequences, including premature closure of the cranial sutures (cranial synostosis), failure to thrive, and developmental delay.

The half-life of TSH receptor Abs is 1–2 weeks. The duration of neonatal hyperthyroidism, a function of Ab potency and metabolic clearance rate, is usually 2–3 months but may be longer.

Laboratory evaluation

Because of the importance of early diagnosis and treatment, fetuses and infants at risk for neonatal hyperthyroidism should undergo both clinical and biochemical assessment. A high index of suspicion is necessary in babies of women who have had thyroid ablation because a high titer of TSH receptor Abs would not be evident clinically. Similarly, women with persistently elevated TSH receptor Abs and with a high requirement for antithyroid medication during pregnancy are at an increased risk of having an affected child.

The diagnosis of hyperthyroidism is confirmed by the demonstration of an increased concentration of T_4, free T_4, T_3, and free T_3 accompanied by a suppressed TSH. Fetal ultrasonography may help to detect a goiter and monitor fetal growth. If necessary, blood can be obtained by cordocentesis, and results compared with normal values during gestation. Demonstration of a high titer of TSH receptor Abs in the baby or mother will confirm the etiology of the hyperthyroidism and, in babies whose thyroid function testing is normal initially, indicate the degree to which the baby is at risk. In general, babies likely to become hyperthyroid have the highest TSH receptor Ab titer whereas, if TSH receptor Abs are not detectable, the baby is most unlikely to become hyperthyroid. In the latter case, it can be anticipated that the baby will be euthyroid, have transient hypothalamic–pituitary suppression, or have a transiently elevated TSH, depending on the relative contribution of maternal hyperthyroidism vs. the effects of maternal antithyroid medication. Therapy is rarely necessary, whether TSH receptor Abs are measured by radioreceptor assay or by bioassay. On the other hand, if TSH receptor Ab potency is intermediate, it is likely that the baby will be euthyroid, have a transiently elevated T_4, or have transient hypothalamic–pituitary suppression.

The sensitivity of the different TSH receptor Ab assays varies, so specific values that are recommended in the literature should be interpreted with caution. Close follow-up of all babies with abnormal thyroid function tests or detectable TSH receptor Abs is mandatory.

Therapy

Treatment of the fetus is accomplished by maternal administration of antithyroid medication. The minimal dosage of PTU or MMI necessary to normalize the fetal heart rate and render the mother euthyroid or slightly hyperthyroid is usually chosen. In the neonate, treatment is expectant. Either PTU (5–10 mg/kg/day) or MMI (0.5–1.0 mg/kg/day) can be used initially in three divided doses. If the hyperthyroidism is severe, a strong iodine solution (Lugol's solution or SSKI, one drop every 8 h) is added to block the release of thyroid hormone immediately because the effect of PTU and MMI may be delayed for several days. Therapy with both PTU and iodine is adjusted subsequently, depending on the response. Propranolol (2 mg/kg/day in two or three divided doses) is added if sympathetic overstimulation is severe, particularly in the presence of pronounced tachycardia. If cardiac failure develops, treatment with digoxin should be initiated, and propranolol should be discontinued.

Rarely, prednisone (2 mg/kg/day) is added for immediate inhibition of thyroid hormone secretion and decreased generation of T_3 from T_4 in peripheral tissues. Alternatively, sodium ipodate (0.5 g every 3 days), an iodine-containing radiocontrast material that inhibits both thyroid hormone secretion and the conversion of T_4 to T_3, has been used successfully as the sole treatment of neonatal hyperthyroidism. Measurement of TSH receptor Abs in treated babies may be helpful in predicting when antithyroid medication can be safely discontinued. Lactating mothers on antithyroid medication can continue nursing as long as the dosage of PTU or MMI does not exceed 400 mg or 40 mg respectively. As the milk–serum ratio of PTU is one-tenth that of MMI, a consequence of pH differences and increased protein binding, PTU is preferable to MMI although relatively low doses of MMI can be given to nursing mothers with no adverse effects on the baby. At higher dosages of antithyroid medication, close supervision of the infant is advisable.

Permanent neonatal hyperthyroidism

Rarely, neonatal hyperthyroidism is permanent and is due to a germline mutation in the TSH receptor resulting in its constitutive activation. A gain-of-function mutation of the TSH receptor should be suspected if persistent neonatal hyperthyroidism occurs in the absence of detectable TSH receptor Abs in the maternal circulation. Most cases result from a mutation in exon 10, which encodes the transmembrane domain and intracytoplasmic tail (Fig. 12.2) [5]. Less

frequently, a mutation encoding the extracellular domain has been described. An autosomal-dominant inheritance has been noted in many of these infants, but other cases have been sporadic, arising from a *de novo* mutation. Early recognition is important because the thyroid function of affected infants is frequently difficult to manage medically. When diagnosis and therapy are delayed, irreversible sequelae, such as cranial synostosis and developmental delay, may result. For this reason, early, aggressive therapy with either thyroidectomy or even radioablation has been recommended.

Thyroid disease in childhood and adolescence

Hypothyroidism

Chronic lymphocytic thyroiditis

The causes of hypothyroidism after the neonatal period are listed in Table 12.6. The most frequent cause is chronic lymphocytic thyroiditis (CLT), an autoimmune disease that is closely related to Graves' disease. In both CLT and Graves' disease both a background inherited predisposition to autoimmunity and additional environmental and hormonal factors that trigger and modulate the disease process appear to be involved [57]. In CLT, lymphocyte- and cytokine-mediated thyroid destruction predominates whereas, in Graves' disease, Ab-mediated thyroid stimulation occurs, but overlap may occur in some patients. Both a goitrous (Hashimoto's thyroiditis) and a non-goitrous (primary myxedema) variant of thyroiditis have been distinguished. The disease has a striking predilection for females, and a family history of autoimmune thyroid disease (both CLT and Graves' disease) is found in 30–40% of patients. During childhood, the most common age at presentation is adolescence, but the disease may occur at any age, even infancy.

Patients with insulin-dependent diabetes mellitus, 20% of whom have positive thyroid Abs and 5% of whom have an elevated serum TSH level, have an increased prevalence of CLT, which may also occur as part of an autoimmune polyglandular syndrome (APS). In APS 1, also called APECED (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy) syndrome, CLT is found in 10% of patients. APS 1 is associated with defective cell-mediated immunity and presents in childhood. It results from a mutation in the AIRE (autoimmune regulator) gene. CLT and diabetes mellitus with or without adrenal insufficiency (APS 2, also referred to as Schmidt syndrome) tend to occur later in childhood or in the adult. In addition to these polyglandular syndromes, there is an increased incidence of CLT in patients with Down, Turner, Klinefelter, and Noonan syndromes. CLT may be associated with chronic urticaria and with immune complex glomerulonephritis.

Table 12.6. Differential diagnosis of juvenile hypothyroidism.

Primary hypothyroidism
Chronic lymphocytic thyroiditis
Goitrous (Hashimoto's)
Atrophic (primary myxedema)
Congenital abnormality
Thyroid dysgenesis
Inborn error of thyroid hormonogenesis
Iodine deficiency (endemic goiter)
Drugs or goitrogens
Antithyroid drugs (PTU, MMI, carbimazole)
Anticonvulsants
Other (lithium, thionamides, aminosalicylic acid, aminoglutethimide)
Goitrogens (cassava, water pollutants, cabbage, sweet potatoes, cauliflower, broccoli, soybeans)
Miscellaneous
Cistinosis
Histiocytosis X
Irradiation of the thyroid
Radioactive iodine
External irradiation of non-thyroid tumors
Surgery
Mitochondrial disease
Infantile hemangioma
Secondary or tertiary hypothyroidism
Congenital abnormality
Acquired
Hypothalamic or pituitary tumor (especially craniopharyngioma)
Treatment of brain and other tumors
Surgery
Radiation

Antibodies to Tg and TPO ("microsomal"), the thyroid Abs measured in routine clinical practice, are detectable in over 95% of patients with CLT. They are useful as markers of underlying autoimmune thyroid damage, TPO Abs being more sensitive and specific. TSH receptor Abs are also found in a small proportion of patients. When stimulatory TSH receptor Abs are present, they may give rise to a clinical picture of hyperthyroidism, the co-existence of CLT and Graves' disease being known as hashitoxicosis. Blocking Abs, on the other hand, have been postulated to underlie both the hypothyroidism and the absence of goiter in some patients with primary myxedema, but are detectable in only a minority of children. In rare instances, the disappearance of blocking Abs has been associated with a normalization of thyroid function in previously hypothyroid patients.

Goiter, present in approximately two-thirds of children with CLT, results primarily from lymphocytic infiltration and, in some patients, from a compensatory increase in TSH. The role of Abs in goitrogenesis is controversial. Contrary to previous

beliefs, accumulating evidence now suggests that primary myxedema arises as a result of independent immune mechanisms and does not represent the "burned out" phase of CLT.

Children with CLT may be euthyroid or may have compensated or overt hypothyroidism. Rarely, they may experience an initial thyrotoxic phase due to the discharge of preformed T_4 and T_3 from the damaged gland. Alternatively, as indicated above, thyrotoxicosis may be due to concomitant thyroid stimulation by TSH receptor stimulatory Abs (hashitoxicosis).

Long-term follow-up studies of children with CLT have suggested that, while most children who are hypothyroid initially remain hypothyroid, spontaneous recovery of thyroid function may occur, particularly in those with initial compensated hypothyroidism. On the other hand, some initially euthyroid patients will become hypothyroid with observation. Therefore, whether or not treatment is initiated (see below), close follow-up is necessary.

Other causes of acquired hypothyroidism

Thyroid dysgenesis and inborn errors of thyroid hormonogenesis

Occasionally, patients with thyroid dysgenesis will escape detection by newborn screening and present later in childhood with non-goitrous hypothyroidism or with an enlarging mass at the base of the tongue or along the course of the thyroglossal duct. Similarly, children with inborn errors of thyroid hormonogenesis may only be recognized later in childhood because of the detection of a goiter.

Iodine and other micronutrient deficiency; natural goitrogens

Iodine deficiency continues to be a major public health problem. Endemic cretinism, the most serious consequence of iodine deficiency, occurs only in areas where the problem is most severe. Hypothyroidism in older infants, children, and adults is seen in regions of moderate iodine deficiency. It develops when adaptive mechanisms fail and may be exacerbated by the coincident ingestion of goitrogen-containing foods, such as cassava, soybeans, broccoli, cabbage, sweet potatoes, and cauliflower, or by certain water pollutants. Iodine deficiency can be due to dietary restriction (for multiple food allergies) or the result of a fad. Thiocyanate-containing foods (broccoli, sweet potatoes, and cauliflower) block trapping and subsequent organification of iodine. Iodine deficiency may also be exacerbated by lack of selenium, a component of the selenocysteine thyroid hormone deiodinases.

Drugs

A number of drugs used in childhood may affect thyroid function. These include antithyroid medication, certain anticonvulsants, lithium, thionamides, aminosalicylic acid, and aminoglutethimide.

Secondary or tertiary hypothyroidism

Secondary or tertiary hypothyroidism may be recognized later in childhood. They may develop as a result of acquired damage to the pituitary or hypothalamus by tumors (particularly craniopharyngioma), granulomatous disease, head irradiation, infection (meningitis), surgery, or trauma. Other pituitary hormones are often affected, particularly growth hormone and gonadotropins.

Thyroid hormone resistance

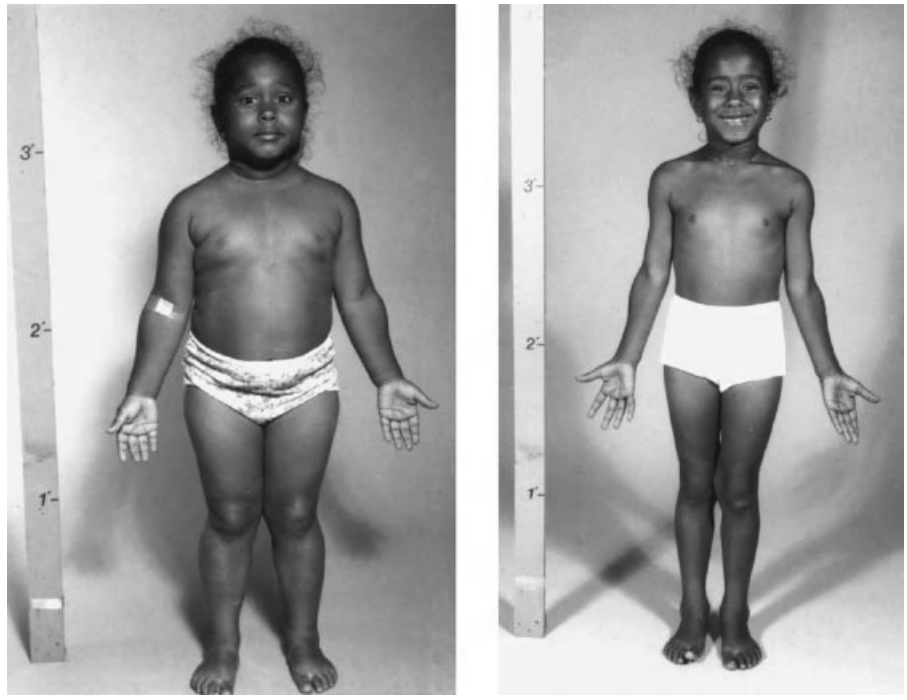
Children with thyroid hormone resistance usually come to attention when thyroid function tests are performed because of poor growth, hyperactivity, a learning disability, or other non-specific signs or symptoms. A small goiter may be present. The presentation is highly variable, and some individuals may be completely asymptomatic, whereas others may have symptoms of both thyroid hormone deficiency and excess. In the past, some individuals have been classified as having selective pituitary resistance as distinct from generalized resistance to thyroid hormone because they appeared to have evidence of peripheral hypermetabolism in response to the elevated thyroid hormone levels. However, variable levels of expression of the mutant allele have not been demonstrated. Thus, it has been suggested that the variable clinical manifestations of this syndrome are a result of the genetic heterogeneity of the many cofactors that modulate TR expression.

Thyroid hormone resistance is most frequently caused by a point mutation in the hinge region or ligand-binding domain of the TRβ gene (Fig. 12.4). As a consequence, there is a dramatic reduction in T_3 binding. Less frequently, it results from impaired interaction with one of the cofactors involved in the mediation of thyroid hormone action. Because these mutant TRs interfere with the function of the normal TRs, a dominant pattern of inheritance is seen. In contrast, in the single family with a deletion of all coding sequences of the TRβ gene, only homozygotes manifest resistance.

Rarely, thyroid hormone resistance may be found in patients with cystinosis.

Miscellaneous causes of acquired hypothyroidism

The thyroid gland may be involved in generalized infiltrative (cystinosis), granulomatous (histiocytosis X), or infectious disease processes that are of sufficient severity to result in a disturbance in thyroid function. Hypothyroidism may also occur in patients with mitochondrial disease [58]. Rarely in infancy, a large hemangioma with high D3 activity can be associated with rapid inactivation of T_4 and severe hypothyroidism [59]. Extremely high T_4 replacement doses may be required.



(a) (b)

Fig. 12.11. Ten-year-old girl with severe I^- hypothyroidism due to primary myxedema before (a) and after (b) treatment. Presenting complaint was poor growth. Note the dull facies, relative obesity, and immature body proportions prior to treatment. At age 10 years, she had not lost a deciduous tooth. After treatment was initiated, she lost six teeth in 10 months and had striking catch-up growth. Bone age was 5 years at a chronologic age of 10 years. TSH receptor-blocking antibodies were negative.

Both mantle irradiation for Hodgkin disease or lymphoma and external irradiation of brain tumors may result in hypothyroidism. In the former case, primary hypothyroidism develops; in the latter case, both primary and secondary hypothyroidism may occur because of the inclusion of the neck in the radiation field.

Clinical manifestations

The onset of hypothyroidism in childhood is insidious. Affected children are usually recognized either because of the detection of a goiter on routine examination or because of poor growth, sometimes for several years prior to diagnosis. Because linear growth tends to be more affected than weight, affected children are relatively overweight for their height, although they are rarely significantly obese (Fig. 12.11). If the hypothyroidism is severe and longstanding, immature facies with an underdeveloped nasal bridge and immature body proportions (increased upper–lower body ratio) may be noted. Dental and skeletal maturation are delayed, the latter often significantly. Patients with secondary or tertiary hypothyroidism tend to be even less symptomatic than those with primary hypothyroidism.

The classical clinical manifestations of hypothyroidism can be elicited on careful evaluation; however, they are often not the presenting complaints. They include lethargy, cold intolerance, constipation, dry skin or hair texture, and periorbital edema. School performance is not usually affected, in contrast to the severe irreversible neuro-intellectual sequelae that occur in inadequately treated babies with CH.

Causes of hypothyroidism associated with a goiter (CLT, inborn errors of thyroid hormonogenesis, thyroid hormone resistance) should be distinguished from non-goitrous causes (primary myxedema, thyroid dysgenesis, secondary or tertiary hypothyroidism). The typical thyroid gland in CLT is diffusely enlarged and has a rubbery consistency. Although the surface is classically described as "pebbly" or bosselated, asymmetric enlargement occurs occasionally and must be distinguished from thyroid neoplasia. An enlarged pyramidal lobe or Delphian lymph node superior to the isthmus can be found and may be confused with a thyroid nodule. A delayed relaxation time of the deep tendon reflexes may be appreciated in more severe cases.

In patients with severe hypothyroidism of longstanding duration, the sella turcica may be enlarged due to thyrotrope hyperplasia. There is an increased incidence of slipped femoral capital epiphyses in hypothyroid children. The combination of severe hypothyroidism and muscular hypertrophy, which gives the child a "Herculean" appearance, is known as the Kocher–Debre–Semelaign syndrome.

Puberty tends to be delayed in hypothyroid children, although sexual precocity has been described in longstanding, severe hypothyroidism. Females may menstruate but commonly have breast development with little sexual hair. Ovarian cysts may be demonstrated on ultrasonography due to FSH secretion. Galactorrhea due to hyperprolactinemia

may occur occasionally. In boys, isolated testicular enlargement may be found.

Laboratory evaluation

Measurement of TSH is the best initial screening test for the presence of primary hypothyroidism. If the TSH is elevated, measurement of free T_4 will distinguish whether the child has compensated (normal free T_4) or overt (low free T_4) hypothyroidism.

Measurement of TSH is not helpful in secondary or tertiary hypothyroidism. Hypothyroidism in these cases is demonstrated by the presence of a low free T_4 with a low TSH. A hypothalamic vs. pituitary origin of the hypothyroidism can sometimes be distinguished by TRH testing, but the value of this procedure has been questioned recently. In hypopituitarism, there is little or no TSH response to TRH. Occasionally, mild TSH elevation is seen in individuals with hypothalamic hypothyroidism, a consequence of the secretion of a TSH molecule with impaired bioactivity but normal immunoreactivity. Thyroid hormone resistance is characterized by elevated levels of free T_4 and T_3 and an inappropriately normal or elevated TSH concentration.

CLT is diagnosed by elevated titers of Tg and/or TPO Abs. Ancillary investigations (thyroid ultrasonography and/or thyroid scintigraphy) may be performed if thyroid Ab tests are negative or if a nodule is palpable, but are rarely necessary. The typical picture of spotty uptake of radioactive iodine that is seen in adults is rare in children. If thyroid Ab tests are negative and no goiter is present, thyroid ultrasonography and/or scan identify the presence and location of thyroid tissue, and thereby distinguish primary myxedema from thyroid dysgenesis. Inborn errors of thyroid hormonogenesis beyond a trapping defect are usually suspected by an increased radioiodine uptake and a large gland on scan.

Therapy

In contrast to CH, rapid replacement is not essential in the older child. This is particularly true in children with longstanding, severe thyroid underactivity in whom rapid normalization may result in unwanted side-effects (deterioration in school performance, short attention span, hyperactivity, insomnia, and behavior difficulties). Replacement doses should be increased very slowly over several weeks to months. Severely hypothyroid children should also be observed closely for complaints of severe headache when therapy is initiated because of the rare development of pseudotumor cerebri. In contrast, full replacement can be initiated at once without much risk of adverse consequences in children with mild hypothyroidism.

Treatment of children with compensated hypothyroidism, also called mild thyroid failure (normal T_4, elevated TSH) is controversial. Some physicians treat all such patients while others choose to reassess thyroid function in 3–6 months before initiating therapy because of the possibility that the thyroid abnormality will be transient. In adults, compensated hypothyroidism has been associated in some patients with a variety of systemic hypothyroid or neuropsychiatric complaints and with mild lipid abnormalities [60]. Treatment has been advocated both for symptom relief and because of the risk of progression to overt hypothyroidism, a risk particularly in older individuals with a positive titer of anti-TPO Abs [61]. An expert US panel recently recommended observation without treatment of adult patients whose TSH level was < 10 mU/L regardless of Ab titer [62]. When signs and symptoms suggestive of hypothyroidism are present, a trial of L-T_4 therapy can be tried.

The typical replacement dose of L-T_4 in childhood is approximately 100 Bg/m^2 or 4–6 Bg/kg for children 1–5 years of age, 3–4 Bg/kg for those aged 6–10 years, and 2–3 Bg/kg for those 11 years of age and older. In patients with a goiter, a somewhat higher L-thyroxine T_4 dosage is used in order to keep the TSH in the low normal range (0.3–1.0 mU/L in an ultrasensitive assay) and thereby minimize its goitrogenic effect. Whether and how patients with thyroid hormone resistance should be treated is controversial [63].

After the child has received the recommended dosage for at least 6–8 weeks, T_4 and TSH should be measured. Once a euthyroid state has been achieved, patients should be monitored every 6–12 months. Close attention is paid to interval growth and bone age as well as to the maintenance of a euthyroid state. Some children with severe, longstanding hypothyroidism at diagnosis may not achieve their adult height potential even with optimal therapy, emphasizing the importance of early diagnosis and treatment. Treatment is usually continued indefinitely.

Asymptomatic goiter

Goiter occurs in 4–6% of schoolchildren in iodine-sufficient areas. Like thyroid disease in general, there is a female preponderance, the female–male ratio being 2 to 3:1. Patients with goiter may be euthyroid, hypothyroid, or hyperthyroid, euthyroid goiters being by far the most common. The most frequent cause of asymptomatic goiter is CLT.

Colloid or simple (non-toxic) goiter

Colloid goiter is another important cause of euthyroid thyroid enlargement in childhood. Not infrequently, there is a family history of goiter, CLT, and Graves' disease, leading to the suggestion that colloid goiter, too, might be an autoimmune disease. Thyroid growth immunoglobulins have been identified in a proportion of patients with simple goiter, but their etiological role is controversial. It is important to distinguish patients with colloid goiter from CLT because of

the risk of developing hypothyroidism in patients with CLT, but not colloid goiter. Whereas many colloid goiters regress spontaneously, others appear to undergo periods of growth and regression, ultimately resulting in the large nodular thyroid glands later in life.

Clinical manifestations and laboratory investigation

Evaluation of thyroid function by measurement of the serum TSH concentration is the initial approach to diagnosis. In euthyroid patients, the most common situation, CLT should be distinguished from colloid goiter. Clinical examination in both instances reveals a diffusely enlarged thyroid gland. Therefore, the distinction is dependent upon the presence of elevated titers of TPO and/or Tg Abs in CLT but not colloid goiter. All patients with negative thyroid Abs initially should have repeat examinations because some children with CLT will develop positive titers with time.

Therapy

Thyroid suppression in children with a euthyroid goiter is controversial. There is no evidence of efficacy in CLT, and no long-term studies are available in children with colloid goiter. A therapeutic trial may be tried when the goiter is large. In some cases, surgery may be required for cosmesis.

Painful thyroid

Painful thyroid enlargement is rare in pediatrics and suggests the probability of either acute (suppurative) or subacute thyroiditis. Occasionally, CLT may be associated with intermittent pain and be confused with these disorders. In acute thyroiditis, progression to abscess formation may occur rapidly so prompt recognition and antibiotic therapy are essential. Recurrent attacks and involvement of the left lobe suggest a pyriform sinus fistula between the oropharynx and the thyroid as the route of infection. In the latter case, surgical extirpation of the pyriform sinus will frequently prevent further attacks.

Subacute thyroiditis, unusual in childhood, is characterized by fever, general malaise, thyroid enlargement, and tenderness. Thyroid function may be normal or elevated, the result of the release of preformed T_4 and T_3 into the circulation. Unlike Graves' disease, radioactive iodine uptake is low or absent. A low titer of TPO and Tg Abs may be found, and the sedimentation rate is elevated. Characteristically, the initial thyrotoxic phase persists for 1–4 weeks and is followed by a period of transient hypothyroidism as the thyroid gland recovers. Treatment is supportive and includes large doses of acetylsalicylic acid or other anti-inflammatory drugs. In severe cases, corticosteroid medication may be helpful. Antithyroid medication is not indicated.

Table 12.7. Causes of thyrotoxicosis in childhood.

Hyperthyroidism
Diffuse toxic goiter (Graves' disease)
Nodular toxic goiter (Plummer disease)
TSH-induced hyperthyroidism
TSH producing pituitary tumor
"Selective pituitary resistance" to thyroid hormone
Thyrotoxicosis without hyperthyroidism
Chronic lymphocytic thyroiditis
Subacute thyroiditis
Thyroid hormone ingestion

Hyperthyroidism

Graves' disease

The causes of hyperthyroidism in childhood and adolescence are indicated in Table 12.7. More than 95% of cases of hyperthyroidism are due to Graves' disease, an autoimmune disorder that, like CLT, occurs in a genetically predisposed population. Genetic predisposition, which has been estimated to account for 70% of the risk, consists of a series of interacting susceptibility alleles of several different genes important in antigen recognition and/or immune modulation. A number of genes, each with small effect, have been implicated [57]. These include genes on the major histocompatibility (MHC) locus (HLA-B8, HLA-DR-3, and possibly HLA-DQA1*0501) and polymorphisms of cytotoxic lymphocyte antigen (CTLA)-4, an immunoregulatory molecule that is expressed on the surface of activated lymphocytes and inhibits T-lymphocyte activation. In addition, several putative novel loci have been identified by whole genome scanning. There is a strong female predisposition, the female–male ratio being 6 to 8:1. Graves' disease is much less common in childhood than in the adult, although it can occur at any age, especially in adolescence. Prepubertal children tend to have more severe disease, require longer medical therapy, and achieve a lower rate of remission compared with pubertal children. This appears to be particularly true in children who present at < 5 years of age.

Graves' disease has been described in children with other autoimmune diseases, both endocrine and non-endocrine. These include diabetes mellitus, Addison's disease, vitiligo, systemic lupus erythematosis, rheumatoid arthritis, myasthenia gravis, periodic paralysis, idiopathic thrombocytopenia purpura, and pernicious anemia. There is an increased risk of Graves' disease in children with Down syndrome (trisomy 21) and DiGeorge syndrome (22q11 deletion) [15].

Unlike CLT, in which thyrocyte damage is predominant, the major clinical manifestations of Graves' disease are hyperthyroidism and goiter. Graves' disease is caused by

TSH receptor Abs that mimic the action of TSH. Binding of ligand results in stimulation of adenyl cyclase with subsequent thyroid hormonogenesis and growth. TSH receptor-blocking Abs inhibit TSH-induced stimulation of adenyl cyclase. Both stimulatory and blocking TSH receptor Abs bind to the extracellular domain of the receptor and appear to recognize apparently discrete linear epitopes in the context of a three-dimensional structure, but the specific epitope(s) with which they interact is different. Stimulatory Abs bind to the amino-terminal portion of the extracellular domain, while blocking Abs bind to the carboxy-terminal domain. Studies using monoclonal TSH receptor Abs cloned from patients' peripheral lymphocytes and recombinant mutant TSH receptor have demonstrated that multiple TSH receptor Abs exist, each with different specificities and functional activities. In general, blocking Abs are more potent inhibitors of TSH binding than are stimulatory ones.

The clinical assessment of TSH receptor Abs takes advantage of their ability to bind to the TSH receptor [binding assay, e.g. radioreceptor assay, coated tube chemiluminescent assay, or enzyme-linked immunosorbent assay (ELISA)] or to stimulate (or inhibit) TSH-induced stimulation of adenyl cyclase (bioassay). In general, binding assays detect the presence of Abs interacting at the receptor whatever their biological activity. Results of bioassays that utilize the rat FRTL-5 cell line, commonly used in the past, should be interpreted with caution as these cells lose sensitivity with repeated passage. Technical improvements, including the development of reliable commercial chemiluminescent assays and bioassays utilizing recombinant human TSH receptor, have greatly improved both the sensitivity and the specificity of these assays [64,65]. Most children with Graves' disease also have TPO and/or Tg Abs in their sera, but measurement of the latter is less sensitive and less specific than measurement of TSH receptor Abs.

Rarer causes of hyperthyroidism

Hyperthyroidism may be caused by a functioning thyroid adenoma, by constitutive activation of the TSH receptor, or may be part of the McCune–Albright syndrome (Table 12.6). Rarely, hyperthyroidism may be due to inappropriately elevated TSH secretion, the result of either a TSH-secreting pituitary adenoma or pituitary resistance to thyroid hormone.

Miscellaneous causes of thyrotoxicosis without hyperthyroidism include the toxic phase of CLT, subacute thyroiditis, and thyroid hormone ingestion (thyrotoxicosis factitia). Thyroxine may be abused by adolescents trying to lose weight or may inadvertently be eaten by toddlers. When the resultant thyrotoxicosis is severe, treatment with iopanoic acid may be effective.

Clinical manifestations

All but a few children with Graves' disease present with some degree of thyroid enlargement, and most have symptoms and signs of excessive thyroid activity, such as tremors, inability to fall asleep, weight loss despite an increased appetite, proximal muscle weakness, heat intolerance, headache, and tachycardia. Often the onset is insidious. Shortened attention span and emotional lability may lead to severe behavioral and school difficulties. Some patients complain of polyuria and of nocturia, the result of an increased glomerular filtration rate. Acceleration in linear growth may occur, often accompanied by advancement in skeletal maturation (bone age). Adult height is not affected. In the adolescent child, puberty may be delayed. If menarche has occurred, secondary amenorrhea is common. If sleep is disturbed, the patient may complain of fatigue.

Physical examination reveals a diffusely enlarged, soft or "fleshy" thyroid gland, smooth skin and fine hair texture, excessive activity, and a fine tremor of the tongue and fingers. A thyroid bruit may be audible. The finding of a thyroid nodule suggests the possibility of a toxic adenoma. The hands are often warm and moist. Tachycardia, a wide pulse pressure, and a hyperactive precordium are common. Café-au-lait spots, particularly in association with precocious puberty, on the other hand, suggest a possible diagnosis of McCune–Albright syndrome but, if a goiter is absent, thyrotoxicosis factitia should be considered. Severe ophthalmopathy is considerably less common in children than in adults, although a stare and mild proptosis are frequently observed.

Laboratory evaluation

The clinical diagnosis of hyperthyroidism is confirmed by the finding of increased concentrations of circulating thyroid hormones. Demonstration of a suppressed TSH excludes much rarer causes of thyrotoxicosis, such as TSH-induced hyperthyroidism and pituitary resistance to thyroid hormone in which the TSH is inappropriately "normal" or slightly elevated. If these diseases are suspected, the free α-subunit, which is elevated in patients with a pituitary TSH-secreting tumor, should be measured. Alternatively, elevated levels of T_4 in association with inappropriately "normal" levels of TSH may be due to an excess of thyroxine-binding globulin (either familial or acquired) or to increased T_4 binding by a mutant albumin ("familial dysalbuminemic hyperthyroxinemia"). In the latter cases, both free T_4, total and free T_3, and serum TBG concentration will be normal. An important acquired cause of thyroxine-binding globulin excess is estrogen excess, e.g. secondary to oral contraceptive use or pregnancy.

If the diagnosis of Graves' disease is unclear, TSH receptor Abs should be measured. A binding assay, preferably utilizing one of the newer, chemiluminescent, coated tube methodologies, is appropriate for initial screening because it is sensitive and technically relatively simple, rapid, and reproducible. Bioassay, although of no advantage in screening, may be useful in the occasional Graves' disease patient who is negative in the binding assay or in treated patients whose clinical picture is discordant with results in the binding assay. Some individuals, initially negative in the radioreceptor assay, become positive several weeks later. It has been hypothesized that TSH receptor Ab synthesis in these patients is restricted at first to lymphocytes residing within the thyroid gland itself or, alternatively, that TSH receptor Abs escape detection because of binding by soluble TSH receptor circulating in serum. Alternatively, negative results may have been due to relative assay insensitivity. Measurement of TSH receptor Abs may be particularly useful in distinguishing the toxic phase of CLT and subacute thyroiditis (TSH receptor Ab negative) from patients with both CLT and Graves' disease ("hashitoxicosis," TSH receptor antibody positive). As noted above, Tg and/or TPO Abs are often present but are less sensitive and specific than TSH receptor Abs in the diagnosis of Graves' disease in childhood. Radioactive iodine uptake and scan are necessary to confirm the diagnosis of Graves' disease only in atypical cases (for example, if measurement of TSH receptor Abs is negative and if the thyrotoxic phase of either CLT or subacute thyroiditis or a functioning thyroid nodule is suspected).

Therapy

The choice of which of the three therapeutic options (medical therapy, radioactive iodine, or surgery) to use should be individualized and discussed with the patient and his/her family. Each approach has its advantages and disadvantages. Medical therapy with one of the thiouracil derivates (PTU or MMI) is the initial choice of most pediatricians, although radioiodine is gaining increasing acceptance, particularly in non-compliant adolescents, in children who are mentally retarded, and in those about to leave home (for example to go to college).

Medical therapy

PTU, MMI, and carbimazole (converted to MMI) exert their antithyroid effect by inhibiting the organification of iodine and the coupling of iodotyrosine residues on the Tg molecule to generate T_3 and T_4. MMI is preferred by many pediatric endocrinologists because, for an equivalent dose, it requires taking fewer tablets and has a longer half-life, an advantage in non-compliant adolescents. In addition, MMI is associated with a more rapid resolution of the hyperthyroidism, with MMI, but not PTU, the rate of minor side-effects appears to be dose related, and the severe side-effects are seen almost exclusively in patients taking PTU [61]. On the other hand, PTU, but not MMI, inhibits the conversion of T_4 to the more active isomer T_3, a potential advantage if the thyrotoxicosis is severe. The usual initial dosage of MMI is 0.5 mg/kg/day (given once or twice daily) and that of PTU is 5 mg/kg/day given thrice daily. Carbimazole is best given in a dose of 10–20 mg twice or thrice daily depending on the concentration of free T_4. Recently, the European Multicentre Trial has demonstrated that, in adults, a low initial MMI dose (10 mg daily) is almost as effective as a high dose (40 mg daily) in normalizing thyroid function tests within 3–6 weeks. Extrapolating these data to children, it is possible that starting doses of MMI lower than those usually recommended may be equally effective and preferable for children with mild to moderate disease in view of the decreased likelihood of side-effects. In severe cases, a beta-adrenergic blocker (propranolol, 0.5–2.0 mg/kg/day given every 8 h) can be added to control the cardiovascular overactivity until a euthyroid state is obtained.

The serum concentrations of T_4 and T_3 normalize in 3–6 weeks, but the TSH concentration may not return to normal until several months later. Therefore, measurement of TSH is useful as a guide to therapy only after it has normalized but not initially. Once the T_4 and T_3 have normalized, one can either decrease the dose of thioamide drug by 30–50% or, alternatively, wait until the TSH begins to rise and add a supplementary dose of L-thyroxine in a block replacement regimen. Advocates of the block replacement regimen cite the fewer hospital visits, but a larger MMI dose is required, perhaps resulting in a higher incidence of side-effects. Also, initial studies suggesting that combined therapy might be associated with an improved rate of remission have not been confirmed. Maintenance doses of PTU may be given twice daily and of MMI once daily.

In adults, there does not appear to be any advantage in treating most patients for more than a year. The optimum duration of therapy in children and adolescents is not known. Approximately 50% of children will go into long-term remission within 4 years, with a continuing remission rate of 25% every 2 years for up to 6 years of treatment. Lack of eye signs, small goiter, and, in patients treated with antithyroid drugs alone, a small drug requirement are favorable indicators that drug therapy can be tapered gradually and withdrawn. Lower initial degree of hyperthyroxinemia [T_4 < 20 Bg/dL (257.4 nmol/L); T_3/T_4 ratio < 20], body mass index, and older age (pubertal vs. prepubertal age) have been associated with an increased likelihood of permanent remission. Persistence of TSH receptor Abs, on the other hand, indicates a high likelihood of relapse.

Toxic drug reactions [erythematous rashes, urticaria, arthralgias, transient granulocytopenia, (< 1500 granulocytes/mm^3)] have been reported in 5–14% of children. Rarely, hepatitis, a lupus-like syndrome, thrombocytopenia,

and agranulocytosis, (< 250 granulocytes/mm^3) may occur. Most reactions are mild and do not contraindicate continued use. In more severe cases, switching to the other thioamide is frequently effective. The risk of hepatitis and agranulocytosis appears to be greater within the first 3 months of therapy; there is some evidence that close monitoring of the white blood cell count during this initial time period may be useful in identifying agranulocytosis prior to the development of a fever and infection. Many authors also recommend checking the white blood cell count and liver function tests before therapy because Graves' disease itself can be associated with abnormalities in these parameters. It is important to caution all patients to stop their medication immediately and consult their physician should they develop unexplained fever, sore throat, gingival sores, or jaundice. Approximately 10% of children treated medically will develop long-term hypothyroidism later in life, a consequence of coincident cell- and cytokine-mediated destruction and/or the development of TSH receptor-blocking Abs.

Radioactive iodine

Definitive therapy with either medical (radioactive iodine) or surgical thyroid ablation is usually reserved for patients who have failed drug therapy, developed a toxic drug reaction, or are non-compliant. Radioactive iodine (RAI) is being favored increasingly in some centers, even as the initial approach to therapy [66]. The advantages are the relative ease of administration, the reduced need for medical follow-up, and the lack of demonstrable long-term adverse effects. On the other hand, as the goal of therapy is thyroid ablation, daily medication with L-T_4 rather than MMI is nonetheless necessary.

Radioactive iodine therapy should be used with caution in children < 10 years of age and particularly in those 5 years of age or less because of the increased susceptibility of the thyroid gland in the young to the proliferative effects of ionizing radiation. Almost all patients who developed papillary thyroid cancer after the Chernobyl disaster were children less than 10 years old at the time of the reactor malfunction. Similarly, the risk of benign thyroid nodules following radioactive iodine therapy for Graves' disease is greatest in the first decade of life.

Although a dose of 50–200 μCi of ^{131}I/estimated gram of thyroid tissue has been used, the higher dosage is recommended, particularly in younger children, in order completely to ablate the thyroid gland and thereby reduce the risk of future neoplasia. The size of the thyroid gland is estimated, based on the assumption that the normal gland is 0.5–1.0 g/year of age, maximum 15–20 g. The formula used is:

(estimated thyroid weight in grams × 50–200 μCi ^{131}I)/(fractional ^{131}I 24-h uptake).

Pretreatment with antithyroid drugs prior to RAI therapy is not necessary unless the hyperthyroidism is very severe.

Thyroid hormone concentrations may rise transiently 4–10 days after RAI administration owing to the release of preformed hormone from the damaged gland. Beta blockers may be useful. Analgesics may be necessary for the discomfort of radiation thyroiditis. Other acute complications of RAI therapy (nausea, significant neck swelling) are rare. One usually sees a therapeutic effect within 6 weeks to 3 months.

Worsening of ophthalmopathy, described in adults after RAI, does not appear to be common in childhood but, if significant ophthalmopathy is present, RAI therapy should be used with caution, and treatment with corticosteroids for 6–8 weeks after RAI administration may be wise. Alternatively, another permanent treatment modality (surgery) should be considered. In approximately 1000 children with Graves' disease treated with RAI and followed for < 5 to > 20 years to date, there did not appear to be any increased rate of congenital anomalies in offspring nor of thyroid cancer. However, the numbers of younger children treated with RAI and followed long term are small.

Surgery

Surgery is performed less frequently now than previously. Its major advantage is the rapid resolution of the hyperthyroidism. Near-total or subtotal thyroidectomy is performed depending on whether the goal is to minimize the risk of recurrence or render the patient euthyroid respectively. Surgery is appropriate for patients who have failed medical management, those who have a markedly enlarged thyroid, those who refuse radioactive iodine therapy, and for the rare patient with significant eye disease in whom radioactive iodine therapy is contraindicated. Because of the potential complications of transient hypocalcemia, recurrent laryngeal nerve paralysis, hypoparathyroidism, and, rarely (as with all forms of surgery), death, this therapy should be performed only by an experienced pediatric thyroid surgeon. Occasionally, unsightly keloid formation occurs at the site of the scar.

The child must be euthyroid before surgery. Iodides (Lugol's solution, 5–10 drops twice a day; or potassium iodide, 2–10 drops daily) are added for 7–14 days before surgery in order to decrease the vascularity of the gland.

After both medical and surgical thyroid ablation, most patients become hypothyroid and require lifelong thyroid replacement therapy. On the other hand, if therapy is inadequate, hyperthyroidism may recur.

Thyroid nodules

Thyroid nodules occur in only 0.05–1.8% of children and adolescents and, as such, are rare in the first two decades of life in iodine-sufficient populations. However, in comparison with adults in whom thyroid nodules are much more common (incidence 50% after the sixth decade of life), they are much

more likely to be carcinomatous. Follicular adenomas and colloid cysts account for the majority of benign thyroid nodules. Other causes of benign nodular enlargement include CLT and embryological defects, such as intrathyroidal duct cysts or unilateral thyroid agenesis. The most common form of cancer is papillary thyroid carcinoma, but other histological types found in the adult, such as follicular and anaplastic carcinomas, may also occur. Thyroid lymphomas are rare.

Clinical evaluation

A high index of suspicion is necessary if the nodule is painless, of firm or hard consistency, or if it is fixed to surrounding tissues, especially if it has undergone rapid growth, or if there is cervical adenopathy, hoarseness, or dysphagia. Children whose thyroids have been exposed to irradiation comprise a particularly high-risk group. Medullary thyroid carcinoma should be considered if there is a family history of thyroid cancer or pheochromocytoma and/or if the child has multiple mucosal neuromas and a marfanoid habitus, findings consistent with multiple endocrine neoplasia, types IIa and/or IIb respectively. Extrathyroidal manifestations suspicious of other syndromes associated with nodular thyroid disease (Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome, familial adenomatous polyposis) should be sought [67].

Laboratory evaluation

The initial investigation includes evaluation of thyroid function and measurement of anti-TPO and anti-Tg Abs. A suppressed serum TSH concentration, accompanied by an elevation in the circulating T_4 and/or T_3 suggests the possibility of a functioning nodule. Positive Abs, although indicating the presence of underlying CLT, does not exclude the possibility of co-existent thyroid cancer. Evidence of autoimmune thyroiditis was obtained in seven of 39 (18%) pediatric papillary thyroid cancer patients in one recent study. Serum calcitonin should be measured if medullary thyroid carcinoma, of C-cell origin, is a concern, such as in familial cases of thyroid cancer, but its routine measurement in the evaluation of thyroid nodules is controversial. Genetic screening for a mutation of the RET proto-oncogene should be performed if multiple endocrine neoplasia type 2a is suspected [68].

Ultrasound

Ultrasound examination has replaced thyroid scintiscan as the preferred imaging procedure to confirm and evaluate the morphological characteristics of a thyroid nodule [69]. Nodules that are cystic or homogeneously hyperechoic are reputed to carry a lower risk of malignancy. Conversely, a solid hypoechoic echotexture, calcifications, irregular shape, or absence of a halo are features associated with malignant nodules. Ultimately, there are no sonographic findings that reliably predict the likelihood of malignancy, and so biopsy is indicated for all thyroid nodules ≥ 1 cm. Performance of thyroid ultrasonography by individuals experienced in this procedure facilitates both the accurate identification of true thyroid nodules and their subsequent follow-up. Often, additional non-palpable nodules are discovered by ultrasonography and are later found to be malignant. Similarly, ultrasonography is helpful in the non-invasive monitoring of nodules too small to biopsy or those with benign cytology, and in surveillance for local recurrence in patients diagnosed with thyroid carcinoma.

Fine-needle aspiration

Fine-needle aspiration biopsy, popular in the investigation of thyroid carcinoma in adults, has been used increasingly, particularly in older children. Ultrasound guidance improves the diagnostic accuracy and safety of this procedure [60]. This is important, as up to 20% of fine-needle aspirations are insufficient or non-diagnostic. Repeat aspiration of non-diagnostic fine-needle aspirations is usually successful. Papillary thyroid cancer, the most common malignant tumor of the thyroid in childhood, is readily identifiable on cytology by the presence of characteristic nuclear abnormalities. However, follicular carcinoma is difficult to differentiate from follicular adenoma, and so documentation of capsular and/or vascular invasion is required. Benign cytology obviates surgical resection. Conversely, in patients with atypical cytology, the degree and type of cytological abnormality allow a more specific assignment of cancer risk and facilitate the discussion of surgical options with the family. If the child is very young or very anxious, open excisional biopsy is a suitable alternative.

Therapy

Excision of the tumor or lobe is the appropriate treatment for benign tumors and cysts. Total or near-total thyroidectomy with preservation of the parathyroid glands and recurrent laryngeal nerves is the optimal therapy for malignant thyroid tumors as it facilitates radioiodine ablation and subsequent monitoring for recurrence and disease progression [70,71]. However, one can reserve initial bilateral surgery for patients at high risk for malignancy, such as those whose cytology predicts a > 50% likelihood of differentiated thyroid cancer or who have bilateral nodules with abnormal cytology. For other patients, thyroid lobectomy can be performed initially, followed by completion thyroidectomy only if lobectomy confirms the diagnosis of cancer. This approach reduces the risk of complications for the majority of patients with benign lesions. Referral to a surgeon with a low personal complication rate and extensive experience with thyroidectomy is required even for lobectomy as surgeon experience is the primary determinant of operative morbidity [72]. Although

unilateral vocal cord paralysis or parathyroid injury may not compromise activities of daily life, these complications increase the risk of permanent morbidity with future surgeries (completion thyroidectomy or neck dissection for local recurrence) in those patients who are found to have cancer at lobectomy.

Children with thyroid nodules < 1 cm or with benign cytology should be followed chronically by serial ultrasound every 6–12 months, and ultrasound-guided fine-needle aspiration should be repeated if significant interval growth or other concerning sonographic features develop.

Radioactive iodine

Even after total thyroidectomy, radioiodine uptake usually persists in the thyroid bed as a result of residual normal thyroid tissue. Ablation of this thyroid remnant with radioactive iodine (RAI) has been shown to lower recurrence rates and, in some series, to reduce cancer mortality, presumably because of the destruction of malignant or premalignant thyrocytes within the macroscopically normal remnant [70,71]. Similar to completion thyroidectomy, radioiodine remnant ablation also facilitates disease surveillance by increasing the specificity of Tg measurements and the sensitivity of diagnostic whole body scans. Remnant radioablation, defined as the destruction of residual macroscopically normal thyroid tissue after surgical thyroidectomy, should be distinguished from RAI therapy, which describes the use of higher ^{131}I doses to destroy local or distal differentiated thyroid cancer. Dosimetric guidelines for adults for both remnant ablation and RAI therapy of nodal metastases have been published [73]. For any given administered dose, the absorbed radiation dose to normal tissues will be higher in young children secondary to their smaller organ volumes and increased cross-radiation due to the shorter distances between organs. Formulae for the estimation of relative pediatric doses should be consulted [74]. Both formal quantitative dosimetry and standardized, empiric, fixed dose methods have been used. An interval of at least 12 months between ^{131}I treatments is recommended to minimize the risk of leukemia. Pulmonary fibrosis is another potential consequence of RAI.

The efficacy of radiation therapy is enhanced by clinical interventions that increase thyroidal iodine uptake, such as an elevated circulating TSH concentration and a low iodine diet for 1–2 weeks before the procedure. L-T_4 withdrawal is the standard method of achieving an elevated TSH. In adults, recombinant TSH avoids the discomfort of hypothyroidism after L-T_4 withdrawal and appears to provide equivalent results, but similar studies have not been performed in children. Prepubertal children are more likely to experience nausea and vomiting with ^{131}I therapy so antiemetic medications should be available. After RAI therapy, the dosage of L-T_4 replacement is adjusted to keep the serum TSH concentration suppressed (between 0.05 and 0.1 mU/L in sensitive assays). Measurement of serum Tg, a thyroid follicular cell-specific protein, is used to detect evidence of metastatic disease in differentiated forms of thyroid cancer, such as papillary or follicular cancer. This is best performed after a period of L-T_4 withdrawal or after exogenous administration of recombinant TSH.

Follow-up treatment and surveillance

After radioiodine therapy, the dosage of L-T_4 is adjusted to keep the serum TSH concentration suppressed (± 0.1 mU/L in sensitive assays). The suppression of endogenous TSH secretion reduces cancer recurrence and, in some series, cancer-related death. Measurement of serum Tg, a thyroid follicular cell-specific protein, is used to detect evidence of metastatic disease in differentiated forms of thyroid cancer, such as papillary or follicular cancer. However, it should be noted that Tg is also produced by normal thyroid tissue. Therefore, the utility of this test is best after thyroidectomy and remnant ablation. Approximately 15–30% of patients with differentiated thyroid cancer possess circulating anti-Tg Abs that can confound commercially available Tg assays. Accordingly, serum should be screened for the presence of Abs every time Tg is measured. Serum Tg Abs may become negative with time after thyroidectomy, however, so serum Tg should be monitored every 6–12 months even in those children who have interfering Abs at the time of diagnosis. As with radionuclide scanning, the sensitivity of Tg measurements is greatest when the TSH is elevated, either after a period of L-T_4 withdrawal or after exogenous administration of recombinant TSH.

Based upon data in adults, 68% of differentiated thyroid cancer recurrences are local (cervical or mediastinal) [71]. Accordingly, surveillance should include annual neck imaging. Thyroid ultrasound is a sensitive and, when coupled with ultrasound-guided fine-needle aspiration, a specific modality to screen for local recurrence. Computed tomography (CT) or MRI are alternative modalities. Local recurrences that are palpable or easily visualized with ultrasound or CT should be excised surgically rather than treated with radioactive iodine. L-T_4 withdrawals or recombinant TSH-stimulated serum Tg measurements and diagnostic whole body scanning should be performed at yearly intervals as indicated throughout childhood. ^{123}I is the preferred agent for diagnostic whole body scanning as it is a pure gamma emitter, minimizing the patient's radiation exposure and avoiding the theoretical concern of "stunning."

Prognosis

Based upon the retrospective analyses of large patient cohorts, primary tumor size > 1.5 cm, local tumor invasion, regional lymph node metastases, age over 40 years, and delay in therapy by > 12 months are poor prognostic factors.

Conversely, the likelihood of cancer death is decreased by female sex, surgery more extensive than lobectomy, and adjunctive therapy with RAI and TSH suppression. In comparison with adults, pediatric thyroid cancers are characterized by high rates of regional lymph node (74%) and distal metastases (25%). Rates of recurrence (13–42%) are also higher in children, illustrated in one series of 50 children, in which 28% developed distant metastases, 8% upon initial presentation and 20% as recurrences [70,71,75]. However, reports of cause-specific mortality vary widely from 0% to 18%, a consequence of both the rarity of differentiated thyroid cancer in childhood and the relatively shorter duration of reported follow-up. Therefore, contrary to popular opinion, when controlled for duration of follow-up and staging, most published pediatric cause-specific mortality rates are actually higher than for the vast majority of adult thyroid cancer patients.

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13 The parathyroid and disorders of calcium metabolism

Jeremy Allgrove

Introduction

Calcium plays a part in human physiology that is unique in many ways. Not only does it have an important role in maintaining normal neuromuscular function, but it is also an important component of bone and therefore has a structural role as well. The mechanisms that exist to juggle these two processes are complex and can be disrupted by a wide variety of causes.

Physiology of calcium metabolism

Calcium

Calcium circulates in plasma in three fractions. The most important is the ionized fraction, which constitutes about 50% of the total and is maintained at a concentration of between 1.1 and 1.3 mmol/L. It is this that determines optimal neuromuscular function and is itself maintained by the various endocrine factors responsible for its stability. Most of the remainder, approximately 40%, circulates bound to albumin, and conditions associated with hypoalbuminemia may reduce the total circulating concentration without affecting the ionized calcium. The remainder of the total calcium circulates complexed to other molecules such as citrate and sulfate.

It is possible to measure ionized calcium directly, although this is not routinely available in most laboratories, which usually measure total calcium and albumin, often making an adjustment to allow for the albumin concentration. In practice, these adjustments usually have little clinical relevance unless severe hypoalbuminemia is present. A suitable correction factor is:

$$Ca_{CORR} = Ca_{TOTAL} + (41 - [Alb])*0.017$$

where Ca_{TOTAL} is the observed total calcium and [Alb] is the plasma albumin in g/L.

Calcium is absorbed in the upper small bowel via an active transcellular transport mechanism that is stimulated by the action of 1α,25-dihydroxyvitamin D (1α,25$(OH)_2$D) and is saturable and a smaller passive paracellular mechanism. Absorption of calcium may be reduced in the presence of large quantities of calcium-binding agents such as phytate or oxalate. Excretion is mainly via the kidney and is influenced by a number of dietary factors including sodium, protein, and acid load, all of which increase calcium excretion [1]. Most reabsorption occurs in the proximal tubule (70%) with a further 20% in the ascending loop of Henle. Only 5–10% is reabsorbed in the distal tubule, but it is this that is mainly under hormonal control [2]. During childhood, particularly during the phases of rapid growth in infancy and adolescence, calcium absorption exceeds excretion sufficiently to allow for bone mineralization, and it is at these times that the highest proportion of ingested calcium is absorbed.

Urinary calcium excretion is most easily measured by assessing the ratio of calcium to creatinine (Ca/Cr) in a mid-morning urine specimen, which should not normally exceed 0.7 mmol/mmol [3]. Excess calcium excretion occurs in hypercalcemic conditions associated with hyperparathyroidism and vitamin D excess, as well as in those not associated with hypercalcemia such as activating mutations of the calcium-sensing receptor and distal renal tubular acidosis, all of which may cause nephrocalcinosis. In contrast, hypercalcemia associated with inactivating mutations of the calcium-sensing receptor or hypocalcemia caused by hypoparathyroidism results in low urinary calcium excretion.

Phosphate

Phosphate circulates in plasma in the form of phospholipids, phosphate esters, and free inorganic phosphate (P_i). Plasma P_i concentrations are not tightly controlled and reflect the fluxes of phosphate entering and leaving the extracellular pool. In contrast to calcium, phosphate concentrations in plasma vary considerably during life being highest during phases of rapid growth. Thus, phosphate concentrations in

premature infants are normally above 2.0 mmol/L, falling to 1.3–2.0 mmol/L during infancy and childhood and to 0.7–1.3 mmol/L in young adults.

Phosphate is readily absorbed throughout the small bowel by both passive and active mechanisms. The total amount absorbed is largely dependent on the dietary phosphate load but may be inhibited by phosphate-binding agents such as calcium carbonate. This is of value in hyperphosphatemic states such as chronic renal failure when phosphate absorption needs to be limited.

Regulation of plasma phosphate occurs principally in the renal tubule. Between 85% and 98% of the filtered load of phosphate is reabsorbed, mainly by the proximal renal tubule. This represents about 10 times the amount absorbed via the intestine. The tubular reabsorption of phosphate by the renal tubule (TRP) is a saturable process determined both by the filtered load, which is itself determined by glomerular filtration rate (GFR) and plasma concentration, and by hormonal factors, particularly parathyroid hormone (PTH), which increases phosphate excretion.

Assessment of phosphate excretion is crucial to diagnosing some conditions, particularly hypophosphatemic rickets. It is most easily assessed by measuring the fractional excretion of phosphate (FE_{PO4}). This is best described as the ratio of the clearance of phosphate to the clearance of creatinine, which therefore requires estimation of plasma and urine phosphate and creatinine on single samples taken simultaneously. It makes the assumption that creatinine clearance approximates to GFR but does not require any timed urine samples. FE_{PO4} is calculated according to the formula:

$$FE_{PO4} = [U_{PO4}]/[P_{PO4}] \times [P_{Creat}]/[U_{Creat}]$$

where all the results are expressed in mmol/L.

TRP is $1–FE_{PO4}$ and is usually expressed as a percentage. Values for TRP are normally in excess of 85% and frequently approach 98% in children. In hyperphosphaturic conditions, the value is frequently below 50%, but this parameter is dependent on the filtered load of phosphate: the lower the plasma concentration, the greater the proportion that can be reabsorbed. A more precise measure of renal tubular phosphate handling, which eliminates any effect of plasma phosphate, can be obtained by calculating the theoretical tubular maximal phosphate threshold as a function of GFR ($TmPO_4$/GFR). This is most easily obtained by the nomogram of Walton and Bijvoet [4] from the plasma phosphate concentration and the FE_{PO4}. $TmPO_4$/GFR is reduced in hyperparathyroidism and phosphate-losing conditions and increased in hypoparathyroidism. It is higher in children and adolescents than in adults [5].

Intrinsic control of phosphate excretion is determined by two genes, PHEX and FGF23. The PHEX (phosphate-regulating gene with homology to endopeptidases on the X chromosome) gene encodes a 749-amino-acid membrane glycoprotein and is present in several tissues but not kidney [6]. It is located on the X chromosome, and mutations in this gene cause classical autosomal-dominant X-linked hypophosphatemic rickets. To date, 179 mutations have been described, and an online database has been established (http://phexdb.mcgill.ca). It is not clear how it causes excess phosphaturia, but it may do so by humoral factors, possibly by interaction with FGF23.

The FGF23 gene is located on chromosome 12p13 and encodes a 251-amino-acid peptide that is further processed to amino- and carboxy-terminal fragments. Mutations in this gene are thought to prevent this processing, and the presence of FGF23 appears to be responsible for the excess phosphate wasting seen in autosomal-dominant hypophosphatemic rickets (ADHR) via an ill-understood mechanism, possibly by interaction with the PHEX gene endopeptidase [7]. Excess FGF23 may also be responsible for the hyperphosphaturia seen in some cases of oncogenic hypophosphatemic osteomalacia.

Excess phosphate excretion is seen in several primary renal tubular abnormalities, such as the Fanconi syndrome (whatever the cause), and in a rare form of hereditary hypophosphatemic rickets with hypercalciuria (HHRH), the cause of which is unknown. Some cases of McCune–Albright polyostotic fibrous dysplasia also have excess phosphate excretion, possibly as a result of a phosphaturic factor.

Alkaline phosphatase

This enzyme is present in several tissues and exists in three main isoforms, intestinal (IAP), placental (PLAP), and tissue non-specific (TNAP). A gene on chromosome 2q34–37 codes for the first two, and a gene on chromosome 1p36.1–p34 codes for the last [8]. Different post-translational modifications of TNAP enzyme result in three tissue-specific forms found in bone, liver, and kidney that can be distinguished by their different isoelectric points and heat lability, the bone-specific form (bTNAP) being the least stable.

bTNAP is present in osteoblasts and promotes bone mineralization. Circulating TNAP is largely derived from liver and bone. Levels in plasma during childhood reflect growth rate [9] and are also raised in the presence of rickets. Low levels are seen in hypophosphatasia, which results from mutations in the TNAP gene. A database that keeps track of these mutations (currently 167) has been established and can be accessed at http://www.sesep.uvsq.fr/Database.html.

Magnesium

This cation is intimately involved in calcium metabolism. It circulates in plasma in a concentration of 0.7–1.2 mmol/L and is important because adequate magnesium is required for normal PTH secretion.

It is absorbed in the small intestine via a specific active transport mechanism. Excretion occurs along several sites in

the nephron, particularly the ascending loop of Henle and distal convoluted tubule. Renal tubular transport occurs by both paracellular and transcellular mechanisms, and defects in these mechanisms can lead to excessive urinary losses.

Fetal and neonatal calcium metabolism

The parathyroid glands are active in the human fetus from about 12 weeks of gestation. Thereafter, a positive gradient of calcium of 0.25–0.5 mmol/L is maintained across the placenta. Studies of PTH have demonstrated that very little is detectable in fetal plasma using immunoassays. This was at variance with bioassays that showed significant bioactivity [10]. The principal factor responsible for this bioactivity is probably parathyroid-related peptide (PTHrP) rather than PTH itself.

The normal full-term infant contains approximately 27 g of calcium, most of it acquired during the last trimester, and the net transfer of calcium across the placenta is 300–400 mg/day at term. Turnover of calcium amounts to more than 1% per day of total body calcium, compared with about 1/50th of this rate in adults. Fetal bone is therefore very active.

Following birth, the supply of calcium from the mother is terminated. This results in a rapid fall in plasma calcium, which declines to a nadir of 1.8–2.0 mmol/L by 48 h. The normal concentration of 2.2–2.6 mmol/L is achieved toward the end of the first week as the supply of calcium is resumed in milk, and the normal physiological mechanisms are established.

Post-neonatal calcium, phosphate, and magnesium metabolism

Following the establishment of normal physiological concentrations of calcium in plasma, there is very little variation throughout life despite a 50-fold increase in bone mineral to about 1200 g by adulthood. In contrast, concentrations of phosphate vary considerably, being highest during periods of greatest demand for bone mineral, particularly during the neonatal period and adolescence. Concentrations of magnesium are also maintained within a narrow range of 0.6–1.2 mmol/L with little variation.

Four factors maintain normal calcium physiology, PTH, vitamin D and its metabolites, PTHrP, and calcitonin. The first two are the most important outside fetal life. Magnesium has an important part to play as deficiency interferes with PTH secretion.

Calcium homeostasis

The concentration of plasma ionized calcium is controlled by a cascade of events (Fig. 13.1). The calcium concentration is detected by a calcium-sensing receptor located on the surface of the parathyroid glands. This is linked to secretion of PTH via an adenylate cyclase system. PTH acts via receptors on the various target organs. These actions are effected mainly via adenylate cyclase, to which it is linked by G-proteins. Defects in any part of this cascade can give rise to hyper- or hypocalcemia. Other factors (vitamin D, magnesium, and PTHrP) have an impact on the cascade and interact with it.

The calcium-sensing receptor (CaSR)

PTH is secreted in response to changes in ionized calcium. A CaSR is present in many tissues, particularly the parathyroid glands and renal tubule, as well as bone, cartilage, and other tissues [11]. The gene for CaSR is located on chromosome 3q13–21, and the CaSR is a large molecule consisting of 1078 amino acid residues. Approximately 610 of these form the extracellular calcium-binding domain, 250 comprise the seven-transmembrane domain, and another 210 the intracellular cytosolic component. Ca^{2+} binds to the extracellular domain in a complex manner and influences PTH secretion via both phospholipase Cβ and G-protein second messengers. As a consequence, PTH secretion changes in a sigmoidal fashion in response to acute changes in plasma calcium (Fig. 13.2), and there is a continuous tonic secretion of PTH, which maintains plasma-ionized calcium at whatever level is "set" by the CaSR [12]. Magnesium also binds to the CaSR and influences PTH secretion in a fashion similar to that of calcium. However, severe magnesium deficiency inhibits PTH secretion, probably because the adenylate cyclase coupled to the G-protein is itself magnesium dependent.

Two other loci, located on chromosomes 19p and 19q13, respectively, have been identified by family linkage studies. The precise nature of the gene products of these loci remains uncertain, but mutations within these loci result in clinical syndromes that are very similar to those resulting from inactivating mutations of the CaSR itself.

Mutations within the CaSR gene result in either inactivation or activation of the receptor, which result in hyper- and hypocalcemia respectively. Inactivating mutations cause insensitivity to calcium, which shifts the curve of PTH secretion in response to plasma calcium to the right (Fig. 13.2). As a consequence, PTH secretion is switched off at a higher concentration than normal, and hypercalcemia results [11]. The receptors are also present in the renal tubule, and renal calcium excretion is thereby reduced. The resulting condition is known as familial benign hypercalcemia (FBH) or familial hypocalciuric hypercalcemia (FHH). In contrast, activating mutations of the receptor shift the PTH secretion curve to the left (Fig. 13.2) causing chronic hypocalcemia and hypercalciuria, a condition known as autosomal-dominant hypocalcemia (ADH).

Many of the mutations found in FBH are clustered around the aspartate- and glutamate-rich regions of the extracellular domain of the molecule, and it has been postulated that this region contains low-affinity binding sites for calcium. Many

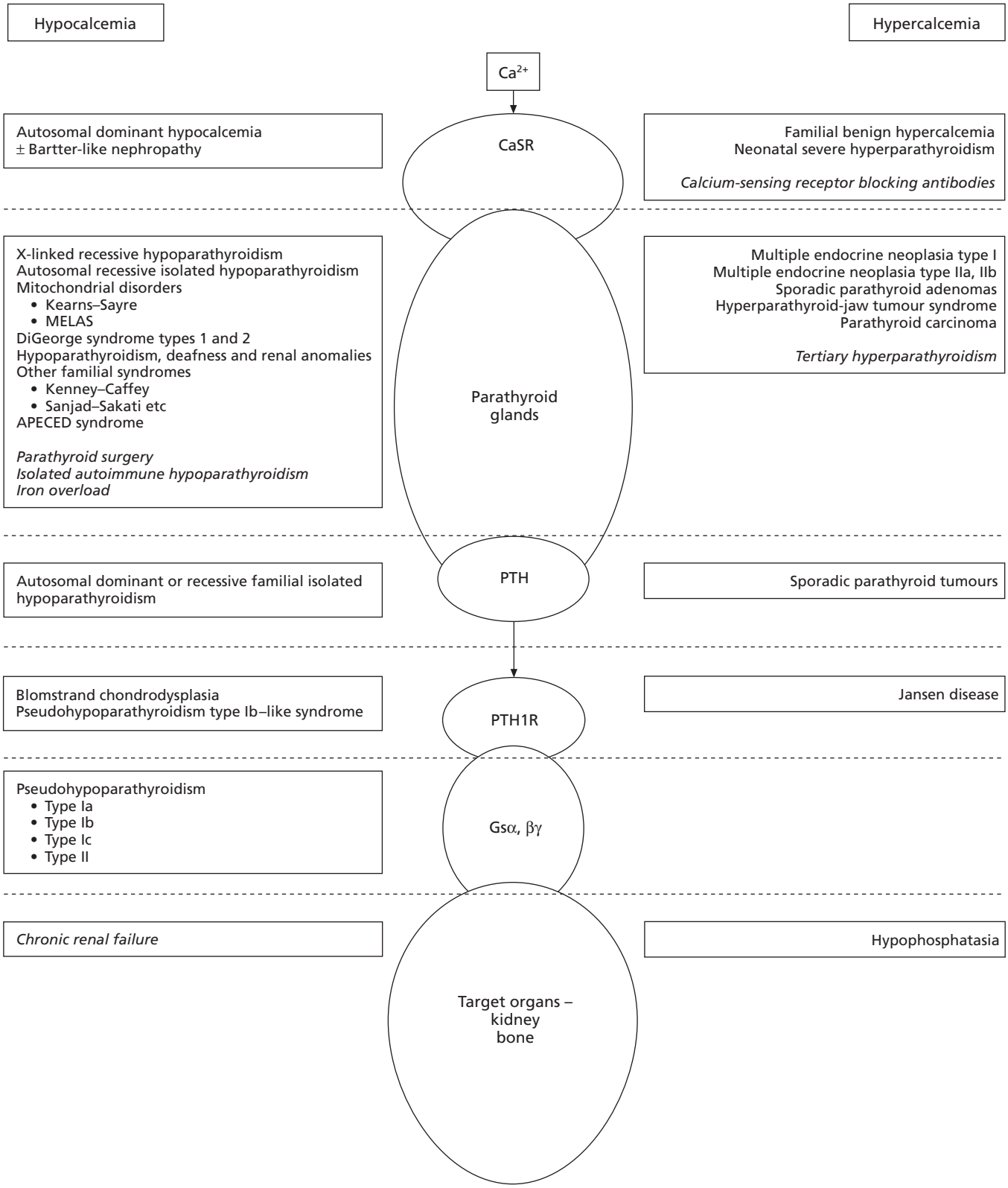

Fig. 13.1. Diagrammatic representation of the calcium cascade showing the various components and the points along the cascade at which abnormalities can occur. Genetic conditions are shown in normal type and acquired conditions in italics.

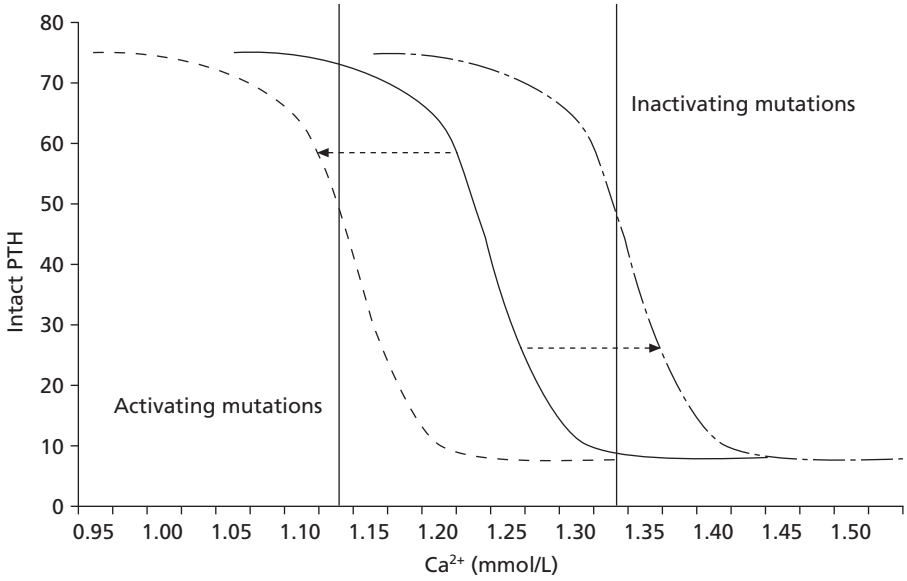

Fig. 13.2. Schematic representation of the sigmoidal relationship between ionized Ca (Ca^{2+}) and intact PTH secretion. The vertical lines represent the normal range of Ca^{2+}. Also shown is the effect of inactivating mutations (right shift) and activating (left shift) of the CaSR. The degree to which these shifts occur is dependent on the mutation involved. Adapted from [12].

of the FBH kindreds have been found to have unique mutations. Mutations have also been detected within the transmembrane domain but only rarely within the intracellular domain. Similarly, most activating mutations that cause ADH are present within the extracellular calcium-binding domain. Eighty-five mutations have been described so far, and an online database has been established to keep track of them (http://www.casrdb.mcgill.ca).

Not all families with FBH have mutations within the CaSR gene, and it has been suggested that there may be abnormalities either within the CaSR gene promoter or within one of the two loci found on chromosome 19. The three variants of FBH linked to chromosome 3q, 19p and 19q, have therefore been referred to as FBH types 1–3.

The parathyroid (PT) glands

The parathyroid (PT) glands, usually four in number, are derived embryologically from the third (lower glands) and fourth (upper glands) branchial arches. Several transcription factors are involved in their development [13]. Some, such as Hoxa3 (thyroid and thymus) and GATA3 (sensorineural deafness, renal anomalies, chromosome 10p13–14), are involved in the development of other structures. At least four genes, Tbx1 (thymus, cardiac outflow tract, and the face, chromosome 22q11), *rnex40*, *nex2.2-nex3*, and UDF1L, are located on the long arm of chromosome 22. Mutations within the genes responsible for these factors result in congenital hypoparathyroidism, which may be isolated or associated with other conditions such as the hypoparathyroidism, deafness, renal anomalies (HDR) syndrome and the CATCH 22 complex, of which the DiGeorge syndrome (DGS) is part. In addition, destruction of the glands may occur as a result of surgery, autoantibodies, or infiltration e.g. with iron.

Parathyroid hormone (PTH)

PTH is a single-chain, 84-amino-acid, polypeptide hormone encoded by a gene on chromosome 11. It is synthesized by the parathyroid glands from prepro-PTH, which has an additional 31 amino acids. Synthesis occurs in the ribosomes, where the initial 25-amino-acid "pre" sequence acts as a signal peptide to aid transport through the rough endoplasmic reticulum. The "pre" sequence is cleaved, and pro-PTH then travels to the Golgi apparatus where the 6-amino-acid "pro" sequence is cleaved to yield the mature hormone, which is stored in secretory vesicles that fuse with the plasma membrane prior to secretion of the hormone [14]. Very little PTH is stored within the glands, and most of the secreted hormone is newly synthesized. Mutations in the PTH gene have been described.

Only the first 34 N-terminal amino acids are required for full activity, and the function of the remainder of the molecule is not understood. The half-life of PTH in the circulation is 1–2 min [14]. The molecule is cleaved at various sites, which results in a number of fragments that can be identified in the circulation. The best modern assays of PTH measure "intact" 1–34 PTH, are able to measure physiological concentrations of PTH, correlate well with bioactivity, and ignore the inactive fragments.

Mechanisms of action of PTH 1: the PTH receptor

PTH acts via two receptors. The first and principal is PTH1R (also called PTH/PTHrP) receptor, which has equal affinity for both PTH and PTHrP. It consists of 593 amino acids coded by a gene on the long arm of chromosome 3 [15]. It has an extracellular binding domain of 190 residues, a seven-transmembrane domain, and a cytosolic component of 134

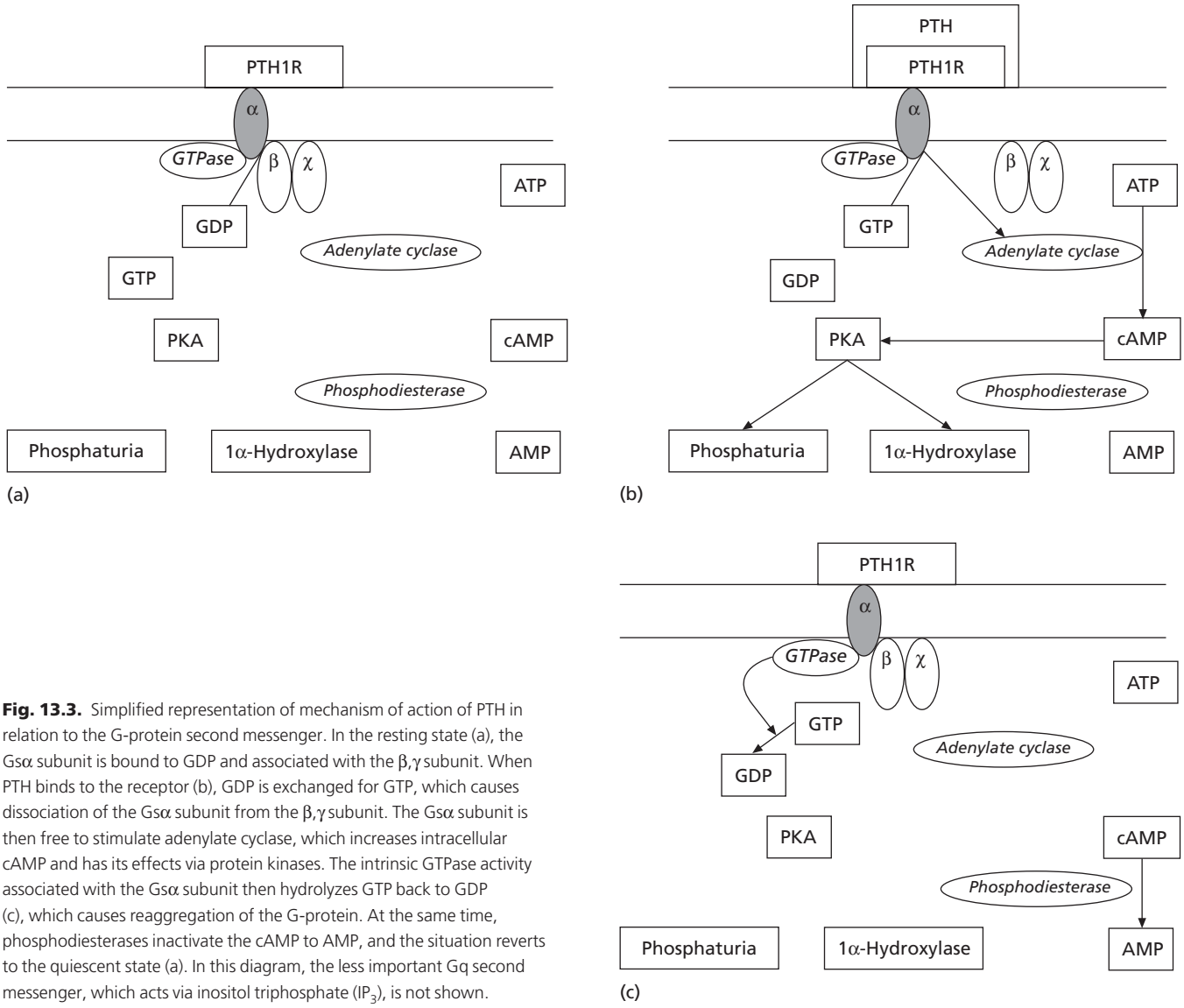

Fig. 13.3. Simplified representation of mechanism of action of PTH in relation to the G-protein second messenger. In the resting state (a), the Gsα subunit is bound to GDP and associated with the β,γ subunit. When PTH binds to the receptor (b), GDP is exchanged for GTP, which causes dissociation of the Gsα subunit from the β,γ subunit. The Gsα subunit is then free to stimulate adenylate cyclase, which increases intracellular cAMP and has its effects via protein kinases. The intrinsic GTPase activity associated with the Gsα subunit then hydrolyzes GTP back to GDP (c), which causes reaggregation of the G-protein. At the same time, phosphodiesterases inactivate the cAMP to AMP, and the situation reverts to the quiescent state (a). In this diagram, the less important Gq second messenger, which acts via inositol triphosphate (IP_3), is not shown.

residues. Both inactivating and activating mutations of the PTH1R have been described. These result in the very rare conditions of Blomstrand lethal chondrodysplasia and Jansen disease respectively. A second PTH2 receptor (PTH2R) is present in the central nervous system. PTHrP is not a ligand for it.

Mechanisms of action of PTH 2: intracellular signaling

Intracellular signaling occurs principally by coupling of the cytosolic component of the PTH1R to G-protein second messengers, Gs and Gq [16]. These are heterotrimeric, consisting of α, β, and γ subunits. In the resting state, they are associated, and the Gsα subunit is bound to GDP. Binding of the ligand with the receptor results in GDP being exchanged for GTP and dissociation of the Gsα subunit from the β,γ complex. The Gsα is then free to stimulate adenylate cyclase, which results in an increase in intracellular cAMP, which activates the various actions of PTH via specific protein kinases. Intrinsic GTPase activity associated with the Gsα subunit hydrolyzes GTP to GDP, which causes reassociation of the components of the G-protein. At the same time, phosphodiesterases inactivate the cAMP to AMP, and the cell reverts to its resting state (Fig. 13.3). This mechanism is common to several hormones, including thyroid-stimulating hormone (TSH), gonadotropins, and growth hormone-releasing hormone (GHRH) [16].

The Gsα subunit is coded by a gene, GNAS1, located on chromosome 20q13.3. This complex gene contains 13 exons that code for the Gsα subunit itself plus another seven exons which, by alternative promoter use and splicing, results in at least four different mRNA transcripts. In most tissues,

these show biallelic expression, but some of the transcripts are derived either from the maternal or paternal alleles. The significance of this is discussed later (see under Pseudohypoparathyroidism). The Gqα subunit activates phospholipase Cβ to generate inositol triphosphate (IP_3), although this is a lesser effect than cAMP generation.

Several factors modify responsiveness to PTH. Mutations within the GNAS1 gene for the Gsα subunit of the G-protein second messenger cause resistance by preventing activation of adenylate cyclase. This results in some of the forms of pseudohypoparathyroidism. PTH can also modify responsiveness to itself. Acute infusions of PTH cause desensitization by uncoupling the receptor from the G-protein. Alternatively, in the presence of chronic hyperparathyroidism, downregulation of the receptors occurs as a result of reduction in the number of receptors.

Resistance to PTH has been assessed in earlier times by examining the effects of PTH on phosphate excretion (the Ellsworth–Howard test) or on cAMP production in either urine or plasma. It has not been possible recently to undertake such stimulation tests as PTH has not been available for use as a test agent. Now that synthetic PTH has been introduced for use in involutional osteoporosis, it may be possible to use it for such purposes again, although, as understanding of the genetic mechanisms underlying many of these conditions has advanced, the need for stimulation tests has declined.

PTH has two principal target organs, bone and kidney. In bone, PTH promotes bone mineralization by an action on osteoblasts when present in physiological concentrations. During phases of hypocalcemia, when PTH concentrations rise, its main target cell in bone is the osteoclast, where it activates bone resorption via the receptor activator of the nuclear factor KB ligand (RANKL/RANK system), a receptor in the tissue necrosis factor (TNF) gene family involved in osteoclast differentiation. The two processes do not occur independently, and increases in bone resorption are accompanied by stimulation of osteoblast activity via a series of paracrine and autocrine mechanisms that result in an increase in bone turnover.

In the nephron, most of the filtered calcium is reabsorbed passively in the proximal tubule via a paracellular mechanism. In the convoluted and straight parts of the proximal renal tubule, PTH also acts to stimulate the action of 25-hydroxyvitamin D 1α-hydroxylase (1α-hydroxylase), the enzyme that converts vitamin D to its active metabolite, 1α,25-dihydroxyvitamin D (1α,25$(OH)_2$D). PTH actively promotes calcium and magnesium transcellular reabsorption in the distal nephron. Phosphate excretion increases in response to PTH, which allows excess phosphate removed from bone with calcium following PTH-stimulated bone resorption to be excreted. PTH also stimulates renal excretion of bicarbonate and amino acids. Thus, hyperparathyroidism results in a mild acquired form of the Fanconi syndrome.

Parathyroid hormone-related peptide (PTHrP)

Following the observation that some cancers are associated with hypercalcemia with undetectable PTH, it became apparent that another factor sharing many of the properties of PTH was the cause of the hypercalcemia. It is now known that PTHrP is secreted by many of these tumors [17]. PTHrP is a polypeptide with considerable homology to PTH, particularly at the N-terminal end. It is secreted as a prohormone, which is cleaved into several fragments. The N-terminal fragment binds with the PTH1R in a way similar to PTH and has similar actions.

PTHrP does not circulate in amounts detectable in plasma and has no significant classical hormonal actions in postnatal life, but it may have important paracrine effects, particularly in bone. It is of importance as the factor that promotes and maintains the positive gradient of calcium across the placenta in fetal life [10]. It is also secreted by the lactating breast and may play an important part in calcium homeostasis during lactation. Women with primary hypoparathyroidism may become hypercalcemic while breast-feeding and require a reduction in their dose of vitamin D analog. This effect is thought to be caused by PTHrP.

Vitamin D

Vitamin D is a secosteroid that exists in two forms. Cholecalciferol is synthesized as a result of the action of ultraviolet (UV) light on 7-dehydrocholesterol. The UV light breaks the B ring of the steroid molecule to produce previtamin D, which is then converted to native vitamin D (cholecalciferol) by the action of body heat. Ergocalciferol is synthesized by plants and differs slightly in structure but is equipotent with cholecalciferol and metabolized in a similar fashion. The generic term vitamin D is used here to include both compounds. Under normal circumstances, about 80% of vitamin D consists of cholecalciferol synthesized in skin, the remainder being acquired from dietary sources as both chole- and ergocalciferols. However, the amount of vitamin D synthesized in skin is dependent upon skin color and exposure. Following synthesis, it becomes bound to a specific vitamin D-binding protein (DBP) and passes to adipose tissue and the liver for storage and further metabolism.

Vitamin D does not have significant biological activity. Its activity requires metabolism via two hydroxylation steps, initially at the 25 and subsequently at the 1 position (Fig. 13.4) [18]. The first step is catalyzed by vitamin D 25 hydroxylase. There are probably two distinct enzymes distinguishable by their different affinities and capacities and by their intracellular localization. Only one has been cloned, a cytochrome P450 low-affinity, high-capacity enzyme (CYP27) located in mitochondria, which also catalyzes hydroxylation of other steroid molecules. It is likely that a second, high-affinity, low-capacity enzyme, which may be of greater physiological significance,

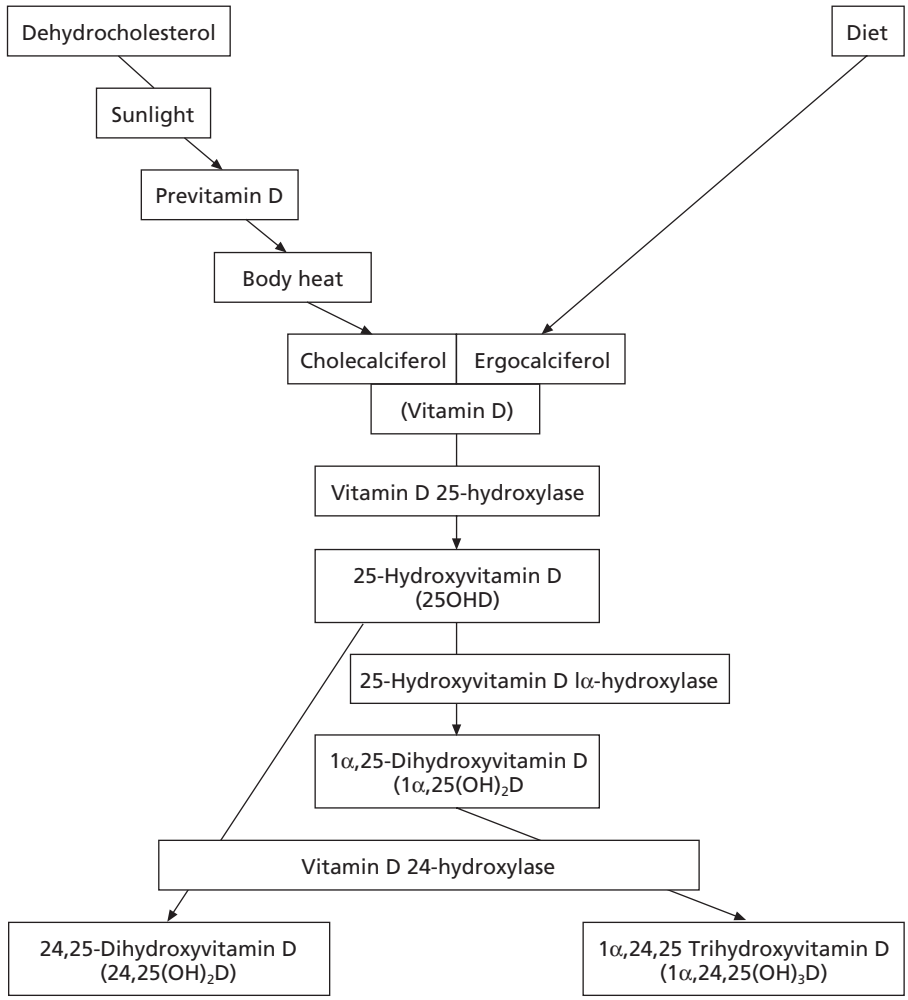

Fig. 13.4. Diagrammatic representation of the principal steps involved in vitamin D metabolism.

is located within hepatic microsomes. It is also a cytochrome P450 enzyme but has not yet been fully characterized.

The resulting product, 25-hydroxyvitamin D (25OHD), circulates in plasma bound to the DBP and is the most abundant vitamin D metabolite in plasma, circulating in nanomolar concentrations. Assay of this compound gives a measure of vitamin D status. Its level varies depending on the supply of vitamin D and shows a considerable annual variation with a peak about 6 weeks after maximal exposure to sunlight. It has some weak activity, which is not normally of clinical significance, but may become so in the presence of vitamin D excess. Vitamin D 25 hydroxylase also catalyzes the conversion of the synthetic vitamin D analog, 1α-hydroxy-cholecalciferol (alfacalcidol), to 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D].

25OHD is metabolized to its active hormone 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D] by 25-hydroxyvitamin D 1α-hydroxylase, which is active only against metabolites that are already hydroxylated at position 25 [19]. A single enzyme has been identified located in convoluted and straight portions of the proximal renal tubule. Activity is also present in osteoblasts, keratinocytes, and lymphohematopoietic cells, where 1α,25$(OH)_2$D may have an autocrine or paracrine role. During fetal life, 1α-hydroxylase activity is found in the placenta. In pathological states, it is present in the macrophages of sarcoid tissue and subcutaneous fat necrosis. It is a mitochondrial cytochrome P450 enzyme (CYP27B1) consisting of 508 amino acids with considerable homology to other P450 enzymes. It is encoded by a single gene on chromosome 12.13.1–13.3. Mutations in this gene are responsible for the condition known variously as pseudo-vitamin D deficiency rickets (PDDR), vitamin D-dependent rickets type I (VDDR-I), or 1α-hydroxylase deficiency.

Activity of 1α-hydroxylase is stimulated by PTH via its cAMP/protein kinase actions. Hypocalcemia stimulates 1α-hydroxylase activity, but this effect is mediated via PTH and not directly. Plasma phosphate has a direct effect on 1α-hydroxylase activity, although there is some evidence to suggest that this may be modulated by growth hormone (GH); calcitonin may also regulate the enzyme.

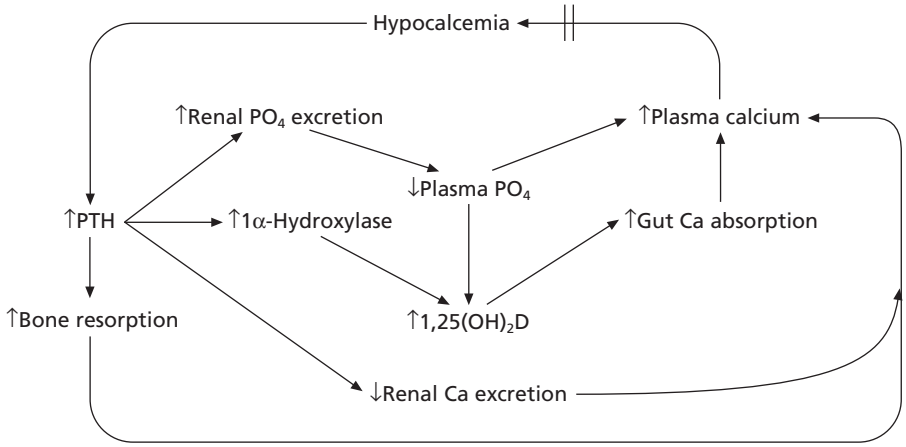

Fig. 13.5. Diagrammatic representation of the principal responses to a hypocalcemic stimulus. Reprinted from [59] with permission from Elsevier.

1α,25$(OH)_2$D is a highly potent compound that circulates in picomolar concentrations. Its synthesis is tightly controlled by the plasma calcium concentration. In order to insure that changes in 1α,25$(OH)_2$D can occur rapidly, a second enzyme, 25-hydroxyvitamin D 24-hydroxylase, exists. This is yet another cytochrome P450 molecule that can use both 25OHD and 1α,25$(OH)_2$D as substrates to form 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] and 1α,24,25-trihydroxyvitamin D [1α,24,25$(OH)_3$D] respectively. The role of this enzyme is probably to divert metabolism of 25OHD away from 1α,25$(OH)_2$D synthesis when this is not needed and to participate in the degradation of existing 1α,25$(OH)_2$D. It is inhibited by PTH and stimulated by 1α,25$(OH)_2$D.

1α,24,25$(OH)D_3$ has limited potency [about 10% of 1α,25$(OH)_2$D] and is probably an intermediate degradation metabolite of 1α,25$(OH)_2$D. The role, if any, of 24,25$(OH)_2$D is uncertain. Some authors have argued that it has no role to play whereas others have suggested that it may influence bone mineralization.

1α,25$(OH)_2$D acts via a specific vitamin D receptor [20]. It is a member of the steroid–thyroid–retinoid superfamily of nuclear receptors and, in many respects, is typical of this group with ligand binding, DNA binding, dimerization, and transcriptional activation domains. It is encoded by a gene on chromosome 12 near the 1α-hydroxylase gene. The receptors are widely distributed in gut, parathyroid glands, chondrocytes, osteoblasts, and osteoclast precursors. 1α,25$(OH)_2$D plays a critical role in promoting calcium absorption in the small intestine, suppresses PTH secretion from the parathyroids, influences growth plate mineralization, and stimulates differentiation of osteoclasts. In addition, there are receptors present in many tissues that are not directly related to calcium homeostasis such as skin, breast, prostate, colon, etc., and it has been postulated that 1α,25$(OH)_2$D may play a part in preventing cancers of these tissues. Mutations in the vitamin D receptor occur throughout the molecule but particularly in either the ligand-binding (ligand binding negative) or the DNA-binding (ligand binding positive) domains. These mutations cause severe rickets, and many individuals also have alopecia. Originally referred to as vitamin D-dependent rickets type II (VDRR-II), it is now more properly called hereditary 1α,25$(OH)_2$D-resistant rickets (HVDRR).

Calcitonin

Calcitonin (CT) is a polypeptide hormone secreted by the C-cells of the thyroid. Embryologically, these are derived from the ultimobranchial bodies that become incorporated into the thyroid. It is secreted in response to hypercalcemia and acts via specific receptors mainly to counteract the effects of PTH in osteoclasts. It therefore has a calcium-lowering effect, which wanes in the presence of sustained CT secretion. Secretion also occurs in response to a specific tetrapeptide sequence present on, among other molecules, glucagon. CT acts via a receptor, the gene for which is located on chromosome 7q21.

The physiological role of CT has been difficult to establish, but it may play a part in moderating bone turnover and may well be of greater importance to the developing skeleton than to the mature one. In practice, CT appears to have little clinical significance except as a therapeutic agent for acute hypercalcemia and as a tumor marker for medullary carcinoma of the thyroid (MCT).

Interactions between calciotropic agents

The interactions between the various influences on calcium metabolism are complex. The primary aim is to maintain plasma-ionized calcium within narrow limits, an aim that is normally achieved very successfully. At the same time, bone metabolism must be allowed to proceed satisfactorily so that adequate calcium and phosphate accumulation and bone remodeling can occur during growth.

The hormone factors responsible for calcium homeostasis are summarized in Figure 13.5.

Disorders of calcium metabolism

Hypocalcemia

Although the plasma concentration of ionized calcium is normally maintained within narrow limits, symptoms of hypocalcemia do not usually occur until they fall to significantly lower values. It is unusual for symptoms to occur until total calcium falls below 1.8 mmol/L, and some patients remain asymptomatic with a plasma calcium as low as 1.2 mmol/L.

Symptoms include muscle twitching and spasms, which can be very painful, apnea, stridor, carpopedal spasms, and focal or generalized seizures. A measurement of plasma calcium should always be part of the investigation of unexplained fits to avoid confusion with epilepsy. Clinical examination may reveal positive Chvostek or Trousseau signs, and chronic hypocalcemia may cause calcification of the lens of the eye. In infants whose hypocalcemia is secondary to vitamin D deficiency, a form of hypertrophic cardiomyopathy may develop. This does not occur with other causes of hypocalcemia (e.g. hypoparathyroidism) and is probably the result of a direct effect of the vitamin D deficiency on cardiac muscle. The prognosis for the cardiomyopathy is good although it may take several months to recover completely. Some syndromes are associated with specific dysmorphic features. In addition, signs of rickets may be present in some instances. Soft tissue calcification is sometimes present in conditions such as pseudohypoparathyroidism, and computed tomographic (CT) scanning of the brain may reveal the presence of basal ganglion and frontal lobe calcification (Fig. 13.6).

Investigation of disorders of calcium metabolism is summarized in Table 13.1. First-line investigations should include measurement of total (and, if available, ionized) calcium, phosphate, albumin, magnesium, alkaline phosphatase, creatinine, PTH, and 25OHD in blood, a sample of which should also be stored for future measurement of $1\alpha,25(OH)_2D$ later if this is relevant, particularly if rickets is also present. Urine should be taken for measurement of calcium, phosphate, and creatinine. More detailed investigation includes markers of bone formation (osteocalcin or procollagen type 1 C-peptide) in blood and of bone resorption in urine (hydroxyproline, pyridinoline, deoxypyridinoline) or blood [C-terminal telopeptide of type 1 collagen (1CTP) and N- or C-terminal telopeptides].

X-rays will reveal the presence of rickets, skeletal dysplasias (e.g. in pseudohypoparathyroidism), hyperparathyroid bone disease, or soft tissue calcification. They are relatively insensitive in detecting intracranial calcification, for which CT scanning is most appropriate. Early nephrocalcinosis can best be detected by ultrasonography. X-rays are less sensitive but, if nephrocalcinosis is demonstrated, this may require further investigation and management of renal function.

Where a genetic cause for a disorder of calcium metabolism is suspected (Table 13.2), blood should be taken and DNA

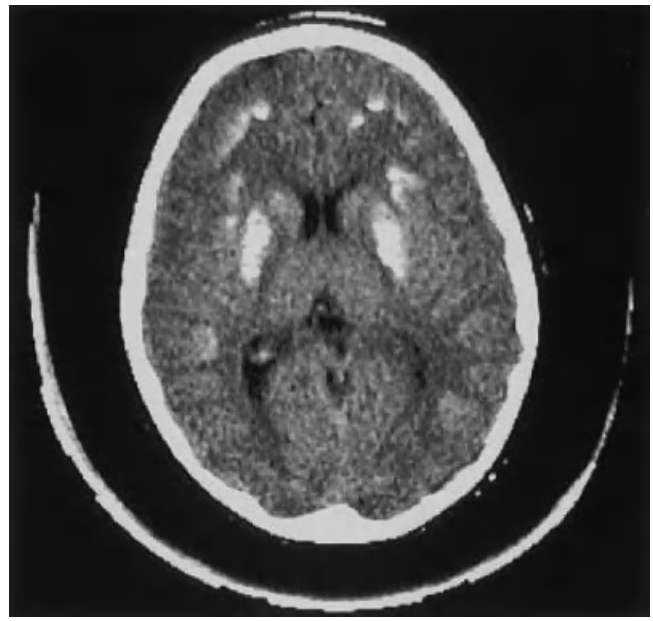

Fig. 13.6. Computed tomography image of basal ganglion and frontal lobe calcification in pseudohypoparathyroidism type Ia.

Table 13.1. Table of investigations that are indicated when a patient presents with a disorder of calcium metabolism. Not all investigations are indicated in all patients.

Blood – initial investigations	*Urine – initial investigations*
Calcium	Calcium
Phosphate	Phosphate
Albumin	Creatinine
Alkaline phosphatase	
Creatinine	*Calculate:*
25OHD	Ca/creatinine ratio
Intact PTH	Fe_{PO4} and TRP
Save serum for $1\alpha,25(OH)_2D$	T_mPO_4/GFR
Subsequent investigations as necessary	*Subsequent investigations as necessary*
Blood gases	Glucose and amino acids
$1\alpha,25(OH)_2D$	Bone turnover and markers
DNA analysis for genetic abnormalities	
PTHrP	
Bone turnover markers	
Radiology and nuclear medicine	
Hand and knee for rickets	
Skeletal survey for bone abnormalities	
Renal ultrasound for nephrocalcinosis	
Parathyroid ultrasound for parathyroid tumors	
CT scan for intracranial calcification	
SestaMIBI scan for PT gland localization	

Table 13.2. Genetic conditions that cause hypocalcemia. The conditions are classified according to which part of the calcium cascade they affect and, where known, the gene location, gene product, inheritance, and principal clinical features are shown.

Location in calcium cascade	Metabolic abnormality	Chromosome location	Gene/gene product	Inheritance	Principal clinical features
Calcium-sensing receptor	Autosomal-dominant hypocalcemia	3q13–21	CaSR	AD	(Symptomatic) hypocalcemia, hypercalciuria, nephrocalcinosis
	Autosomal-dominant hypocalcemia with Bartter-like features	3q13–21	CaSR	AD	
The parathyroid glands	X-linked recessive hypoparathyroidism	Xq26–27	?	XLR	Infantile onset hypoparathyroidism
	Autosomal-recessive isolated hypoparathyroidism	6p23–24	GCMB	AR	Isolated hypoparathyroidism
	Mitochondrial disorders				
	Kearns–Sayre	Mitochondrial gene deletion	various	Maternal	Hypoparathyroidism, progressive ophthalmoplegia, pigmentary retinopathy, heart block or cardiomyopathy, short stature, primary gonadal failure, sensorineural deafness, proximal myopathy, diabetes mellitus
	MELAS	Mitochondrial gene point mutation	various	Maternal	Hypoparathyroidism, mitochondrial encephalopathy, lactic acidosis, stroke-like episodes, proximal myopathy, diabetes mellitus
	DiGeorge syndrome – type I	22q11.2	*rnex40 nex2.2-nex3*	Sporadic or AD or unbalanced translocation	Neonatal hypoparathyroidism, thymic aplasia, ear, nose, and mouth deformities, aortic arch abnormalities – truncus arteriosus, right-sided aortic arch, etc.
	DiGeorge syndrome – type II	10p13–14	?	Sporadic	Neonatal hypoparathyroidism, immune deficiency
	Hypoparathyroidism, deafness, renal anomalies	10p14-10pter	GATA3	AD	Hypoparathyroidism, sensorineural deafness, cystic renal changes
	Other familial syndromes				
	Autosomal-dominant Kenny–Caffey	?	?	AD	Similar to AR Kenney–Caffey
	Autosomal-recessive Kenny–Caffey	1q43-44	TCBE	AR	Hypoparathyroidism, extreme short stature, cortical thickening and medullary stenosis of tubular bones, normal bone age, absent diploic space, delayed closure of anterior fontanelle, normal intelligence
	Sanjad–Sakati, and Richardson and Kirk	1q43-44	TCBE	AR	Hypoparathyroidism, deep-set eyes, microcephaly, thin lips, long philtrum, beaked nose, external ear anomalies, micrognathia, depressed nasal bridge, mental retardation
	Shaw and Haigh	?	?	AR	Congenital hypoparathyroidism, early death
	Barakat	?	?	?AR	Hypoparathyroidism, sensorineural deafness, nephrotic syndrome leading to renal failure
	Dahlborg and Borer	?	?	AR or XLR	Hypoparathyroidism, congenital lymphedema, nephropathy, mitral valve prolapse, brachtelephalagy
	Pluriglandular autoimmune hypoparathyroidism (APECED)	21q22.3	AIRE-1	AR	Mucocutaneous candidiasis, hypoparathyroidism, adrenal insufficiency, hypogonadism, diabetes mellitus, nail pitting, keratopathy, alopecia, hepatitis, intestinal malabsorption
PTH	Familial isolated hypoparathyroidism	11p15	PTH	AD	Hypoparathyroidism
	Familial isolated hypoparathyroidism	11p15	PTH	AR	Hypoparathyroidism

Table 13.2. (*continued*)

Location in calcium cascade	Metabolic abnormality	Chromosome location	Gene/gene product	Inheritance	Principal clinical features
PTH/PTHrP receptor	Blomstrand chondrodysplasia	3p21.1–p22	PTH1R	AR	Advanced bone maturation, accelerated chondrocyte maturation, increased bone density, poor bone modeling, rapidly lethal
	Pseudohypoparathyroidism type Ib-like syndrome	3p21.1–p22	PTH1R	?AR	Hypoparathyroidism with raised PTH
Post-receptor events	Pseudohypoparathyroidism type Ia	20q13.2–13.3	Gsα	AD paternally imprinted	Hypoparathyroidism with raised PTH, short stature, round facies, short metacarpals and metatarsals (Albright's hereditary osteodystrophy), mild hypothyroidism, disturbance of ovarian function, mild developmental delay
	Pseudopseudohypoparathyroidism	20q13.2–13.3	Gsα	AD maternally imprinted	As above but with no hypoparathyroidism
	Pseudohypoparathyroidism with testotoxicosis	20q13.2–13.3	Gsα – differential heat sensitivity	AD paternally imprinted	As for PHP-Ia but with testotoxicosis
	Pseudohypoparathyroidism type Ib	?20q13	?	AD ?paternally imprinted	Hypoparathyroidism with raised PTH but no features of AHO. May retain bone sensitivity
	Pseudohypoparathyroidism type Ic	?	?	?AD	Multiple hormone resistance with AHO
	Pseudohypoparathyroidism type II	?	?	?	Hypoparathyroidism with normal cAMP but impaired phosphaturic response
Magnesium deficiency	Familial primary hypomagnesemia	9q12–22	?	?	Isolated defect of magnesium transport in gut
	Familial hypomagnesemia with hypercalciuria and nephrocalcinosis	3q	PCLN-1 (or CLDN16)/ Paracellin-1	AR	Hypermagnesuria, hypercalciuria, hypomagnesemia, nephrocalcinosis, renal failure, ocular abnormalities
	Isolated renal magnesium wasting	11q23	FXYD2/Na, K-ATPase γ	AD	Hypermagnesuria with hypomagnesemia
	Isolated renal magnesium wasting	?	?	AD	Hypermagnesuria with hypermagnesemia
	Gitelman syndrome	16q13	Na/K cotransporter	AR	Hypomagnesuria, hypokalemic alkalosis, chronic dermatitis

extracted for analysis in an appropriate laboratory. Many of these are research procedures and may require liaison with a local clinical genetics department.

Neonatal hypocalcemia

Hypocalcemia may occur early in the neonatal period (within the first 2–3 days) or later (toward the end of the first week). In the former, the physiological fall in plasma calcium is exaggerated, especially in the preterm infant, following birth asphyxia, in "sick" infants, and in those born to diabetic mothers. The mechanisms for neonatal hypocalcemia are unclear but may represent a delayed response to the rise of PTH following hypocalcemia. There may be an exaggerated response of calcitonin, especially where hypoglycemia is present because of the secretagog effect of glucagon. This is unlikely to be the explanation in the infant of a diabetic mother as glucagon responses to hypoglycemia are known to be impaired, presumably because of chronic hyperglycemia *in utero*. Magnesium deficiency may be a factor, particularly if the mother's diabetes has been poorly controlled, and measurement of magnesium should be included in the investigation of neonatal hypocalcemia and corrected if necessary. Early-onset hypocalcemia usually corrects itself spontaneously within the first week, but additional calcium supplements may be required if symptoms persist. Hypophosphatemia is frequently present in preterm infants and may contribute to the development of bone disease of prematurity.

Late neonatal hypocalcemia is usually symptomatic. This can be the first manifestation of hypoparathyroidism, but vitamin D deficiency or primary hyperparathyroidism in the mother must be considered. In the former, hypocalcemia can present at any time after birth depending on the severity of the deficiency and is not necessarily associated with radiological evidence of rickets, particularly if the presentation is soon

after birth. It is almost entirely confined to infants of mothers from ethnic minority groups, and routine vitamin D supplementation of 400 IU/day may not be sufficient to prevent neonatal hypocalcemia in these infants. Hyperparathyroid mothers may be asymptomatic, and the presence of hypocalcemia in the infant may be a clue to maternal disease. Measurement of vitamin D in mother and infant and of bone profile in the mother should form part of the investigation of late neonatal hypocalcemia.

Neonatal hypocalcemia that is symptomatic requires treatment initially with intravenous 10% calcium gluconate (0.225 mmol/mL) given as a slow infusion of 1–3 mL/kg. This can be continued as an infusion of 1–2 mmol/kg/day or as oral supplements. It is important to insure that the infusion is given into a secure intravenous site as extravasation causes unsightly burns. In the event of vitamin D deficiency, additional vitamin D supplements of 1000–1500 IU/day are required.

Childhood hypocalcemia

Hypocalcemia in childhood can result from defects in any part of the calcium metabolic cascade. Causes of hypocalcemia that affect the early part of the cascade are generally associated with low PTH, and those affecting the latter part with high PTH. The reverse is true of hypercalcemic conditions. Many of these are genetic in origin (Table 13.2).

Disorders of the calcium-sensing receptor (CaSR)

Autosomal-dominant hypocalcemia (ADH) is caused by an activating mutation of the CaSR gene [11]. Calcium is sensed as being "normal" at subphysiological levels, and PTH secretion is therefore switched off inappropriately causing hypoparathyroidism. The extent of the resulting hypoparathyroidism is determined by how much the mutation shifts the calcium response curve to the left (Fig. 13.2). Patients may or may not be asymptomatic. Several mutations have been described and, although there is no genotype–phenotype correlation, symptoms are related to the degree of hypocalcemia, which remains fairly constant within individuals. Inheritance is usually autosomal dominant, but sporadic cases have been described.

It can be difficult to distinguish ADH from isolated hypoparathyroidism in the absence of a family history, and germline mosaicism in an apparently unaffected parent can confuse the issue further and make prediction of recurrence difficult. Diagnosis depends on demonstrating hypocalcemia with normal PTH levels. In contrast to hypoparathyroidism, urinary calcium excretion is relatively high, and these patients are susceptible to nephrocalcinosis, especially if treated to prevent symptoms.

The need for treatment largely depends on whether or not symptoms are present. In patients whose plasma calcium is above 1.95 mmol/L, treatment is unnecessary and, in those with a lower level of calcium, treatment is required only if symptoms are present. 1αOHCC should be used cautiously and in the smallest dose required to prevent symptoms. It is not necessary to restore plasma calcium to normal. Urinary calcium excretion should be monitored carefully to avoid nephrocalcinosis, and regular renal ultrasonography can be helpful in detecting early changes. If it proves difficult to prevent symptomatic hypocalcemia without causing nephrocalcinosis, thiazide diuretics may also be used. Selective CaSR blocking agents may become available in the future.

Another phenotype associated with activating mutations of the CaSR has been described recently and probably represents a more extreme example of the same condition [21]. These patients presented with apparent hypoparathyroidism during the neonatal period and were treated as such with calcium and vitamin D or calcitriol. They subsequently presented in late childhood, adolescence, or early adulthood with a Bartter-like syndrome of impaired renal function, hypercalciuria, nephrocalcinosis, hyper-reninemia, hypokalemia, and hyperaldosteronism in various degrees. In all cases, activating mutations of the CaSR were identified resulting in a marked left shift of the dose–response curve for extracellular calcium signaling. The mechanism of these biochemical changes is not entirely clear, but it has been suggested that the CaSR defect prevents calcium and magnesium reabsorption, which normally takes place in conjunction with sodium. This leads to sodium wasting and a concentrating defect that stimulates hyper-reninemia and hyperaldosteronism and leads to increased potassium losses. At the same time, the distal convoluted tubule (DCT) then has to work overtime to compensate for the sodium losses, and this leads to further calcium excretion and hypokalemic alkalosis. Caution is therefore urged in the use of thiazide diuretics, which might theoretically worsen the calcium excretion.

Disorders of the parathyroid (PT) glands

In X-linked recessive familial isolated hypoparathyroidism (FIH), a mutant gene on chromosome Xq26–q27 has been described in two families [22]. The nature of the gene product is not known, but mitochondrial DNA studies have shown both families to be related. Affected males suffer infantile onset of hypoparathyroidism.

Autosomal-recessive isolated hypoparathyroidism presents with severe hypoparathyroidism at an early age [22]. In two cousins, a mutation was found in the GCMB gene located on chromosome 6p23–24. This gene is expressed only in PT glands and appears to have a role in PT gland development.

The Kearns–Sayre Syndrome (KSS) comprises hypoparathyroidism with progressive external ophthalmoplegia, pigmentary retinopathy, heart block or cardiomyopathy, and proximal myopathy. It may also be associated with diabetes mellitus. It overlaps with the MELAS syndrome in which

hypoparathyroidism is associated with a childhood onset of mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes [22]. Proximal myopathy and diabetes mellitus have also been described with this condition. Mutations in the mitochondrial genome have been reported in some of these patients, although the role of these mutations is not well understood.

The DiGeorge syndrome (DGS) consists of a tetrad of parathyroid gland hypoplasia, thymic immunodeficiency, congenital heart disease, and facial anomalies, structures all derived from the third and fourth branchial pouches [23]. It is related to several other conditions, including the velocardiofacial (VCFS) and conotruncal anomaly facial (CTAFS) syndromes, and a number of non-syndromic cardiac conditions, such as pulmonary atresia with ventricular septal defect, Fallot's tetralogy, truncus arteriosus, and interrupted aortic arch. Only the DGS includes hypoparathyroidism.

They are linked under the umbrella of the CATCH22 syndrome (cardiac anomalies, abnormal facies, thymic hypoplasia, cleft palate, and hypocalcemia associated with microdeletions in the long arm of the number 22 chromosome). The very variable nature of these conditions is seen from their original clinical descriptions.

Most cases of DGS arise *de novo* and are associated with deletions of variable size in chromosome 22q11.2. Autosomal-dominant transmission has been described in association with an unbalanced translocation and deletion involving the same chromosomal area [24]. In all patients described so far, two genes, *rnex40* and *nex2.2-nex3*, are deleted. These are contained within a 250-kb minimal critical region. The precise role of the gene products is not well understood, but they are probably DNA-binding proteins [25]. Another gene, UDF1L, is also located within the 22q11 region, and deletions of this gene have been found in all patients with the CATCH22 syndrome [26]. Not all patients with DGS have been shown to have mutations in the 22q region. Those that have are designated as DGS1. Mutations in a second locus on chromosome 10p13–14 have also been seen in association with hypoparathyroidism and immune deficiency, which has been designated DGS2.

In DGS, the emphasis is on the PT and thymus glands and the cardiac anomalies. The severity of the condition varies, but most infants with this syndrome present with cardiac abnormalities, which may require urgent attention. Often the developing hypocalcemia does not become immediately apparent and is frequently overlooked. Thymus gland aplasia is suspected by the absence of a thymic shadow on chest X-ray and can be confirmed by a low T-cell count, although the total lymphocyte count may be normal. Late-onset DGS has also been described. These patients present with hypocalcemia in late childhood or adolescence and have only minor dysmorphic features. Microdeletions of the 22q11 chromosome have also been identified in them [27].

Autosomal-dominant hypoparathyroidism, deafness, and renal anomalies (HDR) was first described in 1992 [22]. The hypoparathyroidism is associated with low or inappropriately normal levels of PTH with normal responsiveness to PTH, the deafness is sensorineural, and the renal anomalies consist of cystic changes that lead to renal impairment in some patients. Cytogenetic abnormalities of chromosome 10p14–10pter have been identified in these patients. This region does not overlap the DGS2 region and contains a gene, GATA3, that is involved in the developing kidney, otic vesicles, and parathyroid glands.

The autosomal-recessive Kenny–Caffey [22] and Sanjad–Sakati [22] syndromes as well as that described by Richardson and Kirk [28] are probably all variants of the same condition. Hypoparathyroidism is associated with short stature and developmental delay. They have been described mainly in consanguineous families from Saudi Arabia and Kuwait, and mutations have been mapped to chromosome 1q42–43. Other familial syndromes are also described, although the chromosomal locations and gene defects have not been identified. For details, see Table 13.2. For further details and references to these conditions, see Bassett and Thakker [22].

The autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) syndrome, also known as the polyglandular autoimmune type 1 syndrome, is an evolving association between mucocutaneous candidiasis and hypoparathyroidism that usually develops in mid-childhood [29]. About 70% of patients develop adrenal insufficiency and other endocrinopathies, such as hypogonadism and hypothyroidism; diabetes mellitus may develop in later life. Other associated features include nail pitting, keratopathy, alopecia, hepatitis, and intestinal malabsorption, all of which are autoimmune mediated.

Several mutations in the autoimmune regulator (AIRE-1) gene have been recognized. This gene is located on chromosome 21q22.3. Its role in regulating immune function is not known, but it is a 545-amino-acid protein that probably acts as a transcriptional factor. The condition is particularly prominent in Finnish families, in which a mutation at codon 257 (Arg→stop) has been identified in 82% of subjects. It has also been identified in Iranian Jews.

Patients usually present with mucocutaneous candidiasis and later develop hypoparathyroidism followed by other features. Adrenal insufficiency should be suspected if hypercalcemia supervenes in a previously stable patient. This is probably because of changes in renal calcium reabsorption following the hypovolemia associated with mineralocorticoid deficiency. These patients require careful follow-up in order to identify adrenal insufficiency.

Other acquired non-genetic forms of hypoparathyroidism include parathyroid gland destruction during thyroid surgery or following parathyroidectomy. Isolated autoimmune hypoparathyroidism may also occur, and destruction of the PT glands can occur following iron overload with multiple transfusions in β-thalassemia major.

Disorders of PTH

Autosomal-dominant familial isolated hypoparathyroidism (FIH) has been described in a patient in whom a single base substitution was found in exon 2 of the PTH gene. This results in a single base substitution (arginine for cysteine) that impedes processing of the mutant prepro- sequence. In two families, different mutations, also involving the prepro-sequence, have been found to cause autosomal-recessive FIH.

Disorders of the PTH/PTHrP receptor

Blomstrand's chondrodysplasia is a rare autosomal-recessive condition that results in advanced bone maturation, accelerated chondrocyte maturation, increased density of the skeleton, increased ossification, and poor bone modeling, particularly of the long bones [30]. It is rapidly lethal. Mutations of the PTH/PTHrP receptor have consisted of nucleotide exchanges in the maternal allele (the paternal allele not being well expressed), single nucleotide insertions or deletions resulting in frameshifts, or nonsense mutations resulting in a truncated protein.

Three siblings in one family have been described who presented with features very similar to those of pseudohypoparathyroidism type Ib (see below) and were found to have a single amino acid deletion (del382Ile) in the C-terminal end of the PTH/PTHrP receptor [31]. This mutation appears to uncouple the PTH/PTHrP receptor from the Gsα while not affecting other hormones.

Treatment of hypoparathyroidism

Treatment is aimed at maintaining plasma calcium levels within the lower part of the normal range without causing hypercalciuria. The mainstay of treatment is vitamin D either in its active form, 1α,25$(OH)_2$D (calcitriol), or the analog 1α-hydroxycholecalciferol (alfacalcidol). The dose of calcitriol is usually 15–30 ng/kg/day to maintain normocalcemia but requires twice-daily dosage. Alfacalcidol usually requires about twice the dose but, because it has to be metabolized first, it has a longer half-life and needs to be given only once daily. Calcium supplements are usually required, which may enable the dose of alfacalcidol to be reduced. This is a particular advantage in hypoparathyroid disorders in which the renal tubular reabsorptive effects of PTH are lacking and hypercalciuria may supervene. In those patients in whom cardiac failure is also present (e.g. DiGeorge syndrome), loop diuretics such as frusemide should be used with caution as the hypercalciuric effects of these agents may precipitate symptomatic hypocalcemia. Regular renal ultrasound examinations are useful in detecting early nephrocalcinosis. Synthetic PTH (1–34) has recently become available, but there is no experience of its use in children for hypoparathyroidism. Potent PTH analogs may become available in the future.

Hypomagnesemia

Magnesium is a ligand for the CaSR and, if plasma magnesium levels fall, PTH secretion is stimulated in a manner similar to that of hypocalcemia. Hypomagnesemia (< 0.5 mmol/L) inhibits PTH secretion in response to hypocalcemia. Initially, this inhibition is incomplete, and PTH remains elevated but not as high as would be expected from the degree of hypocalcemia. As levels fall further to 0.2–0.3 mmol/L, PTH secretion is inhibited completely, and a state of hypoparathyroidism then exists [32]. During the initial phase of mild hypomagnesemia, when PTH levels are still elevated, resistance to the action of PTH, caused by either desensitization or downregulation, worsens the hypocalcemia. Thus, hypocalcemia secondary to hypomagnesemia is resistant to treatment until magnesium levels have been restored to normal.

Hypomagnesemia is rare in childhood. It usually arises as a result of impaired intestinal absorption or increased urinary losses, which can be distinguished by measuring the urinary magnesium–creatinine ratio. An autosomal-recessive primary defect in magnesium absorption can occur in consanguineous kindreds, which leads to chronic hypomagnesemic hypocalcemia. Acquired malabsorption of magnesium can also occur as a result of gastrointestinal pathology.

Increased urinary losses of magnesium occur as a primary defect of renal tubular function or secondary to renal tubular damage. Autosomal-dominant hypermagnesuria is caused by mutations in the Na,K-ATPase γ in the DCT. A more severe form of autosomal-recessive magnesium wasting is seen in the familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) syndrome caused by a defect in the paracellin-1 magnesium transport protein in the thick ascending loop of Henle. It often leads to renal failure and is associated with gouty arthritis, chondrocalcinosis, and rickets. Hypermagnesuria also occurs in Gitelman's syndrome in association with hypocalciuria and hypokalemic alkalosis. Acquired tubulopathies leading to hypermagnesuria may occur in diabetic ketoacidosis, chronic alcoholism, or following the chronic use of various drugs, such as loop diuretics, cyclosporin A, aminoglycoside antibiotics, and cisplatin.

Treatment of hypomagnesemia is aimed at restoring plasma magnesium concentrations to normal to prevent inhibition of PTH secretion. In the acute state, intramuscular magnesium may be given as a 50% solution of $MgSO_4 \cdot 7H_2O$, which contains 2 mmol/mL. This may be repeated until magnesium levels are satisfactory. Intravenous infusion should be avoided as magnesium is an intense vasodilator and can cause cardiac arrhythmias. Normal magnesium levels can usually be maintained by oral supplementation, although this may need to be given several times a day. Magnesium

glycerophosphate is a useful preparation as it does not cause as much diarrhea as some other preparations.

Parathyroid hormone resistance

This group of conditions, which occurs as a result of defects toward the end of the calcium cascade, is characterized by hypocalcemia, usually but not always accompanied by hyperphosphatemia and raised PTH. The two most important are pseudohypoparathyroidism and deficiencies in the supply or metabolism of vitamin D.

Pseudohypoparathyroidism type Ia (PHP-Ia) is an autosomal-dominant condition characterized by the biochemical features of hypoparathyroidism (hypocalcemia and hyperphosphatemia) but with raised levels of PTH. Resistance to the action of PTH can be confirmed by demonstrating lack of cyclic AMP or phosphaturic responses to PTH infusion. A characteristic set of features includes short stature, round facies, shortening of the metacarpals and metatarsals, particularly the fourth and fifth, and obesity [collectively termed Albright's hereditary osteodystrophy (AHO)] (Fig. 13.7). Other features include intracranial calcification (Fig. 13.6), sensorineural deafness, and a poor sense of smell. Resistance to other cyclic AMP-dependent hormones, especially TSH and gonadotropins, may be present, leading to mild hypothyroidism and menstrual irregularity. This syndrome is referred to as PHP-1a. Following the discovery of the Gsα subunit of the G-protein, it was recognized that inactivating mutations within the gene are responsible for the PTH resistance [33].

Ten years after the original description of PHP, a second syndrome was described in which AHO was present without an abnormality of calcium metabolism. This was termed pseudopseudohypoparathyroidism (PPHP). It subsequently became apparent that both conditions can occur within the same family but not within the same sibship. While both conditions are associated with the skeletal manifestations, it emerged that, when hypocalcemia was present, the gene had been inherited from an affected mother; paternal transmission of the gene did not result in PTH resistance, despite the fact that identical mutations could be demonstrated within families. Gene imprinting was suspected [33] and subsequently confirmed by detailed genetic studies.

The GNAS1 gene has 13 exons that code for the Gsα subunit plus an additional seven exons including those that code for transcripts known as A/B, XL, and NESP55. By a complex arrangement of splicing, four different mRNA transcripts are known to result (Fig. 13.8). All have the products of codons 2–13 in common.

Native Gsα also contains exon 1, and this mRNA is expressed in most tissues in a biallelic manner. However, the transcripts containing A/B and XL are expressed only by the paternal allele, because the maternal allele is methylated, resulting in inactivation; the NESP55 allele is expressed only

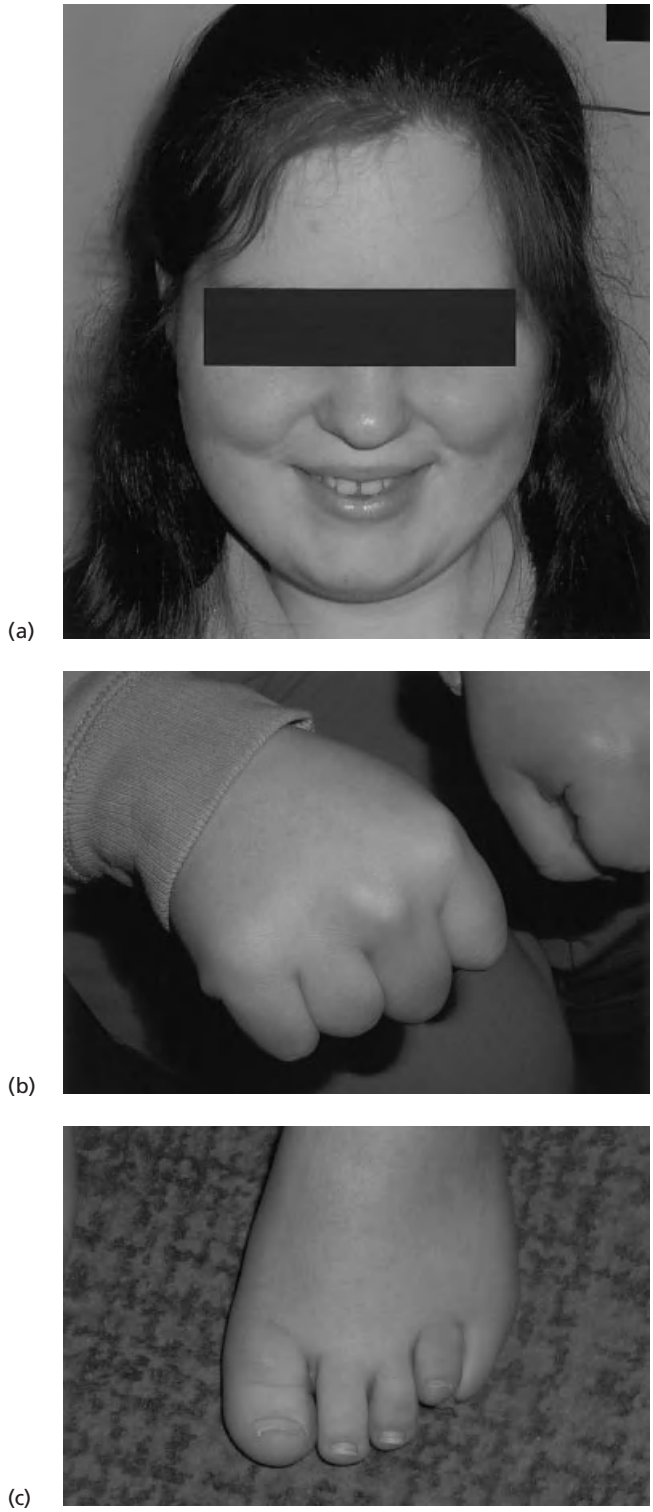

Fig. 13.7. Photographs of the face (a), showing the typical rounded facies, and of the right hand (b) and left foot (c) of a patient with pseudohypoparathyroidism type Ia showing the typical shortening of the metacarpals and metatarsals seen as part of Albright's hereditary osteodystrophy (AHO). She had presented with short stature. A mutation in the GNAS1 gene has been demonstrated in this patient. Reproduced with the kind permission of the patient.

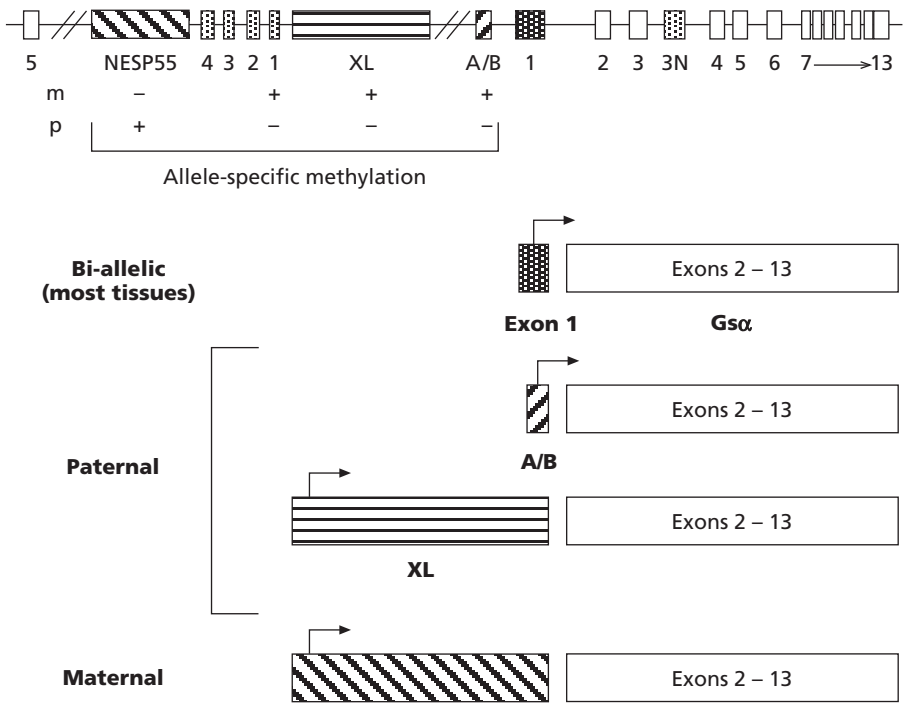

Fig. 13.8. Diagrammatic representation of the intron/exon organization of the GNAS1 gene showing the different mRNAs that are derived as a result of alternative splicing. Native Gsα is thought to be expressed in most tissues and to be biallelic. Mutations result in AHO. The A/B and XL alternative transcripts are principally expressed in the paternal allele whereas the NESP55 transcript, which is mainly found in the kidney, is expressed from the maternal allele. Therefore, paternally acquired mutations result in pseudopseudohypoparathyroidism while pseudohypoparathyroidism results from maternally acquired mutations. Adapted and reprinted from [43] with permission from Elsevier.

in the maternal allele, the paternal allele being methylated [34]. Some tissues, including the renal tubule, express only the maternal allele, and it is proposed that it is this that distinguishes the phenotypes. Thus, if a mutation occurs within the maternal allele, PTH resistance and hypocalcemia are present. Hypocalcemia is absent if the paternal allele is mutated.

Several mutations have been described throughout the GNAS1 gene, but most frequently in exon 7. There is no obvious phenotype–genotype correlation, apart from a missense mutation at codon 366 consisting of an Ala→Ser substitution, which results in a temperature-sensitive Gsα mutant. At 37°C, it is inactivated, resulting in PHP; at 34°C, it is activated and results in testotoxicosis.

In pseudohypoparathyroidism type Ib (PHP-Ib), features of AHO are absent, but PTH resistance is present. Renal resistance to PTH can be demonstrated by impaired cAMP and phosphaturic responses. Some patients exhibit hyperparathyroid bone disease, indicating that some measure of bone sensitivity to PTH is retained. These patients have been variously referred to as pseudohypohyperparathyroidism or pseudohypoparathyroidism with raised alkaline phosphatase. The term PHP-1b is preferred. The etiology is not clear. No mutations in the GNAS1 gene have been detected, but linkage studies have suggested that the gene responsible is located on chromosome 20q13 near to or in the same position as the GNAS1 gene. PHP-1b appears to be paternally imprinted in the same way as PHP-1a [35]. It is possible that tissue- or cell-specific promoters or enhancers of GNAS1 may be responsible. Recent studies have suggested that a microdeletion in the STX16 gene, which lies downstream from the GNAS gene, may be the crucial factor that results in differential loss of methylation of the A/B exon of the GNAS gene, thus resulting in renal resistance but without causing AHO [36].

Pseudohypoparathyroidism type Ic (PHP-Ic) is characterized by multiple hormone resistance, including PTH, together with features of AHO. No defect in Gsα has been demonstrated, and the genetic defect is not known, although it is presumed to reside somewhere within the adenylate cyclase–receptor system.

Pseudohypoparathyroidism type II (PHP-II) is reserved for a small group of patients who have PTH resistance without features of AHO. The PTH resistance is confined to the phosphaturic response, whereas cAMP responses are normal. The defect, which has not been identified, presumably lies beyond the adenylate cyclase system.

Treatment of PHP

The principles of treatment of PHP are similar to those of hypoparathyroidism. Alfacalcidol (1–3 μg/day) is usually sufficient to maintain normocalcemia. Hypercalciuria is less likely to occur than in primary hypoparathyroidism, and the plasma calcium concentration can usually be kept well within the normal range. Patients with PHP-1a or PHP-1c may have resistance to other hormones. TSH is frequently slightly raised, and thyroxine is required to suppress this and insure optimum thyroid function. Menstrual irregularities may require estrogen therapy. The role of growth hormone for short stature is controversial but has been used in some patients with variable effect. If resistance to GRF can be

demonstrated, there is some logic to this therapy. No treatment has a significant effect on the AHO.

Disorders associated with abnormal supply or metabolism of vitamin D

Vitamin D deficiency

Vitamin D in its active form is the second hormone essential for calcium homeostasis. Deficiency of vitamin D or defects in its metabolism result in inadequate amounts of $1\alpha,25(OH)_2D$, which leads to reduced calcium absorption and defective mineralization. Calciopenic osteomalacia, which manifests itself as rickets in growing bone, is frequently the result.

Vitamin D deficiency (nutritional rickets) remains a significant cause of abnormalities of calcium metabolism. Following the recognition of the importance of vitamin D, rickets was virtually eliminated in western societies with fortification of foods and administration of vitamin D supplements to children. A resurgence of vitamin D deficiency was seen following the increase in immigration, particularly from the Caribbean and Indian subcontinent in the 1950s and 1960s. Various campaigns, such as the Glasgow "Stop Rickets" campaign during the 1970s, reduced the incidence of rickets and vitamin D deficiency but, recently, there has been a "third wave" seen in both the UK and the USA. Vitamin D deficiency remains the single most common cause of rickets in the UK. Vitamin D deficiency can also arise as a result of impaired absorption of vitamin D in gastrointestinal disorders, such as celiac disease, especially if the supply of vitamin D from sunlight is restricted.

The biochemical abnormalities that develop in vitamin D deficiency occur in three stages [37]. In stage 1, hypocalcemia and hyperphosphatemia are present, bone turnover is increased, and alkaline phosphatase is usually raised. PTH is raised secondary to the hypocalcemia, and these patients can present with severe hypocalcemic symptoms. Rickets may not be present at this stage. Infants under the age of 1 year and adolescents appear to be most at risk of developing symptomatic hypocalcemia, and it has been postulated that this is because demand for calcium for bone mineralization is greatest during these periods because of the rapid growth rate [38]. The presence of hypocalcemia and hyperphosphatemia with raised PTH resembles an acquired PHP-like state and, indeed, PTH resistance, as demonstrated by cAMP responses to PTH, are impaired. Because of this, it is not possible to define a cause for hypocalcemia until vitamin D deficiency has been excluded or corrected. In some parts of the UK, vitamin D deficiency remains the most common cause of hypocalcemia outside the neonatal period.

In stage 2, the biochemical changes alter such that plasma calcium is only slightly low and hypophosphatemia supervenes. At the same time, rickets develops. The hypophosphatemia is presumably a response to the hyperparathyroidism, which becomes more marked during this phase.

In stage 3, hypocalcemia worsens and may again become symptomatic, whereas the hypophosphatemia persists. Rickets becomes worse. It should be noted that these three stages are not clearly defined, and considerable overlap occurs between them.

In vitamin D deficiency, 25OHD levels are usually low. However, a normal level does not exclude the diagnosis, especially if vitamin D has been administered or sunlight exposure obtained prior to presentation. Similarly, $1\alpha,25(OH)_2D$ levels (if measured) are usually low but may be normal or even elevated if vitamin D treatment has already begun. This is because $1\alpha,25(OH)_2D$ rises to supraphysiological concentrations in response to the high PTH following administration of vitamin D and falls to physiological levels only as the rickets heal [39].

Treatment of vitamin D deficiency is best undertaken with vitamin D and not one of its analogs. A dose of 1500 IU/day in infants and 3000 IU/day for 3 months in older children is usually sufficient to correct the biochemistry and restore vitamin D stores satisfactorily [40]. Acute symptomatic hypocalcemia may require temporary calcium infusions until symptoms subside. It is also advisable to give oral calcium supplements as well as vitamin D. The use of alfacalcidol should be avoided as it does not correct the vitamin D deficiency. Furthermore, if used in "physiological" doses, it may be ineffective in healing the rickets because it seems that the supraphysiological levels needed are required for adequate healing.

Disorders of vitamin D metabolism

The biochemical changes seen in rickets associated with abnormalities of vitamin D metabolism are similar to those seen in vitamin D deficiency, with the exception of the vitamin D metabolites. Chronic liver disease may affect 25-hydroxylation of vitamin D, but this is not usually of clinical significance; patients with chronic liver disease are usually given vitamin D supplements. In very low birthweight infants (23–25 weeks' gestation), who often develop a degree of hepatitis, poor 25-hydroxylation may be significant, and these infants sometimes require treatment with calcitriol rather than alfacalcidol to overcome this defect.

In vitamin D-dependent rickets type 1 (VDRR-I, 1α-hydroxylase deficiency), $1\alpha,25(OH)2D$ levels are low or only just within the normal range despite very adequate levels of 25OHD and high PTH. In HVDRR [hereditary $1\alpha,25(OH)_2D$-resistant rickets], both 25OHD and $1\alpha,25(OH)_2D$ levels are greatly elevated, particularly if treatment with alfacalcidol or calcitriol has been instituted.

Alfacalcidol is the treatment of choice in VDRR-I. Large doses (150–200 ng/kg/day) may be used in the first instance

until the rickets heal. This mimics the supraphysiological levels of $1\alpha,25(OH)_2D$ that occur during the initial phase of treatment of vitamin D deficiency. These patients need to be monitored and the dose reduced to prevent hypercalciuria or hypercalcemia as the bones heal. Treatment of HVDRR can prove very difficult. Some patients respond to very large doses of calcitriol whereas others prove almost completely resistant.

Systemic conditions associated with hypocalcemia

Tumor lysis syndrome occurs in about 30% of children during the initial phases of treatment of some hematological tumors. The release of large quantities of phosphate, potassium, and uric acid results in a syndrome characterized biochemically by hyperphosphatemia, hyperuricemia, hyperkalemia, uremia, and hypocalcemia [41]. The hypocalcemia is largely consequent upon the hyperphosphatemia, which itself occurs secondarily to the acute renal failure of hyperuricemia. The condition can largely be prevented by a combination of forced alkaline diuresis and the use of the recombinant urate oxidase inhibitor, rasburicase, which has been found to be useful and cost-effective.

Chronic renal failure (CRF) has a serious impact on calcium metabolism. Reduced GFR results in retention of phosphate, plasma levels of which begin to rise once GFR falls below 30 mL/min/1.73 m^2. As the kidney is the only site of 1α-hydroxylase activity, levels of $1\alpha,25(OH)_2D$ fall. Metabolic acidosis, either directly as a result of the CRF or caused by renal tubular disorders that may have led to the CRF, is often a factor. Hypocalcemia results, which induces secondary hyperparathyroidism. Renal osteodystrophy therefore consists of a spectrum of both high turnover resulting from the hyperparathyroidism and low turnover secondary to the osteomalacia [42]. Additional factors influencing renal osteodystrophy include calcium, phosphorus, vitamin D analogs, and aluminum.

The principles of minimizing renal osteodystrophy depend upon preventing hyperphosphatemia and reversing the effects of the reduced 1α-hydroxylase activity. Oral phosphate-binding agents are used for the former, and calcium carbonate is the most commonly used agent. Alfacalcidol or calcitriol is used to maintain $1\alpha,25(OH)_2D$ levels but must be monitored carefully to prevent hypercalciuria or hypercalcemia, which might worsen the renal failure.

Childhood hypercalcemia

The symptoms of hypercalcemia in childhood are age dependent. Mild hypercalcemia may be asymptomatic but, as the calcium concentration rises above 3.0 mmol/L, symptoms become more common. Infants present with failure to thrive, vomiting, and constipation. Muscle hypotonia, lethargy, anorexia, abdominal pain, and constipation may be present in older children. Polyuria and polydipsia result from a concentrating defect in the renal tubule, and longstanding hypercalciuria can lead to nephrocalcinosis, kidney stones, and renal failure. Occasionally, psychiatric disturbance accompanies hypercalcemia and reverses when calcium returns to normal.

Disorders of the calcium-sensing receptor

Although not as common as the disorders causing hypocalcemia, many of these are also genetic in origin (Table 13.3).

Familial benign hypercalcemia (FBH) or familial hypocalciuric hypercalcemia (FHH) was described in 1966 when a patient who was initially thought to have primary hyperparathyroidism remained hypercalcemic despite subtotal parathyroidectomy. Seventeen other asymptomatic family members in three generations were subsequently found to be hypercalcemic. Following the identification of the CaSR gene, inactivating mutations were shown to be the cause [11].

This is an autosomal-dominant condition, and most of the patients are heterozygous. It is often identified incidentally or as a result of investigation of FBH kindreds. In some families, there is a history of parathyroidectomy for presumed hyperparathyroidism. Plasma calcium usually remains elevated throughout life. There is a high degree of penetrance, and hypercalcemia has usually developed before 10 years of age but often much earlier. Most patients remain asymptomatic, although some infants may develop mild symptoms during the first year. Pancreatitis has been described as a rare complication in FBH. It is not clear whether or not this is a true association or whether the hypercalcemia itself may be the cause. Some mutations may confer susceptibility to pancreatitis in a subgroup of patients.

FBH must be distinguished from primary hyperparathyroidism. Although plasma calcium is elevated, sometimes above 3.0 mmol/L, PTH remains normal unless attempts have been made to reduce the plasma calcium with low-calcium diets, etc. The PTH has normal biological activity. However, in contrast to hyperparathyroidism, plasma magnesium is usually slightly elevated, urinary calcium excretion is inappropriately low for the degree of hypercalcemia, and nephrocalcinosis does not develop. Treatment of FBH is usually unnecessary and, when the condition is diagnosed in a child of a kindred known to carry the gene, reassurance is all that is required.

Neonatal severe primary hyperparathyroidism (NSPHT) is usually caused by a homozygous inactivating mutation in the CaSR gene and occurs mostly in consanguineous families [11]. Newborns fail to thrive, feed poorly, and suffer constipation and atonia shortly after birth. Gross hypercalcemia, often in excess of 5.0 mmol/L, and hypophosphatemia are present. PTH is markedly elevated, and hyperparathyroid

Table 13.3. Genetic conditions that cause hypercalcemia. The conditions are classified according to which part of the calcium cascade they affect and, where known, the gene location, gene product, inheritance, and principal clinical features are shown.

Location in calcium cascade	Metabolic abnormality	Chromosome location	Gene/gene product	Inheritance	Principal clinical features
Calcium sensing receptor	Familial benign hypercalcemia	3q13–21	CaSR	AD	Asymptomatic hypercalcemia with hypocalciuria. Occasional pancreatitis
	Neonatal severe primary hyperparathyroidism	3q13–21	CaSR	AD – homozygous	Severe neonatal hyperparathyroidism with grossly elevated calcium and PTH
The parathyroid glands	Multiple endocrine neoplasia type 1	11q13	MENIN	AD	Parathyroid adenomas with hyperparathyroidism, pancreatic tumors, anterior pituitary tumors
	Multiple endocrine neoplasia type 2a	10cen–10q11.2	*c-ret* proto-oncogene	AD	Parathyroid tumors, medullary carcinoma of the thyroid, pheochromocytomas
	Multiple endocrine neoplasia type 2b	10cen–10q11.2	*c-ret* proto-oncogene	AD	Medullary carcinoma of the thyroid, mucosal neurofibromas, intestinal autonomic ganglion dysfunction
	Medullary carcinoma only	10cen–10q11.2	*c-ret* proto-oncogene	AD	Medullary carcinoma of the thyroid
	Sporadic parathyroid adenomas	1p32–ter	?tumor suppressor gene	Sporadic	Isolated hyperparathyroidism
	Hyperparathyroid–jaw tumor syndrome	1q21–31	HRPT2 gene ?Parafibromin	AD	Parathyroid adenomas and carcinomas, mandibular and maxillary jaw tumors
	Familial isolated primary hyperparathyroidism	Various	Various	AD	Isolated hyperparathyroidism (occasionally carcinoma)
	Parathyroid carcinomas	13q14	Retinoblastoma		
PTH	Sporadic parathyroid adenomas	11p15/11q13	PRAD1 cyclin D1	Sporadic	Primary hyperparathyroidism
PTH/PTHrP receptor	Jansen's disease	3p21.1–p22	PTH1R	AD	Neonatal hyperparathyroidism with low PTH, short stature, abnormal chondrocyte proliferation
Target organs	Hypophosphatasia	1p36.1–p34	TNAP	AR	Hypercalcemia, hyperphosphatemia, severe undermineralization of bone, variable severity depending on age of presentation
Abnormal vitamin D metabolism	Williams syndrome	7q11.23	Elastin gene LIM-kinase	AD but usually sporadic	Failure to thrive, poor feeding, irritability, "elfin" facies, radioulnar synostosis, "cocktail party" conversation

bone disease develops such that respiratory distress necessitating assisted ventilation may result from poor rib compliance. The bones become thin and develop a "moth-eaten" appearance because of the severe hyperparathyroidism (Fig. 13.9), which can be identified by the presence of microcysts in the subperiosteal areas. Multiple fractures may occur and be mistaken for rickets. Once the diagnosis has been established, total parathyroidectomy is required to eliminate the hypercalcemia. As sometimes happens with primary hyperparathyroidism, a "hungry bone" condition usually develops, which requires infusion of large quantities of intravenous calcium to prevent hypocalcemia until such time as the bones recover sufficiently. Prior to surgery, bisphosphonates may be useful temporarily to restore normocalcemia.

NSPHT may also develop in infants who have a heterozygous mutation of the CaSR gene. The reasons for this are not clear but may be related to the maternal calcium concentration. If the infant has inherited the gene from an affected father and the mother is normocalcemic, the fetus may sense the maternal calcium as low and develop a degree of secondary hyperparathyroidism that settles progressively. Alternatively, the degree of set point abnormality or bone responsiveness to PTH may be responsible. In these cases, conservative management may be sufficient until the hypercalcemia settles spontaneously to a level at which it becomes asymptomatic.

Another recently described phenotype similar to FBH is associated with the presence of CaSR blocking antibodies that lead to secondary hyperparathyroidism [21]. Mutational analysis of the CaSR was negative in all cases, most of whom had other autoimmune conditions such as hypothyroidism or celiac disease. The principal difference between this and

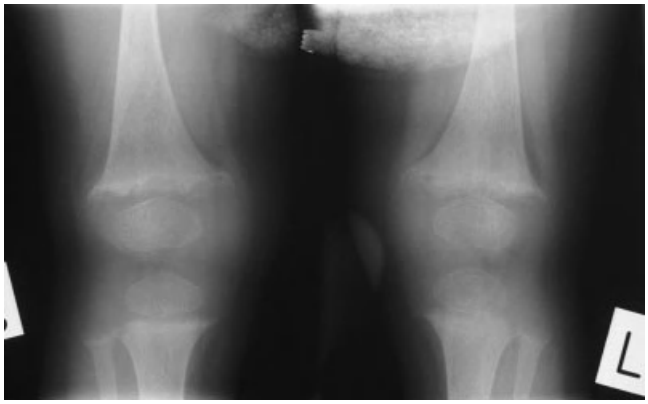

Fig. 13.9. Radiographic image of lower end of femur and upper tibia in an infant suffering from severe neonatal primary hyperparathyroidism. Note the "moth-eaten" appearance and the similarity to that of rickets. This infant's chest radiograph showed the presence of multiple rib fractures and cystic areas, and he was ventilator dependent for several weeks prior to parathyroid gland removal. He then developed a severe "hungry bone" syndrome requiring infusion of large quantities of calcium to prevent symptomatic hypocalcemia. Reproduced by kind permission of Dr William Van't Hoff.

primary hyperparathyroidism was the absence of hypercalciuria, the raised plasma magnesium, and the normal PTH levels. The natural history of this condition is not known, but there is a likelihood that it may remit spontaneously as the antibody levels decline.

Disorders of the parathyroid glands

Primary hyperparathyroidism can result from generalized PT gland hyperplasia or single adenomas. They may be isolated and sporadic or form part of one of the inherited multiple tumor syndromes. In many instances, in both the sporadic and the inherited forms of tumor, mutations in one of the oncogenes, tumor suppressor genes, or the PTH gene have been demonstrated. In sporadic cases, a "single hit" mutation affects a proto-oncogene such as PRAD1 (parathyroid adenoma 1), resulting in preferential growth of a single cell line. In the familial syndromes, a germline "first hit" mutation affects a tumor suppressor gene and makes the parathyroid (and other) glands susceptible to a "second hit" [43].

Multiple endocrine neoplasia type 1 (MEN1) is characterized by a combination of parathyroid (90% of patients), pancreatic endocrine (40%), and anterior pituitary (30%) tumors. Adrenocortical and carcinoid tumors as well as lipomas, angiofibromas, and collagenomas may also occur [44]. The etiology is probably an inactivating mutation of the MEN1 gene, located on chromosome 11q13, which normally codes for a tumor suppressor protein MENIN. Nonsense mutations, deletions, insertions, donor-splice site mutations, and missense mutations have all been described. Parathyroid tumors are usually the first to present, generally in late adolescence or the twenties or thirties.

Three different variants of multiple endocrine neoplasia type 2 (MEN2) are described. In the most common variant, MEN2a, parathyroid tumors (20%) are associated with medullary carcinoma of the thyroid (MCT) and pheochromocytomas [45]. MEN2b is not usually associated with parathyroid tumors but has an association with pheochromocytomas, mucosal neurofibromas, and intestinal autonomic ganglion dysfunction. In the third variant, MCT-only, no tumors other than MCT occur.

All three are linked by mutations in a gene that maps to chromosome 10cen–10q11.2. This contains the *c-ret* proto-oncogene. Different mutations have been found in all three variants, and identification of these mutations is useful in the diagnosis and management of family members at risk of developing these tumors.

Allelic loss of chromosome 1p32–pter has been found in a number of cases of isolated sporadic parathyroid adenomas [46]. This region contains a putative tumor suppressor gene, but the gene product is unidentified.

Hyperparathyroid–jaw tumor syndrome (HYP-JT) is an autosomal-dominant syndrome. Parathyroid adenomas and carcinomas are associated with mandibular and maxillary jaw tumors that are fibro-osseous in nature. Inactivating mutations of the HRPT2 gene, located on chromosome 1q21–q31, are thought to be responsible [47]. This gene codes for a parafibromin protein that acts as a tumor suppressor.

Familial isolated primary hyperparathyroidism (FIHP) has also been described in several families. In some it appears to be a variant of either MEN1, FBH or occasionally HYP-JT. In others, no genetic abnormalities have been detected and it is not clear if this is a separate entity.

Parathyroid carcinoma can be difficult to distinguish histologically from parathyroid adenoma, unless metastases are present. All cases have been associated with allelic deletions of the retinoblastoma (Rb) gene, which has three polymorphic markers, located on chromosome 13q14. Loss of heterozygosity (LOH) of at least one of the markers at the Rb locus of the gene occurs in all cases of carcinoma but also occurs in some cases of parathyroid adenoma. However, all parathyroid adenomas show some positivity for the retinoblastoma protein (pRb) whereas this is lacking in all carcinomas [48]. Lack of translation of the pRb seems to be the distinguishing feature (48). Treatment is surgical.

Disorders of the PTH gene

Sporadic parathyroid tumors may result from mutations within the PTH gene itself. The PRAD1 gene is derived from a rearrangement of exon 1 of the PTH gene, which is normally not translated, with new non-PTH DNA located on chromosome 11q13. It normally encodes for a 295-amino-acid protein, cyclin D1 [49]. Mutations resulting in overexpression

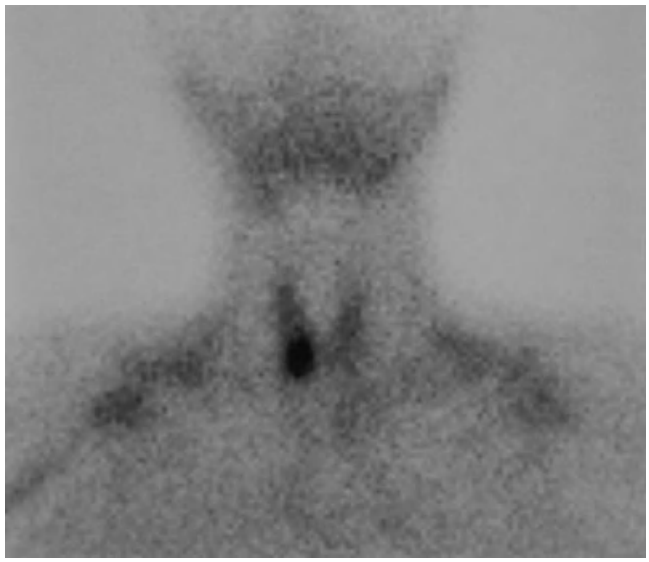

Fig. 13.10. SestaMIBI scan taken in a child with a single right lower parathyroid adenoma. She presented with hypercalcemia. A single adenoma was removed at operation and she remains normocalcemic. No mutations of the MEN1 or HYP-JT genes have been demonstrated.

of this gene cause parathyroid cell proliferation and hyperparathyroidism. The mutations appear to be somatic and are therefore not inherited.

More recently, a 22-bp deletion within exon 2 of the PTH gene has been described [50]. It is possible that silencing of the PTH gene leads to overexpression of the PRAD1/cyclin D1 gene, thus causing the parathyroid tumors seen in this condition. This may cause confusion when assaying PTH as it may not react in all immunoassays.

Diagnosis and treatment of hyperparathyroidism

Hypercalcemia and hypophosphatemia are associated with raised PTH levels. Urinary calcium excretion is also raised, and a partial Fanconi syndrome (generalized aminoaciduria and mild metabolic acidosis) is usually present. Plasma magnesium is often slightly low, in contrast to FHB. Radiological examination may reveal the presence of subperiosteal microcysts, and severe hyperparathyroidism can be confused with rickets.

Localization of parathyroid tumors is best undertaken with the aid of radionuclide scanning with ^{99m}Tc-MIBI (methoxyisobutyl isonitrile) or ^{99m}Tc-tetrofosmin (Fig. 13.10). These methods are more sensitive than either ultrasonography or magnetic resonance imaging (MRI) scanning and have proved invaluable in locating persistent tumors, especially after primary surgery has failed to eradicate the problem. They are sometimes combined with thyroid subtraction scintigraphy and, if performed shortly before surgery, can be combined with the use of a handheld gamma camera to pinpoint the tumor at operation.

Treatment consists of surgical removal of the tumors, which should be undertaken only by those experienced in the procedure. It may be necessary to control the hypercalcemia before surgery by the use of forced diuresis and frusemide. Failing this, bisphosphonates (e.g. pamidronate given in a dose of 0.5 mg/kg daily for 2–3 days) is usually sufficient to restore plasma calcium to normal. Plasma calcium usually declines post-operatively within a few hours, and the patient may become hypocalcemic and remain so for some while if hyperparathyroidism has been longstanding. In this case, a "hungry bone" syndrome develops that requires infusion of calcium in large quantities.

Disorders of the PTH1R

Jansen's disease is an autosomal-dominant condition that presents in the neonatal period with apparent hyperparathyroidism but without detectable PTH or PTHrP. It is characterized by short-limbed short stature caused by abnormal regulation of chondrocyte proliferation and differentiation in the metaphyseal growth plate. It is caused by mutations of the PTH/PTHrP receptor, which autoactivate in the absence of either hormone [51]. Treatment is difficult, but the condition is said to respond to calcitonin and, theoretically, bisphosphonates should be of value.

Hypercalcemia associated with abnormal vitamin D metabolism

Subcutaneous fat necrosis usually occurs in term infants who have suffered a mild degree of birth asphyxia. Firm lumps appear in the subcutaneous tissues and may be multiple in number. Hypercalcemia develops within the first few weeks after birth and is accompanied by hypercalciuria and nephrocalcinosis [52]. The skin lesions are invaded by macrophages, and the etiology of the hypercalcemia is thought to be inappropriate activation of 1α-hydroxylase within the macrophages, which results in high concentrations of circulating $1\alpha,25(OH)_2D$. The condition is self-limiting within a few weeks, but steps may need to be taken in the meantime to reduce the plasma calcium level. Calcium and vitamin D restriction, steroids, and bisphosphonates may be of value.

A similar process is thought to occur in sarcoidosis and other granulomatous diseases, which are very rare in childhood. Approximately 30–50% of children with sarcoidosis develop hypercalcemia, which may be precipitated by sunlight. Others have hypercalciuria without hypercalcemia. Other granulomatous diseases, including tuberculosis and cat-scratch disease, may also cause hypercalcemia via a similar mechanism [53]. The hypercalcemia usually resolves with treatment of the underlying condition.

In very large doses, vitamin D may cause hypercalcemia, mainly because of the high concentrations of 25OHD that

result from this consequent upon the uncontrolled metabolism of vitamin D by 25-hydroxylase. Although 25OHD has limited activity, high concentrations cause increased bone resorption. A more common cause of hypercalcemia as a result of vitamin D excess is seen in patients treated with excess doses of either alfacalcidol or calcitriol. The symptoms are those typical of hypercalcemia from other causes. Complications of prolonged hypercalcemia are ectopic calcification, nephrocalcinosis, and impaired renal function. Hypercalcemia following excess vitamin D is usually more prolonged than that caused by the vitamin D metabolites because vitamin D itself is stored in fat, whereas the metabolites have a much shorter half-life. Treatment is directed toward restricting the source of excess vitamin D. If acute symptoms are present, steroids or bisphosphonates may be of value.

Williams syndrome is autosomal dominant but usually sporadic. During infancy, patients have a characteristic phenotype consisting of "elfin facies" caused by periorbital fullness, a long philtrum, malar hypoplasia, and an open-mouthed appearance caused by an arched upper lip and full lower lip. As they get older, features change, become coarsened, and some skeletal abnormalities such as radioulnar synostosis may develop. Many patients develop hypercalcemia during infancy, which rarely lasts beyond the first year. Subsequently, cardiac anomalies are often present. These manifest themselves particularly as subvalvar aortic stenosis or peripheral pulmonary stenosis. Developmental delay is a feature, and patients develop a tendency to "cocktail party" conversation as children and young adults, in which it appears that they are conducting an intelligent conversation which, on reflection, is largely meaningless.

The etiology of the hypercalcemia is not clear. Some patients have been thought to have abnormalities of vitamin D metabolism or CT deficiency while hypercalcemic, whereas others have been found to have no identifiable defect in any of the parameters of calcium metabolism after the hypercalcemia resolves. Most cases have a microdeletion of chromosome 7q11.23, which encompasses the elastin gene [54]. A second gene, termed LIM-kinase, which is expressed in the central nervous system (CNS), is also present in the same region of chromosome 7 and has been implicated in some of the abnormalities. Mutations of the CT receptor gene (7q21) are not thought to be responsible. How these mutations affect calcium metabolism is uncertain.

Infant patients present with failure to thrive, poor feeding, and irritability. Treatment of the hypercalcemia consists of giving a low-calcium diet. A low-calcium milk (Locasol) is useful in this respect. It should be noted that, where patients live in hard water areas, there may be sufficient calcium in the water to negate the effect of Locasol. If symptoms are severe, a short course of prednisolone, 1 mg/kg/day, is useful and can usually be stopped after a few weeks. Correcting the hypercalcemia does not seem to have any effect on the progress of the other features of the disease, which may evolve without hypercalcemia ever having been present.

Idiopathic infantile hypercalcemia (IIH) was originally described in infants born to mothers who had been ingesting large quantities of vitamin D, and the incidence declined with a general reduction in vitamin D supplementation. However, some cases continued to occur with no evidence of excess vitamin D intake. Familial cases have been described. Some of the features of this condition show similarities to Williams syndrome and can include hypertension, strasbismus, and radioulnar synostosis with failure to thrive, poor feeding, etc. The dysmorphic features are usually absent, and correction of the hypercalcemia allows normal development, although the tendency to hypercalcemia may last beyond the first year. Lack of mutations in the elastin gene allows this condition to be distinguished from Williams syndrome [55].

The etiology of this condition is uncertain. It has been suggested that an intrinsic hypersensitivity to vitamin D is present, and elevated levels of N-terminal PTHrP were demonstrated during hypercalcemia in one series. Treatment consists of lowering the plasma calcium with a calcium- and vitamin D-restricted diet, steroids, and bisphosphonates if necessary. Cellulose phosphate has been used to limit calcium absorption.

Other causes of hypercalcemia in childhood

Immobilization

Hypercalcemia occurs in a small proportion of patients who are immobilized following quadriplegia or other neurological insults [56]. It is more common in adolescents whose bone turnover is naturally more rapid than in adults. The symptoms are non-specific and consist of lethargy, mood changes, nausea, vomiting, and anorexia, but may be overlooked in the context of the other problems. They usually arise within a few days or weeks of the original insult. Hypercalcemia and hypercalciuria are present, and nephrocalcinosis can result. Bone biopsy shows loss of trabecular volume, increased osteoclast and decreased osteoblast activity with an overall increase in bone turnover as demonstrated by raised bone turnover markers. The etiology is uncertain and is probably multifactorial, including lack of mechanical stress, poor vascularity, metabolic changes in bone, and denervation [56]. If remobilization is not possible and conventional treatment with intravenous fluids and loop diuretics (which may increase urinary calcium excretion) are ineffective in controlling the hypercalcemia, infusion of pamidronate, 0.5 mg/kg daily for 2–3 days is usually effective in correcting the hypercalcemia. This effect may last for several weeks. Calcitonin has been used, but the effect is not so rapid, and it has to be given in divided daily doses for more prolonged periods.

Hypercalcemia of malignancy

Hypercalcemia is a rare complication of malignancy in childhood and has been reported to occur in about 0.4% of cases [57]. It may be a presenting feature of leukemia but also occurs in Hodgkin disease, non-Hodgkin lymphoma, and a variety of solid tumors, such as rhabdomyosarcoma, hepatoblastoma, neuroblastoma, and angiosarcoma.

As with other conditions complicated by hypercalcemia, the symptoms may be overlooked. The cause is usually related to excess secretion of PTHrP, and PTH levels are low. Bone turnover is increased and, if the hypercalcemia does not remit on treatment of the underlying malignancy, it usually responds well to bisphosphonate therapy as for immobilization.

Hypophosphatasia

This rare condition is usually autosomal recessive and is caused by mutations in the TNAP gene. Four different forms are described depending on the age of presentation and severity [58]. Hypercalcemia and hyperphosphatemia occur in the more severe forms. In perinatal hypophosphatasia, infants are born with undermineralized bones with rachitic changes, have a high-pitched cry and unexplained fevers and seizures, and usually die shortly after birth. It has been suggested that this is the homozygous form of the condition.

In the infantile form, hypercalcemia and its attendant symptoms together with bone abnormalities develop during the first 6 months after birth. If they survive, there tends to be a gradual improvement with time, and the prognosis is relatively good. The childhood form must be distinguished from rickets but tends to improve at adolescence, although osteomalacia may reappear later in life, and the adult form is relatively mild. There is no specific treatment. Bisphosphonates have been tried in the infantile form but without success.

Tertiary hyperparathyroidism

This occasionally occurs in children after chronic hyperstimulation of the PT glands, particularly in CRF. PTH levels are usually very elevated, and the hyperplastic glands become susceptible to developing autonomous nodules. It is not clear whether or not this adenomatous formation is polyclonal or monoclonal in origin, but recent studies have suggested, somewhat surprisingly, that the latter may be present in a majority of cases. Treatment consists of parathyroidectomy.

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14 Disorders of bone metabolism

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Bone structure

Long bones have three parts. The widened ends are the *epiphyses*, the tubular middle is the *diaphysis*, and the part in between the epiphysis and the diaphysis is the *metaphysis*. In growing children, the fourth region, the *growth plate*, is the area between the epiphysis and metaphysis and consists of epiphyseal cartilage, which is uncalcified; this is where growth occurs. When growth ceases, the growth plate is calcified and disappears.

There are two types of bone, *cortical bone* and *trabecular* (or *cancellous*) *bone*. The cortical bone or *cortex* is the outer layer of bone. In all, 80–90% of cortical bone is calcified, and this densely calcified bone is prominent in the diaphysis. On the other hand, only 15–35% of trabecular bone is calcified and is made up of a lattice of calcified trabeculae surrounded by bone marrow. Trabecular bone is more prominent in the metaphysis and epiphysis.

Bone matrix

Bone is composed of a scaffolding, the *bone matrix*, and mineral, which is the *hydroxyapatite* crystals that fill in the scaffolding. The bone matrix is made up of collagen fibers and non-collagenous proteins [1]. The most abundant protein in bone matrix is type I collagen. Each type I collagen molecule is composed of two α1(I) chains and one α2(I) chain, encoded by the COL1A1 and COL1A2 genes respectively. The three chains form a triple helix. The collagen monomers then come together to form fibrils, thus providing the tensile strength needed. Type I collagen is found in a variety of other tissues, such as ligaments, tendons, dermis, sclera, dentin, fascia, and the viscera. Mutations in COL1A1 or COL1A2 result in the disorder *osteogenesis imperfecta*.

There are a number of other types of collagen in bone [1]. Type V collagen is also found as trimers of α1(V) and α2(V) chains encoded by COL5A1 and COL5A2 chains, respectively, and co-localizes with type I collagen. Type II collagen is the primary collagen in cartilage, including the growth plate cartilage. Type II collagen is found as homotrimers of α1(II) chains, encoded by the COL2A1 gene. Type IX collagen is found in hyaline cartilage. Type IX collagen is made up of heterotrimers of α1(IX), α2(IX), and α3(IX) chains, encoded by the COL9A1, COL9A2, and COL9A3 genes respectively. Type XI collagen is composed of three chains, α1(XI), α2(XI), and α3(XI). The α1(XI) and α2(XI) chains are encoded by the COL11A1 and COL11A2 genes, respectively, whereas the α3(XI) chain is a variant product of the COL2A1 gene. Absence of any one of the three chains results in *spondyloepiphyseal dysplasia*. Type XI collagen combines with other collagen molecules, including type II and IX collagen, to form fibrils in bone and cartilage. Type X collagen, composed of homotrimers of α1(X) chains encoded by the COL10A1 gene, are produced by the hypertrophic chondrocytes of the growth plate. Mutations in type X collagen lead to *Schmid metaphyseal chondrodysplasia*.

Non-collagenous proteins account for about 10–15% of the protein in bone [2]. They can be roughly divided into proteoglycans, glycosylate proteins, and γ-carboxylated (gla) proteins [1,2]. Proteoglycans include a number of macromolecules. Aggrecan is one of a group of chondroitin sulfate proteoglycans that binds hyaluronate. These proteoglycans aggregate and fill in the spaces between collagen fibrils in the matrix. There is a group of "small, leucine-rich interstitial proteoglycans," a family of extracellular matrix glycoproteins, and proteoglycans that bind to collagens and to growth factors [1]. Members of this family include decorin, biglycan, fibromodulin, lumican, osteomodulin, epiphycan, and chondroadherin.

Glycoproteins in bone include thrombospondins 1–5, glycoproteins that bind to other macromolecules and to cell surface receptors. Thrombospondin 5 is also known as cartilage oligomeric matrix protein (COMP). Cell-surface interactions with the thrombospondins may be important in maintaining cell adhesion, migration, and cell shape. Mutations in the COMP gene are responsible for the skeletal dysplasias *pseudoachondroplasia* and *multiple epiphyseal dysplasia*. Osteonectin

is another glycoprotein and is the most abundant non-collagenous protein in mineralized bone matrix. Osteonectin binds calcium, hydroxyapatite, and collagen. Osteonectin inhibits cell spreading and cell adhesion.

Gla proteins account for about 10% of non-collagenous protein in bone and require vitamin K as a cofactor. The gla proteins may function to inhibit mineral deposition. The gla-containing proteins include matrix gla protein (MGP) and osteocalcin, also called bone Gla protein. Osteocalcin is expressed exclusively by osteoblasts and is important in bone formation and in bone mineral maturation.

Bone sialoprotein accounts for about 12% of non-collagenous protein in bone and binds to calcium and hydroxyapatite cells, as well as collagen. Osteopontin is a secreted glycoprotein and is a potent inhibitor of apatite formation and growth.

Bone cells

Osteoblasts

Osteoblasts are cells that line the surfaces of the bone and synthesize the organic bone matrix [3,4]. Osteoblasts produce alkaline phosphatase at high concentrations, as well as collagen and non-collagenous proteins. Once osteoblasts have completed producing matrix, they either die (apoptosis) or differentiate either into osteocytes enclosed in the bone matrix in small lacunae or into lining cells that line 80–95% of bone surfaces. The remaining 5–20% of the bone surface that is not lined by lining cells is covered by osteoblasts or osteoclasts. The uncalcified bone matrix is called *osteoid*. The osteoid then mineralizes, forming bone. Osteoblasts have receptors for parathyroid hormone (PTH), estrogen, vitamin D, prostaglandins, adhesion molecules, and cytokines. Osteoblasts produce colony-stimulating factor -1 (CSF-1) and receptor activator of NF-kappa B ligand (RANKL), which both activate osteoclast differentiation. Osteoprotegerin, also produced by the osteoblasts, acts as a decoy for the RANK receptor.

Osteoclasts

Osteoclasts are the bone-resorbing cells [3]. They start off as mononucleated cells derived from hemopoietic stem cells and fuse with each other or with existing osteoclasts to form giant multinucleated cells. They are characterized by staining positive for tartrate-resistant acid phosphatase (TRAP). Osteoclasts have high concentrations of proton pumps, proteolytic enzymes, and cathepsin K. Osteoclasts dissolve bone by attaching to the bone surface, sealing off the area, and acidifying the sealed-off area. Protons generated by carbonic anhydrase II dissolve the mineral in bone, and other enzymes secreted by osteoclasts degrade the organic matrix of bone. Osteoclasts have receptors for calcitonin, which can inactivate the osteoclast. Osteoclasts also have RANK (the receptor for RANKL) and M-CSF receptors. Binding of RANKL and CSF (secreted by osteoblasts) to their respective receptors on the osteoclasts leads to osteoclast differentiation. In this way, osteoblast activity is coupled to osteoclast activity. Osteoprotegerin (OPG), secreted by the osteoblast, is a soluble decoy receptor for RANKL, interfering with RANKL activation of RANK and thus inhibiting osteoclast differentiation [3]. Thus, the interplay between OPG and RANKL and RANK determines osteoblast formation.

Bone turnover

Osteoblasts and osteoclasts work together in the formation and resorption of bone, which is critical for bone remodeling, growth, and the adaptation of the skeleton to the demands place upon it. In children, bone formation exceeds bone resorption, leading to skeletal growth. In the bone, the sites of bone remodeling are the bone remodeling units (BMUs). In the first phase of bone remodeling, osteoclasts attach to the bone surface, fuse into multinucleated cells, and resorb bone. In the second phase, phagocytes and/or other cells deposit a *cement line*. In the third phase, osteoblasts come in and deposit osteoid and mineral. Finally, there is a resting phase where there is no osteoid left between the lining cells and mineralized bone.

Bone growth

There are two mechanisms of bone growth, *endochondral* and *intramembranous* bone formation [5]. *Endochondral ossification* is the most common mechanism of bone formation, bone being formed from a matrix that is synthesized by chondrocytes. In endochondral ossification, mesenchymal cells differentiate into chondroblasts that secrete a cartilaginous matrix. The chondroblasts become surrounded in lacunae, at which point they are called chrondrocytes. *Indian hedgehog* (*IHH*) and *parathyroid hormone-related peptide* (*PTHRP*) are the primary molecules involved in chondrocyte regulation. At the center of the cartilaginous matrix, the chondrocytes no longer proliferate and hypertrophy, thus becoming hypertrophic chondrocytes. As the matrix starts to mineralize, preosteoblasts and blood vessels invade, the hypertrophic chondrocytes undergo apoptosis, and the preosteoblasts differentiate into osteoblasts. The osteoblasts lay down a matrix on the one that was left behind by the hypertrophic chondrocytes, called the *primary spongiosa*. After further remodeling, the *secondary spongiosa* is formed, which will become mature trabecular bone. Near the ends of the bone, a similar process occurs in the secondary ossification centers. The area between the primary and secondary ossification centers is the *growth plate*.

In *intramembranous bone formation*, there is no cartilage matrix, and mesenchymal cells differentiate directly into osteoblasts. Bones grow as the preosteoblasts proliferate

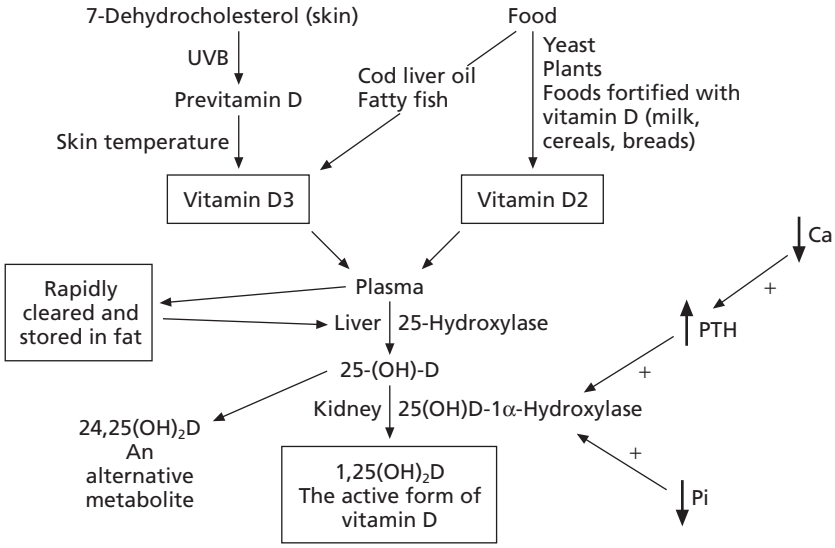

Fig. 14.1. Vitamin D metabolism.

and differentiate at the perimeter of the bone. Sutures form as the perimeters of the bones approach each other. Intramembranous bone formation is primarily responsible for the formation of the flat bones of the skull.

Endocrine and paracrine factors important in the control of bone growth include growth hormone, insulin-like growth factor (IGF)-1, the fibroblast growth factors (FGFs) and their receptors (FGF-R1–3), IHH, bone morphogenetic proteins (BMPs), members of the Wnt signaling pathway (e.g. LRP5), PTH, PTHRP, thyroid hormone, estrogen, and androgens [5].

Vitamin D

Vitamin D is the hormone responsible for calcium absorption in the intestine. Although termed a "vitamin," vitamin D is not a true vitamin as it is produced endogenously in the skin after sun exposure [6].

Sources of vitamin D

Vitamin D is produced in the skin after exposure to sunlight. 7-Dehydrocholesterol is converted into previtamin D by UV-B radiation. Previtamin D then undergoes thermal isomerization to vitamin D3 (*cholecalciferol*). Vitamin D3 is made in fish and mammals. Vitamin D2 (*ergocalciferol*) is made in yeast and plants. Milk may be fortified with vitamin D2. The minor differences between vitamin D3 and D2 do not affect function, and the term "vitamin D" is usually used to refer to both vitamins D2 and D3.

Activation of vitamin D (Fig. 14.1 [6,7])

Vitamin D binds to the vitamin D-binding protein (DBP) and is transported to the liver where it is converted by vitamin D-25-hydroxylase to 25-hydroxyvitamin D [25(OH)D] by the addition of a hydroxyl group to carbon 25. The vitamin D-25-hydroxylase is not tightly regulated; as a result, an increase in vitamin D will lead to an increase in 25(OH)D. Thus, measuring serum 25(OH)D concentrations is a good way to determine overall vitamin D status [7,8]. 25(OH)D is transported to the kidney where it is hydroxylated at the 1α position to 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] by the enzyme 25(OH)D-1α-hydroxylase. The primary site for 1α-hydroxylation of 25(OH)D is the proximal tubule of the renal cortex. 1α-Hydroxylase activity is stimulated by PTH and indirectly by hypocalcemia through PTH. Hypophosphatemia also stimulates 1α-hydroxylase activity, as does calcitonin. 1,25$(OH)_2$D in turn inhibits PTH and 1α-hydroxylase gene expression, thus preventing sustained production of 1,25$(OH)_2$D that would lead to hypercalcemia. 1,25$(OH)_2$D also induces the expression of 25(OH)D-24-hydroxylase. 24-Hydroxylation of 25(OH)D and of 1,25$(OH)_2$D leads to the formation of 24,25-dihydroxyvitamin D and 1,24,25-trihydroxyvitamin D, respectively, which are both much less active metabolites of vitamin D.

1,25$(OH)_2$D binds to the vitamin D receptor, VDR. VDR heterodimerizes with the retinoic acid receptor, RXR, as it binds 1,25$(OH)_2$D. It then interacts with target genes, regulating their transcription [7]. The affinity of VDR for 1,25$(OH)_2$D is about 1000 times greater than for 25(OH)D.

The main action of 1,25$(OH)_2$D in calcium homeostasis is in the small intestine, where it stimulates intestinal absorption of calcium. 1,25$(OH)_2$D activates calbindin gene transcription [6,7]. Calbindin binds calcium and may play a role in calcium transport. 1,25$(OH)_2$D may also facilitate calcium transport in the small intestine through an epithelial calcium channel [9]. In the absence of vitamin D, 10–15% of the calcium and 60%

of the phosphorus in the intestines is absorbed, whereas in the vitamin D-replete state, 30% of calcium and 70–80% of phosphorus is absorbed [7]. $1,25(OH)_2D$ also has an antiproliferative effect on parathyroid cells and inhibits PTH gene transcription, and thus PTH synthesis and PTH secretion [6,7].

VDR is also expressed on osteoblasts, and the binding of $1,25(OH)_2D$ to osteoblasts results in expression of RANKL on the osteoblast surface. RANKL from the osteoblast interacts with RANK (the receptor) on the osteoclast, stimulating the formation of mature osteoclasts [6,7]. Osteoclasts then resorb bone, increasing calcium concentrations. $1,25(OH)_2D$ is also involved in regulating bone matrix proteins, downregulating transcription of the α1(I) collagen gene and activating transcription of the osteocalcin and osteopontin genes [6]. $1,25(OH)_2D$ does not appear to play a direct role in mineralization. $1,25(OH)_2D$ also has roles other than in mineral homeostasis. For example, $1,25(OH)_2D$ has antiproliferative effects on some malignant cells and induces the differentiation of keratinocytes [6,7].

Peak bone mass

Peak bone mass (PBM) is defined as the amount of bone that has been accumulated by the time the skeleton has matured. PBM appears to have a direct effect on the risk of fractures, and a 10% increase in PBM decreases the risk of fractures by 50% [10]. As a result, recent attention has been placed on identifying the determinants of PBM with the hope of decreasing the risk of fractures. Some 25% of peak bone mass is accumulated during the 2 years surrounding the peak height velocity in adolescence [11].

Bone mass does not differ between males and females until puberty. The longer period of maturation in boys vs. girls likely explains the differences in PBM between the sexes [12]. In females, gains in bone mass decrease significantly after menarche [13], whereas bone mass accumulation continues in males until late adolescence. Males have a greater increase in bone size and cortical thickness than females, but there does not appear to be a difference in volumetric trabecular density at the end of puberty [14]. The higher incidence of fractures during peak growth velocity in boys and girls may be due to transient fragility because of the lag in the growth of the bone mineral mass relative to the rapid increase in bone size with the growth spurt [10].

Genetic and environmental factors determine an individual's PBM. A large proportion of the variance in PBM is genetically determined [10] and is expressed before puberty [15]. Compared with adult bone, growing bones are particularly responsive to physical activity. It appears that calcium intake, especially if < 400–500 mg/day, could be deleterious to bone mass gain [10]. Calcium supplementation appears to be most effective in prepubertal children.

Pediatric osteoporosis

Definitions

Osteoporosis and osteopenia are both due to a decrease in bone formation or an increase in bone resorption. Osteomalacia, which is also associated with a low bone mass, is due to a mineralization defect of bone so that unmineralized bone matrix (osteoid) accumulates. Thus, osteoporosis and osteomalacia are both associated with a low bone mass, but the mechanisms that lead to the low bone mass are different.

The diagnosis of osteoporosis and osteopenia in children is difficult because measurements of bone mineral density (BMD), by which they are defined in adults, are unreliable in children. In adults, the definitions of these conditions are based on bone densitometry (as measured by a dual-energy X-ray absorptiometry or DEXA scan). The World Health Organization (WHO) defines osteopenia in post-menopausal women by a BMD T-score (standard deviation score compared with peak bone mass) of –1.0 SD to –2.5 SD and osteoporosis by a T-score of less than –2.5 SD. A decrease in BMD as measured by a DEXA scan in an adult of 1.0 SD is associated with a two- to threefold increase in the risk of fracture. There are no comparable data in children. In addition, DEXA scans measure areal BMD in g/cm^2, which is not a true volumetric measurement. The areal BMD is highly dependent on bone size and thus increases with age as the size of the bones increases. Thus, instead of comparing BMD with peak bone mass (T-score), bone density in children should be compared only with that of age-matched control subjects (Z-score). This means that the WHO definition of osteoporosis and osteopenia, which uses T-scores and is based on a known fracture threshold, is inappropriate.

One approach to interpreting BMD data in children is to use the normal distribution curve and to consider "osteopenia" as a BMD Z-score (compared with age-matched control subjects) of –1.0 to –2.0 SD and "osteoporosis" as a Z-score of –2.0 SD or less [16]. However, many would argue that the terms "osteopenia" and "osteoporosis" should never be used in pediatrics and, instead, that bone density should be characterized as normal or low. The International Society for Clinical Densitometry (at the 2004 Position Development Conference) recommended that it can be stated that the bone density is "low for chronologic age" if the BMD Z-score is –2.0 SD or less [17,18]. Given the few data on bone density and risk of fracture, the diagnosis of osteoporosis should be made only in the presence of a low-trauma or atraumatic fracture [16]. Bone densitometry should be use to monitor gains in BMD and/or response to therapy and should also be interpreted in the context of the patient's age, bone age, pubertal status, height, weight, and ethnicity/race.

Primary osteoporosis

Primary osteoporosis results from intrinsic skeletal defects. In children, most of these are heritable disorders of connective tissue, with the exception of idiopathic juvenile osteoporosis [16].

Osteogenesis imperfecta (OI) is the most common cause of primary osteoporosis in children and is divided into up to seven types (types I–VII) [19–21]. Types I–IV are due to mutations in the COL1A1 and COL1A2 genes that encode the type Iα collagen chains. The defects in the more recently described (and much rarer) types V and VI are unknown. Type II is the lethal neonatal form, and type III is the classic severe OI, with multiple fractures, blue sclerae, and skeletal deformities. Type IV and especially the mildest form, type I, may be difficult to diagnosis clinically, as blue sclerae (particularly in type IV) and skeletal deformities (particularly type I) may be absent. In all forms of OI, fractures tend to decrease in frequency in puberty. A mild form of OI (type I or IV) should be suspected in anyone with a low BMD, low-trauma fractures, and no secondary causes of osteoporosis. In order to make the diagnosis, the mobility of collagen from a fibroblast culture from a skin biopsy can be studied. A genetic diagnosis can also be made by mutational analysis of the COL1A1 or COL1A2 genes. Treatment is generally palliative, although cyclic pamidronate has been shown to increase BMD, decrease fractures, and improve bone pain and mobility in children with OI [22].

Other connective tissue disorders that lead to primary osteoporosis include Marfan [23] and Ehlers–Danlos syndrome [24].

Idiopathic juvenile osteoporosis is a rare cause of primary osteoporosis in children that presents in previously healthy prepubertal children (usually 2–3 years before puberty) and resolves spontaneously over 2–5 years [25,26]. The disorder is not inherited, and the cause is unknown. Pain and fractures, including vertebral compression factures, are common. The bone density is markedly decreased. There is no treatment, other than optimizing vitamin D and calcium intake [25,26]. Bisphosphonates may be considered under certain circumstances.

Secondary osteoporosis

Secondary osteoporosis is due to an underlying disorder or its treatment. Any disorder resulting in decreased mobilization leads to secondary osteoporosis. Under these conditions, osteoporosis results from chronic disuse and lack of mechanical stress on bone. In children, the result is lack of adequate accrual of bone.

Causes include cerebral palsy, primary disorders of muscle, such as muscular dystrophy, and a number of chronic illnesses. These include hematological (leukemia), gastrointestinal (anorexia nervosa, inflammatory bowel disease), pulmonary (cystic fibrosis), neuromuscular, rheumatological and endocrine diseases, and organ transplantation. Lack of normal activity, poor nutrition, and treatment with medications such as glucocorticoids may all contribute to the low BMD. Individuals with seizures are often on anticonvulsants that interfere with vitamin D metabolism. Endocrine disorders, such as growth hormone deficiency, Turner syndrome, delayed puberty, hyperthyroidism, and diabetes mellitus, are all associated with decreases in BMD, although the clinical significance of the changes in bone mass are not clear.

Some causes of secondary osteoporosis can be treated, such as growth hormone deficiency and hypogonadism associated with Turner syndrome. However, many of the causes cannot be reversed, and prevention of secondary osteoporosis often involves the recognition and treatment of contributing factors, such as inadequate nutritional intake, especially of calcium and vitamin D, and lack of exercise. Vitamin D deficiency should be excluded. Drugs that interfere with bone mass accumulation, such as glucocorticoids, should be avoided whenever possible, and individuals on anticonvulsants should be on twice the recommended daily intake of vitamin D. Bisphosphonates increase the BMD of children with secondary osteoporosis [27], but more studies are required before they can be recommended routinely [28].

Rickets

Rickets is a disorder of the growth plate defined by a decrease in the endochondral calcification at the growth plate, which results in growth plate deformities, decreased growth rate, and skeletal deformities. Rickets can occur only in growing children, but is associated with osteomalacia in adults and children. Osteomalacia is defined by a decrease in the mineralization of osteoid on the trabecular and cortical surfaces at sites of bone turnover.

Rickets can be broadly classified into calciopenic and phosphopenic rickets (Table 14.1). *Calciopenic rickets* is characterized by osteopenia and hypocalcemia leading to secondary hyperparathyroidism, which results in excessive bone resorption and decreased bone mass. Symptoms result from hypocalcemia and include irritability, tetany, and seizures. *Phosphopenic rickets* is characterized by increased undermineralized osteoid. Osteopenia does not occur, and thus secondary hyperparathyroidism is not a feature; as a result, there is no excessive resorption of bone, and the bone mass is not decreased. Hypophosphatemia is associated with few symptoms, other than bone pain sometimes, and patients are generally less symptomatic than those who have calciopenic rickets.

There is another group of disorders that are not considered classic forms of rickets but in which mineralization is inhibited and thus rachitic features occur. Examples include hypophosphatasia and fluoride toxicity.

Table 14.1. Biochemical findings in calciopenic vs. phosphopenic rickets.

	Serum						Urine	
	Calcium	**Phosphorus**	**PTH**	**Alkaline phosphatase**	**25(OH)D**	**1,25(OH)$_2$D**	**TRP**	**Amino acids**
Calciopenic rickets								
Vitamin D deficiency	Normal	Normal or low	High or low	High	Low	Low, normal, or high	Low	Negative
Vitamin D 1α-hydroxylase deficiency	Low	Normal or low	High	High	Normal	Low	Low	Negative
Hereditary 1,25(OH)$_2$D-resistant rickets (HVDRR)	Low	Low	High	High	Normal	Very high	Low	Negative
Phosphopenic rickets								
Familial hypophosphatemic rickets	Normal	Low	Normal	High	Normal	Normal	Low	Negative
Hereditary hypophosphatemic rickets with hypercalciuria	Normal	Low	Normal	High	Normal	High	Low	Negative
Fanconi syndrome	Normal	Low	Normal	High	Normal	High	Low	High

Manifestations of rickets

Clinical

The manifestations of rickets result from the hypocalcemia or hypophosphatemia, the resulting osteomalacia, and the effects of the underlying etiology (e.g. vitamin D deficiency) on other organ systems. The age of the child and the bones that sustain the most stress also determine the presentation.

Presenting complaints include decreased growth rate, skeletal deformities, and delayed standing or walking. Young children often do not present with rickets until they start walking and the weight-bearing on the legs leads to bowing. In the case of hypocalcemia, muscle weakness, lethargy, irritability, tetany, and seizures may be present.

The physical manifestations of rickets are somewhat age dependent. The most common findings are due to swelling around growth plates, especially at wrists and ankle, as these are the sites where growth velocity is greatest. The linear growth rate decreases. The skeletal deformities that occur are due to mechanical stresses on undermineralized bone. Genu varum (bowlegs) and tibial and femoral torsion are common and develop with ambulation in young children with rickets, often leading to a waddling gait. In older children, in whom physiological bowing has disappeared, genu valgum (knock-knees) or a windswept deformity (genu varum on one leg and genu valgum on the other) can occur. In children with severe rickets, coxa vara of the femoral neck can occur and lead to pelvic deformities that may persist into adulthood.

Chest deformities include rachitic rosary due to swelling of the costrochondral junction of ribs, which appears as beading along the anterolateral aspect of the ribs. Harrison sulci (grooves) represent indentations where the muscular diaphragm attaches to and pulls on the lower ribs. Eventually, with longstanding rickets, as the ribs become softer, the chest narrows as a result of negative intrathoracic pressure. Combined with muscle weakness, there can be significant respiratory distress. Scoliosis, kyphosis, and lordosis can occur as a result of muscle weakness and are especially common in adolescents with rickets.

In young children, closure of the fontanelles may be delayed. Craniotabes occurs when there is softening of the skull in the occipital region behind the ears. There may be delayed eruption of primary teeth.

Additional manifestations include muscle weakness, presenting as hypotonia and a delay in the acquisition of gross motor skills in young children, and a proximal myopathy in older patients. This is thought to be due to the effects of vitamin D on muscle, in which there are 1,25(OH)$_2$D receptors [29–31], and not of hypocalcemia per se. Electrocardiographic changes and left ventricular dysfunction are probably directly related to hypocalcemia.

In children with vitamin D-deficient rickets, there can be manifestations related to the effects of vitamin D on immune function. There may be an increase in respiratory infections [32] due to this, exacerbated by muscle weakness and a small chest size, resulting in poor lung function. In addition, there appears to be an increase in gastrointestinal infections in children with vitamin D deficiency.

Radiographic findings

The radiographic features of rickets are due to lack of endochondral calcification at the growth plate (rickets), decreased mineralization of osteoid at sites of bone turnover (osteomalacia), and secondary hyperparathyroidism [33].

The first radiographic sign is loss of the demarcation between the growth plate and the distal end of metaphysis as a result of loss of the provisional zone of calcification at the distal metaphysis [33]. The growth plate widens, and

the distance from epiphyseal center to metaphysis increases. Widening of the growth plate is clinically evident as widening of the ends of the long bones (especially a widened wrist) and at the costrochondral junctions (rachitic rosary). As the disease progresses, the metaphyses become flared, cupped, and widened with irregular outlines. The epiphyses are indistinct or invisible. As the growth plates of the long bones are most affected, the wrist (distal radius and ulna) in young children and knees in older children are the best sites for obtaining radiographs to document rickets.

The radiographic findings of osteomalacia are more difficult to detect. Generalized demineralization or osteopenia is seen. Looser zones (pseudofractures) are not as common in children as in adults with osteomalacia. Pathological fractures can occur, as can vertebral compression fractures in longstanding rickets. Genu varum (bowlegs) and genu valgum (knock-knees) are due to a combination of osteomalacia of the shaft of the long bones and deformation occurring at the unmineralized growth plate.

Secondary hyperparathyroidism contributes to osteopenia, but the pathognomanic features of hyperparathyroidism are much less commonly seen in rickets. A coarse trabecular pattern can be seen at the ends of the long bones.

Healing rickets also has typical radiographic findings with a transverse line of calcification at the end of the metaphysis usually seen within 1 month of starting treatment [33]. The metaphyses mineralize, the coarse trabecular pattern fills in, and the unmineralized osteoid mineralizes and appears as periosteal new bone formation. The entire healing process takes several months, although the skeletal deformities may take years to normalize.

Calciopenic rickets

Calciopenic rickets results from deficiency in calcium or in vitamin D intake or action (Fig. 14.2). Calcium deficiency can be due to inadequate calcium intake or to the use of calcium chelators. Alterations in vitamin D can be due to lack of dietary intake of vitamin D and/or lack of exposure to the sun. Secondary vitamin D deficiency can be due to fat malabsorption as a result of gastrointestinal or hepatobiliary diseases. Decreased production of $1,25(OH)_2D$ can occur as a result of renal osteodystrophy or 25(OH)D-1α-hydroxylase deficiency. Increased turnover of vitamin D occurs with anticonvulsant use. Nephrotic syndrome leads to increased loss of vitamin D in urine with protein. Finally, there can be resistance to $1,25(OH)_2D$, termed vitamin D-resistant rickets.

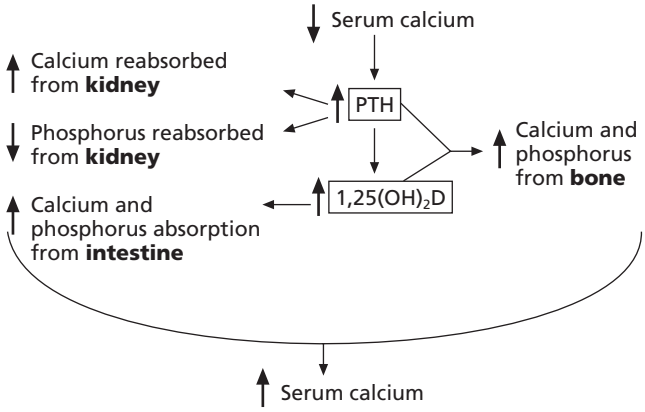

Fig. 14.2. Hypocalcemia.

Nutritional rickets

Nutritional rickets spans a spectrum from vitamin D deficiency to calcium deficiency, with combinations of relative vitamin D insufficiency and reduced intake of calcium in the middle [33]. Vitamin D deficiency is by far the most common cause of nutritional rickets in developed countries.

Causes of nutritional rickets

Vitamin D-deficient rickets results from a combination of lack of vitamin D formation in the skin and of adequate vitamin D intake. The amount of UV-B radiation that reaches the skin and other factors influence the amount of vitamin D made in the skin. In the winter months, at latitudes that are far from the equator, little vitamin D is synthesized in the skin. For example, in Boston in the winter (42° N), there is insufficient UV-B radiation to convert 7-dehydrocholesterol to previtamin D [34]. Air pollution decreases the amount of UV-B radiation available, and the amount of skin exposed and the time the skin is exposed to UV-B radiation also influences the amount of vitamin D made. Sunscreens interfere with sunlight exposure, as do greater amounts of skin melanin [35].

Vitamin D-deficient rickets is most common in children between 3 and 18 months of age [33]. Breastfed infants are at particularly high risk, as breast milk has only about 20–60 IU/L vitamin D, which is insufficient to maintain normal vitamin D concentrations in the infant [36], and 25(OH)D concentrations in breastfed infants primarily reflect their exposure to sunlight [37]. Thus, children at highest risk of nutritional rickets are breastfed infants who do not receive vitamin D supplementation and who are not exposed to adequate sunlight. African-American infants, especially those who are breastfed, are at particularly high risk of developing nutritional rickets, in part because they have increased melanin in the skin resulting in decreased exposure to UV-B radiation. It has been estimated that breastfed infants in the northern USA during the summer months need 30 min/week exposure to the sun in a diaper only and no sunscreen, or 2 h/week if fully clothed but not wearing a hat [38]. Infant formulas contain 400 IU/L vitamin D, and 500 mL/day thus provides 200 IU/day vitamin D, which is considered to be adequate.

Diets low in calcium may exacerbate the effects of vitamin D deficiency. It has been proposed that poor calcium intake

Table 14.2. 25(OH)D, calcium, PTH, phosphorus, and alkaline phosphatase in the three stages of rickets.

	Stage 1	Stage 2	Stage 3
25(OH)D	Decreased	Further decreased	Further decreased
Calcium	Decreases	Increases to low–normal range	Decreases
PTH	Starts to rise	Elevated	Increases further
Phosphorus	Normal	Decreases	Low
Alkaline phosphatase	Increases	Increases	Increases

leads to increased catabolism of 25(OH)D by increasing PTH, which increases $1,25(OH)_2D$ production [33]. $1,25(OH)_2D$ may increase the clearance of 25(OH)D in the liver, thus worsening 25(OH)D deficiency [39]. This mechanism of the development of rickets may be common in African-Americans in the USA and may be a common cause of rickets in adolescents, in whom calcium intake is low, while calcium requirements are increasing as the skeleton is growing.

Calcium deficiency alone is an uncommon cause of rickets and is found primarily in developing countries.

Stages of vitamin D-deficient rickets

The progression of vitamin D-deficient rickets can be broken down into three stages (Table 14.2), each with characteristic biochemical findings [33]. Stage 1 is characterized by hypocalcemia. The decreased amount of 25(OH)D results in decreased $1,25(OH)_2D$, which impairs intestinal calcium absorption. Stage 1 is usually transient and can be mild. The hypocalcemia leads to an increased alkaline phosphatase and secondary hyperparathyroidism, which characterizes stages 2 and 3. Clinical manifestations in infants can include apnea, seizures, tetany, or stridor. Bone changes have not had time to be manifest.

In stage 2, the hypocalcemia leads to secondary hyperparathyroidism, which results in normalization of the calcium concentrations. Calcium is mobilized from bone and reabsorbed from kidney, leading to further increases in alkaline phosphatase. In addition, the elevated PTH concentrations stimulate increased conversion of 25(OH)D to $1,25(OH)_2D$, increasing intestinal absorption of calcium. These effects together normalize serum calcium concentrations. Secondary hyperparathyroidism also results in hypophosphatemia due to the action of PTH on the kidney. During stage 2, radiographic and clinical manifestations of rickets emerge.

In stage 3, the rickets becomes severe. The homeostatic mechanisms in place to normalize calcium fail, and hypocalcemia recurs as the secondary hyperparathyroidism progresses, but with diminishing effect. Depletion of calcium from the bone results in decreased PTH-stimulated calcium release from bone, despite high PTH concentrations. Further depletion of 25(OH)D results in relative deficiency of $1,25(OH)_2D$. The hypocalcemia combined with hypophosphatemia leads to the severe manifestations of rickets.

Laboratory changes in nutritional rickets

Low concentrations of 25(OH)D define vitamin D-deficient rickets. However, there is some debate as to what are normal concentrations. Although the normal range in many laboratories is 9–74 ng/mL, many consider a level of < 15 ng/mL to represent vitamin D deficiency and a level of 15–20 ng/mL to represent vitamin D insufficiency. In vitamin D-deficient rickets, the 25(OH)D concentrations are usually < 15 ng/mL and are often < 9 ng/mL or "undetectable." Once the clinical diagnosis of rickets is made, finding a low 25(OH)D level confirms the diagnosis of vitamin D deficiency.

$1,25(OH)_2D$ concentrations are more problematic and variable in vitamin D-deficient rickets and cannot be used as a diagnostic tool. $1,25(OH)_2D$ concentrations can be elevated because of increased conversion of 25(OH)D to $1,25(OH)_2D$ by PTH. As $1,25(OH)_2D$ is the active form of vitamin D, it is puzzling that rickets occurs when the $1,25(OH)_2D$ concentrations are elevated. It may be that, in stage 1 with low 25(OH)D concentrations, concentrations of $1,25(OH)_2D$ decrease, leading to hypocalcemia. Secondary hyperparathyroidism then occurs in stage 2, increasing the conversion of 25(OH)D to $1,25(OH)_2D$ and thus correcting the hypocalcemia by increasing intestinal absorption of calcium and increasing bone resorption. By stage 3, there is insufficient 25(OH)D to maintain normal $1,25(OH)_2D$ concentrations, and the concentrations may be inappropriately low given the degree of elevation of PTH.

Calcium concentrations in nutritional rickets are often normal. Although hypocalcemia does occur in stages 1 and 3, hypocalcemia is not evident in many children with rickets, and calcium concentrations may be in the low–normal range. However, even the mild, transient hypocalcemia in stage 1 leads to secondary hyperparathyroidism, which characterizes nutritional rickets. As a result of hyperparathyroidism, other abnormalities occur that are also characteristic of rickets. Hypophosphatemia occurs because of the effect of PTH on the kidney to decrease tubular reabsorption of phosphate and increase phosphate loss. However, phosphate concentrations are often normal or elevated, which may be a result of PTH resistance [33]. Urinary calcium excretion is decreased, and there can be amino aciduria and increased loss of bicarbonate in the urine.

Alkaline phosphatase concentrations are increased, even though osteoblastic activity is decreased. Caution must be taken in interpreting alkaline phosphatase concentrations, as the liver isoenzyme represents a large portion of the total alkaline phosphatase, and liver disease can also raise alkaline phosphatase concentrations. In addition, alkaline phosphatase concentrations vary with age, pubertal status, growth rate, and nutritional status, further confounding interpretation. There are a number of other markers of bone formation and resorption. However, there tends to be variation with

age, pubertal status, and even circadian rhythm, limiting their use in pediatrics. Alkaline phosphatase remains the best marker to follow the progression of rickets with therapy.

Treatment and prevention

Vitamin D deficiency is treated with oral vitamin D2 or D3. Treatment regimens include 5–15 000 IU/day for 4–8 weeks or a single oral or intramuscular dose of 2–600 000 IU. The improvement in biochemical parameters is somewhat faster (4–7 days) when a large oral dose of 600 000 IU of vitamin D is given [40], compared with the smaller daily dose (2–3 weeks) [41]. There is no evidence that the large oral dose leads to vitamin D intoxication in the context of vitamin D deficiency. One advantage of the single, large, oral dose is that compliance is not an issue. Whatever regimen used, 25(OH)D concentrations should be repeated after several months and, if the response has been inadequate, the treatment should be repeated. Calcium supplementation should also be given, especially if the intake of calcium is less than 600–1000 mg/day (depending on the size of the child). Symptomatic hypocalcemia should be treated with a slow intravenous infusion of calcium gluconate, 1–2 mg/kg of a 10% solution [33].

Sun exposure will prevent vitamin D deficiency, but there may be barriers to adequate exposure to UV irradiation, such as latitude, religious customs, use of sunscreens and hats, increased melanin pigmentation, overcrowding, and living in an urban environment. Thus, oral intake of vitamin D is the best method to assure adequate vitamin D and prevent rickets. Breastfed infants are at particularly high risk of developing vitamin D deficiency as vitamin D concentrations in breast milk are inadequate. To address the issue of vitamin D deficiency in infants due to inadequate vitamin D concentrations, the American Academy of Pediatrics has recently issued new guidelines [42]. They recommend that "all infants, including those who are exclusively breastfed, have a minimum intake of 200 IU of vitamin D per day beginning during the first 2 months of life. In addition, it is recommended that an intake of 200 IU of vitamin D per day be continued throughout childhood and adolescence, because adequate sunlight exposure is not easily determined for a given individual" [42]. It is likely that 200 IU/day is the minimum; doses of 400–600 IU/day are safe and will not cause vitamin D intoxication.

Genetic forms of calciopenic rickets

Vitamin D 1α-hydroxylase deficiency

Vitamin D 1α-hydroxylase converts 25(OH)D to the active form, $1,25(OH)_2D$. Vitamin D 1α-hydroxylase deficiency occurs when loss-of-function mutations in the renal 1α-hydroxylase gene render it inactive [43]. This form of rickets has variably been referred to as pseudo-vitamin D-deficient rickets and vitamin D-dependent rickets type I. Now that the underlying genetic cause is known, the term vitamin D 1α-hydroxylase deficiency is most appropriate. Children usually present in the first 2 years of life with symptoms and signs of severe rickets. Laboratory findings are similar to those seen in vitamin D-deficient rickets, including hypocalcemia, hypophosphatemia, elevated alkaline phosphatase, and elevated PTH. However, the distinguishing feature is a very low $1,25(OH)_2D$ level and normal 25(OH)D [44]. Treatment is straightforward, and calcitriol ($1,25(OH)_2D$) at 0.25–2.0 Bg/day cures the disease, although treatment must be lifelong. An adequate intake of calcium must be maintained, especially during the healing phase. Calcium concentrations should be maintained in the low–normal range, and PTH concentrations should be allowed to remain in the high–normal range to avoid hypercalciuria and nephrocalcinosis.

Hereditary $1,25(OH)_2D$-resistant rickets

Hereditary $1,25(OH)_2D$-resistant rickets (HVDRR) has also been referred to under various names, including pseudo-vitamin D-deficient rickets type II, vitamin D-dependent rickets type II, and calcitriol-resistant rickets. The genetic cause is now known, and mutations in the vitamin D receptor (VDR) lead to resistance of target tissues to vitamin D [45]. Thus, HVDRR is the most appropriate term for this disorder. Patients present with symptoms and signs similar to those with nutritional rickets and vitamin D 1α-hydroxylase deficiency, but many patients have alopecia, which is a distinguishing feature. Biochemical features are similar to those with the other forms of calciopenic rickets but, unlike other forms of calciopenic rickets, $1,25(OH)_2D$ concentrations are vastly elevated, and the 25(OH)D concentrations are normal [44]. Treatment is more difficult than for the other forms of calciopenic rickets because of the resistance to vitamin D. High doses of 25(OH)D or $1,25(OH)_2D$ are moderately effective. Patients with alopecia are the least responsive to therapy, probably because the presence of alopecia is a marker for a more severe defect. Infusions of large doses of calcium have been effective in some patients with refractory rickets but are complicated by cardiac arrhythmias and nephrolithiasis [46].

Phosphopenic rickets

Phosphopenic rickets can be due to dietary phosphorus deficiency or to impaired absorption of phosphate by the intestine, either as a result of disorders of the gastrointestinal system that affect phosphate absorption or of phosphate binders, such as aluminum salts or calcium carbonate. By far the most common cause is a defect that leads to renal phosphate wasting. Genetic causes of renal phosphate loss include familial hypophosphatemic rickets (FHR), including X-linked due to abnormalities in the PHEX gene, or autosomal-dominant hypophosphatemic rickets (ADHR) due to mutations in FGF23. FHR is the second most common cause of rickets after nutritional rickets. Hereditary hypophosphatemic rickets with hypercalciuria is another

genetic cause, which is much rarer than FHR; the underlying etiology is unknown. Tumor-induced osteomalacia (TIO) is a condition that has all the same features as FHR but is acquired and associated with the presence of a tumor.

Other disorders of the kidney can lead to phosphopenic rickets. Fanconi syndrome, which involves multiple defects of the proximal renal tubule and renal tubular acidosis, causes phosphate wasting. Inborn errors of metabolism, such as glycogen storage disease, galactosemia, cystinosis, tyrosinemia, and hereditary fructose intolerance, lead to phosphopenic rickets. Hypophosphatemia can also be seen in polyostotic fibrous dysplasia.

Familial hypophosphatemic rickets

X-linked hypophosphatemic rickets

X-linked hypophosphatemic rickets (XLH) is the prototype for the familial hypophosphatemic rickets [47] and is relatively common in children. XLH is characterized by renal phosphate wasting, hypophosphatemia, and inappropriately normal concentrations of $1,25(OH)_2D$ in the face of hypophosphatemia. Children develop skeletal deformities, short stature, and dental abscesses.

Hypophosphatemia is the hallmark of XLH. Phosphate concentrations are normally higher in children than in adults and, thus, it is important to know the normal range for age, in order not to miss the diagnosis. Hypophosphatemia results from decreased tubular reabsorption of phosphate (TRP), the fraction of phosphate that is reabsorbed by the kidney:

$$TRP = [1 - (\text{urine phosphorus} \times \text{serum creatinine})]/(\text{serum phosphorus} \times \text{urine creatinine}).$$

The normal range is 0.85–1.0. From the TRP, the tubular threshold maximum for phosphorus per glomerular filtration rate (TMP/GFR) is calculated using a nomogram [48,49]. The normal range in adults is 2.5–4.2 mg/dL and is higher in children. Thus, hypophosphatemia and a low TRP and TMP/GFR are consistent with renal phosphate wasting being responsible for the low serum phosphate concentrations.

Another hallmark of XLH is the normal or mildly decreased $1,25(OH)_2D$ concentrations. Hypophosphatemia normally stimulates 1α-hydroxylase activity, leading to increased concentrations of $1,25(OH)_2D$. The lack of elevation in $1,25(OH)_2D$ in XLH is related to the underlying genetic defect, and the etiology is unclear. The lack of elevation of $1,25(OH)_2D$ also distinguishes XLH from renal tubular disorders that lead to hypophosphatemia, where the $1,25(OH)_2D$ concentrations are elevated.

Alkaline phosphatase concentrations are high, but not as high as in other forms of rickets. The alkaline phosphatase concentrations go down with treatment and are a good way of assessing healing rickets. Calcium and 25(OH)D concentrations are normal, and PTH is normal or slightly increased. These findings all distinguish XLH from calciopenic rickets.

The clinical manifestations of XLH are similar to those of nutritional rickets but persist through childhood. Unlike other forms of rickets, dental abscesses are common. The primary defect is undermineralized dentin, leading to expansion of the pulp chambers and weakening of the enamel barrier. This results in penetration by microorganisms and abscesses, often in the absence of dental caries. Treatment, which has a positive impact on most features of XLH, does not affect the dental manifestations.

Short stature is common. As adults, individuals with XLH continue to have bone and joint problems, including decreased joint mobility, degeneration of knee joints, bone and joint pain, pseudofractures, and enthesopathy.

Treatment of XLH is palliative. Elemental phosphorus (K-phos Neutral or Neutraphos) and calcitriol form the mainstays of therapy. The treatment regimen is difficult, as phosphorus must be taken frequently, and compliance is almost never complete. Treatment usually improves the radiographic findings, and the alkaline phosphatase decreases. Complications of therapy with calcitriol include hypocalcemia and hypercalciuria, and excessive phosphate can result in hyperparathyroidism. Nephrocalcinosis is also a common complication, the pathogenesis of which is not clear. Frequent monitoring of PTH and urinary calcium excretion and monitoring for nephrocalcinosis by renal ultrasound is crucial. Serum phosphate concentrations should never be used to make changes in phosphorus dosing, as serum phosphate concentrations virtually never normalize. If phosphate concentrations are normal, the dose of phosphorus is probably too high.

XLH results from mutations in PHEX (phosphate-regulating gene with homologies to endopeptidases, on the X chromosome) [50]. Despite the identification of PHEX, it is unclear exactly how PHEX leads to phosphate wasting, and a treatment for XLH based on the identification of PHEX has been elusive.

Autosomal-dominant hypophosphatemic rickets

Autosomal-dominant hypophosphatemic rickets (ADHR) is a rare disorder with clinical manifestations similar to XLH. However, unlike XLH, the penetrance is variable [51]. Within the same family, some members with the disorder present in childhood, some in adulthood, and some obligate carriers never manifest the disease. In addition, some individuals lose the phosphate-wasting defect [51].

ADHR results from mutations in FGF23, a novel member of the fibroblast growth factor family [52]. Mutations that lead to ADHR are in a region of the protein that is a recognition sequence for proteolytic enzymes and thus may protect FGF23 from degradation. It is hypothesized that FGF23 is a "phosphotonin" and lowers phosphate concentrations by increasing renal phosphate excretion. Mutations in FGF23 lead to an increase in circulating concentrations of FGF23 in patients with ADHR and thus higher concentrations of a

protein that causes phosphate wasting. One proposal is that PHEX, an endopeptidase, acts normally to cleave FGF23. Mutations in PHEX also lead to elevated concentrations of FGF23 and phosphate wasting by inhibiting this activity.

Tumor-induced osteomalacia

Tumor-induced osteomalacia (TIO) is an acquired condition that mimics XLH. Symptoms and signs are usually more severe than in XLH, with lower phosphate concentrations and lower $1,25(OH)_2D$ concentrations and more bone pain and muscle weakness. TIO results from tumors that appear to secrete a substance that produces a picture similar to XLH. Most tumors are benign mesenchymal tumors, although other tumors have been described, and not all are benign. The disease is cured by removal of the tumor.

The cause of TIO is not entirely clear. There is strong evidence that FGF23 is the cause [53–55], although there are other candidates, including MEPE (matrix extracellular phosphoglycoprotein) [56] and FRP4 (frizzled-related protein 4) [57], both of which have been shown to be excreted from TIO tumors.

Hereditary hypophosphatemic rickets with hypercalciuria

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is another rare form of hypophosphatemic rickets initially described in a Bedouin tribe with a high degree of consanguinity [58]. HHRH is similar to XLH in that there is hypophosphatemia, renal phosphate wasting, and an elevated alkaline phosphatase. Unlike XLH, there is hypercalciuria, suppressed PTH, and an elevated $1,25(OH)_2D$ (an appropriate response to the hypophosphatemia). The high $1,25(OH)_2D$ leads to hyperabsorption of calcium from the intestinal epithelium. Renal stones occur, probably secondary to the hypercalciuria. The skeletal phenotype consists of osteopenia and osteomalacia. Treatment is with oral phosphorus. By increasing the serum phosphate concentrations, $1,25(OH)_2D$ concentrations go down, the hyperabsorption of calcium and hypercalciuria improve. The osteopenia also improves.

McCune–Albright syndrome

McCune–Albright syndrome (MAS) is due to gain-of-function mutations in GNAS, the gene that encodes for the $G_{s\alpha}$ subunit of the GTP protein that couples hormone receptors to the activation of adenlyate cyclase. The mutation results in constitutive activation of cAMP-PKA. Fibrous dysplasia of bone is a common finding. Osteomalacia has also been noted and has been found to be due to hypophosphatemia secondary to renal phosphate wasting in a number of patients [59]. The underlying etiology of the phosphate wasting in MAS is unclear.

Renal tubular disorders – Fanconi syndrome

Fanconi syndrome is a renal tubular disorder characterized by defects in the reabsorption of a number of ions, organic solutes, and proteins, including magnesium, phosphate, and calcium, as well as sodium, potassium, bicarbonate, glucose, and amino acids. Causes of Fanconi syndrome include hereditary disorders, such as cystinosis, Lowe syndrome, type I tyrosinemia, galactosemia, and mitochondrial disorders. Acquired causes include nephrotic syndrome, amyloidosis, paroxysmal nocturnal hemoglobinuria, and a variety of drugs, heavy metals, or other toxin exposures. Osteomalacia with or without rickets is universal [60]. The primary cause of the bone disease is hypophosphatemia secondary to renal phosphate wasting. Abnormalities in vitamin D metabolism are common in Fanconi syndrome and may contribute to the bone disease. In particular, 1α-hydroxylation of 25-(OH)D may be impaired on account of the renal tubular disease. Treatment includes oral phosphate and usually calcitriol.

Hypophosphatasia

Hypophosphatasia is included as it can present with rickets in children and with osteomalacia [61,62]. Teeth are also affected, and there is absence of dental cementum leading to premature loss of teeth. Dentin is not well formed. Hypophosphatasia is caused by a genetic defect in the tissue-non-specific alkaline phosphatase (liver/bone/kidney), termed TNSALP [63]. Osteoid volume is decreased and correlates with the decrease in the alkaline phosphatase. Biochemically, hypophosphatasia is characterized by low alkaline phosphatase activity in the blood, although alkaline phosphatase activity can be low in other conditions. Urinary phosphoethanolamine is elevated, although an elevated phosphoethanolamine can be found in other conditions and is not always found in hypophosphatasia. The most sensitive marker of hypophosphatasia may be an elevated serum pyridoxal-5′-phosphate (PLP) [64]. Unlike rickets, calcium and phosphorus concentrations are normal or elevated, and vitamin D metabolites are usually normal. PTH may be suppressed as a result of the hypercalcemia.

Hypophosphatasia is classified into several forms, depending on age at presentation: perinatal, infantile, childhood, or adult onset. In the perinatal form, the lack of mineralization *in utero* is severe, long bones are deformed, and the cranium is severely undermineralized. Children are often stillborn or live only a few days because of pulmonary and/or neurological compromise.

In the infantile form, infants often appear normal at birth but, by 6 months of age, rickets and failure to thrive are present. Hypocalcemia occurs and may be symptomatic. Radiographs show demineralization and metaphyseal changes characteristic of rickets. The chest is often deformed, which may lead to respiratory difficulties and pneumonia.

The sclerae can be blue. Craniosynostosis may occur because of premature closure of the sutures. If children survive, the long-term prognosis is reasonably good.

The childhood form of hypophosphatasia usually presents in the second or third year of life with signs of rickets on physical examination and radiographically. However, there are clinical features that distinguish hypophosphatasia from rickets. Exfoliation of the teeth is common. Craniosynostosis is also common and involves all sutures, leading to shallow orbits and ocular prominence. The bone disease may remit in adolescence, but may recur in adulthood.

The adult form of hypophosphatasia is mild but can be debilitating. Osteomalacia can be associated with pseudofractures, bone pain, and traumatic fractures. Dental disease is also common.

In odontohypophosphatasia, only the dental disease is present, and there is no bone disease. Loss of teeth occurs, including early loss of deciduous teeth and unexplained loss of permanent teeth. Excessive bone resorption leads to a reduction in the alveolar ridge. The pulp chambers enlarge, and there is hypomineralization of the dentin.

There is no good treatment for hypophosphatasia. Because hypocalcemia can be a problem, especially in the infantile form where hypocalcemia may be severe, treatment for rickets and osteomalacia (vitamin D and calcium) should be avoided. Bone marrow transplantation has been used [65].

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15 The adrenal cortex and its disorders

Walter L. Miller

Embryology, anatomy, and history

The adrenal cortex produces three categories of steroid hormones. *Mineralocorticoids*, principally aldosterone, regulate renal retention of sodium, which influences electrolyte balance, intravascular volume, and blood pressure. *Glucocorticoids*, principally cortisol, are named for their carbohydrate-mobilizing activity but influence a wide variety of bodily functions. *Adrenal androgens* modulate the mid-childhood growth spurt and regulate some secondary sexual characteristics in women; their overproduction may result in virilism.

Embryology

The cells of the adrenal cortex are mesodermal, in contrast to the ectodermal adrenal medulla. Between the fifth and sixth week of fetal development, the "gonadal ridge" develops near the rostral end of the mesonephros. These cells give rise to the steroidogenic cells of the gonads and the adrenal cortex. The adrenal and gonadal cells separate, with the adrenal cells migrating retroperitoneally and the gonadal cells migrating caudally. Between the seventh and eighth weeks, the adrenal cells are invaded by sympathetic neural cells that give rise to the adrenal medulla. By the end of the eighth week, the adrenal has become encapsulated and is clearly associated with the upper pole of the kidney, which at this time is much smaller than the adrenal.

The fetal adrenal cortex consists of an outer "definitive" zone, the principal site of glucocorticoid and mineralocorticoid synthesis, and a much larger "fetal" zone that makes androgenic precursors for the placental synthesis of estriol. The fetal adrenal gland is huge in proportion to other structures. At birth, the adrenals weigh 8–9 g, roughly twice the size of adult adrenals, and represent 0.5% of total body weight, compared with 0.0175% in the adult.

Anatomy

The adrenals derive their name from their anatomical location, located on top of the upper pole of each kidney. Unlike most other organs, the arteries and veins serving the adrenal do not run in parallel. Arterial blood is provided by several small arteries arising from the renal and phrenic arteries, the aorta, and, sometimes, the ovarian and left spermatic arteries. The veins are more conventional, with the left adrenal vein draining into the left renal vein and the right adrenal vein draining directly into the vena cava. Arterial blood enters the sinusoidal circulation of the cortex and drains toward the medulla, so that medullary chromaffin cells are bathed in very high concentrations of steroid hormones.

The adrenal cortex consists of three histologically recognizable zones: the *glomerulosa* is immediately below the capsule, the *fasciculata* is in the middle, and the *reticularis* lies next to the medulla, constituting 15%, 75%, and 10%, respectively, of the adrenal cortex in the older child and adult. The zones appear to be distinct functionally as well as histologically, but considerable overlap exists, and immunocytochemical data show that the zones physically interdigitate. After birth, the large fetal zone begins to involute and disappears by 1 year of age. The definitive zone simultaneously enlarges, but two of the adult zones, the glomerulosa and the fasciculata, are not fully differentiated until about 3 years of age, and the reticularis may not be fully differentiated until about 15 years of age.

History

The adrenal glands were first described in 1563 by the Italian anatomist Bartolomeo Eustaccio, better known for the Eustachian tube of the ear [1]. Medical interest in them as something other than an anatomical curiosity began in the mid-nineteenth century with Addison's classic description of adrenal insufficiency and Brown-Sequard's experimental creation of similar disorders in animals subjected to

Table 15.1. Physical characteristics of human genes encoding steroidogenic enzymes.

Enzyme	Number of genes	Gene size (kb)	Chromosomal location	Exons (n)	mRNA size (kb)
P450scc	1	> 20	15q23–q24	9	2.0
P450c11	2	9.5	8q21–22	9	4.2
P450c17	1	6.6	10q24.3	8	1.9
P450c21	2	3.4	6p21.1	10	2.0
P450aro	1	> 52	15q21.2	10	3.5, 2.9
3β-HSD-I and -II	2	8	1p13	4	1.7
11β-HSD-I	1	7	1	6	1.6
11β-HSD-II	1	6.2	16p22	5	1.6
17β-HSD-I	2	3.3	17q21	6	1.4, 2.4
17β-HSD-II	1	> 40	16q24	5	1.5
17β-HSD-III	1	> 60	9q22	11	1.4
Adrenodoxin	1	> 30	11q22	5	1.0, 1.4, 1.7
Adrenodoxin reductase	1	11	17q24–q25	12	2.0
P450 oxidoreductase	1	< 22	7p15→q35	15	2.5
5α-Reductase – type 1	1	> 35	5p15	5	2.4
5α-Reductase – type 2	1	> 35	2p23	5	2.4

adrenalectomy. The signs and symptoms of glucocorticoid excess due to adrenal tumors were well known by 1932, when Cushing described the pituitary tumors that cause what is now known as Cushing syndrome [2]. Effects of adrenalectomy on salt and water metabolism were reported in 1927 and, by the late 1930s, Selye [3] had proposed the terms "glucocorticoid" and "mineralocorticoid" to distinguish the two broad categories of actions of adrenal extracts.

Numerous adrenal steroids were painstakingly isolated and their structures determined during the 1930s in the laboratories of Reichstein [4] and Kendall [5], leading to their sharing the 1950 Nobel Prize for medicine. Many of these steroids were synthesized chemically, providing pure material for experimental purposes. The observation in 1949 that glucocorticoids ameliorated the symptoms of rheumatoid arthritis [6] greatly stimulated interest in synthesizing new pharmacologically active analogs of naturally occurring steroids. The structures of the various adrenal steroids suggested precursor–product relationships, leading in 1950 to the first treatment of congenital adrenal hyperplasia (CAH) with cortisone by both Wilkins *et al*. [7] and Bartter *et al*. [8]. This opened a vigorous era of clinical investigation of the pathways of steroidogenesis in a variety of inherited adrenal and gonadal disorders. The association of cytochrome P450 with 21-hydroxylation was made in 1965 [9], and some of the steroidogenic enzymes were then isolated in the 1970s. It was not until the genes for most of these enzymes were cloned in the 1980s that it became clear which proteins participated in which steroidal transformations [10]. The identification of these genes (Table 15.1) then led to an understanding of the genetic lesions causing heritable disorders of steroidogenesis. At the same time, studies of steroid hormone action led to the discovery of steroid hormone receptors in the 1960s, but it was not until they were cloned that their biology was understood [11].

Steroid hormone synthesis

Early steps: cholesterol uptake, storage, and transport

The adrenal gland can synthesize cholesterol *de novo* from acetate, but most of its cholesterol comes from plasma low-density lipoproteins (LDL) derived from dietary cholesterol [12]. Adequate concentrations of LDL will suppress 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, the rate-limiting enzyme in cholesterol synthesis. Adrenocorticotrophic hormone (ACTH), which stimulates adrenal steroidogenesis, also stimulates the activity of HMGCoA reductase, LDL receptors, and uptake of LDL-cholesterol. LDL-cholesterol esters are taken up by receptor-mediated endocytosis and are then stored directly or converted to free cholesterol and used for steroid hormone synthesis [13]. Storage of cholesterol esters in lipid droplets is controlled by the action of two opposing enzymes, cholesterol esterase (cholesterol ester hydrolase) and cholesterol ester synthetase. ACTH stimulates the esterase and inhibits the synthetase, thus increasing the availability of free cholesterol for steroid hormone synthesis [14].

Steroidogenic enzymes

Cytochrome P450

Most steroidogenic enzymes are members of the cytochrome P450 group of oxidases. Cytochrome P450 is a generic term

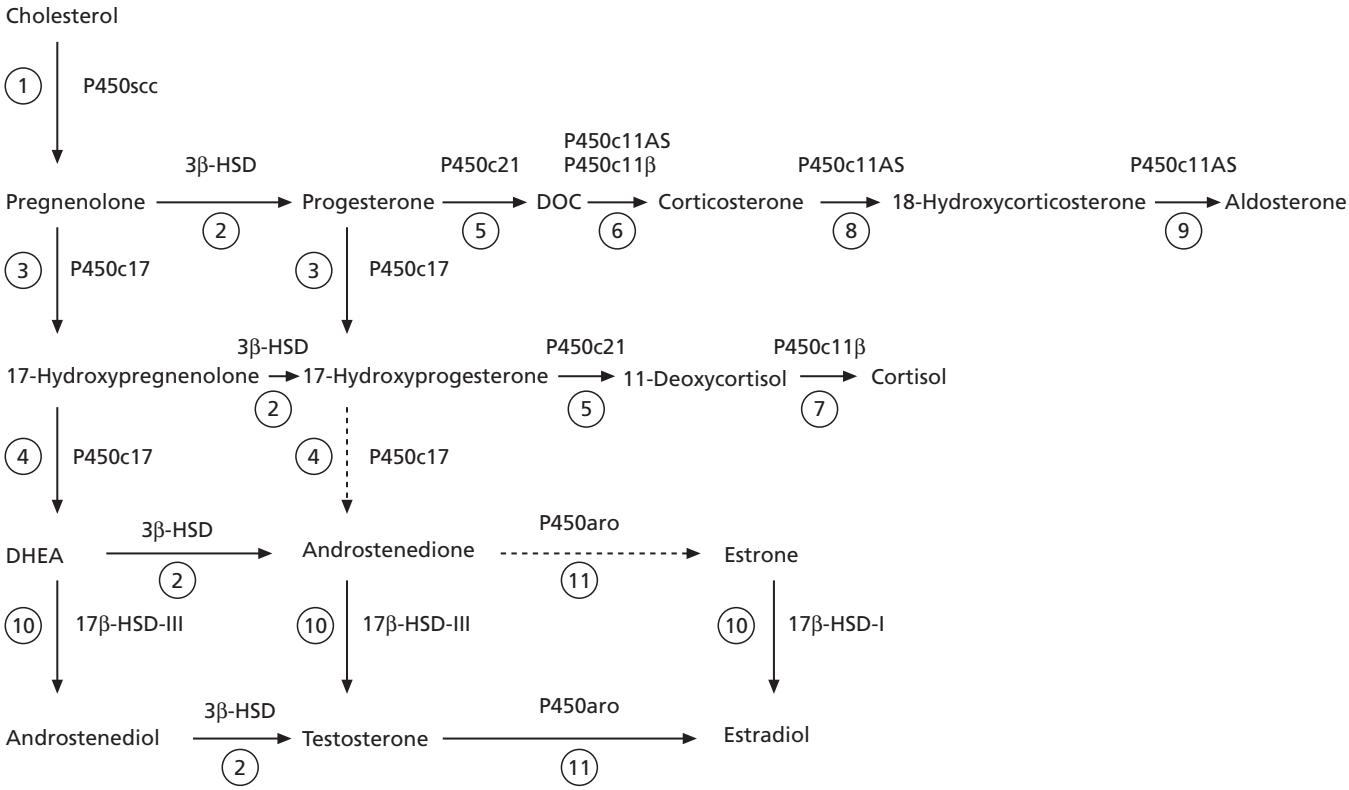

Fig. 15.1. Principal pathways of human adrenal steroid hormone synthesis. Other quantitatively and physiologically minor steroids are also produced. The names of the enzymes are shown by each reaction, and the traditional names of the enzymatic activities correspond to the circled numbers. Reaction 1, mitochondrial cytochrome P450scc mediates 20α-hydroxylation, 22-hydroxylation, and cleavage of the C20–22 carbon bond. Reaction 2, 3β-HSD mediates 3β-hydroxysteroid dehydrogenase and isomerase activities, converting Δ^5 steroids to Δ^4 steroids. Reaction 3, P450c17 catalyzes the 17α-hydroxylation of pregnenolone to 17-OH-pregnenolone and of progesterone to 17-OH-progesterone. Reaction 4, the 17,20-lyase activity of P450c17 converts 17-OH-pregnenolone to DHEA; only insignificant amounts of 17-OH-progesterone are converted to Δ^4 androstenedione by human P450c17, although this reaction occurs in other species. Reaction 5, P450c21 catalyzes the 21-hydroxylation of progesterone to DOC and of 17-OH-progesterone to 11-deoxycortisol. Reaction 6, DOC is converted to corticosterone by the 11-hydroxylase activity of P450c11AS in the zona glomerulosa and by P450c11β in the zona fasciculata. Reaction 7, 11-deoxycortisol undergoes 11β-hydroxylation by P450c11β to produce cortisol in the zona fasciculata. Reactions 8 and 9, the 18-hydroxylase and 18-oxidase activities of P450c11AS convert corticosterone to 18-OH-corticosterone and aldosterone, respectively, in the zona glomerulosa. Reactions 10 and 11 are found principally in the testes and ovaries. Reaction 10, 17β-HSD-III converts DHEA to androstenediol and androstenedione to testosterone, while 17β-HSD-I converts estrone to estradiol. Reaction 11, testosterone may be converted to estradiol and androstenedione may be converted to estrone by P450aro.

for a large number of oxidative enzymes, all of which have about 500 amino acids and contain a single heme group [15]. They are termed P450 (pigment 450) because all absorb light at 450 nm in their reduced states [16,17]. It is sometimes stated that certain steroidogenic enzymes are P450-dependent enzymes. This is a misnomer, because it implies a generic P450 cofactor to a substrate-specific enzyme; the P450 *is* the enzyme binding the steroidal substrate and catalyzing the steroidal conversion on an active site associated with the heme group. Most cytochrome P450 enzymes are found in the endoplasmic reticulum of the liver, where they metabolize countless endogenous and exogenous toxins, drugs, xenobiotics, and environmental pollutants. Despite this huge variety of substrates, the Human Genome Project has shown that humans have only 57 distinct P450 genes. The overwhelming majority of drugs that undergo hepatic degradation are metabolized by only eight P450 enzymes. Thus, most, if not all, P450 enzymes can metabolize multiple substrates, catalyzing a broad array of oxidations. This theme recurs with each adrenal P450 enzyme.

Five distinct P450 enzymes are involved in adrenal steroidogenesis (Fig. 15.1). P450scc, found in adrenal mitochondria, is the cholesterol side-chain cleavage enzyme catalyzing the series of reactions formerly termed 20,22-desmolase. Two distinct isozymes of P450c11, P450c11β and P450c11AS, also found in mitochondria, catalyze 11β-hydroxylase, 18-hydroxylase, and 18-methyl oxidase activities. P450c17, found in the endoplasmic reticulum, catalyzes both 17α-hydroxylase and 17,20-lyase activities, and P450c21 catalyzes the 21-hydroxylation of both glucocorticoids and mineralocorticoids. In the gonads (and elsewhere), P450aro in the endoplasmic reticulum catalyzes aromatization of androgens

to estrogens, and four other P450 enzymes, P450clα, P450c24, P450c27, and P4502R1, are responsible for the activation and degradation of vitamin D [18].

Hydroxysteroid dehydrogenases

In addition to the cytochrome P450 enzymes, a second class of enzymes termed hydroxysteroid dehydrogenases (HSDs) is also involved in steroidogenesis [19]. These enzymes have molecular masses of about 35–45 kDa, do not have heme groups, and require NAD^+ or $NADP^+$ as cofactors. Whereas most steroidogenic reactions catalyzed by P450 enzymes result from the action of a single form of P450, each of the reactions catalyzed by HSDs can be catalyzed by at least two, often very different, isozymes. Members of this family include the 3α- and 3β-hydroxysteroid dehydrogenases, the two 11β-hydroxysteroid dehydrogenases, and a series of 17β-hydroxy-steroid dehydrogenases; the 5α-reductases are unrelated to this family.

P450scc

Conversion of cholesterol to pregnenolone in mitochondria is the first, rate-limiting, and hormonally regulated step in the synthesis of all steroid hormones. This involves three distinct chemical reactions, 20α-hydroxylation, 22-hydroxylation, and cleavage of the cholesterol side-chain to yield pregnenolone and isocaproic acid. Early studies showed that 20-hydroxycholesterol, 22-hydroxycholesterol, and 20,22-hydroxycholesterol could all be isolated from adrenals in significant quantities, suggesting that three separate and distinct enzymes were involved. However, protein purification studies and *in vitro* reconstitution of enzymatic activity show that a single protein, termed P450scc (where scc refers to the *s*ide-*c*hain *c*leavage of cholesterol), encoded by a single gene on chromosome 15 [20], catalyzes all the steps between cholesterol and pregnenolone [21–23]. These three reactions occur on a single active site [24] that is in contact with the hydrophobic bilayer membrane [16]. Deletion of the gene for P450scc in the rabbit eliminates all steroidogenesis [25], indicating that all steroidogenesis is initiated by this one enzyme.

Transport of electrons to P450scc: adrenodoxin reductase and adrenodoxin

P450scc functions as the terminal oxidase in a mitochondrial electron transport system. Electrons from NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) are accepted by a flavoprotein, termed adrenodoxin reductase, that is loosely associated with the inner mitochondrial membrane [26]. Adrenodoxin reductase transfers the electrons to an iron–sulfur protein termed adrenodoxin, which is found in the mitochondrial matrix [27] or loosely adherent to the inner mitochondrial membrane [28]. Adrenodoxin then transfers the electrons to P450scc (Fig. 15.2). Adrenodoxin reductase and adrenodoxin serve as generic electron transport proteins for all mitochondrial P450s and not just for those involved in steroidogenesis; hence, these proteins are also termed ferredoxin oxidoreductase and ferredoxin. Adrenodoxin forms a 1:1 complex with adrenodoxin reductase, then dissociates and subsequently reforms an analogous 1:1 complex with P450scc or P450c11, thus functioning as an indiscriminate diffusible electron shuttle mechanism [29–31]. Adrenodoxin reductase is a membrane-bound mitochondrial flavoprotein that receives electrons from NADPH [26]. The single human adrenodoxin reductase gene [32,33] and the single functional adrenodoxin gene [34] are expressed in all human tissues [35,36], indicating that there are generic mitochondrial electron transfer proteins with roles that are not limited to steroidogenesis.

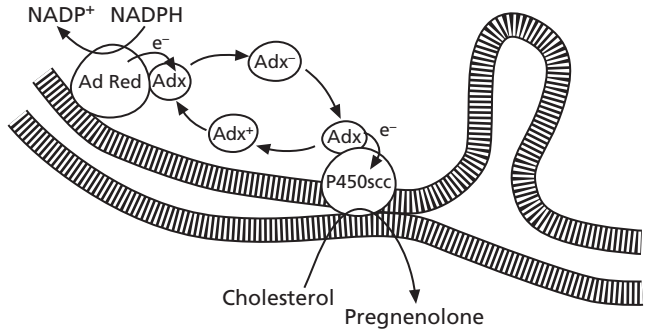

Fig. 15.2. Electron transport to mitochondrial forms of cytochrome P450. Adrenodoxin reductase (Ad Red), a flavoprotein loosely bound to the inner mitochondrial membrane, accepts electrons (e^-) from NADPH, converting it to $NADP^+$. These electrons are passed to adrenodoxin (Adx), an iron–sulfur protein in solution in the mitochondrial matrix that functions as a freely diffusible electron shuttle mechanism. Electrons from charged adrenodoxin (Adx^-) are accepted by any available cytochrome P450 such as P450c11 or P450scc shown here. The uncharged adrenodoxin (Adx^+) may then be bound again to adrenodoxin reductase to receive another pair of electrons. For P450scc, three pairs of electrons must be transported to the P450 to convert cholesterol to pregnenolone. The flow of cholesterol into the mitochondria is facilitated by StAR, which is not shown in this diagram.

Cholesterol transport into mitochondria

The chronic regulation of steroidogenesis by ACTH is at the level of gene transcription [37,38], but the acute regulation, in which cortisol is released within minutes of a stimulus, is at the level of cholesterol access to P450scc. When either steroidogenic cells or intact rats are treated with inhibitors of protein synthesis, such as cycloheximide, the acute steroidogenic response is eliminated, suggesting that a short-lived, cycloheximide-sensitive protein acts at the level of the mitochondrion [23] as the specific trigger to the acute steroidogenic response [39,40]. Several candidate factors were proposed for this acute trigger, including the peripheral

benzodiazepine receptor complex (PBR) and its endogenous ligand, endozepine (also termed diazepam-binding inhibitor, DBI) (for a review, see [41]). The role of the PBR/endozepine system remains uncertain; substantial data indicate that this system participates in the slow, chronic delivery of cholesterol to the inner mitochondrial membrane (for a review, see [42]), but it is clear that the "acute trigger" of steroidogenesis is the steroidogenic acute regulatory protein (StAR) [41].

StAR was first identified as short-lived 30- and 37-kDa phosphoproteins that were rapidly synthesized when steroidogenic cells were stimulated with trophic hormones [43–45]. Mouse StAR was then cloned from MA-10 Leydig cells in 1994 and could induce steroidogenesis when transfected back into these cells [46]. The central role of StAR was definitively proven by showing that it promoted steroidogenesis in non-steroidogenic COS-1 cells co-transfected with StAR and the cholesterol side-chain cleavage enzyme system, and by finding that mutations of StAR caused the most severe disorder of human steroidogenesis, congenital lipoid adrenal hyperplasia [47]. Thus, StAR is the acute trigger that is required for the rapid flux of cholesterol from the outer to the inner mitochondrial membrane, which is needed for the acute response of aldosterone to angiotensin II, of cortisol to ACTH, and of sex steroids to a luteinizing hormone (LH) pulse.

Some steroidogenesis is independent of StAR; when non-steroidogenic cells are transfected with the P450scc system, they convert cholesterol to pregnenolone at about 14% of the StAR-induced rate [47,48]. Furthermore, some steroidogenic tissues, including the placenta and the brain, utilize mitochondrial P450scc to initiate steroidogenesis [49,50] but do not express StAR [51]. The mechanism of StAR-independent steroidogenesis is unknown. It is possible that it occurs spontaneously, without any triggering protein, or that some other protein may exert StAR-like activity to promote cholesterol flux without StAR's rapid kinetics. The mechanism of StAR's action is unknown, but it is clear that StAR acts exclusively on the outer mitochondrial membrane and does not need to enter the mitochondria to be active [52,53,54]. StAR appears to undergo structural changes while interacting with the outer mitochondrial membrane [55].

3β-Hydroxysteroid dehydrogenase/$\Delta^5 \rightarrow \Delta^4$ isomerase

Once pregnenolone is produced from cholesterol, it may undergo 17α-hydroxylation by P450c17 to yield 17-hydroxypregnenolone, or it may be converted to progesterone, the first biologically important steroid in the pathway. A single 42-kDa microsomal enzyme, 3β-hydroxysteroid dehydrogenase (3β-HSD), catalyzes both the conversion of the hydroxyl group to a keto group on carbon 3 and the isomerization of the double bond from the B ring (Δ^5 steroids) to the A ring (Δ^4 steroids) [56–58]. Thus, a single enzyme converts pregnenolone to progesterone, 17α-hydroxypregnenolone to 17α-hydroxyprogesterone, dehydroepiandrosterone (DHEA) to androstenedione, and androstenediol to testosterone. As is typical of hydroxysteroid dehydrogenases, there are two isozymes of 3β-HSD, encoded by separate genes. The enzyme catalyzing 3β-HSD activity in the adrenals and gonads is the type II enzyme [59,60]; the type I enzyme, encoded by a closely linked gene with identical intron/exon organization, catalyzes 3β-HSD activity in placenta, breast, and "extraglandular" tissue enzymes [59,61].

P450c17

Both pregnenolone and progesterone may undergo 17α-hydroxylation to 17α-hydroxypregnenolone and 17α-hydroxyprogesterone (17-OHP) respectively. 17α-Hydroxyprogesterone may also undergo cleavage of the C17,20 carbon bond to yield DHEA; however, very little 17-OHP is converted to androstenedione because the human P450c17 enzyme catalyzes this reaction at only 3% of the rate for conversion of 17α-hydroxypregnenolone to DHEA [62,63]. These reactions are all mediated by P450c17. This P450 is bound to smooth endoplasmic reticulum, where it accepts electrons from P450 oxidoreductase. As P450c17 has both 17α-hydroxylase activity and C-17,20-lyase activity, it is the key branch point in steroid hormone synthesis. If neither activity of P450c17 is present, as in the zona glomerulosa, pregnenolone is converted to mineralocorticoids; if 17α-hydroxylase activity is present but 17,20-lyase activity is not, as in the zona fasciculata, pregnenolone is converted to cortisol; if both activities are present, as in the zona reticularis, pregnenolone is converted to precursors of sex steroids (Fig. 15.1).

17α-Hydroxylase and 17,20-lyase were once thought to be separate enzymes. The adrenals of prepubertal children synthesize ample cortisol but virtually no sex steroids (i.e. they have 17α-hydroxylase activity but not 17,20-lyase activity) until adrenarche initiates the production of adrenal androgens (i.e. turns on 17,20-lyase activity) [64]. Furthermore, patients have been described lacking 17,20-lyase activity but retaining normal 17α-hydroxylase activity [65]. However, purification of pig testicular microsomal P450c17 to homogeneity and *in vitro* reconstitution of enzymatic activity show that both 17α-hydroxylase and 17,20-lyase activities reside in a single protein [66,67], and cells transformed with a vector expressing P450c17 cDNA acquire both 17α-hydroxylase and 17,20-lyase activities [68,69]. P450c17 is encoded by a single gene on chromosome 10q24.3 [70,71] that is structurally related to the genes for P450c21 (21-hydroxylase) [72].

Thus, the distinction between 17α-hydroxylase and 17,20-lyase is functional and not genetic or structural. The factors involved in determining whether a steroid molecule will remain on the single active site of P450c17 and undergo 17,20 bond cleavage after 17α-hydroxylation remain unknown. P450c17 prefers Δ^5 substrates, especially for 17,20 bond

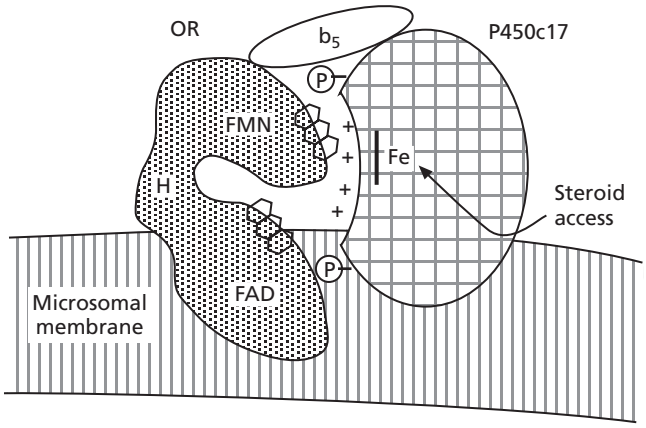

Fig. 15.3. Electron transport to microsomal forms of cytochrome P450. This figure shows the interactions of P450c17 with its redox partners; the interactions of other P450 proteins may be simpler, as most microsomal P450 proteins are not phosphorylated and do not interact with cytochrome b_5. The FAD moiety of P450 oxidoreductase (OR) picks up electrons from NADPH (not shown) and transfers them to its FMN (flavin mononucleotide) moiety. The FAD and FMN groups are in distinct protein domains connected by a flexible protein hinge (H). The FMN domain approaches the redox partner binding site of the P450, shown as a concave region. The active site containing the steroid lies on the side of the plane of the heme ring (Fe) opposite from the redox partner binding site. Cytochrome b_5 allosterically facilitates the interaction between OR and P450c17 to favor 17,20-lyase activity. Phosphoserine residues (P) also promote 17,20-lyase activity.

cleavage, consistent with the large amounts of DHEA secreted by both fetal and adult adrenal. Furthermore, the 17α-hydroxylase reaction occurs more readily than the 17,20-lyase reaction. An additional important factor is the abundance of electron donors for P450c17.

Electron transport to P450c17: P450 oxidoreductase and cytochrome b_5

All microsomal forms of cytochrome P450, including P450c17 and P450c21, receive electrons from a membrane-bound flavoprotein, termed P450 oxidoreductase, which is a different protein from the mitochondrial flavoprotein, adrenodoxin reductase. P450 oxidoreductase receives two electrons from NADPH and transfers them one at a time to the P450 [73,74]. The first electron is transferred rapidly, but transfer of the second is slow [75]. Electron transfer for the lyase reaction is promoted by the action of cytochrome b_5 as an allosteric factor rather than as an alternative electron donor [63]. 17,20-Lyase activity also requires the phosphorylation of serine residues on P450c17 by a cyclic adenosine monophosphate (cAMP)-dependent protein kinase [76,77] (Fig. 15.3). Because the adrenal endoplasmic reticulum contains many more molecules of P450c17 and of P450c21 than of P450 oxidoreductase, the P450s compete with one another for the reducing equivalents provided by the reductase.

The availability of electrons appears to determine whether P450c17 performs only 17α-hydroxylation or also performs 17,20 bond cleavage, as increasing the ratio of P450 oxidoreductase or cytochrome b_5 to P450c17 *in vitro* or *in vivo* increases the ratio of 17,20-lyase activity to 17α-hydroxylase activity [62,63,78]. Competition between P450c17 and P450c21 for available 17-hydroxyprogesterone does not appear to be important in determining whether 17-OHP undergoes 21-hydroxylation or 17,20 bond cleavage [78]. However, increasing the ratio of P450 oxidoreductase to P450c17 increases 17,20-lyase activity (the testis contains three to four times more P450 oxidoreductase activity than does the adrenal) [78]. Thus, the regulation of 17,20-lyase activity, and consequently of DHEA production, depends on factors that facilitate the flow of electrons to P450c17. These are high concentrations of P450 oxidoreductase, the presence of cytochrome b_5, and serine phosphorylation of P450c17 [79]. The essential role of P450 oxidoreductase in mammalian biology is underscored by the demonstration that mice lacking a functional gene for P450 oxidoreductase are malformed and die *in utero* [79,80]. Nevertheless, mutations in human P450 oxidoreductase can cause a picture of combined 17α-hydroxylase and 21-hydroxylase deficiencies, often in combination with the Antley–Bixler skeletal malformation syndrome [81].

P450c21

After the synthesis of progesterone and 17-hydroxyprogesterone, these steroids are hydroxylated at the 21 position to yield deoxycorticosterone (DOC) and 11-deoxycortisol respectively (Fig. 15.1). The nature of the 21-hydroxylating step has been of great clinical interest because disordered 21-hydroxylation causes more than 90% of all cases of CAH. The clinical symptoms associated with this common genetic disease are complex and devastating. Decreased cortisol and aldosterone synthesis often leads to sodium loss, potassium retention, and hypotension, which will lead to cardiovascular collapse and death within the month after birth if not treated appropriately. Decreased synthesis of cortisol *in utero* leads to overproduction of ACTH and consequent overstimulation of adrenal steroid synthesis; as the 21-hydroxylase step is impaired, 17-OHP accumulates because P450c17 converts only miniscule amounts of 17-OHP to androstenedione. However, 17-hydroxypregnenolone also accumulates and is converted to DHEA and subsequently to androstenedione and testosterone, resulting in severe prenatal virilization of female fetuses [82–84].

CAH has been extensively studied clinically. Variations in the manifestations of the disease, and especially the number of patients without apparent defects in mineralocorticoid activity, suggested that there were two separate 21-hydroxylating enzymes that were differentially expressed in the zones of the adrenal specifically synthesizing aldos-

terone or cortisol. However, characterization of the P450c21 protein [85] and gene cloning show that there is only one 21-hydroxylase encoded by a single functional gene on chromosome 6p21 [86–88]. As this gene lies in the middle of the major histocompatibility locus, disorders of adrenal 21-hydroxylation are closely linked to specific human leukocyte antigen (HLA) types [89].

Adrenal 21-hydroxylation is mediated by P450c21 found in smooth endoplasmic reticulum. P450c21 uses the same P450 oxidoreductase used by P450c17 to transport electrons from NADPH. 21-Hydroxylase activity has also been described in a broad range of adult and fetal extra-adrenal tissues [90]. However, extra-adrenal 21-hydroxylation is not mediated by the P450c21 enzyme found in the adrenal [91]; the nature of the enzyme(s) responsible for extra-adrenal 21-hydroxylation is unknown. As a result, patients with absent adrenal 21-hydroxylase activity may still have appreciable concentrations of 21-hydroxylated steroids in their plasma.

P450c11β and P450c11AS

Two closely related enzymes, P450c11β and P450c11AS, catalyze the final steps in the synthesis of both glucocorticoids and mineralocorticoids [92,93]. These two isozymes have 93% amino acid sequence identity [94] and are encoded by tandemly duplicated genes on chromosome 8q21–22 [95]. Like P450scc, the two forms of P450c11 are found on the inner mitochondrial membrane and use adrenodoxin and adrenodoxin reductase to receive electrons from NADPH [96]. By far the more abundant of the two isozymes is P450c11β, which is the classic 11β-hydroxylase that converts 11-deoxycortisol to cortisol and 11-deoxycorticosterone to corticosterone. The less abundant isozyme, P450c11AS, is found only in the zona glomerulosa, where it has 11β-hydroxylase, 18-hydroxylase, and 18-methyl oxidase (aldosterone synthase) activities; thus, P450c11AS is able to catalyze all the reactions needed to convert DOC to aldosterone [97,98].

P450c11β, which is principally involved in the synthesis of cortisol, is encoded by a gene (*CYP11B1*) primarily induced by ACTH via cAMP and suppressed by glucocorticoids such as dexamethasone. The existence of two distinct functional genes is confirmed by the identification of mutations in each that cause distinct genetic disorders of steroidogenesis. Thus, patients with disorders in P450c11β have classic 11β-hydroxylase deficiency but can still produce aldosterone [99], whereas patients with disorders in P450c11AS have rare forms of aldosterone deficiency (so-called corticosterone methyl oxidase deficiency) while retaining the ability to produce cortisol [100–102].

17β-Hydroxysteroid dehydrogenase

Androstenedione is converted to testosterone, DHEA to androstenediol, and estrone to estradiol by a series of short-chain dehydrogenases called 17β-hydroxysteroid dehydrogenases (17β-HSDs), sometimes also termed 17-oxidoreductase or 17-ketosteroid reductase [103,104]. The terminology for this enzyme varies, as this is the only readily reversible step in steroidogenesis; hence, different names are used, depending on the direction of the reaction being studied. This is the most complex and confusing step in steroidogenesis because there are several different 17β-HSD enzymes encoded by distinct genes: some are preferential oxidases, whereas others are preferential reductases; they differ in their substrate preference and sites of expression; there is inconsistent nomenclature, especially with the rodent enzymes; and some proteins termed 17β-HSD actually have very little 17β-HSD activity and are principally involved in other reactions [19].

Type I 17β-HSD (17β-HSD-I), also known as estrogenic 17β-HSD, is a cytosolic protein first isolated and cloned from the placenta, where it produces estriol, and is expressed in ovarian granulosa cells, where it produces estradiol [57,105–107]. 17β-HSD-I is not reversible and does not participate in androgen metabolism. 17β-HSD-I uses NADPH as its cofactor to catalyze its reductase activity. The three-dimensional structure of human 17β-HSD-I has been determined by X-ray crystallography [108,109]. No genetic deficiency syndrome for 17β-HSD-I has been described.

17β-HSD-II is a microsomal oxidase that uses NAD^+ to inactivate (oxidize) estradiol to estrone and testosterone to Δ^4 androstenedione. 17β-HSD-II is found in the placenta, liver, small intestine, prostate, secretory endometrium, and ovary. In contrast to 17β-HSD-I, which is found in placental syncytiotrophoblast cells, 17β-HSD-II is expressed in endothelial cells of placental intravillous vessels, consistent with its apparent role in defending the fetal circulation from transplacental passage of maternal estradiol or testosterone [110]. No deficiency state for 17β-HSD-II has been reported.

17β-HSD-III, the androgenic form of 17β-HSD, is a microsomal enzyme that is apparently expressed only in the testis [111] where it converts androstenedione to testosterone. This is the enzyme that is disordered in the classic syndrome of male pseudohermaphroditism, which is often termed 17-ketosteroid reductase deficiency [111,112].

An enzyme termed 17β-HSD-IV was initially identified as an NAD^+-dependent oxidase with activities similar to 17β-HSD-II [113], but this peroxisomal protein is primarily an enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase [114,115].

17β-HSD-V, originally cloned as a 3α-hydroxysteroid dehydrogenase [116], catalyzes the reduction of Δ^4 androstenedione to testosterone [117], but its precise role is unclear, and no deficiency state has been described. It is widely expressed in peripheral tissues and is probably the enzyme responsible for "peripheral conversion" of androstenedione to testosterone and of DHEA to androstenediol, accounting for the efficacy of those steroids as anabolic agents.

Steroid sulfotransferase and sulfatase

Steroid sulfates may be synthesized directly from cholesterol sulfate or may be formed by sulfation of steroids by cytosolic sulfotransferases. Steroid sulfates may also be hydrolyzed to the native steroid by steroid sulfatase. Deletions in the steroid sulfatase gene on chromosome Xp22.3 cause X-linked ichthyosis [118,119]. In the fetal adrenal and placenta, diminished or absent sulfatase deficiency reduces the pool of free DHEA available for placental conversion to estrogen, resulting in low concentrations of estriol in the maternal blood and urine. The accumulation of steroid sulfates in the stratum corneum of the skin causes the ichthyosis. Steroid sulfatase is also expressed in the fetal rodent brain, possibly converting peripheral DHEA sulfate (DHEAS) to active DHEA [120,121].

Aromatase: P450aro

Estrogens are produced by the aromatization of androgens, including adrenal androgens, by a complex series of reactions catalyzed by a single microsomal aromatase, P450aro [122,123]. This typical cytochrome P450 is encoded by a single, large gene on chromosome 15q21.1. This gene uses several different promoter sequences, transcriptional start sites, and alternatively chosen first exons to encode aromatase mRNA in different tissues under different hormonal regulation. Aromatase expression in the extraglandular tissues, especially adipose tissue, can covert adrenal androgens to estrogens. Aromatase in the epiphyses of growing bone can convert testosterone to estradiol, accelerating epiphyseal maturation and terminating growth [123]. Although it has traditionally been thought that aromatase activity is needed for embryonic and fetal development, infants and adults with genetic disorders in this enzyme have been described, showing that fetoplacental estrogen is not needed for normal fetal development [124,125].

5α-Reductase

Testosterone is converted to the more potent androgen dihydrotestosterone by 5α-reductase, an enzyme found in testosterone's target tissues. There are two distinct forms of 5α-reductase. The type I enzyme, found in the scalp and other peripheral tissues, is encoded by a gene on chromosome 5; the type II enzyme, the predominant form found in male reproductive tissues, is encoded by a structurally related gene on chromosome 2p23 [126]. The syndrome of 5α-reductase deficiency, a disorder of male sexual differentiation, results from a wide variety of mutations in the gene encoding the type II enzyme [127]. The type 1 and 2 genes show an unusual pattern of developmental regulation of expression. The type 1 gene is not expressed in the fetus; it is expressed briefly in the skin of the newborn and then remains unexpressed until its activity and protein are again found after puberty. This probably explains the lack of peripheral virilization of male fetuses despite the presence of testosterone concentrations equivalent to adult male levels. The type 2 gene is expressed in fetal genital skin, in the normal prostate, and in prostatic hyperplasia and adenocarcinoma. Thus, the type I enzyme may be responsible for the pubertal virilization seen in patients with classic 5α-reductase deficiency, and the type II enzyme may be involved in male pattern baldness [126].

11β-Hydroxysteroid dehydrogenase

Although certain steroids are categorized as glucorticoids or mineralocorticoids, cloning and expression of the "mineralocorticoid" (glucocorticoid type II) receptor shows that it has equal affinity for both aldosterone and cortisol [128]. However, cortisol does not act as a mineralocorticoid *in vivo*, even though cortisol concentrations can exceed aldosterone concentrations by 100- to 1000-fold. In mineralocorticoid-responsive tissues, such as the kidney, cortisol is enzymatically converted to cortisone, a metabolically inactive steroid [129]. The interconversion of cortisol and cortisone is mediated by two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD), each of which has both oxidase and reductase activity, depending on the cofactor available [130].

The type I enzyme (11β-HSD-I) is expressed mainly in glucocorticoid-responsive tissues, such as the liver, testis, lung, and proximal convoluted tubule. 11β-HSD-I can catalyze the oxidation of cortisol to cortisone using $NADP^+$ as its cofactor (K_m 1.6 μM) or the reduction of cortisone to cortisol using NADPH as its cofactor (K_m 0.14 μM); the reaction catalyzed depends on which cofactor is available, but the enzyme can only function with high (micromolar) concentrations of steroid [131,132]. 11β-HSD-II catalyzes only the oxidation of cortisol to cortisone using NADH and can function with low (nanomolar) concentrations of steroid (K_m 10–100 nM) [133,134]. 11β-HSD-II is expressed in mineralocorticoid-responsive tissues and thus serves to "defend" the mineralocorticoid receptor by inactivating cortisol to cortisone, so that only "true" mineralocorticoids, such as aldosterone or deoxycorticosterone, which are not substrates for 11β-HSD-II, can exert a mineralocorticoid effect. Thus, 11β-HSD-II prevents cortisol from overwhelming renal mineralocorticoid receptors [129]. In the placenta and other fetal tissues, 11β-HSD-II also inactivates cortisol [135,136]. The placenta also has abundant $NADP^+$, favoring the oxidative action of 11β-HSD-I, so that, in the placenta, both enzymes protect the fetus from high maternal concentrations of cortisol [130], thus ensuring that the fetus develops in an environment with very little glucocorticoid.

Fetal adrenal steroidogenesis

Adrenocortical steroidogenesis begins early in embryonic life, probably around week 6 of gestation (8 weeks after the

mother's last menstrual period). Fetuses affected with genetic lesions in adrenal steroidogenesis can produce adrenal androgen sufficient to virilize a female fetus to a nearly male appearance, and this masculinization of the genitalia is complete by the 12th week of gestation [83,137]. The definitive zone of the fetal adrenal produces steroid hormones according to the pathways in Figure 15.1. In contrast, the large fetal zone of the adrenal is relatively deficient in 3β-HSD-II activity because it contains very little mRNA for this enzyme [138,139]. The fetal adrenal has relatively abundant 17,20-lyase activity of P450c17; low 3β-HSD and high 17,20-lyase activity account for the huge amount of DHEA and DHEAS produced by the fetal adrenal for conversion to estrogens by the placenta.

The fetal adrenal has considerable sulfotransferase activity but little steroid sulfatase activity, which favors conversion of DHEA to DHEAS. The resulting DHEAS cannot be a substrate for adrenal 3β-HSD-II; instead, it is secreted, 16α-hydroxylated in the fetal liver, and then acted on by placental 3β-HSD-I, 17β-HSD-I, and P450aro to produce estriol; the substrates can also bypass the liver to yield estrone and estradiol. Placental estrogens inhibit adrenal 3β-HSD activity, providing a feedback system to promote the production of DHEAS [140]. Fetal adrenal steroids account for 50% of the estrone and estradiol and 90% of the estriol in the maternal circulation.

Although the fetoplacental unit produces huge amounts of DHEA, DHEAS, and estriol, as well as other steroids, they do not appear to serve an essential role. Successful pregnancy is wholly dependent on placental synthesis of progesterone, which suppresses uterine contractility and prevents spontaneous abortion, but fetuses with genetic disorders of adrenal and gonadal steroidogenesis develop normally, reach term gestation, and undergo normal delivery.

Mineralocorticoid production is required postnatally, estrogens are not required, and androgens are needed only for male sexual differentiation [141]. It is not clear whether human fetal development requires glucocorticoids but, if so, the small amount of maternal cortisol that escapes placental inactivation suffices [141–143].

The regulation of steroidogenesis and growth of the fetal adrenal are not fully understood, but both are related to ACTH. ACTH stimulates steroidogenesis by fetal adrenal cells *in vitro* [144,145], and excess ACTH is clearly involved in the adrenal growth and overproduction of androgens in fetuses affected with CAH. Prenatal treatment of such fetuses by administering dexamethasone orally to the mother at 6–10 weeks' gestation can significantly reduce fetal adrenal androgen production and thus reduce the virilization of female fetuses.

The hypothalamo-pituitary–adrenal axis functions very early in fetal life [146], but anencephalic fetuses, which lack pituitary ACTH, have adrenals that contain a fairly normal complement of steroidogenic enzymes and retain their capacity for steroidogenesis. Thus, it appears that fetal adrenal steroidogenesis is regulated by both ACTH-dependent and ACTH-independent mechanisms.

Regulation of steroidogenesis

The hypothalamo-pituitary–adrenal axis

The principal steroidal product of the human adrenal is cortisol, which is mainly secreted in response to ACTH (corticotrophin) produced in the pituitary; secretion of ACTH is stimulated mainly by corticotrophin-releasing factor (CRH) from the hypothalamus. Hypothalamic CRH is a 41-amino-acid peptide synthesized mainly by neurones in the paraventricular nucleus. These same hypothalamic neurones also produce arginine vasopressin (AVP, also known as antidiuretic hormone or ADH) [147]. Both CRH and AVP are proteolytically derived from larger precursors, with the AVP precursor containing the sequence for neurophysin, which is the AVP-binding protein. CRH and AVP travel through axons to the median eminence, which releases them into the pituitary portal circulation, although most AVP axons terminate in the posterior pituitary [148]. Both CRH and AVP stimulate the synthesis and release of ACTH, but they appear to do so by different mechanisms. CRH functions principally by receptors linked to the protein kinase A pathway, stimulating production of intracellular cAMP, whereas AVP appears to function via protein kinase C and intracellular Ca^{2+} [149]. It is fairly clear that CRH is the more important physiological stimulator of ACTH release, although maximal doses of AVP can elicit a maximal ACTH response. When given together, CRH and AVP act synergistically, as would be expected from their independent mechanisms of action.

ACTH and pro-opiomelanocortin (POMC)

Pituitary ACTH is a 39-amino-acid peptide derived from POMC, a 241-amino-acid protein. POMC undergoes a series of proteolytic cleavages, yielding several biologically active peptides [150,151] (Fig. 15.4). The N-terminal glycopeptide (POMC 1–75) can stimulate steroidogenesis and may function as an adrenal mitogen [152]. POMC 112–150 is ACTH 1–39; POMC 112–126 and POMC 191–207 constitute α- and β-MSH (melanocyte-stimulating hormone) respectively. POMC 210–241 is β-endorphin. POMC is produced in small amounts by the brain, testis, and placenta, but this extrapituitary POMC does not contribute significantly to circulating ACTH. Malignant tumors will commonly produce "ectopic ACTH" in adults and rarely in children; this ACTH derives from ectopic biosynthesis of the same POMC precursor [150]. Only the first 20–24 amino acids of ACTH are needed for its full biological activity, and synthetic ACTH 1–24 is widely used in diagnostic tests of adrenal function. The shorter

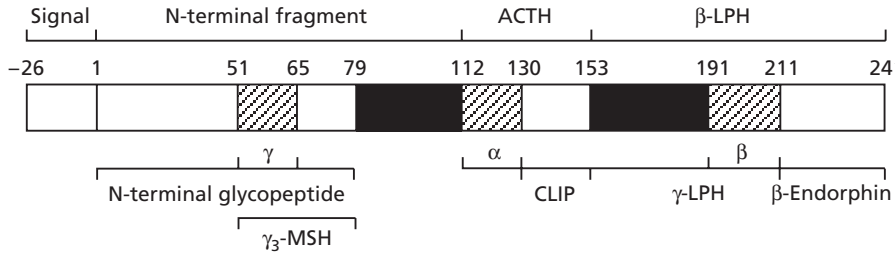

Fig. 15.4. Structure of human prepro-opiomelanocortin. The numbers refer to amino acid positions, with no. 1 assigned to the first amino acid of POMC after the 26-amino-acid signal peptide. The α-, β- and γ-MSH regions, which characterize the three "constant" regions, are indicated by diagonal lines; the "variable" regions are solid. The amino acid numbers shown refer to the N-terminal amino acid of each cleavage site; because these amino acids are removed, the numbers do not correspond exactly to the amino acid numbers of the peptides as used in the text. CLIP, corticotrophin-like intermediate lobe peptide.

forms of ACTH have a shorter half-life than native ACTH 1–39. POMC gene transcription is stimulated by CRH and is inhibited by glucocorticoids [153].

Actions of ACTH

ACTH stimulates steroidogenesis by interacting with receptors that stimulate the production of cAMP, which elicits acute and long-term effects. ACTH, acting via cAMP, stimulates the biosynthesis of LDL receptors and the uptake of LDL, which provides most of the cholesterol used for steroidogenesis [12]. ACTH via cAMP also stimulates transcription of the gene for HMGCoA reductase, the rate-limiting step in cholesterol biosynthesis, but adrenal biosynthesis of cholesterol is quantitatively much less important than the uptake of LDL-cholesterol [13].

Cholesterol is stored in steroidogenic tissues as cholesterol esters in lipid droplets. ACTH stimulates the activity of cholesterol esterase while inhibiting cholesterol ester synthetase, thus increasing the intracellular pool of free cholesterol, the substrate for P450scc [14,154]. The esterase is similar to gastric and lingual lipases [155]. Finally, ACTH facilitates transport of cholesterol into mitochondria by stimulating the synthesis and phosphorylation of StAR, thus increasing the flow of free cholesterol into the mitochondria. All these actions occur within minutes and constitute the acute effect of ACTH on steroidogenesis. The adrenal contains relatively modest amounts of steroid hormones; thus, release of preformed cortisol does not contribute significantly to the acute response to ACTH, which occurs by the rapid provision of large supplies of cholesterol to mitochondrial P450scc [41,156].

Long-term chronic effects of ACTH are mediated directly at the level of the steroidogenic enzymes. ACTH via cAMP stimulates the accumulation of the steroidogenic enzymes and their mRNAs by stimulating the transcription of their genes [38]. Thus, ACTH increases both the uptake of the cholesterol substrate and its conversion to steroidal products. The stimulation of this steroidogenesis occurs at each step in the pathway, not only at the rate-limiting step, P450scc.

The role of ACTH and other peptides derived from POMC in stimulating growth of the adult adrenal remains uncertain [157,158]. However, in the fetal adrenal, ACTH stimulates the local production of insulin-like growth factor (IGF) II [145,159], basic fibroblast growth factor [160] and epidermal growth factor [161]. These, and possibly other factors, work together to mediate ACTH-induced growth of the fetal adrenal [162].

Diurnal rhythms of ACTH and cortisol

Plasma concentrations of ACTH and cortisol are high in the morning and low in the evening. Peak ACTH levels are usually seen at 04.00–06.00 h and peak cortisol levels follow at about 08.00 h. Both ACTH and cortisol are released episodically in pulses every 30–120 min throughout the day, but the frequency and amplitude are greater in the morning. The basis of this diurnal rhythm is complex and incompletely understood. The hypothalamic content of CRH itself shows a diurnal rhythm, with peak content at about 04.00 h. At least four factors appear to play a role in the rhythm of ACTH and cortisol. These interdependent factors include intrinsic rhythmicity of synthesis and secretion of CRH by the hypothalamus, light–dark cycles, feeding, and inherent rhythmicity in the adrenal, possibly mediated by adrenal innervation [163].

Dietary rhythms may play as large a role as light–dark cycles [164,165], as animal experiments show that altering the time of feeding can overcome the ACTH/cortisol periodicity established by a light–dark cycle. In normal human subjects, cortisol is released before lunch and supper, but not at these times in persons eating continuously during the day. Thus, glucocorticoids, which increase blood glucose, appear to be released at times of fasting and are inhibited by feeding [166,167].

As all parents know, infants do not have a diurnal rhythm of sleep or feeding. They acquire such behavioral rhythms in response to the environment long before they acquire a rhythm of ACTH and cortisol. The diurnal rhythms begin to be established at 6–12 months but are often not well established until after 3 years of age [168]. Once the rhythm is well

established in the older child or adult, it is changed only with difficulty. When people move time zones, ACTH/cortisol rhythms generally take 15–20 days to adjust.

Physical stress (major surgery, severe trauma, blood loss, high fever, or serious illness) increases the secretion of both ACTH and cortisol, but minor surgery and minor illnesses (upper respiratory infections) have little effect [169,170]. Infection, fever, and pyrogens can stimulate the release of interleukin 1 (IL-1) and IL-6, which stimulate secretion of CRH, and also IL-2 and tumor necrosis factor (TNF), which stimulate release of ACTH, providing further stimulus to cortisol secretion during inflammation [171]. Most psychoactive drugs, such as anticonvulsants, neurotransmitters, and antidepressants, do not affect the diurnal rhythm of ACTH and cortisol, although cyproheptidine (a serotonin antagonist) suppresses ACTH release.

Adrenal–glucocorticoid feedback

The hypothalamo-pituitary–adrenal axis is a classic example of an endocrine feedback system. ACTH increases the production of cortisol, and cortisol decreases the production of CRH and ACTH [153,172]. Like the acute and chronic phases of the action of ACTH on the adrenal, there are acute and chronic phases of the feedback inhibition of ACTH (and presumably CRH) [172]. The acute phase, which occurs within minutes, inhibits release of ACTH (and CRH) from secretory granules. With prolonged exposure, glucocorticoids inhibit ACTH synthesis by directly inhibiting the transcription of the gene for POMC. Some evidence also suggests that glucocorticoids can inhibit steroidogenesis at the level of the adrenal fasciculata cell itself, but this appears to be a physiologically minor component of the regulation of cortisol secretion.

Mineralocorticoid secretion: the renin–angiotensin system

Renin is a serine protease enzyme synthesized primarily by the juxtaglomerular cells of the kidney. It is also produced in a variety of other tissues, including the glomerulosa cells of the adrenal cortex [173]. The role of adrenally produced renin is not well established; it appears to maintain basal levels of P450c11AS, but it is not known whether angiotensin II is involved in this action [174]. Renin is synthesized as a precursor (406 amino acids) that is cleaved to pro-renin (386 amino acids) and, finally, to the 340-amino-acid protein found in plasma [175]. Decreased blood pressure, upright posture, sodium depletion, vasodilatory drugs, kallikrein, opiates, and β-adrenergic stimulation all promote the release of renin. Renin enzymatically attacks angiotensinogen, the renin substrate, in the circulation.

Angiotensinogen is a highly glycosylated protein and therefore has a highly variable molecular weight, from 50 000 to 100 000 Da. Renin proteolytically releases the amino-terminal 10 amino acids of angiotensinogen, referred to as angiotensin I. This decapeptide is biologically inactive until converting enzyme, an enzyme found primarily in the lungs and blood vessels, cleaves off its two carboxy-terminal amino acids to produce an octapeptide termed angiotensin II. Converting enzyme can be inhibited by captopril and related agents useful in the diagnosis and treatment of hyperreninemic hypertension.

Angiotensin II has two principal actions, both of which increase blood pressure. It directly stimulates arteriolar vasoconstriction within a few seconds, and it stimulates the synthesis and secretion of aldosterone within minutes [176]. Increased plasma potassium is a powerful and direct stimulator of aldosterone synthesis and release [177,178].

Aldosterone, secreted by the glomerulosa cells of the adrenal cortex, has the greatest mineralocorticoid activity of all naturally occurring steroids. It causes renal sodium retention and potassium loss, with a consequent increase in intravascular volume and blood pressure. Angiotensin II functions through receptors that stimulate the production of phosphatidylinositol, mobilize intracellular and extracellular Ca^{2+}, and activate protein kinase C [179]. These intracellular second messengers then stimulate transcription of the P450scc gene by means independent of those used by ACTH and cAMP [180]. Potassium ions increase uptake of Ca^{2+}, with consequent hydrolysis of phosphoinositides to increase phosphatidylinositol. Thus, angiotensin II and potassium work at different levels of the same intracellular second-messenger pathway, but these differ fundamentally from the action of ACTH.

Although the renin–angiotensin system is clearly the major regulator of mineralocorticoid secretion, ACTH, and possibly other POMC-derived peptides such as γ_3-MSH, can also promote secretion of aldosterone when used in high concentrations in animal systems [181,182]. The relevance of physiological concentrations in human beings has not been established. Ammonium ions, hyponatremia, dopamine antagonists, and some other agents can also stimulate secretion of aldosterone; atrial natriuretic factor is a potent physiological inhibitor of aldosterone secretion [183].

Adrenal androgen secretion and the regulation of adrenarche

DHEA, DHEAS, and androstenedione, which are almost exclusively secreted by the adrenal zona reticularis, are generally referred to as adrenal androgens because they can be converted peripherally to testosterone. These steroids have little if any capacity to bind to and activate androgen receptors and are hence only androgen precursors, not true androgens. The fetal adrenal secretes large amounts of DHEA and DHEAS, and these steroids are abundant in the newborn; their concentrations fall rapidly as the fetal zone of the adrenal involutes after birth.

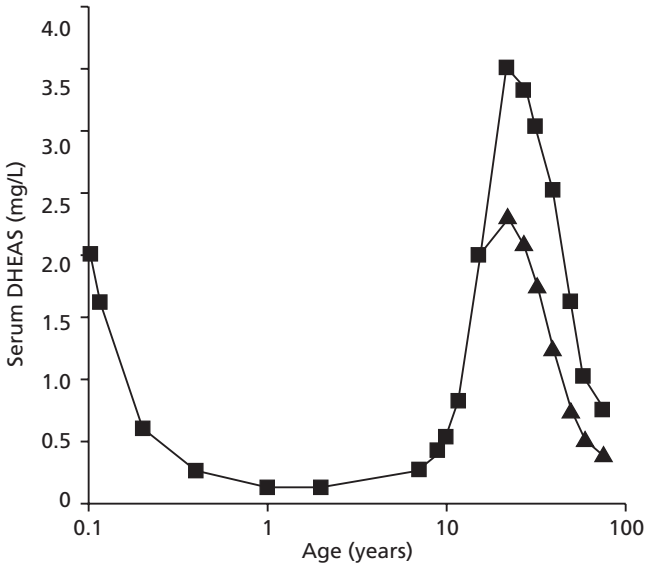

Fig. 15.5. Concentrations of DHEAS as a function of age. Note that the *x*-axis is on a log scale. Squares, males; triangles, females.

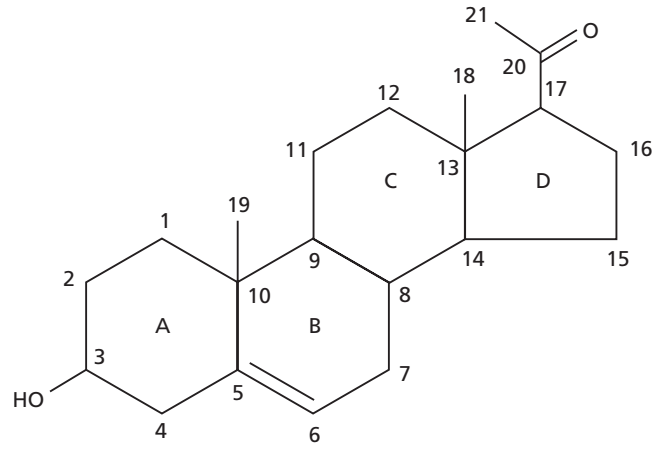

Fig. 15.6. Structure of pregnenolone. The carbon atoms are indicated by numbers, and the rings are designated by letters according to standard convention. Pregnenolone is derived from cholesterol, which has a six-carbon side-chain attached to carbon 21. Pregnenolone is a "Δ^5 compound," having a double bond between carbons 5 and 6; the action of 3β-hydroxysteroid dehydrogenase/isomerase moves this double bond from the B ring to carbon numbers 4 and 5 in the A ring, forming Δ^4 compounds. All the major biologically active steroid hormones are Δ^4 compounds.

After the first year of life, the adrenals of young children secrete small amounts of DHEA, DHEAS, and androstenedione until the onset of adrenarche, usually around age 7–8 years, preceding the onset of puberty by about 2 years. Adrenarche is independent of puberty, the gonads, or gonadotropins, and the mechanism by which its onset is triggered remains unknown [64]. The secretion of DHEA and DHEAS continues to increase during and after puberty and reaches maximal values in young adulthood, after which there is a slow, gradual decrease in the secretion of these steroids in elderly people ("adrenopause") (Fig. 15.5) [184]. Despite the increases in the adrenal secretion of DHEA and DHEAS during adrenarche, circulating concentrations of ACTH and cortisol do not change with age. Thus, ACTH plays a permissive role in adrenarche but does not trigger it. Searches for hypothetical polypeptide hormones that might specifically stimulate the zona reticularis have been unsuccessful [185,186].

Recent studies of adrenarche have focused on the roles of 3β-HSD and P450c17. The abundance of 3β-HSD protein in the zona reticularis appears to decrease with the onset of adrenarche [187–189], and the adrenal expression of cytochrome b_5, which fosters the 17,20-lyase activity of P450c17, is confined almost exclusively to the zona reticularis [190,191]. Both these factors would strongly favor the production of DHEA [192]. Exaggerated adrenarche has been found in association with insulin resistance, and girls with this condition appear to be at a much higher risk of developing the polycystic ovarian syndrome as adults [193–195]. Recent evidence suggests that infants born small for gestational age may be at increased risk of this syndrome [196]. Evidence is accumulating to suggest that replacement of DHEA after adrenopause may improve memory and a sense of well-being in elderly people [197].

Plasma steroids and their disposal

Structure and nomenclature

All steroid hormones are derivatives of pregnenolone (Fig. 15.6). Pregnenolone and its derivatives that contain 21 carbon atoms are often termed C_{21} steroids. Each carbon atom is numbered, indicating the location at which the various steroidogenic reactions occur (e.g. 21-hydroxylation, 11-hydroxylation). The 17,20-lyase activity of P450c17 cleaves the bond between carbon atoms 17 and 20, yielding C_{19} steroids, which include all the androgens. P450aro converts C_{19} androgens to C_{18} estrogens. With the exception of estrogens, all steroid hormones have a single unsaturated carbon–carbon double bond. Steroids having this double bond between carbon atoms 4 and 5, including all the principal biologically active steroids, are termed Δ^4 steroids; their precursors, having a double bond between carbon atoms 5 and 6, are termed Δ^5 steroids. The two isozymes of 3β-HSD convert Δ^5 to Δ^4 steroids.

A rigorous, logically systematic, and unambiguous chemical terminology has been formulated to describe accurately the structure of all the steroid hormones and all their conceivable derivatives. However, this terminology is unbelievably cumbersome (e.g. cortisol is 11β,17α,21-trihydroxy-pregn-4-ene-3,20-dione and dexamethasone is 9α-fluoro-11β,17α,

21-trihydroxyprena-1,4-diene-3,20-dione). Therefore, we use only the standard trivial names.

Before the structures of the steroid hormones were determined in the 1930s, Reichstein, Kendall, and others identified them as spots on paper chromatograms and designated them A, B, C, etc. Unfortunately, some persist in using this outmoded terminology more than 70 years later, so that corticosterone is sometimes termed "compound B," cortisol "compound F," and 11-deoxycortisol "compound S." This archaic terminology obfuscates the precursor–product relationships of the steroids, confuses students, and should not be used.

Circulating steroids

Although over 50 different steroids have been isolated from adrenocortical tissue, the main pathways of adrenal steroidogenesis include only a dozen or so steroids, of which only a few are secreted in sizable quantities. The adult secretion of DHEA and cortisol is each about 20 mg/24 h, and the secretion of corticosterone, a weak glucocorticoid, is about 2 mg/24 h [198]. Although glucocorticoids, such as cortisol, and mineralocorticoids, such as aldosterone, are both needed for life and hence are of "equivalent" physiological importance, diagrams such as Figure 15.1 fail to indicate that these steroids are not secreted in molar equivalents. The adult secretion rate of aldosterone is only about 0.1 mg/24 h. This 100- to 1000-fold molar difference in the secretory rates of cortisol and aldosterone must be borne in mind when considering the effects of steroid-binding proteins in plasma and when conceptualizing the physiological manifestations of incomplete defects in steroidogenesis due to single amino acid changes causing the partial loss of activity of a steroidogenic enzyme.

Most circulating steroids are bound to plasma proteins, including corticosteroid-binding globulin (CBG, also termed transcortin), albumin, and α_1-acid glycoprotein [199,200]. CBG has a very high affinity for cortisol but a relatively low binding capacity; albumin has a low affinity and high capacity; α_1-acid glycoprotein is intermediate for both variables. The result is that about 90% of circulating cortisol is bound to CBG and a little more is bound to other proteins. These steroid-binding proteins are not transport proteins, as the biologically important steroids are water soluble in physiologically effective concentrations, and absence of CBG does not cause a detectable physiological disorder. However, these plasma proteins do act as a reservoir for steroids. This insures that all peripheral tissues will be bathed in approximately equal concentrations of cortisol, which greatly diminishes the physiological effect of the great diurnal variation in cortisol secretion.

Synthetic glucocorticoids do not bind significantly to CBG and bind poorly to albumin, partially accounting for their increased potencies, which are also associated with increased receptor-binding affinities. Aldosterone is not bound well by any plasma protein; hence, changes in plasma protein concentration do not affect plasma aldosterone concentrations but greatly influence plasma cortisol concentrations. Estradiol and testosterone bind strongly to a different plasma protein termed sex steroid-binding globulin and also bind weakly to albumin.

Because steroids are hormones, it is often thought that the concentration of "free" (i.e. unbound) circulating steroids determines biological activity. However, the target tissues for many steroid hormones contain enzymes that modify those steroids. Thus, many actions of testosterone are actually due to dihydrotestosterone produced by local 5α-reductase; cortisol will have differential actions on various tissues as a result of the presence or absence of 11β-HSD, which inactivates cortisol to cortisone. Similar peripheral metabolism occurs via "extraglandular" 21-hydroxylase, P450aro, 3β-HSD, and 17β-HSD. Thus, circulating steroids are both classic hormones and precursors to locally acting autocrine or paracrine factors.

Steroid catabolism

Only about 1% of circulating plasma cortisol and aldosterone is excreted unchanged in the urine; the remainder is metabolized by the liver. A large number of hepatic metabolites of each steroid is produced, most containing additional hydroxyl groups and linked to a sulfate or glucuronide moiety, rendering them more soluble and readily excretable by the kidney. A great deal is known about the various urinary metabolites of the circulating steroids because their measurement in pooled 24-h urine samples has been an important means of studying adrenal steroids. Although the measurement of urinary steroid metabolites by modern mass spectrometric techniques remains an important research tool [201–203], the development of separation techniques and of specific and highly sensitive radioimmunassays for each of the steroids in plasma has greatly reduced the need to measure their excreted metabolites in clinical practice.

Clinical and laboratory evaluation of adrenal function

Clinical evaluation

Primary adrenal deficiency or hypersecretion is generally evident before performing laboratory tests. Patients with chronic adrenal insufficiency have weakness, fatigue, anorexia, weight loss, hypotension, and hyperpigmentation. Patients with acute adrenal insufficiency have hypotension, shock, weakness, apathy, confusion, anorexia, nausea, vomiting, dehydration, abdominal or flank pain, hyperthermia, and hypoglycemia.

Early signs of glucocorticoid excess include increased appetite, weight gain, and growth arrest without a concomitant delay in bone age. Chronic glucocorticoid excess in children results in typical cushingoid facies, but the buffalo hump and centripetal distribution of body fat characteristic of Cushing disease in adults are seen only in long-standing undiagnosed disease.

Mineralocorticoid excess is characterized by hypertension, but patients receiving very low sodium diets (e.g. the newborn) are not hypertensive, as mineralocorticoids increase blood pressure primarily by retaining sodium and thus increasing intravascular volume.

Deficient adrenal androgen secretion will compromise the acquisition of virilizing secondary sexual characteristics (pubic and axillary hair, comedones, axillary odor) in female adolescents. Moderate hypersecretion of adrenal androgens is characterized by mild signs of virilization, whereas substantial hypersecretion of adrenal androgens is characterized by accelerated growth with a disproportionate increase in bone age, increased muscle mass, acne, hirsutism, deepening of the voice, and more profound degrees of virilism. A key feature of any physical examination of a virilized male is careful examination and measurement of the testes. Bilaterally enlarged testes suggest true (central) precocious puberty; unilateral testicular enlargement suggests testicular tumor; prepubertal testes in a virilized male indicate an extratesticular source of androgen, such as the adrenal.

Imaging studies are of limited use in adrenocortical disease. Computed tomography (CT) rarely detects pituitary tumors secreting ACTH, although recent advances in magnetic resonance imaging (MRI) may detect many of these with gadolinium enhancement. The small size, odd shape, and location near other structures compromise the use of imaging techniques for the adrenals. Patients with Cushing disease or CAH have modestly enlarged adrenals, but these are often not detectable by imaging with any useful degree of certainty. The gross enlargement of the adrenals in congenital lipoid adrenal hyperplasia, their hypoplasia in adrenal hypoplasia congenita, or in the hereditary ACTH unresponsiveness syndrome can be imaged, as can many malignant tumors, but most adrenal adenomas are too small to be detected. Thus, imaging studies may establish the presence of pituitary or adrenal tumors but never exclude them.

Laboratory evaluation

Steroid measurements

Plasma cortisol is measured by a variety of techniques including radioimmunoassay, immunoradiometric assay, and high-performance liquid chromatography (HPLC). Other procedures, such as fluorimetric assays and competitive protein-binding assays, are useful research tools but are not in general clinical use. It is of considerable importance to know what procedure one's laboratory is using and precisely what it is measuring, because laboratories may have different normal values, and most central hospital and commercial laboratories are designed primarily to serve adult, rather than pediatric, patients. Tables 15.2 and 15.3 summarize the normal plasma concentrations for a variety of steroids.

All immunoassays have some degree of cross-reactivity with other steroids, and most cortisol immunoassays detect cortisol and cortisone, which are readily distinguished by HPLC. As the newborn's plasma contains mainly cortisone rather than cortisol during the first few days of life, comparison of newborn data obtained by HPLC with published standards obtained by immunoassays may incorrectly suggest adrenal insufficiency.

With the notable exception of DHEAS, most adrenal steroids exhibit a diurnal variation based on the diurnal rhythm of ACTH. Because the stress of illness or hospitalization can increase adrenal steroid secretion and because diurnal rhythms may not be well established in children under 3 years of age, it is best to obtain two or more samples for the measurement of any steroid [204,205].

Plasma renin

Renin is not generally measured directly but is assayed by its enzymatic activity. Plasma renin activity (PRA) is simply an immunoassay of the amount of angiotensin I generated per milliliter of serum per hour at 37°C. In normal serum, the concentration of both renin and angiotensinogen (the renin substrate) is limiting. Therefore, another test, plasma renin content (PRC), measures the amount of angiotensin I generated in 1 h at 37°C in the presence of excess concentrations of angiotensinogen. Immunoassays for renin itself are beginning to enter clinical practice.

Plasma renin activity is sensitive to dietary sodium intake, posture, diuretic therapy, activity, and sex steroids. Because PRA values can vary widely, it is best to measure renin twice, once in the morning after overnight supine posture and then again after maintenance of upright posture for 4 h [206]. A simultaneous 24-h urine for total sodium excretion is generally needed to interpret PRA results. Decreased dietary and urinary sodium, decreased intravascular volume, diuretics, and estrogens will increase PRA. Sodium loading, hyperaldosteronemia, and increased intravascular volume decrease PRA.

The greatest use of renin measurements is in the evaluation of hypertension and in the management of CAH. However, several additional situations require assessment of the renin–angiotensin system. Children with simple virilizing adrenal hyperplasia who do not have clinical evidence of urinary salt wasting (hyponatremia, hyperkalemia, acidosis, hypotension, shock) may nevertheless have increased PRA, especially when dietary sodium is restricted. This was an early clinical sign that this form of 21-hydroxylase deficiency (21-OHD)

Table 15.2. Mean sex steroid concentration in infants and children.

	PROG	17-OHP	DHEA	DHEAS	Δ4 A	E1	E2	T		DHT	
								M	F	M	F
Cord blood	1100	62	21	6400	3.0	52	30	1.0	0.9	0.2	0.2
Premature babies	11	8.1	28	11 000	7.0			4.2	0.4	1.0	0.1
Term newborns		1.1	20	4400	5.2			6.9	1.4	0.9	0.3
Infants	1.0	1.0	3.8	820	0.7	< 0.1	< 0.1	6.6	< 0.4	1.4	< 0.1
Children											
1–6 years			1.0	270	0.9	< 0.1	< 0.1	0.2		0.1	
6–8 years			3.1	540	0.9	< 0.1	< 0.1	0.2		0.1	
8–10 years			5.6	1400	0.9	< 0.1	< 0.1	0.2		0.1	
Males											
Pubertal stage I	0.6	1.3	5.6	950	0.9	0.0	0.0	0.2		< 0.1	
Pubertal stage II	0.6	1.6	10	2600	1.6	0.1	0.0	1.4		0.3	
Pubertal stage III	0.8	2.0	14	3300	2.4	0.1	0.1	6.6		0.7	
Pubertal stage IV	1.1	2.6	14	5400	2.8	0.1	0.1	13		1.2	
Pubertal stage V	1.3	3.3	17	6300	3.5	0.1	0.1	19		1.6	
Adult	1.1	3.3	16	7300	4.0	0.1	0.1	22		1.7	
Females											
Pubertal stage I	0.6	1.0	5.6	1100	0.9	0.1	0.0	0.2		0.1	
Pubertal stage II	1.0	1.6	11	1900	2.3	0.1	0.1	0.7		0.3	
Pubertal stage III	1.3	2.3	14	2500	4.2	0.1	0.1	0.9		0.3	
Pubertal stage IV	9.2	2.9	15	3300	4.5	0.1	0.2	0.9		0.3	
Pubertal stage V	5.1	3.6	19	4100	6.0	0.2	0.4	1.0		0.3	
Adult											
Follicular	1.0	1.5	16	4100	5.8	0.2	0.2	1.0		0.3	
Luteal	24	5.4	16	4100	5.8	0.4	0.5	1.0		0.3	

PROG, progesterone; 17-OHP, 17-hydroxyprogesterone; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; Δ4 A, androstenedione; E1, estrone; E2, estradiol; T, testosterone; DHT, dihydrotestosterone; M, male; F, female.

All values are in nmol/L.

Data adapted from Endocrine Sciences, Tarzana, CA, USA.

Table 15.3. Mean glucocorticoid and mineralocorticoid concentrations.

	Cortisol	DOC	Corticosterone	18-OH-corticosterone	Aldosterone	Plasma renin activity
Cord blood	360	5.5	19		2.4	50
Premature babies	180			5.5	2.8	222
Newborns	140		6.6	9.7	2.6	58
Infants	250	0.6	16	2.2	0.8	33
Children (08.00 h)						
1–2 years	110–550			1.8	0.8	15
2–10 years	As adults	0.3		1.2	0.3→0.8*	8.3
10–15 years	As adults			0.7	0.1→0.6*	3.3
Adults (08.00 h)	280–550	0.2	12	0.6	0.2→0.4*	2.8→4.0*
Adults (16.00 h)	140–280		3.8			

DOC, deoxycorticosterone.

All values in nmol/L except plasma renin activity (μg/L/s).

*Two values separated by an arrow indicate those in supine and upright posture.

was simply a milder form of the more common, severe, salt-wasting form. Treatment of simple virilizing 21-OHD with mineralocorticoid sufficient to suppress PRA into the normal range will reduce the child's requirement for glucocorticoids, thus maximizing final adult height. Children with CAH need to have their mineralocorticoid replacement therapy monitored routinely by measuring PRA. Measurement of angiotensin II is also possible in some research laboratories, but most antibodies to angiotensin II cross-react strongly with angiotensin I. Thus, PRA remains the most useful way of evaluating the renin–angiotensin–aldosterone system.

Urinary steroid excretion

The measurement of 24-h urinary excretion of steroid metabolites is one of the oldest procedures for assessing adrenal function and is still useful. Examination of the total 24-h excretion of steroids eliminates the fluctuations seen in serum samples as a function of time of day, episodic bursts of ACTH, and steroid secretion and transient stress (such as a visit to the clinic or difficult venepuncture). Collection of a complete 24-h urinary sample can be difficult in the infant or small child. Two consecutive 24-h collections should be obtained. Because of the diurnal and episodic nature of steroid secretion, one should never obtain 8- or 12-h collections and attempt to infer the 24-h excretory rate from such partial collections.

Urinary 17-hydroxycorticosteroids, assayed by the colorimetric Porter–Silber reaction, measures 17,21-dihydroxy-20-ketosteroids by the generation of a colored compound after treatment with phenylhydrazine [207]. The reaction is highly specific for the major urinary metabolites of cortisol and cortisone. It will also measure metabolites of 11-deoxycortisol.

Measurement of 17-hydroxycorticosteroids is being replaced by measurement of urinary free cortisol, thus avoiding the non-specificity and drug interference problems inherent in 17-hydroxycorticosteroids. In adults, the test is highly reliable in the diagnosis of Cushing syndrome. Free cortisol is extracted from the urine and measured by immunoassay or HPLC, providing the advantage of specificity; furthermore, unlike 17-hydroxycorticosteroids, urinary free cortisol is not increased in exogenous obesity [208]. The upper limit of normal for urinary free cortisol excretion for children is 80 $\mu g/m^2$/day and that for 17-hydroxycorticosteroids is 5 mg/m^2/day [209]. Some clinical experience indicates that urinary 17-hydroxycorticosteroids may be more reliable for the diagnosis of Cushing disease in children, possibly because of greater experience with 17-hydroxycorticosteroids [210].

Urinary 17-ketosteroids, assayed by the Zimmerman reaction, measure 17-ketosteroids by the generation of a colored compound after treatment with *meta*-dinitrobenzine and acid [211]. The reaction principally measures metabolites of DHEA and DHEAS and thus correlates with adrenal androgen production. Androstenedione will contribute significant 17-ketosteroids and, if an alkali extraction is not used, estrone will also contribute. The principal androgens, testosterone and dihydrotestosterone, have hydroxyl rather than keto groups on carbon 17; hence, their metabolic products are not measured as 17-ketosteroids. A wide variety of drugs, including penicillin, nalidixic acid, spironolactone, and phenothiazines, as well as non-specific urinary chromogens can spuriously increase values of 17-ketosteroids. Measurement of urinary 17-ketosteroids remains a useful, inexpensive screening test, and some clinicians prefer to follow 17-ketosteroids to monitor therapy of CAH, but measurements of plasma steroids have now replaced the use of urinary 17-ketosteroids in most centers.

Urinary 17-ketogenic steroids are occasionally confused with urinary 17-ketosteroids because of the similarity of the names; however, 17-ketogenic steroids are used to measure urinary metabolites of glucocorticoids, not sex steroids. Although some laboratories continue to perform measurements of 17-ketogenic steroids, this obsolete assay no longer has a place in modern pediatric practice.

Plasma ACTH and other POMC peptides

Accurate immunoassay of plasma ACTH is available in most centers, but its measurement remains more difficult and variable than the assays for most other pituitary hormones [212]. Samples must be drawn into a plastic syringe containing heparin or ethylenediamine tetraacetic acid (EDTA) and transported quickly in plastic tubes on ice, as ACTH adheres to glass and is quickly inactivated. Elevated plasma ACTH concentrations can be informative, but most assays cannot detect low or low–normal values, and such values can be spurious if the samples are handled badly. In adults and older children with well-established diurnal rhythms of ACTH, normal 08.00 h values rarely exceed 50 pg/mL, whereas 20.00 h values are usually undetectable. Patients with Cushing disease often have normal morning values, but consistently elevated afternoon and evening ones can suggest the diagnosis. Patients with the ectopic ACTH syndrome have values from 100 to 1000 pg/mL.

The carboxy portion of POMC [β-lipotrophic hormone (β-LPH), POMC 153–241] is released from POMC in equimolar amounts with ACTH. β-LPH has a longer circulating plasma half-life than ACTH, is more stable in the laboratory, and is easier than ACTH to assay [213]. Such assays may be a useful adjunct to ACTH assays, but routine measurement of β-LPH is not available.

Secretory rates

The secretory rates of cortisol and aldosterone (or other steroids) can be measured by administering a small dose of tritiated cortisol or aldosterone and measuring the specific

Table 15.4. Responses of adrenal steroids to a 60-min ACTH test.

	Infants		Prepubertal		Pubertal	
	Basal	Stimulated	Basal	Stimulated	Basal	Stimulated
17-OH-pregnenolone	6.8		1.7	9.6	3.6	24
17-OHP	0.8	5.8	1.5	5.8	1.8	4.8
DHEA	1.4		2.4	4.3	9.0	19
11-Deoxycortisol	2.3		1.8	5.8	1.7	4.9
Cortisol	280	830	360	830	280	690
DOC	0.6	2.4	0.2	1.7	0.2	1.7
Progesterone	1.1	3.2	1.1	4.0	1.9	4.8

All values are mean values in nmol/L.
Data adapted from Endocrine Sciences, Tarzana, CA, USA.

activity of one or more known metabolites in a 24-h urine collection. This procedure permitted the measurement of certain steroids, such as aldosterone, before specific immunoassays became available. The procedures have provided much information about the normal rate of production of various steroids. On the basis of this procedure, most authorities previously concluded that children and adults secrete about 12 mg of cortisol per square meter of body surface area per day. More recent studies indicate a rate of 6–9 mg/m^2 in children and adults [214,215]. Such differences are of considerable importance in estimating physiological replacement doses of glucocorticoids.

Dexamethasone suppression test

Administration of small doses of dexamethasone, a potent synthetic glucocorticoid, will suppress secretion of pituitary ACTH and of adrenal cortisol. Originally described by Liddle [216] in 1960, the dexamethasone suppression test remains the most useful procedure for distinguishing whether glucocorticoid excess is due primarily to pituitary disease or adrenal disease. As dexamethasone also suppresses adrenal androgen secretion, this test is useful for distinguishing between adrenal and gonadal sources of sex steroids. A dexamethasone suppression test requires the measurement of basal values and those obtained in response to both low- and high-dose dexamethasone. Variations of the test are common, notably the single 1.0-mg dose in adults [217] or 0.3 mg/m^2 in children [218]. This is a useful outpatient screening procedure for distinguishing Cushing syndrome from exogenous obesity. It can be useful for the same purpose in adolescents and older children but is otherwise of limited utility in pediatrics. An overnight high-dose dexamethasone suppression test is probably more reliable than the standard 2-day, high-dose test in differentiating adults with Cushing disease from those with the ectopic ACTH syndrome. The usefulness of this test in pediatric patients has not been established.

Stimulation tests

Direct stimulation of the adrenal with ACTH is a rapid, safe, and easy way to evaluate adrenocortical function. The original ACTH test consisted of a 4- to 6-h infusion of 0.5 units/kg of ACTH (1–39) to stimulate adrenal cortisol secretion maximally. It diagnoses primary adrenal insufficiency (Addison disease). In secondary adrenal insufficiency, some steroidogenic capacity is present, and some cortisol is produced in response to the ACTH.

This ACTH test has been replaced in clinical practice by the 60-min test, in which a single bolus of ACTH (1–24) is administered intravenously, and cortisol values are measured at 0 and 60 min [219]. Normal responses are shown in Table 15.4 [220]. Synthetic ACTH (1–24) (cosyntropin) is preferred as it has a more rapid action and shorter half-life than ACTH (1–39). The usual dose is 0.1 mg in newborns, 0.15 mg in children up to 2 years of age, and 0.25 mg for children over the age of 2 years and adults. All these doses are pharmacological.

A very low-dose (1 μg) test may be useful in assessing adrenal recovery from glucocorticoid suppression [221]. Newer data show that maximal steroidal responses can be achieved after only 30 min, but the best available standards are for a 60-min test.

One of the widest uses of intravenous ACTH tests in pediatrics is in diagnosing CAH. Stimulating the adrenal with ACTH increases steroidogenesis, resulting in the accumulation of steroids proximal to the disordered enzyme. For example, inspection of Figure 15.1 shows that impaired activity of P450c21 (21-hydroxylase) should lead to the accumulation of progesterone and 17-hydroxyprogesterone (17-OHP). However, progesterone does not accumulate in appreciable quantities, because it, too, is converted to 17-OHP. Measuring the response of 17-OHP to a 60-min or 6-h challenge with ACTH is the single most powerful and reliable means of diagnosing 21-OHD. Comparing the patient's basal and ACTH-stimulated values of 17-OHP against those from large

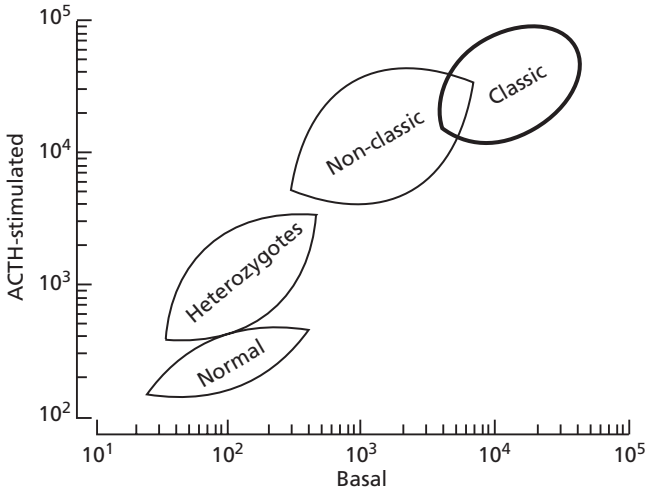

Fig. 15.7. 17-OHP values before and after stimulation with ACTH in normal subjects, patients with CAH, and heterozygotes. Smoothed data redrawn from [222].

numbers of well-studied patients usually permits the discrimination of normal persons, heterozygotes, patients with non-classic CAH, and patients with classic CAH, although there is inevitably some overlap between groups [222] (Fig. 15.7). Measurement of testosterone or Δ^4 androstenedione in response to ACTH can distinguish normal persons from patients with classic CAH, but heterozygotes and patients with cryptic CAH have values overlapping both normal and classic CAH (Fig. 15.7).

Longer ACTH tests of up to 3 days have also been used to evaluate adrenal function, but it is important to remember that ACTH has both acute and chronic effects. Thus, short tests measure only the acute effects of ACTH, the maximal stimulation of pre-existing steroidogenic machinery. A 3-day test will examine the more chronic effects of ACTH to stimulate increased capacity for steroidogenesis by increasing the synthesis of steroidogenic machinery. Few situations exist in which a 3-day intramuscular ACTH test is indicated, although it is useful in diagnosing the rare syndrome of hereditary unresponsiveness to ACTH [223].

Insulin-induced hypoglycemia is another commonly used test. The hypoglycemia stimulates the release of counter-regulatory hormones (ACTH and cortisol, growth hormone, epinephrine, and glucagon) that have actions to increase plasma glucose concentrations. Most patients experience hunger, irritability, diaphoresis, and tachycardia; when these are followed by drowsiness or sleep, blood sugar levels are probably below acceptable limits. If this occurs, a blood sample should be obtained and 2 mL/kg 25% glucose given intravenously to a maximum of 100 mL [224].

Metyrapone test

Metyrapone blocks the action of P450c11β and, to a much lesser extent, P450scc. It is thus a chemical means of inducing a transient deficiency of 11-hydroxylase activity, which results in decreased cortisol secretion and subsequent increase in ACTH secretion. Metyrapone testing is done to assess the capacity of the pituitary to produce ACTH in response to a physiological stimulus. This test is useful in evaluating the hypothalamo-pituitary axis in the presence of central nervous system lesions after neurosurgery or long-term suppression by glucocorticoid therapy [225]. Patients with a previous history of hypothalamic, pituitary, or adrenal disease or those who have been withdrawn from glucocorticoid therapy should be re-evaluated with a metyrapone test or with an insulin tolerance test. A normal response indicates recovery of the hypothalamo-pituitary–adrenal axis and predicts that the patient will respond normally to the stress of surgery.

Metyrapone is generally given orally as 300 mg/m^2 every 4 h for a total of six doses (24 h). Unlike many other drugs, it is appropriate to continue to increase the dose in older or overweight patients, but the total dose should not exceed 3.0 g [226]. Blood should be obtained for cortisol, 11-deoxycortisol, and ACTH before and after the test, and a 24-h urine collection should be obtained before and during the test for 17-hydroxycorticosteroids. In a normal response to metyrapone, cortisol decreases, ACTH increases, and 11-deoxycortisol (the substrate for P450c11β) increases greatly to about 5 μg/dL. Metabolites of 11-deoxycortisol result in a doubling in urinary 17-hydroxycorticosteroid excretion. Adults and older children can be tested with the administration of a single oral dose of 30 mg/kg at midnight, given with food to reduce the gastrointestinal irritation [226]. Blood samples are drawn at 08.00 h on the mornings before and after administering the drug.

CRH testing

CRH is now generally available as a test of pituitary ACTH reserve [227]. It remains experimental in adults, and little experience has been gained from children. Early data suggest that it may be useful for distinguishing hypothalamic from pituitary causes of ACTH deficiency, and may also be a useful adjunct in establishing the diagnosis of Cushing disease [228].

Genetic lesions in steroidogenesis

Autosomal-recessive disorders disrupt each of the steps in the pathway shown in Figure 15.1. Most result in diminished synthesis of cortisol. In response to adrenal insufficiency, the pituitary synthesizes increased amounts of POMC and ACTH, which promotes increased steroidogenesis; ACTH and possibly other peptides derived from the amino-terminal end of POMC also stimulate adrenal hypertrophy and hyperplasia. Thus, the term congenital adrenal hyperplasia (CAH) refers to a group of diseases traditionally grouped together on the basis of the most prominent finding at autopsy.

Table 15.5. Clinical and laboratory findings in the congenital adrenal hyperplasias.

Enzyme	Presentation	Laboratory findings	Therapeutic measures deficiency
Lipoid CAH	Salt-wasting crisis Male pseudohermaphroditism	Low/absent levels of all steroid hormones Decreased/absent response to ACTH Decreased/absent response to hCG in male pseudohermaphroditism and salt supplementation	Glucocorticoid and mineralocorticoid (StAR) replacement Estrogen replacement at age 12 years Gonadectomy of male pseudohermaphrodite
3β-HSD	Salt-wasting crisis ↑Δ^5/Δ^4 serum steroids Male and female pseudohermaphroditism	↑ACTH and PRA ↑Δ^5 steroids before and after ACTH Suppression of elevated adrenal steroids after glucocorticoid administration ↑ACTH and PRA	Glucocorticoid and mineralocorticoid replacement Salt supplementation Surgical correction of genitalia Sex hormone replacement as necessary
P450c21	*Classic form*: Salt-wasting crisis Female pseudohermaphroditism Pre- and postnatal virilization *Non-classic form*: Premature adrenarche, menstrual irregularity, hirsutism, acne, infertility	↑17-OHP before and after ACTH ↑Serum androgens and urine 17-ketosteroids Suppression of elevated adrenal steroids after glucocorticoid treatment ↑ACTH and PRA	Glucocorticoid and mineralocorticoid replacement Salt supplementation Surgical repair of female pseudo-hermaphroditism
P450c11β	Female pseudohermaphroditism Postnatal virilization in males and females	↑11-deoxycortisol and DOC before and after ACTH ↑Serum androgens and urine 17-ketosteroids Suppression of elevated steroids after glucocorticoid administration ↑ACTH and ↓PRA Hypokalemia	Glucocorticoid administration Surgical repair of female pseudohermaphroditism
P450c11AS	Failure to thrive Weakness Salt loss	Hyponatremia, hyperkalemia ↑Corticosterone ↓Aldosterone and ↑PRA	Mineralocorticoid replacement Salt supplementation
P450c17	Male pseudohermaphroditism Sexual infantilism Hypertension	↑DOC, 18-OHDOC, corticosterone, 18-hydroxycorticosterone Low 17α-hydroxylated steroids and poor response to ACTH Poor response to hCG in male pseudohermaphroditism Suppression of elevated adrenal steroids after glucocorticoid administration ↑ACTH and ↓PRA Hypokalemia	Glucocorticoid administration Surgical correction of genitalia and sex steroid replacement in male pseudohermaphroditism consonant with sex of rearing Estrogen replacement in female at 12 years Testosterone replacement if reared as male (rare)
P450 oxidoreductase	Infants with Antley–Bixler syndrome plus genital anomaly and adrenal insufficiency; maternal aromatase deficiency. Adults with infertility.	↑Prog, 17OHP, ACTH ↓DHEA, androstenedione testosterone, estradiol Poor cortisol response to ACTH Normal mineralocorticoids	Glucocorticoid, mineralocorticoid, and sex steroid replacement.

In theory, CAH is easy to understand. A genetic lesion in one of the steroidogenic enzymes interferes with normal steroidogenesis. The signs and symptoms of the disease derive from deficiency of the steroidal endproduct and the effects of accumulated steroidal precursors proximal to the blocked step. Thus, reference to the pathways in Figure 15.1 and a knowledge of the biological effects of each steroid should permit one to deduce the manifestations of the disease.

In practice, CAH can be confusing, both clinically and scientifically. The key clinical, laboratory, and therapeutic features of each form are summarized in Table 15.5. Because each steroidogenic enzyme has multiple activities and many

extra-adrenal tissues contain enzymes that have similar activities, the complete elimination of a specific adrenal enzyme may not result in the complete elimination of its steroidal products from the circulation. In the past, disorders of steroidogenic enzymes had to be studied by examining their steroid metabolites in serum and urine, an indirect approach that led to numerous misconceptions about the steroidogenic processes. The cloning of the genes for the steroidogenic enzymes has now permitted the direct study of these diseases, altering traditional views substantially.

Congenital lipoid adrenal hyperplasia

Lipoid CAH, the most severe genetic disorder of steroid hormone synthesis, is characterized by the absence of significant concentrations of all steroids, high basal ACTH, and plasma renin activity. Steroid responses to long-term treatment with high doses of ACTH or human chorionic gonadotropin (hCG) are absent. The adrenals are grossly enlarged with cholesterol and cholesterol esters [229–232]. These findings indicate a lesion in the first step in steroidogenesis, the conversion of cholesterol to pregnenolone, and have led to other names for lipoid CAH, including 20,22-desmolase deficiency and P450scc deficiency because other errors in steroidogenesis were in steroidogenic enzymes [232–237]. However, the P450scc gene is normal in these patients [237], as are the mRNAs for adrenodoxin reductase and adrenodoxin [237]. The normal P450scc system plus the accumulation of cholesterol esters in the affected adrenal suggested that the lesion lay in a factor involved in cholesterol transport to the mitochondria. After initial unsuccessful searches for this factor [237,238], the steroidogenic regulatory protein (StAR) was cloned in 1994 [46] and was quickly identified as the disordered step in lipoid CAH [47,239]. Thus, lipoid CAH was the first disorder in steroid hormone biosynthesis identified that is not caused by a disrupted steroidogenic enzyme.

Lipoid CAH provided a gene knockout of nature, elucidating the complex physiology of the StAR protein [240]. Transfection of non-steroidogenic cells with the P450scc system with and without StAR showed that StAR promotes steroidogenesis by increasing the movement of cholesterol into mitochondria but that, in the absence of StAR, steroidogenesis proceeds at about 14% of the StAR-induced level [47,48,156,239]. This observation led to the two-hit model of lipoid CAH [48]. The first hit is the loss of StAR itself, leading to a loss of most but not all steroidogenesis, with a compensatory rise in ACTH and LH. These hormones increase cellular cAMP, which increases biosynthesis of LDL receptors, their uptake of LDL-cholesterol, and *de novo* synthesis of cholesterol. In the absence of StAR, this increased intracellular cholesterol accumulates as in a storage disease causing the second hit, which is the mitochondrial and cellular damage caused by the accumulated cholesterol, cholesterol esters, and their auto-oxidation products [48].

The two-hit model explains the unusual clinical findings in lipoid CAH. In the fetal testis, which is steroidogenically very active under the trophic stimulation of hCG [241], the Leydig cells are destroyed early in fetal life, eliminating testosterone biosynthesis. An affected 46,XY fetus does not undergo normal virilization and is born with female external genitalia and a blind vaginal pouch. The Sertoli cells remain undamaged and continue to produce Müllerian inhibitory hormone, so that the phenotypically female 46,XY fetus has no cervix, uterus, or Fallopian tubes. The steroidogenically active fetal zone of the adrenal is similarly affected, eliminating most fetal adrenal DHEA biosynthesis and the fetoplacental production of estriol; mid-gestation maternal and fetal estriol levels are thus very low [242].

The definitive zone of the fetal adrenal, which differentiates into the zonae glomerulosa and fasciculata, normally produces very little aldosterone and, as fetal salt and water metabolism are maintained by the placenta, stimulation of the glomerulosa by angiotensin II generally does not begin until birth. Consistent with this, many newborns with lipoid CAH do not have a salt-wasting crisis until after several weeks of life, because StAR-independent aldosterone synthesis initially suffices, but chronic stimulation by angiotensin II eventually leads to cellular damage [48]. However, patients with lipoid CAH born in hot, dry climates tend to develop a salt-wasting crisis very early, presumably reflecting chronic compensated hypovolemia in the mother with secondary stimulation of the fetal renin–angiotensin system, so that damage to the glomerulosa begins before birth [48].

The two-hit model also explains the spontaneous feminization of affected 46,XX females who are treated in infancy and reach adolescence. The fetal ovary makes no steroids and contains no steroidogenic enzymes [241]; consequently, the ovary remains undamaged until it is first stimulated by gonadotropins at the time of puberty, when it produces some estrogen by StAR-independent steroidogenesis. Continued stimulation results in cholesterol accumulation and cellular damage, so that biosynthesis of progesterone in the latter part of the cycle is impaired. Because gonadotropin stimulation recruits individual follicles and does not promote steroidogenesis in the whole ovary, most follicles remain undamaged and available for future cycles. Cyclicity is determined by the hypothalamo-pituitary axis and remains normal. With each new cycle, a new follicle is recruited and more estradiol is produced by StAR-independent steroidogenesis. Although net ovarian steroidogenesis is impaired, enough estrogen is produced (especially in the absence of androgens) to induce breast development, general feminization, monthly estrogen withdrawal, and cyclic vaginal bleeding [48,243]. However, progesterone synthesis in the latter half of the cycle is disturbed by the accumulating cholesterol esters so that the cycles are anovulatory. Measurements of estradiol, progesterone, and gonadotropins throughout the cycle in affected adult females with lipoid CAH confirm this model [244].

Similarly, examination of StAR knockout mice confirms the two-hit model [245,246]. Thus, examination of patients with lipoid CAH has elucidated the physiology of the StAR protein in each steroidogenic tissue.

Genetic analysis of patients with lipoid CAH has revealed numerous mutations in the StAR gene [48,240,247,248]. These data reveal two distinct genetic clusters. As first reported in 1985 [232], lipoid CAH is common in Japan, and about 65–70% of affected Japanese alleles and virtually all affected Korean alleles carry the mutation Q258X [47,48,247,249]. The carrier frequency for this mutation appears to be about 1 in 300 [48,249], so that one in every 250 000–300 000 newborns in these countries is affected, giving a total of about 500 patients in Japan and Korea. A second genetic cluster is found among Palestinian Arabs, most of whom carry the mutation R182L [48].

Many other mutations have been found throughout the gene, but all amino acid replacement (missense) mutations are found in the carboxy-terminal 40% of the protein, suggesting that this is the biologically important domain [48,240]. Spectroscopic analysis of these mutant proteins indicates that they have lost activity because they are substantially misfolded [250]. Deletion of only 10 carboxy-terminal residues reduces StAR activity by half [53], and deletion of 28 carboxy-terminal residues by the common Q258X mutation eliminates all activity. In contrast, deletion of the first 62 amino-terminal residues has no effect on StAR activity, even though this deletes the entire mitochondrial leader sequence and forces StAR to remain in the cytoplasmic compartment [53]. Physical studies and partial proteolysis indicate that residues 63–193 of StAR (i.e. the domain that lacks most of the crucial residues identified by missense mutations) are protease resistant; this constitutes a "pause–transfer" sequence, which permits the bioactive loosely folded carboxy-terminal molten globule domain to have increased interaction with the outer mitochondrial membrane [55]. Studies of StAR's mitochondrial import confirm that StAR acts exclusively on the outer mitochondrial membrane and that its level of activity is proportional to the length of time it resides there [54].

Fetoplacental P450scc is needed for placental synthesis of progesterone, which is required to suppress uterine contractility and maintain pregnancy in the first trimester [251]. This observation led to the presumption that human P450scc deficiency is incompatible with term gestation. Two reports describe individuals with clinical features of late-onset lipoid CAH: one was heterozygous for an inactivating mutation in P450scc [252], and the other was a compound heterozygote but only one of the mutations was inactivating [253]. In both cases, the inactivating lesion was the result of a *de novo* mutation not found in either parent. The pathophysiology of these cases is also explained by the two-hit model [252]. As P450scc is the slowest steroidogenic enzyme, haploinsufficiency yields insufficient steroidogenesis, especially in times of stress. Insufficient cortisol and aldosterone result in hypersecretion of ACTH and angiotensin II, promoting LDL uptake to a degree beyond the adrenal capacity for steroidogenesis, resulting in lipid accumulation and eventual cell death and adrenal insufficiency [252]. As this lesion affects the gonads similarly, such individuals are infertile and only *de novo* mutations are seen.

Treatment of lipoid CAH is straightforward if the diagnosis is made. Physiological replacement with glucocorticoids, mineralocorticoids, and salt permit survival to adulthood [231,232]. The differential diagnosis includes 3β-HSD deficiency and adrenal hypoplasia congenita (AHC). The glucocorticoid requirement is less than in the virilizing adrenal hyperplasias because it is not necessary to suppress excess adrenal androgen production. Growth in these patients should be normal [232]. Genetic males have female external genitalia and should undergo orchidectomy and be raised as females [48,231,232].

3β-Hydroxysteroid dehydrogenase deficiency

3β-HSD deficiency is a rare cause of glucocorticoid and mineralocorticoid deficiency that is fatal if not diagnosed early [254]. In its classic form, genetic females have cliteromegaly and mild virilization because the fetal adrenal overproduces large amounts of DHEA, a small portion of which is converted to testosterone by extra-adrenal 3β-HSD type I. Genetic males also synthesize some androgens by peripheral conversion of adrenal and testicular DHEA, but the concentrations are insufficient for complete male genital development so that these males have a small phallus and severe hypospadias.

There are two functional human genes for 3β-HSD: the type I gene is expressed in the placenta and peripheral tissues [59,61] and the type II gene in the adrenals and gonads [255,256]. Genetic and endocrine studies of 3β-HSD deficiency show that both the gonads and the adrenals are affected as a result of a single mutated 3β-HSD-II gene that is expressed in both tissues. However, considerable hepatic 3β-HSD activity persists in the face of complete absence of adrenal and gonadal activity as a result of the enzyme encoded by the 3β-HSD-I gene, thus complicating the diagnosis of 3β-HSD deficiency. Genetic studies have identified numerous mutations causing 3β-HSD deficiency, all in the type II gene [257–261]. Mutations have never been found in 3β-HSD-I, presumably because this would prevent placental biosynthesis of progesterone, resulting in a spontaneous first-trimester abortion.

The presence of peripheral 3β-HSD activity complicates the diagnosis of this disease. Affected infants should have low concentrations of 17-OHP, but some newborns with 3β-HSD deficiency have very high concentrations of serum 17-OHP, approaching those seen in patients with classic 21-OHD [262]. These are due to extra-adrenal 3β-HSD-I. The adrenal of a patient with 3β-HSD-II deficiency will secrete

very large amounts of the principal Δ^5 compounds, pregnenolone, 17-hydroxypregnenolone, and DHEA. Some of the secreted 17-hydroxypregnenolone is converted to 17-OHP by 3β-HSD-I. This 17-OHP is not effectively picked up by the adrenal for subsequent conversion to cortisol because the circulating concentrations are below the K_m of P450c21. The ratio of the Δ^5 to the Δ^4 compounds remains high, consistent with the adrenal and gonadal deficiency of 3β-HSD [262]. Thus, the principal diagnostic test in 3β-HSD deficiency is intravenous administration of ACTH with measurement of the three Δ^5 compounds and their corresponding Δ^4 compounds.

Mild or "partial" defects of adrenal 3β-HSD activity have been reported on the basis of ratios of Δ^5 steroids to Δ^4 steroids after an ACTH test that exceed 2 or 3 SD (standard deviations) above the mean. The patients are typically young girls with premature adrenarche or young women with a history of premature adrenarche and complaints of hirsutism, virilism, and oligomenorrhea [263–265]. The 3β-HSD-II genes are normal in these patients, and even patients with mild 3β-HSD-II mutations have ratios of Δ^5 to Δ^4 steroids that exceed 8 SD above the mean [260,266–268]. The basis of the mildly elevated ratios of Δ^5 to Δ^4 steroids in these hirsute individuals with normal 3β-HSD genes is unknown. These patients prove that hormonal studies alone may be insufficient to make a diagnosis of a specific form of CAH. In adult women, the hirsutism can be ameliorated and regular menses restored by suppressing ACTH with 0.25 mg of dexamethasone given orally each day, but such treatment is contraindicated in girls who have not yet reached final height.

17α-Hydroxylase/17,20-lyase deficiency

P450c17 is a single enzyme that has both 17α-hydroxylase and 17,20-lyase activities. Deficient 17α-hydroxylase activity and deficient 17,20-lyase activity have been described as separate genetic diseases, but it is now clear that they represent different clinical manifestations of different lesions in the same gene. P450c17 deficiency is fairly rare, although over 120 cases have been reported on clinical grounds [269,270]. A recent study showed that this disorder is surprisingly common in Brazil, due to genetic founder effects, with the mutation R362C predominating among individuals of Portuguese ancestry and W406R predominating among those of Spanish descent [271]. Deficient 17α-hydroxylase activity results in decreased cortisol synthesis, overproduction of ACTH, and stimulation of the steps proximal to P450c17. The patients may have mild symptoms of glucocorticoid deficiency, but this is not life-threatening as the lack of P450c17 results in the overproduction of corticosterone, which also has glucocorticoid activity. This is similar to the situation in rodents, the adrenals of which lack P450c17 [272] and consequently produce corticosterone as their glucocorticoid. Affected patients overproduce DOC in the zona fasciculata, which causes sodium retention, hypertension, and hypokalemia and also suppresses plasma renin activity and aldosterone secretion from the zona glomerulosa. When P450c17 deficiency is treated with glucocorticoids, DOC secretion is suppressed, and plasma renin activity and aldosterone concentrations rise to normal [270, 273].

The absence of 17α-hydroxylase and 17,20-lyase activities in complete P450c17 deficiency prevents the synthesis of adrenal and gonadal sex steroids. As a result, affected females are phenotypically normal but fail to undergo adrenarche and puberty [274]; genetic males have absent or incomplete development of the external genitalia [275]. The classic presentation is a teenage female with sexual infantilism and hypertension. The diagnosis is made by finding low or absent 17-hydroxylated C_{21} and C_{19} plasma steroids and low urinary 17-hydroxycorticosteroids and 17-KS, which respond poorly to stimulation with ACTH. Serum levels of DOC, corticosterone, and 18-hydroxy-corticosterone are elevated, show hyper-responsiveness to ACTH, and are suppressible with glucocorticoids.

The single gene for P450c17 has been cloned [72] and localized to chromosome 10q24.3 [70,71]. The molecular basis of 17α-hydroxylase deficiency has been determined in several patients by cloning and sequencing of the mutated gene, identifying nearly 40 distinct mutations [270,271].

Selective deficiency of the 17,20-lyase activity P450c17 has been reported in about a dozen cases [269], which initially led to the incorrect conclusion that 17α-hydroxylase and 17,20-lyase are separate enzymes. One of the original patients had two wholly inactivating mutations [276], which led to a corrected diagnosis of the patient as having complete 17α-hydroxylase deficiency [277]. Thus, because both 17α-hydroxylase and 17,20-lyase activities of P450c17 are catalyzed by the same active site, it was not clear that a syndrome of isolated 17,20-lyase deficiency could exist until two patients with genital ambiguity, normal excretion of 17-hydroxycorticosteroids, and markedly reduced production of C_{19} steroids were studied [278]. One was homozygous for the P450c17 mutation R347H and the other homozygous for R358Q. Both mutations changed the distribution of surface charges in the redox partner binding site of P450c17 [278]. When expressed in transfected cells, both mutants retained nearly normal 17α-hydroxylase activity but had no detectable 17,20-lyase activity [278,279]. Enzymatic competition experiments proved that the mutations did not affect the substrate binding site [279]. When an excess of both P450 oxidoreductase and cytochrome b_5 was provided, some 17,20-lyase activity was restored, demonstrating that the loss of lyase activity was caused by impaired electron transfer [279]. The diagnosis of isolated 17,20 lyase deficiency is difficult, requiring accurate hormonal assays and sophisticated cell biology approaches [280]. Four additional well-characterized patients have been reported, three carrying mutations in R347 or R358 [281] and are carrying the novel active-site mutation E305 G [282].

A detailed computer graphic model of P450c17 has been built and confirmed by computational, enzymatic, and mutagenesis approaches [283]. This model accurately predicts the effects of all known mutations, including those with partial retention of both activities and those causing selective 17,20-lyase deficiency. The model identifies both Arg-347 and Arg-358 and several other arginine and lysine residues in the redox partner binding site; mutations of these residues all cause varying degrees of selective loss of 17,20-lyase activity [276,277,283,284]. Another example of the critical nature of redox partner interactions comes from the sole reported case of cytochrome b_5 deficiency; this patient was a male pseudohermaphrodite but was not evaluated hormonally [285]. The central role of electron transfer in 17,20-lyase activity is now well established.

21-Hydroxylase deficiency

21-OHD results from mutations in the gene encoding adrenal P450c21. It is one of the most common inborn errors of metabolism and accounts for about 95% of CAH cases. Because of success in diagnosis and treatment in infancy, many patients with severe forms of 21-OHD have reached adulthood, so management issues in CAH concern physicians dealing with all age groups. Detailed reviews of the complex physiology and molecular genetics of this disorder have appeared [82–84,286–287], and a Consensus Statement on its management has been endorsed by the world's leading Pediatric Endocrine societies [288].

Pathophysiology

For patients with a complete absence of P450c21, the clinical manifestations can be deduced from Figure 15.1. Inability to convert progesterone to DOC results in aldosterone deficiency causing severe hyponatremia (Na^+ often below 110 mmol/L), hyperkalemia (K^+ often above 10 mmol/L), and acidosis (pH often below 7.1) with concomitant hypotension, shock, cardiovascular collapse, and death. As the control of fluids and electrolytes in the fetus can be maintained by the placenta and the mother's kidneys, the salt-losing crisis develops only after birth, usually during the second week of life.

The inability to convert 17-OHP to 11-deoxycortisol results in cortisol deficiency, which impairs postnatal carbohydrate metabolism and exacerbates cardiovascular collapse because a permissive action of cortisol is required for full pressor action of catecholamines. Although a role for cortisol in fetal physiology is not established [141], cortisol deficiency is also manifested prenatally. Low fetal cortisol stimulates ACTH secretion, which stimulates adrenal hyperplasia and transcription of the genes for all the steroidogenic enzymes, especially for P450scc, the rate-limiting enzyme in steroidogenesis. This increased transcription increases enzyme production and activity, with consequent accumulation of non-21-hydroxylated steroids, especially 17-OHP. As the pathways in Figure 15.1 indicate, these steroids are converted to testosterone.

In the male fetus, the testes produce large amounts of mRNA for the steroidogenic enzymes, and concentrations of testosterone are high in early to mid-gestation [241]. This testosterone differentiates external male genitalia from the pluripotential embryonic precursor structures. In the male fetus with 21-OHD, the additional testosterone produced in the adrenals has little if any phenotypic effect. In a female fetus, the ovaries lack steroidogenic enzyme mRNAs and are quiescent [241]; no sex steroids or other factors are needed for differentiation of the female external genitalia [289]. The testosterone inappropriately produced by the adrenals of the affected female fetus causes varying degrees of virilization of the external genitalia. This can range from mild cliteromegaly, with or without posterior fusion of the labioscrotal folds, to complete labioscrotal fusion that includes a urethra traversing the enlarged clitoris (Fig. 15.8). These infants have normal ovaries, Fallopian tubes, and a uterus but have ambiguous external genitalia or may be sufficiently virilized that they appear to be male, resulting in errors of sex assignment at birth.

The diagnosis of 21-OHD is suggested by genital ambiguity in females, a salt-losing episode in either sex, or rapid growth and virilization in males. Plasma 17-OHP is markedly elevated and hyper-responsive to stimulation with ACTH (Fig. 15.7). Measurement of 11-deoxycortisol, 17-OHP, DHEA, and androstenedione is important to distinguish from other forms of CAH and because adrenal or testicular tumors can also produce 17-OHP [290]. High newborn 17-OHP values that rise further after ACTH can also be seen in 3β-HSD and P450c11 deficiencies [260]. 17-OHP is normally high in cord blood but falls to normal newborn levels after 12–24 h (Fig. 15.9) so that assessment of 17-OHP should not be made in the first 24 h of life. Premature infants and term infants under severe stress (e.g. with cardiac or pulmonary disease) may have persistently elevated 17-OHP concentrations with normal 21-hydroxylase.

Clinical forms of 21-OHD

The broad spectrum of clinical manifestations of 21-OHD depends on the mutations of the P450c21 alleles. The different forms are not different diseases but a spectrum of manifestations, ranging from severe salt wasting to clinically unapparent forms that may be normal variants. Thus, the disease forms described are mainly for clinical convenience.

Salt-wasting 21-OHD

Salt wasting is due to a complete deficiency of P450c21 activity, effectively eliminating both glucocorticoid and mineralocorticoid synthesis. Females are frequently diagnosed at birth

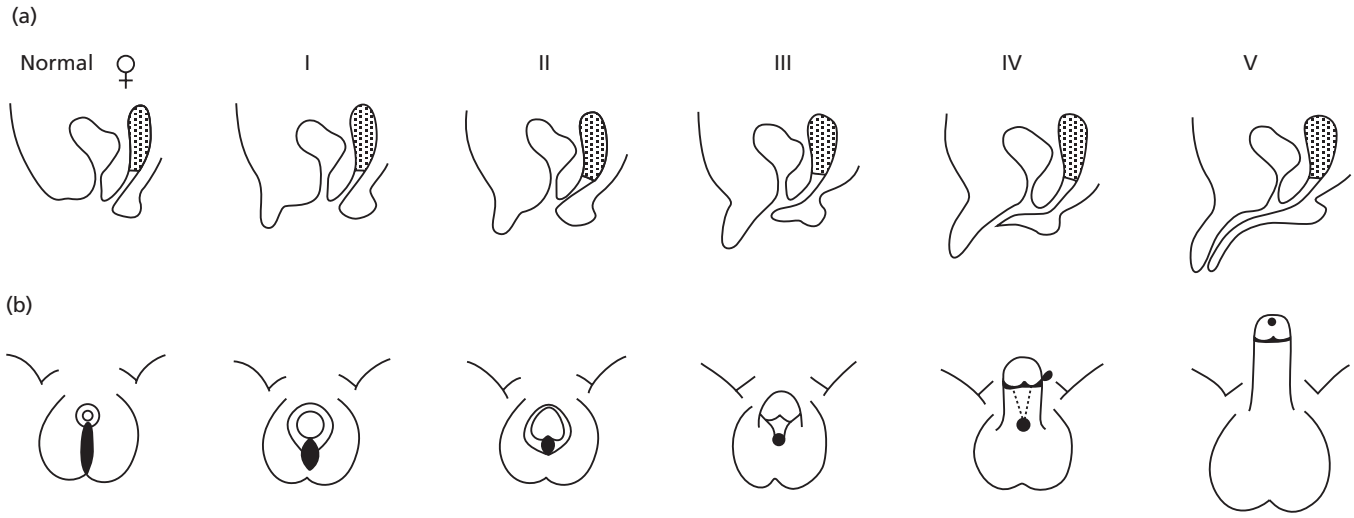

Fig. 15.8. Virilization of the external genitalia. A continuous spectrum is shown from normal female to normal male in both sagittal section (a) and perineal views (b), using the staging system of Prader. Disorders of external genitalia can occur either by the virilization of a normal female, as in congenital adrenal hyperplasia, or because of an error in testosterone synthesis in the male. In females with congenital adrenal hyperplasia due to 21-OHD, the degree of virilization correlates poorly with the presence or absence of clinical signs of salt loss.

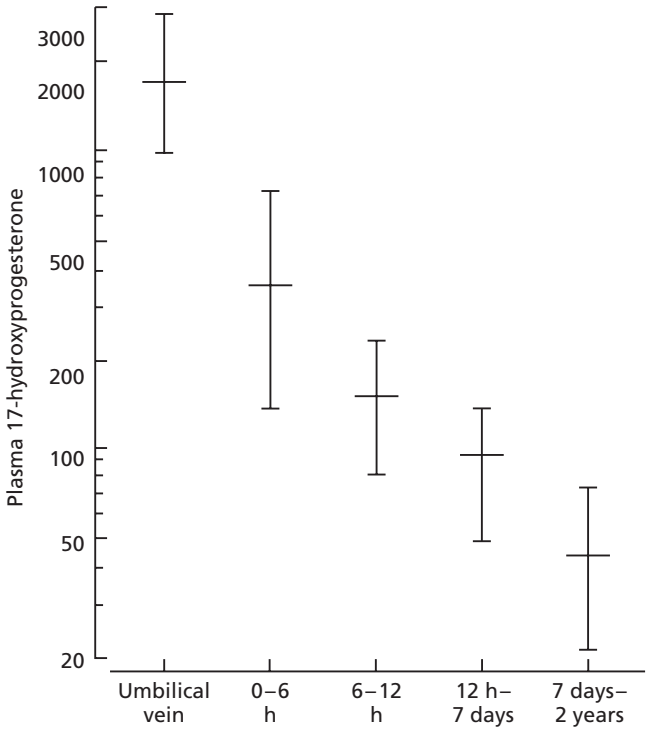

Fig. 15.9. Means and ranges of 17-OHP in normal newborns (ng/100 mL). Note that values can be very high and quite variable for the first 24 h of life.

because of masculinization of the external genitalia. After appropriate resuscitation of the cardiovascular collapse, acidosis, and electrolyte disorders, the mineralocorticoids and glucocorticoids can be replaced orally, and the ambiguous genitalia can be corrected with a series of plastic surgical procedures. The management of steroid replacement is difficult because of the rapidly changing needs of a growing infant or child. Drug doses must be adjusted frequently, and there is considerable individual variability in what constitutes physiological replacement. As underdosage of glucorticoids can be life-threatening, especially during illness, most pediatricians have tended to err on the safe side, so children have received inappropriately large doses. It is not possible to compensate for growth lost during the first 2 years of life, when it is fastest, so these children almost always end up short. Female survivors may have sexual dysfunction, marry with a low frequency, and have decreased fertility [291–295]. Males are not generally diagnosed at birth, and they come to medical attention either during the salt-losing crisis that follows 5–15 days later, or they die, invariably having been diagnosed incorrectly.

Simple virilizing 21-OHD

Virilized females with elevated concentrations of 17-OHP but who do not suffer a salt-losing crisis have long been recognized as having the simple virilizing form of CAH. The existence of this clinical variant first led to the incorrect belief that there were distinct 21-hydroxylases in the zona glomerulosa and in the zona fasciculata. Males often escape diagnosis until age 3–7 years, when they develop pubic, axillary, and facial hair and phallic growth. The testes remain of prepubertal size in CAH, whereas gonadotrophic stimulation in true precocious puberty results in pubertal-sized testes. The children grow rapidly and are tall for age when diagnosed, but their bone age advances at a disproportionately rapid rate so that adult height is compromised.

Untreated or poorly treated children may fail to undergo normal puberty, and boys may have small testes and azoospermia because of the feedback effects of the adrenally produced testosterone. When treatment is begun at several years of age, suppression of adrenal testosterone secretion may remove tonic inhibition of the hypothalamus, occasionally resulting in true central precocious puberty requiring treatment with a gonadotropin-releasing hormone (GnRH) agonist. High concentrations of ACTH in some poorly treated boys may stimulate enlargement of adrenal rests in the testes. These enlarged testes are usually nodular, unlike the homogeneously enlarged testes in central precocious puberty. Because the adrenal normally produces 100–1000 times as much cortisol as aldosterone, mild defects (amino acid replacement mutations) in P450c21 are less likely to affect mineralocorticoid than cortisol secretion. Thus, patients with simple virilizing CAH simply have a less severe disorder of P450c21. This is reflected by increased plasma renin activity seen after moderate salt restriction.

Non-classic 21-OHD

Many people have very mild forms of 21-OHD. These may be evidenced by mild to moderate hirsutism, virilism, menstrual irregularities, and decreased fertility in adult women (so-called late-onset CAH) [296–298], but there may be no phenotypic manifestations at all, other than an increased response of plasma 17-OHP to an intravenous ACTH test (so-called cryptic CAH) [299]. Despite the minimal manifestations of this disorder, these individuals have hormonal evidence of a mild impairment in mineralocorticoid secretion, as predicted from the existence of a single adrenal 21-hydroxylase [300].

There has been considerable debate about how to classify patients, principally because each diagnostic category represents a picture in a spectrum of disease resulting from a spectrum of lesions in the P450c21 gene. Furthermore, because many different mutant P450c21 alleles are common in the general population, most patients are compound heterozygotes, carrying a different mutation in the alleles inherited from each parent. Finally, many factors other than the specific mutations found in P450c21 influence the clinical phenotype, including the presence of extra-adrenal 21-hydroxylases (other than P450c21), undiagnosed P450c21 promoter mutations, and variations in androgen sensitivity. Discordances between genotype and phenotype are to be expected.

Incidence of 21-OHD

Perinatal screening for elevated concentrations of serum 17-OHP in several countries yielded an incidence of 1 in 14 000 for salt-wasting and simple virilizing CAH and 1 in 60 for heterozygous carriers [301]. This calculation has now been confirmed through the screening of 1.9 million newborns in Texas [302]. The overall incidence was 1 in 16 000 and, because of the large numbers involved, an ethnic breakdown was possible showing an incidence of 1 in 15 600 Caucasians, 1 in 14 500 Hispanics (primarily Mexican Americans of indigenous American ancestry), and 1 in 42 300 African-Americans [302]. Because about 20% of the African-American gene pool is of European descent, the calculated incidence in individuals of wholly African ancestry is about 1 in 250 000.

Non-classic 21-OHD is much more common, but the data vary from 1 in 27 for Ashkenazi Jews, 1 in 53 for Hispanics, 1 in 63 for Yugoslavs, 1 in 333 for Italians to 1 in 1000 for other whites [303–305]. This indicates that one-third of Ashkenazi Jews, one-quarter of Hispanics, one-fifth of Yugoslavs, one-ninth of Italians, and one-fourteenth of other Caucasians are heterozygous carriers. However, carrier rates of 1.2% [292] to 6% [292,295] for Caucasian populations that were not subdivided further have been recorded. These differences reflect the small populations examined, the restricted and geographic localities involved, and the errors that arise when hormonal data are used to distinguish individuals with non-classic CAH from heterozygous carriers of classic CAH. This error can be ameliorated by careful measurement of 17-OHP before and after stimulation with ACTH (Fig. 15.7) [83].

In homozygotes for both classic and non-classic CAH, serum concentrations of 21-deoxycortisol rise in response to ACTH, but ACTH-induced 21-deoxycortisol remains normal in heterozygotes for both classic and non-classic CAH [308]. However, these studies have classified individuals by hormonal phenotype without examining the P450c21 genes directly to establish these incidences. Therefore, the diagnosis of non-classic CAH requires family studies, as the hormonal data (17-OHP responses to ACTH) in these individuals may be indistinguishable from those for unaffected heterozygous carriers of the more severe forms. The high incidence, lack of mortality, and lack of decreased fertility in most individuals with non-classic CAH indicate that this is probably a variant of normal and not a disease in the classic sense. Nevertheless, patients may seek help for virilism and menstrual disorders.

Genetics of the 21-hydroxylase locus

21-Hydroxylase genes

There are two 21-hydroxylase loci, containing a functional gene (formerly termed *CYP21A2*) and a non-functional pseudogene (formerly termed *CYP21A1P*) [309]. These genes, P450c21B (functional gene) and P450c21A (pseudogene), are duplicated in tandem with the *C4A* and *C4B* genes encoding the fourth component of serum complement [310,311] (Fig. 15.10). Although the P450c21A locus is transcribed into two alternately spliced, adrenal-specific mRNAs, neither encodes a protein [312]: only the P450c21B gene encodes adrenal 21-hydroxylase. The P450c21 genes consist of 10 exons, are about 3.4 kb long, and differ in only 87 or 88 of these bases [86–88]. This high degree of sequence similarity indicates that the two genes are evolving in tandem through intergenic exchange of

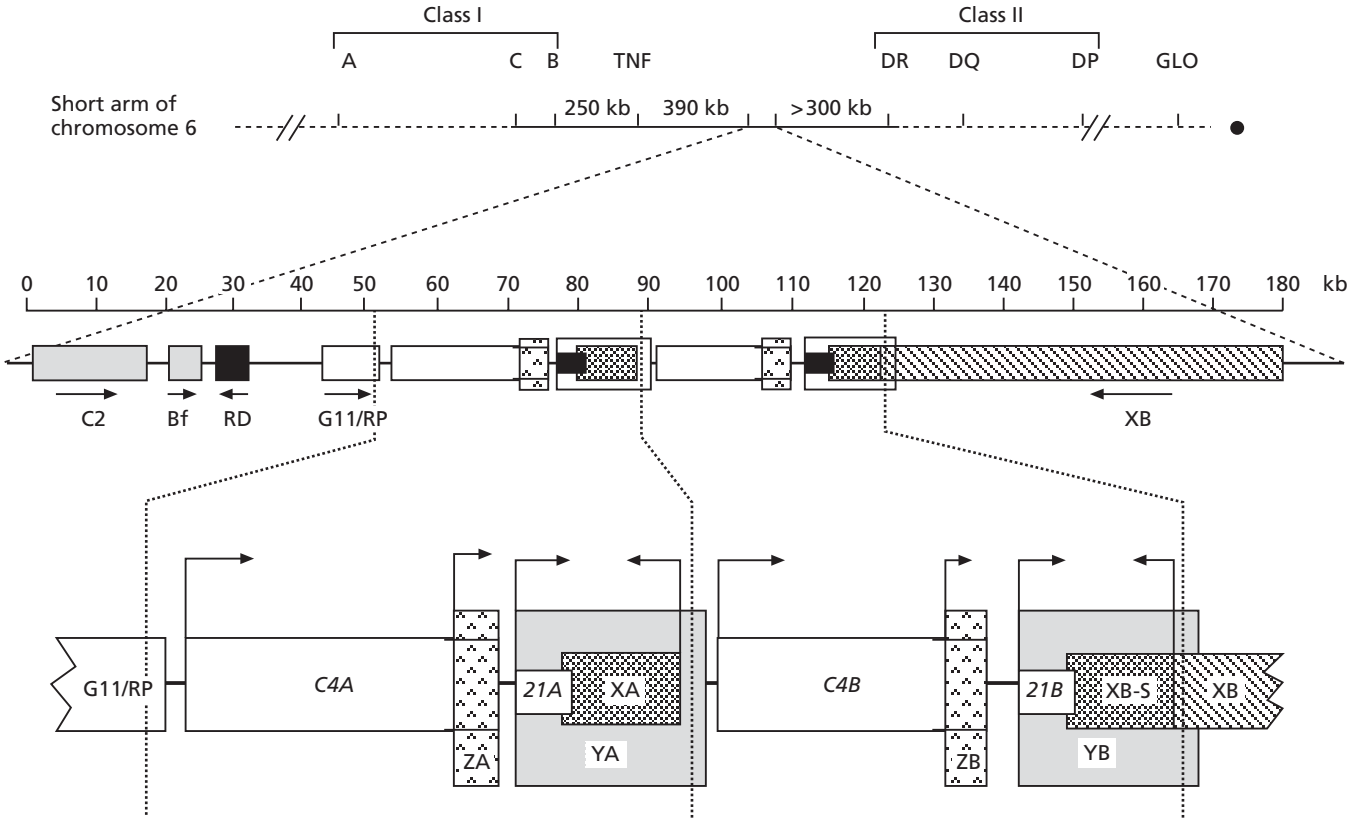

Fig. 15.10. Genetic map of the HLA locus containing the genes for P450c21. The top line shows the p21.1 region of chromosome 6, with the telomere to the left and the centromere to the right. Most HLA genes are found in the class I and class II regions; the class III region containing the P450c21 genes lies between these two. The second line shows the scale (in kilobases) for the diagram immediately below, showing (from left to right) the genes for complement factor C2, properedin factor Bf, and the RD and G11/RP genes of unknown function; arrows indicate transcriptional orientation. The bottom line shows the 21-hydroxylase locus on an expanded scale, including the C4A and C4B genes for the fourth component of complement, the inactive *CYP21A* gene (21A), and the active *CYP21B* gene (21B) that encodes P450c21. XA, YA, and YB are adrenal-specific transcripts that lack open reading frames. The *XB* gene encodes the extracellular matrix protein tenascin-X; *XB-S* encodes a truncated adrenal-specific form of the tenascin-X protein whose function is unknown. ZA and ZB are adrenal-specific transcripts that arise within the *C4* genes and have open reading frames, but it is not known whether they are translated into protein; however, the promoter elements of these transcripts are essential components of the CYP21A and CYP21B promoters. The arrows indicate transcriptional orientation. The vertical dotted lines designate the boundaries of the genetic duplication event that led to the presence of A and B regions.

DNA. The P450c21 genes of mice [313] and cattle [314,315] are also duplicated and linked to leukocyte antigen loci, but only P450c21B functions in humans, only P450c21A functions in mice [316,317], but both function in cattle [318]. Sequencing of the gene duplication boundaries shows that the human locus, duplicated after mammalian speciation [319], is consistent with data indicating that other mammals have single P450c21 gene copies [320].

HLA linkage

The 21-hydroxylase genes lie within the class III region of the human major histocompatibility complex (MHC) (Fig. 15.10). The P450c21 locus lies about 600 kb from HLA-B and about 400 kb from HLA-DR. HLA typing has been widely used for prenatal diagnosis and to identify heterozygous family members. Statistical associations (linkage disequilibrium) are well established between CAH and certain specific HLA types. Salt-losing CAH is associated with HLA-B60 and HLA-40 in some populations [321], and the rare HLA type Bw47 is very strongly associated with salt-losing CAH [322,323]. HLA-Bw51 is often associated with simple virilizing CAH in some populations [324], and 30–50% of haplotypes for non-classic CAH carry HLA-B14 [325]. HLA-B14 is often associated with a duplication of the *C4B* gene [326,327]. In contrast, all HLA-B alleles can be found linked to CAH. HLA-identical individuals in a single family may have different clinical features of 21-OHD despite HLA identity [328–331], possibly representing extra-adrenal 21-hydroxylation, *de novo* mutations, or multiple genetic crossover events. In one family with clinically non-concordant HLA-identical siblings, Southern blotting studies showed a within-generation genetic rearrangement [330].

C4 genes

The tandemly duplicated *C4A* and *C4B* loci produce proteins that can be distinguished functionally and immunologically; the C4B protein has substantially more hemolytic activity, despite greater than 99% sequence identity with C4A [332,333]. The *C4A* gene is always 22 kb long, but there are long (22 kb) and short (16 kb) forms of *C4B* because of a variation in one intron [334,335]. The 3′ ends of the *C4* genes are only 2466 bp upstream from the transcriptional start sites of the P450c21 genes. Promoter sequences needed for the transcription of the human P450c21B gene lie within intron 35 of the *C4B* gene [336] and also initiate the transcription of the "Z transcripts," which have an open reading frame but whose translational status is unclear [337].

Other genes in the 21-hydroxylase locus

In addition to the P450c21 and C4 genes, there are numerous other genes within 100 kb of the P450c21 gene (Fig. 15.10). The genes for complement factor C2 and properdin factor Bf lie 80–100 kb 5′ to the P450c21 gene and have the same transcriptional orientation (i.e. lie on the same strand of DNA). Lying just 3′ of the Bf gene is the RD gene, so called because it encodes a protein with a long stretch of alternating arginine (R) and aspartic acid (D) residues [338]. This gene lies on the opposite strand of DNA from the complement and P450c21 genes and is expressed in all tissues, but its function is unknown [339]. Thirteen other putative genes have been identified lying between the gene for TNF and C4A, but no functions have been ascribed to them [340].

A pair of genes, XA and XB, is duplicated with the C4 and P450c21 genes. These lie on the strand of DNA opposite the C4 and P450c21 genes and overlap the 3′ end of P450c21. The last exon of XA and XB lies within the 3′ untranslated region of exon 10 in P450c21A and P450c21B respectively [338]. Although the human XA locus was truncated during the duplication of the ancestral C4–P450c21–X genetic unit, the XA gene is abundantly transcribed in the adult and fetal adrenal [341]. In contrast, the XB gene encodes a large extracellular matrix protein (tenascin X) that is expressed in a wide variety of adult and fetal tissues, especially connective tissue [342,343]. The XB gene spans about 65 kb of DNA and includes 43 exons encoding a 12-kb mRNA [342,344]. The XB gene also encodes a short truncated form of tenascin X with unknown function and arising from an intragenic promoter [345].

Identification of a patient with a "contiguous gene syndrome" comprising a deletion of both the P450c21B and XB genes demonstrated that deficiency of tenascin X results in Ehlers–Danlos syndrome (EDS) [346]. EDS from tenascin X deficiency is autosomal recessive and typically more severe than the common dominant form of EDS caused by mutations in collagen V [347]. Haploinsufficiency of tenascin X is associated with joint hypermobility [348]. Studies in cattle indicate that tenascin X is associated with and stabilizes collagen fibrils [349], thus explaining the related phenotype of mutations in tenascin X and collagen-associated genes. Although the various transcripts from the XA and XB genes are complementary to the mRNA for P450c21 and the other transcripts that arise from the P450c21 promoters [312], these RNAs do not form RNA/RNA duplexes *in vivo* [350] and hence do not regulate P450c21.

P450c21 gene lesions causing 21-OHD

21-OHD can be caused by P450c21B gene deletions, gene conversions, and apparent point mutations. Most of the point mutations in the P450c21B gene are actually small gene conversion events [82,84,351], so that gene conversions account for about 85% of the lesions in 21-OHD. The P450c21 genes are autosomal; hence, each person has two alleles, one contributed by each parent. Most patients with 21-OHD are compound heterozygotes, having different lesions on their two alleles. Because gene deletions and large conversions eliminate all P450c21B gene transcription, these lesions cause salt-losing 21-OHD in the homozygous state. Some microconversions, such as those creating premature translational termination, are also associated with salt-losing CAH. Milder forms (simple virilizing and non-classic 21-OHD) are associated with amino acid replacements in the P450c21 protein caused by gene microconversion events. Patients with these forms of CAH are usually compound heterozygotes bearing a severely disordered allele and a mildly disordered allele so that the clinical manifestations are based on the nature of the mildly disordered allele.

Mapping of P450c21 genes in normal subjects and in 21-OHD

Although the P450c21B and P450c21A loci differ by only 87 or 88 nucleotides, they can be distinguished by restriction endonuclease digestion and Southern blotting. The P450c21A locus is characterized by 3.2- and 2.4-kb *Taq*I fragments and 12-kb *Bgl*II fragments, whereas the functional P450c21B gene is characterized by 3.7- and 2.5-kb *Taq*I fragments and 11-kb *Bgl*II fragments. Two unusual and related features of the 21-hydroxylase locus complicate its analysis. First, the gene deletions in this locus are most unusual in that they extend 30 kb from one of several points in the middle of P450c21A to the precisely homologous point in P450c21B. Thus, the 15% of alleles that carry deletions do not yield a typical Southern blotting pattern with a band that is a different size from that of the normal, unless one uses very rare cutting enzymes and analyses the resulting large DNA fragments by pulsed-field gel electrophoresis. The second unusual feature of this locus is that gene conversions are extremely common [352,353].

Gene conversions

If a segment of gene A replaces the corresponding segment of the related gene B, the structure of recipient gene B is said

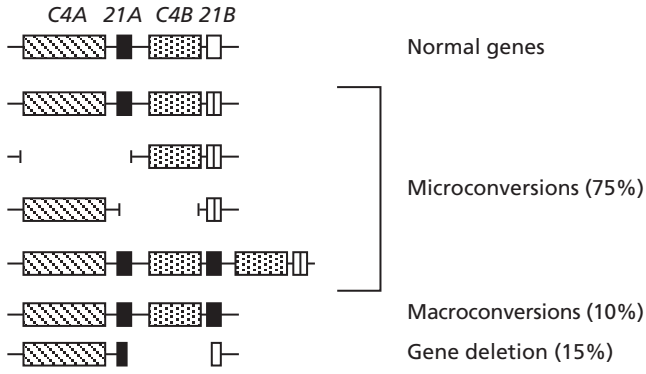

Fig. 15.11. Classes of genetic rearrangements causing 21-OHD. Deletions or duplications of the *C4A* and *C4B* genes can occur with or without associated lesions in the P450c21B gene. Note that all "point mutations" in P450c21B are actually "microconversion." Many authors combine the "gene deletion" and "macroconversion" groups because these are difficult to distinguish by Southern blotting, as both result in a loss of the P450c21B gene, but the genotypes are clearly distinct, as shown.

Table 15.6. Microconversions of the P450c21B gene that cause 21-hydroxylase deficiency.

Mutation	Location	Associated phenotypes	Activity
Pro-30→Leu	Exon 1	NC/SV	30–60%
A→G	Intron 2	SV/SW	Minimal
8-bp deletion	Exon 3	SW	0
Ile-172→Asn	Exon 4	SV	3–7%
Ile-236→Asp			
Val-237→Glu	Exon 6	SW	0
Met-239→Lys			
Val-281→Leu	Exon 7	NC	18 ± 9%
Gly-292→Ser	Exon 7	SW	
T insertion at 306	Exon 7	SW	0
Gly-318→Stop	Exon 8	SW	0
Arg-339→His	Exon 8	NC	20–50%
Arg-356→Trp	Exon 8	SV/SW	2%
Pro-453→Ser	Exon 10	NC	20–50%
GG→C at 484	Exon 10	SW	0

to be "converted" to that of donor gene A. The hallmark of gene conversion is that the number of closely related genes remains constant, but their diversity decreases. Two types of gene conversions commonly cause 21-OHD, large gene conversions that can be mistaken for gene deletions and small microconversions that resemble point mutations. When a large gene conversion causes 21-OHD, the *Taq*I digestion pattern of the P450c21B gene is converted to that of the P450c21A. This conversion changes the 5′ end of the P450c21B sequence to the 5′ end of P450c21A.

The relative frequency of large gene conversions compared with gene deletions in 21-OHD was formerly controversial, principally because initial studies used relatively small groups of patients from single locations or ethnic groups. A study of 68 French patients showed that 12.5% of the mutant alleles had large gene conversions, 12.5% had gene deletions, and 75% had microconversions [327]. A compilation of the world literature on the genetics of 21-OHD found that 19% of mutant alleles had gene deletions, 8% large gene conversions, 67% microconversions, and 6% uncharacterized lesions [84] (Fig. 15.11). Such statistics must be viewed with caution because there is considerable ascertainment bias in favor of the more severely affected patients [324] and because some studies excluded mildly affected patients. Thus, the above statistics are weighted in favor of gene deletions and large conversions, which can only yield a phenotype of salt-wasting 21-OHD.

Point mutations (microconversions) causing 21-OHD

About 75% of mutated P450c21 genes appear to be structurally intact by Southern blotting and thus appear to carry point mutations [327,351]. Many mutant P450c21B genes causing 21-OHD have been cloned and sequenced (Table 15.6), revealing that a relatively small number of mutations cause the condition, virtually all of which are also found in the P450c21A pseudogene. These observations indicate that most CAH alleles bearing apparent point mutations actually carry microconversions [82,84,351].

Effects of known point mutations on 21-hydroxylase activity

There are three changes in the P450c21A pseudogene that render its product non-functional. Each results in an altered reading frame and/or premature stop codon, hence eliminating all activity; all of these, the C→T transition at codon 318, the 8-bp deletion in exon 3, and the T insertion in exon 7, have been found in P450c21B alleles that cause severe salt-losing 21-OHD. Three closely clustered base changes alter the normal amino acid sequence Ile–Val–Glu–Met at codons 236–239 in exon 6 to Asn–Glu–Glu–Lys in both P450c21A and in a small number of genes causing severe salt-losing 21-OHD. There is no assayable 21-hydroxylase activity when this sequence is expressed *in vitro*.

The most common lesion in classic 21-OHD is an A→G change in the second intron, 13 bases upstream from the normal 3′ splice acceptor site of this intron, a microconversion found in over 25% of severely affected alleles. This intronic mutation causes abnormal splicing of the mRNA precursor, destroying activity. However, a small portion of this mRNA may be spliced normally in some patients so that the phenotypic presentation is variable; most such patients are salt losers, but some are not salt losing. This intron 2 microconversion is often associated with the Ser/Thr polymorphism at codon 268; this is a true polymorphism as S268T does not alter enzymatic activity [354]. The microconversion R356W, which is found in about 10% of severely affected alleles [355],

eliminates all detectable activity [356], apparently because it changes a residue in the binding site for P450 oxidoreductase [283]. This mutation may retain slight activity and has been found in simple virilizing cases. Other, extremely rare mutations have been described in single individuals [357–359].

Missense mutations causing simple virilizing 21-OHD

The microconversion I172N is the most common cause of simple virilizing 21-OHD [356,360,361]. Ile-172 is conserved in the other known mammalian P450c21 genes and may contribute to the hydrophobic interactions needed to maintain the correct conformation of the enzyme. When Ile-172 was changed to Asn, Leu, Gln, or His and the constructed mutants were expressed in mammalian cells, the mutant constructions yielded only 3–7% of the 21-hydroxylase activity of normal P450c21 [356,362]. The intron 2 microconversion is occasionally seen in simple virilizing cases. The microconversion P30L is generally associated with non-classic 21-OHD but is found in some patients with the simple virilizing form.

Missense mutations causing non-classic 21-OHD

The most common mutation causing non-classic 21-OHD is V281L. This microconversion is seen in all patients with the non-classic form linked to HLA-B14 and HLA-DR1, but is also found in patients with other HLA types. This mutation does not alter the affinity of the enzyme for substrate but drastically reduces its V_{max} [363]. The microconversion P30L is found in about 15–20% of non-classic alleles. In addition, the mutations R339H and P453S have been associated with the non-classic form [364,365]. Initial surveys of the mutations in P450c21A failed to reveal these mutations, suggesting that they are bona fide point mutations rather than gene microconversions. Examination of large numbers of P450c21A pseudogenes shows that at least the P453S mutation is polymorphic in about 20% of P450c21A pseudogenes, and hence also represents a microconversion event.

Structure–function inferences from P450c21 mutations

Each P450c21 missense mutation appears to occur in a functional domain of P450c21. By analogy with the membrane-anchoring domain of hepatic P450IIB, amino acids 167–178 of P450c21, including the crucial Ile-172 residue, appear to constitute a similar domain [366]. By analogy with the computationally inferred structure of the closely related enzyme P450c17 [283], Arg-356 may be part of the redox partner binding site, Val-281 appears to participate in co-ordinating the heme moiety, and Cys-428 is the crucial cystine residue in the heme binding site found in all cytochrome P450 enzymes. All these mutations can arise by gene microconversions. The N-terminal region of P450c21, including Pro-30, appears to be required for membrane insertion and enzyme stability [366]. Finding most mutations in the amino-terminal portion of P450c21 is consistent with finding most gene conversion and gene deletion events occurring in exons 1–8 of the P450c21B gene. Changes in exons 9 and 10 are very rare, possibly as a result of evolutionary pressure to retain the 3′ untranslated and 3′ flanking DNA of the P450c21B gene, as this DNA also contains the 3′ end of the XB gene [341,342].

Prenatal diagnosis and treatment of 21-OHD

The prenatal diagnosis and therapy of 21-OHD are being actively pursued, but prenatal therapy remains experimental and controversial [287,288,367–370]. The fetal adrenal is active in steroidogenesis from early in gestation, so a diagnosis can be made by amniocentesis and measurement of amniotic fluid 17-OHP [371–373]. Concentrations of Δ^4 androstenedione are also elevated in the amniotic fluid of fetuses with 21-OHD, providing a potentially useful adjunctive assay [374]. However, amniotic fluid concentrations of 17-OHP and Δ^4 androstenedione are reliable only for identifying fetuses affected with severe salt-losing 21-OHD, because these steroids may not be elevated above the broad range of normal in the non-salt-losing or non-classic forms [375,378].

If a fetus is known to be at risk because the parents are known heterozygotes, 21-OHD can be diagnosed by HLA typing of fetal amniocytes or by analysis of fetal amniocyte DNA. If the fetus has the same HLA type as the previously affected child, the fetus will be affected; a fetus that shares one parent's HLA type with the index case will be a heterozygous carrier, and a fetus having both haplotypes differing from the index case will be unaffected. HLA typing of cultured amniocytes requires previous linkage analysis of the affected child and parents. Only HLA-A and HLA-B can be reliably determined in cultured amniocytes, although some HLA-B alleles are expressed weakly in amniocytes. There is a relatively high incidence of HLA-B homozygosity among 21-OHD patients. HLA-B loci are frequently identical between parents and patients, some HLA-B antigens may cross-react, and amniocytes may not express HLA-DR antigens, further limiting the usefulness of HLA typing.

Experimental prenatal treatment requires early and accurate prenatal diagnosis. Female fetuses affected with 21-OHD begin to become virilized at about 6–8 weeks' gestation at the same time that a normal male fetal testis produces large amounts of testosterone, causing fusion of the labioscrotal folds, enlargement of the genital tubercle into a phallus, and the formation of the phallic urethra [289]. The adrenals of affected female fetuses can produce concentrations of testosterone that may approach those in a normal male, resulting in varying degrees of masculinization of the external genitalia. If fetal adrenal steroidogenesis is suppressed in an affected fetus, the virilization can be reduced or eliminated. Several studies have reported the successful application of this approach by administering dexamethasone to the mother as soon as pregnancy is diagnosed [377–381]. This can be done only when the parents are known to be heterozygotes

by having already had an affected child. However, even in such pregnancies, only one in four fetuses will have CAH. Furthermore, as no prenatal treatment is needed for male fetuses affected with CAH, only one in eight pregnancies of heterozygous parents would harbor an affected female fetus that might potentially benefit from prenatal treatment, and seven would have been treated unnecessarily.

The efficacy, safety, and desirability of such prenatal treatment remain highly controversial [84,146,287,367–370,373,380–383]. It is not known precisely when the fetal hypothalamus begins to produce CRH, when the fetal pituitary begins to produce ACTH, whether all fetal ACTH production is regulated by CRH, or whether these hormones are suppressible by dexamethasone in the early fetus. Although there is considerable evidence that pharmacological doses of glucocorticoids do not harm pregnant women, no such data exist for the fetus. Pregnant women with diseases such as nephrotic syndrome and systemic lupus erythematosus are generally treated with prednisone, which does not reach the fetus because it is inactivated by placental 11β-HSD. Treatment of a fetus requires the use of fluorinated steroids that escape metabolism by these enzymes, and few data are available about the long-term use of such agents throughout gestation. The available preliminary studies indicate that the response of the fetal genital anatomy to treatment is generally good if the treatment is started very early (before week 6); thereafter, the virilization is reduced but may not be eliminated, so that at least one reconstructive surgical procedure may still be needed in the infant [146,372–384].

Successful treatment requires dexamethasone doses of 20 μg/kg maternal body weight. For a 70-kg woman, this is 1.4 mg, which is equivalent to that in the low-dose dexamethasone suppression test. As the physiological replacement dose of dexamethasone is less than 0.2 mg/m^2 body surface area [385], this dose is three to six times the physiological replacement dose. The fetus normally develops in the presence of very low cortisol concentrations (less than 100 nmol/L, 3.6 μg/dL) [143], i.e. about 10% of the corresponding maternal level. Thus, the doses used in prenatal treatment appear to achieve effective concentrations of active glucocorticoid that may be up to 60 times physiological for the fetus. Treatment of pregnant rats with 20 μg/kg dexamethasone predisposes the fetuses to hypertension in adulthood [386], and some studies indicate that even moderately elevated concentrations of glucocorticoids can be neurotoxic [387–391]. Thus, prenatal treatment of CAH remains an experimental and controversial therapy that should be done only in research centers. Follow-up studies of very long duration are needed to evaluate its effects fully, especially on the seven fetuses treated unnecessarily.

Diagnosis

The key diagnostic maneuver in all forms of 21-OHD is the measurement of the 17-OHP response to intravenous synthetic ACTH. Individual patient responses must be compared with age- and sex-matched data from normal children [220] (Table 15.4 and Fig. 15.7). Other ancillary tests are listed in Table 15.5.

Plasma renin activity and its response to salt restriction constitute an especially useful test. Most patients with simple virilizing 21-OHD have high plasma renin activity, which increases further on sodium restriction, confirming that these patients are partially mineralocorticoid deficient and can maintain a normal serum sodium only by hyperstimulation of the zona glomerulosa. Mineralocorticoid therapy in these patients returns plasma volume to normal and eliminates the hypovolemic drive to ACTH secretion. Thus, mineralocorticoid therapy often permits the use of lower doses of glucocorticoids in patients with simple virilizing CAH, optimizing growth in children, and diminishing unwanted weight gain in adults.

Long-term management is difficult and requires clinical and laboratory evaluation. Growth should be measured at 3- to 4-month intervals, along with an annual assessment of bone age. Each visit should be accompanied by measurement of urinary 17-KS and serum Δ^4 androstenedione, DHEA, DHEAS, and testosterone. Measurement of 3α-androstenediol glucuronide may also be useful. In general, plasma 17-OHP is a suboptimal indicator of therapeutic efficacy because of its great diurnal variation and hyper-responsiveness to stress (e.g. clinic visits).

Treatment

Although Wilkins *et al*. [7] and Bartter *et al*. [8] first demonstrated effective treatment of 21-OHD with cortisone in 1950, the management of this disorder remains difficult. Overtreatment with glucocorticoids causes delayed growth, even when the degree of overtreatment is insufficient to produce signs and symptoms of Cushing syndrome. Undertreatment results in continued overproduction of adrenal androgens, which hastens epiphyseal maturation and closure, again resulting in compromised growth and other manifestations of androgen excess.

Doses of glucocorticoids should be based on the expected normal cortisol secretory rate. Widely cited classic studies have reported that the secretory rate of cortisol is 12.5 ± 3 mg/m^2/day [392–394] and have led most authorities to recommend doses of 10–20 mg/m^2/day hydrocortisone (cortisol). However, the cortisol secretory rate is actually substantially lower, at 6–7 ± 2 mg/m^2/day [214,215]. Newly diagnosed patients, especially newborns, do require substantially higher initial dosages to suppress their hyperactive CRH/ACTH/adrenal axis: simple physiological replacement is usually insufficient to suppress adrenal androgens.

The glucocorticoid used is important. Most tables of glucocorticoid dose equivalences are based on their equivalence in anti-inflammatory assays. However, the growth-suppressant equivalences of various glucocorticoids do not parallel their

Table 15.7. Potency of various therapeutic steroids (set relative to the potency of cortisol).

Steroid	Anti-inflammatory glucocorticoid effect	Growth-retarding glucocorticoid effect	Salt-retaining mineralocorticoid effect	Plasma half-life (min)	Biological half-life (h)
Cortisol (hydrocortisone)	1.0	1.0	1.0	80–120	8
Cortisone acetate (oral)	0.8	0.8	0.8	80–120	8
Cortisone acetate (IM)	0.8	1.3	0.8		18
Prednisone	3.5–4	5	0.8	200	16–36
Prednisolone	4		0.8	120–300	16–36
Methyl prednisolone	5	7.5	0.5		
Betamethasone	25–30		0	130–330	
Triamcinolone	5		0		
Dexamethasone	30	80	0	150–300	36–54
9α-Fluorocortisone	15		200		
DOC acetate	0		20		
Aldosterone	0.3		200–1000		

IM, intramuscularly.

anti-inflammatory equivalences [395]. Thus, long-acting synthetic steroids such as dexamethasone have a disproportionately greater growth-suppressant effect and must be avoided when treating growing children and adolescents (Table 15.7). Most authorities favor the use of oral hydrocortisone or cortisone acetate in three divided daily doses in growing children. However, adults and older teenagers who have already fused their epiphyses may be managed very effectively with prednisone or dexamethasone.

Only one oral mineralocorticoid preparation, fludrocortisone (9α-fluorocortisol), is generally available. It must be given as crushed tablets, not as a suspension, which delivers the medication unreliably. When the oral route is not available in severely ill patients, mineralocorticoid replacement is achieved through intravenous hydrocortisone plus sodium chloride. Hydrocortisone (20 mg) has a mineralocorticoid effect of about 100 μg of 9α-fluorocortisol (Table 15.7). Mineralocorticoids are unique in pharmacology in that their doses are not based on body mass or surface area. In fact, newborns are quite resistant to mineralocorticoids, as reflected by their high serum aldosterone concentrations (Fig. 15.12), and require larger doses (100–200 μg/day) than do adults. In older children, the replacement dose of 9α-fluorocortisol is 50–150 μg daily. A mineralocorticoid is useless unless adequate sodium is presented to the renal tubules. Thus, additional salt supplementation, usually 1–2 g of NaCl per day in the newborn, is also needed. Patients with severe salt-losing CAH can sometimes discontinue mineralocorticoid replacement and salt supplementation as adults. They certainly need lower doses, possibly because they become more sensitive to the mineralocorticoid action of hydrocortisone via a developmental decrease in renal 11β-HSD activity, which normally inactivates cortisol to cortisone.

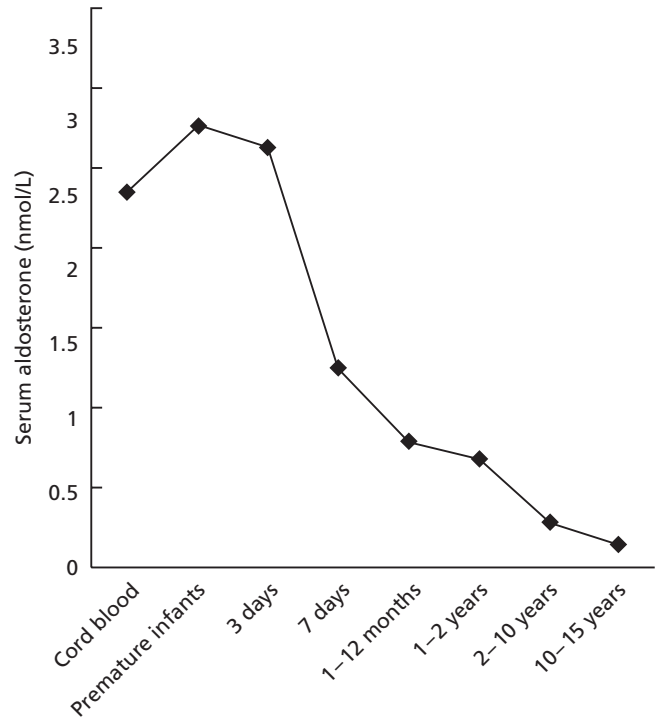

Fig. 15.12. Concentrations of aldosterone as a function of age.

P450 oxidoreductase deficiency – a disorder affecting multiple P450 enzymes

Beginning with a report in 1985 [396], several patients have been described with clinical and hormonal findings suggesting partial deficiencies of both 17α-hydroxylase and 21-hydroxylase. Some of these individuals were born to mothers

who had become virilized during pregnancy, suggesting fetal–placental aromatase deficiency, and many also had the Autley–Bixler congenital malformation syndrome characterized by craniosynostosis and radioulnar synostosis [397]. Although mutations of P450 oxidoreductase were suggested as the cause for this disorder when the first case was reported [398], the P450 oxidoreductase gene was not examined until recently. About half of patients with Antley–Bixler syndrome have normal steroidogenesis and normal genitalia; these patients have mutations in the gene for fibroblast growth factor receptor 2 (FGFR2) [397]. However, patients with Antley–Bixler syndrome who also have genital anomalies and disordered steroidogenesis do not have FGFR2 mutations [397]. Three such patients with Antley–Bixler syndrome, genital ambiguity, and hormonal findings suggesting partial deficiencies of 17α-hydroxylase and 21-hydroxylase, and a fourth patient who was phenotypically normal but had a similar hormonal profile, were found to have missense (amino acid replacement) mutations in P450 oxidoreductase [81]. One of these patients was born to a woman who had become virilized during the pregnancy, suggesting partial feto-placental aromatase deficiency. Four biochemical assays of the mutant proteins created in microorganisms showed that the mutations in the Antley–Bixler patients had severely impaired, but not totally absent, activity, whereas the mutations found in the phenotypically normal person were less severe [81]. It is unlikely that patients will be found who are homozygous for mutations that destroy all activity, as knockout of P450 oxidoreductase in mice causes embryonic lethality [79,80]. As P450 oxidoreductase is required for the activities of all hepatic drug-metabolizing P450 enzymes, it is likely that such patients will also have abnormal drug metabolism. While this issue has not yet been studied directly, reports of Antley–Bixler syndrome in some infants of mothers who ingested fluconazole (an antifungal agent that interferes with the fungal P450 catalyzing lanosterol-14-demethylase activity) suggest that defective drug metabolism due to defective P450 oxidoreductase may result in abnormal metabolism of otherwise benign drugs, thus rendering them teratogenic [81].

Lesions in isozymes of P450c11: 11β-hydroxylase deficiency, corticosterone methyl oxidase deficiency, and glucocorticoid-suppressible hypertension

There are two distinct forms of 11-hydroxylase. P450c11β mediates the 11β-hydroxylation of 11-deoxycortisol to cortisol and that of DOC to corticosterone in the zonae fasciculata and reticularis. P450c11AS, aldosterone synthase, is found only in the zona glomerulosa and mediates 11β-hydroxylation, 18-hydroxylation, and 18-oxidation; thus, it is the sole enzyme required to convert DOC to aldosterone. Deficient P450c11β activity is a rare cause of CAH in persons of European ancestry but accounts for about 15% of cases in both Moslem and Jewish Middle Eastern populations [399]. Severe deficiency of P450c11β decreases the secretion of cortisol, causing CAH and virilization of affected females. However, because one of the steroids that accumulates in P450c11β deficiency, DOC, is a mineralocorticoid, these patients can retain sodium.

Although DOC is less potent than aldosterone, it is secreted at high levels in 11β-hydroxylase deficiency, so that salt is retained and the serum sodium remains normal. Overproduction of DOC frequently leads to hypertension; as a result, 11β-hydroxylase deficiency is often termed "the hypertensive form of CAH." However, newborns often manifest mild, transient salt loss [399,400], presumably as a result of the normal newborn resistance to mineralocorticoids (Fig. 15.12); this may lead to incorrect diagnosis and treatment. Thus, there may be a poor correlation between DOC concentrations, serum potassium, and blood pressure or between the degree of virilization in affected females and the electrolyte and cardiovascular manifestations [83]. The diagnosis is established by demonstrating elevated basal concentrations of DOC and 11-deoxycortisol, which hyperrespond to ACTH; a normal or suppressed plasma renin activity is also a hallmark of this disease [401].

The genetic lesions causing 11β-hydroxylase deficiency are in the *CYP11B1* gene that encodes P450c11β. In a study of Sephardic Jews of Moroccan ancestry, 11 of 12 affected alleles bore the mutation R448H [99], but at least two frameshifts, four premature stop codons, and five amino acid replacement mutations have also been described in other populations [92]. A milder, non-classic form of 11β-hydroxylase deficiency, analogous to non-classic 21-OHD, has been reported in otherwise asymptomatic women with hirsutism, virilism, and menstrual irregularities [399,402]. However, true non-classic 11β-hydroxylase deficiency is rare; only two of five hyperandrogenemic women who had 11-deoxycortisol values more than three times higher than the 95th percentile in response to stimulation with ACTH had mutations of P450c11β, all of which retained 15–37% of normal activity [403]. Repeated ACTH testing in two of the three women who lacked mutations showed much lower (but still elevated) 11-deoxycortisol values. Thus, just as in the case of non-classic 3β-HSD deficiency, an abnormal steroid response to ACTH is not sufficient to diagnose a genetic lesion.

P450c11AS, the isozyme of P450c11β that is 93% identical in its amino acid sequence, is expressed exclusively in the zona glomerulosa, where it catalyzes 11β-hydroxylase, 18-hydroxylase, and 18-methyl oxidase activities. Both P450c11AS and P450c11β are expressed in the human zona glomerulosa, and both can convert DOC to corticosterone, but the conversion of corticosterone to 18-hydroxycorticosterone and subsequently to aldosterone is performed exclusively by P450c11AS. Disorders of P450c11AS cause the so-called corticosterone methyl oxidase (CMO) deficiencies,

in which aldosterone biosynthesis is impaired while the zonae fasciculata and reticularis continue to produce corticosterone and DOC. The absence of aldosterone biosynthesis will generally result in a salt-wasting crisis in infancy, at which time the normal secretory rate of DOC is insufficient to meet the newborn's mineralocorticoid requirements (similarly to the newborn with P450c11β deficiency).

These infants typically present with hyponatremia, hyperkalemia, and metabolic acidosis, but the salt-wasting syndrome is typically less severe than in patients with 21-OHD or lipoid CAH because of the persistent secretion of DOC. The patients may recover spontaneously and grow to adulthood without therapy. This probably reflects the increasing sensitivity to mineralocorticoid action with advancing age in childhood, as reflected by the usual age-related decrease in serum aldosterone (Fig. 15.12). Consistent with this, plasma renin activity is markedly elevated in affected children but may be normal in affected adults [404].

CMOI deficiency results from a complete loss of P450c11AS activity so that no 18-hydroxylase or 18-methyl oxidase activity persists, eliminating the biosynthesis of 18OH-corticosterone and aldosterone while preserving the biosynthesis of corticosterone by P450c11β. Thus, the diagnosis for CMOI deficiency is usually based on an increased ratio of corticosterone to 18OH-corticosterone [101]. Only three cases of CMOI deficiency have been fully characterized genetically, including a frameshift mutation [405], a premature stop codon [406], and the missense mutation R384P [407].

CMOII deficiency results from amino acid replacement mutations in P450c11AS that selectively delete the 18-methyl oxidase activity while preserving the 18-hydroxylase activity. The diagnosis of CMOII deficiency requires an increased 18OH-corticosterone and very low aldosterone concentration. CMOII deficiency is common in Sephardic Jews of Iranian origin, where all affected individuals appear to be homozygous for two different mutations, R181W and V385A [100]. Family members who were homozygous for only one of these mutations were clinically unaffected; both mutations are required to cause disease.

The distinction between CMOI and CMOII is not always clear. One patient with the clinical history and hormonal phenotype of CMOII had two mutations on each parental allele [102]. The mother's allele carried R181W and a deletion/frameshift mutation that deleted all activity; the father's allele carried T318M and V386A. Thr-318 is predicted to be the universally conserved Thr residue in all P450 enzymes that participates in cleavage of the dioxygen bond of O_2 to create the iron–oxy intermediate required for P450 catalysis. When the T318M/V386A double mutant was recreated *in vitro* there was no detectable activity [102]. These data would have predicted the CMOI phenotype instead of the patient's CMOII phenotype.

Another patient was homozygous for the missense mutations E198A and V386A. When recreated *in vitro*, the double mutant enzyme behaved similarly to the mutant enzyme found in the Iranian Jewish CMOII patients, but the patient's clinical phenotype was CMOI [408]. This patient also carried R173K, which is a normally occurring polymorphism that has no affect on the enzyme's K_m or V_{max} [409]. Thus, the distinction between CMOI and CMOII is not precise, and these disorders should be regarded as different degrees of severity on a clinical spectrum, just as the various forms of 21-OHD.

Rats have four *CYP11B* genes encoding three P450c11 enzymes, but there are only two *CYP11B* genes in the human genome, encoding P450c11β and P450c11AS [410]. This genetic anatomy is reminiscent of the P450c21A and P450c21B genes. Although gene conversion can cause CMOII deficiency [411], gene conversion appears to be much rarer than in the P450c21 locus. This may be due to the higher recombinational frequency in the HLA region carrying the P450c21 genes, or it may be related to the abundant antisense transcripts produced in the P450c21 locus [312,350].

Although gene conversion events in the P450c11 locus are rare, an unusual gene duplication causes glucocorticoid-suppressible hyperaldosteronism [412–414]. This homologous recombination event creates a third P450c11 gene, which fuses the 5′ flanking DNA of the P450c11β gene on to the gene for P450c11AS. In two such patients, the genetic crossover occurred in intron 2, and in intron 3 or exon 4 in two other patients [413]. All these hybrid genes produce a hybrid P450c11 that retains aldosterone synthase activity; however, as the hybrid gene has P450c11β regulatory regions, its transcription is induced by ACTH and cAMP, as is the normal P450c11β gene. Thus, these patients make P450c11AS in response to physiology that should stimulate P450c11β. The excess P450c11AS causes hyperaldosteronism and hypertension, which is then suppressible by glucocorticoid suppression of ACTH, which normally suppresses P450c11β.

It is conceivable that localized microconversions, similar to those that cause most cases of 21-OHD, could insert sequences crucial for aldosterone synthase activity into the P450c11β gene. Expression of chimeric proteins produced *in vitro* identified the residues Ser-288 and Val-320 as important for this activity [415]. Similarly, activating mutations that increase the aldosterone synthase activity of P450c11AS have been created *in vitro* [416]. However, examination of large numbers of patients with low-renin hypertension has failed to show mutations in this gene system, other than the gene conversions that cause glucocorticoid-suppressible hypertension [409–418].

Adrenal insufficiency

Besides CAH, many other conditions cause adrenal insufficiency, including ACTH deficiency and primary adrenal disorders. Primary adrenal insufficiency is commonly termed Addison disease, a vague term that encompasses many

Table 15.8. Causes of adrenal insufficiency.

Primary adrenal insufficiency
Autoimmune adrenalitis
Autoimmune polyglandular syndromes (types I and II)
Tuberculosis, fungal infections
Sepsis
AIDS
Congenital adrenal hyperplasia
Adrenal hemorrhage or infarction
Congenital adrenal hypoplasia
Adrenoleukodystrophy
Primary xanthomatosis
Unresponsiveness to ACTH
Secondary adrenal insufficiency
Withdrawal from glucocorticoid therapy
Hypopituitarism
Hypothalamic tumors
Irradiation of the CNS

Table 15.9. Signs and symptoms of adrenal insufficiency.

Features shared by acute and chronic insufficiency
Anorexia
Apathy and confusion
Dehydration
Fatigue
Hyperkalemia
Hypoglycemia
Hyponatremia
Hypovolemia and tachycardia
Nausea and vomiting
Postural hypotension
Salt craving
Weakness
Features of acute insufficiency (adrenal crisis)
Abdominal pain
Fever
Features of chronic insufficiency (Addison disease)
Decreased pubic and axillary hair
Diarrhea
Hyperpigmentation
Low-voltage electrocardiogram
Small heart on X-ray
Weight loss

disorders (Table 15.8). Up to World War II, most patients with "Addison disease" had tuberculosis of the adrenal, but over 80% of contemporary adult patients have autoimmune adrenalitis, and the term *Addison disease* is now widely used to indicate an autoimmune or idiopathic cause.

Chronic primary adrenal insufficiency

Autoimmune adrenalitis

Autoimmune adrenalitis is most commonly seen in 25- to 45-year-old adults, about 70% of whom are women. The incidence in adults is about 1 in 25 000. The incidence in children is unknown but much less. Boys constitute about 75% of patients [419]. Chronic adrenal insufficiency is suggested by poor weight gain or weight loss, weakness, fatigue, anorexia, hypotension, hyponatremia, hypochloremia, hyperkalemia, frequent illnesses, nausea, and vague gastrointestinal complaints (Table 15.9), reflecting chronic deficiency of both glucocorticoids and mineralocorticoids. Early in the course of autoimmune adrenalitis, one may see signs of glucocorticoid deficiency (weakness, fatigue, weight loss, hypoglycemia, anorexia) without signs of mineralocorticoid deficiency [hyponatremia, hyperkalemia, acidosis, tachycardia, hypotension, low voltage on electrocardiogram (ECG), small heart on chest X-ray] or evidence of mineralocorticoid deficiency without glucocorticoid deficiency. Thus, an initial clinical presentation that spares one category of adrenal steroids does not mean it will be spared in the long run. The symptoms listed in Table 15.9 can be seen in chronic adrenal insufficiency that is either primary or secondary.

In primary chronic adrenal insufficiency, the low concentrations of plasma cortisol stimulate the hypersecretion of ACTH and other POMC peptides, including the various forms of melanocyte-stimulating hormone (MSH), which is characterized by hyperpigmentation of the skin and mucous membranes. Such hyperpigmentation is most prominent in skin exposed to sun and in extensor surfaces such as knees, elbows, and knuckles. The diagnosis is suggested by the signs and symptoms, verified by a low morning cortisol level with a high ACTH, and confirmed by a minimal response of cortisol to a 60-min intravenous ACTH test. Associated findings may include the appearance of a small heart on chest X-ray, anemia, azotemia, eosinophilia, lymphocytosis, and hypoglycemia. Treatment consists of physiological glucocorticoid and mineralocorticoid replacement therapy.

The diagnosis of an autoimmune cause is based on finding circulating antiadrenal antibodies. In many cases, the adrenal antigens are the steroidogenic P450 enzymes, especially P450c21 [420,421]. It is not clear how these enzymes reach immune cells to elicit an antibody response, or whether there are clinical correlations with the spectrum of which enzymes become the antigens giving rise to a cytotoxic immune response. Autopsy studies show lymphocytic infiltration of the adrenal cortex. Thus, it is likely that the primary process is initiated by T lymphocytes, and that the antibodies to steroidogenic P450 enzymes are secondary markers, analogous to the antibodies to insulin and glutamic acid decarboxylase seen in type 1 diabetes mellitus.

Autoimmune dysfunction of other endocrine tissues is frequently associated with autoimmune adrenalitis [422]. Approximately half of adult patients with lymphocytic

adrenalitis also have disease of another endocrine system and high titers of antibodies specific to the affected tissues. The term Schmidt syndrome refers to the relatively common association of thyroiditis and/or diabetes mellitus with autoimmune adrenal insufficiency [423]. This disease triad, which is seen mainly in adults, is sometimes termed type 2 autoimmune polyglandular syndrome and is linked to HLA-DR3 and HLA-DR4 [424]. In older children and adults, primary ovarian failure (but not primary testicular failure) is seen in about one-quarter of patients with primary lymphocytic autoimmune adrenalitis.

Hypoparathyroidism, pernicious anemia, and chronic mucocutaneous candidiasis are often seen without adrenalitis in girls [422] but, when adrenalitis is also present, boys and girls are affected equally. This associated group of disorders is more common in children than in adults and is sometimes termed type 1 autoimmune polyglandular syndrome. It may also include atrophic gastritis, hypergonadotrophic hypogonadism, chronic active hepatitis, alopecia, or vitiligo. Unlike type 2 autoimmune polyglandular syndrome, a specific HLA association has not been found. This disorder is caused by mutations in a gene called AIRE (autoimmune regulator), which is widely expressed in developing immune tissues, but the mechanistic link to the various findings in type 1 autoimmune polyglandular syndrome is not clear [425,426].

Metabolic causes

Metabolic disorders cause chronic primary adrenal insufficiency, including adrenoleukodystrophy (Schilder disease), primary xanthomatosis (Wolman disease), cholesterol ester storage disease, hereditary unresponsiveness to ACTH, and adrenal hypoplasia congenita.

Adrenoleukodystrophy is caused by mutations in a gene on chromosome Xq28a termed ALDP, but the mechanism by which these mutations cause the disease is unclear [427,428]. This X-linked disorder is seen almost exclusively in males, although a rare severe infantile autosomal-recessive form also occurs. The disease is characterized by high ratios of C_{26} to C_{22} very-long-chain fatty acids in plasma and tissues, permitting diagnosis of carriers and affected fetuses as well as individual patients [429]. Symptoms commonly develop in mid-childhood, but a variant of the disorder, adrenomyeloneuropathy, presents in early adulthood [430]. Both adrenoleukodystrophy and adrenomyeloneuropathy are caused by mutations in the gene for ALDP. The same mutation causes both forms of the disease, so it is likely that other genetic loci are also involved [431].

Earliest findings are associated with the central nervous system leukodystrophy and include behavioral changes, poor school performance, dysarthria, and poor memory, progressing to severe dementia. Symptoms of adrenal insufficiency usually appear after symptoms of white-matter disease, but adrenal insufficiency may be the initial finding in some children [432]. In contrast, adrenomyeloneuropathy begins with adrenal insufficiency in childhood and adolescence, and signs of neurological disease follow 10–15 years later. Dietary therapy with so-called Lorenzo's oil has not been effective [431].

Wolman disease and cholesterol ester storage disease appear to be two allelic variants in the secreted form of lysosomal acid lipase (cholesterol esterase) that mobilizes cholesterol esters from adrenal lipid droplets [433]. The gene for this enzyme on chromosome 10q has been cloned, and the mutations in it causing one case of Wolman disease have been identified [434]. Because insufficient free cholesterol is available to P450scc, there is adrenal insufficiency. The disease is less severe than congenital lipoid adrenal hyperplasia with respect to steroidogenesis, and patients may survive for several months after birth. However, the disease affects all cells, not just steroidogenic cells, as all cells must store and utilize cholesterol; hence, the disorder is relentless and fatal.

Vomiting, steatorrhea, failure to thrive, hepatosplenomegaly, and adrenal calcification are the usual presenting findings. The diagnosis is established by bone marrow aspiration yielding foam cells containing large lysosomal vacuoles engorged with cholesterol esters, and is confirmed by finding absent cholesterol esterase activity in fibroblasts, leukocytes, or marrow cells. Cholesterol ester storage disease appears to be a milder defect in the same enzyme, generally presenting in childhood or adolescence among the 10 reported cases.

Hereditary unresponsiveness to ACTH (familial glucocorticoid deficiency) can present as an acute adrenal crisis precipitated by an intercurrent illness in an infant or with the signs and symptoms of chronic adrenal insufficiency in childhood [223]. Unlike patients with autoimmune adrenalitis or other forms of destruction of adrenal tissue, patients with hereditary unresponsiveness to ACTH continue to produce mineralocorticoids normally because production of aldosterone by the adrenal zona glomerulosa is regulated principally by the renin–angiotensin system. Thus, the presenting picture consists of failure to thrive, lethargy, pallor, hyperpigmentation, delayed milestones, and hypoglycemia (often associated with seizures), but serum electrolytes are normal, and dehydration is seen only as part of the precipitating intercurrent illness. The disorder is transmitted as an autosomal-recessive trait. Some but not all affected patients have mutations of the gene encoding the ACTH receptor (melanocortin 2 receptor, MC2R) on chromosome 18p11, indicating the heterogeneous nature of the defect [435,436].

Triple A (Allgrove) syndrome is a rare disorder consisting of ACTH-resistant adrenal (glucocorticoid) deficiency, achalasia of the cardia, and alacrima [437]. The disorder appears to be autosomal dominant and resembles ACTH resistance, but mutations in the ACTH receptor have been excluded [223]. Many patients also have progressive neurological symptoms, including intellectual impairment, sensorineural

deafness, peripheral neuropathies, and autonomic dysfunction [223,438]. The disorder is caused by mutations in a gene called ALADIN, which encodes a WD-repeat protein, the precise function of which remains unclear [439,440].

Adrenal hypoplasia congenita (AHC, congenital adrenal hypoplasia) generally affects males because the principal form is caused by mutations of the DAX-1 gene on chromosome Xp21 [441]. This gene encodes a nuclear transcription factor that participates at various steps in the differentiation of adrenal and gonadal tissues, as well as in gonadotropin expression, so that successfully treated children may not enter puberty [442]. In this disorder, the definitive zone of the fetal adrenal does not develop, and the fetal zone is vacuolated and cytomegalic. Poor function of the fetal zone results in low maternal estriol concentrations during pregnancy, but parturition is normal. Neonatal glucocorticoid and mineralocorticoid deficiencies manifest with a typical salt-wasting crisis and respond well to replacement therapy. Deletions of the *DAX-1* gene may also encompass adjacent genes, causing glycerol kinase deficiency, Duchenne muscular dystrophy, and mental retardation [441,442]. Genetic 46,XY males with adrenal hypoplasia have normal male external genitalia but, in 46,XX females, the distinction between adrenal hypoplasia congenita and congenital lipoid adrenal hyperplasia cannot be made hormonally and requires imaging of the adrenals, which are small in adrenal hypoplasia and large in lipoid CAH. A much rarer miniature form has autosomal-recessive inheritance, but the gene responsible has not been identified.

Other causes

Chronic adrenal insufficiency may result from causes other than these. Adrenal hypoplasia, hemorrhage, and infections, all discussed below as causes of acute primary adrenal insufficiency, may spare some adrenal tissue, leaving severely compromised, rather than totally absent, adrenal function. The result, as with autoimmune adrenalitis, is a chronic disorder with insidious onset of the broad range of non-specific findings described above. Tuberculosis, fungal infections, and amyloidosis may cause a similar clinical picture.

Acute primary adrenal insufficiency

Acute adrenal crisis occurs most commonly in the child with undiagnosed chronic adrenal insufficiency who is subjected to an additional stress such as major illness, trauma, or surgery. The major presenting symptoms and signs include abdominal pain, fever, hypoglycemia with seizures, weakness, apathy, nausea, vomiting, anorexia, hyponatremia, hypochloremia, acidemia, hyperkalemia, hypotension, shock, cardiovascular collapse, and death. Treatment consists of fluid and electrolyte resuscitation, ample doses of glucocorticoids, chronic glucocorticoid and mineralocorticoid replacement, and treatment of the precipitating illness.

Massive adrenal hemorrhage with shock due to blood loss can occur in large infants who have had a traumatic delivery [443]. A flank mass is usually palpable and can be distinguished from renal vein thrombosis by microscopic rather than gross hematuria. The diagnosis is confirmed by computed tomography or ultrasonography [444]. Massive adrenal hemorrhage is more commonly associated with meningococcemia (Waterhouse–Fridrichsen syndrome). Meningitis is often, but not always, present. The characteristic petechial rash of meningococcemia can progress rapidly to large ecchymoses; the blood pressure drops and respirations become labored, frequently leading rapidly to coma and death. Immediate intervention with intravenous fluids, antibiotics, and glucocorticoids is not always successful. A similar adrenal crisis may also occur rarely with septicemia from *Streptococcus*, *Pneumococcus*, or diphtheria.

Secondary adrenal insufficiency

Chronic adrenal insufficiency may result from insufficient trophic stimulation of the adrenal and tissue insensitivity to adrenal steroids. Insufficient trophic stimulation of the adrenal can be due to idiopathic hypopituitarism, central nervous system tumors that damage the cells producing CRH and/or POMC, or chronic suppression of these cells by long-term glucocorticoid therapy.

Idiopathic hypopituitarism (multiple anterior pituitary hormone deficiency) is a hypothalamic rather than a pituitary disorder. The deficient secretion of growth hormone, gonadotropins, thyroid-stimulating hormone, and ACTH is due to insufficient stimulation of the pituitary by the corresponding hypothalamic hormones. Isolated growth hormone deficiency, a common disorder, and isolated ACTH deficiency, a rare disorder, are variants of this theme. In hypopituitarism from most causes, growth hormone secretion is generally lost first, followed in order by gonadotropins, thyroid-stimulating hormone (TSH), and ACTH. Combined deficiency of growth hormone and ACTH will strongly predispose the patient to hypoglycemia, as both hormones act to raise plasma glucose. Patients with ACTH deficiency, either with or without deficiency of other anterior pituitary hormones, have a relatively mild form of adrenal insufficiency. Mineralocorticoid secretion is normal, whereas cortisol secretion is reduced but not absent. However, adrenal reserve is severely compromised by the chronic understimulation of biosynthesis of the steroidogenic enzymes.

Because some cortisol synthesis continues, the diagnosis may not be apparent unless a CRH or metyrapone test of pituitary ACTH production capacity and an intravenous ACTH test of adrenal reserve are performed. This can be especially true when TSH deficiency is a component of hypopituitarism. The hypothyroidism resulting from TSH deficiency will result in slowed metabolism of the small amount of cortisol produced, which therefore protects

the patient from the symptoms of adrenal insufficiency. Treatment of the hypothyroidism with thyroxine will accelerate metabolism of the small amounts of cortisol, thus unmasking adrenal insufficiency due to ACTH deficiency and, on occasion, precipitating an acute adrenal crisis. Careful evaluation of the pituitary–adrenal axis is required in hypopituitarism with secondary hypothyroidism. Many clinicians will choose to "cover" a patient with small doses of glucocorticoids (one-quarter to one-half of physiological replacement) during initial treatment of such secondary hypothyroidism.

Hypothalamic and pituitary tumors, such as craniopharyngioma [445], are associated with ACTH deficiency in about 25% of patients, perhaps more in tumors such as germinoma and astrocytoma [446]. Adrenal insufficiency is rarely the presenting complaint but may contribute to the clinical picture. After surgery and radiotherapy, the great majority of these patients have ACTH deficiency as part of their pituitary damage, and all patients should receive glucocorticoid coverage during treatment, irrespective of the status of the hypothalamo-pituitary–adrenal axis at the time the tumor is identified. Cortisol is required for the kidney to excrete free water. Treatment of secondary adrenal insufficiency in some central nervous system tumors can unmask a previously unapparent deficiency of antidiuretic hormone (ADH) and thus precipitate diabetes insipidus.

Long-term glucocorticoid therapy can suppress POMC gene transcription and the synthesis and storage of ACTH. Furthermore, long-term therapy apparently decreases the synthesis and storage of CRH and diminishes the abundance of receptors for CRH in the pituitary. Therefore, recovery of the hypothalamo-pituitary axis from long-term glucocorticoid therapy entails recovery of multiple components in a sequential cascade and often requires considerable time. Patients successfully withdrawn from glucocorticoid therapy or successfully treated for Cushing disease may exhibit a fairly rapid normalization of plasma cortisol values while continuing to have diminished adrenal reserve for over 6 months.

Table 15.10. Etiology of Cushing syndrome in infancy.

	Males	Females
Adrenal tumors ($n = 48$)		
Carcinoma	5	20
Adenoma	4	16
Not defined	2	1
Ectopic ACTH syndrome	1	1
Nodular adrenal hyperplasia	1	4
Undefined adrenal hyperplasia	2	2
ACTH-producing tumor	1	0
Total	16	44

Data from [449].

Glucocorticoid therapy of pregnant women can suppress the fetal adrenal. Treatment of pregnant women with cortisone or prednisone will result in minimal suppression of the fetal adrenal, because placental 11β-HSD converts the biologically active form of these steroids, cortisol and prednisolone, back to their biologically inactive parent compounds. Thus, when radiolabeled cortisol or prednisolone is administered to a pregnant woman, the equilibrium concentrations in maternal plasma are 10 times higher than those in cord plasma. However, dexamethasone is a poor substrate for 11β-HSD, so that administration of low doses to a pregnant woman can affect fetal adrenal steroidogenesis.

Adrenal excess

Cushing syndrome

The term *Cushing syndrome* describes any form of glucocorticoid excess. *Cushing disease* designates hypercortisolism due to pituitary overproduction of ACTH. The related disorder caused by ACTH of non-pituitary origin is termed the *ectopic ACTH syndrome*. Other causes of Cushing syndrome include adrenal adenoma, adrenal carcinoma, and multinodular adrenal hyperplasia. All these are distinct from *iatrogenic Cushing syndrome*, which is the clinical constellation resulting from administration of supraphysiological quantities of ACTH or glucocorticoids.

Although generally described in great detail and illustrated with striking photographs in endocrine texts, Cushing disease is rare in adults [447], but 25% of patients referred to large centers are children, so it is clear that the disorder is more common in children than previously recognized. Many patients first seen as adults actually experienced the onset of symptoms in childhood or adolescence. Harvey Cushing's original patient was a young woman of only 23 years whose history and clinical features indicated longstanding disease [2]. In adults and children over 7 years of age, the most common cause of Cushing syndrome is Cushing disease [448]. In infants and children under 7 years, adrenal tumors predominate. Among 60 infants under 1 year of age with Cushing syndrome, 48 had adrenal tumors [449] (Table 15.10).

Clinical findings

The physical features of Cushing syndrome are familiar. Central obesity, "moon facies," hirsutism, and facial flushing are seen in over 80% of adults. Striae, hypertension, muscular weakness, back pain, buffalo hump fat distribution, psychological disturbances, acne, and easy bruising are also very commonly described (35–80%). These are the signs of advanced Cushing disease. When annual photographs of such patients are available, it is often apparent that the features can take 5 years or longer to develop. Thus, the classic cushingoid

Table 15.11. Findings in 39 children with Cushing disease.

Sign/symptom	Number of patients	%
Weight gain	36/39	92
Growth failure	31/37	84
Ostopenia	14/19	74
Fatigue	26/39	67
Hypertension	22/35	63
Delayed or arrested puberty	21/35	60
Plethora	18/39	46
Acne	18/39	46
Hirsutism	18/39	46
Compulsive behavior	17/39	44
Striae	14/39	36
Bruising	11/39	28
Buffalo hump	11/39	28
Headache	10/39	26
Delayed bone age	2/23	13
Nocturia	3/39	8

Data from [450].

appearance will usually not be the initial picture seen in the child with Cushing syndrome.

The earliest, most reliable indicators of hypercortisolism in children are weight gain and growth arrest [450] (Table 15.11); any overweight child who stops growing should be evaluated for Cushing syndrome. The obesity of Cushing disease in children is initially generalized rather than centripetal, and a buffalo hump is evidence of long-standing disease. Psychological disturbances, especially compulsive overachieving behavior, are seen in about 40% of children and adolescents with Cushing disease [450] and are distinctly different from the emotional lability and depression typically seen in adults [451]. An underappreciated aspect is the substantial degree of bone loss and undermineralization in these patients[450,452]. It is likely that Cushing disease is generally regarded as a disease of young adults because the diagnosis was missed, rather than absent, during adolescence. Rarely, Cushing syndrome caused by adrenal carcinoma and the ectopic ACTH syndrome can produce a rapid fulminant course.

Cushing disease

The recent development of trans-sphenoidal surgical approaches to the pituitary has led to pituitary exploration in large numbers of patients with Cushing disease. Among adults, over 90% of such patients have identifiable pituitary microadenomas [451,453], which are generally 2–10 mm in diameter, are not encapsulated, have ill-defined boundaries, and are frequently detectable with a contrast-enhanced pituitary MRI. They are often identifiable only by minor differences in their appearance and texture from surrounding tissue, so the frequency of surgical cure is correlated with the technical skill of the surgeon. Although histological techniques may not distinguish the tumor from normal tissue, molecular biological techniques confirm increased synthesis of POMC in these tissues [454]. Among children and adolescents, about 80–85% of those with Cushing disease have surgically identifiable microadenomas [210,455]. Although removal of the tumor usually appears to be curative, 20% of such "cured" patients suffer relapse and manifest Cushing disease again within about 5 years, so that the net cure rate is 70–75% [450,456]. Trans-sphenoidal surgery offers the best initial approach for rapid and complete cure of most patients, thus maximizing final height, which is typically reduced by 1.5–2.0 SD by the long-term hypercortisolism [450,457].

The high cure rate of trans-sphenoidal microadenomectomy in Cushing disease indicates that the majority of patients have primary disease of the pituitary itself, rather than secondary hyperpituitarism resulting from hyperstimulation of the pituitary by CRH or other agents. Careful follow-up studies of these patients confirm this [450,455,456]. In most post-operative patients, the circadian rhythms of ACTH and cortisol return to normal, ACTH and cortisol respond appropriately to hypoglycemia, cortisol is easily suppressed by low doses of dexamethasone, and the other hypothalamo-pituitary systems return to normal.

Some patients with Cushing disease have no identifiable microadenoma, and some "cured" patients relapse. This suggests that this smaller population of patients may have a primary hypothalamic disorder. Effective treatment of Cushing disease with cyproheptidine, a serotonin antagonist, has been reported in adults, further suggesting a hypothalamic disturbance. Thus, present clinical investigation suggests that Cushing disease is usually caused by a primary pituitary adenoma but that sometimes it is caused by hypothalamic dysfunction. Microsurgery can be curative in the former but not in the latter. Unfortunately, no diagnostic maneuver is available to distinguish the two possibilities, so trans-sphenoidal exploration remains the preferred initial therapeutic approach to the patient with Cushing disease.

Other therapeutic approaches include hypophysectomy, pituitary irradiation, cyproheptidine, adrenalectomy, and drugs that inhibit adrenal function. All have significant disadvantages, especially in children. Hypophysectomy eliminates pituitary secretion of growth hormone, TSH, and gonadotropins, causing growth failure, hypothyroidism, and failure to progress in puberty.

Pituitary irradiation has been touted to avoid many of these problems, but the deficiency of various pituitary hormones may be obscured by delayed onset, and the delayed onset in elimination of the hypersecretion of ACTH will further compromise the final adult height of the child with Cushing disease. Furthermore, large doses of radiation increase the risk of cerebral arteritis, leukoencephalopathy, leukemia, glial neoplasms, bone tumors involving the skull, and congenital defects in subsequent offspring.

Cyproheptidine has met with virtually no success in pediatric Cushing disease, in part because of the unacceptable side-effects (weight gain, irritability, hallucinations) often seen with the doses needed.

Laparascopic adrenalectomy is the preferred approach when two trans-sphenoidal procedures fail. In addition to the obvious effects of eliminating normal production of glucocorticoids and mineralocorticoids, removal of the adrenal eliminates the physiological feedback inhibition of the pituitary. In some adults, this results in the development of pituitary macroadenomas, producing very large quantities of ACTH. These can expand and impinge on the optic nerves and can produce sufficient POMC to yield enough melanocyte-stimulating hormone (MSH) to produce profound darkening of the skin (Nelson syndrome), but this is rarely seen in children. There is little pediatric experience with ketoconazole and other drugs that inhibit steroidogenesis, but these may provide a useful form of therapy for selected patients. Metyrapone is not useful for long-term therapy. *Ortho*, *para*-DDD (mitotane), an adrenolytic agent, may be used to effect a chemical adrenalectomy, but its side-effects of nausea, anorexia, and vomiting are severe.

Other causes of Cushing syndrome

The ectopic ACTH syndrome is commonly seen in adults with oat cell carcinoma of the lung, carcinoid tumors, pancreatic islet cell carcinoma, and thymoma. Ectopically produced POMC and ACTH are derived from the same gene that produces pituitary POMC [150], but it is not sensitive to glucocorticoid feedback in the malignant cells. This phenomenon permits distinction between pituitary and ectopic ACTH by suppressibility of the former by high doses of dexamethasone. Although the ectopic ACTH syndrome is rare in children, it has been described in infants younger than 1 year of age. Associated tumors have included neuroblastoma, pheochromocytoma, and islet cell carcinoma of the pancreas [453]. The ectopic ACTH syndrome is typically associated with ACTH concentrations 10–100 times higher than those seen in Cushing disease.

Adults and children with this disorder may show little or no clinical evidence of hypercortisolism, probably because of the typically rapid onset of the disease and the general catabolism associated with malignancy. Unlike patients with Cushing disease, patients frequently have hypokalemic alkalosis, presumably because the extremely high levels of ACTH stimulate the production of DOC by the adrenal fasciculata and may also stimulate the adrenal glomerulosa in the absence of hyper-reninemia [458].

Adrenal tumors, especially adrenal carcinomas, are the more typical cause of Cushing syndrome in infants and small children [459] (Table 15.10). They occur with much greater frequency in girls for unknown reasons [460]. Adrenal adenomas almost always secrete cortisol with minimal secretion of mineralocorticoids or sex steroids. In contrast, adrenal carcinomas tend to secrete both cortisol and androgens [461]. Congenital bodily asymmetry (hemihypertrophy) may be associated with adrenal adenoma or carcinoma, with or without association with the Beckwith–Wiedemann syndrome. CT and MRI are useful in the diagnosis of adrenal tumors. The treatment is surgical, although the prognosis for adrenal carcinoma is generally poor. A few patients have done well with adjunctive therapy with *ortho*, *para*-DDD. Size is the best guide to differentiating adenoma (< 10 cm) from carcinoma.

ACTH-independent multinodular adrenal hyperplasia is a rare entity characterized by the secretion of both cortisol and adrenal androgens [462]. It is seen in infants, children, and young adults, with females affected more frequently. Familial instances have been seen [463], and many of these have an autosomal-dominant disorder (Carney complex), consisting of pigmented lentigines and blue nevi on the face, lips, and conjunctivae atrial myxomas, and a variety of other tumors including schwannomas and Sertoli cell tumors [464]. Carney complex, which accounts for up to 80% of patients with *bilateral* micronodular adrenal hyperplasia, is linked to two distinct genetic loci. The PRKAR1A gene on chromosome 17q22–24, which encodes regulatory subunit 1A of cyclic AMP-dependent protein kinase A (PKA), is mutated in about half of affected patients. A second locus on chromosome 2p16 appears to account for most, and possibly all, patients lacking PRKAR1A mutations, but the responsible gene remains uncertain [465–468]. Because the hypercortisolism is resistant to suppression with high doses of dexamethasone and because both glucocorticoids and sex steroids are produced, this entity was difficult to distinguish from the ectopic ACTH syndrome before plasma ACTH assays became available. Adrenalectomy is usually indicated, although some successes have been reported with subtotal resections. A form of multinodular adrenal hyperplasia is occasionally seen in the McCune–Albright syndrome, suggesting that this form of adrenal hyperfunction may be associated with a G-protein defect.

Differential diagnosis

The suspicion of Cushing syndrome in children is usually raised by weight gain, growth arrest, mood change, and change in facial appearance (plethora, acne, hirsutism). The diagnosis may be subtle and difficult when it is sought early in the natural history of the disease. Absolute elevations of concentrations of plasma ACTH and cortisol are often absent. Rather than finding morning concentrations of cortisol > 20 μg/dL or of ACTH > 50 pg/mL, it is more typical to find mild, often equivocal elevations in the afternoon and evening values. This loss of diurnal rhythm, evidenced by continued secretion of ACTH and cortisol throughout the afternoon, evening and night-time, is usually the earliest reliable laboratory index of Cushing disease. Values for

Table 15.12. Diagnostic values in various causes of Cushing syndrome.

Test	Values	Normal	Adrenal carcinoma	Nodular adrenal adenoma	Adrenal hyperplasia	Cushing disease	Ectopic ACTH syndrome
Plasma cortisol	AM	> 14	↑	↑	↑	±	↑↑
concentration	PM	< 8	↑	↑	↑	↑	↑↑
Plasma ACTH	AM	< 100	↓	↓	↓	↑	↑↑
concentration	PM	< 50	↓	↓	↓	↑	↑↑
Low-dose dex	Cortisol	< 3	No Δ	No Δ	No Δ	*	No Δ
suppression	ACTH	< 30	No Δ	No Δ	No Δ	*	No Δ
	17-OHCS	< 2	No Δ	No Δ	No Δ	*	No Δ
High-dose dex	Cortisol	↓↓	No Δ	No Δ	†	↓	No Δ
suppression	ACTH	↓↓	No Δ	No Δ	†	↓	No Δ
	17-OHCS	↓↓	No Δ	No Δ	†	↓	No Δ
IV ACTH test	Cortisol	> 20	No Δ	±↑	±↑	↑	No Δ
Metyrapone test	Cortisol	↓	±↓	No Δ	±↓	↓	±↓
	11-Deoxycortisol	↑	±↑	No Δ	±↑	↑	±↑
	ACTH	↑	No Δ	No Δ	±↑	↑	No Δ
	17-OHCS	↑	No Δ	No Δ	±	↑	No Δ
24-h urinary excretion	17-OHCS		↑↑	↑	↑	↑	↑ (basal)
	17-Ketosteroids		↑↑	±↑	↑	↑	↑
Plasma concentration	DHEA or DHEAS		↑↑	↓	±↑	↑	↑

Dex, dexamethasone. Cortisol concentration in mg/dL. ACTH concentration in pg/mL. 17-OHCS in mg/24 h.
*Incomplete response, i.e. ±.
†Usually no Δ.

ACTH and cortisol are typically extremely high in the ectopic ACTH syndrome, whereas cortisol is elevated but ACTH suppressed in adrenal tumors and in multinodular adrenal hyperplasia (Table 15.12).

The performance of low- and high-dose dexamethasone suppression tests can be useful. Two days of baseline (control) data should be obtained. Low-dose dexamethasone (20 μg/kg/day) should be given, divided into equal doses given every 6 h for 2 days followed by high-dose dexamethasone (80 μg/kg/day) given in the same fashion. Values at 08.00 h and 20.00 h for ACTH and cortisol and 24-h urine collections for 17-OHS, 17-ketosteroids, free cortisol, and creatinine (to monitor the completeness of the collection) should be obtained on each of the 6 days of the test. Because of variations due to episodic secretion of ACTH, 08.00 h and 20.00 h blood values should be drawn in triplicate: on the hour and 15 and 30 min after. In patients with exogenous obesity or other non-Cushing disorders, cortisol, ACTH, and urinary steroids will be suppressed readily by low-dose dexamethasone. Plasma cortisol should be less than 5 μg/100 mL, ACTH less than 20 pg/mL, and 24-h urinary 17-OHS less than 1 mg/g of creatinine. Patients with adrenal adenoma, adrenal carcinoma, or the ectopic ACTH syndrome will have values relatively insensitive to both low- and high-dose dexamethasone, although some patients with multinodular adrenal hyperplasia may respond to high-dose suppression. Patients with Cushing disease classically respond with a suppression of ACTH, cortisol, and urinary steroids during the high-dose treatment but not during the low-dose treatment. However, some children, especially those early in the course of their illness, may exhibit partial suppression in response to low-dose dexamethasone. Thus, if the low dose that is given exceeds 20 μg/kg/day or if the assays used are insufficiently sensitive to distinguish partial from complete suppression, false-negative tests may result. In general, the diagnosis of Cushing disease is considerably more difficult to establish in children than in adults.

Virilizing and feminizing adrenal tumors

Most virilizing adrenal tumors are carcinomas producing a mixed array of androgens and glucocorticoids. Virilizing and feminizing adrenal adenomas are rare. Virilizing tumors in boys have a presentation similar to that of simple virilizing CAH [469] with phallic enlargement, erections, pubic and axillary hair, increased muscle mass, deepening of the voice, acne, and scrotal thinning; testicular size will be prepubertal. Elevated concentrations of testosterone in young boys alter behavior, with increased irritability, rambunctiousness, hyperactivity, and rough play without evidence of libido. Diagnosis is based on hyperandrogenemia that is insuppressible by glucocorticoids. The treatment is surgical; all such

tumors should be handled as if they are malignant, with care exerted not to cut the capsule and seed cells on to the peritoneum. The pathological distinction between adrenal adenoma and carcinoma is difficult.

Feminizing adrenal tumors are extremely rare. P450aro, the enzyme aromatizing androgenic precursors to estrogens, is not normally found in the adrenals, but is found in peripheral tissues such as fat. It is not known whether most feminizing adrenal tumors exhibit ectopic adrenal production of this enzyme, whether some other enzyme mediates aromatization in the tumor, or whether these are truly androgen-producing, virilizing tumors occurring in a setting where there is unusually effective peripheral aromatization of adrenal androgens. Feminizing adrenal (or extra-adrenal) tumors can be distinguished from true (central) precocious puberty in girls by the absence of increased circulating concentrations of gonadotropins and by a prepubertal response of luteinizing hormone to an intravenous challenge of gonadotropin-releasing hormone (GnRH). In boys, such tumors will cause gynecomastia, which will resemble the benign gynecomastia that often accompanies puberty. However, as with virilizing adrenal tumors, testicular size and the gonadotropin response to GnRH testing will be prepubertal. The diagnosis of a feminizing tumor in a pubertal boy can be extremely difficult but is usually suggested by an arrest in pubertal progression and can be proved by the persistence of circulating plasma estrogens after the administration of testosterone.

Conn syndrome

Conn syndrome, characterized by hypertension, polyuria, hypokalemic alkalosis, and low plasma renin activity due to an aldosterone-producing adrenal adenoma, is well described in adults but is exquisitely rare in children. The diagnostic task is to differentiate primary aldosteronism from physiological secondary hyperaldosteronism occurring in response to another physiological disturbance. Any loss of sodium, retention of potassium, or decrease in blood volume will result in hyper-reninemic secondary hyperaldosteronism. Renal tubular acidosis, treatment with diuretics, salt-wasting nephritis, or hypovolemia due to nephrosis, ascites, or blood loss are typical settings for physiological secondary hyperaldosteronism. Primary aldosteronism is characterized by hypertension and hypokalemic alkalosis. The cause is a small adrenal adenoma, usually confined to one adrenal. Both adrenals need to be explored surgically because adrenal vein catheterization is not possible in children and is difficult in adults.

Glucocorticoid therapy and withdrawal

Since their introduction into clinical medicine in the early 1950s, glucocorticoids have been used to treat virtually every known disease [470]. At present, their rational use falls into two broad categories, replacement in adrenal insufficiency and pharmacotherapeutic use. The latter category is largely related to the anti-inflammatory properties of glucocorticoids but also includes their actions to lyze leukemic leukocytes, lower plasma calcium concentrations, and reduce increased intracranial pressure. Virtually all these actions are mediated through glucocorticoid receptors, which are found in most cells. Because there appears to be only one major type of glucocorticoid receptor, all glucocorticoids affect all tissues containing such receptors. Thus, with the exception of the distinction between glucocorticoids and mineralocorticoids, tissue-specific, disease-specific, or response-specific analogs of naturally occurring glucocorticoids cannot be produced. The only differences among the various glucocorticoid preparations are their ratio of glucocorticoid to mineralocorticoid activity, their capacity to bind to various binding proteins, their molar potency, and their biological half-life. Dexamethasone is commonly used in reducing increased intracranial pressure and brain edema. Neurosurgical experience indicates that the optimal doses are 10–100 times those that would thoroughly saturate all available receptors, suggesting that this action of dexamethasone may not be mediated through the glucocorticoid receptor.

Glucocorticoids are so termed because of their major actions to increase plasma concentrations of glucose. This occurs by their induction of the transcription of the genes encoding the enzymes of the Embden–Myerhoff glycolytic pathway and other hepatic enzymes that divert amino acids, such as alanine, to the production of glucose. Thus, the coordinated action to increase the transcription of these genes can result in increased plasma concentrations of glucose, obesity, and muscle wasting. The other features of Cushing syndrome are similarly attributable to the increased transcriptional activity of specific glucocorticoid-sensitive genes.

Replacement therapy

Glucocorticoid replacement therapy is complicated by undesirable side-effects with even minor degrees of overtreatment or undertreatment. Overtreatment can cause the signs and symptoms of Cushing syndrome, and even minimal overtreatment can impair growth. Undertreatment will cause the signs and symptoms of adrenal insufficiency (Table 15.9) only if the extent of undertreatment (dose and duration) is considerable. However, undertreatment may impair the individual's capacity to respond to stress.

To optimize pediatric glucocorticoid replacement therapy, physicians have gauged their therapy to resemble the endogenous secretory rate of cortisol. Most authorities recommend treatment equivalent to a secretory rate of 12.5 mg of cortisol per square meter of body surface area per day, 9.5–15.5 mg/m^2/day. The time-honored value of 12.5 mg/m^2 may be too high, and appropriate replacement may be as

low as 6 mg/m^2 in younger children and 9 mg/m^2 in older children and adolescents [214,215].

The management of the delicate balance between over- and undertreatment is confounded by considerable variation in the normal cortisol secretory rate among different children of the same size and the probability that most conventional guidelines err on the side of overtreatment. Additional factors must, however, be considered in tailoring a specific child's glucocorticoid replacement regimen.

The specific form of adrenal insufficiency influences therapy. When treating autoimmune adrenalitis or any other form of Addison disease, it is prudent to err slightly on the side of undertreatment. This will eliminate the possibility of glucocorticoid-induced iatrogenic growth retardation and permit the pituitary to continue to produce normal to slightly elevated concentrations of ACTH. This ACTH will continue to stimulate the remaining functional adrenal steroidogenic machinery and provide a convenient means of monitoring the effects of therapy. In contrast, when treating CAH, the adrenal should be suppressed more completely, as any adrenal steroidogenesis will result in the production of unwanted androgens, with their consequent virilization and rate of advancement of bony maturation that is more rapid than the rate of advancement of height.

The presence or absence of associated mineralocorticoid deficiency is important. Children with mild degrees of mineralocorticoid insufficiency, such as those with simple virilizing CAH, may continue to have mildly elevated ACTH values, suggesting insufficient glucocorticoid replacement in association with elevated PRA. In some children, the ACTH is elevated in response to chronic, compromised hypovolemia, attempting to stimulate the adrenal to produce more mineralocorticoid. In these children, who do not manifest overt signs and symptoms of mineralocorticoid insufficiency, treatment with mineralocorticoid replacement may permit one to decrease the amount of glucocorticoid replacement needed to suppress plasma ACTH and urinary 17-ketosteroids. This reduction in glucocorticoid therapy reduces the likelihood that adult height will be compromised.

The specific formulation of glucocorticoid is of great importance. Potent, long-acting glucocorticoids, such as dexamethasone or prednisone, are preferred in the treatment of adults but are rarely appropriate for children. Small, incremental dose changes are more easily carried out with weaker glucocorticoids. It is easy to change from 25 to 30 mg of hydrocortisone but virtually impossible to change from an equivalent 0.5 to 0.6 mg of dexamethasone. The efficacy of attempting to mimic the physiological diurnal variation in steroid hormone secretion remains controversial. As ACTH and cortisol concentrations are high in the morning and low in the evening, it is intellectually and logically appealing to attempt to duplicate this circadian rhythm in replacement therapy. However, the results do not indicate clearly that better growth is achieved by giving relatively larger doses in the morning and lower doses at night.

This probably reflects the fact that ACTH and cortisol secretion are episodic throughout the day and that this well-established circadian variation is not smooth. The pattern of high in the morning and low in the evening is only an averaged result. Furthermore, the adrenal releases cortisol episodically throughout the day in response to various physiological demands (hypoglycemia, exercise, stress, etc.); thus, under normal circumstances, the plasma concentrations are high when the clearance and disposal rates are also high. A planned program of replacement therapy cannot possibly anticipate these day-to-day variations.

Finally, dosage equivalents among various glucocorticoids can be misleading (Table 15.7) because most preparations of glucocorticoids are intended for pharmacotherapeutic use rather than replacement therapy and because the most common indication for pharmacological doses of glucocorticoids is for their anti-inflammatory properties.

All these variables explain why there is little unanimity in recommendations for designing a glucocorticoid replacement regimen. An understanding of them will permit appropriate monitoring of the patient and encourage the physician to vary the treatment according to the responses and needs of the individual child.

Commonly used glucocorticoid preparations

Numerous chemical derivatives and variants of the naturally occurring steroids are commercially available in a huge array of dosage, forms, vehicles, and concentrations, all carrying confusing and uninformative brand names. Choosing the appropriate product can be simplified by considering only the most widely used steroids listed in Table 15.7. There are four relevant considerations.

First, the glucocorticoid potency of the various drugs is generally calculated and described according to the anti-inflammatory potency. The pharmaceutical industry has chosen this standard for convenience and because the majority of their sales are to physicians using pharmacological doses of these steroids to achieve anti-inflammatory effects.

Second, the growth-suppressant effect of a glucocorticoid preparation may be significantly different from its anti-inflammatory effect. This results from differences in half-life, metabolism, and protein-binding and receptor affinity (potency), but it is not due to receptor specificity as all known receptor-mediated effects of glucocorticoids are mediated through a single type of receptor.

Third, the mineralocorticoid activity of various glucocorticoid preparations varies widely. Both glucocorticoid and mineralocorticoid hormones can bind to both glucocorticoid (type I) and mineralocorticoid (type II) receptors, and most authorities now regard these as two different types of glucocorticoid receptors and find that there is no true specific

mineralocorticoid receptor. Mineralocorticoid activity is intimately related to the activity of 11β-HSD, which metabolizes glucocorticoids but not mineralocorticoids to a form that cannot bind the receptor. Thus, the relative mineralocorticoid potency of various steroids is determined by both their affinity for the type II receptor and their resistance to the activity of 11β-HSD. An understanding that some commonly used glucocorticoids, such as cortisol, cortisone, prednisolone, and prednisone, have significant mineralocorticoid activity is especially important when large doses are used as stress doses in a patient on replacement therapy. Stress doses of the glucocorticoid preparation may provide sufficient mineralocorticoid activity to meet physiological needs, so mineralocorticoid supplementation is not needed.

Fourth, the plasma half-life and biological half-life of the various preparations may be discordant and vary widely. This is mainly related to binding to plasma proteins, hepatic metabolism, and hepatic activation. For example, cortisone and prednisone are biologically inactive (and even have mild steroid antagonist actions) until they are metabolized by hepatic 11β-HSD-I to their active forms, cortisol and prednisolone. Thus, the relative glucocorticoid potency of these preparations will also be affected by hepatic function. Cortisone and prednisone are cleared more rapidly in patients receiving drugs such as phenobarbital or phenytoin, which induce hepatic enzymes, and are cleared more slowly in patients with liver failure.

In addition to these chemical considerations, the route of administration is critical. Glucocorticoids are available for oral, intramuscular, intravenous, intrathecal, intra-articular, inhalant, and topical use on skin, mucous membranes, and conjunctivae. Each preparation is designed to deliver the maximal concentration of steroid to the desired tissue while delivering less steroid systemically. All the preparations are absorbed to varying extents, so that the widely used inhalant preparations used to treat asthma can, in sufficient doses, cause growth retardation and other signs of Cushing syndrome.

In general, and in contradistinction to many other drugs, orally administered steroids are absorbed rapidly but incompletely, whereas intramuscularly administered steroids are absorbed slowly but completely. Thus, if the secretory rate of cortisol is 8 mg/m^2 body surface area, the intramuscular or intravenous replacement dose of cortisol (hydrocortisone) would be 8 mg/m^2. However, because only about half of an oral dose is absorbed intact, the oral equivalent would be about 15–20 mg of hydrocortisone. The efficiency of absorption of glucocorticoids can vary considerably depending on diet, gastric acidity, bowel transit time, and other individual factors. Thus, the dosage equivalents listed in Table 15.7 are only general approximations. The equivalences shown are estimated biological equivalences with a broad range of variability and are not physical chemical equivalents.

ACTH can also be used for glucocorticoid therapy by its action to stimulate endogenous adrenal steroidogenesis. Although intravenous and intramuscular ACTH are useful in diagnostic tests, the use of ACTH as a therapeutic agent is no longer favored, principally because it will stimulate synthesis of mineralocorticoids and adrenal androgens as well as glucocorticoids. Furthermore, the need to administer ACTH parenterally further diminishes its usefulness.

Intramuscular ACTH (1–39) in a gel form is the treatment of choice for infantile spasms (West syndrome) and possibly also for other forms of epilepsy in infants resistant to conventional anticonvulsants. Whether this action is mediated by ACTH itself, by other peptides in the biological preparation, by ACTH-induced adrenal steroids, or by ACTH-responsive synthesis of novel "neurosteroids" [471] in the brain has not been determined. When pharmacological doses of ACTH are used therapeutically, as in infantile spasms, the patient should be given a low-sodium diet to ameliorate steroid hypertension.

Although greatly elevated concentrations of ACTH, as in the ectopic ACTH syndrome, cause pituitary suppression, treatment with daily injections of ACTH results in less hypothalamo-pituitary suppression than treatment with equivalent doses of oral glucocorticoids, presumably because the effect on the adrenal is transient. Adrenal suppression obviously does not occur in ACTH therapy. Because the effects of ACTH on adrenal steroidogenesis are highly variable, it is even more difficult to determine dosage equivalences for ACTH and oral steroid preparations than it is among the various steroids. A very rough guide from studies in adults is that 40 units of ACTH (1–39) gel is approximately equivalent to 100 mg of cortisol.

Pharmacological steroid therapy

Pharmacological doses of glucocorticoids are used in an endless variety of clinical situations. The choice of glucocorticoid preparation to be used is guided by pharmacological parameters (described above and in Table 15.7) and by custom (e.g. the use of betamethasone rather than dexamethasone to induce fetal lung maturation in impending premature deliveries).

Pharmacological doses of glucocorticoids administered for more than 1 or 2 weeks will cause signs and symptoms of iatrogenic Cushing syndrome. These are similar to the glucocorticoid-induced findings in Cushing disease but may be more severe because of the high doses involved (Table 15.13). Iatrogenic Cushing syndrome is not associated with adrenal androgen effects, and mineralocorticoid effects are rare.

Alternate-day therapy can decrease the toxicity of pharmacological glucocorticoid therapy, especially suppression of the hypothalamo-pituitary–adrenal axis and growth. The basic premise of alternate-day therapy is that the disease state can be suppressed with intermittent therapy, while there is significant recovery of the hypothalamo-pituitary–adrenal axis during the "off" day. Alternate-day therapy requires

Table 15.13. Complications of high-dose glucocorticoid therapy.

Short-term therapy	Long-term therapy
Gastritis	Gastric ulcers
Growth arrest	Short stature
Increased appetite	Weight gain
Hypercalciuria	Osteoporosis, fractures
Glycosuria	Slipped epiphyses
Immune suppression	Ischemic bone necrosis
Masked symptoms of infection, especially fever and inflammation	Poor wound healing
	Catabolism
Toxic psychoses	Cataracts
	Bruising (capillary fragility)
	Adrenal/pituitary suppression
	Toxic psychosis

the use of a short-acting glucocorticoid administered once in the morning of each therapeutic day to ensure that the "off" day is truly "off." Long-acting glucocorticoids, such as dexamethasone, should not be used for alternate-day therapy; results are best with oral prednisone or methyl prednisolone.

Withdrawal of glucocorticoid therapy

Withdrawal of glucocorticoid therapy can lead to symptoms of glucocorticoid insufficiency. When glucocorticoid therapy has been used for only 1 week or 10 days, therapy can be discontinued abruptly, even if high doses have been used [472]. Although only one or two doses of glucocorticoid are needed to suppress the hypothalamo-pituitary–adrenal axis, this axis recovers very rapidly from short-term suppression. When therapy has persisted for 2 weeks or longer, recovery of hypothalamo-pituitary–adrenal function is slower, and tapered doses of glucocorticoids are indicated. Acute discontinuation of therapy in such patients will lead to symptoms of glucocorticoid insufficiency, the so-called steroid withdrawal syndrome. This symptom complex does not include salt loss, as adrenal glomerulosa function regulated principally by the renin–angiotensin system remains normal. However, blood pressure can fall abruptly, as glucocorticoids are required for the action of catecholamines in maintaining vascular tone.

The most prominent symptoms of the steroid withdrawal syndrome include malaise, anorexia, headache, lethargy, nausea, and fever. In reducing pharmacological doses of glucocorticoids, it might appear logical to reduce the dosage precipitously to physiological replacement doses. This is rarely successful and occasionally disastrous. Even when given physiological replacement, patients who have been receiving pharmacological doses of glucocorticoids experience steroid withdrawal.

Although the mechanism is not known, it is most likely that long-term pharmacological glucocorticoid therapy inhibits transcription of the gene(s) for glucocorticoid receptors, thus reducing the number of receptors per cell. If this is so, physiological concentrations of glucocorticoids will elicit subphysiological cellular responses, resulting in the steroid withdrawal syndrome. Thus, it is necessary to taper gradually from the outset. The duration of glucocorticoid therapy is a critical consideration in designing a glucocorticoid withdrawal program. Therapy for a couple of months will completely suppress the hypothalamo-pituitary–adrenal axis but will not cause adrenal atrophy. Therapy of years' duration may result in almost total atrophy of the adrenal fasciculata/reticularis, which may require a withdrawal regimen that takes months.

Procedures for tapering steroids are empirical. Their success is determined by the length and mode of therapy and by individual patient responses. Patients who have been on alternate-day therapy can be withdrawn more easily than those receiving daily therapy, especially daily therapy with a long-acting glucocorticoid such as dexamethasone. In patients on long-standing therapy, a 25% reduction in the previous level of therapy is generally recommended weekly. When withdrawal is done with steroids other than cortisone or cortisol, measurement of morning cortisol values can be a useful adjunct. Morning cortisol values of 10 μg/dL or more indicate that the dose can be reduced safely.

Even after the successful discontinuation of therapy, the hypothalamo-pituitary–adrenal axis is not wholly normal and may be incapable of responding to severe stress for 6–12 months after successful withdrawal from long-term, high-dose glucocorticoid therapy. Evaluation of the hypothalamus and pituitary by a CRH or metyrapone test and evaluation of adrenal responsiveness to pituitary stimulation with an intravenous ACTH test should be done at the conclusion of a withdrawal program and 6 months thereafter. The results of these tests will indicate if there is a need for steroid cover in acute surgical stress or illness.

Stress doses of glucocorticoids

The cortisol secretory rate increases significantly during physiological stress such as trauma, surgery, or severe illness. Patients receiving glucocorticoid replacement therapy or those recently withdrawn from pharmacological therapy need cover with stress doses. The indications for this cover and the appropriate dose are controversial and difficult to establish; most practitioners prefer to err on the safe side of steroid overdosage. This is a good tactic in the short term but can have a significant effect on growth over a period of years.

It is generally said that doses 3–10 times physiological replacement are needed for the stress of surgery. The stress accompanying a surgical procedure can vary greatly. Modern techniques of anesthesiology, better anesthetic, analgesic, and muscle-relaxing drugs, and increased awareness of the particular needs of children in managing intraoperative fluids and electrolytes have greatly reduced the stress of

surgery. In the past, a significant portion of such stress had to do with pain and hypovolemia, but these should be minimized in contemporary practice. Similarly, part of the stress of acute illness is fever and fluid loss, factors now familiar to all pediatricians. Although it remains appropriate and necessary to give about three times physiological requirements during such periods of stress, it is probably not necessary to give much higher doses. Similarly, it is not necessary to triple a child's physiological replacement regimen during simple colds, upper respiratory infection, otitis media, or after immunizations.

The preparation of the hypoadrenal patient on replacement therapy for surgery is simple if planned in advance. Although stress doses of steroids can be administered intravenously by the anesthetist during surgery, this may be suboptimal. Doses administered as an intravenous bolus are short acting and may not provide cover throughout the procedure. The transition from ward to operating room to recovery room usually involves a transition among three or more teams of personnel, increasing the risk for error. Because intramuscularly administered cortisone acetate has a biological half-life of about 18 h, we recommend intramuscular administration of twice the day's physiological requirement at 18 h before surgery and again at 8 h before surgery. This provides the patient with a body reservoir of glucocorticoid throughout the surgical and immediate post-operative period. Regular therapy at two to three times physiological requirements can then be reinstituted on the day after the surgical procedure.

Mineralocorticoid replacement

Replacement therapy with mineralocorticoids is indicated in salt-losing CAH and in syndromes of adrenal insufficiency that affect the zona glomerulosa. Only one mineralocorticoid, 9α-fluorocortisol (Fluorinef), is currently available. There is no parenteral mineralocorticoid preparation, so hydrocortisone and salt must be used.

Mineralocorticoid doses used are essentially the same irrespective of the size or age of the patient. Newborns are quite insensitive to mineralocorticoids and may require larger doses than adults. The replacement dose of 9α-fluorocortisol is usually 50–100 μg daily; sodium must be available to the nephrons for mineralocorticoids to promote reabsorption of sodium.

Cortisol has significant mineralocorticoid activity and, when given in stress doses, provides adequate mineralocorticoid activity so that mineralocorticoid replacement can be interrupted. Because 9α-fluorocortisol can be administered only orally and because this may not be possible in the postoperative period, the appropriate drug for glucocorticoid replacement is cortisol or cortisone, which have mineralocorticoid activity, rather than a synthetic steroid such as prednisone or dexamethasone, which have little mineralocorticoid activity.

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16 Polyglandular syndromes

Catherine J. Owen, Tim D. Cheetham, and Simon H.S. Pearce

The autoimmune polyglandular endocrinopathy syndromes (APS) encompass a wide clinical spectrum of disease of monogenic and complex genetic etiologies. The first manifestation of the disorders is frequently in childhood or adolescence, and their presentation is heterogeneous.

Autoimmune polyglandular syndrome type I (APS1)

Definition

APS1, known as the autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy syndrome (APECED), is a rare and frequently debilitating disorder of childhood. It is inherited as an autosomal-recessive condition; heterozygotes have no manifestations. The female–male ratio is close to 1. The clinical diagnosis of APS1 requires the presence of two of the three cardinal components: chronic mucocutaneous candidiasis, autoimmune hypoparathyroidism, and autoimmune adrenal failure [1–6]. Only one of these manifestations is required if a sibling has the syndrome [1]. There is a spectrum of associated minor components, which include endocrine and non-endocrine manifestations.

Large cohorts of APS1 patients have been reported from several countries including Finland [2,6], Norway [5], Israel [3], Sardinia [7], northern Italy [4], and northern America [1]. Although a rare disorder in most countries (about two or three cases per million in the UK [8]), it shows a founder effect leading to a much higher prevalence in certain populations: Finns 1:25 000 [2], Iranian Jews 1:9000 [3], and Sardinians 1:14 500 [7]. There are also differences in the phenotype between different populations: for example, chronic mucocutaneous candidiasis and adrenal failure are among the commonest manifestations in most patients of European descent but are present in only about 20% of Iranian Jews [3,6].

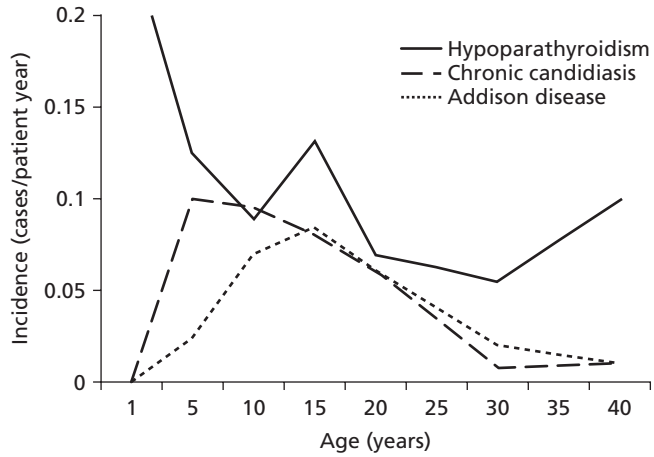


Fig. 16.1. Incidence of the three most common components of APS1 according to age (modified from [6]).

Clinical features and course

The first manifestation is typically mucocutaneous candidiasis, which develops in infancy or early childhood. Hypoparathyroidism characteristically develops around the age of 7 years and adrenocortical failure by the age of 13 years (Fig. 16.1) [4,8,9]. The complete evolution of the three cardinal features usually occurs in the first 20 years, with additional minor manifestations continuing to appear at least until the fifth decade [1]. Although this temporal sequence of appearance of the major manifestations is frequently observed in childhood, APS1 subjects not uncommonly present in other ways, either with one cardinal feature and several minor manifestations or with several minor manifestations and characteristic ectodermal dystrophy.

The median number of disease components is four, with up to 10 manifestations in some subjects. The cardinal triad occurs in around 60% of subjects, and there may be a delay in diagnosis in the early years when rarer components may dominate the clinical picture. Patients who present initially

Table 16.1. Frequencies of the major and main minor components of APS1.

Disease	Frequency (%)
Main manifestations	
Chronic mucocutaneous candidiasis	72–100
Autoimmune hypoparathyroidism	76–93
Autoimmune adrenal failure	73–100
Common minor manifestations	
Autoimmune endocrinopathies	
Hypergonadotrophic hypogonadism	17–61
Autoimmune thyroid disease	4–18
Type 1 diabetes mellitus	0–23
Pituitary defects	7
Gastrointestinal components	
Pernicious anemia	13–31
Malabsorption	10–22
Cholelithiasis	44
Chronic active hepatitis	5–31
Skin autoimmune diseases	
Vitiligo	8–26
Alopecia	29–40
Urticarial-like erythema with fever	9
Ectodermal dysplasia	
Nail dystrophy	10–52
Dental enamel hypoplasia	40–77
Tympanic membrane calcification	33
Other manifestations	
Keratoconjunctivitis	2–35
Asplenia	15

Data from European and North American patients [1,2,4,5,6,9]. Iranian Jews have distinctly different frequencies from the other populations and have been excluded.

with adrenal insufficiency rather than candidiasis tend to develop fewer components than others [2,6]. It has also been reported that the earlier the first component presents, the more likely that multiple components will develop [1,4]. Table 16.1 lists the cardinal and more common minor manifestations together with their frequency.

Cardinal manifestations

Chronic mucocutaneous candidiasis (CMC)

Chronic or periodic mucocutaneous candidiasis is commonly the first manifestation of the syndrome, occurring as early as 1 month of age, but more typically in the first 2 years of life. It is frequently mild or intermittent and responds well to periodic systemic anti-candidal treatment. In some subjects, CMC does not develop until adulthood [1,2], but it is the most frequently occurring cardinal manifestation, present in 73–100% of patients [1,2,4–6]. It is considered to be the clinical expression of dysfunctional presentation of *Candida albicans* antigens to T lymphocytes. Oral candidiasis is the commonest presentation, but esophagitis is also found, causing substernal pain and odynophagia. Infection of the intestinal mucosa leads to abdominal discomfort and diarrhea. Candidal infection can also affect the vaginal mucosa, nails, and skin.

Hypoparathyroidism

This is frequently the first endocrine feature of APS1 [1–6,10], with a peak incidence between 2 and 11 years of age. Hypoparathyroidism occurs in around 75–95% [1,2,4–6,9], although there appears to be a slightly reduced penetrance in males [11]. Hypoparathyroidism may be asymptomatic but presents typically with tetany and grand mal seizures. Presentation may be precipitated by factors such as fasting, low calcium, or high phosphate intake. The diagnosis is confirmed by a low or undetectable plasma parathyroid hormone (PTH) level in the presence of hypocalcemia. Hyperphosphatemia and hypomagnesemia are common, with low urinary calcium excretion. Autopsy studies of parathyroid glands from these patients show atrophy and an infiltration of the parathyroids with mononuclear cells [10].

Adrenal failure

Autoimmune adrenal failure (Addison disease) is typically the third of the cardinal manifestations to present in APS1, with a peak incidence around 13 years [1–6,9]. In most populations of APS1 patients, it occurs less frequently than the other major components (72–100%), [1,2,4–6]. Destruction of the adrenal cortex may develop gradually, and deficiencies of cortisol and aldosterone can appear in either order up to 20 years apart [6]. At autopsy, the adrenals of these patients are atrophic, with the adrenal cortex being almost completely destroyed and having an extensive inflammatory cell infiltrate. Diagnosis of adrenal insufficiency is confirmed by a normal or low cortisol concentration with increased adrenocorticotrophic hormone (ACTH) and a subnormal cortisol response to ACTH stimulation. A temporary hypermineralocorticoid-like state is seen in some patients with cortisol deficiency, paradoxically leading to hypokalemia [6]. Deficiency of aldosterone may be heralded by salt craving and is confirmed by a raised plasma renin activity even before the development of overt electrolyte disturbance.

Minor manifestations

Autoimmune endocrinopathies

Primary hypogonadism is the commonest minor manifestation of APS1, occurring in 17–61% of cases [1–6,9,12]. It is almost invariably accompanied by adrenal failure. About

half of APS1 females with hypogonadism present with primary amenorrhea, and the remainder have secondary amenorrhea. Male hypogonadism has been reported from puberty onwards [6]. One male patient has been reported with azoospermia and possible antisperm autoimmunity [6].

Type 1 diabetes mellitus is relatively infrequent in APS1 compared with other polyendocrinopathy syndromes. There is an age-related penetrance with a peak presentation in the teenage years [6]. There is a wide range in the reported prevalence between different APS1 populations from 0 to 23%, depending on age of the cohort [1–6,9].

Destructive autoimmune thyroid diseases (Hashimoto thyroiditis or primary atrophic thyroiditis) are relatively uncommon in APS1, occurring in 4–18% of cases. The age of presentation varies from around 10 years for Hashimoto thyroiditis to 17 years for primary atrophic thyroiditis [1,2,4]. Hyperthyroidism is very rare.

Pituitary defects such as lymphocytic hypophysitis or autoimmune pituitary disease have occasionally been described (≈ 5%) and can induce single or multiple hormonal defects [9]. Cases of secondary hypogonadism [2], growth hormone deficiency [13], and idiopathic diabetes insipidus [4] have been reported.

Gastrointestinal components

Chronic atrophic gastritis affects up to a third of patients with APS1, with a peak incidence at 10–20 years [1–6,9]. It can lead to a megaloblastic anemia due to vitamin B12 deficiency (pernicious anemia) or a microcytic anemia because of iron deficiency.

Malabsorption occurs in 10–22% of cases [1–6,9] and can be due to a variety of causes including villous atrophy, exocrine pancreatic insufficiency, intestinal infections (*Giardia lamblia* or *Candida*), defective bile acid reabsorption, and intestinal lymphangiectasia [4,6,14]. Autoimmune destruction of the enterochromaffin cells of the small intestine leading to deficiency of cholecystokinin and serotonin has been implicated [15]. The malabsorption presents with periodic or chronic diarrhea, usually with steatorrhea, but may be associated with constipation. It can be a characteristic feature of an early "atypical" presentation of APS1 in the first year of life, being part of the initial manifestations in around 10%. There is a strong association with the hypocalcemia of hypoparathyroidism, as hypocalcemia impairs the secretion of cholecystokinin leading to a failure of normal gall bladder contraction and pancreatic enzyme secretion.

Cholelithiasis is present in up to 40% by ultrasonography [9], is frequently asymptomatic, and is thought to be secondary to disruption of the enterohepatic circulation.

Chronic active hepatitis develops in 5–30% of cases [1–6,9]. The clinical course varies from chronic but asymptomatic in the majority of cases to the development of cirrhosis or fulminant hepatic failure with a potentially fatal outcome in some [1,16]. It may present in early childhood and can be the first manifestation of APS1. Elevation of serum alanine aminotransferase for more than 3 months, when no other cause such as viral or drug-induced hepatitis can be found, is an indication for liver biopsy [2]. Clinicians should be particularly vigilant in the early weeks after the identification of abnormal liver function in APS1 subjects as rapid decompensation to frank liver failure may occur.

Skin autoimmune diseases

Vitiligo can appear at any age but most commonly in childhood [1,9], affecting up to a quarter of APS1 patients [1–6,9]. It is highly variable in extent and often worsens with time.

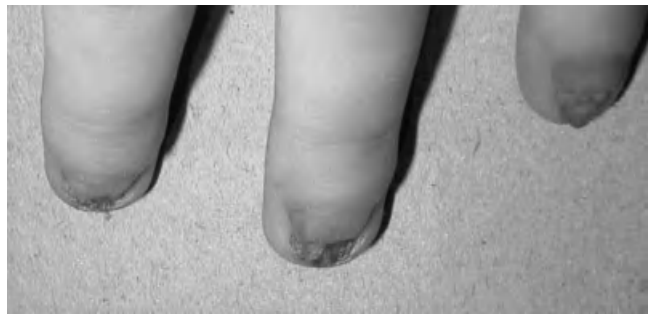
Alopecia affects about a third of patients and can involve all body sites in varying degrees [1–6,9]. It can develop rapidly and at any age.

Recurrent urticaria with fever has been reported as an unusual manifestation in about 10% of patients during childhood. It may persist for many years and is strongly associated with uveitis. High levels of immunoglobulin G (IgG) and circulating immune complexes are found, and skin biopsy reveals a lymphoplasmacytic vasculitis [6].

Other manifestations

Ectodermal dystrophy affects the nails and tooth enamel (Fig. 16.2). The pitted nails are unrelated to candidal infection

(a)



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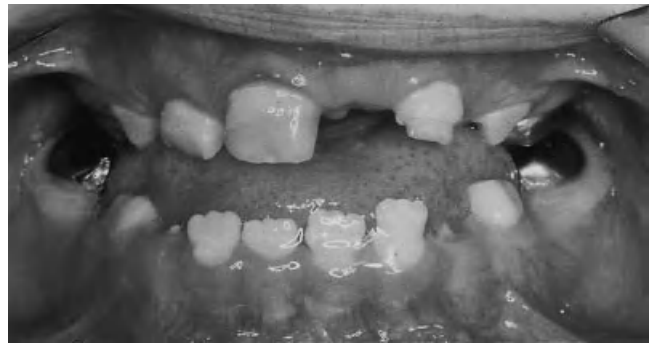

Fig. 16.2. Ectodermal features of APS1 illustrating the nail dystrophy and the dental enamel hypoplasia.

Table 16.2. Rarer minor manifestations having reported association with APS1 [1–6,9].

Rare components of APS1
Immunological
Selective IgA deficiency
Hypergammaglobulinemia
Tuberculin anergy
Renal
Interstitial nephritis
Hypercalcemia-based nephrocalcinosis
Neurological
Intracranial calcification
Progressive myopathy
Connective tissue
Sjögren's syndrome
Cutaneous vasculitis
Scleroderma
Rheumatoid arthritis
Lupus-like panniculitis
Hematological
Pure red cell aplasia
Autoimmune hemolytic anemia
Malignant
Oral squamous cell carcinoma
Esophageal carcinoma
Adenocarcinoma of the stomach
Ophthalmic
Iridiocyclitis
Optic nerve atrophy
Retinal degeneration
Other
Metaphyseal dysplasia
Primary pulmonary hypertension
Lymphocytic myocarditis
Bronchiolitis obliterans pneumonia

and can be an important clue to the diagnosis of APS1. Dental enamel hypoplasia has been reported in 40–75% of patients [1–6,9], although deciduous teeth are never affected. Enamel hypoplasia can precede hypoparathyroidism and is unrelated to serum calcium concentrations. Even in the absence of ear infection, a third of patients have calcified plaques on the tympanic membranes [2,6].

Keratoconjunctivitis incidence varies from 10% to 40% between reports [1–6,9,10]. It is the first manifestation of APS1 in some cases. The initial symptoms are intense photophobia, blepharospasm, and lacrimation; permanent visual impairment and even blindness is not infrequent [6]. Some patients enter a quiescent phase around 10 years after onset.

Asplenia or hyposplenism has been documented by ultrasonography or suggested by hematological parameters in up to 15% of APS1 cases [6]. It may be congenital or acquired, secondary to progressive autoimmune-mediated destruction or vascular insult to the spleen. It is suspected by a typical blood smear including Howell–Jolly bodies and thrombocytosis. It causes an additional secondary immunodeficiency, rendering subjects susceptible to pneumococcal sepsis.

Rarer associations: several cases of selective IgA deficiency and hypergammaglobulinemia have been reported [14]. Many patients have tuberculin anergy but whether this indicates an abnormal susceptibility to tuberculosis is unclear. Impairment of renal function, due to interstitial nephritis or iatrogenic nephrocalcinosis, was reported in more than 5% of Finnish cases and necessitates transplantation in some cases [6]. Neoplasia, most commonly squamous carcinoma of the oral mucosa (in subjects with chronic oral *Candida* who smoke cigarettes) and adenocarcinoma of the stomach, is also seen. Other rare manifestations are listed in Table 16.2.

Sudden death is well recognized in established APS1 patients, their siblings, and from post-mortem studies of subjects in whom the diagnosis was not suspected [2,5,8]. It is presumed that these deaths are due to undiagnosed adrenal failure, fulminant sepsis, hypoparathyroidism, or a combination of these.

Genetics

The gene defective in APS1 was identified by positional cloning in 1997 and is located on chromosome 21q22.3. It is named the autoimmune regulator or *AIRE* gene [17,18]. *AIRE* encodes a putative nuclear protein, containing several motifs suggestive of a transcription factor, including two zinc fingers. It is expressed in a variety of tissues of the immune system but particularly in the epithelial antigen-presenting cells in the thymus, where it is thought to play an important role in the central induction of self-tolerance, being involved in the negative selective of potentially autoreactive thymocytes [5,19].

Over 45 different disease-causing mutations have now been described in the *AIRE* gene [2,4,5,7,17,18,20]. These include point mutations, insertions, and deletions, and are spread through the whole coding region of the gene. Mutations affecting splice sites have also been reported. The most frequent *AIRE* mutations include the founder Finnish mutation in exon 6 (R257X) [17,18] and the common northern European mutation in exon 8 (964del13) [20]. This 13-bp deletion is seen frequently in Norwegian patients and in whites from the USA and UK [20], where it accounts for more than 70% of all mutant *AIRE* alleles. Many patients with this 13-bp deletion carry the same haplotype over the 21q.22 region, which is evidence for a founder effect. The common Finnish *AIRE* mutation is also fairly prevalent (5–30%) in other subjects of white European ancestry [4,5]. Additional common mutations are found in isolated populations such as a mutation in exon 3 (R139X) found in Sardinians [7] and a mutation in exon 2 (Y85C) in the Iranian Jewish population [2]. In several instances, only one mutant allele of the *AIRE* gene has been reported in typical APS1 patients, suggesting that the second

mutation might be located in the regulatory regions of the gene. Most of these mutations are believed to form null alleles that lead either to the synthesis of a truncated product or to the production of a nonsense transcript with a rapidly degraded mRNA. Missense mutations in the amino terminus of the protein may inhibit function by preventing dimerization [21].

It is possible that the specific manifestations that develop in a particular APS1 patient may depend on alleles at other loci such as human leukocyte antigens (*HLA*), because the same *AIRE* mutations are associated with varying phenotypes. No consistent associations between APS1 manifestations and *HLA* alleles have been found, but *HLA-A28* shows a weak association with hypoparathyroidism, keratopathy, and alopecia in this disease [2], and *HLA-A3* with ovarian failure [22]. Addison disease has been associated with *HLA DRB1*03* and alopecia with *HLA DRB1*04*, *DQB1*0302*. Type 1 diabetes shows negative correlation with *DRB1*15*, *DQB1*0602* [23]. Thus, *HLA* polymorphisms may explain some of the variability in phenotype seen in APS1. No correlation between cytotoxic T lymphocyte antigen 4 (*CTLA4*) gene polymorphisms and APS1 have been found to date [9]. The factors determining an individual phenotype are not understood, and it is likely that there are several loci involved.

Autoantibodies and pathogenesis

The pathogenesis of many of the manifestations of APS1 is unclear, but autoimmunity is involved in the development of the endocrinopathies, and patients have circulating autoantibodies to a variety of antigens from other affected tissues.

Steroid 21-hydroxylase (P450c21) and cholesterol side-chain cleaving enzyme (P450scc) are the major adrenal autoantigens; P450scc is the major gonadal autoantigen in APS1 patients [24]. Antibodies against at least one of P450scc, steroid 17α-hydroxylase (P450c17), and P450c21 were found in 81% of APS1 patients with and in 21% of those without adrenal failure. The presence of antibodies for at least one of these three enzymes correlates significantly with gonadal failure in female but not male patients [4,5]. This is possibly due to the blood–testis barrier protecting the steroidogenic cells of the testis from immunological attack. Adrenal cell autoantibodies are frequently detectable in patients with candidiasis or hypoparathyroidism without adrenal failure. These patients are almost certain to develop adrenal failure [9].

Autoantibodies to the extracellular domain of the calcium-sensing receptor have been reported in idiopathic hypoparathyroidism including some subjects with APS1 [25]. This has not been replicated in several other studies [11,24], probably because of differences in assay technique [26].

Glutamic acid decarboxylase (GAD) 65 autoantibodies have been found in 75% of patients with diabetes up to 8 years before the onset, but these are non-specific and are also found in 40% of non-diabetic APS1 patients [27]. Antibodies against the IA-2 tyrosine phosphatase-like protein and insulin are less common in these patients compared with non-APS1-associated type 1 diabetes but have higher specificity (96–100%) [11]. Circulating antithyroid antibodies have been found to be a poor marker for predicting hypothyroidism in APS1 [4].

The main autoantigens for hepatitis in APS1 appear to be cytochrome P450 1A2 (CYP1A2), P450 2A6 (CYP2A6), and aromatic L-amino acid decarboxylase (AADC) [28]. CYP1A2 in particular appears to be a highly specific but insensitive marker for APS1 hepatitis [28]. Liver–kidney microsomal (LKM) autoantibodies have been found in 50% of APS1 patients with chronic active hepatitis and 11% of APS1 patients without increased levels of hepatic enzymes [28]. Other hepatic autoantigens associated with non-APS1 autoimmune hepatitis, such as smooth muscle and antinuclear antibodies, are not found [28]. Tryptophan hydroxylase autoantibodies have been found to be the best predictor of autoimmune hepatitis in APS1 [24]. Although a rise in antibody titers to liver antigens may predate biochemical evidence of liver disease, raised autoantibodies are not found in all APS1 patients with autoimmune hepatitis at biopsy [16]. This, together with the broad spectrum of autoantigens found, suggests heterogeneity in pathogenesis, as well as outcome.

Anti-parietal cell and intrinsic factor autoantibodies precede parietal cell atrophy. Villous atrophy is associated with reticulin and/or endomysial autoantibodies [4]. Gastrointestinal dysfunction has been associated with autoantibodies to tryptophan hydroxylase (48% cases), histidine decarboxylase, and GAD 65 [24]. Vitiligo in APS1 is associated with the presence of complement-fixing melanocyte autoantibodies [4] and has been associated with antibodies to the transcription factors SOX9, SOX10, [29] and AADC autoantibodies [24]. Antibodies to tyrosine hydroxylase are found in APS1 subjects with alopecia areata [9].

Measurement of autoantibodies is of limited use in patients with APS1 in determining their risk of developing new components because the sensitivity of the antibody test may frequently be less than the patient's pre-existing risk of the complication. There are, however, certain autoantibodies that are almost exclusive to APS1, particularly AADC, CYP1A2, tyrosine hydroxylase, and tryptophan hydroxylase. This unique spectrum of autoantibodies can thus help to differentiate APS1 and other autoimmune diseases [24] (Table 16.3).

Diagnosis of APS1

Perheentupa found the classic criterion (two out of three cardinal manifestations) to be fulfilled by 5 years in only 22% cases, by 10 years in 67%, by 20 years in 89% and by 30 years in 93.5% [6]. In order to make a prompt diagnosis, all the different disease components should be considered. Suspicion should be high in patients under 30 years with mucocutaneous candidiasis, hypoparathyroidism, adrenal

Table 16.3. The identified autoantigens in APS1 for the commoner disease components.

Disease component	Autoantigens
Major manifestations	
Addison disease	P450c21, P450scc, P450c17
Hypoparathyroidism	Possibly the calcium-sensing receptor
Minor manifestations	
Gonadal failure	P450c17, P450scc
Type 1 diabetes	GAD65, insulin, 1A-2
Hashimoto thyroiditis	Thyroid peroxidase, thyroglobulin
Graves' disease	TSH receptor, thyroid peroxidase
Autoimmune hepatitis	CYP1A2*, CYP2A6, AADC*, LKM
Autoimmune gastritis/pernicious anemia	H/K-ATPase of gastric parietal cells, intrinsic factor
Celiac disease	Transglutaminase, gliadin
Gastrointestinal dysfunction	TPH*, histidine decarboxylase, GAD65
Vitiligo	SOX9 and SOX10, AADC*
Alopecia	Tyrosine hydroxylase*

Those marked * are almost exclusive to APS1 and thus helpful in differentiating APS1 from other autoimmune diseases [4,5,9,11,16,24,25,27–29].
P450c21, steroid 21-hydroxylase; P450scc, cholesterol side-chain cleaving enzyme; P450c17, steroid 17α-hydroxylase; GAD65, glutamic acid decarboxylase 65; 1A-2, tyrosine phosphatase-like protein 1A-2; CYP1A2, cytochrome P450 1A2; CYP2A6, cytochrome P450 2A6; AADC, aromatic L-amino acid decarboxylase; LKM, liver–kidney microsomal; TPH, tryptophan hydroxylase; SOX, transcription factors.

failure, ectodermal dystrophy, keratoconjunctivitis, prolonged diarrhea, vitiligo, or non-infectious hepatitis. Such patients should be checked for other manifestations, particularly the sometimes subtle nail signs of ectodermal dystrophy, oral, or ophthalmic components. DNA screening for *AIRE* mutations and an autoantibody screen should be considered in subjects with an atypical presentation.

There is often no clinical value in DNA analysis in subjects with two or more cardinal features, but the molecular findings in a proband will be of value in counseling and for screening siblings. All patients with established APS1 and those with one or more suspicious features need close follow-up for the development of new components. Their siblings should also be examined, as one of the cardinal manifestations or definite ectodermal components is diagnostic.

Diagnosis is often delayed, perhaps because of the long interval between development of the first and second manifestation. Up to two-thirds of patients are not diagnosed until admission to hospital with acute adrenal insufficiency or hypocalcemic crisis, and nearly half of these already have one major component of APS1 present [5]. Increased awareness of APS1 is essential to prevent fatalities. Mutational analysis has aided the early diagnosis of APS1, but it must be remembered that there are a large number of possible mutations and, in the UK, only the commonest two are routinely screened.

Table 16.4. Investigations recommended in the routine follow-up of APS1 patients to attempt to identify early development of new complications.

Disease component	Blood screening investigation
Major manifestation	
Addison disease	U&E, ACTH, plasma renin activity, annual synacthen test
Hypoparathyroidism	Serum calcium, phosphate, and magnesium
Minor manifestation	
Hypogonadism	Gonadotropin levels
Type 1 diabetes	Glycosylated hemoglobin
Autoimmune thyroid disease	fT3, fT4, and TSH
Autoimmune hepatitis	Liver function tests
Atrophic gastritis/pernicious anemia	FBC*
Hypo/asplenism	FBC*, blood smear†

*The presence of anemia on full blood count (FBC) results needs further investigation with ferritin, transferrin, and serum iron levels if the anemia is microcytic, and vitamin B12 levels if macrocytic.
†A blood smear indicating hypo/asplenism (Howell–Jolly bodies, anisocytes, poikilocytes, target cells, and burr cells) and/or the presence of thrombocytosis needs follow-up with an abdominal ultrasound to assess spleen presence and size.

Thus, APS1 is not excluded by negative routine DNA analysis, and the presence of one abnormal allele in a child with a major or minor manifestation makes the diagnosis highly likely. The individual disease components of APS1 should be recognized by the standard endocrine surveillance methods. The search for antibodies predicting new diseases can be an additional tool in aiding early diagnosis (Table 16.3).

Follow-up

The most important goal of this is the recognition of new disease components, which is essential as some manifestations are life threatening. These patients should be seen three or four times a year. Each visit requires a thorough history and examination, particularly for oral mucocutaneous candidiasis and signs of evolving adrenal insufficiency, such as postural change in blood pressure. Blood should be taken for basal hormone, hematological, and biochemical markers, and an occasional antibody screen performed (Table 16.4). This, together with a high index of clinical suspicion, allows earlier diagnosis and treatment of additional components as they develop.

The early diagnosis of Addison disease is of particular importance. Individuals at risk need an annual measurement of ACTH until adrenocortical failure develops [2]. Plasma renin activity should be measured at the same time. Adrenal failure can evolve rapidly in APS1, and annual assessment may not be sufficient to prevent acute presentations. The patient, immediate family, and primary health care team

must be made aware of the signs and symptoms of adrenal failure [8]. Postural blood pressure and serum electrolytes should be determined at each clinic visit, together with periodic screening for 21-hydroxylase autoantibodies.

Treatment

Treatment of the individual disorders is no different from treating patients with the isolated disorders, except that polypharmacy is the rule, and malabsorption may complicate therapy. The different endocrine failures are managed by conventional hormonal replacement, which may be complex when a patient has several endocrine deficiencies. Immunosuppressive treatment with glucocorticoids can also complicate matters. Professional psychological support is needed for many patients. A high rate of depression, social isolation, alcoholism, and substance misuse is reported, particularly as patients reach adulthood.

Mucocutaneous candidiasis is treated with local and/or systemic antifungal drugs, dental care, and oral hygiene, with expert oral surgical follow-up for refractory cases. Suppression of oral candidiasis is important because of the risk of oral carcinoma. Fluconazole or ketoconazole is indicated if topical treatments fail. Itraconazole is preferable to treat nail candidiasis but requires a course of 4–6 months [4]. These drugs can cause transient elevation of liver enzymes and occasionally hepatitis, so close monitoring is required. Ketoconazole is a global P450 cytochrome inhibitor and so can precipitate decompensation in patients with marginal adrenal reserve.

Patients with adrenal failure and/or hypoparathyroidism are managed as described in Chapters 15 and 13 respectively. The serum calcium levels of APS1 patients appear to be labile compared with non-APS1 hypoparathyroidism, and serious hypercalcemia can occur despite previous long periods of normocalcemia. Although standard doses of calcitriol or alfacalcidol can be used initially (20–50 ng/kg/day), patients with APS1 often require much larger doses of vitamin D analogs to maintain eucalcemia (3–5 μg/day being not unusual). This is presumed to be due to malabsorption, and the intermittent nature of this can lead to marked hypercalcemia with rapid onset of renal impairment.

Our practice is to monitor serum calcium and phosphate levels 8-weekly with regular determinations of urinary calcium excretion. Standard treatment with vitamin D analogs often leads to hypercalciuria, so serum calcium levels need to be maintained at around the lower end of the normal range (2.0–2.2 mmol/L total serum calcium). The vicious cycle of hypocalcemia and malabsorption can usually be broken by an increased oral dose, but parenteral therapy may be required in severe situations. Refractory cases may benefit from monthly intramuscular (IM) injections of calciferol to maintain basal levels, in addition to the daily use of a short-acting sterol. Hypomagnesemia may contribute to resistance and require treatment. Owing to the prevalence of nephrocalcinosis, our practice is to perform occasional (≈ 3- to 5-yearly) renal ultrasonography in subjects with hypoparathyroidism, taking the additional opportunity to assess the gallbladder and the size of the spleen. In patients with adrenal insufficiency, alteration of the cortisol dose will lead to an alteration in calcium absorption. Also of note is that unexplained hypercalcemia may be the first sign of the development of adrenal failure.

There is a lack of prospective data regarding the treatment and outcome of APS1-associated hepatitis. Autoimmune hepatitis is treated with immunosuppressive therapy, most experience being with the use of prednisolone and/or azathioprine. Liver transplantation has occasionally been reported in APS1-associated hepatitis [16]. Immunosuppressive therapy may increase the risk of *Candida*-related cancer and predispose the patient to generalized candidal infection [4]. Immunosuppressants are occasionally required for severe intestinal dysfunction with diarrhea, and there can be an associated improvement in control of serum calcium levels. The use of prednisolone with azathioprine, methotrexate, or cyclosporin A has been reported with varying symptomatic benefits [6]. Milder diarrhea has been found to respond to gut motility-reducing agents such as loperamide. Oral bile acid replacement therapy may help with fat malabsorption in patients with steatorrhea resulting from cholecystokinin deficiency [15].

Live vaccines must be avoided in view of the underlying immunodeficiency [6] but, as splenic atrophy is a common component, all APS1 patients should receive polyvalent pneumococcal vaccine with measurement of antibody response 6–8 weeks later. Non-responders or those who are asplenic should receive prophylactic daily antibiotics [8].

Prognosis

Many patients feel chronically unwell, and the physical and psychological impact of the multiple problems should not be underestimated. Despite improved survival, mortality rates are still high at 10–20%. Death is from a variety of causes including adrenal crisis, diabetic ketoacidosis, fulminant hepatic failure, oral carcinoma, septicemia, hypocalcemia, generalized candidal infection during immunosuppressive treatment, complications of kidney failure, and alcoholism [2,4,6]. Around 3% die before the diagnosis of APS1 has been made, with adrenal failure the likely cause. Suicide is high among this patient group. Working capacity may be maintained in subjects with a limited number of manifestations, but many are significantly incapacitated [6].

Summary

The clinical presentation of APS1 is very variable. Diagnosis can be difficult initially when only one manifestation is

Table 16.5. Classification of APS3 [9].

Autoimmune thyroid disease plus	Autoimmune endocrinopathy excluding Addison disease, e.g. type 1 diabetes, premature ovarian failure, lymphocytic hypophysitis	3A
	Autoimmune gastrointestinal disease, e.g. pernicious anemia, celiac disease, autoimmune hepatitis	3B
	Skin or neurological manifestations, e.g. alopecia, vitiligo, myasthenia gravis	3C
	Connective tissue disease, e.g. SLE, rheumatoid arthritis, Sjögren syndrome	3D

SLE, systemic lupus erythematosus.

present, and it often takes years for others to appear. Increased awareness of the condition, combined with analysis of specific autoantibodies and mutational analysis of the *AIRE* gene, should help to diagnose this condition earlier and prevent serious and fatal complications.

Autoimmune polyglandular syndrome type 2 and associated disorders

Definition

APS2 is defined by the presence of primary adrenocortical insufficiency with either autoimmune thyroid disease or type 1 diabetes in the same individual. An autoimmune origin of all the major components should be demonstrated for the correct diagnosis of APS2. The association of autoimmune Addison disease and autoimmune thyroid disease is known as Schmidt syndrome, and the association of Addison disease with type 1 diabetes is also called Carpenter syndrome. Other endocrine and non-endocrine autoimmune disorders occur with increased frequency in these individuals and their families [1].

APS3 is defined as the association between autoimmune thyroid disease and an additional autoimmune disease other than Addison disease [9]. Many clinical combinations can be found in APS3, and it can therefore be subdivided into 3A to D, depending on the associated conditions (Table 16.5) [9]. Some authors use the term APS4 to encompass an association of autoimmune diseases not falling into the categories APS1–3 [9]. Many of these patients develop more classical APS2/3 manifestations later, and this classification describes an extremely heterogeneous group of patients. We feel that there is little clinical benefit in its use, and that it is generally more helpful to describe the individual components.

APS2

Clinical features and course

APS2 is rare, with an estimated prevalence of 4–5/100 000 [30,31]. Clinical presentation can be at any age but is most frequently in early adulthood, with a peak onset in the fourth decade. It is recognized less commonly in children and adolescents. It affects both sexes, with a female–male ratio of 3:1 [30].

Major manifestations

By definition, Addison disease is present in 100% of APS2 cases. Autoimmune thyroid disease occurs in 70–80% and type 1 diabetes in 30–50% [1,9,32]. Only about 10% have the complete triad [9]. Adrenal failure is the first endocrine abnormality in around 50%, but several minor APS2 components are often present at the diagnosis of adrenal failure, raising the possibility of APS2. On presentation with Addison disease, type 1 diabetes already exists in around 20% and autoimmune thyroid disease in around 30%, but they may present more than 20 years before the diagnosis of adrenal failure. Autoimmune thyroid disease encompasses a variety of thyroid disorders, including Hashimoto thyroiditis, atrophic hypothyroidism, Graves' disease, and post-partum thyroiditis. Hypothyroidism is commoner than Graves' disease, but Graves' disease tends to present at a younger age in the context of APS2.

Delayed diagnosis and preventable deaths still occur in patients with undiagnosed adrenal failure. Signs and symptoms are often vague and non-specific until an adrenal crisis ensues. Low morning serum cortisol concentrations and electrolyte abnormalities (hyponatremia and hyperkalemia) represent late changes, occurring at or just before the onset of clinical adrenal insufficiency. Hyperpigmentation may be observed but may be absent in fair or red-headed subjects. Adrenal insufficiency often presents as hypoglycemic seizures in children.

In those who already have type 1 diabetes, deterioration of glycemic control with recurrent hypoglycemia can be the presenting sign. The onset of autoimmune hyperthyroidism or thyroxine replacement for newly diagnosed hypothyroidism leads to enhanced cortisol clearance and can precipitate adrenal crisis in subjects with subclinical adrenocortical failure. Clinicians should maintain a high degree of alertness for underlying adrenal failure before initiating thyroid hormone replacement. Conversely, cortisol inhibits thyrotrophin release, so thyroid-stimulating hormone (TSH) levels are often high at the initial diagnosis of adrenal insufficiency (typically 5–10 mU/L) but return to normal after initiation of glucocorticoid replacement in the absence of co-existent thyroid disease. Adrenal insufficiency can mask the hyperglycemia of type 1 diabetes.

Table 16.6. Minor manifestations frequently associated with APS2 [9,32,33].

	Frequency (%)
Minor manifestation	
Pernicious anemia	1–25
Gonadal failure	
Females	3.5–10
Males	1–2
Vitiligo	4–11
Alopecia	2–5
Autoimmune hepatitis	4
Malabsorption (including celiac disease)	1–2
Sjögren syndrome	1
Neoplasias	3
Rarer manifestations	
Endocrine	*Neurological*
Pituitary involvement	Myositis
Hypophysitis	Myasthenia gravis
Empty sella syndrome	Neuropathy
Late-onset hypoparathyroidism	Stiff man syndrome
Gastrointestinal	*Other*
Ulcerative colitis	Sarcoidosis
Primary biliary cirrhosis	Serositis
Dermatological	Selective IgA deficiency
Granuloma annulare	Idiopathic heart block
Dermatitis herpetiformis	Idiopathic thrombocytopenia purpura

Minor manifestations

These are listed in Table 16.6 together with their frequency. All these associated autoimmune disorders are present at lower frequency in APS2 compared with APS1, and they are usually associated with their respective immunological markers. Primary hypogonadism is one of the commonest minor manifestations in APS2/3 females, with premature ovarian failure leading to secondary amenorrhea in around 10% of women under 40 years. Gonadal failure is very rare among males with APS2/3 [33]. Pituitary involvement is very occasionally seen in APS2/3, with lymphocytic hypophysitis leading to empty sella syndrome, panhypopituitarism, or isolated failure of any of the anterior pituitary hormones [34].

In contrast to APS1, hypoparathyroidism is very rare in APS2/3. If hypocalcemia does occur in APS2, celiac disease is the most likely reason, and the finding of an elevated PTH concentration in the latter will distinguish the two. Hypoparathyroidism has been described in a few adult patients with parathyroid-suppressing antibodies [25,33], often co-existing with autoimmune thyroid disease. Autoimmune hypoparathyroidism in childhood is almost pathognomic of APS1.

Incomplete APS2

Patients with autoimmune thyroid disease or type 1 diabetes and adrenal autoantibodies in the serum or patients with Addison disease and either thyroid and/or islet cell autoantibodies are sometimes classified as incomplete APS2 [9]. Self-evidently, these patients may develop APS2 in the future, particularly those with evidence of subclinical disease such as an elevated TSH or impaired glucose tolerance. Annual screening by ACTH and renin measurement, together with education about the likely presentation of adrenal failure, is recommended for such individuals. About 30% of subjects with positive adrenal antibodies progress to adrenal failure over a 6-year period [35]. Patients with either autoimmune thyroid disease or type 1 diabetes alone, but who have a sibling with APS2, are also classified by some authors as having incomplete APS2, because of their possible higher risk of adrenal failure [9].

APS3

APS3 is defined as the association between autoimmune thyroid disease and autoimmune disorders other than Addison disease. Hashimoto thyroiditis is the commonest form of autoimmune thyroid disease, although Graves' disease and post-partum thyroiditis are also seen. Autoimmune thyroid diseases tend to increase in incidence in the teenage years, with a peak in the fourth decade for Graves' disease and in the fifth and sixth decades for autoimmune hypothyroidism. Autoimmune thyroid disease is most commonly isolated, and polyglandular involvement in the form of APS3 or APS2 is rare (≈ 5%). Only 1% of patients with isolated autoimmune thyroid disease have adrenal autoantibodies (with risk of APS2), whereas 3–5% have either pancreatic islet autoimmunity and/or clinical type 1 diabetes [36].

Autoimmune thyroid disease is more commonly associated with pernicious anemia, vitiligo, alopecia, myasthenia gravis, and Sjögren syndrome, and autoimmune thyroid disease should be sought prospectively in patients with these conditions. Around 30% of subjects with vitiligo have another autoimmune disorder, with autoimmune thyroid disease and pernicious anemia being the most common. Many patients with vitiligo are asymptomatic, and other autoimmune diseases are diagnosed only by prospective screening, including evaluation of autoantibody status [33,37]. Up to 15% of patients with alopecia and nearly 30% of those with myasthenia gravis have autoimmune thyroid disease.

Genetics

APS2 is a genetically complex and multifactorial disease. It aggregates in families and appears to show an autosomal-dominant pattern of inheritance with incomplete penetrance in some [38]. Susceptibility is determined by multiple genetic loci that interact with environmental factors. Only two

genes have shown consistent association with APS2, *HLA* and *CTLA4*. Of these, *HLA* appears to have the strongest gene effect [1].

HLA and APS2

Many of the component disorders in APS2, including autoimmune thyroid disease, type 1 diabetes, Addison disease, celiac disease, myasthenia gravis, selective IgA deficiency, and dermatitis herpetiformis, are associated with the same extended *HLA* haplotype: *HLA-A1*, *HLA-B8*, *HLA DR3*, *DQA1*0501*, *DQB1*0201* (*DQ2*). Thus, unsurprisingly, *HLA DR3*, *DQB1*0201* is associated with APS2 [9,39]. Type 1 diabetes and, to a lesser extent, Addison disease also show association with *HLA DR4*, *DQA1*0301*, *DQB1*0302* (*DQ8*) [39,40], and *HLA DR5* shows association in patients with a combination of Addison disease and autoimmune hypothyroidism [9]. Some 35% of individuals with type 1 diabetes are heterozygous for the *HLA DR3*/*DR4* combination, with about 50% of children developing type 1 diabetes under 5 years having this combination of haplotypes.

Although specific *HLA* haplotypes influence susceptibility to APS component disorders, others appear to be protective. The haplotype *DR2* (*DRB1*1501*), *DQA1*0102*, *DQB1*0602* appears to provide dominant protection against type 1 diabetes, even in the presence of insulin autoantibodies [38]. Similarly patients with P450c21 autoantibodies and *DRB1*0401* and *DRB1*0402* appear to progress to adrenal failure less often [41].

CTLA4

CTLA4 encodes an important negative regulator of T-cell activation that is expressed on the surface of activated T lymphocytes. Alleles of *CTLA4* have been linked primarily to autoimmune thyroid disease, both Graves' disease and Hashimoto thyroiditis [42,43], but there is also a weaker effect in type 1 diabetes [40,44]. Addison disease (either isolated or as part of APS2) has been shown to be associated with *CTLA4* alleles, particularly in a subgroup of patients carrying *HLA DQA1*0501* [42,45]. Other studies have shown association with Addison disease in certain populations only [40].

The association of the component disorders in APS2 is therefore, in part, related to the shared susceptibility alleles of *HLA* and *CTLA4* conferring risk to the different diseases. It is highly likely that there is a complex interaction between these and other unidentified loci and environmental factors.

Autoantibodies and pathogenesis

The pathogenesis of autoimmunity in APS2 is considered as a multifactorial or complex genetic trait, similar to that of the individual disease components. There are several hypotheses to explain why autoimmunity occurs against multiple organs in individuals with APS. It has been suggested that this may result from a shared epitope(s) between an environmental agent and a common antigen present in several endocrine tissues [46], or that the organs derived from the same germ layer expressing common germ layer-specific antigens could serve as targets for the autoimmune response in APS [47]. More likely, there is a subtle thymic defect of negative selection of autoreactive T cells, caused either by a defect in T-cell apoptosis or by a problem in thymic antigen presentation. This may be most severe for low-abundance, specialist antigens, such as those needed for the biosynthesis, secretion, and regulation of the various hormones.

At the onset of autoimmune adrenal failure, adrenal cell autoantibodies or P450c21 autoantibodies are detectable in > 90% of patients [9,31]. P450c21 has been identified as the major adrenal antigen in autoimmune adrenalitis, and these antibodies are present in 80–90% of patients with disease duration under 15 years, declining to 60% with disease duration over 15 years. These P450c21 autoantibodies are highly specific, being found in only 0.5% of healthy subjects and those with other autoimmune diseases. Some 40–50% of patients with such adrenal autoantibodies have abnormal ACTH stimulation tests. Thus, P450c21 autoantibodies have a high predictive value for clinical Addison disease [31]. Spontaneous disappearance of adrenal antibodies has been reported in up to 20% of cases [31], but disease is permanent in patients who have an abnormal ACTH stimulation test.

Other steroid-producing cell autoantibodies (SCA), such as P450c17 and P450scc, are present in 20–30% patients with Addison disease and are more frequent in females than males [31,48]. There is a strong association between the presence of SCA and ovarian failure in women with APS2/3, but SCA are extremely rare in women with ovarian failure with no signs of adrenal autoimmunity [31,48]. Because of the shared antigens of the steroidogenic enzymes, adrenal autoimmunity is more common (≈ 10%) in those subjects with established gonadal failure.

Autoimmune thyroid disease or type 1 diabetes is a frequent component of APS2. Thyroperoxidase (TPO) and thyroglobulin (TG) are the major thyroid antigens. In Hashimoto thyroiditis, TPO autoantibodies are found in 90–100% and TG autoantibodies in 60–70%. They are both also frequently found in Graves' disease, where TSH receptor autoantibodies are found in ≈ 90% of cases [33]. Many patients with thyroid autoantibodies alone progress slowly to clinical disease [49].

Islet cell autoantibodies are found in around 80% of new-onset type 1 diabetes patients [31]. The main islet autoantigens are insulin, GAD65, and the tyrosine phosphatase-related protein IA-2. Among recently diagnosed subjects with type 1 diabetes, the prevalence of antibodies to insulin and IA-2 is dependent on age, being detected in most children and adolescents with type 1 diabetes, but less than 30% with adult onset. The frequency of antibodies to GAD65 is 70–80% and is not influenced by age; this therefore gives the

highest diagnostic sensitivity in adulthood [50]. In one investigation, all APS2 patients with type 1 diabetes were positive for GAD65 antibodies, but only 54% of those with antibodies had type 1 diabetes. In comparison, IA-2 antibodies are less sensitive but more specific for type 1 diabetes [31].

Gastric parietal cell autoantibodies are found in about 90% of patients with chronic autoimmune gastritis or pernicious anemia [51], and in 30% of their non-anemic first-degree relatives. The major autoantigen is gastric H/K-ATPase. Around 70% of patients with pernicious anemia are also positive for intrinsic factor autoantibodies that block the binding of vitamin B12 to intrinsic factor [33]. Tissue transglutaminase (TTG) is the major autoantigen in celiac disease. IgA TTG antibodies are more specific for celiac disease than IgG, but both have a high diagnostic sensitivity and specificity. There is good correlation between endomysial autoantibodies and TTG antibodies [33].

Diagnosis and follow-up

Once APS2/3 is suspected, a full assessment of endocrine function is needed. The number of disorders that will develop and the age at which they will present is unpredictable, so long-term follow-up is needed. A high clinical index of suspicion needs to be maintained, particularly in those subjects who have yet to develop adrenal failure or diabetes. Presymptomatic recognition of autoimmune disease minimizes associated morbidity and mortality. There is a clear link between the presence of organ-specific autoantibodies and the progression to disease, although there is often an asymptomatic latent period of months or years. The absence of autoantibodies does not exclude the risk of a disease component.

In any patient with clinical and biochemical signs of adrenal insufficiency, determination of P450c21 autoantibodies demonstrates the autoimmune nature of the disease [33]. An etiological diagnosis should be sought in all subjects, but the presence of autoimmune disorders in family members is suggestive of autoimmunity. In all patients with Addison disease, there is a need to screen for other endocrine disorders, particularly autoimmune thyroid disease and type 1 diabetes. At diagnosis, screening for TPO and GAD65 autoantibodies is worthwhile. If negative, this should be repeated occasionally, perhaps every 2–3 years. In children or adolescents with Addison disease, determination of insulin and IA-2 autoantibodies is a sensitive predictor of type 1 diabetes, particularly if both autoantibodies are present. If these β-cell autoantibodies are found, an assessment of fasting blood glucose and, in some cases, an oral glucose tolerance test are required.

The determination of thyroid function should be carried out at least annually for early recognition of thyroid disease in all subjects with type 1 diabetes and Addison disease. The determination of P450c17 and P450scc antibodies in females with Addison disease and APS2 may identify subjects at high risk from primary hypogonadism before gonadotropins become elevated. Such subjects may be suitable for cryopreservation of ovarian material.

The determination of P450c21 autoantibodies should be performed in children presenting with type 1 diabetes as positive adrenal autoantibodies are highly predictive of future adrenal insufficiency [31]. In subjects with P450c21 autoantibodies, an ACTH stimulation test, determination of electrolytes and plasma renin activity enables identification of patients with preclinical adrenal dysfunction. If normal, the ACTH stimulation test should be repeated yearly with interval determination of postural blood pressure and electrolytes. Regardless of antibody status, patients with persistent or worsening symptoms after treatment of autoimmune thyroid disease and subjects with type 1 diabetes who have brittle control or persistent lethargy or those with unexplained vague symptoms should be screened biochemically for Addison disease.

An increased frequency of IgG–TTG antibody has been found in type 1 diabetes children, but the prevalence in adult Addison or type 1 diabetes patients is the same as in the healthy population. Thus, they should be included in APS2/3 screening of children but limited in adults to cases with clinical or laboratory signs of malabsorption. Positive levels in children require follow-up with an intestinal biopsy to confirm the diagnosis of celiac disease. The predictive value of gastric parietal cell or intrinsic factor autoantibodies for autoimmune gastritis and pernicious anemia is limited by the frequent occurrence of these in healthy first-degree relatives and in the general population (≈ 5–10%). A blood count to detect macrocytosis is a more useful routine investigation, although neurological features of vitamin B12 deficiency can be present in the absence of anemia. Thus, vitamin B12 levels should be measured urgently if clinically suspected.

Screening for APS2-associated disorders should also be performed in women with primary or secondary amenorrhea or premature ovarian failure and young patients with vitiligo. As APS2 shows strong familial tendencies, family members should also be checked for features of associated endocrine conditions.

Management

Hormone replacement or other therapies for the component diseases of APS2 are similar whether the disease occurs in isolation or in association with other conditions, and disorders should be treated as they are diagnosed. However, certain combinations of diseases require specific attention. Most importantly, thyroxine therapy for hypothyroidism can precipitate a life-threatening adrenal crisis in a patient with untreated and unsuspected adrenal insufficiency. Thus, to avoid adrenal crisis, clinicians should maintain a high

degree of suspicion for co-existing adrenal failure in subjects who are hypothyroid. Hyperthyroidism increases cortisol clearance so, in patients with adrenal insufficiency who have unresolved hyperthyroidism, glucocorticoid replacement should be at least doubled until the patient is euthyroid. Decreasing insulin requirements or increasing occurrence of hypoglycemia in type 1 diabetes can be one of the earliest indications of adrenocortical failure. One of the most important aspects of managing these patients is to be continually alert to the possibility of the development of further endocrinopathies to insure early diagnosis and treatment.

Prognosis

Mortality in patients with primary adrenal insufficiency has not been thoroughly studied. Life expectancy is often reduced as a consequence of unrecognized adrenal crisis, underlying illness, and as yet unidentified other causes. Despite adequate hormonal replacement, quality of life is often impaired in these patients, with predominant complaints being unpredictable fatigue, lack of energy, depression, and anxiety. It has been shown that the number of patients receiving disability pensions is two- to threefold higher than the general population in certain countries [52].

Summary

A high index of suspicion needs to be maintained whenever one organ-specific autoimmune disorder is diagnosed in order to prevent morbidity and mortality from the index disease as well as associated diseases. Further definition of susceptibility genes and autoantigens, as well as a better understanding of the pathogenesis, is required to improve the diagnosis and management of these patients.

Miscellaneous disorders with autoimmune endocrinopathies

Immune dysregulation, polyendocrinopathy, and enteropathy (X-linked) syndrome (IPEX)

IPEX is a rare and devastating X-linked condition of male infants, affecting immune regulation and resulting in multiple autoimmune disorders. The first feature is commonly intractable diarrhea and failure to thrive due to autoimmune enteropathy occurring around 3–4 months of age. Type 1 diabetes and autoimmune hypothyroidism develop in the first year of life in around 90% and 50% of males respectively. Additional clinical features include eczema, autoimmune hemolytic anemia, autoimmune thrombocytopenia, recurrent infections, lymphadenopathy, membranous nephropathy, and striking growth retardation. Other autoimmune features are less frequent [53]. Sepsis may result from a primary defect in immune regulation but is exacerbated by autoimmune neutropenia, immunosuppressive drugs, malnutrition, enteropathy, and eczema.

The condition is heterogeneous in its presentation, with the occasional case not presenting until later childhood or adulthood [54]. Diabetes or eczema is a not infrequent initial presentation, but any of the disease components can present first. There are no estimates of incidence, but it is likely to be underdiagnosed because of the clinical variability in presentation and the presence of frequent new mutations. Intermittent eosinophilia and raised IgE levels are found in many patients, but there is an absence of any other consistent features of immunodeficiency. The presence of autoantibodies appears to be variable. The most consistent pathological finding is total villous atrophy of the small intestine, with inflammatory cell infiltration of the lamina propria. Diagnosis relies on the clinical presentation, family history, and elimination of other diagnoses with similar presentations. Genetic screening has proved useful in some cases. There is a high mortality in these infants, many succumbing to the untreatable diarrhea, malnutrition, and superimposed infections by 24 months of age. Survival into adolescence is occasionally seen with the use of aggressive immunosuppression and parenteral feeding, although symptoms are rarely entirely relieved [53,55]. There are increasing reports of the use of bone marrow transplantation in these infants, but experience is very limited [53].

IPEX was first reported more than 20 years ago in a large family with typical X-linked recessive inheritance [54]. IPEX appears to be mediated by an abnormality in $CD4^+$ T-cell regulation, with evidence for increased T-cell activation and overproduction of cytokines. In comparison with a murine model, mutations in the *FOXP3* gene, located at Xp11, encoding a transcription factor belonging to the forkhead/winged-helix family, were found in IPEX boys [53]. An increasing number of mutations have been reported, mainly in the coding region of *FOXP3*, although one mutation in the regulatory region has also been found [53,55]. *FOXP3* is specifically expressed in naturally arising $CD4^+CD25^+$ regulatory T cells and appears to convert naïve T cells to this regulatory phenotype. Thus, *FOXP3* is a critical regulator of $CD4^+CD25^+$ T-cell development and function [56]. In a few cases, no mutation has been identified. Although female carriers of *FOXP3* mutations appear to be healthy, a small number of cases of an IPEX-like syndrome have been reported recently in families with affected girls in whom no mutation was found [55]. The molecular etiology is unclear, but it is possible that there may be an autosomal locus accounting for the problem in some families. This genetic heterogeneity may explain some of the clinical variation seen in this syndrome but, as yet, no obvious genotype–phenotype relationship has been identified, and other modifying genes, such

as *HLA*, as well as environmental factors may influence the outcome.

Autoimmune lymphoproliferative syndrome (ALPS)

ALPS was first described in 1967, although the etiology and pathogenesis of the condition were unknown [57]. Onset is usually in the first 2 years of life, and the characteristic feature in all cases is massive generalized lymphadenopathy. Hepatosplenomegaly and hematological autoimmunity (hemolytic anemia and thrombocytopenia) are also frequent manifestations. Other autoimmune conditions, including thyroid autoimmunity and type 1 diabetes, have occasionally been reported as part of this syndrome [58]. Characteristically, fever, infections, or immunosuppressive therapy lead to a decrease in the degree of lymphadenopathy and hepatosplenomegaly and an improvement in the autoimmune phenomena. ALPS tends to follow a chronic course, with the response to immunosuppressive drugs varying. Splenectomy is usually performed to reduce the lymphadenopathy and improve the thrombocytopenia and hemolytic anemia, although this leads to an increased risk of infections in patients who are often neutropenic. Long-term outcome is variable, although survival into adulthood has been reported, when an increase in malignancy is seen [59]. Allogenic bone marrow transplantation has been found to be a successful treatment in a few children.

Mutations of the Fas receptor or of its ligand FasL are responsible for ALPS type 1a and ALPS type 1b respectively [59]. ALPS type 2 is a clinical variant caused by mutations in the caspase-10 gene. Fas is a key receptor in the apoptotic pathway, and the binding of FasL to Fas leads to apoptosis by activating a series of events involving a group of proteases called caspases. The defective apoptotic function in ALPS leads to an accumulation of lymphocytes (particularly $CD3^+CD4^-CD8^-$), including potentially autoreactive cells.

Kabuki make-up syndrome (KMS)

KMS is a syndrome of unknown cause, although probably genetic, consisting of five characteristic manifestations: (1) dysmorphic face with eversion of the lower lateral eyelid, arched eyebrows with sparseness of their lateral one-third, long palpebral fissures with long eyelashes, depressed nasal tip, and prominent large ears (100%); (2) unusual dermatoglyphic patterns (96%); (3) skeletal abnormalities and hypermobile joints (88%); (4) mild to moderate mental retardation (84%); and (5) postnatal growth retardation with short stature (55%) [60]. Other well-recognized features include dental abnormalities, susceptibility to infections, particularly recurrent otitis media, cardiovascular anomalies, renal and urinary tract anomalies, biliary atresia, diaphragmatic hernia, and anorectal anomalies. Less common associations include growth hormone deficiency, primary ovarian dysfunction, Hashimoto thyroiditis, and vitiligo [58,60]. It has been recognized most commonly within the Japanese population (incidence 1:32 000), but it is now recognized in all countries. Patients often survive with a good prognosis unless they have severe complications such as cardiovascular, hepatic, or renal disease [60]. Males and females are affected equally, and most cases are sporadic, although a few familial cases have been reported. KMS may be inherited as an autosomal-recessive disorder. As yet, there is no evidence or clues to the underlying cause of the syndrome. The endocrinopathies should be treated along standard lines.

Conclusion

Diagnosis and management of the polyglandular syndromes has many dimensions. In the modern era, we should aim to use the powerful combination of clinical skills, autoantibody assays, and molecular genetic investigation, along with basal and dynamic endocrine testing, to institute early diagnosis and therapy for these clustering conditions. In the future, more accurate disease prediction may allow us to counsel individuals and families with greater certainty. Ultimately, when pathogenesis is more clearly understood, specific intervention to prevent endocrinopathy in those at high risk may become more than a theoretical possibility.

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17 Disorders of water balance

Rebecca P. Green, Joseph A. Majzoub, and Louis J. Muglia

Introduction

While water intake and excretion can vary widely in normal infants, children, and adults, plasma osmolality is maintained strictly within the range of 275–295 mOsm/kg. Plasma osmolality above or below the range results in alterations in intracellular solute concentrations, patterns of cellular depolarization, cell morphology, and critical aspects of cell function that can become life-threatening.

To limit excursions in osmolality, sensitive systems regulating water intake and excretion have evolved. Thirst controls water intake, and arginine vasopressin (AVP) controls urine concentration and thereby water excretion. These systems work in concert to preserve fluid balance and maintain plasma osmolality within the narrow range. Intact function of either thirst or vasopressin secretion can maintain normal plasma osmolality independently with adequate access to water. In addition to regulation of plasma osmolality, regulatory networks maintain extracellular and intracellular volumes. The regulation of extracellular fluid volume is primarily under the control of the renin–angiotensin–aldosterone system and occurs by modulation of sodium intake and excretion, in contrast to the regulation of osmolality by water intake and excretion.

Body water and electrolytes

Throughout life, water contributes the largest mass to the human body of any of its chemical components. The relation of water to total body weight changes significantly from birth to childhood and adulthood [1]. In term neonates and young infants, 75–80% of body weight is water, with 45–50% of body weight extracellular water and 30% of body weight intracellular water [2] (Fig. 17.1). During the first few days of life, there is a rapid diuresis of 7% of total body water from the extracellular compartment. This trend slows but continues over the first year of life so that the adult distribution of intracellular, extracellular, and total body water of 40%, 20%, and 60%, respectively, is achieved during childhood.

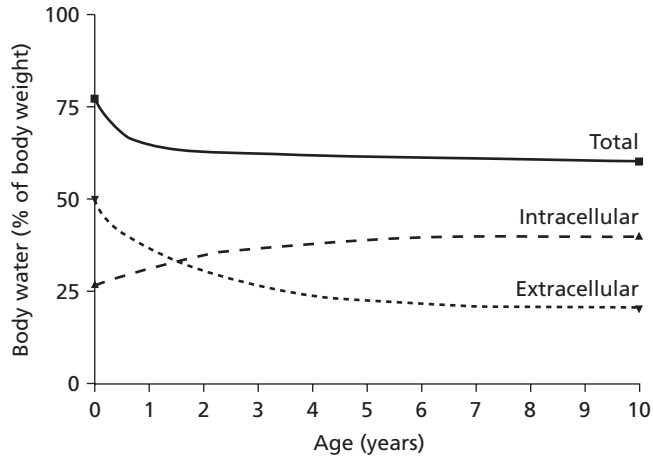

Fig. 17.1. Body water distribution between intracellular and extracellular compartments as a function of body weight in infants and children. Adapted from [1,2].

The large and consistent contribution of water to total body weight in healthy children and adults reflects a dynamic equilibrium achieved by the balance of fluctuating water intake and excretion. Daily water intake and loss can vary 10-fold between individuals and even within a given individual due to changes in diet, environmental conditions, and state of health (for example, increased losses with febrile illness or gastroenteritis). Water losses occur through the respiratory tract and skin, so-called insensible losses, the gastrointestinal tract and urine. Urine volume, and thus water losses, depends upon the amount of solute required to be excreted and the concentration of the urine in which it is excreted. In healthy infants and children, these parameters can vary over a wide range as a result of factors such as changes in the solute composition of infant formula, milk, juice, and other dietary components introduced over the first year of life, together with improved renal concentrating capacity over the first months [3].

Normal daily obligate solute excretion is approximately 500 mOsm/m^2/day. To excrete this solute in urine in the middle of the concentration range (osmolality

Table 17.1. Daily electrolyte requirements (additional adjustments should be made for abnormal losses).

Electrolyte	Amount
Sodium	20–50 mEq/m^2/day
Potassium	20–50 mEq/m^2/day
Calcium	
Term newborns	50–75 mg/kg/day
Infants	600 mg/day
Children	800 mg/day
Adolescents	1200 mg/day

Calcium figures are for oral intake.

500–600 mOsm/kg) would require approximately 900 mL/m^2/day urine. Combined with respiratory and skin losses of 750 mL/m^2/day, gastrointestinal losses of 100 mL/m^2/day, and gain of total body weight due to water of oxidation generated during metabolism of energy sources of 250 mL/m^2/day, this yields an average net loss of approximately 1500 mL/m^2/day. This volume is characteristically considered to be the amount of maintenance fluids to be administered to patients to maintain homeostasis. Under conditions in which urine cannot be diluted or concentrated to the mid-range, this maintenance volume can lead to overhydration or dehydration with resultant abnormalities in plasma osmolality.

When considering the amounts of water needed to maintain osmotic stability, it is also useful to consider daily electrolyte requirements to avoid depletion of electrolyte stores or excess solute diuresis (Table 17.1). This is particularly important when intravenous fluids are the main source of water and electrolytes. During fluid therapy of short duration (hours to a few days), sodium, potassium, and associated anions are the primary electrolytes that should be administered. With intravenous fluid therapy of longer duration, additional electrolytes such as calcium, magnesium, and phosphorus should be added.

Change in the ratio of water to solutes in intracellular and extracellular compartments, thus making a difference in osmolality between the compartments, results in rapid equalization of osmolality between them. Cell membranes are impermeable to electrolytes such as sodium and chloride, which constitute the main extracellular solutes, and potassium and phosphate, which constitute the main intracellular solutes, and they undergo active transport into and out of cells to establish their gradients. While these solutes differ in relative amounts intracellularly and extracellularly, the total solute concentration is the same at equilibrium on account of the unimpeded movement of water across most cell membranes. Thus, a change in the osmolality of one compartment results in an osmotic gradient that is removed by the rapid redistribution of water from one compartment to the other.

For example, the loss of water that exceeds the loss of sodium and chloride relative to the composition of normal plasma during some episodes of gastroenteritis results in a transient increase in extracellular sodium and its anions and in the osmolality of plasma and interstitial fluids. The osmotic gradient introduced between intracellular and extracellular compartments causes the net movement of water from the intracellular space to the extracellular space, evenly distributing the water loss throughout total body water and equalizing the osmotic gradient. Conversely, dilution of plasma sodium and its anions by rapid administration of hypotonic fluids results in the net movement of water from the extracellular compartment to the intracellular compartment to distribute the water gain throughout total body water. Chronic, as opposed to acute, changes in cell osmolality can result in cell adaptation by reversibly increasing or decreasing intracellular, impermeable solutes. Whether these adaptive changes might have occurred has to be considered when instituting therapy designed to correct hyponatremia or hypernatremia.

Physiology of osmotic regulation

To maintain plasma osmolality in the range that allows optimal cellular function requires sensitive mechanisms for detecting deviation from a normal set point and neural and biochemical pathways that implement a means of restoring the system to that normal set point. Osmosensors within the central nervous system modulate two effector pathways to maintain homeostasis, thirst to change water intake and posterior pituitary vasopressin secretion to alter renal water excretion.

Vasopressin and water excretion

Biochemistry

Arginine vasopressin (AVP) is the key hormone that regulates plasma osmolality by controlling renal free water excretion. AVP is a cyclic nonapeptide that, like its evolutionarily related counterpart oxytocin, consists of a six-member disulfide ring and a three-member tail on which the carboxy-terminal group is amidated (Fig. 17.2a). AVP differs from oxytocin only in replacement of phenylalanine for isoleucine at position 3 and of arginine for leucine at position 8 of the molecule. These subtle structural differences allow activation of receptors relatively specific for either AVP or oxytocin and thereby separation of biological effects. While AVP has potency in activating renal V_2-type vasopressin receptors, the affinity of oxytocin is two orders of magnitude lower [4], such that it cannot effectively compensate for loss of vasopressin in promoting free water reabsorption.

AVP is synthesized as a preprohormone composed of a signal peptide followed by the nonapeptide hormone, a tripeptide linker, a binding protein known as neurophysin II, a dipeptide linker, and a glycosylated peptide known as

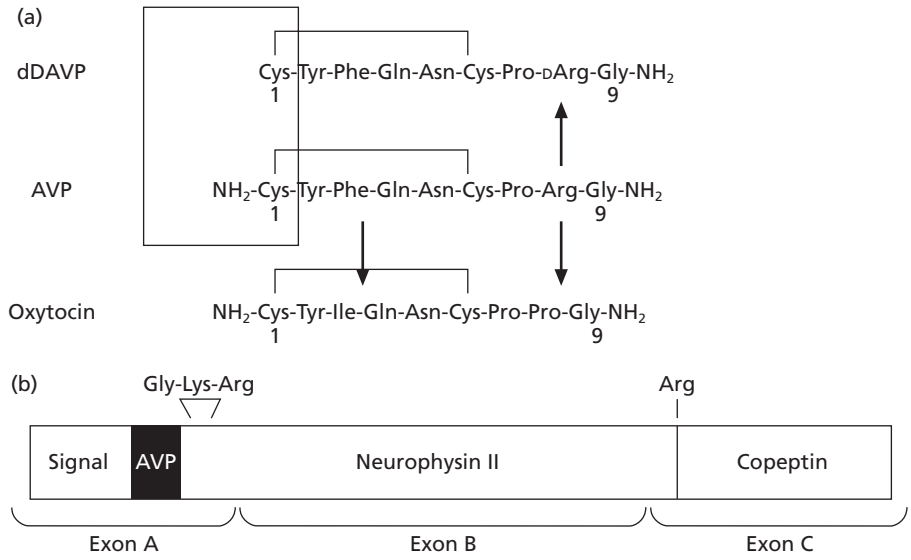

Fig. 17.2. AVP protein and gene structure. (a) Amino acid comparison of AVP and the structurally related molecules oxytocin and dDAVP. Amino acids are numbered from the amino-terminus of each molecule, and differences in amino acids between these molecules are shown by the arrows. The box indicates the deamidation of the amino-terminus in dDAVP compared with AVP. (b) Relationship of AVP to its preproAVP precursor. Intron–exon boundaries of the gene relative to the coding sequences are shown, as are the di- and monobasic cleavage sites essential for protein processing.

copeptin (Fig. 17.2b). The AVP gene, along with the adjacent oxytocin gene, is located on chromosome 20 in humans [5]. The structure of the oxytocin precursor is similar to that of AVP, except that it lacks the copeptin moiety found in provasopressin and encodes neurophysin I instead of neurophysin II. Despite their linkage in mammalian genomes and highest expression within the hypothalamus, the genes encoding AVP and oxytocin are expressed in different neurons [6].

The processing of preprovasopressin to the mature peptide has been studied extensively. During its synthesis, translocation of preprovasopressin into the endoplasmic reticulum first allows cleavage of the signal peptide. The neurophysin component promotes self-association during transition from the Golgi apparatus into the neurosecretory granules, initially as dimers [7]. The importance of neurophysin folding and oligomerization in the trafficking of provasopressin is highlighted by the condition of familial autosomal-dominant neurohypophyseal diabetes insipidus (ADNDI). ADNDI is most frequently due to mutations in neurophysin II that prevent proper targeting of the otherwise normal mature AVP hormone to neurosecretory granules [8]. The efficiency of neurophysin II folding, and hence AVP trafficking, is dependent upon pairing of its seven internal disulfides and binding of the mature hormone moiety [9]. The intermolecular interaction and oligomerization of the neurophysins is enhanced by non-covalent binding of the nonapeptide hormone region to the amino-terminal domain of the neurophysin [10].

As the neurosecretory granules traffic toward the axon terminal, the prohormone is cleaved by endopeptidases and exopeptidases, releasing AVP, neurophysin II, and copeptin. The individual cleaved prohormone components remain noncovalently bound during the transit process. The hormone is amidated at its C-terminus by a monooxygenase and lyase present as insoluble complexes with neurophysin II within granules. The vasopressin-containing granules are stored in the nerve terminals until neuronal activation occurs causing calcium entry into the nerve terminal and subsequent exocytosis.

Once in plasma, the AVP–neurophysin complex dissociates, and the hormone circulates in a free form. Increases in secretion of AVP are coupled to increases in synthesis, but this compensatory response may not always balance the increased rate of release [11]. A chronic severe stimulus, such as prolonged water deprivation or nephrogenic diabetes insipidus (NDI), may thus severely deplete the posterior pituitary stores of vasopressin, as can be seen by an absence of the pituitary bright spot on magnetic resonance imaging (MRI).

Detailed structure–function analyses of specific amino acids within the vasopressin and oxytocin peptides has generated new molecules that are advantageous in the management of states of vasopressin deficiency. For example, replacement of L-arginine with D-arginine at position 8 of vasopressin together with its amino-terminal deamidation resulted in a vasopressin analog with more potent and prolonged antidiuretic activity, which is now widely used clinically [desamino-D-arginine vasopressin (dDAVP), Fig. 17.2] [12].

Anatomy

AVP destined for modulation of renal water handling is synthesized by neurons located mainly in the bilateral hypothalamic supraoptic and paraventricular nuclei. The large magnocellular neurons within these nuclei send axons toward the midline to terminate at various levels within the pituitary stalk or in the posterior pituitary (neurohypophysis) itself (Fig. 17.3). AVP is released from these neurosecretory granule-rich terminals in the posterior pituitary and stalk into the systemic circulation. The superior and

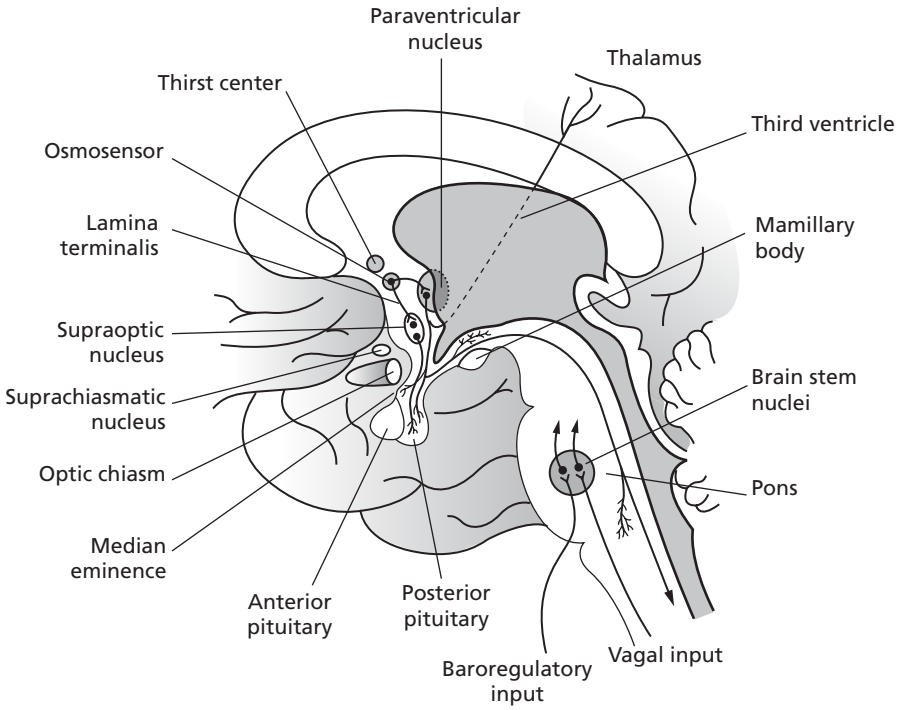

Fig. 17.3. Anatomy of AVP-producing cells in the hypothalamus and their projections to the posterior pituitary. AVP is produced by neurons in the supraoptic, paraventricular, and suprachiasmatic nuclei. The magnocellular neurons located in the supraoptic and paraventricular nuclei send axonal projections to the posterior pituitary for secretion of AVP into the systemic circulation. Reproduced with permission from [110].

inferior hypophyseal arteries, distal branches of the internal carotid artery, provide the blood supply to the posterior pituitary [13]. There is a second group of smaller parvocellular neurons that also synthesize AVP in the paraventricular nucleus of the hypothalamus. In contrast to the magnocellular neurons, the parvocellular neurons give rise to axons that terminate at the median eminence and secrete AVP into the portal-hypophyseal vascular plexus to augment ACTH synthesis and release from anterior pituitary corticotrophs.

Regulation of AVP secretion

Under normal conditions, plasma osmolality is the most important regulator of AVP secretion from nerve terminals in the neurohypophysis. As it increases, more AVP is secreted to increase water retention. While magnocellular AVP-containing neurons in the supraoptic and paraventricular nuclei can depolarize and secrete AVP in response to a hypertonic environment, the primary regulatory centers controlling AVP release are anatomically distinct osmosensing neurons outside the blood–brain barrier in the anterior hypothalamus [14]. These regions include the organ vasculosum of the lamina terminalis and the subfornical organ. In addition to osmotic regulation, substantial changes in intravascular volume, nausea, and drugs can stimulate AVP release by distinct neuronal circuits.

Osmotic regulation

In healthy individuals, the set point for initiation of AVP secretion occurs at a plasma osmolality of 280 mOsm/kg, although this can vary between 275 and 290 mOsm/kg based upon interindividual genetic differences, other hormonal signals, and volume status [15]. For example, during pregnancy or the luteal phase of the menstrual cycle, the osmotic threshold for AVP release and thirst are both decreased by 5–10 mOsm/kg [16] (Fig. 17.4). Human chorionic gonadotrophin elevation during pregnancy and alteration in luteinizing

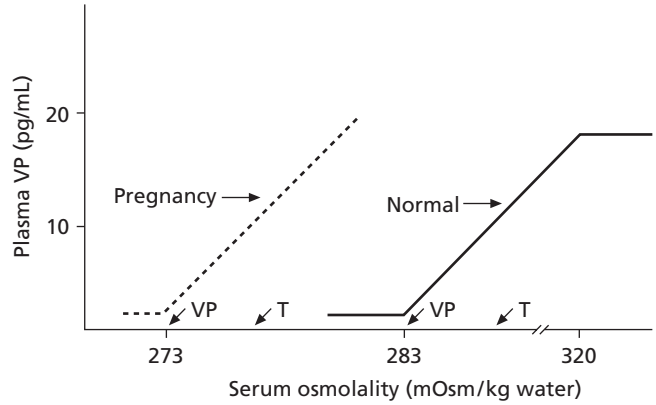

Fig. 17.4. Relationship of osmotic thresholds for activation of AVP secretion and thirst. Note that the threshold for vasopressin release occurs at a lower osmolality than the osmolality required for the sensation of thirst. Under normal circumstances, plasma AVP increases linearly until an osmolality of approximately 320 mOsm/kg and then plateaus. In pregnancy, the set points for both AVP release and thirst are shifted such that induction occurs with similar sensitivity but at a lower threshold. Arrows on the *x*-axis indicate the threshold for AVP secretion (AVP) or thirst sensation (T). Reproduced with permission from [110].

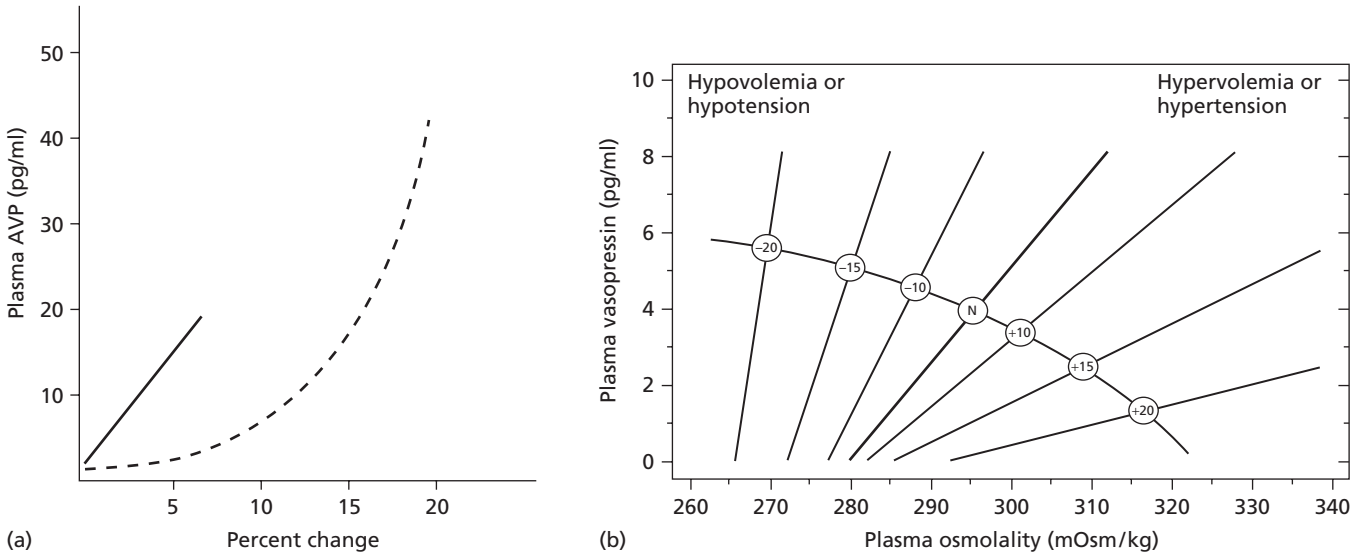

Fig. 17.5. Interactions of osmolality and hemodynamic stimuli in the regulation of AVP secretion. (a) Plasma AVP concentration in relation to percentage increase in blood osmolality (solid line) or percentage decrease in blood volume (dashed line). Adapted from [111]. (b) Changes in hemodynamic status alter the sensitivity of AVP secretion into the plasma. Reproduced with permission from [112].

hormone (LH) secretion during the luteal phase may mediate these changes.

When serum osmolality falls below the osmotic threshold for AVP release, plasma AVP concentration falls below 1 pg/mL, the sensitivity limit of most radioimmunoassays [17,18]. This reduction in AVP action on the kidney promotes excretion of maximally dilute urine. The resultant loss of free water increases serum osmolality and limits further dilution of intracellular and extracellular fluids.

Once serum osmolality exceeds the threshold for AVP release, increasing plasma osmolality 1% increases plasma vasopressin by approximately 1 pg/mL (1 pmol/L), an amount sufficient to alter urine concentration and flow (Fig. 17.5). Peak antidiuresis and production of a maximally concentrated urine occurs at a plasma AVP concentration of 5 pg/mL [17,18]. Osmotic stimulation linearly increases plasma AVP to as high as 20 pg/mL at a plasma osmolality of 320 mOsm/kg or above, but this causes no further change in urine concentrating ability.

Many solutes contribute to plasma osmolality, but sodium and its anions constitute the majority, and they modulate vasopressin release, as controlled by osmosensor neurons [19]. Increases in osmolality by sugars such as mannitol also augment vasopressin release through the osmosensors, but not all solutes that contribute to plasma osmolality have the capacity to stimulate AVP release. Non-stimulatory solutes include glucose and urea in healthy individuals, but hyperglycemia does stimulate AVP release in the context of insulin deficiency, which may possibly exacerbate progression of hyponatremia during treatment of diabetic ketoacidosis [20]. The mechanisms by which plasma solutes are differentially sensed by the osmoregulators have not yet been determined.

Non-osmotic regulation

In addition to plasma osmolality, other homeostatic and environmental factors influence AVP secretion. Of these, acute changes in intravascular volume and pressure are particularly important. Baroreceptors in the cardiac atria and aortic arch are activated by blood vessel wall stretching due to increased intravascular volume and, in turn, activate neurons within the brainstem nucleus tractus solitarius [21]. These neurons synapse upon neurons within the supraoptic and paraventricular nuclei and inhibit vasopressin release. Conversely, when intravascular volume falls or blood pressure decreases, inhibition of vasopressinergic neurons within the hypothalamus is diminished, resulting in augmented AVP release. In contrast to the subtle changes in plasma osmolality that modulate AVP secretion, larger changes in intravascular volume are needed to initiate AVP release. Vasopressin concentration does not increase until intravascular volume deficits exceed 8% [16,22] (Fig. 17.5). When intravascular volume depletion exceeds this threshold, plasma AVP levels increase exponentially so that decreases in intravascular volume of 20–30% increase plasma vasopressin to levels far greater than those required for maximum antidiuresis and maximal levels seen with osmotic stimulation.

Osmotic and hemodynamic stimuli interact to enhance the AVP response generated by each independent stimulus (Fig. 17.5). For example, hypovolemia or hypotension lowers the threshold and increases the gain for AVP release imparted by the osmoregulatory system, increasing the stimulatory effect of a given level of plasma osmolality [16]. Conversely, increased intravascular volume or hypervolemia dampens the AVP response to increases in plasma osmolality. This interaction suggests that the osmo- and baroregulatory

systems, although anatomically distinct, converge upon the same population of neurosecretory neurons [23].

The sensation of nausea strongly promotes vasopressin secretion [24], and circulating levels of AVP may exceed those associated with maximal osmotic stimulation. This augmentation in AVP secretion is probably mediated by afferents from the area postrema of the brainstem, a key emetic center. Nicotine is also a strong stimulus for AVP release [25]. These signals probably do not involve osmosensors or baroreceptors, as pharmacological blockade of an emetic stimulus does not alter the AVP secretory response to increased plasma osmolality or hypotension.

Other non-osmotic stimuli for vasopressin release include physiological stressors, such as acute hypoglycemia, hypoxia, and hypercapnia, as well as many drugs and hormones. AVP secretion is inhibited by glucocorticoids. Thus, with glucocorticoid deficiency, loss of inhibition of AVP release may occur and contribute to hyponatremia [26].

Many drug effects on AVP secretion are thought to occur indirectly by providing hemodynamic or emetic stimuli. Psychological or physiological stress caused by pain, emotion, physical exercise, or other deviations from homeostasis has long been thought to cause the release of vasopressin, but this may be due indirectly to other factors, such as the hypotension or nausea that often accompany vasovagal reactions [16].

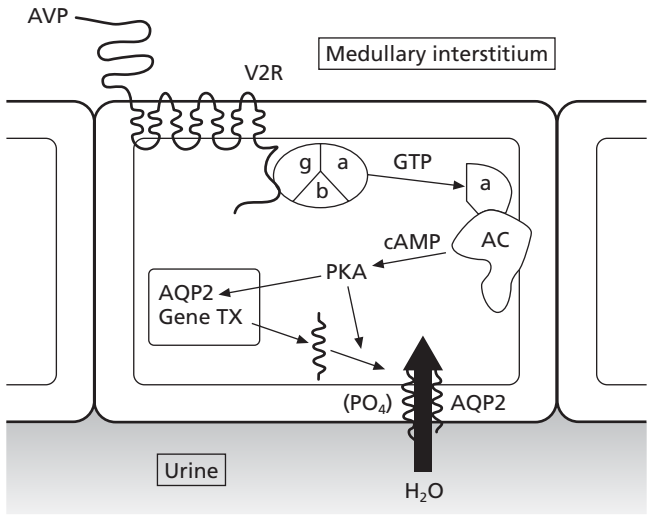

Fig. 17.6. Renal actions of AVP. In the collecting duct epithelium, AVP binding to the V_2 receptor results in Gα-mediated activation of cAMP production from adenylyl cyclases. The elevation in intracellular cAMP causes activation of protein kinase A (PKA), which then phosphorylates AQP-2 at serine 256. This phosphorylation event promotes aggregation of AQP-2 homotetramers in subapical membrane vesicles and their fusion with the apical plasma membrane. The insertion of the water channels into the luminal membrane allows the flow of water from the urine within the duct lumen into the hypertonic medullary interstitium, decreasing free water clearance. Reproduced with permission from [110].

Vasopression metabolism

AVP has a half-life of 5–10 min in the circulation, being quickly degraded by vasopressinase, a cysteine amino-terminal peptidase. Because of its resistance to amino-terminal degradation, the synthetic AVP analog dDAVP has a much longer half-life, 8–24 h. Vasopressinase activity increases during pregnancy because it is synthesized and secreted by the placenta [27]. Pregnant women compensate for the increased clearance of AVP by increasing posterior pituitary vasopressin secretion. During pregnancy, women with subtle, compensated impairment of AVP secretion or action [28] or those with increased concentrations of placental vasopressinase associated with liver dysfunction [29] or multiple gestations [30] may develop DI, which resolves after delivery of the placenta. This form of vasopressinase-dependent pregnancy-associated DI responds well to treatment with dDAVP but not with vasopressin.

Biological action of AVP

The crucial action of AVP in regulation of plasma osmolality or intravascular volume in cases of moderate to severe volume depletion is to limit the excretion of water. It does so by increasing the permeability of the distal nephron to luminal water, thereby increasing reabsorption of free water and reducing urine output. To achieve this antidiuretic action, AVP acts upon specific vasopressin receptors on the serosal surface of the distal and collecting renal tubules (Fig. 17.6). At least three subtypes of vasopressin receptors have been identified, which have been designated V_{1a}, V_{1b} (V_3), and V_2 [31]. Each arises from a different gene coding for a member of the seven-transmembrane G-protein-coupled receptor family [32].

The V_2 receptor shares 60% sequence identity with the V_1 receptor and is the vasopressin receptor in the kidney accounting for the antidiuretic effects of AVP. The gene encoding the V_2 receptor maps to the distal long arm of the X chromosome (Xq28), and mutations in the gene result in X-linked NDI [33] (Fig. 17.7a). The V_2 receptor signals by coupling to adenylyl cyclase through $G_{\alpha s}$ to increase intracellular cAMP concentration. In addition to the distal nephron, V_2 receptors are located in the thick ascending limb of Henle's loop and periglomerular tubules [4,34]. Outside the kidney, V_2 receptors are found on vascular endothelial cells where activation promotes vasodilation [35], action of von Willebrand factor, factor VIIIa, and tissue plasminogen activator. The prothrombotic actions of AVP, and specifically the long-lasting analog dDAVP, have been used to treat bleeding disorders associated with von Willebrand disease and hemophilia.

Action of AVP on V_{1a} receptors mediates extrarenal effects including contraction of vascular smooth muscle, stimulation of hepatic glycogenolysis, and aggregation of platelets [36].

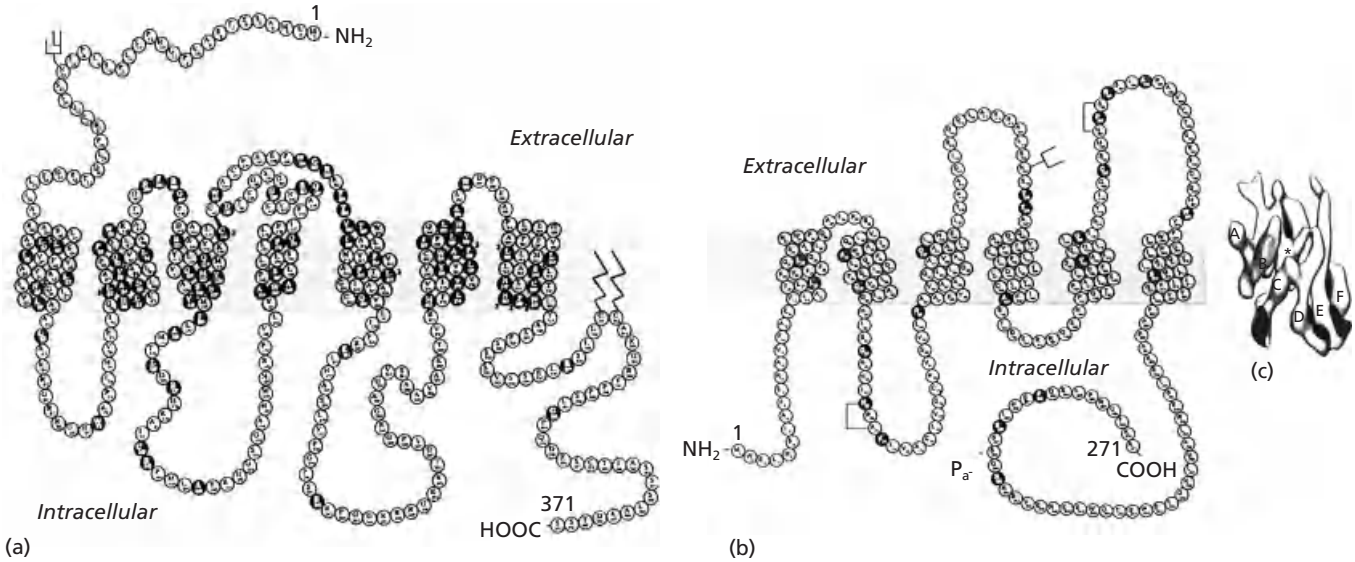

Fig. 17.7. The AVP V_2 receptor and aquaporin-2. (a) Schematic representation of the seven transmembrane V_2 receptor. The locations of known missense or nonsense mutations are indicated by solid circles. A number accompanying a solid circle indicates that there is more then one known mutation in that codon. (b) Schematic representation of the AQP-2 protein. A monomer has six transmembrane helices. The location of the protein kinase A phosphorylation site (Pa) is indicated. Solid symbols indicate the location of known mutations. (c) Three-dimensional representation of the six-helix barrel of aquaporin-2, viewed parallel to the bilayer. Reprinted with permission from [84].

V_{1b} receptors are primarily located on adrenocorticotrophic hormone (ACTH)-producing corticotrophs in the anterior pituitary [37], where activation increases ACTH release during acute and chronic stress. In addition, V_{1b} receptors in the brain may mediate behavioral actions of AVP [38]. In contrast to V_2, neither V_{1a} nor V_{1b} couples to $G_{\alpha s}$ to cause induction of cAMP production. Instead, both couple to phospholipase C with modulation of intracellular calcium and phosphatidylinositol signaling pathways.

In the absence of vasopressin, the luminal surface of the distal nephron epithelial cells is largely impermeable to water and solutes. Dilute tubular fluid traversing from the more proximal nephron passes without substantial additional concentration as dilute urine. Urine is maximally dilute (osmolality < 100 mOsmol/kg), and a relatively high rate of urine flow ensues (at or above 3 L/m^2/day depending on the amount of solute requiring excretion).

In the presence of vasopressin, V_2 receptors are activated and cAMP is generated. This stimulates phosphorylation by cAMP-dependent protein kinase A, which subsequently results in remodeling of cytoskeletal components and the exocytic insertion of preformed, subapical vesicular water channels, aquaporin-2, into the apical membrane [39]. The insertion of these water channels results in a large increase in water permeability of the luminal epithelial membrane (up to 100-fold), allowing diffusion of water along its osmotic gradient from the lumen of the tubule into the hypertonic inner medullary interstitium. This net movement of water in excess of solute allows reduction in urine volume and excretion of concentrated urine.

The water channels themselves belong to a family of related proteins, the aquaporins, which differ in their sites of expression and pattern of regulation. The aquaporins consist of a single polypeptide chain with six membrane-spanning domains thought to function as homotetramers in the plasma membrane [40]. Aquaporin-2 (AQP-2) comprises the specifically vasopressin-regulated water channel in the kidney, its predominant site of expression. At this site, V_2 receptor-mediated protein kinase A activation results in phosphorylation of serine 256 in aquaporin-2, an event required for trafficking to the apical membrane [41]. More prolonged stimulation by vasopressin increases the production of aquaporin-2 which, over the course of several hours, further enhances urinary concentrating capacity [42]. The gene that encodes aquaporin-2 in humans has been localized to chromosome 12q13 and has been found to be mutated in patients with autosomal recessive NDI (Fig. 17.7b and c) [43].

Aquaporins-1, -3, and -4 are also expressed in the kidney but play a less prominent, and AVP-independent, role in water balance. Aquaporins-3 and -4 are expressed on the basolateral, rather than the apical, surface of the collecting duct epithelium. In genetically altered animal models, mutations of aquaporin-3 or -4 result in mild defects in urinary concentrating ability [44,45]. Aquaporin-1 is expressed in the proximal tubule. Defects in aquaporin-1 function result in increased delivery of free water to the distal nephron and impaired urine-concentrating ability [46].

The dose–response analyses of AVP and urine concentration, while variable in adult subjects, have shown

that an increase in plasma vasopressin of 0.5 pg/mL raises urine osmolality by approximately 150–250 mOsm/kg [16]. Maximum antidiuresis occurs when plasma vasopressin concentration reaches 2–5 pg/mL. Vasopressin may also play a role in limiting insensible water loss from the skin and lungs, but this effect is small and easily overcome by changes in environmental conditions such as temperature and humidity, as well as exercise.

Thirst

Restoration of net deficits in body water due to urinary and insensible losses occurs normally by ingestion of water, which prevents dehydration. Thirst, the conscious sensation of the need to drink, is the essential mechanism by which water losses are replaced. Thirst is regulated by many of the same physiological factors that regulate vasopressin release, of which plasma osmolality is the most potent [47]. Anatomically, osmotic regulation of thirst occurs by osmosensors in the anterior hypothalamus and includes modulatory activity by neurons in the ventromedial nucleus of the hypothalamus [48]. Stimulation of thirst is thought to be mediated by angiotensin II [49]. While lesion studies of the anteroventral third ventricle suggest anatomic proximity of osmosensors controlling thirst and vasopressin release, the sensors controlling vasopressin release and thirst are not likely to be identical [50].

For example, the set point for vasopressin release is at a plasma osmolality lower than that required to stimulate thirst. This 10 mOsm/kg difference in threshold for activation proves physiologically advantageous. The initial activation of vasopressin before a thirst-mediated increase in fluid intake allows retention of the fluid ingested with initiation of thirst. Were the set points reversed and thirst activated before vasopressin release, the ingested fluid would not be retained, and a sustained polyuric and polydipsic state would ensue. Even before plasma osmolality changes significantly, drinking water causes vasopressin secretion to diminish and thirst to abate. This negative feedback arm serves to protect from excessive ingestion and overhydration and is thought to arise from chemoreceptors that respond to both the volume and the temperature of the fluid ingested [51].

Hypovolemia and hypotension also increase thirst. The magnitude of intravascular depletion or hypotension needed to stimulate thirst has not been defined in humans but is probably larger than that associated with AVP release. Similar to the consequences for AVP secretion, volume and blood pressure changes alter the threshold set point and gain for thirst [52].

Fluid intake often occurs for reasons other than thirst. These include social cues of others drinking, pleasurable taste, or other effects of an ingested beverage, hunger or dry mouth resulting from factors independent of hydration such as anxiety or medication. When water intake exceeds homeostatic requirements, plasma vasopressin decreases to undetectable concentrations, allowing excretion of the extra water. Thirst and AVP efficiently maintain plasma tonicity in the appropriate range under normal circumstances. With defects in either thirst or urine concentrating ability, plasma tonicity can still be maintained within the normal range. Thus, during the investigation of patients with an inability to concentrate their urine due to deficiencies in vasopressin release or action, random plasma osmolality is usually normal if the patient has adequate access to water as an intact thirst mechanism stimulates water ingestion up to 10 $L/m^2/day$. Similarly, normal vasopressin regulation can mask mild to moderate impairment of thirst, preventing dehydration by avidly retaining water. However, when centers controlling both thirst and vasopressin secretion are disrupted, the occurrence of potentially life-threatening dysregulation of plasma osmolality and intravascular volume is high.

Volume sensor and effector pathways

Renin–angiotensin–aldosterone system

The primary regulator of vasopressin and thirst is osmolality and, although vasopressin and thirst do respond to large changes in intravascular volume, the renin–angiotensin system is the primary regulatory network for maintaining euvolemia. This system acts in a classical endocrine manner, although local renin–angiotensin axes may have paracrine effects in target tissues [53]. Renin, a proteolytic enzyme that catalyzes the cleavage of circulating angiotensinogen, is synthesized in the renal juxtaglomerular cells and released into the circulation in response to a number of stimuli. These include decreased renal arteriolar pressure, which occurs with intravascular volume depletion or hypotension, decreased intratubular fluid sodium concentration, which is sensed by the macula densa, and increased renal sympathetic nerve activation.

The proteolytic action of renin releases the decapeptide angiotensin I from angiotensinogen. The inactive angiotensin I serves as a substrate for angiotensin-converting enzyme in the lungs and other peripheral sites to generate the biologically active octapeptide angiotensin II that can be further metabolized to angiotensin III. The effects of angiotensin II include vascular smooth muscle contraction and blood pressure elevation, and both angiotensin II and III stimulate aldosterone release from the zona glomerulosa of the adrenal.

Aldosterone increases sodium reabsorption and potassium excretion in the distal renal tubules by augmenting the production of sodium channels trafficked to the apical plasma membrane, mitochondrial ATP synthesis, and production of subunits of the Na^+, K^+- ATPase [54]. In addition to stimulating aldosterone-mediated sodium reabsorption, angiotensin II stimulates sodium–hydrogen exchange and bicarbonate

reabsorption in the proximal tubule. Both these increases in active sodium transport produce increases in water absorption, thereby supporting intravascular volume expansion.

In addition to intravascular volume status, aldosterone release from the adrenal zona glomerulosa is stimulated directly by elevated plasma potassium concentration [53]. While acute increases in plasma levels of ACTH or AVP have the capacity transiently to stimulate aldosterone secretion, chronic administration of either does not result in sustained increases in aldosterone release. Aldosterone release is inhibited by atrial natriuretic peptide, somatostatin, and dopamine [55,56].

The natriuretic peptide system

The natriuretic peptides [atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP)] contribute to salt and water balance by promoting renal salt excretion and altering vasopressin secretion from the hypothalamus respectively [57]. These structurally related peptides of 22–32 amino acids interact with three different receptors [58] (Fig. 17.8). Two of them, NPR-A and NPR-B, possess guanylyl cyclase activity. NPR-A binds both ANP and BNP with high affinity, while NPR-B binds CNP with much higher affinity than either ANP or BNP. The third receptor, NPR-C, is not a guanylyl cyclase and clears all three ligands from the circulation.

The physiological effects of ANP were the first to be elucidated [59]. ANP synthesis occurs in both the left and the right atria in response to increasing wall pressure and increased heart rate. Ventricular production of ANP increases with ventricular hypertrophy. ANP produced within the brain is also modulated in a volume-dependent manner. ANP released into the peripheral circulation has a number of renal effects, inhibiting sodium reabsorption in the medullary collecting duct, impairing the salt-retaining actions of angiotensin II on the proximal tubule and the renal effects of vasopressin in water retention [60]. In addition, ANP inhibits aldosterone synthesis in adrenal zona glomerulosa cells by inhibiting actions of aldosterone secretagogues, particularly the action of angiotensin II. ANP reduces plasma renin activity, which reduces the generation of angiotensin II, further diminishing aldosterone secretion and renal salt reabsorption.

BNP is synthesized in the ventricle with production augmented in congestive heart failure and hypertension; it causes renal and adrenal effects similar to ANP [60]. The brain is the primary site of CNP production, and CNP expression overlaps with ANP expression in the hypothalamus [61]. Little CNP is present in plasma normally or in response to volume overload. Experiments in which ANP has been injected into the central nervous system (CNS) demonstrated vasodepression, bradycardia, and inhibition of AVP, ACTH, and gonadotropin-releasing hormone (GnRH) secretion [62]. These results suggest similar net metabolic effects of natriuretic peptides produced within the CNS and periphery and regulatory consequences for salt, water, and hemodynamic balance.

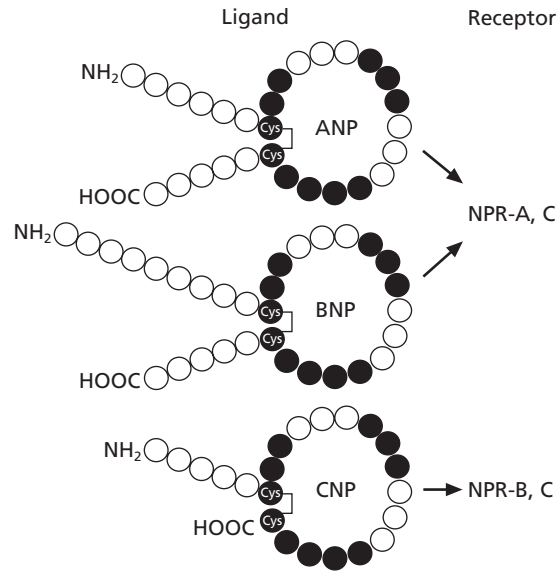

Fig. 17.8. Structure of the natriuretic peptides and their receptor specificity. ANP, BNP, and CNP each consist of a 17-member ring structure closed by a disulfide bond between cysteine residues, along with amino- and carboxy-terminal tails of variable length. Conserved amino acids are indicated by the filled circles. Each family member binds the NPR-C clearance receptor, while ANP and BNP exert their effects through binding NPR-A, and CNP activates NPR-B.

Disorders of water balance

Vasopressin deficiency

Deficiencies in AVP secretion or action result in polyuria, characterized by chronic excretion of abnormally large volumes of dilute urine (exceeding 2 L/m^2/day), and consequent polydipsia. AVP-deficient or -resistant polyuria and polydipsia are known as diabetes insipidus (DI). The signs and symptoms of DI are primarily urinary frequency with high urine output, nocturia in older children and adults, and persistent enuresis or delayed toilet training in younger children. These symptoms are accompanied by thirst and increased fluid intake throughout the day and night. Children with untreated DI crave cold fluids, especially water. Polyuria and polydipsia due to pathological reduction of vasopressin secretion (central DI) or action (NDI) can result in hypernatremia if access to water is restricted or the thirst mechanism is impaired. DI should be differentiated from other causes of urinary frequency. These include a reduced bladder capacity or bladder irritation, in which fluid intake and the 24-h urine volume are normal, and diabetes mellitus (DM) or other forms of solute diuresis, in which fluid

intake and urine output is increased, or psychological increases in water intake (primary polydipsia).

Diagnostic approach to DI

Patients presenting with polyuria and polydipsia should have DM excluded promptly. DM is much more common than DI, and delay in diagnosis places a child at risk of ketoacidosis. When DM has been excluded and the possibility of DI is being considered, fluid intake and output should be established to determine whether polyuria (urine output > 2 L/m^2/day) is indeed present. If possible, an intake/output record should be kept at home to determine the volume of urine in 24 h. If the child is not toilet trained or has a condition that makes it difficult to collect urine, the measurement of fluid intake is an acceptable substitute.

The answers to specific questions can be helpful during the initial assessment: When did the polyuria and polydipsia begin? Does the need to drink and urinate interfere with normal activities? Is nocturia or enuresis present? Does the patient drink at night? Is there a psychological or psychiatric component to the need to drink? What is the preferred drink? What is the color of the patient's urine? Does the history (including longitudinal growth) or physical examination suggest other deficient or excessive pituitary hormone secretion? Are there symptoms or findings suggestive of a CNS mass? Is there bone pain or skin rash? Is the child taking medication that could result in AVP resistance?

If pathological polyuria or polydipsia is present, serum osmolality and concentrations of sodium, potassium, glucose, calcium, and urea should be measured as an outpatient; urinalysis, including measurement of osmolality, specific gravity, and glucose, should be performed. A urine osmolality greater than 600 mOsm/kg excludes DI, and DI is also unlikely if the serum osmolality and sodium are low or low–normal, results most consistent with primary polydipsia. Serum sodium above 145 mmol/L or serum osmolality greater than 300 mOsm/kg with urine osmolality less than 600 mOsm/kg confirms the diagnosis of DI, and a water deprivation test is superfluous.

Because measurement of serum osmolality is often not as accurate as that of serum sodium, interpretation of a single serum osmolality should be made with caution if it is not accompanied by a concordant change in serum sodium. Nevertheless, because intact thirst results in compensatory fluid intake, the majority of patients with DI have serum sodium and osmolality in the normal range on initial testing. Therefore, most patients with dilute urine and intake/output records at home suggesting significant polyuria and polydipsia need a water deprivation test to establish a diagnosis of DI and to differentiate central from nephrogenic causes.

The water deprivation test should be done in a controlled setting, either at an outpatient site appropriate for 8–10 h of observation and assessment or as an inpatient. Because patients with DI can become dehydrated in a few hours, it is not appropriate to begin fluid restriction before the patient arrives for the test. However, if clinical history suggests that the child can comfortably go for a few hours without drinking, the child can be fluid restricted for that length of time before the test begins. During the test, the environment must be controlled to avoid surreptitious water intake, as the intense drive to drink can lead to fluid intake from unusual sources. Physical signs and biochemical parameters are measured hourly (Fig. 17.9). Because measurement of serum osmolality is relatively imprecise, serum sodium is more reliable than osmolality during water deprivation testing. The laboratory should be aware of the need for prompt assessment of the specimens.

A common recommendation is to stop a test based on a 5% loss of body weight, but this may result in the test being stopped before a diagnosis can be made. Unless vital signs or other symptoms suggest significant clinical hypovolemia, the test should proceed until diagnostic results are obtained.

Water deprivation phase

- Obtain initial weight, vitals and document duration of pretest water restriction (if any)
- Place intravenous line to assist with repeated blood drawing, and place foley catheter in children too young to provide hourly voided urine specimens
- Obtain baseline serum Na, vasopressin, urine osmolality, and urine specific gravity
- Begin (or continue) water deprivation
- Measure and record hourly on a flow sheet:
  - Weight, HR, BP, urine output, and urine specific gravity
  - Stat laboratory testing: serum sodium and urine osmolality
- If Na < 145, urine osmolality < 600, and there is no clinical evidence of significant, symptomatic hypovolemia, continue water deprivation
- If urine osmolality is above 1000, or above 600 and stable over two measures, stop test. Patient does not have diabetes insipidus
- If serum osmolality is above 300 and urine osmolality is below 600, the patient has diabetes insipidus. Proceed to vasopressin response phase

Vasopressin response phase

- Collect blood for vasopressin level
- Administer Pitressin, 1 unit/m^2, SQ
- Allow the patient to eat and drink, limiting fluid intake to the volume of urine produced during the entire testing period (water deprivation and vasopressin response)
- 30 and 60 minutes after Pitressin, measure vital signs, urine output, and urine specific gravity, and send urine to lab for osmolality
- A twofold increse in urine osmolality indicates central diabetes insipidus
- An increase of less than twofold in urine osmolality is consistent with nephrogenic diabetes insipidus

Fig. 17.9. Protocol for water deprivation testing for diagnosis of diabetes insipidus.

If a diagnosis of DI is made, subcutaneous aqueous vasopressin (Pitressin) can be used to differentiate central and nephrogenic forms. This is frequently done immediately following the water deprivation test but can be done independently of it. Following administration of vasopressin, fluid restriction should be stopped, as continued restriction in a child with NDI will result in progressive dehydration, but intake should be limited to protect against excessive water consumption with resultant hyponatremia. Owing to its longer duration of action, dDAVP is not recommended for this test, as it can cause water intoxication [63]. Patients with long-standing primary polydipsia may have mild NDI because of dilution of their renal medullary interstitium.

The water deprivation test is adequate to establish the diagnosis of DI in most patients and to differentiate central from N causes. Plasma AVP concentration may be obtained during the procedure; although this is rarely needed for diagnosis, it may be helpful in differentiating partial central from NDI [64]. The various types of DI can also be differentiated by their distinctive responses to a closely monitored therapeutic trial of dDAVP, but care must be taken to avoid water intoxication, particularly in small children or children in whom there is a suspicion of psychogenic polydipsia.

Table 17.2. Causes of central diabetes insipidus.

Genetic
AVP
Neurophysin gene
Autosomal dominant
Autosomal recessive
Wolfram syndrome
Congenital
Septo-optic dysplasia
Midline craniofacial defects
Holoprosencephalic syndromes
Agenesis of the pituitary
Acquired
Neoplasms
Craniopharyngioma
Germinoma
Pinealoma
Leukemia/lymphoma
Inflammatory/infiltrative
Langerhans cell histiocytosis
Systemic lupus erythematosus
Neurosarcoidosis
Lymphocytic neurohypophysitis
Infectious
Meningitis
Encephalitis
Congenital infection
Traumatic injury
CNS surgery
Head trauma
Hypoxic injury
Idiopathic

Central DI (CDI)

The most common form of DI results from deficiency of AVP secretion. Central (neurogenic, pituitary, hypothalamic, cranial, or vasopressin-responsive) DI results when pituitary AVP production is reduced for any of a number of reasons. Losses of up to 90% of normal AVP secretion can occur without overt clinical manifestations in otherwise well individuals. Further loss of AVP secretion results in onset of clinically apparent polyuria and polydipsia. Therefore, onset of symptoms is generally perceived as abrupt, and patients or their families often identify a discrete time of onset. Once symptoms develop, the degree of polyuria and polydipsia depends both on the degree of AVP deficiency and other factors, such as the integrity of thirst, renal function, dietary salt load, and normality of other endocrine systems. Urinary losses as high as 400 $mL/m^2/h$ have been documented. Central DI responds well to AVP replacement therapy with vasopressin or its more stable analog, dDAVP.

Causes of CDI

Genetic causes

The inherited forms of CDI account for less than 10% of cases of DI in most patient populations (Table 17.2).

Familial autosomal-dominant neurohypophyseal DI (*ADNDI*). The most frequent inherited form of DI, it is caused by mutations in the AVP-NPII gene, and more than 40 mutations have been described. The majority are in the neurophysin coding region or in the signal peptide and are presumed to impair processing, folding, or dimerization. Symptoms appear in the first decade of life, usually before 7 years of age, but are not apparent at birth. Vasopressin secretion is normal initially and declines gradually until DI of variable severity supervenes [65].

Cell culture and animal studies have shown that the mutant proteins are trapped in the endoplasmic reticulum (ER), and high-level expression of mutant protein results in abnormal ER morphology and increased cell death [66]. Autopsy studies in individuals with ADNDI reveal a markedly subnormal number of AVP-producing magnocellular neurons in the supraoptic and paraventricular nuclei of the hypothalamus, associated with moderate gliosis [67]. These findings are consistent with degeneration of these neurons and suggest that accumulation of the abnormal vasopressin prohormone in the ER causes neuronal degeneration and cell death. Neurotoxicity with degeneration of the magnocellular neurons due to accumulation of abnormal prohormone would explain both the autosomal-dominant inheritance and the delayed onset of DI.

Autosomal-recessive familial neurohypophyseal DI. A single mutation in the AVP-NPII gene produces a rare autosomal-recessive form of familial DI [68]. A missense mutation at nucleotide 301 changes the proline at position 7 of AVP to a leucine. The mutant hormone is a weak agonist for the V_2 receptor, with approximately 30-fold reduced binding affinity. Affected children are asymptomatic for the first year or more of life, presumably because increased secretion of the mutant hormone is able to compensate for its decreased activity in early life. There is insufficient evidence to determine why the children subsequently develop DI, but it is presumably not due to neurotoxicity of the mutant protein, as the heterozygous carrier parents have no evidence of impaired AVP secretion.

Wolfram syndrome/DIDMOAD. Vasopressin deficiency is part of Wolfram syndrome, a rare, progressive, neurodegenerative condition also known as DIDMOAD (DI, diabetes mellitus, optic atrophy and deafness) [69]. The minimal features required for diagnosis are juvenile-onset insulin-dependent diabetes and optic atrophy. DI, sensorineural deafness, urinary tract atony, ataxia, peripheral neuropathy, mental retardation, and psychiatric illness develop in the majority of patients. Onset of DI is usually in the second decade. The Wolfram syndrome gene, *WFS1*, is located on the short arm of chromosome 4. It codes for an 890-amino-acid transmembrane protein, Wolframin, of unknown function. Wolframin is ubiquitously expressed and predominantly localized to the ER. Mutations in *WFS1* cause isolated, autosomal-dominant, low-frequency hearing loss and have been associated with isolated diabetes mellitus and isolated psychiatric disease.

Congenital intracranial anatomic defects

Midline brain defects are associated with central DI and estimated to account for 5–10% of pediatric cases. DI may become apparent in the first weeks of life, but diagnosis may be delayed. The most frequent defect associated with congenital central DI is septo-optic dysplasia (SOD, De Morsier syndrome). This is characterized by hypoplasia of the optic nerves with other midline cerebral anomalies and pituitary hormone deficiencies. Mutations in the homeobox gene Hesx1 have been associated with some cases of SOD, although most cases do not have a gene defect identified [70].

Other anomalies associated with CDI include nasal encephalocele, porencephaly, holoprosencephaly, hydrocephalus, and hydranencephaly. MRI evaluation generally reveals an absent posterior pituitary bright spot, in addition to the accompanying CNS lesions. Visible midline craniofacial defects associated with congenital CDI include single central incisor, cleft lip or palate, high arch palate, micrognathia, synophrys, hypotelorism, flat nasal bridge, or other midface hypoplasia, but many children with congenital CDI have no external evidence of midline abnormalities. Deficiencies of anterior pituitary hormones and defects in thirst perception are not uncommon. In patients who have cortisol deficiency, symptoms of DI may be masked because cortisol deficiency impairs renal free water clearance. In such cases, glucocorticoid therapy may unmask AVP deficiency and precipitate polyuria.

Acquired central DI

The non-familial, acquired forms of DI account for the majority of central DI cases.

Tumor is the most common cause of CDI. Brain tumors presenting with DI account for 10–15% of acquired DI in children and include germinoma, astrocytoma, pinealoma, CNS lymphoma, glioma, and craniopharyngioma. Although craniopharyngioma infrequently causes DI before surgery, the majority of children develop DI following resection, and craniopharyngiomas ultimately account for the majority of tumor-associated CDI in children. Including those who develop DI following surgery, brain tumors account for up to 50% of acquired DI.

Because hypothalamic AVP neurons are distributed over a large area within the hypothalamus, tumors that cause DI must be large, infiltrative, or located at the point of convergence of the hypothalamo-neurohypophysial axonal tract in the infundibulum. Because germinomas and pinealomas typically arise near the base of the hypothalamus, where AVP axons converge as they enter the posterior pituitary, they are the tumors most commonly associated with DI at diagnosis. Germinomas causing DI can be small and undetectable by MRI for several years following the onset of DI [71]. The β-subunit of human chorionic gonadotropin and alfa-fetoprotein are often secreted by germinomas and pinealomas, and repeated measurements and MRI scans should be performed in children with idiopathic or unexplained DI. Tumor-associated DI is rare before 5 years of age [72].

Neurosurgical intervention is one of the most common causes of CDI. In the post-operative period, it is important to be aware of the risk of DI and to distinguish polyuria associated with DI from polyuria due to the normal diuresis of fluids given during surgery and polyuria associated with cerebral salt wasting. In both DI and normal diuresis, the urine may be very dilute and of high volume but, with post-surgical diuresis, serum sodium and osmolality will be normal, whereas they will be high in DI if the patient does not have free access to water.

In cerebral salt wasting, urine volumes are also high, but urinary sodium concentrations are high, and serum osmolality and sodium are low. Post-surgical DI is characterized by an abrupt onset of polyuria, usually within the first 12–24 h. This phase is often transient, resolving spontaneously in 1–2 days, and is thought to be due either to acute injury to the neurohypophysis with inhibition of AVP secretion or to release of biologically inactive AVP-like peptide hormones from the damaged hypothalamo-neurohypophyseal system that interfere with the binding of AVP to the V_2 receptor [73].

Not infrequently, a "triple-phase" response is seen. Following the initial DI, the syndrome of inappropriate ADH secretion (SIADH) is seen, typically after 4–5 days, which can last for 10 days and is due to the unregulated release of vasopressin from dying neurons. A third phase of permanent DI follows if sufficient numbers of vasopressin-producing cells were destroyed. Although cranial irradiation is associated with the development of anterior pituitary hormone deficiencies, it is never associated with DI.

Infections involving the base of the brain, such as meningococcus, group B streptococci, *Haemophilus influenzae*, *Streptococcus pneumoniae*, cryptococcal and listeria meningitides, congenital cytomegalovirus, tuberculoma, or toxoplasmosis can cause CDI, which may be transient or permanent. When permanent, DI is often combined with anterior pituitary endocrinopathies.

Langerhans cell histiocytosis (LCH) is the most common infiltrative disorder causing CDI and may be responsible for up to 10% of acquired cases. LCH is generally considered as a disease of childhood, although the diagnosis is also made in adults. It is characterized by a clonal proliferation of abnormal dendritic histiocytes (Langerhans cells) with an accompanying infiltration of lymphocytes, eosinophils, and neutrophils. It can involve many body organ systems or tissues, and the disease often targets the posterior hypothalamo-pituitary region.

Some 50% of patients with LCH have DI. A minority has concurrent anterior hormone deficiencies, which can develop many years after the onset of DI but rarely without it. DI associated with LCH is almost always a multisystem disease, with lesions in bone (68%), skin (57%), lung (39%), and lymph nodes (18%) [74]. X-ray evaluation for skeletal lesions and clinical symptoms of multisystem disease should be sought when LCH is considered in a differential diagnosis. Thickening of the pituitary stalk may be seen on cranial MRI, but the pituitary stalk can also appear normal. DI can also be associated with sarcoid, but neurological manifestations of sarcoid are rare in children [75].

Trauma to the base of the brain can cause swelling around or severance of the magnocellular neurons, resulting in DI, even after seemingly minor trauma. As in CNS surgery, the DI associated with trauma may develop rapidly after the injury and can be transient or permanent. Occasionally, onset can be delayed as the magnocellular neurons degenerate.

Autoimmune DI is associated with autoantibodies to AVP-secreting cells; these are present in more than half of adults less than 30 years of age presenting with idiopathic DI and are much more common in individuals with a history of prior autoimmune disease or pituitary stalk thickening [76]. Based on the presence of these antibodies, it has been suggested that autoimmune lymphocytic neurohypophysitis may account for a significant proportion of patients with idiopathic DI, but 16% of patients with non-idiopathic CDI also have the antibodies. It is possible that antibodies directed against vasopressin-containing cells are not pathogenic but are markers of previous neuronal cell destruction. In addition, patients with other autoimmune diseases may have AVP-secreting cell antibodies without evidence of DI [77]. Biopsy-proven lymphatic hypophysitis or infundibuloneurohypophysitis with associated DI has been reported infrequently in children, so the magnitude of its contribution to acquired childhood DI is unknown.

Hypoxic injury caused by carbon monoxide poisoning, smoke inhalation, respiratory failure, cardiopulmonary arrest, septic shock, and sudden infant death syndrome may cause CDI. The interval between the insult and development of DI ranges from a few hours to many days. Because the neurohypophyseal system has a bilateral blood supply and is relatively resistant to hypoxic injury, the appearance of DI following hypoxic injury is ominous and generally indicative of widespread neurological damage. Thus, DI is present in approximately 40% of children with brain death, and CDI should be considered in the differential diagnosis of polyuria occurring in any patient who has suffered hypoxic injury.

Idiopathic DI occurs in 12–20% of cases. MRI may be normal, or the pituitary stalk may be thickened. Thickening is observed in approximately one-third of children with CDI. Some of these have a cause for their DI at presentation, most often LCH, but most have idiopathic DI. Patients with idiopathic DI and thickening of the pituitary stalk appear to be more likely to have or to develop anterior pituitary hormone deficiencies, but all patients should have anterior pituitary function tests at presentation and during follow-up. Patients with or without thickening of the pituitary stalk should be followed with repeated MRI, as DI is the most common initial presentation of germinoma and may occur before radiographic evidence of the tumor is present. Evidence of a tumor is usually within 2.5 years after diagnosis and 1.3 years after thickening of the pituitary stalk is noted on MRI. Gonadotropin deficiency is a marker for organic disease (78).

Management/treatment of central DI

Once central DI has been diagnosed, the management requires a search for a cause, as above. In 80–90% of healthy children and adults, the posterior pituitary emits a hyperintense signal ("bright spot") on T_1-weighted, non-infused midsagittal images (Fig. 17.10). The posterior pituitary bright spot is absent in more than 90% of children with CDI at the time of diagnosis. This is not diagnostic of CDI as the bright spot is also absent in NDI, presumably because of increased release of AVP. The bright spot is notably normal in primary polydipsia. If initial studies do not reveal the etiology, repeat MRI at 6-month intervals is recommended for 2 years. Imaging frequency can then be decreased to yearly if the pituitary is stable or stalk thickening is improving. Anterior pituitary function should also be evaluated. In some cases, a lumbar puncture may be needed to identify a germinoma

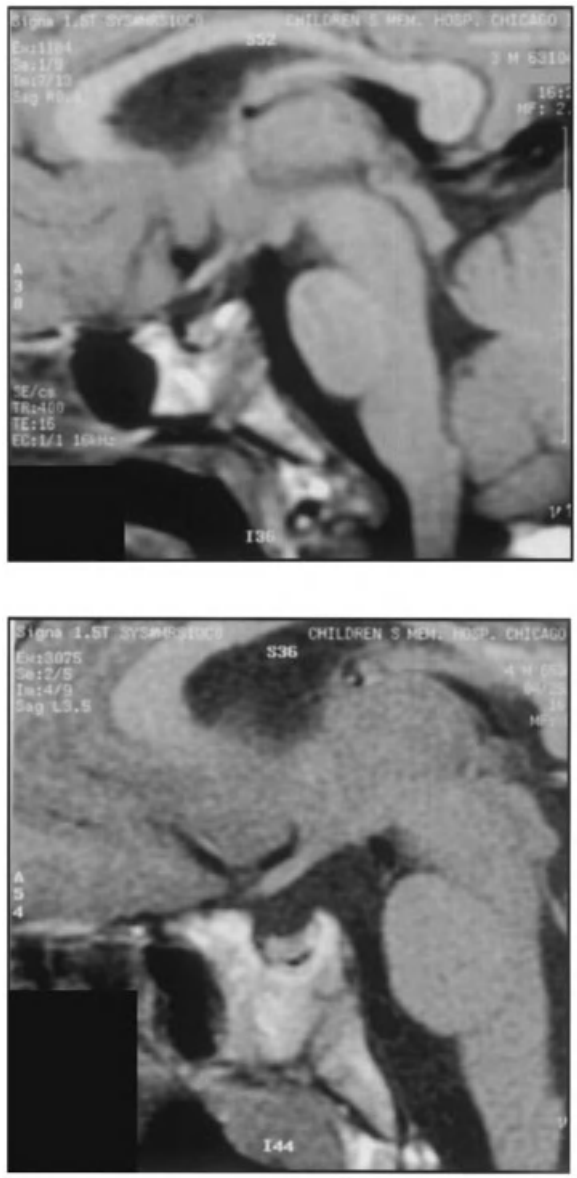

Fig. 17.10. Magnetic resonance imaging of the hypothalamus and pituitary in central DI. Both images are T_1 weighted and obtained before the infusion of gadolinium. (a) This sagittal image is from a healthy 4-year-old boy and shows the hyperintense signal normally emitted by the posterior pituitary. (b) This comparable image from a boy with autosomal-dominant neurohypophyseal DI lacks the posterior pituitary bright spot.

because cerebrospinal fluid (CSF) α-fetoprotein or human chorionic gonadotropin concentrations may be elevated in the absence of an elevation in serum levels.

Treatment of CDI is usually lifelong because recovery from a deficiency lasting more than a week is uncommon, even if the underlying cause is eliminated. With intact thirst and free access to water, an individual with DI will drink sufficiently to maintain normal serum osmolality and high–normal serum sodium. To relieve the symptoms of polyuria and polydipsia, the treatment of choice is administration of dDAVP (desmopressin) (Fig. 17.2). dDAVP (4 μg/mL) is available for subcutaneous injection in doses from 0.1 to 1 μg once or twice per day. dDAVP as a nasal solution (10 μg/0.1 mL) can be delivered in increments of 2.5 μg by tube or by 10-μg increments as spray in the same concentration. It is approximately 10-fold less potent than the injected form. Oral dDAVP is approximately 10- to 20-fold less potent than when given via the intranasal route (100- to 200-fold less potent than injection), but oral dDAVP is highly effective and safe in children in doses of 50–600 μg (0.05–0.6 mg) every 8–12 h.

Treatment should begin with the lowest amount that gives the desired antidiuretic effect. Dosing can be once or twice per day, and patients with intact thirst should be allowed to escape from the antidiuretic effect briefly at least once a day to allow excessive water to be excreted and reduce the risk of water intoxication. The antidiuretic effect of dDAVP is usually apparent within 60–120 min, although the maximum effect may not be achieved until 24–48 h after the first dose because of the blunting of concentrating capacity caused by chronic water diuresis. Antidiuresis is followed promptly by a 1–2% increase in body water and a similar decrease in plasma osmolality and sodium, which relieves thirst and results in a reduction in fluid intake.

dDAVP cannot reduce fluid output below the level of water intake in a standard diet, so hyponatremia should not occur in the absence of excess fluid intake. However, because the level of antidiuresis is not reduced in response to an increase in hydration, dDAVP treatment can result in hyponatremia if fluid intake is excessive. Patients and parents should be educated about the risk of excessive fluid intake and the signs and symptoms of water intoxication. Patients and families should be counseled that intake should be guided solely by thirst, and children should be taught to avoid incidental or social drinking. It may be helpful to suggest limiting the intake of juice, soda, and other non-nutrient drinks and offering plain water to prevent taste-induced drinking.

Treatment of CDI in the setting of hypodipsia or adipsia

The osmoregulation of thirst is normal in more than 90% of patients with pituitary DI, but a few, mostly those with a history of congenital midline CNS malformations, head trauma, hypothalamic surgery, ruptured anterior communicating artery aneurysms, or suprasellar malignancy, have hypodipsia or adipsia. Because thirst adjusts water intake to compensate for changes in urine output and insensible losses, intact thirst protects against dehydration and hypernatremia or overhydration and hyponatremia during treatment of DI. When hypodipsia or adipsia are present, this greatly complicates the management, as changing urine output is no longer compensated by spontaneous adjustment in fluid intake. Many such patients also have significant defects in cognitive function.

Management of DI in this situation requires a fixed dose of dDAVP and a daily water intake to meet fluid needs under usual conditions of treatment, diet, temperature, and activity. This is usually close to 1 L/m^2/day in the absence of significant diuresis. Patients and their families can be taught to adjust the intake from the fixed target amount based on situational changes, which include changes in urine output due to unexpected changes in dDAVP effect or dietary solute load and increased insensible losses secondary to changes in ambient temperature, illness, or physical activity. Frequent monitoring of serum sodium is essential. This has required laboratory testing but, with the availability of small analyzers designed for point-of-care monitoring, families can be taught to do sodium monitoring in the home [79]. Daily weight can be helpful in determining the need to make interval adjustments in the daily fluid intake, but target weights need to be recalibrated periodically to compensate for growth.

Treatment of CDI in infants

Neonates and young infants receive their nutrition in liquid form, necessitating large fluid intakes to deliver adequate calories. Combining this high fluid intake with dDAVP can result in hyponatremia, and neonates are best managed with fluid therapy alone. However, if an infant with DI cannot concentrate urine to reach an osmolality greater than the renal solute concentration of the feed, excessive urine output and hypernatremia will result. Large volumes of added free water required to reduce the renal solute concentration of standard infant formulas may result in inadequate calorie intake and poor growth. Decreasing the solute load with low-solute formula or breast milk can reduce the obligate urine output, allowing fluid balance to be achieved with modest free water supplementation in infants who can concentrate their urine to between 70 and 100 mOsm/L. If DI is severe and urine cannot be spontaneously concentrated to 70 mOsm/L, addition of a thiazide diuretic is helpful in increasing urine osmolality and reducing urine output [80]. This management is comparable to that used for NDI.

Treatment of post-operative CDI

In the acute post-operative management of DI, vasopressin therapy can be used, but hyponatremia can occur if the child is receiving an excessive amount of fluid. Vasopressin will mask the emergence of the SIADH phase of the triple-phase response to neurosurgical injury or resolution of the DI. For these reasons, it is often best to manage post-operative DI in children with fluids alone. When intravenous therapy is used, input is matched with output hourly, with an initial limit imposed on total fluid administration at 3–5 L/m^2/day.

A basal infusion rate of 1 L/m^2/day (40 mL/m^2/h) should be given as 5% dextrose in 0.22% saline. No additional fluid should be administered for hourly urine volumes under 40 mL/m^2/h. For hourly urine volumes above 40 mL/m^2/h, the additional volume should be replaced with 5% dextrose–water to a maximum of 120–200 mL/m^2/h (3–5 L/m^2/day). For urine outputs above 120 mL/m^2/h, the initial total infusion rate should be 120 mL/m^2/h and may be adjusted up to 200 mL/m^2/h if needed. In the presence of DI, this will result in serum sodium in the 150 mmol/L range. This mildly volume-contracted state should produce a prerenal reduction in urine output, generally avoiding the need to give larger volumes of fluid, and will also allow the assessment of thirst and the return of normal vasopressin function or the emergence of SIADH.

Patients may become mildly hyperglycemic with this regimen, particularly if they are also receiving post-operative glucocorticoids. Frequent assessment of fluid balance, urine specific gravity, and serum electrolytes to determine appropriate adjustments in therapy are essential to avoid fluctuations in volume status, particularly in cases where the triple-phase response develops. If the child is awake and able to drink, free access to water based on thirst should be allowed with advice to avoid non-thirst-mediated fluid intake. If there is concern about impaired thirst, oral intake should be matched to urine output using the same parameters given for intravenous fluid administration.

If therapy with AVP or dDAVP is used, fluid intake should be limited to 1 L/m^2/day, unless unusual non-renal fluid losses are anticipated. Therapy with aqueous vasopressin (Pitressin) is preferred, as its effect is more rapidly reversed, and the dose can be titrated to achieve the desired urine output. For intravenous vasopressin therapy, infusion rates around 1.5–2.5 mU/kg/h are appropriate starting doses sufficient to concentrate urine within 1–6 h of initiation [81]. Occasionally, following hypothalamic surgery, higher concentrations of vasopressin are required initially to treat acute DI, which may be attributable to the release of biologically inactive vasopressin-like peptides acting as antagonists to normal vasopressin activity [82]. If dDAVP is used, the need for additional doses should be determined by whether or not polyuria and hypernatremia recur following each dose. Patients treated with vasopressin for post-neurosurgical DI should be switched from intravenous to oral fluid intake at the earliest opportunity, because thirst sensation, if intact, will help to regulate blood osmolality and minimize the risk of significant hypernatremia or hyponatremia.

Nephrogenic DI

Nephrogenic (vasopressin-resistant) diabetes insipidus (NDI) is characterized by impaired urinary concentrating ability despite normal or elevated plasma concentrations of AVP and can be genetic or acquired. Genetic etiologies are diagnosed during childhood and are generally more severe than acquired causes. Acquired NDI can occur as a component of acquired kidney disease, in the setting of a number of metabolic abnormalities, or in response to drugs.

Causes of NDI

Genetic NDI

There are three genetic causes of NDI, all of which are rare (Table 17.3). The symptoms are the same, but they can be differentiated by their pattern of inheritance. In contrast to familial CDI, polyuria and polydipsia are present from birth with NDI, and pregnancies involving affected infants can be complicated by hydramnios. Unless the disease is recognized early, affected children have repeated episodes of dehydration, sometimes complicated by convulsions and death. Affected infants are irritable and present with symptoms such as vomiting, anorexia, failure-to-thrive, fever, and constipation. Serum sodium is generally elevated at presentation. Growth failure in the untreated child may be secondary to the ingestion of large amounts of water, which the child may prefer over other higher calorie substances and/or due to general poor health resulting from dehydration and hypernatremia. Mental retardation of variable severity has historically been the norm, but it is probable that this results from frequent episodes of dehydration and can be prevented by early recognition and appropriate management. In a series of 30 patients from the Netherlands, the average age at diagnosis was 9 months, and a majority had normal intelligence later in childhood [83]. However, even with early institution of therapy, short stature remains common in children with congenital NDI. Some patients develop severe dilation of the urinary tract, which may predispose to rupture after minor trauma. Intracranial calcification has been described in NDI.

Congenital, X-linked NDI accounts for more than 90% of congenital cases. It has an estimated population prevalence of eight per million males, although the frequency is higher in some populations. It is caused by loss-of-function mutations in the AVP V_2 receptor, which is located in the chromosome region Xq28. Although it is X linked, heterozygous females may rarely be affected, presumably as a result of X-chromosome inactivation of the wild-type locus. More than 180 mutations in the V_2 receptor have been identified [84] (Fig. 17.7a). They are mostly single base mutations that result in amino acid substitutions (50%), translational frameshifts (27%), or termination of peptide synthesis (11%). Most of the mutations result in abnormal, misfolded proteins that are trapped in the endoplasmic reticulum, apparently as a result of failed processing through the N-linked glycosylation pathway. A few have been demonstrated to result in proteins with normal abundance on the cell surface but impaired AVP binding or G-protein coupling. Almost all mutations in the V_2 receptor gene result in severe NDI, but there are a few exceptions to this rule, which are associated with a milder phenotype and later presentation.

Autosomal NDI constitutes less than 10% of cases of NDI and is caused by mutations in the aquaporin-2 gene, located in the 12q13 chromosome region. More than 30 mutations have been identified [84] (Fig. 17.7b and c) that impair the ability of the luminal membrane to undergo an increase in water permeability following signaling through the V_2 receptor. Most of the known mutations in AQP-2 result in autosomal-recessive disease. The mutations result in misfolding and misrouting of AQP-2 mutant proteins, with retention of the protein within the endoplasmic reticulum. A rare autosomal-dominant form of NDI has been described in a few families. Oocyte expression studies have shown that the autosomal-dominant protein behaves in a dominant-negative way by forming heteroligomers with wild-type AQP-2 and impairing routing of both wild-type and mutant proteins out of the Golgi apparatus to the plasma membrane [85].

AQP-2 mutations can be distinguished from the V_2 receptor mutation forms by demonstrating the presence of extrarenal V_2-mediated responses to dDAVP. Patients with autosomal NDI show normal increases in von Willebrand factor, factor VIII, and tissue-type plasminogen activator levels in response to dDAVP, while these responses are absent in X-linked NDI. These tests are difficult to do in infants and have been replaced by molecular identification of the mutations in aquaporin-2 or the V_2 receptor. If the family history is suggestive of familial DI, genetic characterization of the defect is appropriate. Patients with a family history of NDI can be evaluated for the disorder in the prenatal or perinatal period by DNA sequence analysis, allowing therapy to be initiated without delay [86].

Table 17.3. Causes of nephrogenic diabetes insipidus.

Genetic
X-linked recessive (AVP-V_2 receptor)
Autosomal recessive (aquaporin-2)
Autosomal dominant (aquaporin-2)
Acquired
Drugs
Lithium
Foscarnet
Demeclocycline
Many others
Metabolic
Hyperglycemia
Hypercalcemia
Hypokalemia
Protein malnutrition
Renal
Chronic renal failure
Ischemic injury
Impaired medullary function
Outflow obstruction

Acquired NDI

Drug-induced NDI is not common, but approximately 50% of patients receiving lithium have impaired urinary concentrating ability, and 10–20% of them develop symptomatic NDI on long-term therapy. Lithium appears to act by decreasing

AQP-2 targeting to the apical membrane. The risk of symptomatic DI increases with duration of lithium therapy, and NDI may be very slow to recover or may persist following discontinuation of lithium therapy.

Other drugs that have been reported more rarely to be associated with NDI include foscarnet, cidofovir, clozapine, fluvoxamine, amphotericin, gentamicin, demeclocycline, cyclophosphamide, isophosphamide, methotrexate, cimetidine, verapamil, methoxyflurane, colchicine, and glyburide. Most of these cause NDI in the setting of severe illness. Foscarnet, the second most common drug associated with NDI, appears to interfere with the transduction of the AVP signal proximal to cAMP, and its effect can be blocked by non-steroidal anti-inflammatory drugs (NSAIDs), suggesting that it may be mediated via prostaglandins. How any of the other agents cause NDI is not known.

Metabolic causes of NDI such as hyperglycemia, hypokalemia, and hypercalcemia are associated with AVP-resistant polyuria and polydipsia. Hyperglycemia causes an osmotic diuresis, preventing normal water reabsorption even in the face of intact signaling. In contrast to other forms of polyuria and polydipsia, osmotic diuresis is associated with increased urine osmolality. In hypokalemia, the DI is not as severe as that seen with familial or lithium-induced NDI but does appear to result from a true reduction in AVP responsiveness, probably by a reduction in total AQP-2. Hypercalcemia-associated polyuria and polydipsia is associated with AQP-2 downregulation and diminished trafficking of AQP-2 to the collecting duct apical membrane.

Kidney disease: impaired urinary concentrating capacity and unresponsiveness to AVP occurs in acute and chronic renal failure. In animal models of ischemic renal failure, aquaporins 1–4 were all decreased. In addition, impairment of the countercurrent concentrating mechanism probably contributes to reduced concentrating ability. Defective medullary countercurrent function resulting in AVP-resistant concentrating defects are also seen in other diseases that cause medullary damage, such as sickle cell disease, Sjögren syndrome, amyloidosis, sarcoid, and cystinosis. Protein malnutrition or low sodium intake can also lead to diminished tonicity of the renal medullary interstitium and diminish the driving force for water reabsorption. Urinary tract obstruction produces polyuria, which appears to be multifactorial but includes decreased AQP-2 [87].

Management of NDI

Once the diagnosis of NDI has been established, management requires a search for the cause. Treatment of acquired NDI focuses on elimination of the underlying disorder or drug, if possible. In the setting of congenital NDI, the main goals are to insure an adequate intake of calories for growth and to avoid severe dehydration. Unlike CDI, the therapies for congenital NDI do not completely eliminate polyuria, but reduction of polyuria to 3–4 L/m^2/day is often achievable.

Foods with the highest ratio of calorie content to osmotic load should be used to maximize growth and minimize the urine volume required to excrete solute. Thiazide diuretics in combination with amiloride or indomethacin are the most useful pharmacological agents in the treatment of NDI. The most commonly used thiazide is hydrochlorothiazide 2–4 mg/kg/day divided two or three times daily. Thiazides inhibit the NaCl co-transporter in the distal convoluted tubule. It has long been held that the resultant increased sodium excretion results in extracellular volume contraction, decreased glomerular filtration rate (GFR), and ultimately an increase in proximal tubule sodium and water reabsorption. This results in decreased delivery of water and sodium to the collecting tubules and therefore decreased urine output. However, recent work indicates that thiazides also directly increase water resorption in the collecting duct [88]. The thiazide-induced increase in water permeability is decreased in the presence of prostaglandins, explaining why indomethacin potentiates the thiazide effect.

Indomethacin (2 mg/kg/24 h) can be associated with significant side-effects, most notably gastrointestinal (GI) bleeding. With the introduction of cyclooxygenase-2 specific inhibitors, it may be possible to reap the benefits of the prostaglandin inhibitory effect, with a reduced risk of side-effects. The combination of thiazide and amiloride diuretics is the most commonly used combination regimen for the treatment of congenital NDI. When thiazides are used alone, potassium depletion can occur, and hypokalemia itself can cause vasopressin-resistant polyuria. Amiloride, used at a dose of 0.3 mg/kg/day divided three times per day, counteracts thiazide-induced hypokalemia, avoids the toxicity associated with indomethacin therapy, and is well tolerated, even with prolonged treatment [89].

Synthetic membrane-permeable AVP antagonists can act as chemical chaperones, increasing the movement of mutant V_2 to the plasma membrane. Once there, some of the mutant proteins are capable of generating AVP-mediated cAMP production and presumably AVP-mediated water uptake [90]. These antagonists offer hope of more effective therapy for at least some forms of X-linked NDI in the future.

Primary polydipsia

Polyuria and polydipsia with low vasopressin levels can result from excessive intake of water and is often mistaken for DI. However, in contrast to DI, hypernatremia is never seen. Excessive intake of water slightly reduces the effective osmotic pressure of body fluids. Inhibition of the secretion of vasopressin allows water diuresis to compensate for the increased intake. This condition is called primary polydipsia, and it can be seen in a number of situations, all of which are uncommon. Because they do occur occasionally and because dDAVP therapy is contraindicated in most cases, it must be distinguished from DI. Therapy with dDAVP may cause

water intoxication to develop rapidly, usually within 24–48 h and characterized by varying levels of hyponatremia as well as central nervous symptoms and signs. Therefore, whenever dDAVP is used to diagnose or treat a patient in whom the diagnosis of primary polydipsia cannot be excluded, the trial should be initiated with close monitoring of fluid balance for the first 24–48 h.

Psychogenic polydipsia

Primary polydipsia can occur as part of a general cognitive defect associated with schizophrenia or other psychiatric disorders, which is usually called psychogenic polydipsia or compulsive water drinking. This is very rare in children but may occur in adolescents. With rare exception, patients do not complain of thirst and usually attribute their polydipsia to disordered beliefs. Water intake in psychogenic polydipsia is often in excess of that necessary to maintain fluid balance, even in DI, and may occasionally exceed the renal capacity to excrete free water, predisposing individuals with this form of primary polydipsia to hyponatremia. Treatment focuses on the underlying psychiatric disorder. If water intake has been sufficient to cause hyponatremia in the absence of dDAVP, the patient may need supervised care to control access to water.

Dipsogenic polydipsia

In dipsogenic polydipsia, increased water consumption is due to an increase in thirst. This can be seen in diseases involving the hypothalamus, although it is most often idiopathic. The management should include a search for the cause and for the presence of associated defects in hypothalamic and anterior pituitary function. Some cases of dipsogenic polydipsia appear to result from resetting the osmotic threshold for thirst below the threshold for vasopressin release. Because vasopressin secretion is suppressed at the thirst osmotic threshold, the ingested water is rapidly excreted and thirst persists. dDAVP therapy may be beneficial in cases where thirst and AVP osmotic threshold are reversed, because it allows the serum osmolality to fall below the threshold for thirst, thereby suppressing water ingestion.

Iatrogenic polydipsia

Primary polydipsia can also be prompted by incorrect advice or incorrect understanding of advice offered by physicians, nurses, folk practitioners, or the lay media. It is usually mild and rarely results in urine outputs of more than 5 L/day. It can be corrected if those involved are amenable to adopting more appropriate fluid intake practices.

Other causes of hypernatremia

Hypernatremia (serum sodium concentration > 145 mmol/L) is caused by loss of water or gain of sodium. Because the increased serum osmolality associated with hypernatremia induces intense thirst, even a modest rise in serum sodium stimulates water ingestion, preventing progression of hypernatremia. Therefore, in a normal ambulatory individual with intact thirst, hypernatremia rarely occurs, even in the setting of DI. A prerequisite for hypernatremia is impaired water intake relative to water requirement.

Adipsic hypernatremia

Primary adipsia is usually caused by lesions in the anterior hypothalamus and is often accompanied by DI with impaired AVP release in response to increasing serum osmolality, but adipsia can occur without DI, as can adipsic hypernatremia. The absence of thirst in a conscious hypernatremic child can be assessed by offering water and observing the response. The water intake associated with a normal diet is insufficient to match obligate renal, bowel, and insensible water losses, and absent thirst can lead to hypernatremic dehydration. Hypernatremia can develop slowly, and even moderate to severe elevations in plasma sodium may be well tolerated and cause no obvious clinical abnormalities. The development of hypernatremia can be accelerated by an increase in urinary or stool water loss or an increase in insensible losses associated with exercise, increase in environmental temperature, or fever. If hypernatremic dehydration develops rapidly or is particularly severe, it usually results in overt clinical signs of hypovolemia, and damage to the brain and other organs can occur.

As with DI, adipsia requires an evaluation of the cause, including cranial MRI to evaluate the hypothalamus. The long-term management of patients with adipsic hypernatremia should prevent or minimize recurrences of hypertonic dehydration by minimizing urinary water losses using dDAVP if DI is present and insuring that fluid intake is sufficient to replace total water output. Even in the absence of dDAVP, caution should be taken to avoid excessive fluid intake as some of these individuals will have an impaired ability to downregulate AVP secretion and, in the setting of increased fluid intake, may be at risk of water intoxication.

Physical obstacles to drinking

Adipsic hypernatremia should be distinguished from the presence of physical obstacles to drinking, such as occur in patients who are debilitated by acute or chronic illness, have neurological impairment, or are at the extremes of age. Immobilized patients who can talk would not be expected to develop hypernatremia because they can indicate their desire to drink, but very young children or those with significant

developmental delay are at risk of dehydration if their obligate fluid losses are not anticipated and replaced.

Excessive free water losses (other than DI)

When an acute illness results in inadequate fluid intake or excessive free water losses, which cannot be compensated by increased fluid intake because of the ongoing disease process, hypernatremia can result. Gastroenteritis with prolonged vomiting and diarrhea is the most common example. In severely ill hospitalized patients, hypernatremia can also be caused by inadequate replacement of increased insensible water loss, which may occur in patients with burns or high fever or by failure to quantitate and replace increased losses from other sources.

Excessive sodium intake

Increased sodium intake can be due to accidental ingestion of large quantities of salt or intravenous administration of hypertonic solutions. Increased water intake corrects the hyperosmolality, and excess sodium is rapidly excreted as long as thirst and renal function are intact, and there are no physical limitations preventing access to water. In infants, severely ill, or debilitated patients, excessive sodium administration can result in persistent hypernatremia until adequate free water is provided to allow salt diuresis.

Correction of hypernatremia

The initial treatment of hypernatremia should replace the water deficiency and minimize further losses by treating DI or diabetes mellitus if either is present. Hypokalemia should also be treated. Free water should be given orally if possible, but it can also be infused intravenously either as 5% dextrose in 0.225% saline or as 5% dextrose–water (if hyperglycemia is not present). The net increase in body water that must be achieved to correct the deficit can be estimated by the formula

$$\Delta H_2O = [(P_{Na} - 140)/140] \times 0.6 \times BW$$

where ΔH_2O is the estimated water deficit in liters, P_{Na} is the plasma sodium concentration expressed in mmol/L, and BW is the body weight in kilograms.

Non-acute hypernatremic dehydration or hypernatremic dehydration of uncertain duration should be corrected gradually. Hypernatremia results in an efflux of fluid from the intracellular space to maintain osmotic equilibrium. This leads to transient intracellular dehydration. The brain significantly increases its content of sodium, potassium, amino acids, and unmeasured organic substances, and regains 98% of its water content within 1 week, despite persistent hypernatremia [91]. Although protective during hypernatremia, these compensatory changes can result in cellular swelling if hypernatremia is corrected too rapidly. Swelling of cells in most organs does not have life-threatening consequences but, when it occurs in the brain, cerebral edema and the resultant herniation can be devastating.

Therefore, when hypernatremia has been present for more then 12 h or the duration is uncertain, the target should be to replace the free water deficit over a minimum of 24–48 h and to avoid changes in sodium concentration exceeding 0.5–1 mmol/L/h. The rate of fluid administration will be determined by the free water deficit, divided by the desired time over which the deficit is to be replaced, added to fluid administration sufficient to replace estimated or measured renal and GI output and estimated insensible losses. During the correction of hypernatremia, fluid intake, output, and plasma sodium (and glucose if hyperglycemia is present) should be monitored frequently, and the treatment plan adjusted as necessary to achieve the desired rate of correction.

Vasopressin excess

The primary defense against developing hyponatremia is the ability to generate dilute urine and excrete free water, a process inhibited by vasopressin. Primary renal disease can prevent free water excretion, independent of vasopressin signaling. If dilute urine cannot be produced and water intake is not reduced sufficiently to match the reduction in urine output, free water accumulates and dilutes body fluids, resulting in hypotonic hyponatremia. The clinical manifestations result from the effects of hyponatremia on the CNS and include anorexia, headache, nausea, vomiting, muscle cramps, and weakness. As hyponatremia advances, symptoms include impaired responsiveness, bizarre behavior, hallucinations, obtundation, incontinence, seizures, and respiratory insufficiency. In its most severe form, hyponatremia may progress to cerebral edema and impending herniation, including decorticate or decerebrate posturing, bradycardia, hyper- or hypotension, altered temperature regulation, dilated pupils, and respiratory arrest. The severity of the neurological effects depends on the degree of hyponatremia, the rate of decline, and the age of the patient. Children appear to be more susceptible to symptomatic hyponatremia after closure of the fontanelles, which leaves less room for brain expansion [92]. Females after menarche and before menopause are at increased risk of developing symptomatic hyponatremia probably because estrogen inhibits compensatory changes in intracellular osmolality. If hyponatremia develops rapidly (over less then 24–48 h) and/or is exceptionally severe (less than 120 mmol/L), it usually results in symptoms and signs.

Determining the etiology of the hyponatremia is critical, as management depends on the diagnosis. The duration and degree of hyponatremia and the presence or absence of symptoms are important considerations. Acute (< 24–48 h) hyponatremia can be corrected quickly, but chronic asymptomatic

hyponatremia should be corrected slowly, as overly rapid correction has been associated with CNS injury. Symptomatic chronic hyponatremia requires a phase of rapid correction, until symptoms remit, followed by further gradual correction.

Diagnostic approach to hyponatremia

Hyponatremia typically occurs with hypotonicity but can also be found in the context of a normal or elevated serum osmolality. The first step in the evaluation of hyponatremia is confirmation that it is associated with hypotonicity by measuring serum osmolality (Fig. 17.11). The most common cause of hypertonic hyponatremia is hyperglycemia. Glucose-induced hyperosmolality causes an osmotic shift of fluid from the intracellular into the extracellular space, resulting in a dilutional decrease in sodium. If hyperglycemia is present, plasma sodium (P_{Na}) should be corrected for dilution using the formula,

$$P\text{-}Na_{Cor} = P_{Na} + [(Glu - 100) \times 1.6]/100$$

where $P\text{-}Na_{Cor}$ is the corrected plasma sodium in mmol/L and Glu is the plasma glucose in mg/dL. Other solutes, such as mannitol, sorbitol, maltose, and radiocontrast, can also produce hypertonic hyponatremia. Normotonic hyponatremia can be seen in severe hyperproteinemia or hyperlipidemia, which cause a displacement of plasma water. When sodium is measured by methods that involve dilution of the specimen before measurement, sodium concentration appears to be low, although if measured directly in the aqueous phase of the serum only, sodium and osmolality are normal. Hyponatremia in this situation is thus a laboratory artifact and can be called pseudohyponatremia.

In true hypotonic hyponatremia, information on intake and output, weight changes, medication, and underlying medical illnesses is required, as is a clinical assessment of volume status to determine whether the patient is hypovolemic, euvolemic, or hypervolemic. The measurement of serum electrolytes, urea nitrogen, and creatinine provides rapid evidence for significant impairment in renal function and may provide additional information in the determination of volume status. Urine osmolality and sodium are also critical. Hyponatremia associated with excessive water intake is associated with low vasopressin levels, large urine volumes, and maximally dilute urine. Hypotonic hyponatremia associated with anything other than maximally dilute urine (< 100 mOsm/L) in the absence of diuretic use reflects either primary renal disease or increased AVP levels. A significant decrease in effective circulating volume induces non-osmotic but appropriate AVP secretion and can result in hypotonic hyponatremia. Therefore, as many forms of hyponatremia have associated increases in AVP levels, it is important to determine whether the increased AVP reflects a

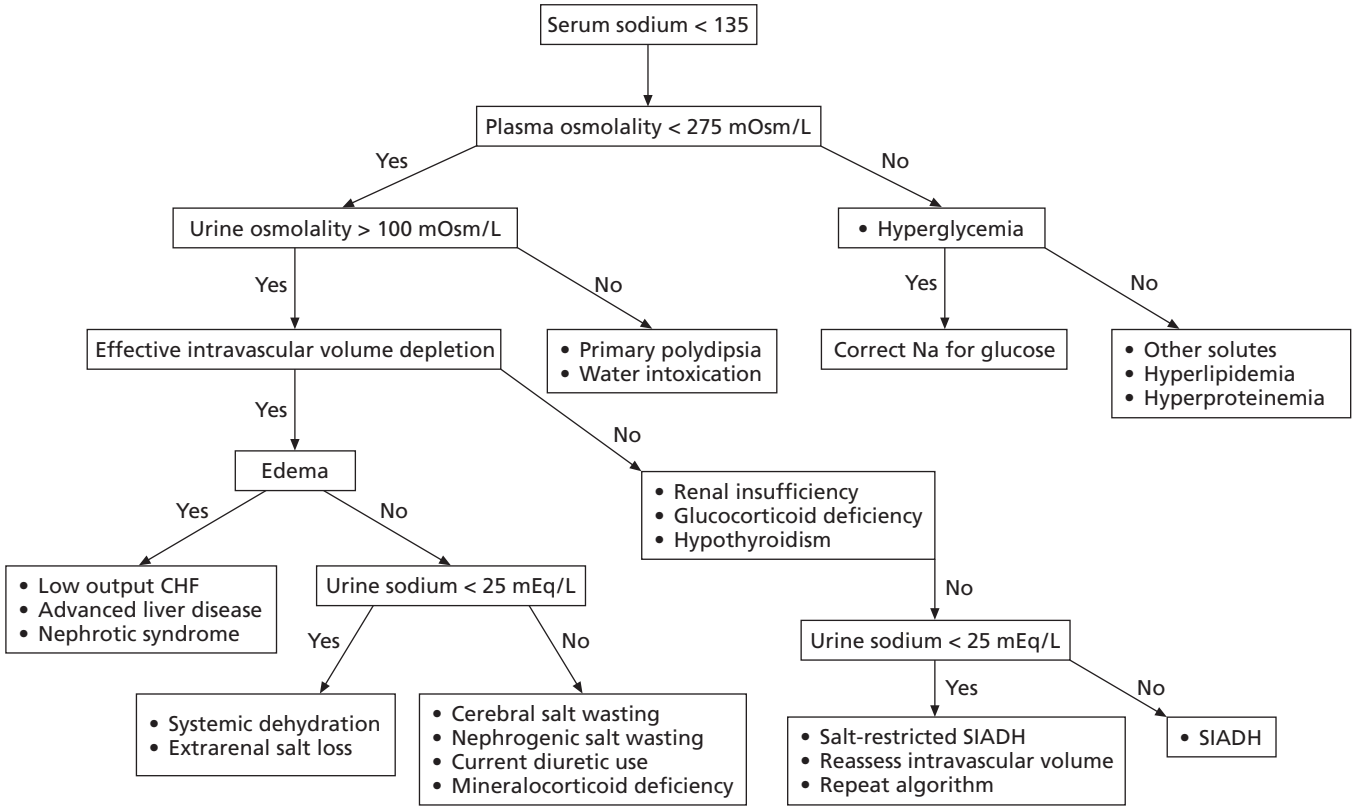

Fig. 17.11. Diagnostic approach to hyponatremia.

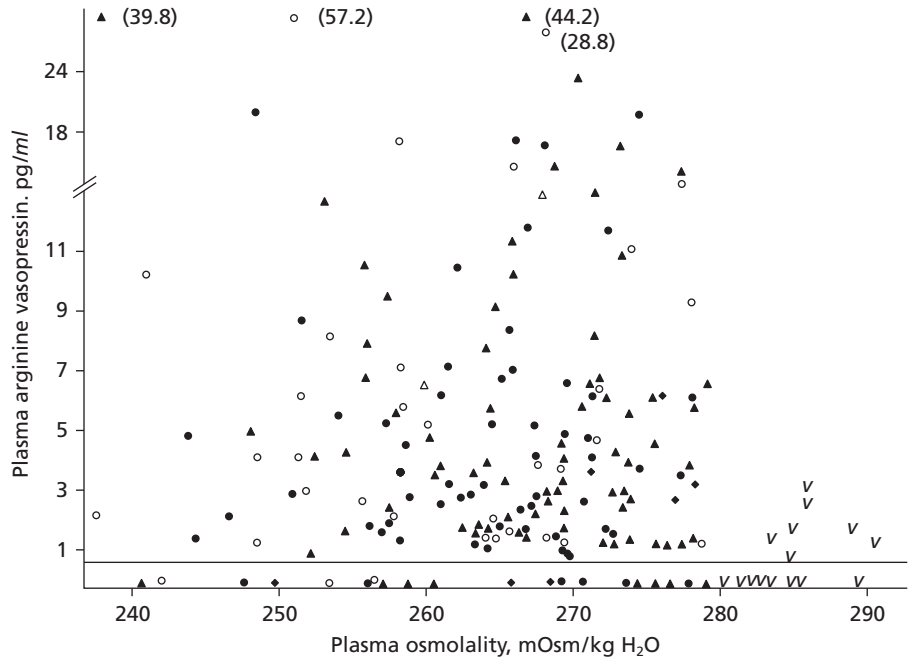

Fig. 17.12. Plasma vasopressin concentration in hyponatremia. It is generally not helpful to measure vasopressin levels during the evaluation of hyponatremia as most patients with hyponatremia have vasopressin levels that are inappropriately elevated for their serum osmolality. Fifteen healthy volunteers (v) who drank 1 L of water per hour for 8–10 h suppressed their vasopressin levels as their plasma osmolality declined and did not develop hyponatremia. In contrast, in 51 patients with CHF-associated hyponatremia (△), 35 patients with cirrhosis-associated hyponatremia (▲), and 55 patients with volume depletion-associated hyponatremia (●), the majority have elevated vasopressin levels despite their hyponatremia. This is presumed to be due to intravascular volume depletion-mediated vasopressin release, and these vasopressin levels are indistinguishable from those seen in 36 individuals with SIADH (○). Nine patients without a known etiology of their hyponatremia are also included (◆). The line at 0.75 pg/mL indicates the minimum detectable vasopressin level in the assay used. The numbers in parentheses at the top indicate the value of plasma vasopressin measurements that are beyond the scale of this diagram. Reproduced with permission from [113].

physiologically appropriate process or is due to inappropriate increases in AVP secretion (Fig. 17.12) [93].

Inappropriate AVP secretion

Osmotically inappropriate AVP secretion that cannot be accounted for by a non-osmotic stimulus implies a primary abnormality in the regulation of vasopressin secretion, the syndrome of inappropriate antidiuretic hormone (SIADH). Patients with SIADH fail to suppress AVP secretion even when plasma osmolality falls below the normal osmotic threshold for stimulated AVP release. This results in impaired renal free water clearance, total body free water excess, and hyponatremia. SIADH is the most common etiology of severe hyponatremia, but the mechanism is not fully understood.

Mechanism of hyponatremia and euvolemia in SIADH

Even maximal antidiuresis by AVP causes little or no decrease in plasma sodium unless fluid intake is maintained at a level greater than total urinary and insensible output. Excessive fluid intake in patients can be due to inappropriate administration of intravenous, nasogastric, or G-tube fluids, non-thirst-mediated drinking, or an associated abnormality in the osmoregulation of thirst. Under these conditions, the excess water accumulates, expanding and diluting body fluids. When the expansion of body water exceeds 5–6%, plasma renin activity and aldosterone are suppressed, atrial natriuretic peptide levels increase, and urine sodium excretion begins to rise. These compensatory mechanisms interrupt further increase in the extracellular volume expansion, preventing extreme volume expansion, but aggravate the hyponatremia.

Because of these compensatory changes, individuals with SIADH are clinically euvolemic and, once they have reached this phase of SIADH, urine free water and sodium excretion rates parallel the rates of water and sodium intake. The increase in urine sodium mediated by the AVP-induced volume expansion helps to distinguish SIADH from other forms of hypotonic hyponatremia. In the absence of diuretic use or renal disease, hyponatremia with low urine output and increased urine sodium (> 20–30 mmol/L) is consistent with SIADH, but SIADH should not be diagnosed if there is clinical evidence of hypovolemia or peripheral edema. Low normal blood urea nitrogen and creatinine and low uric

Table 17.4. Drugs associated with impaired water clearance.

Class	Common drugs
Angiotensin-converting enzyme inhibitors	Lisinopril
Anticonvulsants	Carbamazepine Oxacarbazepine Valproic acid
Anitneoplastics	Cis-platinum Cyclophosphamide Vinblastine Vincristine
Antiparkinsonian	Amantadine Trihexyphenidyl
Antipsychotics	Haloperidol Thioridazine
Antipyretics	Acetaminophen
Hypolipidemics	Clofibrate
Oral hypoglycemics	Chlorpropamide Tolbutamide
Selective serotonin uptake inhibitors	Fluoxetine Sertraline
Tricyclic antidepressants	Imipramine Amitriptyline
Other	Ecstasy

acid can provide additional laboratory evidence of the mild volume expansion expected with SIADH.

Causes of SIADH

Tumors: SIADH was first described with bronchogenic carcinoma and has since been recognized in patients with several other types of tumors, particularly of neuroendocrine origin. Small-cell lung carcinomas are capable of ectopic synthesis and release of AVP. A small percentage of other lung tumors can also produce and release AVP, but they are rare. Head and neck tumors have also been associated with SIADH. Although many SIADH-associated tumors have been shown to produce AVP, a number have not. In those cases, it is presumed that the tumor produces another substance that promotes AVP secretion. Because most SIADH-associated tumors are not seen in children, tumors are a rare cause of SIADH in children.

Drugs are more often the cause of impaired free water clearance, which can result from alteration in vasopressin release, enhanced vasopressin effect at the same plasma vasopressin concentration, or vasopressin-independent changes in distal collecting tubule water permeability. Drugs associated with impaired free water clearance are listed in Table 17.4. Common drugs that have been shown to increase antidiuretic hormone (ADH) secretion and result in hyponatremia include carbamazepine [94], chlorpropamide [95], vinblastine [96], vincristine [97], tricyclic antidepressants [98], and the recreational drug Ecstasy (3,4-methylenedioxymethamphetamine) [99].

CNS disorders include inflammatory processes, such as systemic lupus, sarcoid, and Guillain–Barré syndrome, and infectious causes, such as meningitis and encephalitis. Tuberculous meningitis seems to have an unusually high association with SIADH, with 70% of tuberculous meningitis cases in children affected [100]. SIADH has also been seen in association with CNS mass lesions, such as tumors or brain abscesses, following cerebrovascular accident, and following CNS surgery or traumatic injury. In CNS injury, care must be taken to distinguish SIADH from cerebral salt wasting, which also causes hyponatremia but requires markedly different management.

Non-malignant pulmonary disorders such as hypoxia and hypercapnia elevate plasma AVP levels. SIADH has been seen in advanced chronic obstructive pulmonary disease and several other pulmonary disorders, including tuberculosis, severe asthma, respiratory syncytial virus (RSV) bronchiolitis, cystic fibrosis (CF) exacerbation, and pneumonia. SIADH generally occurs with acute respiratory failure but is limited to the period of respiratory failure. Mechanical ventilation, particularly positive pressure ventilation, has been associated with increased AVP release and SIADH probably because of decreased vascular return to the heart resulting in decreased cardiac output and decreased stretch on the baroreceptors.

Post-operative hyponatremia. It has long been recognized that the use of hypotonic saline for hydration during acute post-surgical management puts patients at risk of hyponatremia and, for this reason, isotonic saline solutions are widely used for fluid support following surgery. However, despite these practices, more than 50% of children sustaining neurological morbidity and mortality associated with hospital-acquired hyponatremia became hyponatremic following minor surgical procedures while receiving hypotonic fluids [101]. The etiology of post-surgical hyponatremia is probably multifactorial but is generally associated with a self-limited increase in AVP levels. Nausea, which is a common post-operative symptom, is a stimulus for AVP secretion as are pain, stress, anxiety, and fever. Whether due to intravascular volume depletion or other stimuli, AVP levels are high in the immediate post-operative period so excessive fluids should be avoided.

Adrenal insufficiency/hypothyroidism: an SIADH-like syndrome occurs in patients with adrenal insufficiency [102], which impairs free water excretion. Cortisol-deficient states are associated with increased AVP levels and also cause antidiuresis in patients with central DI, suggesting that cortisol may also have an effect on urine concentration independent of AVP. As in true SIADH, the development of hyponatremia is dependent upon excessive water intake. Patients with hyponatremia due to isolated glucocorticoid deficiency, as is

seen in secondary adrenal insufficiency, do not have hyperkalemia or signs of hypovolemia and can be reliably differentiated from patients with SIADH only by measuring cortisol. Severe hypothyroidism can also produce an SIADH-like hyponatremia, the etiology of which is unknown. SIADH should not be diagnosed until hypothyroidism and adrenal insufficiency have been excluded.

Management of SIADH

The underlying cause should be identified and treated, and the excess in body water should be corrected. A primary abnormality in vasopressin secretion can often be eliminated when the hormone is made ectopically, given exogenously (dDAVP), results from a deficiency in glucocorticoid or thyroid hormone, or is released from the posterior pituitary in response to drugs that can be discontinued. Much of the time, however, the abnormal vasopressin secretion cannot be corrected and must be allowed to run its course until spontaneous recovery occurs. Fortunately, SIADH is generally temporary, and therapy will be necessary only until it remits.

Asymptomatic hyponatremia

When hyponatremia is not severe (serum sodium > 120 mmol/L) and is asymptomatic, fluid restriction is the optimal therapy. The goal is to promote a gradual increase in sodium, followed by stabilization in the normal range. Rapid correction of hyponatremia has been associated with central pontine myelinolysis, a rare and sometimes fatal neurological disorder characterized by demyelination that involves the central portion of the base of the pons and causes spastic quadriparesis, pseudobulbar paralysis with dysphagia, and dysarthria. Myelinolysis lesions may also be extrapontine, and this syndrome has also been called osmotic demyelinating syndrome.

Patients in whom hyponatremia has been present for more than 48 h may have fewer symptoms but are more likely to have serious complications from rapid correction of hyponatremia. The target is to increase the sodium at not more than 0.5 mmol/h or 12 mmol/day. To promote an increase in serum sodium, fluid restriction to amounts below obligate urine and insensible losses is required, generally 600–800 mL/m^2/day. The expected rate of increase in sodium with such a fluid restriction in place is well below the maximum target of 10–12 mmol/day. Once the sodium has risen into the desired range, maintenance of it in that range can generally be achieved with a fluid restriction of 0.8–1 L/m^2/day. Fluid restriction can be difficult in infants and young children, as the water content of formula or baby food is relatively high, and fluid restriction may result in unintentional calorie deficit. Fluid restriction can also become burdensome to older children when SIADH is prolonged, and additional measures may be needed.

Inhibiting the AVP effect

Body water can also be reduced by inhibiting the antidiuretic effects of vasopressin using demeclocycline, a tetracycline derivative. At conventional doses of up to 1.2 g/day in adults, it causes a reversible form of NDI in almost all patients with SIADH in a week or more. Its potential renal toxicity may cause a rise in plasma urea, which is reversible when the drug is stopped. Its efficacy and safety in treating infants and children with SIADH is unknown, and its use should be monitored closely and restricted to patients with chronic SIADH not amenable to fluid restriction.

Lithium carbonate also causes a form of NDI but, at conventional doses, this effect is inconsistent and, with its narrow therapeutic window, it is prone to produce undesirable side-effects. For these reasons, lithium should not be used for treatment of SIADH in children. Potent oral non-peptide V_2 receptor antagonists capable of blocking the antidiuretic effect of vasopressin have been developed and tested in humans. Preliminary trials indicate that these antagonists are safe and effective in promoting a water diuresis and raising the plasma sodium in adults with SIADH [103], but they have not been tested in children.

Severe or symptomatic hyponatremia

Brain edema associated with hyponatremia can result in decreased cerebral blood flow, hypoxic brain injury, herniation, and cardiopulmonary arrest. Symptomatic hyponatremia is a medical emergency, requiring prompt treatment to prevent permanent brain damage or death. Fluid restriction should be instituted in all cases of SIADH-induced hyponatremia, but hypertonic saline should be used in addition in symptomatic or severe hyponatremia to raise serum sodium more rapidly. Because of the risk of central pontine myelinolysis, hypertonic saline (3% saline at a rate of 1–2 mL/kg/h) should be used with careful monitoring and only to the extent necessary to raise plasma sodium to asymptomatic levels at a rate of 1–2 mmol/L/h. If hyponatremia is of long standing, hypertonic saline treatment must be undertaken with particular caution, and correction rates of 0.5–1 mmol/h may be more appropriate. Urine output as well as plasma sodium should be monitored at least every 2 h during hypertonic saline infusion and every 2–4 h during subsequent fluid restriction.

Once symptoms associated with hyponatremia have remitted, hypertonic saline infusion should be stopped, and further correction should be achieved with fluid restriction alone, with fluid rates sufficient to produce sodium increases no greater than 0.5 mmol/L/h and limited to 10–12 mmol in any 24-h period. Following the rapid correction phase, this may mean that no further increase in sodium is attempted during the first 24 h of care. Normal saline alone is not appropriate for the treatment of severe hyponatremia in SIADH as the sodium infused will be excreted rapidly while water is retained, potentially worsening the hyponatremia. Loop

diuretic treatment, to increase free water clearance and diminish whole-body volume expansion, has been used to manage SIADH-induced hyponatremia together with hypertonic saline administration.

Overtreatment

During treatment of severe chronic hyponatremia, overtreatment leading to an increase in sodium above 10–12 mmol/L/24 h may occur despite careful monitoring. If overcorrecting is associated with a change in mental state, suggesting possible brain injury, or is greater than 15 mmol/L/24 h, it may be appropriate to lower the sodium again to levels at which the patient's symptoms improve or the daily increase in sodium remains below 10 mmol/L/24 h. This can be accomplished with infusion of 5% dextrose or 5% dextrose following administration of DDAVP if the SIADH has resolved. Once the patient has stabilized at the lower sodium, the process of correction can be restarted at a slower rate.

Appropriately increased secretion of vasopressin

Hypovolemic hyponatremia

Osmotically inappropriate but physiologically appropriate thirst and vasopressin secretion occur during large reductions in extracellular volume. The syndrome of hypovolemic hyponatremia can result from a number of salt- and water-depleting diseases, such as severe gastroenteritis, renal tubular acidosis, medullary cystic disease of the kidney, pyelonephritis, deficiency of aldosterone or aldosterone action, and during diuretic therapy.

During systemic dehydration from diseases such as gastroenteritis, there is a fall in the renal GFR that results in an increase in proximal tubular sodium and water reabsorption, with a concomitant decrease in distal tubular water excretion. This, along with the associated stimulation of the renin–angiotensin–aldosterone system and suppression of atrial natriuretic peptide secretion by decreased vascular volume, results in the excretion of a concentrated urine that is very low in sodium. As dehydration progresses, hypovolemia and/or hypotension become major stimuli for AVP release, even in the presence of hypotonicity. These physiological responses, although they preserve volume, can cause hyponatremia, especially if water replacement in excess of salt replacement is given. Hyponatremia may be evident from physical signs, such as increased heart rate and decreased skin turgor; laboratory studies will show hemoconcentration and elevated blood urea nitrogen. However, the diagnosis may be subtle, and urine sodium concentration can be very helpful: it should be low in dehydration- or salt loss-associated hyponatremia (< 10–20 mmol/L) in the absence of diuretic use or renal disease.

In contrast to the hyponatremia associated with SIADH, patients with systemic dehydration should be rehydrated with isotonic salt-containing fluids, such as normal saline or lactated Ringer's solution. Because of activation of the renin–angiotensin–aldosterone system, the administered sodium will be avidly conserved, and a water diuresis will ensue as volume is restored and vasopressin concentrations fall. Hypernatremic fluid administration should not be needed in this situation. As in SIADH, care must be taken to prevent too rapid correction of hyponatremia to avoid central pontine myelinolysis by controlling the rate of fluid administration if the hyponatremia has been prolonged.

Primary sodium deficiency

When salt loss exceeds intake, sodium deficiency can result in hyponatremia. Salt loss from the kidney can result from primary renal diseases, such as congenital polycystic kidney disease, acute interstitial nephritis, and chronic renal failure, deficient mineralocorticoid secretion or action, and diuretic use. Primary salt loss can also occur from the skin in patients with burns and in cystic fibrosis. An imbalance between sodium intake and output can also occur as a result of insufficient nutritional sodium intake, although this is less common. Early in salt deficiency, hyponatremia is countered by suppression of vasopressin release, which results in increased water excretion. With continuing salt loss, hypovolemia develops, resulting in non-osmotic stimulation of vasopressin. Hypovolemia leads to increased thirst, and the ingestion of fluids with low solute content contributes to the hyponatremia.

Dehydration is often evident, as is the cause of sodium wasting. However, if thirst is intact and fluid intake is not interrupted, oral intake may partially correct the volume deficit, and evidence of hypovolemia may be subtle. If the kidney is the site of salt loss, hyponatremia may be accompanied by high urine sodium content, which would otherwise be unexpected in hypovolemic hyponatremia, so assessment of renal function is a vital component of the evaluation of hyponatremia. Aldosterone deficiency or pseudohypoaldosteronism present a special case of hypovolemic hyponatremia, as hyperkalemia is expected. In addition to congenital pseudohypoaldosteronism, aldosterone resistance can be seen in urinary tract obstruction or infection. As in other forms of renal salt wasting, urinary sodium excretion will be inappropriately high.

Patients with hyponatremia due to salt loss require isotonic saline replacement but also ongoing supplementation with sodium chloride and fluids. Intravenous fluids can be followed by oral salt supplementation and oral hydration when the patient is clinically stable. If salt loss is significant and ongoing and the hyponatremia is severe, hypertonic saline may be used to raise the sodium more quickly. As with SIADH, hypertonic therapy should be used only until the hyponatremia-associated symptoms resolve.

Hypervolemic hyponatremia

Severe low-output congestive heart failure, advanced liver cirrhosis with ascites, and nephrotic syndrome are all characterized by increased total body sodium and water, resulting in peripheral edema. They also have a decrease in "effective" intravascular volume due to decreased cardiac output and/or reduction in blood volume caused by a shift of salt and water from plasma to the interstitial space. As with systemic dehydration, the renin–angiotensin–aldosterone system is stimulated, and water and salt excretion by the kidney is reduced. As the disease progresses, decreases in baroreceptor stimulation result in a compensatory increase in vasopressin secretion, leading to a further reduction in water clearance. If water intake is not restricted, hyponatremia can develop, and hyponatremia in these clinical situations is common, although severe hyponatremia is rare.

In patients with impaired cardiac output and elevated atrial or ventricular volume (e.g. congestive heart failure or lung disease), atrial and/or brain natriuretic peptide concentrations are elevated, which can contribute to hyponatremia by promoting natriuresis. On evaluation of hypervolemic hyponatremia, urinary sodium is usually low, and urea, creatinine, and urate are increased, because of the reduction in effective blood volume and GFR. The presence of a predisposing disease and edema distinguishes this form of hyponatremia from that associated with systemic hypovolemia.

This type of hyponatremia is often mild, may be asymptomatic, and may not need treatment. However, it is associated with a poor prognosis in patients with cirrhosis and congestive heart failure (CHF). Although this may reflect the fact that patients with the most severe disease are at greatest risk of hyponatremia, it also raises the possibility that hyponatremia may itself contribute to a poor outcome. This is particularly true in CHF, where hyponatremia can have direct effects on myocardial contractility. This has resulted in an increased interest in identifying effective ways of treating hypervolemic hyponatremia, which can be difficult to reverse.

When possible, the underlying disease should be treated, but often this is not possible. Fluid and salt restriction and diuretics are commonly used but can worsen hyponatremia. Fluid restriction can be very difficult to maintain in this population because the intravascular volume depletion contributes to increased thirst. Use of twice-daily hypertonic saline infusion in combination with high-dose loop diuretic and fluid restriction to 1 L/day has been shown to increase serum sodium and improve outcome in refractory CHF [104].

A number of AVP V_2 receptor (AVP V_2R) antagonists have been developed, and their use has been studied in the treatment of CHF and cirrhosis-induced hyponatremia. In animal models, the antagonists produce a water diuresis and increase sodium. When studied over short periods in humans, they are effective in increasing urine output and plasma sodium in both CHF and cirrhosis and do so at doses that do not produce hypernatremia or a clinically significant worsening of intravascular volume depletion [105]. However, during therapy for hypervolemic hyponatremia with these antagonists, endogenous AVP levels become further elevated, and additional study is needed to determine whether this leads to increased stimulation of the V_1 receptor, which could be counterproductive in CHF.

dDAVP stimulates von Willebrand factor, factor VIIIa, and tissue plasminogen activator through AVP V_2R, so it is possible that use of the AVP V_2R antagonists might increase the risk of bleeding, which could have severe consequences, particularly in individuals with hepatic disease already at risk because of portal hypertension and/or impaired hepatic protein synthesis.

As with SIADH, acute treatment of symptomatic hyponatremia can be accomplished with hypertonic saline, but the underlying disorder makes it difficult to maintain the administered fluid within the intravascular space. Furthermore, patients with cardiac disease administered hypertonic saline may require concomitant treatment with a diuretic such as furosemide to prevent worsening of heart failure and will already have increased natriuretic peptide levels. Both these increase natriuresis and make correction more difficult. Frequent monitoring of sodium and fluid balance is critical and, once symptoms have been controlled, therapy should be adjusted so that the hyponatremia is corrected slowly to avoid the risk of neurological injury associated with overly rapid correction.

Other causes of hyponatremia

Water intoxication

Water intoxication as a cause of hyponatremia in the absence of any of the disease processes described above is rare. It is seen primarily in pyschogenic polydipsia, because adults with normal solute intake and the ability to produce a maximally dilute urine can theoretically ingest up to 20 L/day without becoming hyponatremic. Infants are at increased risk of water intoxication, because they have a decreased ability to produce a dilute urine. Because infants take their nutrition in liquid form, and hunger can overcome hypotonic inhibition of drinking, hungry infants will accept low-solute fluids even in the face of falling serum osmolality. As a result, infants fed overly dilute formula or water in place of formula are at risk of water intoxication.

A water intoxication/hyponatremia syndrome associated with ultralong-distance running was first recognized in 1981. As marathon participation has become more popular among non-competitive runners, associated hyponatremia has been described with increasing frequency. It is seen most often in those who run at speeds slower than 9 km/h and who maintain high rates of fluid intake. This form of hyponatremia has

also been seen during military training involving prolonged moderate-level physical exertion. Although initially attributed to excessive salt loss, this form of hyponatremia has now been clearly associated with fluid overload [106]. Although this form of water intoxication may ultimately be defined as a form of SIADH, the physiologic mechanism of the failure of free water excretion in this setting has not yet been defined, as AVP levels are not elevated shortly after exercise is discontinued [107]. High rates of fluid intake can be attributed to training dogma, which advocates aggressive, non-thirst-mediated hydration during participation in prolonged physical activity. The recognition that this process is related to excessive water intake has resulted in changes in training recommendations, which should decrease the incidence of hyponatremia in endurance athletic activities.

Cerebral salt wasting

Following CNS injury, a syndrome of hyponatremia associated with increased urine sodium concentration, increased urine volume, and volume depletion known as cerebral salt wasting (CSW) can develop. This is associated with primary renal salt losses in the absence of primary renal disease. It is critical to distinguish CSW from the other two major disturbances of water metabolism that can occur associated with CNS injury, DI and SIADH. Each of these syndromes shares some clinical features (Table 17.5), yet the distinction between the disorders is of considerable clinical importance, given the wholly divergent nature of the treatments. Fluid restriction is the treatment of choice for SIADH, whereas the treatment of CSW involves vigorous sodium and volume replacement, and DI requires volume replacement with fluids of low salt content. DI and CSW can be distinguished easily by measuring serum sodium. Determination of volume status is the key to distinguishing SIADH from CSW.

CSW follows subarachnoid hemorrhage and numerous other CNS perturbations. In children, these include CNS tumor resection, severe closed head injury, craniofacial surgery, hydrocephalus, and stroke. There is now considerable evidence that the hyponatremia associated with CSW is related to hypersecretion of atrial and/or brain natriuretic peptide, which is presumed to drive the inappropriate natriuresis [108]. Inconsistent with clinical evidence of hypovolemia, renin and aldosterone levels are frequently low, presumably because of natriuretic peptide inhibition. Low aldosterone levels could further promote salt loss, but these hormonal changes are also present in SIADH as a result of volume expansion. They are therefore not useful in distinguishing these entities, even if measurements were available immediately. Clinical differentiation of the syndromes requires careful attention to urine output and volume status. Findings that support a diagnosis of CSW include orthostatic changes in blood pressure and pulse, dry mucous membranes, weight loss, and negative fluid balance. Useful laboratory findings are hemoconcentration, with increased hematocrit or albumin, and increased blood urea nitrogen (BUN)/creatinine.

CSW is transient, but appropriate management while it persists involves vigorous administration of intravenous isotonic saline solutions. As this therapy would be expected to worsen hyponatremia in SIADH and worsen hypernatremia in DI, the diagnosis and clinical and laboratory markers of volume status and serum osmolality should be reassessed frequently during therapy. As might be expected with the inappropriately low aldosterone, the efficacy of hypervolemic therapy appears to be improved by administration of fludrocortisone to help inhibit the natriuresis [109].

Table 17.5. Comparison of findings in SIADH, CSW, and CDI.

	SIADH	CSW	CDI
Plasma volume	↑	↓	↓
Clinical evidence of volume depletion	–	+	+
Serum sodium/osmolality	↓	↓	↑
Urine sodium/osmolality	↑	↑↑	↓
Urine flow rate	↓	↑↑	↑
Plasma renin activity	↓	↓	↑
Plasma aldosterone concentration	↓ or →	↓	↑
Plasma AVP concentration	↑	↑ or →	↓
BUN/creatinine	↓/↓	↑/↑	↓/↑
Hematocrit	↓	↑	↑
Albumin concentration	↓	↑	↑
Serum uric acid concentration	↓	↓ or →	↑
Plasma ANP or BNP concentration	↑	↑	↓
Treatment	Fluid restriction	Salt and fluid replacement	Salt-poor fluid replacement

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18 Endocrine programming and the fetal and early-life origins of adult disease

Caroline H.D. Fall

Introduction

The concept that events in early life may have permanent effects on human health and disease is not new. Death rates from all causes, in the UK and Sweden, fell in successive year-of-birth cohorts from 1751 to 1930 [1]. One possible explanation for this trend, that "a more healthy race of children was born in each successive decade," was rejected in favor of the conclusion that improved childhood living conditions, resulting from social reforms, had lifelong benefits for health. A geographical correlation within Norway between coronary heart disease mortality in 1964–67 and infant mortality 70 years earlier suggested that growing up in poverty caused "permanent damage" perhaps because of a "nutritional deficit," which resulted in "lifelong vulnerability" to affluent adult lifestyles and high fat intakes [2].

A series of studies starting in the UK a decade later shifted the focus to adverse events in fetal life and infancy rather than childhood. Regions of the UK with high infant mortality rates in 1921–25 had high death rates from adult coronary heart disease in 1968–78 [3]. Follow-up of the 1946 national birth cohort showed that lower birthweight was associated with higher adult blood pressure [4]. Studies of men and women in the UK county of Hertfordshire, where nurses recorded the birthweight and infant weight of babies born during 1911–30, showed that lower birthweight or weight at the age of 1 year was associated with an increased risk of death from coronary heart disease and stroke [5,6]. There was an approximately twofold increase in risk from the highest to lowest categories of birthweight or weight at 1 year (Fig. 18.1). Based on these findings, Barker suggested that the origins of adult cardiovascular disease lay in the effects of undernutrition during fetal life and infancy and of poverty in mothers [5,7]. The implications of this proposal for public health have stimulated an explosion of research into the effects of the early environment on later outcomes.

Studies around the world have confirmed a link between low birthweight and adult cardiovascular disease [8–10]. Consistent associations with low birthweight have also been shown for hypertension and type 2 diabetes, which are strong risk factors for cardiovascular disease [11,12]. These effects are linear and graded across the whole range of birthweight (Fig. 18.1), and are independent of socioeconomic status [8,10]. Studies using birth records with information on gestational age at birth indicate that it is restricted fetal growth

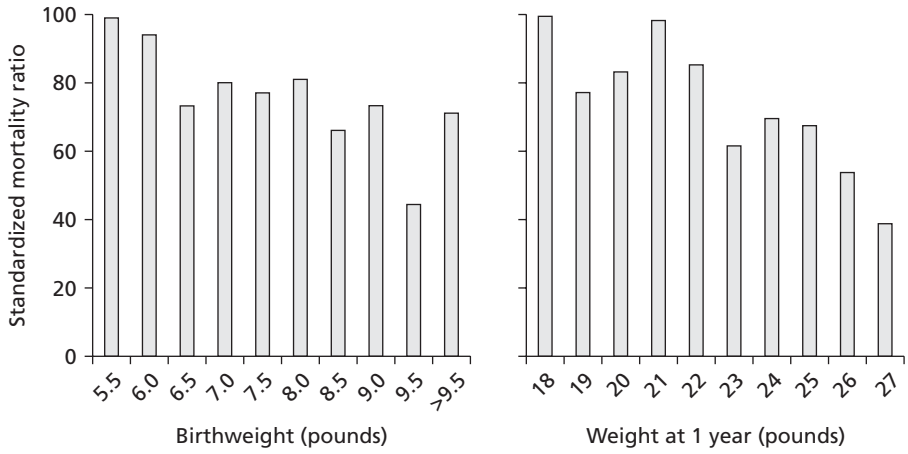

Fig. 18.1. Standardized mortality ratios for cardiovascular disease below the age of 65 years, among men born in Hertfordshire, UK, during 1911–30, according to birthweight and weight at 1 year [6].

rather than preterm birth that carries the risk of cardiovascular disease [10]. The majority of studies have been limited to birthweight as a measure of fetal growth, but there is some evidence that body proportions at birth show stronger associations with disease. For example, low ponderal index at birth (weight/length^3) predicted coronary heart disease mortality better than birthweight alone in Finland [9], and low birthweight relative to head circumference predicted stroke mortality in Sheffield, UK [13]. Old records of weight gain during infancy are rare, but studies in Finland and India have confirmed associations between low infant weight and adult cardiovascular disease and type 2 diabetes [9,14,15].

Barker's interpretation of these findings led to the "fetal origins of adult disease (FOAD)" hypothesis: that undernutrition during critical periods of early development has permanent effects, creating vulnerability to later disease [7]. Inadequate fetal nutrition may result from an impaired nutrient supply (poor placental function or maternal undernutrition) or increased fetal demand (a rapidly growing fetus). The undernourished fetus undergoes physiological changes thought to be adaptive, which include downregulation of growth, prioritization of blood flow to the brain (at the expense of other tissues), and advanced maturation. The FOAD hypothesis proposes that these changes persist postnatally and result in permanently altered body composition, tissue structure, and physiology (Fig. 18.2). These are examples of programming, which is defined as a permanent change in the structure and function of an organism as a result of a transient stimulus or insult occurring at a critical or sensitive period [16].

The mechanisms by which programming could occur at a tissue and cellular level have been reviewed [16,17] and can be divided into *structural* changes, such as permanent reductions in cell numbers in specific tissues, or changes in *cellular homeostatic processes* through alterations in gene expression. Examples of the former are reduced renal nephron numbers, reduced pancreatic beta cell mass, and low muscle mass, all of which have been described in low birthweight individuals, which could increase disease risk later if they persist. High on the list of homeostatic systems that may be programmed by the early environment are endocrine axes. Growth-restricted human fetuses have an altered endocrine profile; for example, they have low circulating insulin and insulin-like growth factor (IGF) concentrations [18]. Programming is well described in endocrine systems. An example is the permanent effects of sex steroids administered in early life on gonadotropin secretion and sexual orientation [16]. Another is vaginal adenocarcinoma in young women, one of the first diseases to be linked to a transient intrauterine exposure (to diethylstilbestrol) and shown to have a long latent period between exposure and disease. Programming effects have been demonstrated in animals in most of the endocrine axes and in response to a variety of stimuli, including fetal undernutrition. It is feasible that some of these occur in humans and contribute to endocrine disease and/or link reduced early growth to later cardiovascular disease.

Some of the strongest evidence for the FOAD hypothesis comes from experiments in animals, which have shown that adult hypertension and glucose intolerance can be produced by alterations in maternal diet during pregnancy. Although there is agreement that associations exist between poor early growth and later disease in humans, there is debate over the extent to which these are nutritionally based, and indeed whether they reflect environmental effects at all or are genetic in origin.

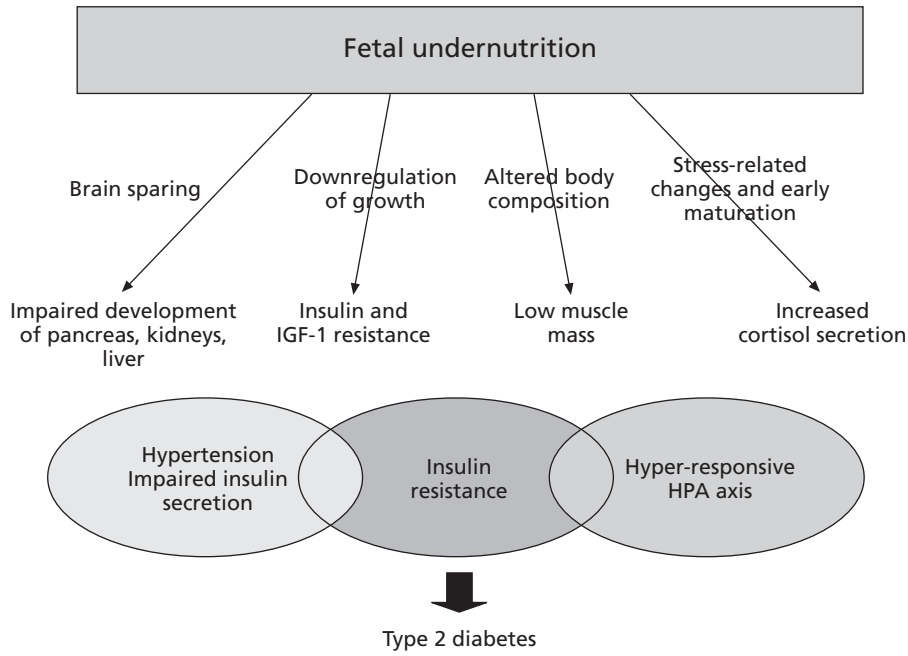

Fig. 18.2. Effects of fetal undernutrition on structure and metabolism, which may lead to later disease.

Postnatal growth is also an important issue. Many of the epidemiological studies have shown that the risk of adult hypertension or type 2 diabetes is greatest in men and women who were small at birth or in infancy but became obese adults. In addition, some show interactions between birthweight/infant weight and adult body mass index (BMI). The adverse effects of adult obesity on blood pressure or glucose tolerance appear to be greater in lower birthweight individuals. This has given rise to the concept that an individual who adapted to become thrifty as a fetus or infant may be unable to maintain homeostasis when exposed to "overnutrition" in later life, sometimes called "adaptation–dysadaptation." Recent data from cohorts that have been measured longitudinally through childhood show that, after infancy, crossing BMI centiles upwards during childhood or adolescence is strongly associated with adult disease [14,15].

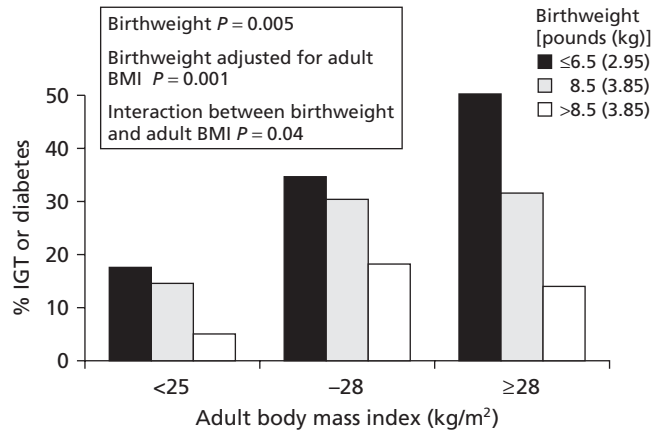

Fig. 18.3. Prevalence of impaired glucose tolerance (IGT) and type 2 diabetes (%) among men born in Hertfordshire, UK, aged 60–71 years (n = 370) . From Haleg CN, Barker DJP, Clark PMS *et al*. fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 1991; 303: 1019–22.

Glucose/insulin metabolism and type 2 diabetes

Low birthweight and infant weight

An association between lower birthweight and an increased risk of impaired glucose tolerance (IGT) and type 2 diabetes was first shown in a sample of 60- to 70-year-old men born during 1920–30 in Hertfordshire [19]. Forty percent of men who weighed less than 5.5 pounds (2550 g) at birth had abnormal glucose tolerance compared with 14% of those who weighed more than 9.5 pounds (4300 g). This has proved to be a robust association in both sexes and in adult birth cohorts worldwide [12]. In Hertfordshire, low weight at the age of 1 year was also associated with increased rates of IGT and diabetes, an association subsequently replicated in cohorts in Finland [14] and India [15]. The Hertfordshire data illustrate the effects of adult obesity (Fig. 18.3). The highest rates of IGT and type 2 diabetes were in men who were small at birth (and/or in infancy) but obese as adults. There was a significant interaction between birthweight and adult BMI; the adverse effect of low birthweight was most marked in obese men, and the adverse effect of adult obesity was strongest in men of low birthweight. Put another way, adult obesity appeared to be of relatively minor importance in men who were heavy at birth, as did low birthweight in men who remained slim as adults.

IGT and type 2 diabetes are thought to result from a combination of insulin resistance and impaired insulin secretion, the latter occurring as a late event, as the β cells become "exhausted." Both insulin resistance and impaired insulin secretion may result from fetal and infant undernutrition. These abnormalities would be least detrimental in people who remained lean throughout their lives but lead to IGT and diabetes in people whose glucose/insulin metabolism is stressed by obesity. This became known as the "thrifty phenotype" hypothesis [20,21]. Researchers have attempted to determine whether the defect linking lower birthweight to adult glucose intolerance is insulin resistance, impaired insulin secretion, or both. Whether using crude measures of insulin resistance, such as fasting insulin concentrations or equations based on fasting insulin and glucose measurements, or better methods such as clamp procedures or insulin tolerance tests, most of these studies have shown that lower birthweight is associated with higher adult insulin resistance [12]. Although there is no association between low birthweight and impaired glucose tolerance in childhood, lower birthweight children are more insulin resistant [12].

Conclusions are less clearcut for insulin secretion. This may be because of a lack of good measures of insulin secretion for use in epidemiological studies. Proxies for insulin secretion include the 30-min insulin increment during an oral glucose tolerance test and fasting concentrations of the insulin precursors proinsulin and 32–33 split proinsulin. Unfortunately, all these tend to be highly correlated with insulin resistance. Thirty-minute insulin increment and fasting proinsulin and 32–33 split proinsulin concentrations were inversely related to birthweight in many studies, but it is not clear whether these measures reflect insulin resistance or defects in insulin secretion [12]. Studies using the better measure of the first-phase insulin response to intravenous glucose have shown inconsistent results; some have shown reduced insulin secretion in men and women of low birthweight, while others have shown no association with size at birth [22–24].

Childhood weight gain and the adiposity rebound

Data from birth cohorts in Finland and India, for whom measurements of weight and height were recorded through-

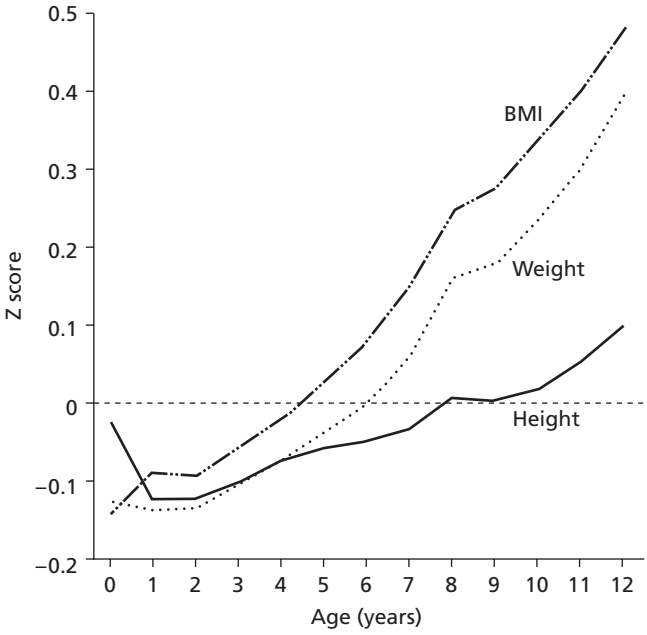

Fig. 18.4. Childhood growth of 290 men and women with type 2 diabetes from a cohort of 8760 born in Helsinki, Finland. Data are presented as SD scores for body mass index (BMI), weight, and height relative to the cohort mean of zero. Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJP. Early adiposity rebound in childhood and risk of type 2 diabetes in adult life. *Diabetologica* 2003; 46: 190–4.

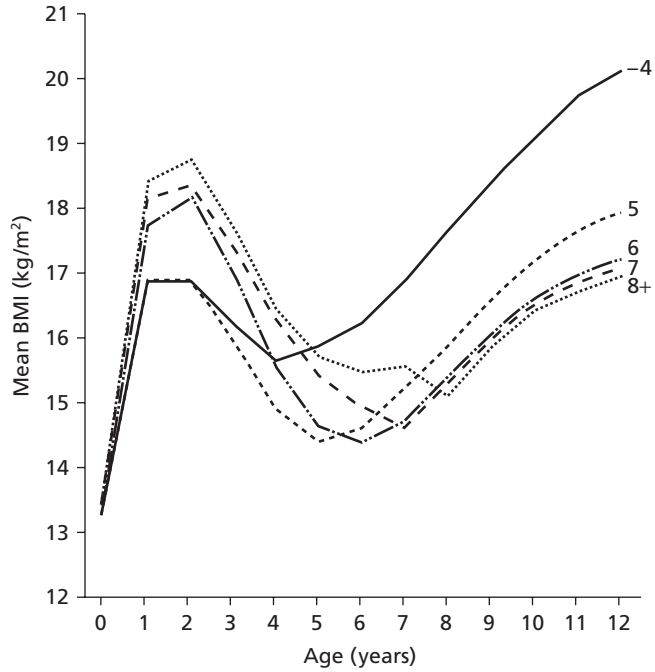

Fig. 18.5. Mean body mass index in 8760 children according to the age of adiposity rebound, Helsinki, Finland. Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJP. Early adiposity rebound in childhood and risk of type 2 diabetes in adult life. *Diabetologica* 2003; 46: 190–4.

out infancy and childhood, have given insight into the relationship between patterns of weight gain in childhood and later risk of type 2 diabetes [14,15]. In both populations, children who developed IGT and diabetes in adult life were characterized by low weight and thinness at birth and in infancy but showed rapid weight gain relative to their peers from the age of 2 or 3 years onwards (Fig. 18.4). They were not obese as children; indeed, they were thinner than the whole cohort population until mid-childhood. The data suggest that measures to prevent later diabetes should start in childhood and may need to be targeted not only to obese children but also to children who started life small and thin and crossed centiles for BMI upwards in childhood. Detection of these children would require serial measurements of BMI in childhood and suitable reference standards against which to assess them [15].

The Finland and India studies have also highlighted the importance of the phenomenon known as the adiposity rebound, the point in early childhood when BMI starts to rise, having fallen during infancy. Earlier adiposity rebound was a strong risk factor for IGT and type 2 diabetes [14,15]. The determinants of age at adiposity rebound are unknown, but low infant weight in Finland and India predicted an earlier rebound (Fig. 18.5). These findings suggest that the rapid childhood weight gain associated with an increased risk for type 2 diabetes may itself be programmed by earlier events, possibly by undernutrition in infancy or prenatally.

There are a number of possible explanations for why weight gain in childhood on a background of fetal restriction might be associated with disease. Low birthweight babies tend to catch-up (compensatory growth), and the rapidity of postnatal growth may indicate simply the severity of growth retardation (in relation to growth *potential*) at birth. Alternatively, the process of catch-up may be disadvantageous in itself. In animals, compensatory growth can lead to adverse short- and long-term effects, operating through a variety of mechanisms [25]. One may be excess demand on tissues that are not capable of compensatory hyperplasia, such as the pancreas. Another may be altered body composition; good nutrition in childhood may enhance the development of fat, which maintains the capacity for growth throughout life, but may not restore muscle tissue, which loses the capacity for cell division in early life. Another possibility is that hormones driving catch-up growth have adverse cardiovascular and metabolic effects [26].

Genes or environment?

Hales and Barker coined the term "thrifty phenotype" [20,21] to contrast with Neel's "thrifty genotype" hypothesis [27]. Neel suggested that type 2 diabetes is caused by "thrifty" genes, which were selected for in mankind's distant past when the supply of food was precarious, but became diabetogenic in a modern setting of plentiful nutrition. In contrast,

the thrifty phenotype hypothesis suggests that the undernourished fetus develops insulin resistance and other metabolic changes as a response to undernutrition, a strategy for immediate survival, for which it pays a price later in life, generally after the reproductive period. Debate continues as to whether the associations between low birthweight and adult diabetes reflect the environment in early life ("thrifty phenotype") or inherited genes ("thrifty genotype"). Both thrifty genes and the thrifty phenotype could become detrimental on exposure to plentiful nutrition. Variations in the concept of the thrifty genotype are currently the strongest alternative to the FOAD hypothesis as an explanation for the associations between low birthweight and cardiovascular risk.

Evidence in support of the environmental side of the debate comes from work in experimental animals. It is possible to induce intrauterine growth retardation in rodents by nutritional restriction of the mother during pregnancy, and the effects of this on glucose/insulin metabolism in the offspring have been well researched [28–32]. Female rats protein-restricted during pregnancy had offspring with enhanced glucose tolerance in early postnatal life but developed insulin resistance, impaired insulin secretion, and diabetes as adults [29]. Tissue-level studies showed widespread effects, suggesting that glucose intolerance resulted from changes in a number of different tissues. Offspring of protein-restricted mothers had reduced pancreatic weight, islet size, and vascularity, low levels of pancreatic glucokinase, and reduced insulin secretion in response to amino acids and glucose. They also had a low muscle mass, and isolated muscle strips and adipocytes showed reduced insulin sensitivity. There were changes in hepatic lobular structure, resulting in altered activity of glycolytic and gluconeogenic enzymes, and in insulin and glucagon receptor expression. Studies using more severe undernutrition of the mothers during pregnancy (30% of normal food intake) showed similar effects on glucose tolerance and suggested yet more possible mediating mechanisms [21,31]. The offspring as adults were more obese than offspring of control mothers, had an increased appetite and food intake postnatally, and were markedly less active. Even the "couch potato syndrome" may have its origins in intrauterine life [31,32]. In both the rat models described, the effects of prenatal undernutrition on later metabolism and behavior were exacerbated in rats fed on a high-calorie diet postnatally.

Obviously, it is not possible to do such elegant experiments with maternal nutrition in humans, but information can be obtained from experiments created by human disasters. During the winter of 1944–45, part of the Netherlands suffered a famine when food supplies were blockaded and rations fell to < 800 calories a day (the "Dutch Hunger Winter"). The population was previously well-nourished, and food supplies were restored quickly after 5 months. Pregnant mothers experienced extreme undernutrition for sharply delineated periods of gestation. Birthweights were normal among women exposed to famine in early gestation but reduced by 350 g in those exposed in late gestation. Cardiovascular disease risk factors were recently measured in men and women born before, during, and after the famine. Exposure to famine in late gestation was associated with glucose intolerance, increased measures of insulin resistance, and a small (nonsignificant) increase in type 2 diabetes compared with subjects conceived after the famine [33].

On the genetic side of the debate, as insulin is a major growth hormone in fetal life, genes associated with either insulin resistance or reduced insulin secretion would lead to reduced fetal growth as well as an increased risk of adult diabetes (the "fetal insulin hypothesis") [34]. No genes that are sufficiently common to explain the observed associations between low birthweight and type 2 diabetes have been identified, but the principle has been established. The presence in the fetal genome of a rare mutation of the glucokinase gene, which is known to be associated with adult-onset diabetes, resulted in a 500-g reduction in birthweight [35]. A number of other rare genetic syndromes causing impaired insulin secretion or insulin resistance are also associated with low birthweight [35]. The fetal insulin hypothesis would predict that low birthweight would be associated with an increased risk of cardiovascular disease and insulin resistance in *fathers*, and such associations have been reported recently [36], although they are not present in all populations [37].

Twin studies have classically been used to distinguish between genetic and environmental effects. A study using the Danish twin registry showed that the smaller of monozygous twin pairs was more likely to become diabetic, supporting the FOAD hypothesis [38]. However, this has not been confirmed in other twin populations. Several features of the biology of fetal growth in twins may limit the conclusions that can be drawn from these studies [39]. The mechanisms underlying the growth restriction of twins probably differ from those limiting growth in singleton fetuses. Higher disease concordance rates for monozygous than dizygous twins may reflect their shared intrauterine environment as well as shared genes. Finally, twin–twin interactions, for example the diffusion of steroid hormones from one twin to another, may reduce within-pair differences in programming effects.

The "genes versus environment" debate is currently stimulating a great deal of hypothesis-testing research. With increasing understanding of epigenetic effects and gene–environment interactions, it is no longer possible to think of disease as being purely either "genetic" or "environmental." In the Finnish birth cohort, the Pro12Pro allele of the peroxisome proliferator-activated receptor gamma (PPAR-γ) gene is associated with increased insulin resistance, but only in men and women of low birthweight, suggesting possible interactions between the fetal genome and environment [40]. Experiments in rats have shown that it is possible to alter

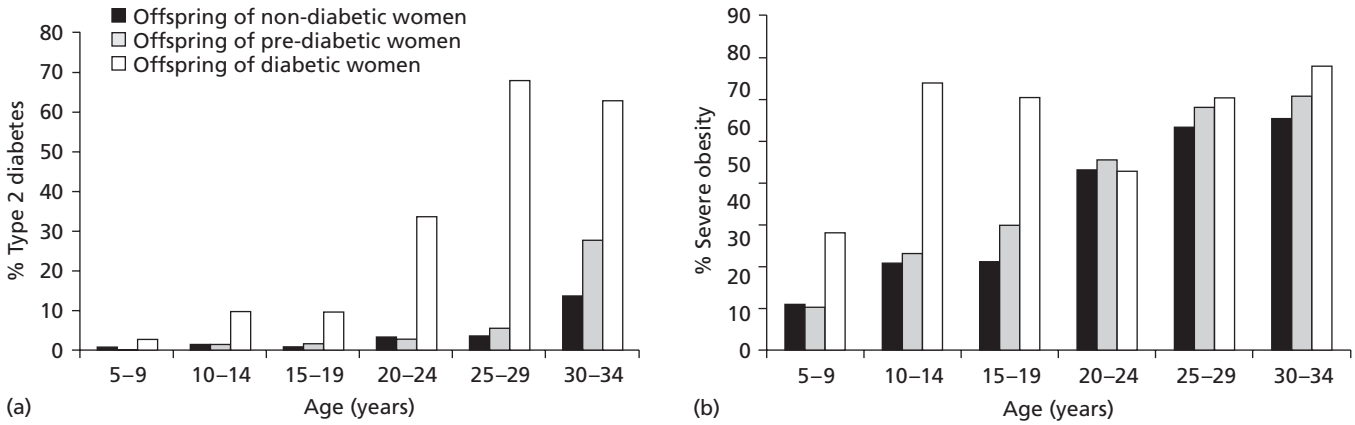

Fig. 18.6. Prevalence of (a) type 2 diabetes and (b) severe obesity in offspring of non-diabetic, prediabetic, and diabetic women (Pima Indians). From Dabelea D, Knowler WC, Pettit DJ. Effect of diabetes in pregnancy and offspring. Follow-up research in the Pima indians. *J Maternal–Fetal Med* 2000; 9: 83–8.

gene expression for components of the insulin-signaling pathway permanently by manipulation of intrauterine nutrition [29]. Such epigenetic effects may underlie the "thrifty phenotype," and it is notable that many of the genes associated with the control of fetal growth (influencing growth factor levels, processes of cell proliferation, apoptosis and placental function, and determining fetal demand) are "imprinted," i.e. modulated by epigenetic processes [41,42]. A maternal low-protein diet during the preimplantation period in rats leads to hypertension and altered organ/body weight ratios in the offspring in adult life [43]. These effects are associated with altered expression of imprinted genes known to control fetal growth. Maternal micronutrient supplementation in "agouti" mice can alter the effects on adult insulin resistance and coat color, which are regulated by well-characterized genes [44]. At this time, it seems likely that the observed associations linking birthweight to adult disease reflect environmental effects, genes, and interactions between the two.

High birthweight

Although the main focus of work on the early-life origins of adult disease has been on low birthweight and fetal growth restriction, maternal diabetes, which results in fetal "overnutrition" and macrosomia, has adverse long-term effects on the offspring. Studies among Pima Indians showed that offspring of diabetic mothers have an increased risk of obesity and type 2 diabetes compared with offspring of non-diabetic mothers or women who became diabetic later [45] (Fig. 18.6). The difference in risk holds true for siblings born before and after the onset of maternal diabetes and is not seen among offspring of diabetic fathers. Among the Pima Indians, who have a high incidence of gestational diabetes, this effect produces a U-shaped relationship between birthweight and adult diabetes, with an increased risk of later diabetes in people at both extremes of the birthweight distribution. The increased risk at high birthweights is attributable to maternal diabetes [46].

Similar data from a large cohort of nurses in the USA show that this effect is also seen in non-Pima populations [47]. Animal experiments have shown that the effects of gestational diabetes can be transmitted across generations, mimicking genetic inheritance. Experimentally induced gestational diabetes leads to an increased risk of gestational diabetes in female offspring when they become pregnant and to diabetes in the third generation [30]. With rising levels of obesity worldwide, maternal diabetes is also increasing. Effects of fetal "overnutrition" may thereby contribute to the rising rates of type 2 diabetes.

Hypothalamo–pituitary–adrenal (HPA) axis

The early environment can permanently modify the HPA axis in animals [48–51]. A variety of pre- and early postnatal stimuli, including subjecting pregnant mothers or newborn pups to stress, manipulating maternal nutrition, and altering fetal and neonatal glucocorticoid exposure, lead to permanent changes in HPA axis function. In most species, maternal stress during pregnancy leads to hyper-responsiveness of the HPA axis in the offspring, with increased peak and prolonged duration of glucocorticoid secretion. Effects are stronger on dynamic than on baseline parameters of HPA function. Different types and timings of maternal stress have different long-term effects, and there may be sexual dimorphism; for example, female rat fetuses are more susceptible to HPA effects induced by maternal stress. The mechanisms involved are not known; stress produces cardiovascular, endocrine [e.g. elevated adrenocorticotrophin hormone (ACTH), glucocorticoids, and catecholamines], nutritional (e.g. loss of appetite), and behavioral changes in the mother. There are also direct effects of maternal stress on placental

function, for example catecholamines cause placental blood vessel constriction.

There has been considerable interest in fetal *glucocorticoid exposure* as a final common pathway that could mediate the effects of various fetal exposures on the HPA axis. The placenta forms a partial barrier to glucocorticoids because of the synthesis of 11β-hydroxysteroid dehydrogenase, which converts cortisol and corticosterone to inactive metabolites. Levels and activity of this enzyme, and thus fetal protection from glucocorticoid exposure, are reduced in animal models of intrauterine growth restriction and maternal undernutrition [48–51,52] and in human pregnancies complicated by intrauterine growth restriction [52]. Pharmacological inhibition of the enzyme during pregnancy leads to elevated baseline and stress-induced HPA responses in the offspring and to high adult blood pressure and hyperglycemia. The enzyme does not block placental transfer of the synthetic glucocorticoids dexamethasone and betamethasone, which have been used to study the effects of increased glucocorticoid exposure on the fetus. Widely differing effects are produced, depending on the dosage and timing in pregnancy, on whether exposure is maternal or fetal, on fetal sex, and on the species studied. These include either increased or decreased HPA axis activity and altered expression of glucocorticoid and mineralocorticoid receptors in the hippocampus and amygdala, which are important sites of negative feedback control of the HPA axis.

Subtle programmed abnormalities of the HPA axis may play a role in the development of hypertension, insulin resistance, and type 2 diabetes in humans and their link with low birthweight [49]. Growth-restricted fetuses have higher circulating cortisol concentrations [53]. Low birthweight has also been associated with elevated fasting plasma cortisol concentrations in several, though not all, adult populations [49]. Among men born in Hertfordshire, cortisol concentrations fell from 408 nmol/L in those who weighed 5.5 lb or less at birth to 309 nmol/L in those who weighed more than 9.5 lb. Cortisol concentrations were in turn related to systolic blood pressure, fasting and 2-h plasma glucose concentration, serum triglyceride concentrations, and insulin resistance. Detailed studies in subsets of these men showed that unstimulated 24-h cortisol profiles showed no differences between men of low or high birthweight [54]. However, men of lower birthweight had higher peak cortisol concentrations, and cortisol concentrations remained elevated for longer in response to ACTH stimulation [49] (Fig. 18.7).

There have been few studies of glucocorticoids in children in relation to early growth. A population-based study of healthy UK children, using 24-h urinary glucocorticoid metabolite excretion, showed a U-shaped relationship with birthweight, with higher excretion in children of both low and high birthweight [55]. Other studies have failed to show associations between size at birth and cortisol profiles in children, either at baseline or during stressful situations

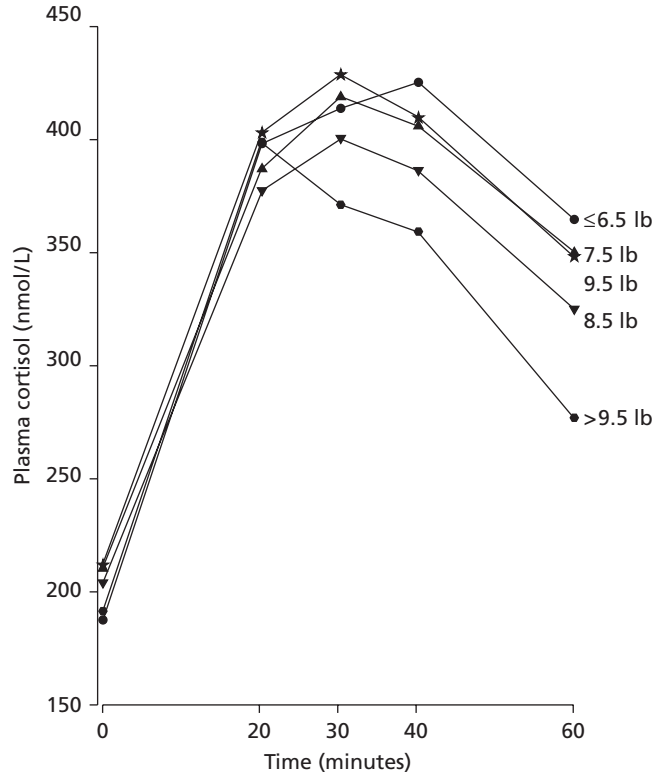

Fig. 18.7. Relationship between birthweight and the cortisol response to an ACTH stimulation (synacthen) test in men aged 67–77 years, born in Hertfordshire, UK. Phillips DIW. Programming of adrenocortical function and the fetal origins of adult disease. *J Endocrinol Invest* 2001; 28: 938–41.

[56,57]. In contrast, several studies of European children aged 5–12 years have shown higher serum adrenal androgen concentrations or higher urinary adrenal steroid excretion in those of lower birthweight [55–58]. In the absence of longitudinal data, it is not known whether this reflects an earlier onset of adrenarche, exaggerated adrenarche, or alterations in the adrenal steroid axis predating adrenarche. An association between low birthweight and a syndrome of exaggerated adrenarche, hyperinsulinemia, precocious puberty, and ovarian hyperandrogenism in girls has been shown [59] (Fig. 18.8). The mechanisms are unknown, but it may indicate either programming by the prenatal environment or a genetic disorder of serine kinase activity, leading to abnormal phosphorylation of hormone receptors.

Altered glucocorticoid responses throughout life may affect other systems, such as immune function and cognitive ability as well as cardiovascular risk, and may also influence ability to handle stress. A retrospective study of Swedish conscripts showed that those of lower birthweight had greater susceptibility to stress as measured by psychological evaluation [49]. Increased vulnerability to stress has been put forward as an explanation for the finding in Finland that poverty was associated with increased cardiovascular mortality only in individuals of low birthweight [60]. There

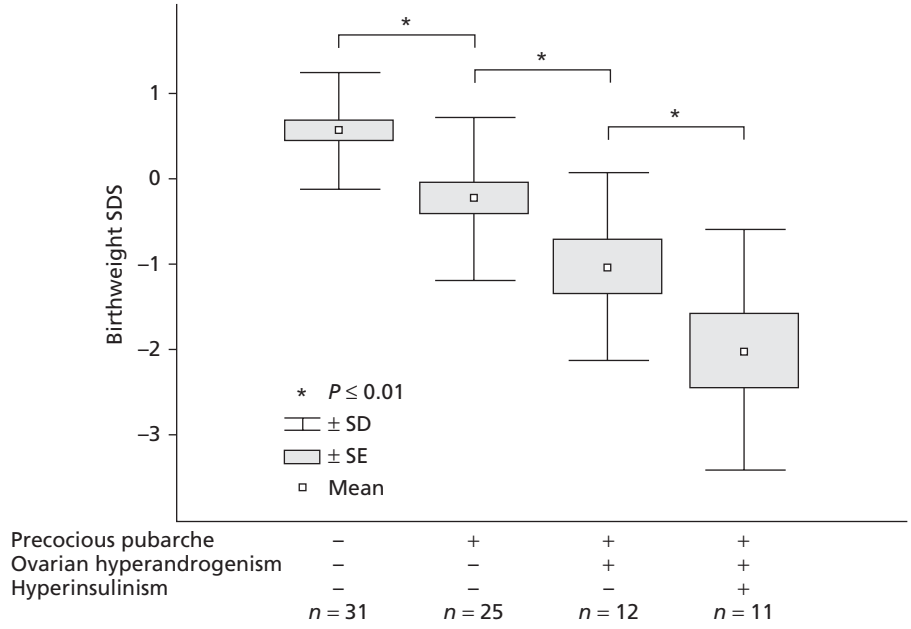

Fig. 18.8. Birthweight SD scores of control girls and girls with a history of precocious adrenarche. Ibanez I, Dimartino-Nardi J, Potau N, Saenger P. Premature adrenarche – normal variant or forerunner of adult disease? *Endocr Rev* 2000; 21: 671–96.

is ongoing research into relationships between early growth and stress-induced responses in adults and children. More research is needed into the effect of maternal factors, such as stress and nutrition, on placental 11β-HSD, on fetal exposure to glucocorticoids, and on the long-term effects of the use of synthetic steroids to promote fetal lung maturation in mothers at risk of preterm delivery.

Growth hormone (GH) and the insulin-like growth factors (IGFs)

The evidence linking fetal growth, the GH–IGF axis, and adult disease has been reviewed recently [61]. The IGFs and their binding proteins (IGFBPs) play a major role in fetal growth and are thought to be nutritionally regulated [42]. Cord blood IGF-I concentrations are correlated with birthweight and are low in growth-restricted fetuses and newborns, while IGFBP-1 concentrations are increased. It is generally believed that GH has a minor role in controlling fetal growth, but length is reduced in congenital GH deficiency, and intrauterine growth restriction is associated with elevated neonatal GH concentrations, suggesting possible GH resistance. There is good evidence in animals that transient events in early postnatal life can have permanent effects on GH secretion. In rats, manipulation of sex steroids in the neonatal period alters the sexually dimorphic pattern of GH secretion after puberty. For example, neonatal administration of testosterone to female rats results in a male pattern of GH secretion in later life. Transient protein restriction in male rats leads to reduced peak and mean GH concentrations in adult life.

The literature relating measurements of the GH–IGF axis to *disease* in humans is confusing and inconsistent. Both acromegaly and GH deficiency are associated with an increased risk of cardiovascular disease. An 18-year prospective study of French policemen showed increased cardiovascular disease mortality in those whose fasting GH was above the median at baseline. On the other hand, in cross-sectional studies, IGF-I concentrations are lower in subjects with clinical symptoms of cardiovascular disease. GH and the IGFs play a major role in postnatal bone growth and have effects on bone metabolism throughout life. Patients with osteoporosis have reduced GH peaks and low serum IGF-I concentrations, and GH deficiency is associated with osteoporosis. Treatment with GH, however, despite increasing bone turnover, appears to be ineffective as a treatment for osteoporosis.

Rather little is known about the relationship between body size in early life and later GH and IGF secretion in humans [61]. This is probably due in part to the difficulties of studying GH secretion because of its pulsatile pattern of secretion. Most data come from follow-up studies of children with intrauterine growth restriction (IUGR) and subsequent short stature. They show persistent abnormalities of the GH–IGF axis with low-amplitude GH peaks and a high baseline GH secretion [62,63]. They also have low serum IGF-I, IGF-II, and IGFBP-3 concentrations compared with non-IUGR children of normal stature [62,64] but higher concentrations than non-IUGR short children, suggesting that they may be IGF resistant as well as GH resistant. In two populations of normal children, serum IGF-I concentrations were inversely related to birthweight [65] (Table 18.1). IGF-I was also directly correlated with the children's current weight, and the highest IGF-I concentrations were in children of below-average birthweight

18.1.1 Pune, India

Birthweight (kg)	Weight at 4 years (kg)				
	≤11.5	>11.5–12.5	>12.5–14.0	>14.0	All
≤2.5	38 (14)	52 (19)	53 (14)	75 (10)	51 (57)
>2.5–2.75	30 (10)	51 (14)	68 (8)	57 (12)	49 (44)
>2.75–3.0	36 (12)	40 (20)	42 (10)	78 (14)	46 (56)
>3.0	28 (5)	48 (8)	48 (14)	48 (16)	43 (43)
All	34 (41)	45 (61)	51 (46)	62 (52)	48 (200)

18.1.2 Salisbury, UK

Birthweight (kg)	Weight at 7 years (kg)				
	≤23.0	>23.0–25.5	>25.5–28.0	>28.0	All
≤3.0	100 (14)	117 (10)	130 (11)	138 (14)	121 (49)
>3.0–3.3	100 (24)	110 (17)	131 (13)	150 (12)	118 (66)
>3.3–3.6	99 (10)	104 (16)	130 (18)	144 (10)	119 (54)
>3.6	103 (9)	108 (20)	109 (20)	125 (26)	114 (75)
All	100 (57)	109 (63)	123 (62)	136 (62)	117 (244)

Table 18.1. Mean plasma IGF-1 concentrations (ng/mL) in healthy 4-year-old children in Pune, India, and 7-year-old children in Salisbury, UK, according to birthweight and current weight; figures in parentheses are numbers of children [65].

and above-average weight when studied. IGF-I concentrations were correlated with systolic blood pressure.

Studies that have examined relationships between birthweight and the adult GH–IGF axis have shown inconsistent results. In young adults in Australia, low birthweight was associated with reduced urinary GH excretion [66]. In contrast, in a small study in Hertfordshire, where serum GH was measured every 20 min for 24 h, there were no correlations between peak, baseline, or median GH with birthweight [67]. Low weight at 1 year of age was associated with low median GH concentrations. In a small study of healthy 18- to 25-year-old women, serum IGF-I concentrations were inversely related to birthweight, but no associations were found in five other adult birth cohorts [68].

Thyroid function

Little work has been done in either humans or animals on the programming of thyroid function. Among women aged 60–71 years, born in Hertfordshire, there was a weak positive association between thyroid-stimulating hormone (TSH) concentrations and birthweight [69]. Thyroglobulin and thyroid peroxidase antibodies were increased in women of lower birthweight in Hertfordshire and among twins in Birmingham in the smaller twin. There were no significant trends with hypothyroidism, although numbers were small [70]. This is an area that warrants further research.

Puberty and reproductive function

Animal breeders know that the timing of pubertal development and subsequent reproductive success are influenced by social, climatic, and nutritional conditions around the time of mating and early development, but little is known about how the prenatal environment influences sexual development and reproductive performance in humans [71].

Several studies have reported earlier menarche in girls of lower birthweight or girls born small for gestational age, although it is not a consistent finding [72]. One study showed an earlier *onset* of puberty in lower birthweight girls, determined by analysis of height curves and the pubertal growth spurt [73]. Follow-up of the 1946 UK birth cohort showed that early menarche was associated with lower birthweight, accelerated growth in infancy, and accelerated BMI gain from birth to 7 years [74]. It is not known how the timing of menarche relates to later health, although there are some data linking early menarche to an increased risk of breast cancer later. There are few data relating the timing of puberty in *boys* to early growth. One study of both sexes showed no association between birthweight and age of onset of puberty in boys, despite a significantly earlier onset of puberty and earlier menarche in lower birthweight girls [73].

Studies examining intergenerational effects on fetal growth have shown that a mother's own birthweight strongly predicts the birthweight of her children [75]. Mean birthweight

of offspring increases by 150–300 g per kg increase in maternal birthweight. A woman born small for gestational age (SGA) has a relative risk of 2.0–4.0 of delivering an SGA baby. The underlying mechanisms are not understood and probably involve both environmental and genetic processes. A genetic component is suggested by the fact that paternal birthweight also predicts birthweight of offspring, although usually less strongly than maternal birthweight (100–200 g/kg paternal birthweight). Environmental mechanisms might include poor maternal nutrition in the same family across generations or permanent effects of a mother's intrauterine experience on her adult size, the development of her reproductive organs, and her hormonal and metabolic systems. Small for gestational age women have been shown to have smaller uteri and ovaries [76]. There are some data in humans showing that month of birth relates to the length of a woman's reproductive life, her age at menopause, and numbers of children and grandchildren [71]. In follow-up studies of women born to mothers who were exposed to famine in the Dutch Hunger Winter, there were no detectable effects of famine on age at menarche or menopause, age at first delivery, proportion having no children, or family size [77]. There was, however, an excess of perinatal deaths among babies born to female offspring of famine-exposed mothers and of lower birthweights in babies born to women whose mothers were exposed to famine in the first trimester of pregnancy [77,78]. There have been fewer studies of reproductive performance in men, but Finnish and English men of lower birthweight were less likely to marry, independently of adult size and socioeconomic status [79].

There is some evidence that prenatal factors play a role in the etiology of polycystic ovarian syndrome. In a follow-up study of women born in Sheffield, UK, Cresswell *et al*. described two types of women with polycystic ovaries [80]. The first had a low adult BMI, high luteinizing hormone (LH) concentrations, but little androgenization (hirsutism and acne). They tended to have been born post term. The second type were overweight, had high LH and testosterone concentrations, and marked androgenization. These women had high birthweights, and their mothers were above average weight in pregnancy. Cresswell suggested that the first group of women have an altered hypothalamo-pituitary "set point" as a result of exposure to sex steroids resulting from placental failure due to prolonged gestation, while the second group had an ovarian defect, either of genetic origin or resulting from some effect of maternal obesity, leading to hypersecretion of androgens.

Hormone-related cancers

Data from animals show that exposures to carcinogens *in utero* and childhood can alter later cancer risk. For example, in animals administration of adult carcinogens or high-fat diets to mothers during pregnancy increases the risk of mammary tumors in the adult offspring [81]. In humans, girls who had a high weight gain in infancy have an increased risk of ovarian cancer [82]. Higher birthweight is associated with an increased risk of breast cancer [relative risk of 1.5–1.7 for birthweights >4000 g compared with normal birthweights (2500–2999 g)] [81]. Being a twin is associated with a higher risk, while pre-eclampsia in the mother and breast-feeding in infancy are associated with a lower risk. It has been suggested that breast cancer risk is related to estrogen exposure *in utero*, which is increased in twin pregnancies and reduced in pre-eclampsia. The data are somewhat inconsistent, and most studies rely on recalled data of poor quality. There is a need for population-based studies with good baseline pregnancy data designed specifically to examine this phenomenon.

Body composition and obesity

Adult obesity and a central body fat distribution (abdominal and truncal) are strong risk factors for hypertension, type 2 diabetes, and cardiovascular disease. Associations between low birthweight and infant weight and increased rates of adult disease have raised the question of whether adult body composition is influenced by the fetal and early post-natal environment. Are growth-restricted fetuses at increased risk of becoming excessively adipose in later life? Some animal models of maternal undernutrition and/or fetal growth restriction have shown increased adiposity in the adult offspring [31,83]. An early follow-up study of men whose mothers were exposed to famine during pregnancy in the Dutch Hunger Winter showed increased obesity in those whose mothers experienced famine during gestation [84].

The evidence for early-life effects on adult obesity in humans has been the subject of two recent reviews [85,86]. If anything, people of lower birthweight tend to become "thinner" adults as measured by BMI. In a large study of Danish conscripts, the prevalence of obesity, defined as a BMI of 30 kg/m^2 or more, rose from 3.5% in those with a birthweight of 2.5 kg or less to 11.4% in those with a birthweight greater than 4.5 kg. [87]. A well-recognized disadvantage of using BMI as an index of adiposity is that it does not distinguish between fat and lean mass. There is good evidence that the positive correlation between birthweight and adult BMI reflects increased lean and muscle mass rather than adiposity. Studies that measured muscle mass (urinary creatinine excretion), lean body mass (DEXA, arm or thigh muscle area), and fat mass (DEXA, skinfolds) have shown that birthweight is positively related to muscle and lean mass but is unrelated or even inversely related to body fat [88–91]. Higher birthweight men had higher lean but not fat mass, measured using DEXA at the age of 70 years, relationships that remained after adjusting for adult height (Fig. 18.9).

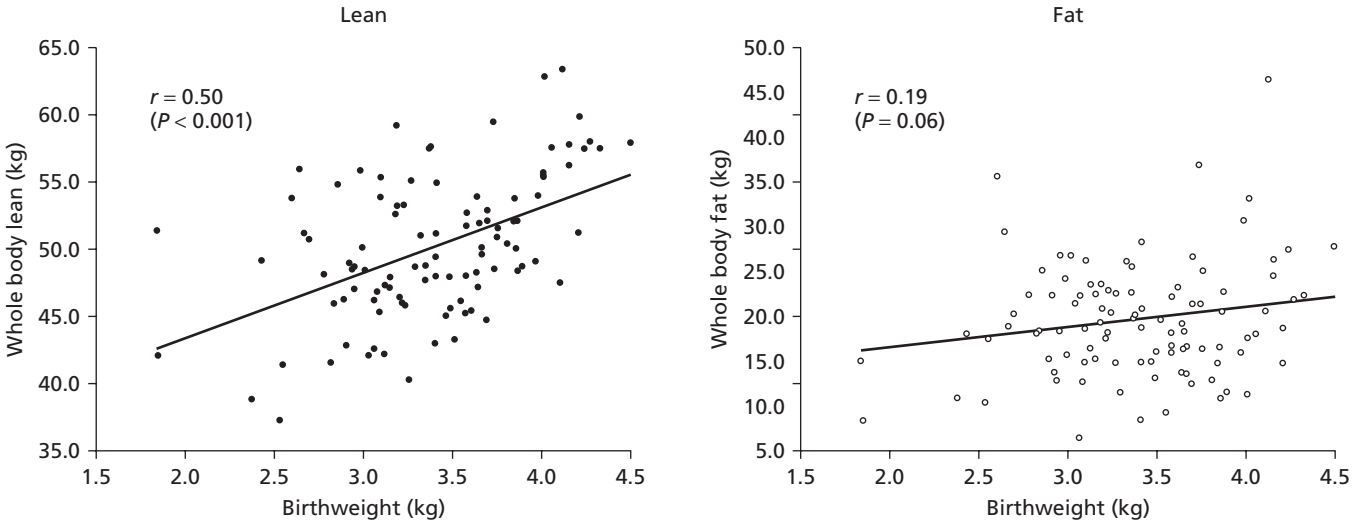

Fig. 18.9. Lean and fat mass measured using dual-energy X-ray absorptiometry (DEXA) scanning in men aged 70–75 years, born in Sheffield, UK. Forsen T, Eriksson JG, Tuomilehto J, Teramo K, Osmond C, Barker DJP, Mother's weight in pregnancy and coronary heart disease in a cohort of Finnish men: follow-up study. *BMJ* 1997; 315: 837–40.

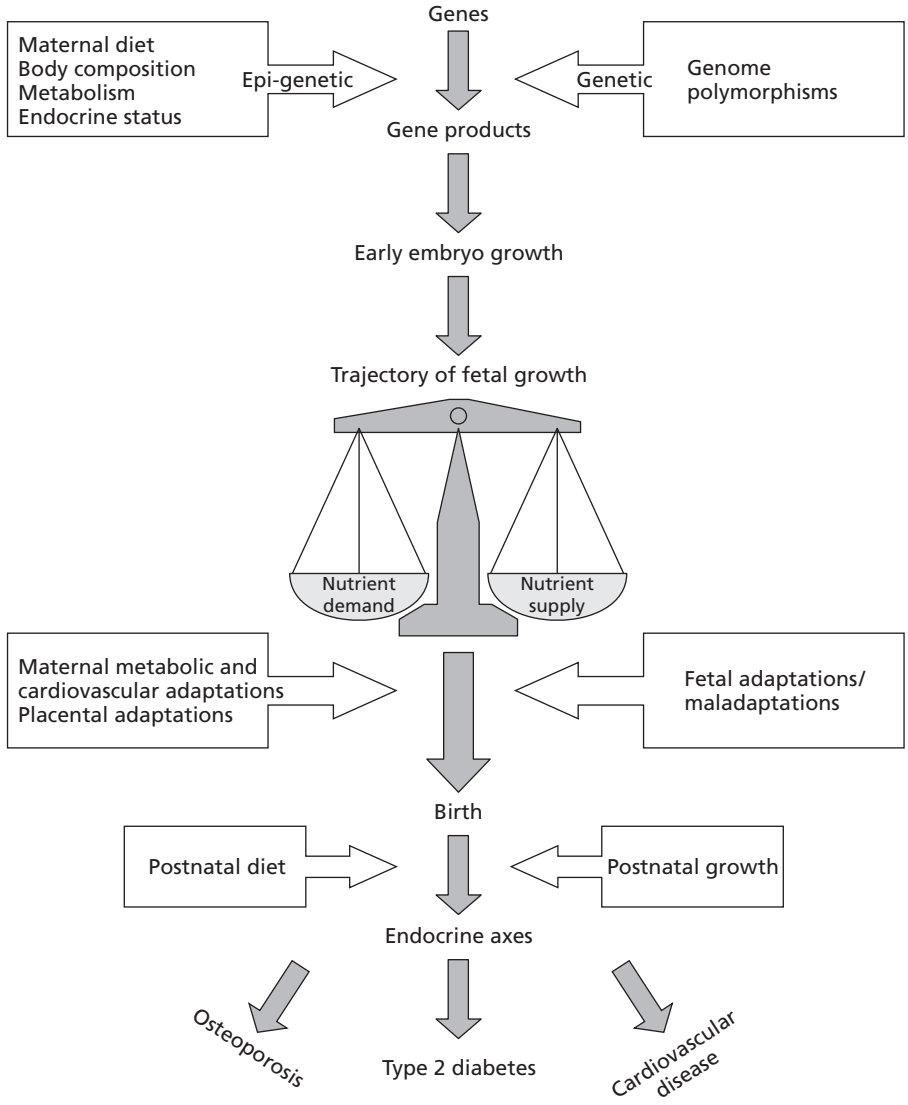

Fig. 18.10. Overview of developmental stages at which programming effects could operate to produce disease in adult life (adapted from ref 28).

Several studies have examined body fat distribution in relation to birthweight. Waist circumference or waist–hip ratio are generally used as indices of abdominal obesity, and the ratio of subscapular to triceps skinfold thickness (SS/TR) as an index of truncal adiposity. These indices are far from ideal, especially in children. In general, inverse associations between birthweight and waist circumference, waist–hip ratio, and SS/TR have been found, but the associations are weak and appear mainly only after adjustment for adult BMI. The associations with waist–hip ratio may reflect positive effects of early growth on the adult hip measurement (a composite of skeleton, muscle, and fat) rather than effects on the waist measurement. There is only one published study of directly measured visceral fat. This was a small study (n=22) in young Korean men and showed no association between birthweight and visceral fat area measured by computed tomography (CT) [92].

Bone metabolism and osteoporosis

Growth-restricted fetuses and children who had a low weight gain in infancy have reduced adult stature, suggesting that human skeletal growth can be influenced permanently by the intrauterine and infant environment. Infantile rickets is a good example of a nutritional impairment in early life that has long-term effects on skeletal structure. Recent studies have started to address the issue of whether fetal growth is linked to osteoporosis [93]. There are associations between low weight at the age of 1 year and adult bone mineral content (BMC) and/or density (BMD). In the Hertfordshire cohort studied at the age of 60–70 years, there were no relationships between BMC or BMD and birthweight. However, there were interactions between birthweight and vitamin D receptor genotype in predicting BMD. Among individuals of lower birthweight, lumbar spine BMD was higher in those with the "BB" genotype than "Bb" or "bb" genotypes; the opposite was true in those of higher birthweight. These findings may reflect gene–environment interactions in early life. Interestingly, among neonates born in Southampton, lower BMC was associated with maternal smoking, low maternal fat stores in mid-gestation, and high maternal physical activity, suggesting a number of environmental effects on early bone growth.

Conclusion

The different developmental stages at which programming could occur, the variety of potentially important environmental influences, and their interactions with both genes and the environment in later life are major challenges for research in humans (Fig. 18.10). The dynamic nature of endocrine systems and the changes in endocrine function during childhood and puberty make the measuring of outcomes especially difficult.

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19 Weight regulation and monogenic obesity

I. Sadaf Farooqi

Introduction

The prevalence of obesity in children is difficult to determine as there is no internationally accepted definition of pathological adiposity. A range of methods is available that estimate total body fat, but none is widely available nor easily applicable to the clinical situation. Body weight is reasonably well correlated with body fat but is also highly correlated with height, so children of the same weight but different heights can have widely differing amounts of fat. Body mass index [BMI: weight (kg) divided by height^2 (m^2)] in adults correlates reasonably well with more specific measurements of body fat. The relation between BMI and body fat in children varies considerably with age and pubertal maturation, but useful centile charts relating BMI to age have been published.

In the 10 years between the National Health and Nutrition Examination Survey (NHANES) II (1976–1980) and NHANES III (1988–1991), the prevalence of overweight in the USA, based on BMI corrected for age and sex, has increased by approximately 40% (to 11% in the 6- to 11-year age group) [1].

Thus, childhood obesity is emerging as a global problem. Its immediate adverse effects include orthopedic complications, sleep apnea, and psychosocial disorders. As obese children are more likely to become obese adults, we may expect to see profound public health consequences as a result of the emergence in later life of associated co-morbidities, such as type 2 diabetes mellitus, ischemic heart disease, and stroke.

Genetic and environmental factors

The rising prevalence of obesity can be explained in part by changes in the environment over the last 30 years, particularly the unlimited supply of convenient, palatable, energy-dense foods, coupled with a lifestyle typified by low physical activity. However, obesity represents the archetypal complex multifactorial disease and arises as a result of behavioural, environmental, and genetic factors that influence individual responses to diet and physical activity.

There is considerable evidence to suggest that weight, like height, is a heritable trait [2]. The most favored traditional model for separation of the genetic component of variance is based on twin studies, as monozygotic co-twins share 100% of their genes and dizygotes 50% on average. Data from twin and adoption studies are consistent with a genetic contribution to the variance of BMI of between 40 and 70% [3]. Different individuals clearly have a genetic propensity to store excessive caloric intake as fat. In one classic study, pairs of monozygotic twins were overfed by precisely calibrated amounts [4]. Different sets of twins showed remarkable differences in the degree to which these calories were stored as fat, but the tendency toward increased adiposity within each set of twins was remarkably similar.

Although the role of genes in body fat regulation is now established, it is safe to assume that the rising prevalence of obesity has not been due to a recent change in the genetics of the western world. It is very likely that the ability to store fat in times of nutritional abundance was a positive trait selected over many thousands of years of human evolution.

The clinical approach

Obesity is a complex phenotype, and the assessment of obese patients should be directed at screening for potentially treatable endocrine conditions and identifying genetic conditions so that appropriate genetic counseling and, in some cases, treatment can be instituted (Table 19.1).

Classically, patients affected by these obesity syndromes have been identified as a result of their association with mental retardation, dysmorphic features, and/or other developmental abnormalities. More recently, several new monogenic disorders have been identified. Obesity is often the predominant presenting feature, although often accompanied by characteristic patterns of neuroendocrine dysfunction. For the purposes of clinical assessment, it remains useful to categorize the genetic obesity syndromes as those with and without associated developmental delay (Figs 19.1 and 19.2).

Table 19.1. Assessment of the obese child/adult.

History

- Age of onset – use of growth charts and family photographs. Early onset (< 5 years of age) suggests a genetic cause
- Duration of obesity – short history suggests endocrine or central cause
- A history of damage to the CNS (e.g. infection, trauma, hemorrhage, radiation therapy, seizures) suggests hypothalamic obesity with or without pituitary growth hormone deficiency or pituitary hypothyroidism. A history of morning headaches, vomiting, visual disturbances, and excessive urination or drinking also suggests that the obesity may be caused by a tumor or mass in the hypothalamus
- A history of dry skin, constipation, intolerance to cold, or fatigue suggests hypothyroidism. Mood disturbance and central obesity suggests Cushing syndrome. Frequent infections and fatigue may suggest ACTH deficiency due to POMC mutations
- Hyperphagia – often denied but sympathetic approach needed and specific questions, such as waking at night to eat and/or demanding food very soon after a meal, suggest hyperphagia. If severe, especially in children, suggests a genetic cause for obesity
- Developmental delay – milestones, educational history, behavioral disorders. Consider craniopharyngioma or structural causes (often relatively short history), and genetic causes
- Visual impairment and deafness can suggest genetic causes
- Onset and tempo of pubertal development – onset can be early or delayed in children and adolescents. Primary hypogonadotropic hypogonadism or hypogenitalism associated with some genetic disorders
- Family history – consanguineous relationships, other children affected, family photographs useful. Severity may differ due to environmental effects
- Treatment with certain drugs or medications. Glucocorticoids, sulfonylureas, MAOIs, oral contraceptives, risperidone, clozapine

Examination

- Document weight and height compared with normal centiles. Calculate BMI and WHR (in adults). In children, obtain parental heights and weights where possible
- Head circumference if clinically suggestive
- Short stature or a reduced rate of linear growth in a child with obesity suggests the possibility of growth hormone deficiency, hypothyroidism, cortisol excess, pseudohypoparathyroidism, or a genetic syndrome such as Prader–Willi syndrome
- Obese children and adolescents are often tall (on the upper centiles), however, accelerated linear growth (height SDS > 2) is a feature of MC4R deficiency
- Body fat distribution – central distribution with purple striae suggests Cushing syndrome. Selective fat deposition (60%) is a feature of leptin and leptin receptor deficiency
- Dysmorphic features or skeletal dysplasia
- Hair color – red hair (if not familial) may suggest mutations in POMC in white Caucasians
- Pubertal development/secondary sexual characteristics. Most obese adolescents grow at a normal or excessive rate and enter puberty at the appropriate age; many mature more quickly than children with normal weight, and bone age is commonly advanced. In contrast, growth rate and pubertal development are diminished or delayed in growth hormone deficiency, hypothyroidism, cortisol excess, and a variety of genetic syndromes. Conversely, growth rate and pubertal development are accelerated in precocious puberty and in some girls with PCOS
- Acanthosis nigricans
- Valgus deformities in severe childhood obesity

Investigations

- Fasting and 2-h post glucose and insulin levels. Proinsulin if PC1 deficiency considered
- Fasting lipid panel for detection of dyslipidemia
- Thyroid function tests
- Serum leptin if indicated
- Karyotype
- DNA for molecular diagnosis
- Bone age
- Growth hormone (GH) secretion and function tests, when indicated
- Assessment of reproductive hormones, when indicated
- Serum calcium, phosphorus, and parathyroid hormone levels to evaluate for suspected pseudohypoparathyroidism
- MRI scan of the brain with focus on the hypothalamus and pituitary, when clinically indicated

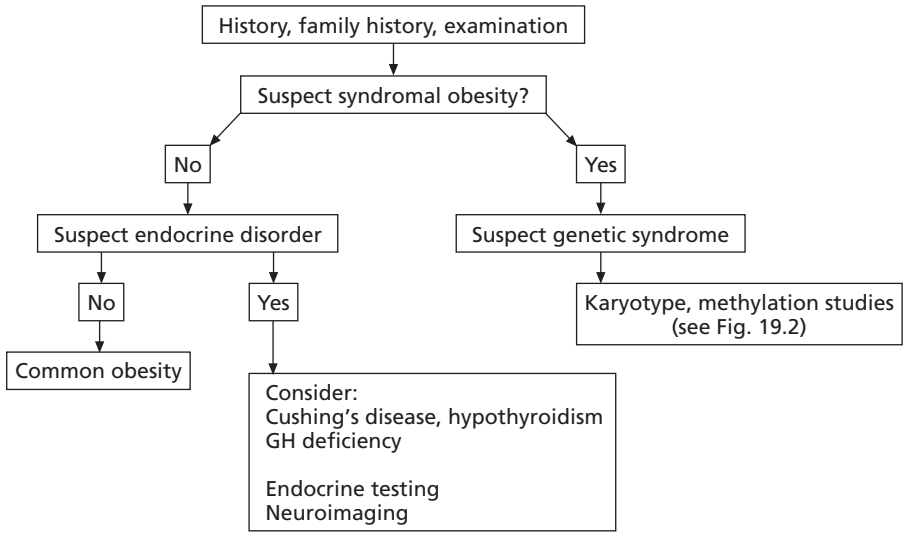

Fig. 19.1. Diagnostic algorithm for childhood obesity.

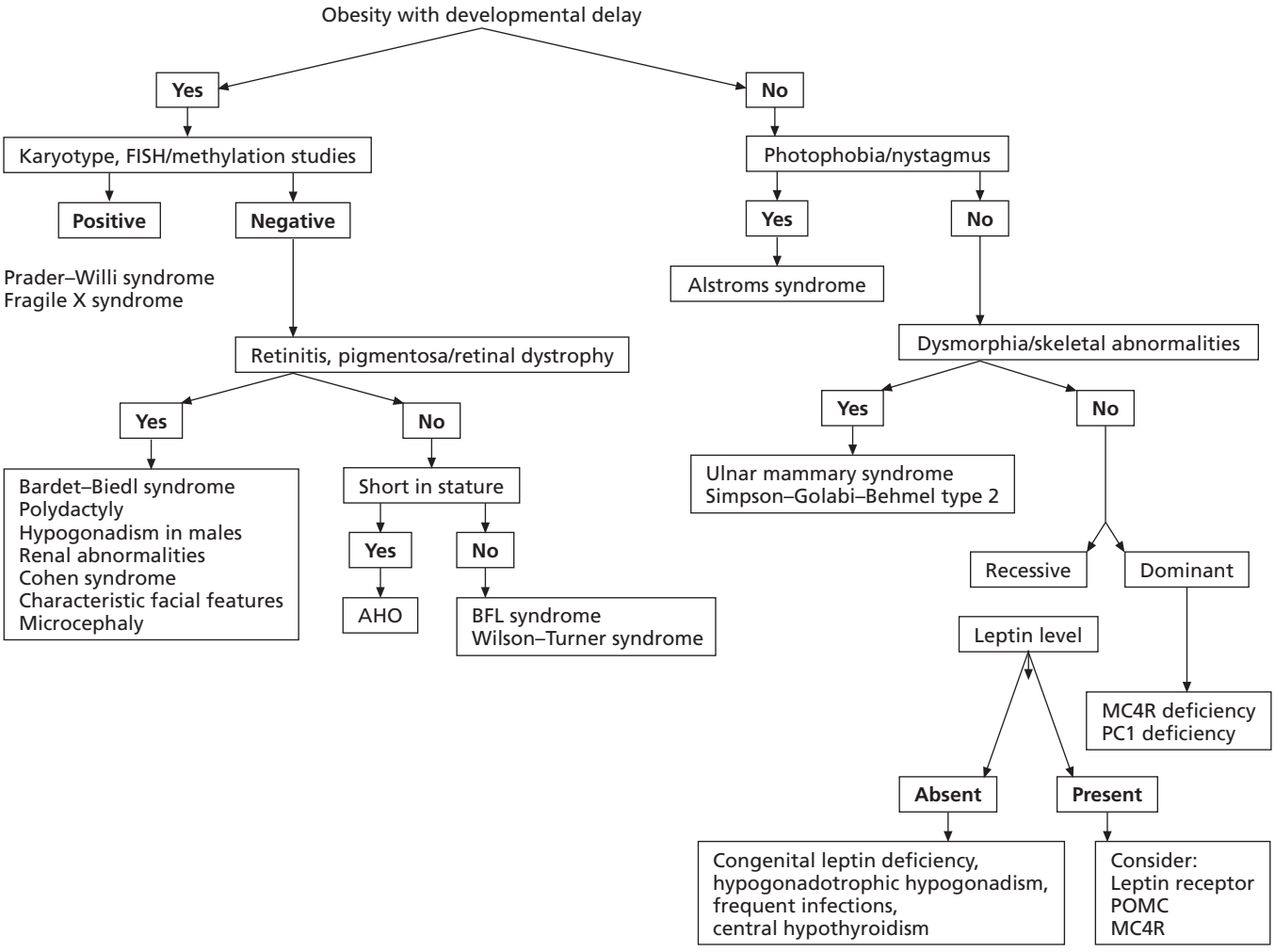

Fig. 19.2. Diagnostic algorithm for childhood obesity with developmental delay.

Pleiotrophic obesity syndromes

Obesity runs in families, although the majority of cases do not segregate with a clear Mendelian pattern of inheritance. There are about 30 Mendelian disorders with obesity as a clinical feature, frequently associated with mental retardation, dysmorphic features, and organ-specific developmental abnormalities (i.e. pleiotrophic syndromes) [5–7]. A number of families with these rare syndromes have been studied by linkage analysis, and the known chromosomal loci for obesity syndromes are summarized in Table 19.2. In a number of cases, mutations in genes have been identified by positional cloning, and the mechanism underlying the development of obesity is becoming clear in some instances [8].

Molecular mechanisms involved in energy homeostasis

The first description of hypothalamic injury associated with obesity was published by Mohr in 1840. This remained unsupported until two landmark papers by Babinski [9] and Frohlich [10] describing tumors in the region of the hypothalamus associated with obesity, gonadal atrophy, decreased vision, and short stature. In 1940, Hetherington and Ranson published their first report demonstrating that electrolytic lesions in rodents involving, but not restricted to, the ventromedial region of the hypothalamus (VMH) were associated with hyperphagia (increased food intake), hyperinsulinemia, and obesity [11]. However, the precise nature of these hypothalamic pathways and the nature of their inputs and outputs was clarified only with the identification and characterization of single gene defects in rodent models of obesity.

Rodent models of obesity

A number of obese inbred strains of mice, both dominant (yellow, Ay/a) and recessive (ob/ob, db/db, fa/fa, tb/tb), have been studied. In the 1990s, the genes responsible for these syndromes were identified, mostly by positional cloning techniques. These observations have given substantial insights into the physiological disturbances that can lead to obesity, the metabolic and endocrine abnormalities associated with the obese phenotype, and the more detailed anatomical and neurochemical pathways that regulate energy intake and energy expenditure [12]. These studies provide the framework upon which the understanding of the more complex mechanisms in humans can be built.

Table 19.2. Obesity syndromes.

Syndrome	Additional clinical features	Locus
Autosomal dominant		
Prader–Willi syndrome	Hypotonia, mental retardation, short stature hypergonadotrophic hypogonadism	Lack of the paternal segment 15q11.2–q12
Albright hereditary osteodystrophy	Short stature, skeletal defects, and impaired olfaction	20q13.2
Ulnar–mammary syndrome	Ulnar defects, delayed puberty, hypoplastic nipples	12q24.1
Autosomal recessive		
Bardet–Biedl syndrome	Mental retardation, dysmorphic extremities, retinal dystrophy, or pigmentary retinopathy, hypogonadism, and structural abnormalities of the kidney, or functional renal impairment	11q13 (BBS1) 16q21 (BBS2) 3p13 (BBS3) 15q22 (BBS4) 2q31 (BBS5) 20p12 (BBS6) 4q27 (BBS7) 14q32 (BBS8)
Alstrom syndrome	Retinal dystrophy, neurosensory deafness, diabetes	2p13
Cohen syndrome	Prominent central incisors, ophthalmopathy, microcephaly	8q22
X linked		
Fragile X syndrome	Mental retardation, hyperkinetic behavior, macroorchidism, large ears, prominent jaw, high-pitched jocular speech	Xq27.3
Borjeson–Forssman–Lehmann syndrome	Mental retardation, hypogonadism, large ears	Xq26
Mehmo syndrome	Mental retardation, epilepsy, hypogonadism, microcephaly	Xp22.13
Simpson–Golabi–Behmel syndrome type 2	Craniofacial defects, skeletal and visceral abnormalities	Xp22
Wilson–Turner syndrome	Mental retardation, tapering fingers, gynecomastia	Xp21.2

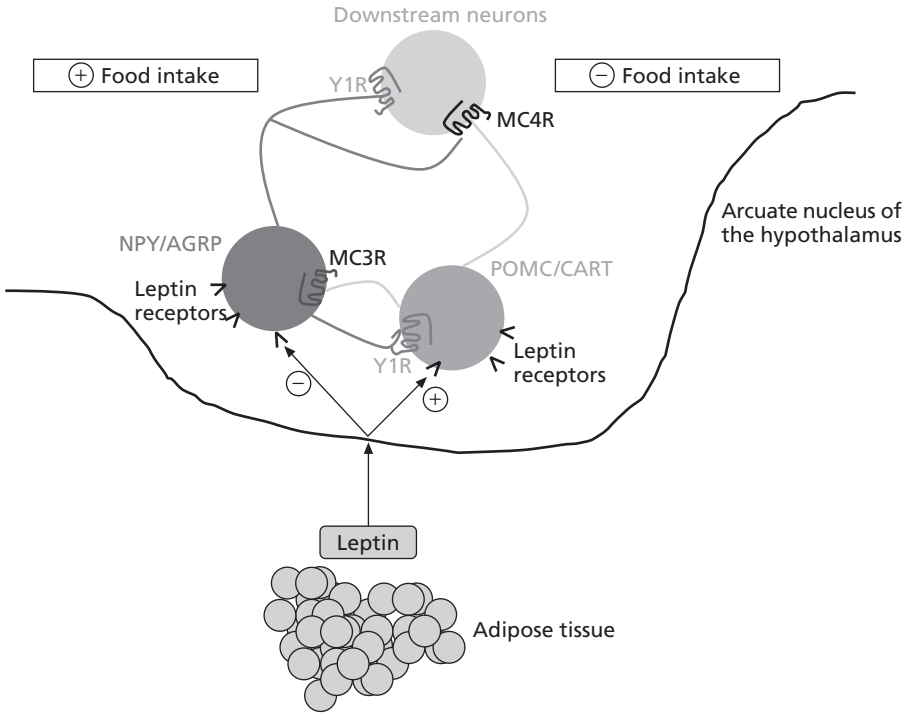

Fig. 19.3. The leptin–melanocortin pathway

Leptin–melanocortin pathway

Initial observations in this field were made as a result of positional cloning strategies in two strains of severely obese mice (ob/ob and db/db). Severely obese ob/ob mice were found to harbor mutations in the ob gene resulting in a complete lack of its protein product leptin [13]. Administration of recombinant leptin reduced the food intake and body weight of leptin-deficient ob/ob mice and corrected all their neuroendocrine and metabolic abnormalities [14–16]. The signaling form of the leptin receptor is deleted in db/db mice, which are consequently unresponsive to endogenous or exogenous leptin [17]. The identification of these two proteins established the first components of a nutritional feedback loop from adipose tissue to the brain [18]. The physiological role of leptin in humans and rodents might be to act as a signal for starvation because, as fat mass increases, further rises in leptin have a limited ability to suppress food intake and prevent obesity [19].

Considerable attention has focused on deciphering the hypothalamic pathways that co-ordinate the behavioral and metabolic effects downstream of leptin [20,21]. The first-order neuronal targets of leptin action in the brain are anorectic (reducing food intake), pro-opiomelanocortin (POMC), and orexigenic (increasing food intake) neuropeptide-Y/Agouti-related protein (NPY/AgRP) neurons in the hypothalamic arcuate nucleus, where the signaling isoform of the leptin receptor is highly expressed (Fig.19.3). Forty per cent of POMC neurons in the arcuate nucleus express the mRNA for the long form of the leptin receptor, and POMC expression is positively regulated by leptin. POMC is sequentially cleaved by prohormone convertases to yield peptides, including α-melanocyte-stimulating hormone (MSH), that have been shown to play a role in feeding behavior [22]. There is clear evidence in rodents that α-MSH acts as a suppressor of feeding behavior, probably through the melanocortin 4 receptor (MC4R). Targeted disruption of MC4R in rodents leads to increased food intake, obesity, severe early hyperinsulinemia, and increased linear growth; heterozygotes have an intermediate phenotype compared with homozygotes and wild-type mice [23].

In the last 7 years, mutations in all the main components of this pathway have been found to result in obesity in humans [6]. The phenotypic characterization of these patients has allowed us to understand the physiological role of these pathways and their interactions with neuroendocrine pathways.

Human monogenic obesity syndromes

Congenital leptin deficiency

In 1997, we reported two severely obese children (an 8-year-old girl weighing 86 kg and her 2-year-old cousin weighing 29 kg) from a highly consanguineous family of Pakistani origin [24]. These children had passed normal developmental milestones and had no dysmorphic features and a normal karyotype. Despite their severe obesity, both children had undetectable concentrations of serum leptin and were found

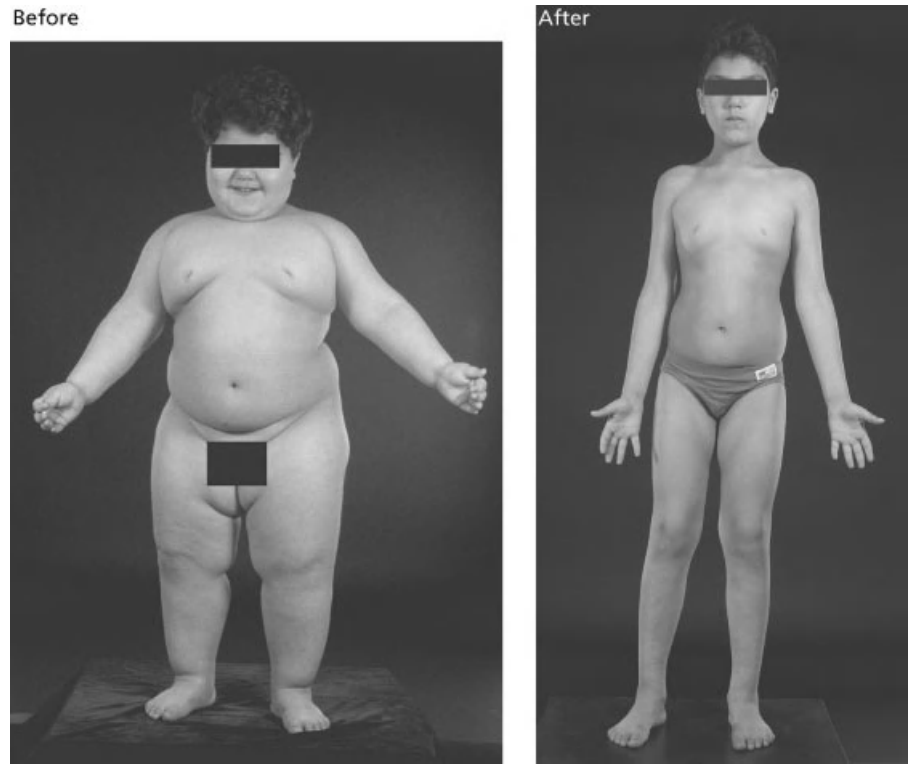

Fig. 19.4. Response to leptin therapy in a child with leptin deficiency.

to be homozygous for a frameshift mutation in the ob gene (ΔG133), which resulted in a truncated protein that was not secreted. We have since identified four further affected individuals from three other families who are also homozygous for the same mutation in the ob gene. All the families are of Pakistani origin but not known to be related over five generations. A large consanguineous Turkish family that carries a homozygous missense mutation has also been described [25].

All subjects in these families are characterized by intense hyperphagia after weaning, waking at night to go looking for food, and demanding food immediately after a meal. The disabling obesity of leptin deficiency is characterized by the selective deposition of fat, and children often develop valgus deformities of the knees by the age of 5–6 years, sleep apnea, and high rates of childhood infection and atopic disease due to abnormalities of T-cell number and function [26]. An advanced bone age is a recognized feature in childhood with a failure to undergo pubertal development due to hypogonadotrophic hypogonadism.

Response to leptin therapy

Congenital leptin deficiency, although rare, is unique in being amenable to therapy [27]. Patients treated with once-daily subcutaneous injections of recombinant human leptin have all lost weight (specifically fat), often with dramatic clinical benefit (Fig. 19.4). The major effect of leptin was on appetite, with normalization of hyperphagia. Leptin therapy reduced energy intake during an 18 MJ *ad libitum* test meal by up to 84% [26]. The administration of leptin permitted progression of appropriately timed pubertal development in the single child of appropriate age and did not cause the early onset of puberty in the younger children (Fig. 19.5) [26].

Free thyroxine and thyroid-stimulating hormone (TSH) concentrations, although usually in the normal range before treatment, had consistently increased at the earliest post-treatment time point and subsequently stabilized at this elevated level. These findings are consistent with evidence from animal models that leptin influences thyrotrophin-releasing hormone (TRH) release from the hypothalamus and from studies illustrating the effect of leptin deficiency on TSH pulsatility in humans [28].

Throughout the trial of leptin administration, weight loss continued in all subjects, albeit with refractory periods that were overcome by increases in leptin dose. All subjects developed anti-leptin antibodies after ≈ 6 weeks of leptin therapy, which interfered with interpretation of serum leptin concentrations and, in some cases, were capable of neutralizing leptin in a bioassay, and were the likely cause of refractory periods occurring during therapy. The fluctuating nature of the antibodies probably reflects the complicating factor that leptin deficiency is itself an immunodeficient state. Administration of leptin leads to a change from the secretion of predominantly Th2 to Th1 cytokines, which may directly influence antibody production [26]. Thus far, we have been

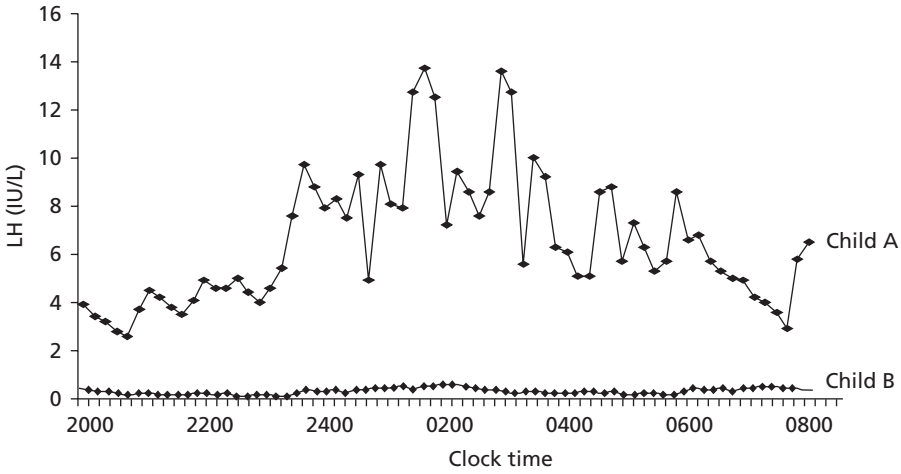

Fig. 19.5. Leptin therapy results in LH pulsatility at an appropriate developmental age in child A compared with child B.

able to regain control of weight loss by increasing the dose of leptin.

Although congenital leptin deficiency is an autosomal-recessive condition, heterozygotes or carriers for the ob mutation have partial leptin deficiency that is associated with a 23% increase in body fat [29].

Leptin receptor deficiency

A mutation in the leptin receptor has been reported in one consanguineous family with three affected subjects [30]. Affected individuals were found to be homozygous for a mutation that truncates the receptor before the transmembrane domain. The mutant receptor ectodomain is shed from cells and circulates bound to leptin, resulting in high leptin concentrations. However, a number of other families with leptin receptor mutations, in whom leptin concentrations are not abnormally high, have been identified.

The phenotype has similarities to that of leptin deficiency. Leptin receptor-deficient subjects were also of normal birthweight but exhibited rapid weight gain in the first few months of life, with severe hyperphagia and aggressive behavior when food was denied. Basal temperature and resting metabolic rate were normal, cortisol concentrations were in the normal range, and all individuals were normoglycemic with mildly elevated plasma insulin concentrations similar to leptin-deficient subjects.

Leptin receptor-deficient subjects had some unique neuroendocrine features not seen with leptin deficiency. Evidence of mild growth retardation in early childhood, with impaired basal and stimulated growth hormone secretion and decreased insulin-like growth factor (IGF)-1 and IGF binding protein (IGFBP)-3 concentrations alongside features of hypothalamic hypothyroidism in these subjects, suggests that loss of the leptin receptor results in a more severe phenotype than loss of leptin itself.

Pro-opiomelanocortin (POMC) deficiency

In 1998, Krude *et al*. reported two unrelated obese German children who were homozygous or compound heterozygous for mutations in POMC. Another five children have subsequently been reported [31]. Initial presentation is in neonatal life with adrenal crisis due to adrenocorticotrophic hormone (ACTH) deficiency, as POMC is a precursor of ACTH in the pituitary, and the children require long-term corticosteroid replacement. The children have pale skin and red hair due to the lack of MSH function at melanocortin 1 receptors in the skin, although this may be less obvious in children from different ethnic backgrounds. POMC deficiency results in hyperphagia and early-onset obesity due to loss of melanocortin signaling at the MC4R. Although trials of treatment have not been performed, it is plausible that selective MC4R agonists will be available for such patients in the near future.

Prohormone convertase 1 deficiency

In 1997, we identified a defect in prohormone processing in a 47-year-old woman with severe childhood obesity, abnormal glucose homeostasis, very low plasma insulin, but with elevated concentrations of proinsulin, hypogonadotrophic hypogonadism, and hypocortisolemia associated with increased concentrations of POMC. She was found to be a compound heterozygote for mutations in prohormone convertase 1 [32] enzyme, which cleaves prohormones at pairs of basic amino acids leaving C-terminal basic residues that are excised by carboxypeptidase E (CPE).

We have recently identified a second child with severe, early-onset obesity who was compound heterozygote for complete loss-of-function mutations in PC1 [33]. As well as a failure to process a number of prohormones, such as preprogonadotropin-releasing hormone (GnRH), preproTRH and

POMC, this patient had a small bowel enteropathy, possibly due to a failure to process gut-derived neuropeptides. Although the inability to cleave POMC is a probable mechanism for obesity in these patients, PC1 cleaves a number of other neuropeptides in the hypothalamus, including glucagon-like peptide 1, which may influence feeding behavior [34].

MC4R deficiency

In 1998, two groups in the UK and France reported heterozygous mutations in the MC4 receptor in humans that were associated with dominantly inherited obesity [35,36]. Since then, heterozygous mutations in MC4R have been reported in obese humans from various ethnic groups with an estimated prevalence of 0.5–1% in obese patients, increasing to 6% in our cohort of severe, early-onset obesity [37]. Thus, MC4R deficiency represents the commonest known monogenic cause of human obesity. While we found a 100% penetrance of early-onset obesity in heterozygous probands, others have described obligate carriers who were not obese.

Given the large number of potential influences on body weight, it is perhaps not surprising that both genetic and environmental modifiers will have important effects in some pedigrees. Indeed, we have now studied six families in whom the probands were homozygotes, and they were all more obese than heterozygotes, some of whom were not obese. This may reflect ethnic-specific effects as all these families were of Indo origin. Taking account of all these observations, co-dominance, with modulation of expressivity and penetrance of the phenotype, is the most appropriate description of the mode of inheritance.

We have recently defined the phenotype in 150 patients with MC4R deficiency [37]. Affected subjects were hyperphagic, but this was not as severe as that seen in leptin deficiency, although it often started in the first year of life. Of particular note is the finding that the severity of receptor dysfunction seen in *in vitro* assays could predict the amount of food ingested at a test meal by the subject harboring that particular mutation. As well as the increase in fat mass, MC4R-deficient subjects also have an increase in lean mass not seen in leptin deficiency and an increase in bone mineral density. Thus, they often appear "big-boned." Linear growth of these subjects is striking, with affected children having a height standard deviation score (SDS) of + 2, whereas the mean height SDS of other obese children in our cohort is + 0.5. MC4R-deficient subjects also have higher concentrations of fasting insulin than age, sex, and BMI SDS-matched children. The accelerated linear growth does not appear to be due to dysfunction of the growth hormone (GH) axis and may be a consequence of the disproportionate early hyperinsulinemia [37].

One notable feature of this syndrome is that the severity of many of the phenotypic features appears to ameliorate with time. Thus, obese adult mutation carriers report less intense feelings of hunger and are less hyperinsulinemic than children with the same mutation.

MC4R mutations appear to be the commonest monogenic cause of obesity described thus far in humans. The maintenance of this reasonably high disease frequency is likely to be partly due to the fact that obesity is expressed in heterozygotes and that there is no evidence of any apparent effect of the mutations on reproductive function.

Conclusion

Although these monogenic syndromes are rare, an improved understanding of the precise nature of the inherited component of severe obesity has undoubted medical benefits and helps to dispel the notion that obesity represents an individual defect in behavior with no biological basis. For individuals at highest risk of the complications of severe obesity, such findings provide a starting point for providing more rational mechanism-based therapies as has successfully been achieved for congenital leptin deficiency.

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20 Metabolic consequences of obesity and their management

Michael Freemark

Introduction

Striking increases in the prevalence of obesity in young people are taking place throughout the developed world. The causes of this "obesity epidemic" are many, encompassing changes in food intake, composition, availability, and cost, as well as modifications of lifestyle that affect energy expenditure. In children, obesity predisposes to insulin resistance and type 2 diabetes, dyslipidemia, hepatic steatosis/steatohepatitis, hypertension and focal glomerulosclerosis, accelerated growth and bone maturation, ovarian hyperandrogenism and gynecomastia, cholecystitis, pancreatitis, and pseudotumor cerebri. Non-metabolic complications include sleep apnea, orthopedic disorders, and stress incontinence. Long-standing obesity and insulin resistance in adults increase markedly the risks of cardiovascular disease, stroke and certain malignancies (Fig. 20.1). Given the human and economic costs associated with the care of obese patients, the condition exacts an enormous toll on the medical, social, and financial communities [1].

In theory, the treatment of obesity and its complications requires a multifaceted approach (Fig. 20.2) designed to: (1) reduce body mass index (BMI) and visceral fat mass; (2) increase the sensitivity of skeletal muscle and adipose tissue to insulin action, decrease hepatic glucose production, and reduce fasting and post-prandial glucose concentrations; (3) decrease circulating insulin concentrations; (4) reduce circulating free fatty acid and triglyceride concentrations and normalize plasma lipids; (5) decrease blood pressure; (6) reduce the expression of inflammatory cytokines; and (7) normalize vascular and endothelial function. These objectives can be achieved in some instances through intensive lifestyle intervention involving caloric restriction and regular exercise. Pharmacologic agents or surgical intervention may reinforce the effects of diet and exercise and reduce the risk of, or even reverse, complications. In all cases the social environment is critical.

Pathogenesis of insulin resistance, hepatic steatosis, and type 2 diabetes in obese subjects

Type 2 diabetes is the endpoint of a process of metabolic decompensation that may evolve over a period of months or years. In most cases in childhood, the disease begins with peripheral resistance to insulin action accompanied by fasting hyperinsulinemia and, extrapolating from animal studies, an increase in islet size and β-cell mass. Progression from insulin resistance to impaired fasting glucose (IFG) and impaired glucose tolerance (IGT or "prediabetes") is accompanied by dysregulation of basal insulin secretion with aberrations in the slow oscillations of insulin release, loss of first-phase glucose-dependent insulin secretion, and altered insulin processing, revealed as an increase in the circulating ratio of proinsulin to insulin [2]. The phenotype of type 2 diabetes is characterized by a decline in total insulin production, relative or absolute hypoinsulinemia, a reduction in β-cell mass, and deposition of amyloid in the pancreatic islets.

The risk of developing type 2 diabetes depends on genetic inheritance, developmental and nutritional factors, and energy expenditure. The disease occurs more commonly among African-Americans, Hispanic-Americans, Native Americans, Pacific Islanders, and (possibly) Asian-Americans than among those of Caucasian background and is far more prevalent among subjects with a family history [2–4]. The prevalence increases with the onset of puberty because of the anti-insulin effects of growth hormone and sex steroids, but risk is modified by events before birth: the disease occurs more frequently in children of diabetic mothers and those born small for gestational age, particularly if there is rapid catch-up growth in early childhood [5–7]. Girls are affected more frequently (1.7-fold) than boys and teenage girls and young women with ovarian hyperandrogenism or the polycystic ovarian syndrome (PCOS) are at high risk; prepubertal girls with adrenarche also appear to be vulnerable [8].

Fig. 20.1. Metabolic complications of obesity. IR, insulin resistance; NASH, non-alcoholic steatohepatitis; CVD, cardiovascular disease; FGS, focal glomerulosclerosis.

The most important modifiable risk factor is obesity. Its critical role was established in the Nurses' Health Study [9]; among nearly 17 000 adult women followed prospectively for 16 years, the risk of developing type 2 diabetes was nearly 40-fold higher among those with the highest BMI than in those with the lowest BMI. Smoking, intake of saturated fats, and a sedentary lifestyle increased diabetes risk only 1.8- to 2.4-fold. Obesity plays a similar role in the pathogenesis of type 2 diabetes in children; with the exception of some teenage girls with PCOS, the overwhelming majority of pediatric patients with type 2 diabetes are obese. Insulin sensitivity correlates inversely with BMI and percentage body fat [8,10,11]. Severe obesity in prepubertal American children and adolescents is commonly associated with IGT (21–25%) and, in some cases (4% of teenagers), with unsuspected type 2 diabetes [3,10]. BMI in childhood (ages 7–13 years) correlates with obesity, hypertension, hyperinsulinemia, and dyslipidemia in adults aged 22–25 years. Obesity and hyperinsulinemia in Pima and African-American children predict the development of type 2 diabetes in adolescence and adulthood [12–18]. Finally, obesity is a common feature of genetic and hormonal conditions associated with IGT and type 2 diabetes such as the Prader–Willi syndrome.

Factors other than the simple accumulation of body fat modulate the risk of glucose intolerance. The distribution of body fat appears to be important. Accumulation of visceral and abdominal subcutaneous fat is associated with insulin resistance [19], while insulin sensitivity correlates less well with stores of femoral and gluteal subcutaneous fat. However, African-American children have relatively less visceral fat than caucasian children but higher rates of type 2 diabetes. This may reflect genetic or environmental differences.

The mechanisms by which fat deposition induces insulin resistance and glucose intolerance are beginning to emerge (Fig. 20.3 and [19–21]). The accumulation of visceral fat is accompanied by adipose tissue resistance to insulin action and heightened sensitivity to catecholamines. Adipose tissue uptake of glucose and free fatty acids (FFA) is reduced, rates of lipolysis are increased, and triglyceride (TG) clearance is impaired because of downregulation of lipoprotein lipase. The resistance to insulin appears to be mediated by changes in the expression of adipocyte cytokines. Tumor necrosis

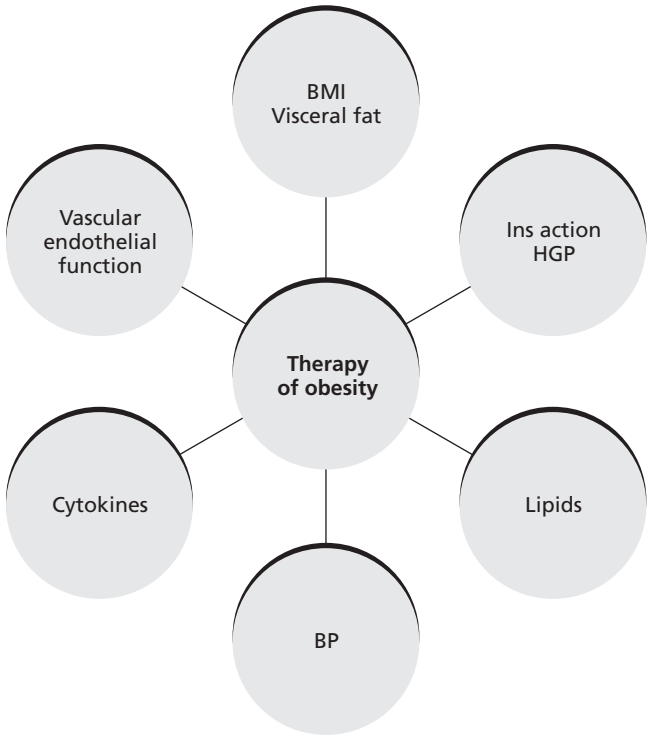

Fig. 20.2. Therapeutic targets in obesity. BMI, body mass index; Ins, insulin; HGP, hepatic glucose production; BP, blood pressure.

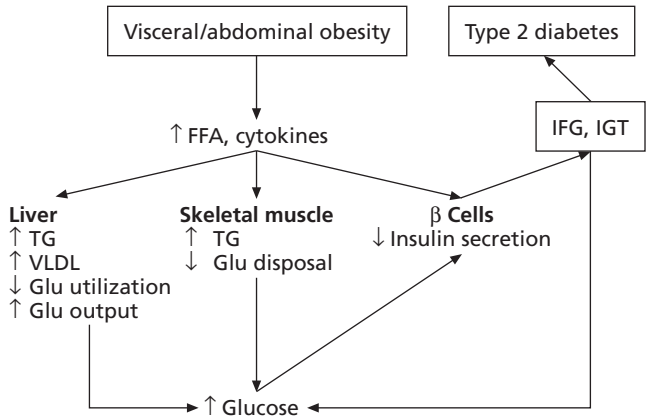

Fig. 20.3. The pathogenesis of glucose intolerance in obese subjects. FFA, free fatty acids; TG, triglycerides; glu, glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

factor-α (TNF-α) and interleukin 6 (IL-6) are overexpressed in adipose tissue of obese subjects, while adiponectin expression is reduced. TNF-α inhibits insulin-mediated glucose and FFA uptake and triglyceride synthesis in fat and, like the catecholamines, induces lipolysis and the release of FFA from adipose stores. The lipolytic effects are potentiated by IL-6, which inhibits lipoprotein lipase and TG deposition in adipose tissue. IL-6 and TNF-α reduce expression of adiponectin in developing preadipocytes, explaining in part the down-regulation of adiponectin in obesity. Plasma adiponectin concentrations are inversely related to BMI, waist circumference, and abdominal fat mass and are higher in females than in males. Adiponectin levels correlate with insulin sensitivity in children as well as adults, and targeted deletion of adiponectin causes diet-dependent resistance to insulin action in skeletal muscle and liver.

TG and FFA diverted or released from fat are stored in extra-adipose tissues including the liver, skeletal muscle, pancreatic β-cell, and heart. Storage of surplus fuel in peripheral tissues is facilitated by a resistance to, or a relative deficiency of, leptin, which normally stimulates tissue fatty acid oxidation and inhibits lipogenesis [22]. The accumulation of TG in liver (hepatic steatosis) may induce hepatic inflammation (steatohepatitis) and a rise in serum transaminases. In rare cases, children with steatohepatitis can develop progressive liver damage, including cirrhosis [23]. Hepatic TG deposition impedes insulin uptake, which contributes to circulating hyperinsulinemia and limits insulin action. Direct effects of TNF-α and IL-6 and reductions in plasma adiponectin concentrations may exacerbate hepatic insulin resistance. The resulting increase in hepatic glucose production, probably mediated by induction of gluconeogenesis, contributes to mild increases in fasting blood glucose concentrations and stimulates pancreatic insulin secretion. Hepatic production of TG is also increased; this exacerbates the rise in circulating TG levels caused by adipose tissue insulin resistance. Exchange of very low-density cholesterol (VLDL)-TG for cholesterol esters in high-density lipoproteins (HDL) increases renal HDL clearance and thereby reduces plasma HDL levels.

The elevations in plasma FFA, TG, and circulating adipocytokines in the setting of leptin resistance have profound effects on insulin action in skeletal muscle. Analysis of muscle biopsies from insulin-resistant adults shows reductions in tyrosine phosphorylation of the insulin receptor and insulin receptor substrate (IRS)-1, decreased IRS-1-associated PI-3 kinase activity, and impaired threonine and serine phosphorylation of Akt. The defects in insulin signaling are thought to be induced by intramyocellular accumulation of TG and other lipid species, including long-chain fatty acyl coA, diacylglycerol, ceramide or β-hydroxy butyrate. The myocellular lipid accumulation may result from adipokine-dependent reductions in fatty acid oxidation and/or inherited defects in mitochondrial oxidative phosphorylation [21]. Inhibition of Akt phosphorylation impairs skeletal muscle glucose uptake by reducing glucose transporter 4 (GLUT-4) expression, translocation, and/or activity [19–21]. The result is a progressive decrease in insulin-stimulated, non-oxidative glucose disposal.

Insulin resistance does not guarantee progression to frank glucose intolerance; indeed, most obese, insulin-resistant subjects never develop type 2 diabetes. The development of glucose intolerance requires β-cell dysfunction and loss of glucose-dependent insulin secretion. Some evidence

suggests that β-cell dysfunction may be a familial or genetic trait that predisposes individuals to type 2 diabetes. Other findings suggest that FFA, cytokines, and glucose may promote β-cell dysfunction in genetically predisposed subjects [19]. Acute elevations of FFA increase β-cell insulin secretion, and the rise in FFA during fasting may sustain basal insulin production and preserve the normal insulin secretory response to glucose. Prolonged administration of FFA, on the other hand, impairs insulin secretion in rodents, but the response to chronic lipids in humans is more variable; in some studies, insulin secretion is maintained or even increased. However, long-term administration of lipids to obese, insulin-resistant adults reduces insulin secretion, although plasma insulin concentrations are increased because insulin clearance is impaired. The response to chronic lipid administration may be conditioned by genetic factors and the prevailing metabolic milieu: in insulin-resistant adult men and women with a strong family history of type 2 diabetes, a 4-day infusion of triglyceride emulsion reduced first- and second-phase insulin secretion and hepatic insulin clearance and increased hepatic glucose production. In contrast, chronic lipid administration increased insulin secretion and had no effect on insulin clearance or hepatic glucose production in normal age- and BMI-matched controls [24]. Thus, chronic elevations in FFA probably contribute to β-cell failure in obese, insulin-resistant subjects predisposed to developing type 2 diabetes.

The mechanisms by which lipids induce toxic effects ("lipotoxicity") on β-cell function remain unclear. FFA and cytokines such as TNF-α and IL-1 might impair β-cell function directly through induction of β-cell apoptosis; alternatively, FFA may induce the production of inflammatory cytokines by macrophages resident within pancreatic islets. Cytokines increase β-cell production of nitric oxide, which inhibits glucose-stimulated insulin secretion and stimulates β-cell apoptosis [25]. Resistance to leptin action may contribute to lipotoxicity because leptin reduces islet expression of nitric oxide synthetase and maintains expression of islet anti-apoptotic genes including Bcl-2 [22]. Beta-cell apoptosis may also result from chronic exposure to elevated glucose concentrations ("glucotoxicity"). Nutrient- and cytokine-dependent loss of β-cell mass and function in an insulin resistant subject lead inexorably to glucose intolerance and ultimately to type 2 diabetes.

Consequences

Hypertension, atherogenesis, and cardiovascular disease in obesity and insulin resistance

The development of insulin resistance and type 2 diabetes has serious implications for long-term cardiovascular health; microvascular complications including neuropathy, retinopathy, and microalbuminuria all occur with increased frequency in adults with IGT as well as diabetes, and rates of myocardial infarction and stroke are increased two- to fivefold [26–29].

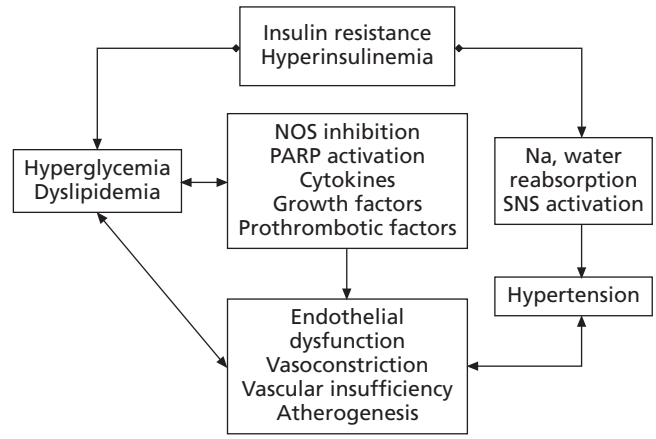

Fig. 20.4. The pathogenesis of vascular disease in patients with insulin resistance. NOS, nitric oxide synthase; PARP, poly(ADPribose) polymerase; SNS, sympathetic nervous system.

Obesity and insulin resistance in childhood predispose to vascular complications in later life. Severe obesity in 9- to 11-year-old children is associated with increased stiffness of the carotid arteries, and obesity in adolescence predisposes to increased carotid intima media thickness (CIMT) in young adulthood. Interestingly, weight loss after adolescence may reduce adult CIMT [30]. Among 93 subjects in the Bogalusa Heart Study who underwent autopsy at age 2–39 years, the prevalence of fatty streaks and fibrous plaques in the aorta and coronary arteries increased with age and correlated positively with standard deviation (z) scores for BMI, serum triglycerides, cholesterol, and blood pressure [31]. The combination of multiple risk factors increased exponentially the extent of arterial intimal surface involvement. Post-mortem analysis of more than 3000 subjects who died of natural causes at 15–34 years of age (Pathological Determinants of Atherosclerosis in Youth [32]) showed that obesity and impaired glucose tolerance were associated with progression of atheromatous lesions in adolescents and young adults. In young men, BMI and abdominal fat mass correlated with the number and size of fatty streaks and raised lesions in the right and left anterior descending coronary arteries. In both women and men, the extent of fatty streaks correlated with glycohemoglobin concentrations. Severe glucose intolerance probably accelerates the progression of vascular disease; a Canadian study [33] of 52 young adults aged 18–33 years who developed type 2 diabetes before age 17 years showed one with a toe amputation and five on dialysis; two of the latter had died and one was blind.

The pathogenesis of vascular disease involves a complex web of hormones, growth factors, vasoactive agents, cytokines, oxygen radicals, and cellular adhesion molecules (Fig. 20.4 [26,34,35]). Under normal conditions, insulin

stimulates vasodilatation through induction of nitric oxide synthase (NOS) and generation of NO in vascular endothelial cells. In obesity and other states associated with insulin resistance, the production of NO is disrupted, leading to vasoconstriction and tissue ischemia. Hyperglycemia contributes to endothelial dysfunction and vascular insufficiency through production of superoxide radicals; reactive oxygen species cause direct endothelial damage and deplete endothelial NO, reducing vascular reactivity. Oxygen radicals also activate poly(ADP ribose) polymerase (PARP), which inhibits glyceraldehyde phosphate dehydrogenase activity and thereby promotes the formation of polyols, glucosamine, and advanced glycation endproducts and the activation of protein kinase C [36]. These endproducts promote the development of microvascular and macrovascular disease.

Glucose-dependent expression of growth factors (such as vEGF, EGF, and IGF-1) and cytokines (IL-1, IL-6, and TNF-α) and a reduction in plasma adiponectin concentrations aggravate these effects by stimulating migration and proliferation of smooth muscle cells and increasing leukocyte adhesion to endothelial surfaces. Reduction in NO availability enhances platelet aggregation and limits fibrinolysis, promoting the progression of atheromatous clots. Increases in the concentrations of the prothrombotic plasminogen activator-1, which is also overexpressed by adipose tissue in obesity, may contribute to fibrin deposition on luminal walls. Production of endothelin-1 in terminal blood vessels is increased, promoting vasoconstriction [35]. These effects are exacerbated by dyslipidemia and hypertension; increases in blood pressure may reflect insulin-dependent increases in sodium and water reabsorption and activation of the sympathetic nervous system [37]. Reductions in tissue perfusion limit insulin-mediated glucose disposal and may increase circulating glucose concentrations, creating a vicious cycle.

Hepatic steatosis, cholecystitis, pancreatitis, and pseudotumor cerebri

Free fatty acids diverted from adipose tissue are synthesized in the liver to triglycerides, leading to fatty infiltration (steatosis). This may lead to cirrhosis [23]. Cholecystitis, pancreatitis, and pseudotumor cerebri occur more frequently in obese than in normal weight children. Among 123 Spanish children (mean age 7.8 years) with cholelithiasis, 12 were obese and four had hypercholesterolemia [38]. Gallstone formation is thought to result from an increased rate of biliary cholesterol excretion relative to that of bile acid or phospholipids. Obesity may place teenage girls at higher risk of idiopathic pancreatitis. The hypertriglyceridemia of obesity/insulin resistance probably plays a pathogenic role.

The pathogenesis of pseudotumor cerebri in obesity remains obscure [39]. Increases in intra-abdominal pressure may increase central venous and intrathoracic pressures and thereby raise intracranial pressure.

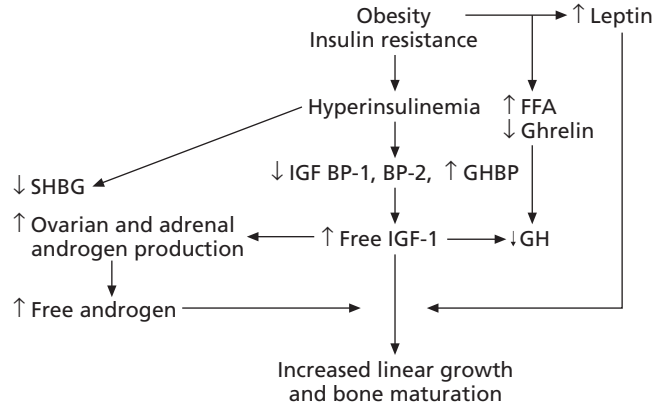

Fig. 20.5. Growth and bone maturation in obesity and insulin resistance. FFA, free fatty acids; GH, growth hormone; IGF, insulin-like growth factor; BP, binding protein; SHBG, sex hormone binding globulin.

Growth and bone maturation, pituitary and adrenal function, and ovarian hyperandrogenism

Rates of linear growth and bone maturation are often increased in obese prepubertal children, despite marked reductions in basal and stimulated plasma growth hormone (GH) concentrations and a reduction in circulating GH half-life (Fig. 20.5 [40]). Reductions in pituitary GH secretion in obesity could in theory reflect increased sensitivity of target tissues to GH, enhanced negative feedback from insulin-like growth factor 1 (IGF-1) and FFA, and/or reductions in circulating concentrations of the GH secretagog ghrelin. Although sensitivity to GH has not been assessed directly in obese, hyperinsulinemic subjects, concentrations of GH binding protein (GHBP) are increased. GHBP represents the circulating form of the extracellular domain of the GH receptor; thus, the increase in serum GHBP concentrations may reflect an increase in tissue GH receptor expression. That hyperinsulinemia may stimulate an increase in tissue GH receptors is suggested by studies demonstrating induction of GH receptor expression in hepatoma cells by insulin [41].

Total IGF-1 and IGF binding protein (BP)-3 concentrations in obese subjects are normal or mildly elevated, but free IGF-1 levels are increased. The latter may reflect reductions in circulating IGFBP-1 and IGFBP-2, which are suppressed by insulin and correlate inversely with insulin sensitivity [42]. Together with nutrient excess, the increase in free IGF-1 levels may accelerate linear growth and bone age.

The effects of IGF-1 on growth and bone maturation in obese subjects may be potentiated by hyperleptinemia. Circulating leptin levels rise in proportion to body (particularly subcutaneous) fat stores and are higher in girls than in boys. Leptin stimulates proliferation of isolated mouse and rat osteoblasts and increases the width of the chondroprogenitor zone of the mouse mandible *in vivo*. In leptin-deficient ob/ob mice, leptin increases femoral length, bone area, and bone mineral content [43]. The effects of leptin may be exerted in

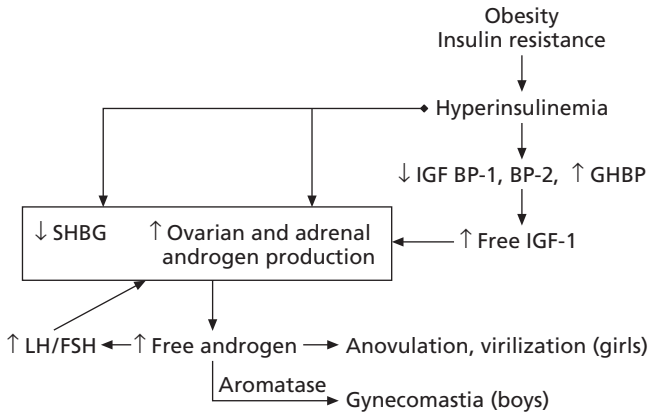

Fig. 20.6. Ovarian hyperandrogenism and gynecomastia in insulin-resistant children. IGF, insulin-like growth factor, BP, binding protein; SHBG, sex hormone binding globulin, LH, luteinizing hormone; FSH, follicle-stimulating hormone.

concert with IGF-1 because leptin increases IGF-1 receptor expression in mouse chondrocytes [44].

A relative excess of sex steroids may also contribute to growth acceleration and bone maturation in obese children (Fig. 20.6). IGF-1 and insulin in excess act in synergy with adrenocorticotrophic hormone (ACTH) and luteinizing hormone (LH) to stimulate the production of androgens from adrenocortical cells and ovarian theca cells respectively. These effects are mediated through induction of P450c17α hydroxylase activity. The biological availability of ovarian and adrenal androgens is increased because insulin suppresses hepatic sex hormone binding globulin (SHBG) expression and reduces plasma SHBG concentrations. Free androgens increase the frequency of gonadotropin-releasing hormone (GnRH) pulses and the ratio of LH to follicle-stimulating hormone (FSH), thereby exacerbating thecal androgen production. The increase in free androgens may induce precocious adrenarche in prepubertal girls and boys and may cause anovulation and hirsutism in adolescent girls and young women [45]. Aromatization of androstenedione in adipose tissue increases plasma estrone concentrations, causing gynecomastia in adolescent boys.

Basal plasma, salivary, and urinary free cortisol concentrations and basal ACTH levels in obese children generally fall within the normal range, and diurnal variation and the response to dexamethasone are maintained. However, body fat mass correlates with total excretion of glucocorticoid metabolites, suggesting that obesity may be related to daily cortisol secretion and turnover. Changes in tissue glucocorticoid metabolism may modulate fat distribution and peripheral insulin sensitivity [46]. In adults with visceral obesity, the production of cortisol from cortisone by adipocytes may be increased through enhanced expression of 11β-hydroxysteroid dehydrogenase type 1 ($11\beta HSD_1$ [46]). Whether or not $11\beta HSD_1$ plays a role in the pathogenesis of childhood obesity is currently unclear.

Renal dysfunction

Increased glomerular filtration rate, renal hypertrophy, and proteinuria may occur in obesity with histological findings of focal segmental glomerulosclerosis, mesangial proliferation, and glomerulomegaly, which differ from the appearances seen in nephrotic syndrome. Obesity-related glomerulopathy is associated with hypertension, hyperinsulinemia, increased free 1GF-1, and hyperlipidemia [47].

Calcium homeostasis and bone mineralization

Morbid obesity in adolescents and adults is accompanied by altered binding of calcium to plasma proteins, low levels of 25OH vitamin D, elevated 1,25 diOH vitamin D and osteocalcin, and secondary hyperparathyroidism [48]. Bone mineral content is variably decreased; fracture rates among obese children are unknown. Alterations in calcium dynamics are reversed by weight loss.

Malignancy

Chronic increases in the circulating concentrations of free IGF-1 and sex steroids may contribute to increased risks of certain malignancies in obese adults ([49], Fig. 20.7). The prevalence of endometrial cancer, cervical cancer, and renal cell carcinoma in women increases in proportion to BMI, while rates of liver cancer (and to a lesser extent gastrointestinal malignancies) are increased markedly in obese men. It is not known whether childhood obesity predisposes to childhood or adult malignancy, although a retrospective study [50] revealed a 9.1-fold (range 1.1–77.5) increase in the incidence of colon cancer among elderly men (not women) who had been obese as adolescents.

Identification and screening of obese children for metabolic complications

Many children and adolescents with insulin resistance and a subset of those with established type 2 diabetes lack classical symptoms of glucose intolerance such as polyuria and polydipsia. Given the high incidence of IGT and undisclosed diabetes among American children with BMI *z*-score exceeding 2 SDs for age and gender [10,11], I recommend screening obese children who have one or more of the risk factors listed in Table 20.1. Alternatively, screening can be conducted in children with BMI > 85th percentile for age and gender with two or more associated risk factors. Particular attention should be focused upon obese children with family histories of type 2 or gestational diabetes and those from high-risk ethnic groups. Progressive abdominal obesity is an ominous finding, and the presence of acanthosis nigricans, which suggests insulin resistance, should raise concern. Given the strong

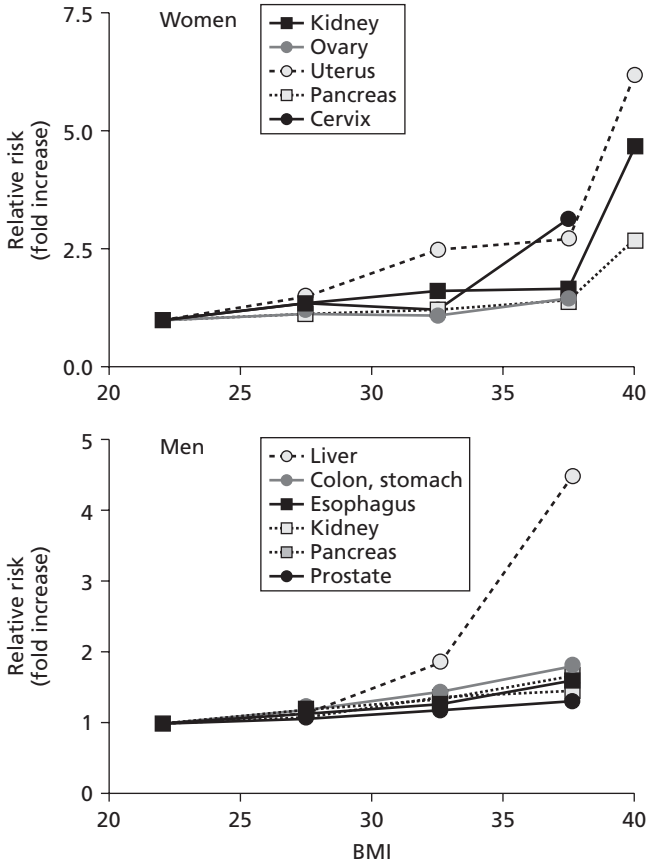

Fig. 20.7. Cancer rates in obese men and women. Adapted from [48] with permission.

association of PCOS with insulin resistance, I recommend screening all girls with ovarian hyperandrogenism, regardless of BMI.

Some investigators recommend a fasting blood glucose level for screening, but isolated measures of fasting blood glucose concentrations miss a significant proportion of children with IGT. Others favor a formal glucose tolerance test, but a modified glucose challenge may better suit the need for population screening (Table 20.1). This would include fasting measures of insulin, glucose, and lipids, with a fingerstick glucose obtained 2 h following the administration of glucola (1.75 g/kg, maximum 75 g). The blood pressure is obtained at the time of the initial blood sample. With this single venipuncture and one fingerstick sample, it is possible to identify patients with undisclosed diabetes, IFG (fasting glucose 111–125 mg%), IGT (2-h glucose 140–199 mg%), dyslipidemia, and hypertension. Patients with insulin resistance but normal glucose tolerance can be identified by calculation of the quantitative insulin sensitivity check {QUICKI = 1/[log fasting insulin (μU/mL) + log fasting glucose (mg%)]}, which correlates strongly with insulin sensitivity measured by insulin clamp techniques in obese adults. Measurement of FFA in the fasting sample (revised QUICKI = 1/[log fasting insulin (μU/mL) + log fasting glucose (mg%) + log FFA (mmol)]} permits detection of insulin resistance even in non-obese subjects.

Table 20.1. Screening for glucose intolerance and the metabolic syndrome in children and adolescents.

High-risk populations
Obese (BMI *z*-score > 95th percentile) children or adolescent, plus
High-risk ethnic group and/or
Family history of type 2 diabetes or GDM and/or
Acanthosis nigricans and/or
Prominent abdominal fat deposition
Ovarian hyperandrogenism
Screening procedures
Blood pressure
Fasting glucose and insulin levels
Fasting lipid panel (+ FFA if possible)
2-h glucose level (+ insulin if possible)
HbA1c (less useful)

BMI, body mass index; GDM, gestational diabetes mellitus; 2-h glucose, plasma glucose 2 h after administration of glucola; HbA1c, hemoglobin A1c.

Management

Given the morbidity associated with insulin resistance and type 2 diabetes, the medical and lay communities face the challenge of preventing, rather than simply treating, chronic metabolic and vascular complications in obese subjects.

Lifestyle intervention

Diet

Mild caloric restriction can be safe and effective when obese children and their families are motivated and encouraged to change longstanding feeding behaviors. Significant reductions in weight are unusual and often transient unless caloric restriction is accompanied by increased energy expenditure. Diets severely restricted in calories produce more dramatic weight loss but cannot be sustained under free-living conditions. Very low-calorie, low-protein diets are potentially dangerous and may precipitate recurrent and futile cycles of dieting and binge eating.

The role of dietary macronutrients in the pathogenesis of obesity, insulin resistance, and type 2 diabetes is controversial. The majority of studies in humans and animals suggest that insulin sensitivity correlates inversely with fat content of the diet [51]. Replacement of saturated fats with polyunsaturated fat and long-chain omega-3 fatty acids can reduce total energy intake, improve insulin sensitivity and, in

combination with exercise, can reduce the risks of type 2 diabetes and cardiovascular disease in adults with IGT.

Obese men and women lost more weight and had more significant reductions in plasma TG concentrations on low-carbohydrate diets than on conventional low-fat diets [52,53]. A review of adult studies [54] suggests that the efficacy of low-carbohydrate diets may be related to decreased caloric intake rather than to reduction in carbohydrate intake. Moreover, the effect of a low-carbohydrate diet may diminish with time.

It is possible that the nature or quality of ingested carbohydrate may modulate weight gain in childhood. The insulin-secretory response to foods containing rapidly absorbed, concentrated carbohydrates (high glycemic index) exceeds the response to foods containing protein, fat, and fiber. The rapid rise and subsequent fall in blood glucose following ingestion of sucrose may precipitate hunger [55], whereas fructose is lipogenic and delays the oxidation of fatty acids, facilitating fat storage [56].

Studies of the effects of glycemic index on weight gain in children are inconclusive. A 19-month study [57] of Massachusetts school children found a positive correlation between BMI and the consumption of sugar-sweetened drinks, and a modified low-glycemic diet (45–50% carbohydrate, 30–35% fat) reduced BMI *z*-score and fat mass in a pilot study of seven obese adolescents [58]. Anecdotal evidence suggests that simple elimination of concentrated soft drinks from the diet can reduce caloric intake in some obese adolescents by as much as 500–1000 kcal/day and thereby facilitate weight reduction.

Other macronutrients, vitamins, and trace elements may contribute to diabetes risk. For example, intake of fiber, particularly whole grains and cereal, correlates inversely with the risks of type 2 diabetes and cardiovascular disease [59]. Insoluble and soluble fiber may limit fat absorption and thereby improve glucose tolerance. The intake of magnesium from whole grains, nuts, and green leafy vegetables and dairy products containing vitamin D and calcium may also correlate inversely with diabetes risk in young adults [60,61].

From the data available, I conclude that caloric restriction and weight reduction by any means will probably reduce the risk of type 2 diabetes and subsequent cardiovascular disease in obese children. Reduced intake of saturated fats will benefit obese adolescents as well as adults. Reduced consumption of high-glycemic foods such as soda, fruit drinks, candy, white bread, pasta, and potatoes will benefit obese children. A balanced diet containing vegetables, fruits, whole grains, fiber, lean meat, fish, and low-fat dairy products is best.

Exercise

A sedentary lifestyle increases the risk of diabetes, while exercise, in combination with caloric and fat restriction, reduces the rate of progression to diabetes in adults with IGT. The Malmo Feasibility Study [62] and the DaQing study [63] demonstrated that lifestyle intervention reduced the rate of progression to diabetes in adults with IGT by 63% and 36% respectively. The Finnish Diabetes Prevention Study [64] randomized individuals with IGT and BMI > 25 kg/m^2 to a control group or an intensive intervention group, which was counseled to reduce total and saturated fat intake, increase fiber intake, reduce body weight, and increase physical activity. No subjects who achieved target goals developed diabetes. In contrast, one-third of those who failed to reach a single target developed diabetes. The primary determinant of diabetes prevention was an increase in insulin sensitivity associated with weight loss. The Diabetes Prevention Program (DPP) [65] compared intensive with standard lifestyle recommendations. Intensive lifestyle changes, which included weight reduction, a decrease in saturated fat intake, and regular exercise (150 min per week) were accompanied by a 58% relative risk reduction in the prevalence of diabetes during a 3-year period.

The mechanisms by which exercise improves insulin sensitivity and glucose tolerance are complex, involving metabolic adaptations in adipose tissue, liver, and skeletal muscle (Fig. 20.8 [66]). Exercise has beneficial effects on fat storage

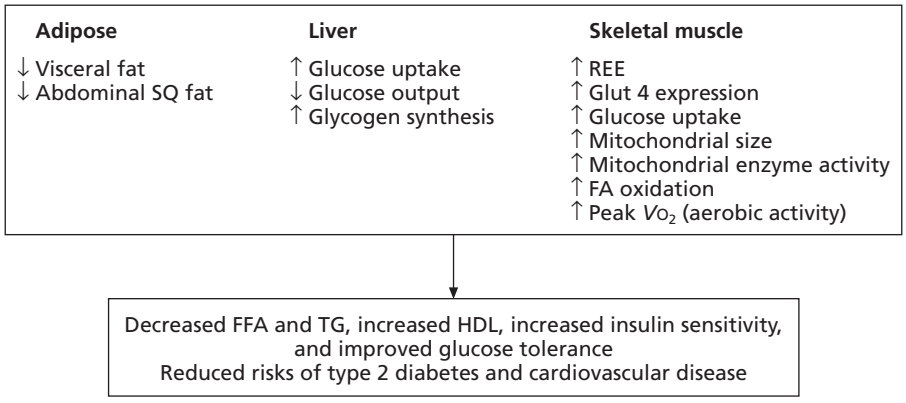

Fig. 20.8. The beneficial effects of exercise training on carbohydrate and lipid metabolism. SQ, subcutaneous; Glut, glucose transporter.

and distribution, with losses of visceral fat depots exceeding those of subcutaneous fat stores. Lean body mass increases, thereby augmenting resting energy expenditure. A reduction in visceral fat mass increases adipose tissue sensitivity to insulin; this explains in part the reductions in fasting and post-prandial free fatty acid, LDL, and TG concentrations and the increase in plasma HDL levels in adults who adhere to a rigorous diet and exercise regimen. The effect of exercise on plasma TG is mediated through induction of lipoprotein lipase and reduction in TG production.

Exercise increases hepatic glucose uptake and glycogen synthesis and decreases hepatic glucose production, thereby reducing fasting glucose and insulin concentrations. In skeletal muscle, exercise stimulates insulin-dependent glucose uptake and thereby reduces post-prandial glucose levels; this action is mediated by increases in muscle GLUT-4 synthesis and induction of GLUT-4 translocation from intracellular pools to the plasma membrane [66]. The induction of GLUT-4 activity may be mediated in turn by an increase in cellular levels of AMP-activated protein kinase (AMPK) [67]. Activation of AMPK after exercise promotes increased cycling of existing GLUT-4 transporters in skeletal muscle as well as enhanced expression of hexokinase II and mitochondrial enzymes.

Several studies suggest that insulin action is related to the oxidative capacity of skeletal muscle. Insulin-resistant individuals, including those with type 2 diabetes, have reduced activities of muscle oxidative enzymes; aerobic exercise training increases muscle oxidative enzyme activity and improves insulin sensitivity by 26–46%. The effect of exercise on oxidative enzyme activity may reflect in part an increase in mitochondrial size [68]. Interestingly, weight loss alone may improve insulin sensitivity but does not alter fasting rates of lipid oxidation. Weight loss coupled with exercise increases fat oxidation.

Few studies have assessed the effects of exercise on insulin resistance in children. Nevertheless, a randomized, modified crossover study of 79 obese children (aged 7–11 years) demonstrated that 4 months of exercise training (40 min of activity on 5 days a week) decreased fasting insulin (10%) and TG (17%) concentrations and reduced percentage body fat (5%) even in the absence of dietary intervention [69]. The effects on plasma insulin and body fat were reversed when training was discontinued. An 8-week trial of cycle ergometry and resistance training in obese adolescents reduced abdominal (7.0%) and trunk (3.7%) fat mass and normalized flow-mediated dilation of the brachial artery [70]. Although preliminary, these findings suggest that exercise has beneficial effects in obese children as well as adults. The capacity for voluntary exercise declines as BMI rises. It is therefore critical to begin regular exercise before the child becomes morbidly obese and functionally immobile.

Benefits from lifestyle intervention are most likely to be reaped when diet and exercise programs are co-ordinated with individual and family counseling and behavior modification. School-based programs, supported by community groups and by state and federal agencies, may assist families and reduce the child's sense of isolation, frustration, and guilt.

Long-term success of lifestyle intervention alone has been disappointing. Rates of obesity and insulin resistance in children and adults continue to increase, despite widespread recognition of the dangers of dietary indiscretion and a sedentary existence. This may reflect the resistance of complex feeding and activity behaviors to change as well as the power of social and economic forces that shape lifestyles. Metabolic and hormonal adaptations to initial weight loss may also create barriers to long-term success; for example, reductions in food intake and body weight decrease the circulating concentrations of triiodothyronine (T_3) and leptin and increase circulating concentrations of ghrelin. The fall in T_3 and leptin levels limits energy expenditure and sympathetic nervous system activity and may facilitate rebound food intake. Hunger may be intensified by the rise in plasma ghrelin, which stimulates food intake [71]. Food restriction also causes a secondary resistance to GH action that may reduce the rates of lipolysis and fat breakdown [40,41]. The obstacles to success with lifestyle intervention have stimulated interest in pharmacologic approaches to the treatment of obesity and the prevention of diabetes and other metabolic complications.

Pharmacotherapy

Anorectic agents

Many drugs initially thought to be safe (for example, diethylpropion, fenfluramine, ephedra, and phenylpronalolamine) have been withdrawn from the commercial market because they caused life-threatening complications. The only anorectic agent currently approved for use in obese adolescents is *sibutramine*, a non-selective inhibitor of neuronal reuptake of serotonin, norepinephrine, and dopamine. In combination with caloric restriction and a comprehensive family-based behavioral program [72], sibutramine reduced BMI by 8.5 ± 6.8% in 43 obese adolescents during an initial 6-month period; a 4.0 ± 5.4% reduction in BMI was achieved in 39 placebo-treated subjects. No additional weight loss occurred during a subsequent 6 months of therapy. Fasting insulin concentrations declined and HDL levels increased. However, 19 out of 43 subjects treated with sibutramine developed mild hypertension and tachycardia, necessitating reduction in drug dose, and five had sustained elevations in blood pressure that required discontinuation of the drug. Other potentially serious complications include insomnia, anxiety, headache, and depression. There is a heightened risk of the serotonin syndrome [73] if sibutramine is used in combination with monoamine oxidase inhibitors, buspirone, lithium,

meperidine, selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, triptans, dextromethorphan, ergot alkaloids, or fentanyl.

Anorectic agents should complement and never replace a diet and exercise program. They have modest effects on total body weight (typically an additional 2–10 kg in obese adults), and responses vary considerably between individuals. Most weight loss from anorectic agents is achieved within the first 4–6 months of treatment; regain of weight is the norm unless drug therapy is maintained.

Drugs that limit nutrient absorption

Orlistat inhibits pancreatic lipase and thereby increases fecal losses of TG. It reduces body weight and total and LDL cholesterol levels and reduces the risk of type 2 diabetes in adults with impaired glucose tolerance. In obese adolescents, the combination of orlistat with lifestyle intervention reduced weight (–4.4 to +4.6 kg), BMI, total cholesterol, LDL, fasting insulin, and fasting glucose concentrations and increased insulin sensitivity during a 3-month trial period [74]. There was considerable variability in response. Variable reductions in body weight (–12.7 to +2.5 kg) and fat mass were also noted in a study of 11 morbidly obese children aged 7–12 years. Side-effects are tolerable as long as subjects reduce fat intake, but vitamin A, D, and E levels may decline, despite multivitamin supplementation. High study dropout rates (25% or more) suggest that long-term fat restriction is a problem for teenagers; dietary non-compliance results in flatulence and diarrhea that ultimately prove unacceptable.

Insulin suppressors and sensitizers

The synthesis and storage of TG in adipose tissue is stimulated by insulin. Thus, increases in nutrient-dependent insulin production and/or fasting hyperinsulinemia may contribute to fat storage and limit fat mobilization. By reducing fasting or post-prandial insulin concentrations, certain pharmacologic agents may prove beneficial in the treatment of obese children and adults.

Metformin is a bisubstituted, short-chain hydrophilic guanidine derivative that works through activation of AMP protein kinase [75]. Its major site of action is the liver: the drug increases hepatic glucose uptake, decreases gluconeogenesis, and reduces hepatic glucose production ([76,77] Table 20.2). Metformin increases insulin receptor binding but has variable and often only minor effects on peripheral insulin sensitivity; there is no effect on skeletal muscle glucose uptake or plasma adiponectin concentrations [76,77]. Major advantages of the drug include decreased food intake, weight loss, decreased fat stores (subcutaneous > visceral), and improved lipid profiles. Of even greater importance, long-term studies suggest that metformin reduces cardiovascular morbidity and mortality in diabetic adults [78].

Table 20.2 Metabolic effects of metformin and the thiazolidinediones (TZDs). HGP, hepatic glucose production; FFA, free fatty acids; TG, triglycerides; glu, glucose; SQ, subcutaneous; visc, visceral.

	Metformin	TZDs
Hepatic glucose uptake	Increased	Variable increase
HGP	Decreased	Variable decrease
Adipose glucose uptake	Variable/negligible	Increased (SQ > visc)
Lipolysis, FFA, and TG	Variable/negligible	Decreased
Fat stores	Decreased SQ > visc	Decreased visc, increased SQ
Adiponectin	No effect	Increased
Skeletal mm glucose uptake	No effect	Increased
Total insulin sensitivity	Variable	Increased
Food intake	Decreased	No effect
Body weight	Mild decrease	Mild–moderate increase
Glucose tolerance	Improved	Improved

Given that most children and adults with insulin resistance or IGT are obese, the effects of metformin on food intake, body weight, and plasma lipid concentrations are of particular interest. In obese adults with normal glucose tolerance, treatment with metformin reduced daily food intake, body weight, body fat, and plasma leptin concentrations, and downward trends were noted in LDL and total plasma cholesterol [79,80].

Metformin also has beneficial effects on body weight and intermediary metabolism in women with PCOS [81]. In trials lasting 6–12 months, metformin reduced BMI, waist circumference, plasma leptin concentrations, and stores of subcutaneous and visceral adipose tissue. Fasting glucose and C-peptide levels and glucose-stimulated insulin levels declined, as did serum testosterone and plasminogen activator-1 levels.

Non-obese women with PCOS, like their obese counterparts, are at increased risk of insulin resistance and type 2 diabetes. In normal weight (BMI ≤ 25), post-menarchal young (13.6–22 years old) women with ovarian hyperandrogenism, hyperinsulinemia, and normal glucose tolerance, metformin increased insulin sensitivity and reduced plasma insulin, TG, and testosterone levels and the ratio of LDL to HDL. The addition of flutamide, an androgen antagonist, potentiated the effects of metformin on TG, LDL/HDL, and adrenal androgen concentrations and markedly reduced hirsutism scores. The combination of metformin and flutamide reduced abdominal and total body fat mass and increased lean body mass despite reductions in plasma GH and IGF-1 levels [82–84].

There have been two randomized, double-blind, placebo-controlled studies of metformin in obese adolescents with insulin resistance, normal glucose tolerance, and a positive

family history of type 2 diabetes. In the first trial (n = 29), metformin reduced BMI z-score (3.6% relative to placebo controls), plasma leptin, and fasting glucose (–9.8 mg%) and insulin (–12 μU/mL), even in the absence of dietary intervention [85]. As increases in BMI and fasting glucose and insulin concentrations predict the development of type 2 diabetes in target populations [3,16,17], these findings suggested that metformin might prove useful in the prevention of glucose intolerance in high-risk adolescents.

In the second trial (n = 24), the combination of a low-calorie diet (1500–1800 calories/day for girls and boys respectively) and metformin reduced weight by 6.5%; diet alone caused a 3.8% weight loss [86]. Patients treated with metformin had greater decline in body fat (–6% vs. –2.7% in the placebo group), a decrease in plasma leptin levels, a 50% decrease in plasma insulin concentrations, and increased insulin sensitivity as determined by fasting and 2-h glucose and insulin levels. Plasma cholesterol and TG levels also declined by 22% and 39% respectively. These findings suggested that metformin and diet may act synergistically to limit weight gain and increase glucose tolerance in obese, insulin-resistant adolescents.

The recently completed Diabetes Prevention Program [65] established the efficacy of metformin in delaying or preventing the onset of type 2 diabetes in adults (aged ≥ 25 years) with IGT. A total of 3234 subjects randomly assigned to one of three interventions included a placebo group that received standard lifestyle recommendations, a metformin (850–1700 mg/day)-treated group that received standard lifestyle recommendations, and a group that received an intensive program of lifestyle modification. A fourth troglitazone-treated group was disbanded after the development of severe hepatotoxicity in a small number of subjects. The experimental groups were studied for 1.8–4.6 years.

Daily energy and fat intake decreased only in the group randomized to intensive lifestyle modification, but patients in the metformin group lost weight, although not as much as those in the intensive lifestyle group. In both groups, weight loss was most significant in the first 6–12 months of the study. The changes in body weight were accompanied by reductions in the rates of progression from IGT to type 2 diabetes. The 3-year cumulative incidence of diabetes was 28.9% in the placebo group, 21.7% in the metformin-treated group, and 14.4% in the intensive lifestyle group. Overall, therefore, intensive lifestyle intervention was more effective than metformin.

Certain subgroups responded preferentially to the various interventions. Metformin was as effective as lifestyle change in subjects with BMI exceeding 34.9 and in those with highest fasting glucose concentrations (Fig. 20.9); these subgroups are at greatest risk for progression to type 2 diabetes. Metformin was also as effective as lifestyle intervention in younger adults aged 25–44 years. On the other hand, treatment effects did not vary according to gender, race, or ethnic group.

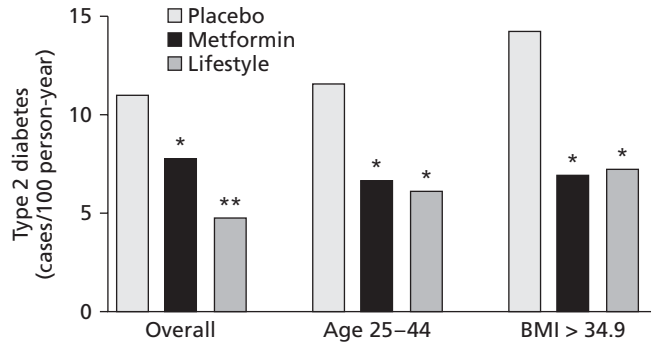

Fig. 20.9. Effects of metformin and intensive lifestyle intervention on rates of type 2 diabetes in adults with IGT. Data adapted from [65] with permission. * Differed significantly from placebo. ** Differed significantly from metformin or placebo.

In addition to reducing the risk of development of type 2 diabetes, intensive lifestyle intervention and metformin also had favorable but small effects on blood pressure and serum lipids. Systolic and diastolic blood pressures were reduced by 1.1 and 3.3 mmHg, respectively, in the metformin and intensive lifestyle groups, while total cholesterol, LDL, and TG concentrations declined by 5 and 8 mg%, 4.0 and 4.3 mg%, and 6 and 23 mg%, respectively, and HDL levels increased slightly (0.5 and 0.8 mg% respectively).

Metformin was well tolerated by the majority of subjects, although many patients had transient abdominal discomfort, which can be prevented by taking the medication with food. There were no instances of hepatic dysfunction or lactic acidosis; nevertheless, the drug should not be administered to patients with underlying cardiac, hepatic, renal, or gastrointestinal disease.

The major effects of lifestyle intervention and metformin were exerted within the first 12–18 months of the study. After the first year, fasting blood glucose concentrations, HbA1c concentrations, and rates of diabetes increased in both the intensive lifestyle and the metformin groups, and the slopes of the intervention and treatment curves after the first year appeared to parallel the slope of the placebo group. This suggests that the interventions may delay rather than prevent the development of type 2 diabetes. Nevertheless, studies performed in non-diabetic subjects 1–2 weeks after the trial's conclusion showed that the protective effect of metformin persisted in three-quarters of the drug-treated subjects, even after discontinuation of medication. The duration of this protective effect is unknown; prolonged treatment with metformin might be necessary to reduce the rate of progression to diabetes in high-risk subjects.

Thiazolidinediones (TZDs) regulate lipid and carbohydrate metabolism through binding to peroxisome proliferator-activated receptor (PPAR)-γ. When activated by TZDs, PPAR-γ heterodimerizes with the retinoid X receptor and

binds to the promoters of target genes including lipoprotein lipase, fatty acid transport protein, acetyl coA-synthase, and aP2 [87]. The major effects of the TZDs are exerted in adipose tissue and skeletal muscle ([19,87] Table 20.2). The drugs induce the differentiation of small insulin-sensitive adipocytes, increase adipose insulin receptor number, adiponectin expression, and adipose tissue glucose uptake, and reduce expression of TNF-α and, in mice, resistin. Rates of lipolysis and FFA release are reduced, TG clearance is enhanced, and hepatic VLDL synthesis decreases. Circulating TG levels decline during treatment with pioglitazone and, to a lesser extent, with rosiglitazone. With adipogenic and anti-lipolytic actions, the TZDs increase total body fat mass and body weight, but the ratio of lower body/SQ fat to upper body/visceral fat may rise [88]. TZDs potentiate the effects of insulin on skeletal muscle glucose uptake through induction of glucose transporters (GLUTs) 1 and 4 [89]. These actions require the presence of insulin and may be mediated by activation of phosphatidylinositol (PI) 3-kinase.

Glucose tolerance improves in type 2 diabetic patients treated with TZDs; plasma insulin concentrations decline while plasma adiponectin levels rise. The rise in adiponectin, in concert with direct effects of the TZDs on the vascular endothelium, reduces carotid intimal medial thickness and increases arterial distensibility. TZDs also reduce plasma C-reactive protein concentrations in diabetic patients and the percentage of small dense LDL levels in diabetic and non-diabetic hypertensive adults [87–89]. In theory, these changes reduce the risk of development and/or progression of atheromatous lesions.

In obese non-diabetic patients [90], troglitazone decreased insulin resistance and improved glucose tolerance. Rates of glucose disposal and the insulin sensitivity index increased, while glycemic responses to oral glucose declined. The mean fasting insulin concentrations decreased by 48%, and the plasma insulin responses to oral glucose and mixed meals decreased by 40% and 41% respectively. Other studies showed that TZDs reduced blood pressure in non-diabetic obese adults. In women with PCOS, a 3-month trial of troglitazone reduced fasting glucose and insulin concentrations and improved, but did not normalize, whole body insulin sensitivity. Troglitazone also improved endothelial function as measured by leg blood flow.

The results of these studies indicate that the TZDs increase insulin sensitivity, improve glucose tolerance, and reduce cardiovascular risk in insulin-resistant adults and in women with PCOS. That the TZDs, like metformin, can reduce the risk of type 2 diabetes in target populations was established in the TRIPOD study [91], a randomized, placebo-controlled, double-blind investigation of women with IGT. The experimental group consisted of Latino women with a history of gestational diabetes and IGT at the time of initiation of the study. During the 30-month investigation, annual diabetes incidence rates were 12.1% in the placebo group and 5.4% in the troglitazone group. Those with an increase in whole body insulin sensitivity following initiation of troglitazone were most likely to benefit.

Interestingly, protection from diabetes persisted for at least 3–8 months after the drug was discontinued. This finding suggested that troglitazone may have altered the natural progression of diabetes and not simply masked progression through a pharmacologic action. As with metformin, the duration of this protective action is currently unknown.

Unfortunately, troglitazone was removed from the commercial market because the drug caused fatal hepatic failure in a small number of subjects. Non-lethal hepatotoxicity has also been reported with other currently available TZDs, although at a far lower frequency than with troglitazone. Hepatic dysfunction must be excluded before TZD therapy is initiated, and liver function tests should be measured monthly for the first 6 months of treatment, every 2 months for the remainder of the first year, and at regular intervals thereafter. Other potential complications of TZD therapy include edema and anemia, so the drug should not be administered to patients with underlying cardiac disease.

Octreotide binds to the somatostatin-5 receptor and thereby impairs closure of the β-cell calcium channel, reducing glucose-dependent insulin secretion. In children with hypothalamic obesity, octreotide reduced insulin secretory responses and rates of weight gain [92]. Unfortunately, the cost of the medication, the need for parenteral administration, and the drug's side-effects, which include gastrointestinal distress, edema, gallstones, suppression of GH and TSH secretion, and cardiac dysfunction, limit its applicability to patients with intractable obesity from hypothalamic injury.

New approaches: metabolic inhibitors and cytokine therapy

Pharmacologic agents currently in use target the complications of insulin resistance indirectly. Animal studies suggest that approaches that target metabolic signaling and cytokine production directly may prove useful in the prevention of type 2 diabetes and cardiovascular disease.

Potential therapeutic candidates [93,94] include adiponectin, the cannabinoid-1 receptor antagonist rimonabant, satiety agents such as extendin 4 and dipeptidyl peptidase IV inhibitors, peptide YY, analogs of amylin, and drugs that modulate 11βHSD activity and glucocorticoid production in adipose tissue. All of these drugs have potentially beneficial effects but also have local and systemic effects that may limit their use, particularly in children.

Bariatric surgery

The long-term success of lifestyle intervention and pharmacotherapy in subjects with severe obesity has been disappoint-

ing. Marked weight loss is unusual and rarely sustained, and metabolic and vascular complications are common, although not universal. Bariatric surgery may be indicated in selected subjects with extreme obesity and serious co-morbidities. The approaches are most commonly laparoscopic gastric banding and the Roux-en-Y gastric bypass.

Laparoscopic adjustable gastric banding (LAGB)

Gastric banding utilizes a prosthetic band to encircle the proximal stomach. The ability to adjust band tension as stomach volume changes provides an important theoretical advantage. Results vary widely. Several small studies [95,96] support the safety and efficacy of LAGB in morbidly obese adolescents: in one study, LABG decreased mean BMI from 46.6 to 32.1 kg/m^2 after 23 months and improved co-morbidities. No patient experienced operative or late complications. In another study, LAGB reduced BMI in seven adolescents (12–19 years old) from 44.7 kg/m^2 to 30.2 kg/m^2 at 24 months.

Gastric banding is less effective than gastric bypass in reducing BMI or reversing co-morbidities. Weight regain is common, possibly because high caloric intake may be maintained through ingestion of concentrated sweet drinks or because the magnitude of decline in plasma concentrations of ghrelin, which stimulates food intake, is less than that in patients subjected to gastric bypass [97]. Gastric banding may cause esophageal dilation and achalasia and may exacerbate gastroesophageal reflux. Other potential complications include port-site malposition or malfunction, balloon rupture, and infection. The potential reversibility of the procedure makes gastric banding attractive in children.

Roux-en-Y gastric bypass (RYGB)

Gastric bypass involves the creation of a small stomach pouch into which a distal segment of jejunum is inserted. This procedure combines the restrictive nature of gastrectomy with the consequences of dumping physiology as a negative conditioning response when high-calorie liquid meals are ingested. Food intake may decline as stomach-derived ghrelin levels fall [97].

In adults with morbid obesity, gastric bypass often causes striking weight loss and may reverse type 2 diabetes, hypertension, dyslipidemia, pseudotumor cerebri, and degenerative joint disease [98]. Pilot studies [99,100] report favorable results of bariatric surgery in morbidly obese adolescents who failed to respond to lifestyle intervention and/or pharmacotherapy. Five years after gastric bypass, body weight had declined from approximately 235% of ideal body weight (IBW) to approximately 180% of IBW in obese adolescents, some of whom have Prader–Willi syndrome. Bariatric surgery was accompanied by marked weight loss (53.6 ± 25.6 kg) in 10 additional severely obese adolescents followed for periods ranging from 8 to 144 (mean 69) months [18]. Weight loss was associated with improvement of co-morbidities including sleep apnea, hypertension, gastroesophageal reflux, type 2 diabetes, and polycystic ovarian disease. Similar findings were noted in a retrospective study of 33 morbidly obese adolescents followed for up to 14 years following bariatric surgery.

Reported complications of RYGB include iron-deficiency anemia (50%), folate deficiency (30%), cholecystitis (20%), wound infections and dehiscence (10%), small bowel or stomach obstruction (5–10%), atelectasis and pneumonia (12%), and incisional hernia (10%). Prophylactic tracheostomy may be required to maintain airway patency and to correct preoperative hypercapnia. Other possible complications include leaks at the junction of stomach and small intestine requiring reanastomosis, acute gastric dilation, which may arise spontaneously or secondary to intestinal obstruction or narrowing of the stoma, pulmonary emboli, and dumping syndrome. Deficiencies of vitamin B12, iron, calcium, and thiamine are common. Mortality rates for RYGB range from 1–5%. Complication rates may be reduced if bariatric procedures are performed through laparoscopy by an experienced surgeon.

Balancing lifestyle intervention, pharmacotherapy, and surgery

All obese children and adolescents require lifestyle intervention. Intensive lifestyle intervention should benefit pediatric patients with IGT or IFG who are at high risk of developing type 2 diabetes and other metabolic complications, but lifestyle intervention remains difficult, time-consuming, and ineffective in many cases. Treatment failure may prolong or exacerbate insulin resistance, dyslipidemia, and glucose intolerance, leading to irreversible β-cell dysfunction, overt type 2 diabetes, and progressive cardiovascular disease.

Pharmacologic therapy should be considered for severely resistant or glucose-intolerant (IFG or IGT) children or adolescents who fail to respond to a 6–12 month trial of lifestyle intervention despite a "good faith effort." This means that the patient has attempted to follow a low saturated fat/low-calorie diet recommended by a dietary counselor and has increased his or her energy expenditure through regular exercise. "Unsuccessful" means that the elevations of fasting or post-prandial glucose persist or worsen despite lifestyle intervention. The decision to initiate drug therapy relieves neither the child nor the physician of the commitment to long-term lifestyle change; thus, diet and exercise regimens should be maintained, even if they had not proved effective in the absence of medication.

Metformin is the drug of choice for treating the obese child with severe insulin resistance, IFG, or IGT. Although lactic acidosis is extraordinarily rare in pediatric patients,

metformin should not be administered to children with underlying cardiac, hepatic, renal, or gastrointestinal disease. Obese subjects with mild elevations in hepatic enzymes (less than threefold higher than established norms) may receive the drug; indeed, some studies suggest that metformin may be useful in the treatment of hepatic steatosis. Concurrent use of a multivitamin seems reasonable because metformin increases urinary excretion of vitamins B1 and B6.

Given the lack of studies of TZDs in insulin-resistant children or adolescents, their potential, albeit rare, for severe hepatic complications and their tendency to cause weight gain, I limit the use of TZDs to adolescents who fail to respond to, or cannot tolerate, metformin. As the danger of hepatic dysfunction with combined therapy in pediatric patients is unknown, the TZDs should not be used in conjunction with metformin in non-diabetic children pending demonstration in long-term studies that the drug combination is safe. TZDs are contraindicated in patients with pre-existing hepatic or cardiac disease.

The use of anorectic drugs for treatment of obese children without glucose intolerance cannot be justified. Common side-effects include hypertension, tachycardia, cognitive dysfunction, and psychological distress, which outweigh any potential long-term benefits of the drugs. Inhibitors of nutrient absorption such as orlistat are not tolerated by many obese children but could be used in selected, highly motivated patients.

It is not possible to provide guidelines for the duration of pharmacologic intervention. A trial off medication may be warranted if glucose tolerance is normalized, particularly if there has been a decline in BMI *z*-score. If IGT persists despite compliance with the medical/pharmacologic regimen, it may be necessary to intensify lifestyle intervention and/or to increase the dose of medication. If glucose tolerance declines or the patient develops overt diabetes, it may be necessary to add insulin or another pharmacologic agent to the therapeutic regimen.

Bariatric surgery should be reserved for treatment of severely obese (BMI > 40), sexually mature adolescents who have failed other treatment approaches and who have established co-morbidities. Surgery should be performed only in medical centers that have considerable expertise in bariatric surgical techniques and longstanding experience in the evaluation and management of obese children. Bariatric surgery requires the support of a multidisciplinary team of specialists in pediatric endocrinology, gastroenterology and nutrition, cardiology, pulmonology, and orthopedics and must be reinforced by presurgical psychological evaluation and long-term psychological and nutritional counseling. Contraindications to bariatric surgery include substance abuse or psychiatric disabilities (including severe eating disorders) that prevent lifelong compliance with nutritional recommendations or medical surveillance.

A multifaceted approach to prevention of complications

The major causes of death in adults with IGT or type 2 diabetes are myocardial infarction and stroke. Although cardiovascular risk in patients with IGT and type 2 diabetes varies with glycemic control, other factors play equal or more important roles. These include obesity, hypertension, smoking, dyslipidemia, ethnic background, and family history. In theory, aggressive lifestyle intervention in children and adolescents should include reduction in BMI *z*-score and abolition of smoking. Pharmacologic therapy may be necessary to reduce blood pressure, control microalbuminuria, and treat dyslipidemia. A multifaceted approach that combined dietary counseling, statins, angiotensin-converting enzyme inhibitors, and low-dose aspirin reduced by 50–60% the long-term (8 year) risks of nephropathy, retinopathy, autonomic neuropathy, and cardiovascular endpoints (myocardial disease and stroke and amputation) in diabetic adults with microalbuminuria [101]. Such an approach may be necessary in the management of obese, insulin-resistant, and glucose-intolerant adolescents, who are commonly hypertensive and hyperlipidemic. The age and intensity of intervention may depend upon the family history of cardiovascular disease as well as the severity of problems in the individual teenager under the physician's care.

Social responsibility

Prevention of metabolic complications necessitates a collaboration between the medical and lay communities to address societal conditions that nurture and sustain childhood obesity and the development of type 2 diabetes. A major effort should be undertaken to eliminate soda and candy from school cafeterias and vending machines. School boards could solicit competitive bids for distributors of "healthy foods," reviewed by committees of local health experts, and could require price reductions for nutritious foods and milk. Schools could also require periods of mandatory exercise for school children of all ages, with facilities to make that exercise participatory and exciting. There should be expansion of community opportunities for exercise and energy expenditure, including development of bike and hiking trails, open and park space, pedestrian walkways, and public transportation. Smoking in public places should be minimized or banned. Federal and local public relations campaigns should publicize the risks of type 2 diabetes in minority populations and those with positive family histories, the hazards of excessive weight gain in childhood, and the benefits of daily exercise and of reducing consumption of concentrated sweets and high-fat foods.

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21 Diabetes mellitus

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Introduction

Diabetes mellitus refers to a number of metabolic diseases characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, blood vessels, and heart [1]. Several pathogenic processes are involved in the development of diabetes, but most cases in children and adolescents fall into one of two broad etiologic categories. Type 1 diabetes mellitus is the most common and is caused by deficiency of insulin secretion; type 2 is caused by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. Abnormalities in carbohydrate, fat, and protein metabolism characteristic of diabetes are attributable to deficiency of insulin action on target tissues.

A difficulty in the diagnosis is the lack of a unique marker that separates all people with diabetes mellitus from all non-diabetic individuals. Diabetic retinopathy best serves the purpose but becomes evident in most individuals with diabetes only years after onset of the disease. The lack of a marker has led to reliance on metabolic abnormalities associated with the disease, such as hyperglycemia measured by fasting plasma glucose (FPG) or 2-h post-prandial venous plasma glucose (PG) concentration, as the most useful diagnostic tests. The diagnostic levels of FPG and 2-h post-prandial PG are based on their association with the risk of having or developing retinopathy (Table 21.1). The disease process may not have progressed sufficiently to cause the sustained hyperglycemia necessary to fulfill the criteria for the diagnosis of diabetes mellitus, but it can cause lesser degrees of impaired glucose regulation, such as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). IGT and IFG are considered to be preclinical states of diabetes associated with increased risk of cardiovascular morbidity [2].

Definition and diagnosis of diabetes in children

Diabetes mellitus is diagnosed in one of three ways:
1 Classic symptoms of diabetes plus casual (defined as any time of day without regard to time since last meal) PG concentration ≥ 11.1 mmol/L (200 mg/dL);
2 Fasting (for at least 8 h) PG ≥ 7.0 mmol/L (126 mg/dL); or
3 2 h post load PG ≥ 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT).

In the absence of unequivocal hyperglycemia with acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day. The OGTT is not recommended for routine clinical use [3] but, when indicated, the test should be performed after at least 3 days of adequate (≥ 150 g per 1.73 m^2) carbohydrate consumption using a

Table 21.1. Biochemical criteria for diabetes mellitus and lesser degrees of impaired glucose regulation [2].

Test	Normal	Impaired fasting glucose (IFG)	Impaired glucose tolerance (IGT)	Diabetes mellitus
FPG mmol/L (mg/dL)	<5.6 (<100)	5.6–6.9 (100–125)		≥ 7.0 (126)
2-h PG mmol/L (mg/dL)	<7.8 (<140)		7.8–11.0 (140–199)	≥ 11.1 (200)
Casual PG mmol/L (mg/dL)	<11.1 (200)			≥ 11.1 (200)

glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water for individuals weighing > 43 kg and 1.75 g/kg for individuals weighing ≤ 43 kg.

Symptoms of hyperglycemia include polyuria, polydipsia, weight loss, sometimes polyphagia, and blurred vision. Chronic hyperglycemia commonly leads to perineal candidiasis in girls and in infants and toddlers of both genders. The diagnosis of type 1 diabetes is usually obvious because children present with classic symptoms that have been present for a few days to a few weeks, accompanied by marked hyperglycemia or with diabetic ketoacidosis (DKA).

These definitions are based on venous plasma glucose levels. Although portable glucose meters are useful for screening purposes in clinics and physicians' offices, the diagnosis of diabetes mellitus should be confirmed by measurement of venous plasma glucose on an analytic instrument in a clinical chemistry laboratory. Precautions should be taken to process the blood sample properly and to deliver it to the laboratory without delay to prevent glucose utilization by leukocytes that could lead to spuriously low PG levels.

Classification

Table 21.2 shows an etiologic classification of diabetes mellitus, and Table 21.3 shows the major clinical characteristics of the most common types in children and adolescents. Regardless of the cause, all forms are associated with long-term risk of microvascular complications. Type 2 diabetes in children and adolescence is a consequence of obesity (see Chapter 20).

Table 21.2. Etiologic classification of diabetes mellitus [3].

I. Type 1 diabetes
   A. Immune mediated
   B. Idiopathic
II. Type 2 diabetes
III. Other specific types
   A. Genetic defects of β-cell function
      MODY
      Mitochondrial DNA
   B. Genetic defects in insulin action
      Type A insulin resistance
      Leprechaunism
      Rabson–Mendenhall syndrome
      Lipoatrophic diabetes
   C. Diseases of the exocrine pancreas
      Cystic fibrosis
      Hemochromatosis
      Pancreatectomy
   D. Endocrinopathies
      Cushing syndrome
      Pheochromocytoma
      Hyperthyroidism
   E. Drug- or chemical-induced
      Glucocorticoids
      Diazoxide
      β-Adrenergic agonists
      Pentamidine
      Nicotinic acid
      α-Interferon
      Tacrolimus
   F. Infections
      Congenital rubella
      Cytomegalovirus
   G. Uncommon forms of immune-mediated diabetes
      "Stiff-man" syndrome
      Anti-insulin receptor antibodies
   H. Other genetic syndromes sometimes associated with diabetes
      Down syndrome
      Turner syndrome
      Klinefelter syndrome
      Wolfram syndrome
      Friedreich ataxia
      Prader–Willi syndrome
      Bardet–Biedl syndrome
      Myotonic dystrophy
IV. Gestational diabetes mellitus

Type 1 diabetes mellitus

Type 1A diabetes results from chronic progressive T-cell-mediated autoimmune destruction of the β cells of the pancreas, leading to severe insulin deficiency manifested by low or undetectable plasma levels of C-peptide. Markers of the process include a variety of islet cell autoantibodies, including autoantibodies to insulin (IAA), to glutamic acid decarboxylase (GAD65), and to the tyrosine phosphatases IA-2 and IA-2β. At least one of these is present in 85–98% of newly diagnosed children. The disease has strong HLA associations, with linkage to the major histocompatibility (MHC) class II genes DQA, DQB, and DRB. Specific HLA-DR/DQ alleles can either predispose to type 1A diabetes or be protective. The rate of β-cell destruction is variable, being especially rapid infants and young children and slower in adolescents and adults, some of whom may retain the ability to secrete insulin for several years [4] (Fig. 21.1). The disease occurs throughout life in genetically predisposed individuals, but is also related to environmental factors that are poorly understood. Type 1A diabetes predominantly affects Europoid Caucasians, is somewhat less frequent in African-Americans, and much less common in Asians and Native North Americans. The classical phenotype used to be that of a thin child with a history of polyuria, polydipsia, and weight loss but, with increasing prevalence of obesity in childhood, 20–25% of newly diagnosed type 1 diabetes patients are obese. Patients with type 1A diabetes are also prone to other autoimmune disorders.

Table 21.3. Characteristics of prevalent forms of primary diabetes mellitus in children and adolescents.

	Type 1A	Type 2	MODY	Atypical DM*
Prevalence	Common	Increasing	≤ 5% in Caucasians	≥ 10% in African-Americans
Age at onset	Throughout childhood	Pubertal	Pubertal	Pubertal
Onset	Acute severe	Insidious to severe	Gradual	Acute severe
Ketosis at onset	Common	≥ 1/3†	Rare	Common
Affected relative	5–15%	75–90%	100%	> 75%
Female:male	1:1	2:1	1:1	Variable
Inheritance	Polygenic	Polygenic	Autosomal dominant	Autosomal dominant
HLA-DR3/4	↑Association	No association	No association	No association
Ethnicity	All, Caucasians	All‡	Caucasian	African-American/Asian
Insulin secretion	Decreased/absent	Variable	Variably decreased	Decreased
Insulin sensitivity	Normal when controlled	Decreased	Normal	Normal
Insulin dependence	Permanent	Episodic	Infrequent	Variable
Obesity	No§	> 90%	Uncommon	Varies with population
Acanthosis nigricans	No	Common	No	No
Pancreatic autoantibodies	Yes¶	No	No	No

*Atypical diabetes mellitus (ADM), also referred to as Flatbush diabetes, type 1.5 diabetes, and idiopathic type 1 diabetes mellitus.
†Reported frequency of ketonuria or ketoacidosis at time of diagnosis of type 2 diabetes varies widely.
‡In North America, type 2 diabetes predominates in African-American, Mexican-American, Native American, Canadian First Nation children and adolescents, and is also more common in Asians and South Asians than in Caucasians.
§With increased prevalence of childhood obesity, 20–25% of newly diagnosed children with type 1 diabetes are obese.
¶Autoantibodies to insulin (IAA), islet cell cytoplasm (ICA), glutamic acid decarboxylase (GAD), or tyrosine phosphatase (insulinoma associated) antibody (IA-2 and IA-2β) at diagnosis in 85–98%.

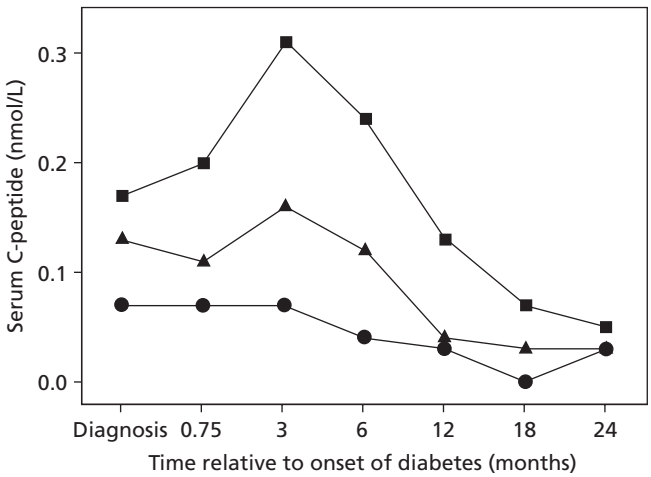

Fig. 21.1. Younger children lose endogenous insulin production more rapidly, as evidenced by plasma C-peptide levels from the time of diagnosis of type 1 diabetes. Data are stratified by age of onset: 5–14.9 years old (squares), 2–4.9 years old (triangles), and < 2 years old (circles). Toddlers have the lowest plasma C-peptide levels at diagnosis. The temporary partial remission experienced by older children is readily apparent and is notably absent in toddlers. Adapted from [4] with permission.

Type 1 diabetes with no known etiology, referred to as type 1B diabetes, accounts for a minority of cases. Although strongly inherited, there is no HLA association or evidence of β-cell autoimmunity.

Atypical diabetes mellitus

Atypical forms of diabetes mellitus have been described in various populations and have been referred to as Flatbush diabetes, atypical diabetes mellitus, idiopathic type 1 diabetes, and type 1.5 diabetes [5]. The hallmark of these atypical forms is a propensity to intermittent ketosis or ketoacidosis and no evidence of autoimmunity. They have been described primarily in individuals of African or Asian ancestry. The relation between them is unclear, and the etiology and genetics are unknown. Characteristics helpful in distinguishing the more common forms are shown in Table 21.3.

Genetic defects of insulin secretion

Maturity-onset diabetes of the young (MODY)

MODY is a form of non-insulin-dependent or "maturity-onset" type diabetes in children and young adults inherited in an autosomal-dominant pattern. MODY, which may account for 1–5% of all cases of diabetes in industrialized countries, is a heterogeneous group of disorders, the primary characteristics of which are shown below (Table 21.4).

Mild asymptomatic hyperglycemia in non-obese children, adolescents, and young adults who have a family history

Table 21.4. Characteristic features of MODY.

- Onset of diabetes usually before age 25–35 years and frequently in childhood or adolescence
- Absence of ketosis
- Absence of obesity
- Autosomal dominant mode of inheritance
- A primary defect in the function of pancreatic β-cells

of diabetes in successive generations is the most common clinical presentation. The diagnosis should be considered whenever three or more consecutive generations are affected by diabetes in an autosomal-dominant fashion. Pancreatic autoantibodies are typically absent. The diagnosis is usually made on clinical grounds. Confirmation of the diagnosis and identification of the specific type of MODY (Table 21.5) requires molecular genetic testing that is currently only available in research laboratories, with the exception of a commercial test (Esoterix, Inc., Calabasas Hills, CA, USA) for MODY3.

Some patients have mild fasting hyperglycemia, whereas others have varying degrees of glucose intolerance for several years before developing persistent fasting hyperglycemia. Because mild hyperglycemia may not cause classic symptoms, diagnosis may be delayed until adulthood. In some cases, progression to overt symptomatic hyperglycemia requiring therapy with an oral hypoglycemic agent or insulin may be rapid.

MODY is caused by mutations of genes expressed in the β-cell. Mutations in six genes have been described [6]. With the exception of MODY2, transcription factor mutations account for five of the six documented causes of classic MODY that have been described, predominantly in Caucasians. These transcription factors are involved in the regulation of insulin gene transcription and may also regulate pancreatic and/or islet or β-cell development [7]. All forms of MODY are the result of varying degrees of insulin deficiency with minimal or no defects in insulin action.

About 15–20% of European and up to 80% of Japanese patients with clinical MODY occur in families whose members have a clinical phenotype compatible with a diagnosis of MODY but do not have mutations in any of the six known MODY-related genes. These patients have been referred to as having MODY X. Additional MODY genes will undoubtedly be discovered to explain the molecular basis of diabetes in these individuals.

Treatment of MODY

Most cases of MODY do not require insulin but do require careful monitoring to insure good glycemic control to avoid complications. Exercise and nutrition to maintain normal weight and insulin sensitivity should be emphasized; pharmacologic treatment, when necessary, is tailored to the patient's specific type of diabetes and level of hyperglycemia.

Mitochondrial diabetes [8]

Diabetes may be the presenting manifestation of syndromes caused by mutations in mitochondrial DNA. *Maternally inherited diabetes and deafness syndrome* (MIDD, MIM#520000) may present in childhood or adulthood. The most common mutation occurs at position 3243 in the tRNA leucine gene, leading to an A-to-G transition. *Kearns–Sayre syndrome* (MIM #530000) is characterized by ophthalmoplegia, retinal pigmentary degeneration, and cardiomyopathy and may include several hormone deficiencies, including diabetes in approximately 13% of cases. Defects in mitochondrial metabolism result in abnormal ATP generation and defective glucose-induced insulin secretion. Diabetes can be treated initially with diet and sulfonylureas but may require insulin. Patients with impaired mitochondrial function are inherently prone to develop lactic acidosis and, therefore, metformin should not be used.

Other molecular disorders

Rare defects in prohormone convertase activity, inherited in an autosomal-dominant pattern, lead to impaired processing of proinsulin and mild glucose intolerance. A few

Table 21.5. Classification of MODY.

Type (MIM#)*	Gene	Frequency	Treatment
MODY1 (MIM#125850)	Hepatocyte nuclear factor-4α	Uncommon	Oral hypoglycemic agent, insulin
MODY2 (MIM#125851)	Glucokinase	Common	Diet and exercise
MODY3 (MIM#600496)	Hepatocyte nuclear factor-1α	Common	Oral hypoglycemic agent, insulin
MODY4 (MIM#606392)	Insulin promoter factor-1	Rare	Oral hypoglycemic agent, insulin
MODY5 (MIM#604284)	Hepatocyte nuclear factor-1β	Rare	Insulin
MODY6 (MIM#606394)	NeuroD1	Rare	Insulin

*MIM, Mendelian inheritance in man.

families have been identified who secrete mutant insulins with impaired ability to bind to the insulin receptor. Glucose metabolism may be normal or only mildly impaired in these individuals.

Impaired insulin sensitivity

Genetic defects of insulin signaling

Several rare insulin resistance syndromes are caused by genetic defects in the insulin receptor or its cellular signaling apparatus. *Leprechaunism* (MIM#246200) is the most severe of these syndromes. It presents at birth with low birthweight, characteristic facial features, nearly total lack of adipose tissue, acanthosis nigricans, and extreme insulin resistance. It is usually fatal in infancy.

Rabson–Mendenhall syndrome (MIM#262190) is characterized by extreme insulin resistance with acanthosis nigricans, abnormalities of the skeleton, teeth, and nails, growth retardation, genitomegaly, and pineal gland hyperplasia.

Type A insulin resistance syndrome presents in thin young women with extreme hyperinsulinism, acanthosis nigricans, glycosuria, hyperandrogenism with virilization, and polycystic ovary syndrome (PCOS).

Inherited lipoatrophic diabetes is associated with widespread loss of adipose tissue and severe insulin resistance. Hyperlipidemia, hepatomegaly, acanthosis nigricans, and elevated basal metabolic rate are common findings. Several forms of lipoatrophic diabetes are due to gene defects.

Seip–Berardinelli syndrome (MIM#269700) is inherited as an autosomal recessive and usually presents in the first year of life with lack of subcutaneous adipose tissue. Insulin resistance, acanthosis nigricans, and diabetes mellitus develop before adolescence.

Familial partial lipodystrophy, Dunnigan syndrome (MIM#151660) is autosomal dominant, as a result of mutation in the lamin A/C gene or peroxisome proliferator-activated receptor gamma gene and presents in adolescence with loss of subcutaneous adipose tissue from the trunk and extremities but with excess adipose tissue on the face and neck. Affected women are more likely to develop diabetes.

Acquired insulin resistance

Severe, generalized, acquired lipoatrophy may present during childhood. Diabetes ensues within a few years of the loss of adipose tissue. Some forms of acquired lipoatrophic diabetes are caused by immune-mediated destruction of adipocytes and are frequently associated with other autoimmune diseases. Some patients with HIV disease treated with protease inhibitors develop partial lipodystrophy. *Type B insulin resistance syndrome* is a rare cause of diabetes caused by circulating antibodies directed against the insulin receptor.

Diabetes as a component of specific genetic syndromes

Wolfram syndrome (MIM#222300) is also known as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness). Insulin-deficient diabetes mellitus is often the presenting characteristic, with a median age at onset of 6 years [9]. Most cases have an identifiable mutation of the Wolframin gene, which is typically inherited in an autosomal-recessive fashion.

Many other syndromes are associated with an increased risk of diabetes (see Table 21.2). Alstrom, Prader–Willi, and Bardet–Biedl syndromes combine severe obesity with insulin-resistant diabetes mellitus.

Secondary causes of diabetes

Cystic fibrosis-related diabetes (CFRD)

The life expectancy of patients with cystic fibrosis has increased dramatically over the past few decades, and CRFD has consequently become more common. Insulinopenia is caused by pancreatic destruction and amyloid deposition in the islets. Insulin resistance may be a prominent feature during exacerbations of pulmonary disease and causes deterioration in glycemia. First-phase insulin release is particularly affected, but ketoacidosis is rare. CFRD can present in the first decade but is usually seen in the second and third decades of life. The development of CFRD is associated with progressive clinical deterioration and increased mortality. Screening for glucose intolerance should begin at the age of 14 years, and hyperglycemia should be aggressively treated [10].

Insulin is the only therapy recommended for CFRD. It prevents protein catabolism, promotes weight gain, and improves pulmonary function. The ideal treatment is a basal-bolus regimen using insulin glargine and rapid-acting insulin. Diet should not be restricted, but patients should be taught carbohydrate counting and how to use rapid-acting insulin with meals. Destruction of the pancreatic α cells results in glucagon deficiency, and chronic use of glucocorticoids can cause adrenocortical insufficiency. Patients with CFRD are therefore at increased risk of severe hypoglycemia owing to malabsorption and impaired counter-regulatory responses.

Hemosiderosis

Frequent blood transfusions and chelation therapy have greatly improved the prognosis of thalassemia major. Adolescents and young adult patients are, however, at increased risk of developing diabetes mellitus because of the effects of iron overload on β-cell function and insulin sensitivity. Insulin is required for treatment.

Drug-induced diabetes

Various pharmacologic agents can cause hyperglycemia [11]. Glucocorticoids induce severe hepatic and peripheral insulin resistance and are potent hyperglycemic agents. Glucocorticoid-induced diabetes is relatively common in children who receive massive doses of glucocorticoids after organ transplantation as a component of chemotherapy for malignancy and in other circumstances in which glucocorticoids are used as anti-inflammatory or immunosuppressive agents. High doses of growth hormone are occasional causes of glucose intolerance or diabetes. Atypical antipsychotic agents increase the risk of diabetes mellitus probably by inducing insulin resistance, sometimes, but not always, associated with weight gain. Antiretroviral protease inhibitors increase the risk of diabetes mellitus associated with features of the metabolic syndrome and lipoatrophic changes. Beta-adrenergic agents, used for treatment of acute asthma, are common causes of transient hyperglycemia. Diazoxide decreases insulin secretion by direct action on the K_{ATP}/Kir6.2 potassium channel involved in the regulation of insulin secretion. Pediatric transplant recipients are especially prone to insulin-requiring diabetes when treated with the calcineurin inhibitor tacrolimus (FK506). Cyclosporin A has also been reported to have toxic effects on the β cell. L-Asparaginase often causes transient insulin-requiring diabetes.

Neonatal diabetes mellitus

The prevalence of insulin-requiring hyperglycemia presenting within the first 4–6 weeks of life is estimated to be 1 in 400 000–500 000 births [12]. Associated findings include intrauterine growth retardation, low birthweight and decreased adipose tissue. Markers of autoimmunity and insulin resistance are typically absent. Various birth defects may be present in about half the cases. A number of cases of transient neonatal diabetes have had macroglossia and umbilical hernia. Abnormalities of the region at 6q24 on chromosome 6 are found in about 50% of cases of transient neonatal diabetes. More than half the cases of neonatal diabetes are transient and resolve within months, but mild non-insulin-dependent diabetes may recur in childhood or early adulthood in a substantial proportion of patients. Long-term surveillance is necessary.

Abnormalities of the thyroid, pancreas, liver, heart, kidney, and skeleton have been variably associated with some cases of permanent neonatal diabetes. Homozygous mutations in MODY genes, glucokinase (MODY2), and IPF-1/PDX1 (MODY4) account for some cases of permanent neonatal diabetes mellitus.

This may also result from heterozygous activating mutations in the gene encoding the Kir6.2 subunit of the ATP-sensitive potassium channel (KCNJ11), in which case there may also be developmental delay, muscular weakness, and epilepsy [13]. Sulfonylureas may be effective in some cases [14]. Two other rare genetic causes of permanent diabetes occasionally present within the first month of life. The IPEX (immunodysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome is characterized by intractable diarrhea, diabetes, and autoimmunity and is often fatal. The Wolcott–Rallison syndrome is characterized by multiple epiphyseal dysplasia and early-onset diabetes.

Epidemiology of type 1 diabetes mellitus

Diabetes mellitus is one of the most common chronic diseases of childhood. The incidence of type 1 diabetes varies widely between geographic regions, with age-adjusted rates as high as 37/100 000 per year in Sardinia and Finland and as low as 0.1/100 000 per year in areas of China and Venezuela [15] (Fig. 21.2). A significant portion of this variation is attributable to differences in the prevalence of protective HLA-DQ alleles among populations. The incidence of type 1 diabetes in children ≤ 14 years of age is similar in the UK and the USA, 15–24 and 12–18 per 100 000 per year respectively [15]. Incidence varies among ethnic subgroups within a given geographical area. Some migrant populations retain their original risk of type 1 diabetes, while others show a trend toward acquiring the diabetes risk of their new location. Secular increases in the incidence of pediatric type 1 diabetes during the twentieth century have been documented in North America and western Europe at rates higher than can

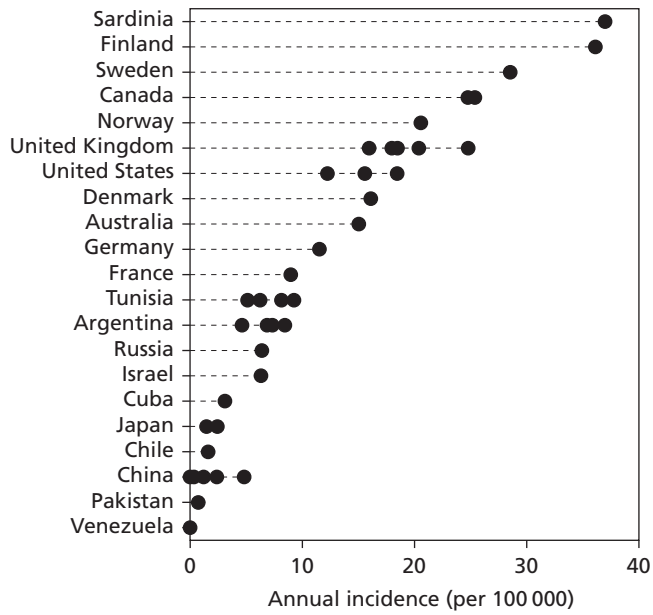

Fig. 21.2. Variations in the incidence of type 1 diabetes in children ≤ 14 years of age among regions of selected countries from six continents. Rates were measured in the early 1990s. Multiple symbols indicate rates measured in different regions within that country. Data from the DiaMond study [13].

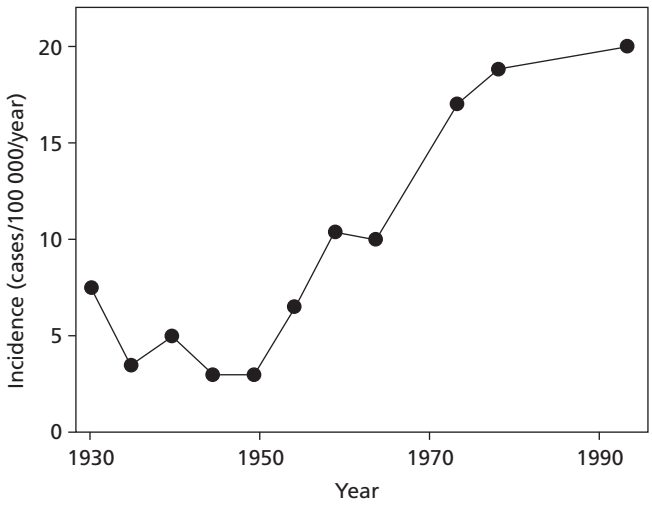

Fig. 21.3. Secular increases in the incidence of childhood type 1 diabetes, as measured among Norwegian children less than 10 years old. Similar trends have been documented in the USA and in many countries in Europe. Adapted from [16] with permission.

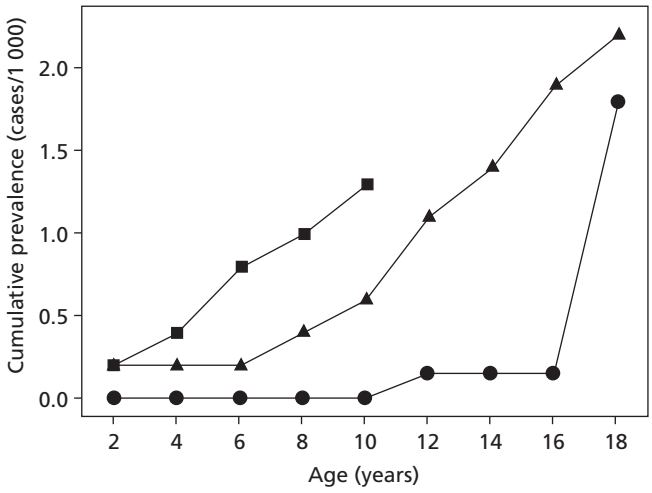

Fig. 21.4. Shifts toward younger age of onset of type 1 diabetes in three UK birth cohorts born in 1946 (circles), 1958 (triangles), and 1970 (squares). Adapted from [16] with permission.

be explained by genetic shifts (Fig. 21.3) [16]. While the increasing incidence of childhood diabetes may be slowing in western countries, it is now being detected in other locations, including several eastern European countries. There has also been an alarming trend toward younger age of onset. Diabetes is no longer uncommon in toddlers and preschool-aged children [16] (Fig. 21.4).

Etiology, genetics, and family risk of type 1A diabetes

Type 1A diabetes mellitus occurs in genetically susceptible individuals as a consequence of chronic T-cell-mediated destruction of β cells of the islets of Langerhans [17]. Autoantibodies against β-cell antigens are detected in 85–98% of newly diagnosed children. Insulitis, characterized by lymphocytic infiltration of the islets of Langerhans, is observed in children who died soon after the onset of type 1 diabetes. There is strong linkage to the MHC locus. Variation in the MHC locus accounts for about half the genetic risk of type 1A diabetes. Increased susceptibility to type 1A diabetes is conferred by certain MHC alleles, such as DRB1*0401/2/5-DQA1*0301-DQB1*0302 (HLA-DR4-DQ8) and DRB1*0301-DQA1*0501-DQB1*0201 (HLA-DR3-DQ2) [18]. Numerous non-MHC genetic loci contribute weakly to type 1A diabetes risk. Conversely, decreased susceptibility to type 1A diabetes is conferred by several MHC alleles, including DRB1*1501-DQA1*0102-DQB1*0602 (HLA-DR2-DQ6) [18].

Approximately 85% of new cases of type 1A diabetes occur in persons without an affected first-degree relative [18]. The risk to siblings of an affected child is approximately 6%. The risk to the child of a parent with type 1A diabetes depends on whether the mother or the father has diabetes and is 1.3–4% or 6–9% respectively. Concordance rates for monozygotic twins are 21–70% and 0–13% for dizygotic twins. These data indicate that both genetic and environmental factors contribute to the pathogenesis of type 1A diabetes. The environmental factor(s) are not known, and there are no interventions that reduce the risk. Possible candidate environmental factors include viral infections, dietary factors, hygiene, and toxins [19]. It is likely that environmental induction of type 1 diabetes relates to the chronology of exposure and interactions with genetic susceptibility.

Prediction and prevention of type 1 diabetes

The onset of diabetes represents the end of an insidious, progressive, immune-mediated attack on β cells, and clinical diabetes occurs when approximately 90% of β cells have been damaged or destroyed. Risk of developing diabetes, both in relatives of a person with type 1 diabetes and in the general population, can be accurately assessed by HLA genotyping and immunologic markers (autoantibodies directed against islet constituents) combined with tests of β-cell function [20]. Autoantibodies restricted to a single antigen have little prognostic value, but an immune response that has spread to multiple antigens and is stable over time is highly predictive of disease. Individuals who have multiple islet autoantibodies are destined to develop immune-mediated diabetes. The latency period between the detection of antibodies and the clinical onset of disease may extend over a period of several years and offers an opportunity to intervene. Parenteral insulin, oral insulin, and nicotinamide have failed to arrest or retard the diabetes disease process. As effective preventive intervention does not exist, it is controversial

whether screening should be performed outside the context of clinical studies.

At the onset of symptoms, about 10% of β cells are viable, and there is good evidence that residual β-cell function has clinical benefit. The Diabetes Complications and Control Trial (DCCT) identified a "virtuous circle" in which residual insulin secretion resulted in better glucose control with less hypoglycemia, slower progression to vascular complications, and prolonged β-cell function [21]. Immune intervention at the time of diagnosis is another route to β-cell rescue, and trials with continuous cyclosporin A administration have demonstrated this. However, the benefit disappeared when treatment was stopped, and the side-effects of cyclosporin A do not justify its long-term use. Other approaches, such as administration of anti-CD3 monoclonal antibody to modulate the T-cell attack on β cells and possibly to induce immune tolerance, are being investigated.

Type 1A diabetes mellitus and other autoimmune diseases

Individuals with type 1A diabetes are at increased risk for several other autoimmune diseases. There are also several autoimmune syndromes whose phenotype includes type 1 diabetes.

Autoimmune thyroid disorders are common in patients with type 1 diabetes [22]. Approximately 22% of patients have thyroid autoantibodies, but the reported prevalence of thyroid dysfunction varies widely, and hypothyroidism can be expected to develop in 6–14% of pediatric patients with type 1 diabetes. Even subclinical hypothyroidism may increase the risk of hypoglycemia. Asymptomatic individuals should be screened annually for thyroid dysfunction with a sensitive thyroid-stimulating hormone (TSH) assay. Alternatively, some endocrinologists determine thyroid autoantibodies and measure serum TSH concentration only in those with autoantibodies [23].

In western Europe, North America, and Australia, the mean prevalence of celiac disease (gluten-induced enteropathy) among children and adults with type 1 diabetes is 4.1% (0–10.4%). Screening studies with anti-endomysial or tissue transglutaminase antibodies show that 3.7–9.9% (mean 7.4%) of children with type 1 diabetes have a positive screening test, and the majority of these have a positive biopsy. Most cases detected by serologic screening (and with biopsy confirmation) of celiac disease have silent disease with villous atrophy only or a subclinical form of the disease with subtle manifestations that may be recognized only in retrospect. It has been suggested that all children with type 1 diabetes should be screened for celiac disease, but the benefits and risks of screening children with diabetes for celiac disease have not been systematically assessed [24]. If screening is not performed routinely, clinicians should consider the possibility of celiac disease in patients with unexplained poor growth, poor glycemic control, diarrhea, abdominal pain, or recurrent hypoglycemia. Screening is performed by measurement of either anti-endomysial or tissue transglutaminase antibodies, but this should be combined with measurement of the serum IgA level to rule out IgA deficiency, which can cause a false-negative serologic result.

Anti-21-hydroxylase antibodies occur in 1.6–2.3% of individuals with type 1 diabetes, but only 1 in 200–300 progresses to develop clinical adrenocortical insufficiency [23]. The risk increases to 1 in 30 in patients with two autoimmune processes (e.g. diabetes and thyroiditis). The development of adrenocortical insufficiency in type 1 diabetes is characterized by recurrent unexplained hypoglycemia and decreasing insulin requirements.

Polyautoimmune disorders associated with type 1 diabetes

Autoiummune polyendocrine syndrome type 1 (APS-1, MIM#-240300), also known as autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) syndrome, is a rare autosomal-recessive disorder caused by mutation of the autoimmune regulator (AIRE-1) gene. At least two of three major criteria, primary hypoparathyroidism, Addison disease, and chronic mucocutaneous candidiasis, are required for the diagnosis. Additional autoimmune features may appear over the course of a patient's life, including autoimmune thyroiditis, type 1A diabetes, vitiligo, autoimmune hepatitis, alopecia, ovarian failure, and hypophysitis.

Immune dysregulation, *polyendocrinopathy*, *enteropathy*, *X-linked syndrome* (IPEX,MIM#304790, caused by mutations in FOXP3, is a rare X-linked recessive disorder characterized by early-onset type 1A diabetes and severe atopy, chronic immune-mediated diarrhea, failure to thrive, thyroiditis, eosinophilia, and hemolytic anemia.

Autoiummune polyendocrine syndrome type 2 (*APS-2*): like type 1A diabetes, genetic susceptibility to APS-2 appears to be conferred by high-risk HLA alleles. Unlike APS-1, it is not a monogenic disorder. Diagnosis of APS-2 requires at least two of three major criteria: Addison disease, autoimmune thyroid disease, and type 1A diabetes. Associated findings may include celiac disease, autoimmune hepatitis, gonadal failure, vitiligo, alopecia, pernicious anemia, and myasthenia gravis.

Presentation of type 1 diabetes mellitus

Children with newly diagnosed type 1 diabetes usually present with classic symptoms for a few days to several weeks. The frequency of diabetic ketoacidosis (DKA) at diabetes onset varies widely by geographic location, ranging from

15% to 67% in Europe and North America and probably more commonly in developing countries. It is more frequent in infants, toddlers, and preschool-aged children, in children who do not have a first-degree relative with type 1 diabetes, and in children whose families are of lower socioeconomic status [25].

The progression of type 1 diabetes tends to follow a characteristic clinical course with an abrupt onset of symptoms over a period of days to a few weeks that disappear after starting insulin replacement therapy. This is often followed by a temporary remission ("honeymoon phase") with partial recovery of endogenous insulin secretion, demonstrable by plasma C-peptide levels (Fig. 21.1). Recurrence or persistence of the autoimmune attack on β cells, however, leads to further β-cell destruction and progressive decline in insulin production until it eventually ceases completely.

Distinguishing between type 1 and type 2 diabetes in children

Both types of diabetes present most frequently during puberty, when a physiologic reduction in insulin sensitivity of approximately 30% occurs. With the current high prevalence of overweight and obesity in children and adolescents, distinguishing the types of diabetes has become more difficult. Some 24% of patients with type 1 diabetes are obese at diagnosis of type 1 diabetes, irrespective of race, gender, and age of onset [26]. In contrast to type 2 diabetes in adults, in which ketonuria is unusual, 33% of adolescents with type 2 diabetes have ketosis at presentation, and up to 25% present in DKA. Insulin requirements typically decrease after several weeks of treatment of type 2 diabetes, which may resemble the remission or "honeymoon" period of type 1 diabetes. Measuring pancreatic autoantibodies and serum insulin and C-peptide levels at the time of diagnosis is recommended to help to distinguish type 1 and type 2 in obese patients. A plasma C-peptide level in the upper portion of the normal range or exceeding the upper limit of normal suggests type 2 diabetes. Plasma C-peptide levels may, however, be temporarily low initially in type 2 diabetes owing to glucotoxicity and lipotoxicity.

Differential diagnosis

Other causes of glycosuria

Diabetes mellitus is occasionally diagnosed in an asymptomatic individual because glycosuria is discovered incidentally. The diagnosis must always be confirmed by at least two independent measurements of plasma glucose concentration. Glycosuria can occur without hyperglycemia because of renal tubular dysfunction (e.g. Fanconi–Bickel syndrome, Fanconi syndrome) or because of an isolated reduction of the renal tubular threshold for glucose reabsorption (benign glycosuria). This disorder is diagnosed by performing an OGTT with simultaneous measurements of plasma and urine glucose concentrations. Glycosuria will be evident with plasma glucose concentrations in the normal range. Hepatic glycogen synthase deficiency (glycogen storage disease type 0) is an uncommon cause of intermittent post-prandial hyperglycemia and glycosuria in children.

Transient hyperglycemia

The incidence of transient hyperglycemia is estimated to be approximately 1 per 8000 pediatric office visits and 1 per 200 emergency department or hospital visits [27]. A minority of children with transient hyperglycemia will develop diabetes mellitus. When transient hyperglycemia is detected in a child who does not have a severe illness, the risk of developing diabetes is much higher than if a severe illness were present. The presence of pancreatic autoantibodies and/or a low first-phase insulin response during an intravenous glucose tolerance test strongly predicts progression to diabetes.

Management of diabetes mellitus

Initial management of newly diagnosed type 1 diabetes mellitus

Whenever possible, the child with DKA should be cared for in a facility that has nursing staff trained in DKA management and access to a clinical chemistry laboratory that can provide frequent and timely measurement of serum chemistries. Children with signs of severe DKA (long duration of symptoms, compromised circulation, depressed level of consciousness) and those who are at increased risk of cerebral edema (< 5 years of age, new-onset diabetes) should be treated in a pediatric intensive care unit or in a children's ward that specializes in diabetes care and can provide comparable resources and supervision of care [25].

The initial goals of management depend on the clinical presentation and are to restore fluid and electrolyte balance, to stabilize the metabolic state with insulin, and to provide basic diabetes education and self-care training for the child (if age and developmentally appropriate) and other caregivers (parents, grandparents, older siblings, daycare providers, and babysitters).

The diagnosis of diabetes in a child is a crisis for the family, who require considerable emotional support and time for adjustment and healing. Shocked, grieving, and overwhelmed parents typically require at least 2–3 days to acquire basic or "survival" skills while they are coping with the emotional upheaval that typically follows the diagnosis of diabetes in a child. Even if they are not acutely ill, children with newly diagnosed type 1 diabetes are usually admitted

to hospital for metabolic stabilization, diabetes education, and self-management training, but outpatient or home-based management has been preferred at some centers with the appropriate resources. Outpatient education and stabilization offers several advantages, which include avoiding the stress of a hospital stay. The outpatient setting or patient's home is a more natural learning environment for the child and family and possibly reduces the cost of care. The sparse literature comparing initial hospitalization with home-based and/or outpatient management of children who are not acutely ill with newly diagnosed type 1 diabetes has been reviewed recently, and the results are inconclusive, but the data suggest that outpatient and/or home initial management of type 1 diabetes in children does not lead to any disadvantages in terms of metabolic control, acute complications, hospitalizations, psychosocial, or behavioral variables. The decision concerning whether or not a child with newly diagnosed diabetes should be admitted to hospital depends on several factors, including the severity of the child's metabolic derangements, a psychosocial assessment of the family, and the resources available at the treatment center. Outpatient management in a comprehensive day-treatment center staffed by a multidisciplinary diabetes team is an appropriate alternative to hospitalization for many newly diagnosed children.

Psychosocial issues [28]

A medical social worker should perform an initial psychosocial assessment of all newly diagnosed patients to identify families who need additional services. Thereafter, patients are referred to the mental health specialist when emotional, social, environmental, or financial concerns are suspected or identified that might interfere with the ability to maintain diabetes control. Some of the more common problems in families that have a child with diabetes include parental guilt resulting in poor adherence to the treatment regimen, difficulty coping with the child's rebellion against treatment, anxiety, depression, fear of hypoglycemia, missed appointments, financial hardship, loss of health insurance affecting the ability to attend scheduled clinic appointments, and/or purchase supplies. Recurrent ketoacidosis is the extreme indicator of psychosocial stress, and management of such patients is incomplete without comprehensive psychosocial assessment.

The treatment of pediatric diabetes is complicated by factors inherent to childhood. Because childhood is characterized by cognitive and emotional immaturity, the involvement of responsible adults is essential to the treatment of pediatric diabetes. Diabetes treatment thus exists within a family dynamic, and treatment-related conflicts are not uncommon, arising in part as a result of natural discord in goals between caretakers and/or the child. Each phase of childhood has characteristics that complicate treatment, such as the unpredictable eating of toddlers and the unscheduled intense physical play of school-aged children that can hinge on irregular factors such as the weather. Adolescence is characterized by multiple physiologic and psychosocial factors that make glycemic control more difficult. Optimal diabetes treatment should thus be tailored to each child and family, based on factors including age, gender, family resources, cognitive faculties, the schedule and activities of the child/family, and the goals and desires of the child and family.

Outpatient diabetes care

The diabetes team

Optimal care of children with type 1 diabetes is complex and time-consuming. Few primary care practitioners or pediatricians have the resources and expertise nor can they devote the time required to provide all the components of an optimal treatment program for children with diabetes. Children with diabetes should be managed by a multidisciplinary diabetes team that provides diabetes education and care in collaboration with the child's primary care physician [29]. The team should consist of a pediatric endocrinologist or pediatrician with training in diabetes, a pediatric diabetes nurse educator, a dietitian, and a mental health professional, either a clinical psychologist or a social worker. A member of the diabetes team should always be available by telephone to respond to metabolic crises that require immediate intervention and to provide guidance and support to parents and patients.

Initial diabetes education

The diabetes education curriculum should be adapted to the individual child and family. Parents and children with newly diagnosed diabetes are anxious and overwhelmed and cannot assimilate a large amount of abstract information. Therefore, the education program should be staged. Initial educational goals should be limited to essential survival skills so that the child can be safely cared for at home and return to his or her daily routine. Initial education and self-management training should include understanding what causes diabetes, how it is treated, how to administer insulin, basic meal planning, self-monitoring of blood glucose and ketones, recognition and treatment of hypoglycemia, and how and when to contact a member of the diabetes team.

Continuing diabetes education and long-term supervision of diabetes care

When the child is medically stable and parents and other care providers have mastered survival skills, the child is discharged from the hospital or ambulatory treatment center. In the first few weeks after diagnosis, frequent telephone contact provides emotional support and helps parents to interpret the results of blood glucose monitoring and adjust

insulin doses if necessary. Within a few weeks of diagnosis, many children enter partial remission evidenced by normal or near normal blood glucose levels on a low dose (<0.25 units/kg/day) of insulin. By this time, most patients and parents are less anxious, have mastered basic diabetes management skills through experience and repetition, and are more prepared to begin to learn the intricate details of intensive diabetes management. At this stage, the diabetes team should begin to provide patients and parents with the knowledge and skills they need to maintain optimal glycemic control while coping with the challenges imposed by exercise, fickle appetite and varying food intake, intercurrent illnesses, and the other variations that normally occur in a child's daily life.

In addition to teaching facts and practical skills, the education program should promote desirable attitudes to health in the young person who has a chronic incurable disease. For some children, this may be best accomplished in a non-traditional educational setting such as summer camp for children with diabetes. The educational curriculum must be concordant with the child's level of cognitive development and has to be adapted to the learning style and intellectual ability of the individual child and family. Parents, grandparents, older siblings, school nurse, and other important people in the child's life are encouraged to participate in the diabetes education program so they can share in the diabetes care and help the child to live a normal life.

In the first month after diagnosis, the patient is seen frequently by the diabetes team to review and consolidate the diabetes education and practical skills acquired in the first few days and to extend the scope of diabetes self-care training. Thereafter, follow-up visits with members of the diabetes team should occur at least every 3 months. Regular clinic visits are to insure that the child's diabetes is being appropriately managed at home and the goals of therapy are being met. A focused history should obtain information about self-care behaviors, the child's daily routines, the frequency, severity, and circumstances surrounding hypoglycemic events, and blood glucose monitoring data should be reviewed. At each visit, height and weight are measured and plotted on a growth chart. The weight curve is especially helpful in assessing adequacy of therapy. Significant weight loss usually indicates that the prescribed dose is insufficient or the patient is not receiving the prescribed doses of insulin. A physical examination should be performed at least twice each year focusing on blood pressure, stage of puberty, evidence of thyroid disease, mobility of the joints in the hands, scarring of the finger tips from frequent lancing, and injection sites for lipohypertrophy or lipoatrophy.

Regular clinic visits also provide an opportunity to review, reinforce, and expand upon the diabetes self-care training begun at the time of diagnosis. The goal at each visit is to reinforce the goals of treatment while increasing the patient's and family's understanding of diabetes management, the interplay of insulin, food, and exercise, and their impact on blood glucose levels. As cognitive development progresses, the child should become more involved in diabetes management and assume increasing age-appropriate responsibility for daily self-care. Parents are encouraged to call for advice if the pattern of blood glucose levels changes between routine visits suggesting the need to adjust the insulin dose or change the regimen. Eventually, when parents and patients have sufficient knowledge and experience, they are encouraged to adjust the insulin dose(s) independently.

Goals of therapy

The DCCT [30,31] and a similar study in Sweden, the Stockholm Diabetes Intervention Study [32], ended the debate about whether the microvascular complications of diabetes are caused by hyperglycemia and can be prevented or ameliorated. Additional evidence for the importance of glycemic control was provided by the UK Prospective Diabetes Study (UKPDS) in adults with type 2 diabetes [33,34]. These trials demonstrated unequivocally the importance of lowering glycated hemoglobin (HbA1c) values to reduce the risk of development and progression of retino pathy, nephropathy, and neuropathy. Treatment regimens that reduce average HbA1c to ≈ 7% (about 1% above the upper limit of normal) are associated with fewer long-term microvascular complications. Moreover, a period of improved glycemic control is associated with a sustained decreased rate of development of diabetic complications [35,36].

The aim of diabetes management is to achieve recommended glycemic targets known to reduce the risk of long-term complications. Treatment goals for adolescents ≥ 13 years of age and for adults with diabetes mellitus are HbA1c < 7% (non-diabetic range 4–6%), preprandial plasma glucose 5–7.2 mmol/L (90–130 mg/dL), and peak post-prandial plasma glucose < 10 mmol/L (180 mg/dL). As there are no clinical trial data available for children < 13 years of age, clinical judgment is required to determine goals appropriate for children of various ages [37].

Management of young children with diabetes, especially those less than 5 years old, must balance opposing risks of hypoglycemia and future vascular complications. The relative contribution of the prepubertal years to the development of microvascular complications has been uncertain. It was commonly believed that prepubertal children were protected from the adverse effects of hyperglycemia on the microvasculature, and it was suggested that the contribution of the prepubertal years of diabetes to long-term prognosis may be minimal. Recent evidence, however, indicates that longer prepubertal duration of diabetes increases the risk of retinopathy and, possibly, microalbuminuria in adolescence and young adulthood, but at a slower rate than the postpubertal years [38].

Table 21.6. Biochemical goals of treatment according to age.

Age (years)	Premeal blood glucose mmol/L (mg/dL)	HbA1c (%)
< 5	5.5–11 (100–200)	≤ 9.0
5–12	4–10 (70–180)	≤ 8.0
≥ 13	4–8 (70–150)	≤ 7.0

Biochemical goals of treatment for children and adolescents were included in the consensus guidelines of The International Society for Pediatric and Adolescent Diabetes (ISPAD). Ideal control is evidenced by HbA1c < 6.05%, optimal < 7.5%, suboptimal 7.6–9.0%, and action is required when the value exceeds 9.0% [29]. The ISPAD guidelines are accompanied by the statement: "for each individual the target should be the lowest achievable HbA1c without the occurrence of frequent or severe hypoglycemia."

The risk of microalbuminuria increases steeply with HbA1c > 8% [39]. Based on these considerations, HbA1c of ≤ 8.0% is a reasonable general goal for children with diabetes but, because of the concern about hypoglycemia in preschool-aged children, we recommend stratifying the biochemical goals based on age (Table 21.6). Biochemical goals should be individualized taking into account both medical and psychosocial considerations. These targets are ideal and should be pursued, provided the child does not experience recurrent or severe hypoglycemia. Less stringent treatment goals are appropriate for preschool-age, children with developmental handicaps, psychosocial problems, lack of appropriate family support, children who have experienced severe hypoglycemia, or those with hypoglycemia unawareness.

Studies performed in the post-DCCT era show that metabolic control of type 1 diabetes in children and adolescents continues to be unsatisfactory. For example, the mean HbA1c of 2873 children from 22 pediatric diabetes centers in 18 countries was 8.6 ± 1.7% (mean ± SD) (equivalent to a mean of 8.3% using the DCCT method) [40] and did not change significantly when re-examined 3 years later despite significant increases in insulin dose and number of daily insulin injections [41] (Fig. 21.5). Mean HbA1c in a nationwide cross-sectional study of 2579 French children with type 1 diabetes was 8.97 ± 1.98% (mean ± SD), of whom 33% had an HbA1c of ≤ 8% [42]. Likewise, a nationwide study of 1755 Scottish children with type 1 diabetes found an average HbA1c of 9.1% [43], and a single diabetes center study of 300 children with type 1 diabetes (aged 7–16 years) in the USA found a mean HbA1c of 8.7 ± 1.2% at baseline and 8.9 ± 1.5 % 1 year later [44].

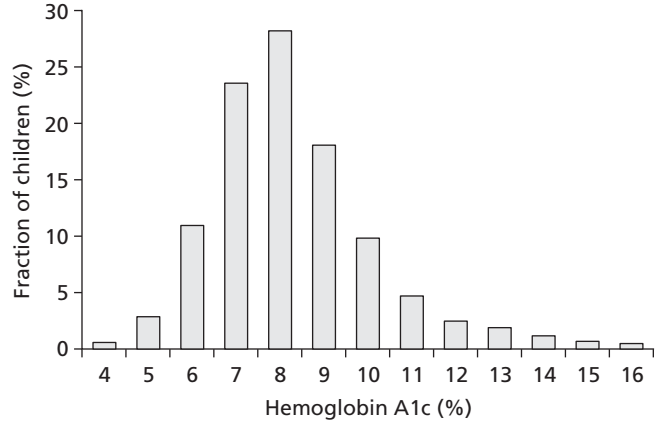

Fig. 21.5. Distribution of hemoglobin A1c in 2873 children and adolescents with type 1 diabetes from 18 countries. Data from [40].

Insulin therapy

Most children with type 1 diabetes are severely insulin deficient and depend on insulin replacement for survival. The aim is to simulate as closely as possible the normal variations in plasma insulin levels that occur in non-diabetic individuals, but physiologic replacement of insulin remains elusive. Practical considerations, including supervision of care, ability and willingness to self-administer insulin several times each day, and difficulty maintaining long-term adherence, make physiologic replacement of insulin challenging. There is no universal insulin regimen that can be used for all children with type 1 diabetes. The diabetes team has to design an insulin regimen that meets the needs of the individual patient and is acceptable to the patient and/or family member(s) responsible for administering insulin to the child or supervising its administration.

The initial route of insulin administration is determined by the severity of the child's condition at presentation. Insulin is preferably given intravenously for treatment of DKA. Children who are metabolically stable without vomiting or significant ketosis may be started with subcutaneous (SC) insulin administration. SC insulin treatment in the newly diagnosed child who has recently recovered from DKA is usually started with either a two or three injections per day regimen consisting of a mixture of human intermediate-acting and rapid- or short-acting insulin (Table 21.7). Some clinicians start basal-bolus insulin therapy at the time of diagnosis, regardless of the severity of presentation or age of the child.

In addition to the severity of metabolic decompensation, the child's age, weight, and pubertal status guide the initial insulin dose selection. When diabetes has been diagnosed early, before significant metabolic decompensation, 0.25–0.5 unit/kg/day is usually an adequate starting dose. When metabolic decompensation is more severe (e.g. ketonuria

Table 21.7. Insulin regimens used to treat children and adolescents.

No. of daily doses	Breakfast	Lunch	Dinner	Bedtime
Two	S/R + N/L*		S/R + N/L	
	S/R + N/L		S/R + UL	
	S/R + UL		S/R + UL	
Three	S/R + N/L		S/R	S/R + N/L
	S/R + N/L	S/R	S/R + N/L	
	S/R + N/L	S/R	S/R + UL	
	S/R + UL	S/R	S/R + UL	
	S/R + N/L		S/R	Glarg†
Four	S/R	S/R	S/R	S/R + N/L
	S/R + N/L	S/R	S/R	S/R + N/L
	S/R	S/R	S/R	S/R + Glarg
	S/R + Glarg	S/R	S/R	S/R
	S/R	S/R	S/R + Glarg	S/R
CSII‡	S/R	S/R	S/R	S/R

S, short-acting insulin (insulin lispro or insulin aspart); R, regular (soluble) insulin; N, neutral protamine Hagedorn (isophane); L, lente (insulin zinc suspension); UL, Ultralente (extended insulin zinc suspension); Glarg, insulin glargine.

*Premixed combinations such as either 70% NPH and 30% regular or 70% neutral protamine aspart (NPA) and 30% insulin aspart, or 75% neutral protamine lispro (NPL) and 25% insulin lispro are usually used in twice-daily fixed dose insulin regimens.

†Insulin glargine is always given as a separate injection and must not be mixed with any other insulin.

‡CSII, continuous subcutaneous insulin infusion (pump), boluses are given with meals and snacks together with basal insulin throughout the day and night. Intensified insulin therapy is defined as the use of at least three daily doses of insulin or CSII.

without acidosis or dehydration), the initial dose is typically at least 0.5 unit/kg/day. After recovery from DKA, prepubertal children usually require at least 0.75 unit/kg/day, whereas adolescents require at least 1 unit/kg/day. In the first few days of insulin therapy, while the focus of care is on diabetes education and emotional support, it is reasonable to aim for premeal blood glucose levels in the range 4.5–11 mmol/L (80–200 mg/dL) and to supplement, if necessary, with 0.05–0.1 unit/kg rapid- or short-acting insulin SC at 3- to 4-h intervals.

Three major categories of insulin preparations, classified according to time course of action, are available (Table 21.8). Various insulin replacement regimens, consisting of a mixture of short- or rapid-acting insulin and an intermediate- or long-acting insulin, are used in children and adolescents (Table 21.7), typically given two to four (or more) times daily. Clear superiority of any one regimen in terms of metabolic outcomes has not been demonstrated [45]. All regimens provide basal insulin throughout the day and night and more insulin with meals and snacks. When a two-dose regimen is used, the total daily dose is typically divided so that about two-thirds is given before breakfast and one-third is given in the evening. With a three-dose regimen, short- or rapid-acting insulin is administered before supper, and the second dose of intermediate-acting insulin is given at bedtime rather than before the evening meal. The initial ratio of rapid- to intermediate-acting insulin at both times is approximately 1:2. Toddlers and young children typically require a smaller fraction of short- or rapid-acting insulin (10–20% of the total dose) and proportionately more intermediate-acting insulin. Regular insulin is given at least 30 min before eating; rapid-acting insulin (lispro insulin, insulin aspart) is given 5–15 min before eating.

The optimal ratio of rapid- or short-acting to intermediate-acting insulin for each patient is determined by the results of frequent blood glucose measurements. At least five daily measurements are required initially to determine the effects of each component of the insulin regimen. The blood glucose concentration is measured before each meal, before the bedtime snack, and once between midnight and 04.00 h. Parents are taught to look for patterns of hyperglycemia or hypoglycemia that indicate the need for an adjustment in the dose. Adjustments are made to individual components of the insulin regimen, usually in 5–10% increments or decrements, in response to patterns of consistently elevated (above the target range for several consecutive days) or unexplained low blood glucose levels respectively. This is referred to as pattern adjustment. The insulin dose is adjusted until satisfactory blood glucose control is achieved with most blood glucose values in or close to the individual child's target range.

At the time of diagnosis, most children have some residual β cells and, within several days to a few weeks, often enter a period of partial remission ("honeymoon"), during which normal or nearly normal glycemic control is relatively easily achieved with a low dose of insulin. At this stage, the dose

Table 21.8. Insulin preparations classified according to their pharmacodynamic profiles.

	Onset of action (h)	Peak action (h)	Duration of action (h)
Rapid-acting			
Insulin lispro*	0.25–0.5	0.5–2.5	≤ 5
Insulin aspart*	< 0.25	1–3	3–5
Short-acting			
Regular (soluble)	0.5–1	2–4	5–8
Intermediate-acting			
NPH (isophane)	1–2	2–8	14–24
Lente (insulin zinc suspension)	1–2	3–10	20–24
Long-acting			
Ultralente	0.5–3	4–20	20–36
Insulin glargine*	2–4	Peakless	20–24
Premixed combinations			
50% NPH, 50% regular	0.5–1	Dual	14–24
70% NPH, 30% regular	0.5–1	Dual	14–24
70% NPA, 30% aspart*	< 0.25	Dual	14–24
75% NPL, 25% lispro*	< 0.25	Dual	14–24

*Insulin analog developed by modifying the amino acid sequence of the human insulin molecule. Data are from the manufacturers. Pharmacodynamic effects of lispro insulin and insulin aspart appear to be equivalent [93]. NPA, neutral protamine aspart; NPL, neutral protamine lispro. Both NPA and NPL are stable premixed combinations of intermediate- and short-acting insulins.

Most of the human insulins and insulin analogs are available in insulin cartridges and/or disposable insulin pens.

These data are for human insulins and are approximations from studies in adult test subjects. Time action profiles are reasonable estimates only. The kinetics of NPH insulin may be more rapid in children [94]. The times of onset, peak, and duration of action vary within and between patients and are affected by many factors, including dose, site and depth of injection, dilution, exercise, temperature.

of insulin should be reduced to prevent hypoglycemia but should not be discontinued. As destruction of the remaining β cells occurs, the insulin dose increases ("intensification phase"), eventually reaching a full replacement dose. The average daily insulin dose in prepubertal children with long-standing diabetes is approximately 0.8 unit/kg/day and in adolescents about 1–1.5 unit/kg/day.

Technical details of insulin therapy in young children

Caring for young children with diabetes is challenging for many reasons, one of which is the need accurately and reproducibly to measure and inject tiny doses of insulin that is supplied in a concentration of 100 units/ml (U100 insulin). To administer a dose of 1 unit requires the ability accurately to measure 10 μL (1/100 mL) of insulin. When the dose is less than 2 U of U100 insulin, neither parents of children with diabetes nor skilled pediatric nurses are able to measure the dose accurately. Furthermore, a dose change of 0.25 U translates into a volume difference of 2.5 μL in a 300 μL (3/10 cc or 30 unit) syringe. When parents attempt to measure insulin doses in increments of 0.25 U of insulin (e.g. 3.0, 3.25, 3.5 U) using a standard commercial 30 unit (300 μL) syringe, they consistently measure more than the prescribed amount. Therefore, to enhance accuracy and reproducibility of small doses, insulin should be diluted to U10 (10 units/mL) with the specific diluent available from the insulin manufacturers. Using U10 insulin, each line ("unit") on a syringe is actually 0.1 U of insulin.

To avoid intramuscular injections in infants and young children with little subcutaneous fat, syringes with 30-gauge 8 mm (short) needles or insulin pens with 31-gauge 5 mm needles should be used to administer insulin. Short needles are also desirable for use in older thin children.

Intensified insulin therapy in children

There is little evidence to guide clinical decisions concerning the risk–benefit ratio of strict control in the preadolescent patient. Clinical trials comparable to the DCCT have not been conducted in prepubertal children; nevertheless, many experts believe that it is reasonable to extrapolate that prepubertal children will also benefit from strict control of their diabetes.

Limitations of twice-daily split-and-mixed insulin regimens

Beyond the remission period, it is not generally possible to achieve near normal glycemia with two injections per day without incurring a greater risk of hypoglycemia, especially during the overnight period. A major problem with the

two-dose "split-and-mixed" regimen (rapid-acting or short-acting insulin combined with intermediate-acting insulin administered before breakfast and before the evening meal, see Table 21.7) is that the peak effect of the predinner intermediate-acting insulin tends to occur at the time of lowest insulin requirement (midnight to 04.00 h), increasing the risk of nocturnal hypoglycemia. Thereafter, insulin action declines from 04.00 h to 08.00 h, when the basal insulin requirement normally increases. Consequently, the tendency for blood glucose levels to rise before breakfast (dawn phenomenon) may be aggravated by waning insulin effect before breakfast and/or by counter-regulatory hormones secreted in response to a fall in blood glucose levels during sleep, post-hypoglycemic hyperglycemia (Somogyi phenomenon). A three-dose insulin regimen with mixed short- or rapid- and intermediate-acting insulins before breakfast, only short- or rapid-acting insulin before dinner, and intermediate-acting insulin at bedtime may significantly reduce these problems (see Table 21.7 and Fig. 21.6) [46]. Intensive insulin regimens that employ intermediate-acting insulin (Fig. 21.5) demand consistency in the daily meal schedule, amounts of food consumed at each meal, and the timing of insulin injections.

Basal-bolus regimens and continuous subcutaneous insulin infusion

Insulin therapy with at least three injections each day or with continuous subcutaneous insulin infusion (CSII) using a portable insulin pump can more closely simulate normal diurnal insulin profiles, overcome many of the limitations inherent in a two-dose regimen, and permit greater flexibility with respect to timing and content of meals. A peakless long-acting insulin, insulin glargine, can be used to provide basal insulin and is used together with short- or rapid-acting insulin injected before each meal (basal-bolus regimen, Fig. 21.6b). Insulin glargine is an insulin analog, produced by recombinant DNA technology, with an approximately 24-h duration of action. It has little peak activity and is typically administered once daily, usually but not invariably at bedtime. It should be injected at about the same time each day, whereas short- or rapid-acting insulin is injected separately before each meal, whenever it is eaten. Insulin glargine has been used safely in children and adolescents [47] and, because it does not have the peak of activity characteristic of NPH, Lente, and Ultralente insulins [48], can reduce nocturnal hypoglycemic episodes without jeopardizing glycemic control [49,50].

In 1996, fewer than 5% of patients starting pump therapy were < 20 years of age. Over the past several years, there has been a worldwide marked increase in the number of children and adolescents using CSII (pump) therapy. An insulin pump has one unique advantage over insulin injections – the ability to program changes in basal dosage to meet an anticipated increase or decrease in need (Fig. 21.6c). This feature

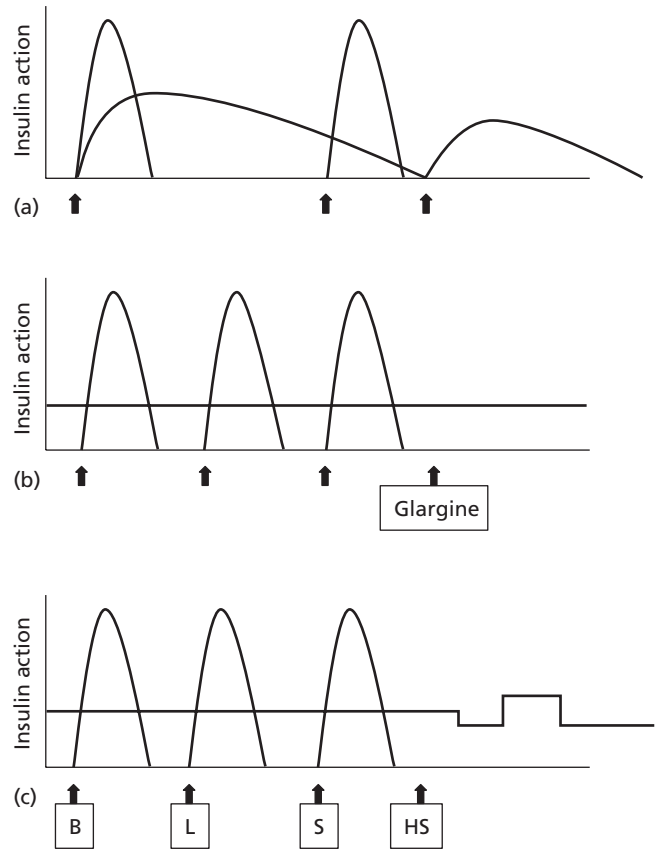

Fig. 21.6. (a) Schematic representation of idealized insulin action provided by a regimen consisting of a mixture of rapid-acting insulin (lispro or aspart) and intermediate-acting insulin (NPH or Lente) before breakfast, rapid-acting insulin (lispro or aspart) before supper, and intermediate-acting insulin (NPH or Lente) at bedtime. (b) Schematic representation of idealized insulin action provided by an insulin regimen consisting of four daily injections: rapid-acting insulin (lispro or aspart) before each meal (B, L, S) and a separate injection of insulin glargine, either at bedtime (as shown here) or at dinner or breakfast. (c) Schematic representation of idealized insulin effect provided by continuous subcutaneous insulin infusion via an insulin pump with insulin aspart or lispro. In this figure, alternative basal rates are illustrated; insulin delivery is shown to decrease from midnight to 03.00 h and to increase before breakfast. B, breakfast; L, lunch; S, supper; HS, bedtime. Arrows indicate times of insulin injection or boluses before meals.

can be advantageous in combating the dawn phenomenon or preventing hypoglycemia during or after exercise. In addition to programming various basal rates, the use of dual-wave and square-wave bolus delivery significantly lowers 4-h post-prandial blood glucose levels. A meta-analysis of randomized controlled clinical trials concluded that CSII resulted in a small (≈ 0.5%) improvement in HbA1c [51].

Although an insulin pump is a complex and sophisticated medical device that requires extensive training in its use, with appropriate education and training and with support from parents and a school nurse, many children can manage the

added responsibility of using an insulin pump and benefit from its advantages [52]. Only short- or rapid-acting insulin is used with CSII; therefore, any interruption in the delivery of insulin rapidly leads to metabolic decompensation. To reduce this risk, meticulous care must be devoted to the infusion system, and blood glucose levels must be measured frequently. Increased lifestyle flexibility, reduced blood glucose variability, improved glycemic control, and reduced frequency of severe hypoglycemia are all documented advantages of CSII. The diabetes team should attempt to select patients who are most likely to benefit from CSII. Success requires motivation to achieve normal blood glucose levels, frequent blood glucose monitoring, record keeping, carbohydrate counting, and frequent contact with the diabetes team. Patients must understand that, to be successful, CSII therapy requires more time, effort, and active involvement in diabetes care by patients and parents and considerable education and support from the diabetes team. The individual who is unable to master a multiple-dose injection regimen is not likely to be successful with CSII. Despite concerns that it might have adverse psychosocial consequences owing to the added burden of treatment, especially in adolescents, the opposite effect has been observed. Short-term studies have shown that more aggressive and successful management of their diabetes by teenagers can be accompanied by enhanced psychosocial well-being. In teenagers, CSII offers a treatment option that can lead to improved control and lower the risk of severe hypoglycemia.

Considerations during puberty

Owing to physiologic peripheral insulin resistance of puberty [53], adolescents require large doses of rapid- or short-acting insulin to control post-prandial blood glucose excursions. However, a large increase in the dose of regular insulin markedly delays its peak effect (to 3–4 h) and prolongs its total duration of action to 6–8 h. Puberty does not cause hepatic insulin resistance; therefore, hyperinsulinemia suppresses hepatic glucose production for several hours and increases the risk of post-prandial hypoglycemia, especially at night between 22.00 h and 02.00 h. This is an important reason to use rapid-acting insulin analogs (insulin lispro or insulin aspart) in preference to regular (soluble) insulin in treating adolescents, most especially before the evening meal.

Technological innovations have provided patients with insulin preparations with pharmacokinetic properties that make it possible crudely to simulate physiologic insulin kinetics. It is now possible for children safely to achieve unprecedented levels of glycemic control without excessive severe hypoglycemia. The diabetes care provider should discuss treatment options with parents and child and explain the advantages and disadvantages of each in attempting to meet the overall goals of treatment. The most suitable regimen for a given child and family should be determined by mutual consent and not by coercion.

Table 21.9. The goals of medical nutrition therapy of children and adolescents.

- To provide adequate macro- and micronutrients to ensure normal growth and physical development
- To integrate insulin regimens into usual eating and physical activity habits
- To provide self-management education for prevention and treatment of hypoglycemia, acute illness, and exercise-related blood glucose perturbations
- To attain and maintain optimal metabolic outcomes, including near to normal blood glucose levels without excessive hypoglycemia and a lipid and lipoprotein profile that reduces the risk for macrovascular disease
- To prevent and treat the chronic complications of diabetes and modify nutrient intake and lifestyle as appropriate for prevention and treatment of obesity, dyslipidemia, hypertension, and nephropathy
- To improve general health through healthful food choices and physical activity
- To address individual nutritional needs taking into consideration personal and cultural preferences and lifestyle while respecting the individual's wishes and willingness to change

Medical nutrition therapy (MNT) [54]

Meal planning continues to be a cornerstone of the management of all types of diabetes mellitus, and nutrition education is an essential component of a comprehensive program of diabetes education for patients and their families [37]. There is no "diabetic diet" *per se*. Nutrition therapy should be individualized, with consideration given to the patient's usual eating habits and other lifestyle factors. Monitoring clinical and metabolic parameters including blood glucose, HbA1c, lipids, blood pressure, and body weight, as well as quality of life, is crucial to insure successful outcomes. Modern diabetes management, combining frequent self-monitoring of blood glucose with intensive insulin therapy and mastery of carbohydrate counting, enables children and adolescents to have considerable dietary flexibility while maintaining glycemic control in the target range.

There is no evidence that the nutritional needs of children with diabetes differ from those of otherwise healthy children. Therefore, nutrient recommendations are based on the requirements of healthy children and adolescents. The total intake of energy must be sufficient to balance the daily expenditure of energy and has to be adjusted periodically to achieve an ideal body weight and to maintain a normal rate of physical growth and maturation. The objective of MNT for patients with type 2 diabetes, most of whom are obese, is to lose weight and then maintain a desirable weight without compromising statural growth and to achieve target blood glucose and HbA1c goals (Table 21.9) [55].

Carbohydrate

Some 60–70% of total energy should be from carbohydrate and monounsaturated fat [56]. Dietary dogma had been to avoid simple sugars and replace them with complex carbohydrates. This belief was based on the assumption that simple sugars are more rapidly digested and absorbed than starches and would aggravate hyperglycemia to a greater degree. The glycemic index (GI), proposed in 1981 as an alternative system for classifying carbohydrate-containing foods, measures the glycemic response after ingestion of carbohydrate. GI is defined as the incremental area under the plasma glucose response curve after consumption of a standard amount of carbohydrate from a test food relative to that of a control food, either white bread or glucose. The glycemic and hormonal responses to a large number of carbohydrates have been systematically examined and their GIs defined. There is a wide spectrum of biologic responses to different complex and simple carbohydrates with so much overlap that they cannot be simply classified into two distinct groups. Even a single food produces a substantially different glycemic response when prepared in different ways. The physical structure and form of a carbohydrate-containing food, in addition to its chemical composition, influences postprandial glycemia by altering its rate of digestion and absorption. Fruits and milk cause a lower glycemic response than most starches, and sucrose causes a glycemic response similar to that of bread, rice, and potatoes. In general, most refined starchy foods eaten in the USA have a high GI, whereas non-starchy vegetables, fruits, and legumes tend to have a low GI.

The usefulness of low-GI diets in individuals with type 1 diabetes continues to be controversial, and studies in children are extremely limited. The literature demonstrates only modest long-term beneficial effects of low-GI diets on blood glucose and lipid concentrations and, at the present time, data are lacking concerning the long-term benefits of low-GI diets in the management of diabetes in children.

The glycemic load of meals and snacks is more important than the source or type of carbohydrate. The glycemic load, defined as the weighted average of the GI of individual foods multiplied by the percentage of dietary energy as carbohydrate, has been proposed as a method to characterize the impact of foods and dietary patterns with different macronutrient composition on glycemic responses. For example, a carrot has a high GI but a low glycemic load, whereas a potato has both a high GI and a high glycemic load. Individuals who use intensive insulin therapy select their premeal insulin doses based on the carbohydrate content of their meals, whereas individuals who receive fixed daily insulin dosages should attempt to maintain day-to-day consistency with respect to the carbohydrate content of their meals and snacks. Although the use of low-GI foods may reduce post-prandial glycemic excursions and may have long-term benefit on HbA1c levels, emphasis should be on the total amount of carbohydrate consumed, and its source should be a secondary consideration [54].

Sucrose as part of the meal plan does not adversely affect blood glucose control in individuals with either type 1 or type 2 diabetes. Sucrose and sucrose-containing foods may be substituted for other carbohydrates. The nutrient content of sucrose-containing foods, as well as the presence of other nutrients frequently ingested with sucrose, such as fat, must be taken into consideration.

Fructose is present as the free monosaccharide in many fruits, vegetables, and honey. About one-third of dietary fructose comes from fruits, vegetables, and other natural sources in the diet, and about two-thirds comes from food and beverages to which fructose has been added. Fructose is absorbed more slowly from the intestinal tract than glucose, sucrose, or maltose and is converted to glucose and glycogen in the liver. Post-prandial plasma glucose levels are reduced when an isocaloric amount of fructose replaces sucrose or starch in the diets of people with diabetes. Fructose has been used in children in amounts up to 0.5 g/kg/day, but the potential benefit is tempered by concern that fructose may have adverse effects on serum lipids, especially low-density lipoprotein (LDL) cholesterol. Consumption of large amounts of fructose [15–20% of daily energy intake (90th centile of usual intake)] increases fasting total and LDL cholesterol in subjects with diabetes and fasting total and LDL cholesterol and triglycerides in non-diabetic subjects. Because of the potential adverse effect of large amounts of fructose on serum lipids, fructose may have no overall advantage over other nutritive sweeteners. There is no reason to avoid naturally occurring sources of fructose.

Carbohydrate counting and exchange lists

Carbohydrate counting is a meal planning method in which the amount of carbohydrate or number of carbohydrate servings eaten at each meal and snack are counted. Carbohydrate is the main nutrient in starches, fruits, milk, and sugar-containing foods and has the greatest effect on blood glucose levels. Therefore, it is the most important macronutrient to control in order to maintain optimal glycemic control. Using exchange lists, one starch choice is considered to be equivalent to either one fruit or milk choice; each contains approximately 15 g of carbohydrate and is equal to one "carbohydrate choice." The "nutrition facts" on food labels list the portion size and total amount of carbohydrate measured in grams per serving. Carbohydrate counting allows flexibility in food choices and minimizes "cheating," as all foods can be included in the meal plan. Table 21.10 shows an example of a patient's daily meal plan incorporating both exchange servings and grams of carbohydrate.

Fiber, which refers to the portion of a plant that is indigestible, markedly influences the digestion, absorption, and

Table 21.10. An example of a patient's daily food allowance distributed among the six food groups.

Group	Exchanges	Carbohydrate (g)	Protein (g)	Fat (g)
Starch	8	120	24	
Fruit	4	60		
Milk	3 low fat (1%)	36	24	9
Vegetables	1	5	2	
Meat	6 medium fat		42	30
Fat	4			20
Grams		221	92	59
Calories (%)		884 (50)	368 (20)	531 (30)

metabolism of many nutrients. Inclusion of plant fiber in the diet may benefit patients with diabetes by diminishing post-prandial glycemia, and certain soluble plant fibers significantly reduce serum cholesterol concentrations and decrease fasting serum triglyceride levels in patients with diabetes who have hypertriglyceridemia. Dietary fiber guidelines for children with diabetes are the same as for non-diabetic children and can be readily achieved by increasing the consumption of minimally processed foods, such as grains, legumes, fruits, and vegetables.

Protein requirements are not increased when diabetes is well controlled with insulin, and children with diabetes should follow the recommended daily allowance guidelines. Physiologic requirements are determined by the amount of protein necessary to sustain normal growth, which is based on ideal weight-for-height and varies with age, being highest in infancy and early childhood. Protein intake should be 0.9–2.2 g/kg body weight per day and constitutes 15–20% of the total daily intake of energy, the same as for non-diabetic children and adolescents. The consumption of saturated fat can be reduced by eating less red meat, whole milk, and high-fat dairy foods and by eating more poultry, fish, and vegetable proteins and drinking more low-fat milk.

A carbohydrate meal that also has a high content of saturated fat significantly increases and prolongs the glycemic effect of the meal and requires anticipatory adjustment of the dose of insulin to combat the effect. Excessive saturated fat, cholesterol, and total energy lead to increased blood levels of cholesterol and triglycerides. Because hyperlipidemia is a major determinant of atherosclerosis and patients with type 1 diabetes eventually develop atherosclerosis and its sequelae, the meal plan should attempt to mitigate this risk factor. Children and adolescents with well-controlled type 1 diabetes are not at high risk of dyslipidemia but should be screened and monitored according to recommended guidelines [57]. Screening should commence with the onset of puberty and, thereafter, should be repeated every 5 years. If there is a family history of hypercholesterolemia or premature atherosclerosis, screening should commence at an earlier age. If the child or adolescent is growing and developing normally and has normal plasma lipid levels, less than 10% of energy should come from saturated fat, the daily intake of cholesterol should be less than 300 mg/day, and consumption of transunsaturated fatty acids should be minimized. Total dietary fat should be reduced in the obese child to reduce total energy consumption. The National Cholesterol Education Program (NCEP) Step II diet guidelines should be implemented in the patient with elevated LDL cholesterol [> 2.6 mmol/L (100 mg/dL)]. Total fat should constitute ≤ 30% of total calories, < 7% of calories from saturated fat, and dietary cholesterol is limited to 200 mg/day. Pharmacologic treatment with a bile acid sequestrant or statin is recommended when LDL cholesterol exceeds 4.1 mmol/L (159 mg/dL) and should be considered when LDL cholesterol is > 3.3 mmol/L (129 mg/dL).

MNT education and formulation of the meal plan

Newly diagnosed children usually present with weight loss. Therefore, the initial meal plan includes an estimation of energy requirements to restore and then maintain an appropriate body weight and allow for normal growth and development. Energy requirements vary with age, height, weight, stage of puberty, and level of physical activity. Because the energy needs of growing children change continuously, the meal plan should be re-evaluated at least every 6 months in young children and annually in adolescents.

MNT begins with an assessment by a dietitian, heeding the ethnic, religious, and economic factors pertaining to the individual patient and family. The meal plan must take account of the child's school schedule, early or late lunches, physical education classes, after-school physical activity, and differences in a child's activities on weekdays compared with weekends and holidays. Young children typically have three meals and two or three snacks daily, depending on the interval between meals, age of the child, and level of physical activity. Although their daily energy intake is relatively constant over time, young children adjust their energy intake at successive meals. The highly variable food consumption from meal-to-meal typical of normal young children is especially challenging when the child has type 1 diabetes. Rapid-acting insulin may be administered after the meal, based on estimation of the actual amount of carbohydrate consumed, and diminishes parental anxiety. The purpose of snacks is to

prevent hypoglycemia and hunger between meals. Patients who use a basal-bolus insulin regimen or insulin pump therapy may not require snacks. Data from pre- and post-prandial blood glucose monitoring and individualized insulin-to-carbohydrate ratios are used to select insulin doses to match anticipated carbohydrate intake.

The dietitian's role is to evaluate the patient's and family's knowledge and understanding of nutrition and to formulate an individualized meal plan. Even intensive insulin replacement regimens are not successful without careful attention to meal planning. Nutrition education, like all aspects of diabetes education, has to be an ongoing process with periodic review and revision of the meal plan and assessment of the child's and parents' levels of comprehension, ability to analyze and solve problems, and adherence to the nutrition goals. The patient with newly diagnosed diabetes and his or her parents should consult with a dietitian several times during the first few days after diagnosis. Within a few weeks of the child resuming his or her usual schedule and activities, the patient and family should review the meal plan with a dietitian, who should also be available to patients for telephone consultation. If the patient's glycemic control is poor, if growth is failing, if weight gain is excessive, or if other problems arise related to MNT, the dietitian should be reconsulted.

The meal plan

The individualized meal plan must be simple, practical, easy to modify, offer foods that are interesting, tasty, and inexpensive. At the authors' institution, meal planning is based on a combination of carbohydrate counting and the traditional exchange system, individualized to meet the ethnic, religious, and economic circumstances of each family. The exchange list system for meal planning is the most widely used substitution system and is based on six exchange lists: milk, fruit, vegetable, starch, meat, and fat. Each list indicates the appropriate size or volume of each food exchange. The meal plan is prescribed in terms of the number of exchanges for each meal and snack, which enables the patient to maintain consistency of total calories and the proportions of nutrients while allowing the patient to select from a wide choice of foods. Accurate estimation of portion sizes has to be learned. Weighing and measuring foods is used to educate and train patients to acquire familiarity with the sizes and amounts of food portions specified in the exchange list. An example of how this system is applied to a hypothetical patient is illustrated below. An 11-year-old girl's height is 144 cm (50th percentile on the Centers for Disease Control and Prevention growth chart) and weight is 37.4 kg (50th percentile). Her daily energy requirement to support growth in the 50th percentile is 1756 calories. An appropriate distribution of macronutrients could be 50% of total calories from carbohydrate, 20% as protein, and 30% as fat (Table 21.10).

Exercise

Children with diabetes are encouraged to participate in sports and make regular exercise a part of their lives. Participation in physical exercise normalizes the child's life, enhances self-esteem, improves physical fitness, helps to control weight, and can improve glycemic control. Regular exercise increases insulin sensitivity, cardiovascular fitness, blood lipid profiles, and lowers blood pressure. For the child with type 1 diabetes, physical exercise is complicated by the need to prevent hypoglycemia but, with proper guidance and preparation, participation in exercise should be a safe and enjoyable experience.

Exercise acutely lowers the blood glucose concentration by increasing utilization of glucose to a variable degree that depends on the intensity and duration of physical activity and the concurrent level of insulin in the blood. It should be noted, however, that increased counter-regulatory hormone secretion in response to acute strenuous exercise may temporarily cause hyperglycemia in type 1 diabetes. Hypoglycemia can be prevented by a combination of anticipatory reduction in pre-exercise insulin dose or temporary interruption of basal insulin infusion (with CSII) and/or supplemental snacks before, during, and after physical activity. The optimal strategy depends on the intensity and duration of the activity and its timing relative to the child's meal plan and insulin schedule. Consideration is given to several factors when selecting the content and size of the snack. Among these are the current blood glucose level, the action of insulin most active during and after the period of anticipated exercise, the interval since the last meal, and the duration and intensity of physical activity. The appropriate amount is learned by trial and error, but a useful initial guide is to provide an additional 15 g of carbohydrate (one bread or fruit exchange) per 30–60 min of vigorous physical activity. Prolonged and strenuous exercise in the afternoon or evening should be followed by a 10–20% reduction in the presupper or bedtime dose of intermediate-acting insulin or equivalent reduction in overnight basal insulin delivery in patients using CSII. In addition, to reduce the risk of nocturnal or early-morning hypoglycemia caused by the lag effect of exercise, the bedtime snack should be larger than usual. Parents should be encouraged to monitor the blood glucose concentration in the middle of the night until they are experienced in modifying the evening dose of insulin after exercise.

Exercising the limb into which insulin has been injected accelerates the rate of insulin absorption. If possible, the insulin injection preceding exercise should be given in a site least likely to be affected by exercise. Because physical training increases tissue sensitivity to insulin, children who participate in organized sports are advised to reduce the dose of the insulin preparation predominantly active during the period of sustained physical activity. The size of such reductions is determined by measuring blood glucose levels before and

after exercise and is generally in the order of 10–30% of the usual dose.

In the child with poorly controlled diabetes, vigorous exercise can aggravate hyperglycemia and ketoacid production and, accordingly, a child with ketonuria should not exercise until satisfactory biochemical control has been restored.

Adjunctive therapy

Metformin

A substantial number of adolescents with type 1 diabetes are overweight or obese and have clinically significant insulin resistance. Adjunctive treatment with metformin, to improve insulin sensitivity and glycemic control without weight gain, modestly improves HbA1c levels [58], but it is not yet clear whether these benefits are sustained. Metformin should not be given to individuals at high risk of ketoacidosis or to patients with impaired renal function.

Monitoring diabetes control

Self-monitoring of blood glucose (SMBG)

SMBG has revolutionized management and is the cornerstone of modern diabetes care. Patients/parents must be taught how to use the data to assess the efficacy of therapy and to adjust the components of their treatment regimen to achieve individual blood glucose goals. For most patients with type 1 diabetes, SMBG should be performed at least four times daily, before each meal and at bedtime. To minimize the risk of nocturnal hypoglycemia, blood glucose (BG) should be measured between midnight and 04.00 h once each week or every other week, and whenever the evening dose of insulin is adjusted. If HbA1c targets are not being met, patients should be encouraged to measure BG levels more frequently, including 90–120 min after meals.

Frequency of BG monitoring is an important predictor of glycemic control in children with type 1 diabetes [44]. The optimal frequency of SMBG for patients with type 2 diabetes is not known but should be sufficient to facilitate attainment of the individual patient's glycemic goals. Children who are able to perform SMBG independently must be properly supervised because it is not unusual for children to fabricate data with disastrous consequences. Common reasons for deterioration of metabolic control are shown in Table 21.11.

Table 21.11. Causes of deterioration of metabolic control in children and adolescents with diabetes mellitus.

Increased insulin requirement
Progressive loss of residual β-cell function
Failure to increase dose with growth
Failure to increase dose during puberty
Increased calorie intake
Illness or significant psychological stress
Diminished physical activity (often seasonal)
Medications that cause insulin resistance (e.g. glucocorticoids)
Insulin omission (inadvertent or deliberate)
"Failure" of administered insulin
Inappropriate timing of insulin in relation to food consumption
Failure completely to suspend intermediate-acting insulin suspension
Lipohypertrophy at site of insulin injection
Loss of insulin potency (frozen, heated, or expired)
Improper injection technique (intramuscular or intraepidermal injection)
Miscellaneous causes
Fabricated blood glucose data
Glucose meter malfunction
Celiac disease
Hyperthyroidism
High-titer insulin antibodies

Continuous glucose monitoring sensors (CGMS)

Glucose sensors have been developed to provide frequent glucose determinations throughout the day and night in patients with diabetes. Two continuous glucose monitors, GlucoWatch® G2™ Biographer, and the continuous glucose monitoring system, CGMS™ System Gold®, are being used in children with diabetes. Both measure interstitial fluid glucose concentrations, which are normalized to approximate serum glucose values using algorithms.

CGMS employs a subcutaneous glucose sensor that measures interstitial glucose concentrations every 5 min for 3 days. Glucose values are not available to the patient in real time but are stored for later retrieval and review in the physician's office. CGMS is useful in identifying recurrent patterns of abnormal glycemia, which may enable the physician to optimize the individual's diabetes treatment plan.

The GlucoWatch® G2™ Biographer is worn on the wrist and uses a weak electric current to draw a small amount of interstitial fluid across the dermis for glucose measurement by the glucose sensor (reverse iontophoresis). Glucose values are displayed every 10 min but there is a 15-min lag time. In short-term studies, this device shows promise as a tool for predicting imminent hypoglycemia. Reports indicate better detection of asymptomatic hypoglycemia, detection of postprandial glycemic excursions, and improvement in glycemic control in children with diabetes. However, relatively large differences between sensor and reference glucose values and frequent false-positive values in the hypoglycemic range have been observed. These devices are not yet being used routinely to manage diabetes in children.

Urine ketone testing

Urine should be tested routinely for ketones during acute illness or stress, when blood glucose levels are persistently elevated (e.g. two consecutive blood glucose values > 300 mg/dL) or when the patient feels unwell, especially with nausea, abdominal pain, or vomiting. False-negative readings may occur when the strips have been exposed to air (e.g. improperly stored) or when urine is highly acidic (e.g. after consumption of large doses of ascorbic acid). Urine ketone tests using nitroprusside-containing reagents can give false-positive results in patients who take valproic acid or any sulfhydryl-containing drug, including captopril.

Blood ketone testing

Home tests to measure blood β-hydroxybutyric acid (βOHB) levels are available but expensive. Quantification of blood βOHB, the predominant ketone body, is preferred over urine ketone testing for diagnosing and monitoring metabolic decompensation, as may occur with illness and in ketoacidosis. Once ketosis has been demonstrated by urine testing, blood ketone testing offers the advantage of accurately assessing improvement. Pooling of urine results in persistent ketonuria after blood levels have begun to improve and could lead to excessive insulin administration after the blood ketone concentration has reverted to normal.

Glycated hemoglobin, hemoglobin A1c (HbA1c)

Blood glucose and blood or urine ketone testing provides useful information for day-to-day management of diabetes, whereas HbA1c provides important information about recent overall glycemic control. HbA1c is a minor fraction of adult hemoglobin, which is formed slowly and non-enzymatically from hemoglobin and glucose. Because erythrocytes are freely permeable to glucose, HbA1c is formed throughout the lifespan of the erythrocyte; its rate of formation is directly proportional to the ambient glucose concentration. The concentration of HbA1c, therefore, provides a "glycemic history" of the previous 120 days, the average lifespan of erythrocytes. Numerous methods are used to measure hemoglobin A_{1c}, which has led to different non-diabetic reference ranges because different glycated hemoglobin fractions are measured [59]. Efforts are being made to standardize hemoglobin A_{1c} assays with results certified as traceable to the DCCT assay. Without such standardization, results between laboratories may not be comparable. The correlation between mean plasma glucose levels (from DCCT capillary plasma glucose data) and HbA1c level is: mean plasma glucose (mg/dL) = [(35.6 × HbA1c)–77.3] [60].

In patients with hemoglobin variants (HbS, HbC, HbF), radioimmunoassay and affinity chromatography methods for measuring glycated hemoglobin must be used instead of conventional high-performance liquid or cation-exchange chromatography, which give spurious values. Several clinical conditions, including uremia and high-dose aspirin, lead to chemical modifications of hemoglobin that can spuriously increase hemoglobin A1c measurements. Average glucose levels are underestimated by hemoglobin A1c in conditions that shorten the average circulating red blood cell lifespan, such as hemolysis, sickle cell disease, transfusion, and iron deficiency anemia. When accurate hemoglobin A1c measurement is not possible, alternative tests of chronic glycemia, such as fructosamine or glycosylated serum albumin, should be used.

HbA1c should be measured approximately every 3 months to determine whether a patient's metabolic control has reached or has been maintained within a target range. The HbA1c test is primarily used to monitor the effectiveness of glycemic therapy and as an indicator for when therapy needs to be modified. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus believes that it is still premature to add A1c to the group of tests used for the definitive diagnosis of diabetes [2].

Acute complications of diabetes

Diabetic ketoacidosis (DKA)

In Canada and Europe, rates of hospitalization for DKA in established and new patients with type 1 diabetes have remained stable at about 10 per 100 000 children over the past 20 years. The risk of DKA in patients with established type 1 diabetes is 1–10% per patient per year [25]. It is increased in children with poor metabolic control or previous episodes of DKA, peripubertal and adolescent girls, children with psychiatric disorders, including those with eating disorders, and those with difficult family circumstances, including lower socioeconomic status and lack of health insurance. In the era of CSII, interruption of insulin delivery, irrespective of the reason, is an important cause of DKA. Children rarely have DKA when insulin administration is closely supervised or performed by a responsible adult. In established patients, most instances of DKA are probably associated with insulin omission or treatment error, while the remainder are due to inadequate insulin therapy during intercurrent illness.

The biochemical criteria for the diagnosis of diabetic ketoacidosis (DKA) include hyperglycemia [blood glucose > 11 mmol/L (≈ 200 mg/dL)] with acidosis (venous blood pH < 7.3 and/or serum bicarbonate ≤ 15 mmol/L), ketonemia with total serum ketones (β-hydroxybutyrate and acetoacetate) > 3 mmol/L, and ketonuria. DKA is generally categorized by the severity of the acidosis, from mild (venous pH < 7.30, bicarbonate < 15 mmol/L) to moderate (pH < 7.2, bicarbonate < 10) to severe (pH < 7.1, bicarbonate < 5).

Table 21.12. Clinical manifestations of diabetic ketoacidosis.

- Dehydration
- Rapid, deep, sighing (Kussmaul) respiration
- Nausea, vomiting, abdominal pain that may mimic an acute abdomen
- Increased leukocyte count with left shift
- Non-specific elevation of serum amylase
- Fever when there is infection
- Progressive obtundation and loss of consciousness

Table 21.13. Usual losses of fluids and electrolytes in diabetic ketoacidosis and normal maintenance requirements.

	Average losses per kg (range)	Maintenance requirements per meter^2
Water	70 mL (30–100)	1500 mL
Sodium	6 mmol (5–13)	45 mmol
Potassium	5 mmol (3–6)	35 mmol
Chloride	4 mmol (3–9)	30 mmol
Phosphate	(0.5–2.5) mmol	10 mmol

These data are from measurements in only a few children and adolescents [23].

Pathophysiology [59]

DKA is the result of absolute or relative deficiency of circulating insulin and the combined effects of increased levels of the counter-regulatory hormones, catecholamines, glucagon, cortisol, and growth hormone. Absolute insulin deficiency occurs in previously undiagnosed type 1 diabetes and when patients on treatment deliberately or inadvertently do not take insulin. Relative insulin deficiency occurs when the concentrations of counter-regulatory hormones increase under conditions of stress such as sepsis, trauma, or gastrointestinal illness with diarrhea and vomiting. Low serum levels of insulin and high concentrations of the counter-regulatory hormones result in an accelerated catabolic state, the effects of which are increased glucose production by the liver and kidney (via glycogenolysis and gluconeogenesis), impaired peripheral glucose utilization resulting in hyperglycemia and hyperosmolality, and increased lipolysis and ketogenesis causing ketonemia and metabolic acidosis. Hyperglycemia and hyperketonemia cause osmotic diuresis, dehydration, and obligatory loss of electrolytes, which is often aggravated by vomiting (Table 21.12).

Ketoacidosis may be aggravated by lactic acidosis from poor tissue perfusion and/or sepsis. DKA is characterized by severe depletion of water and electrolytes; the range of losses is shown in Table 21.13. Despite dehydration, patients continue to have considerable urine output unless they are extremely volume depleted. The magnitude of specific deficits in an individual patient at the time of presentation varies depending upon the duration of illness, the extent to which the patient was able to maintain intake of fluid and electrolytes, as well as the content of food and fluids consumed before presentation.

Management of DKA

Initial evaluation

- Perform a clinical evaluation to establish the diagnosis and determine its cause (especially any evidence of infection). Weigh the patient and measure height or length. Determine body surface area. Assess the patient's degree of dehydration.
- Determine the blood glucose concentration with a glucose meter and the blood or urine ketone concentration.
- Obtain a blood sample for laboratory measurement of glucose, electrolytes, and TCO_2, blood urea nitrogen, creatinine, serum osmolality, venous (or arterial in critically ill patient) pH, pCO_2, pO_2, hemoglobin, hematocrit, total and differential white blood cell count, calcium, phosphorus, and magnesium concentrations.
- Perform a urinalysis and obtain appropriate specimens for culture (blood, urine, throat).
- Perform an electrocardiogram for baseline evaluation of potassium status.

Supportive measures

- Secure the airway and empty the stomach by continuous nasogastric suction to prevent pulmonary aspiration in the unconscious or severely obtunded patient.
- Antibiotics should be given to febrile patients after obtaining appropriate cultures of body fluids.
- Supplementary oxygen should be given to patients with severe circulatory impairment or shock.
- Catheterization of the bladder is not usually necessary but, if the child is unconscious or unable to void on demand (e.g. infants and very ill young children), the bladder should be catheterized.
- A flow chart is essential to record the patient's clinical and laboratory data, including vital signs [heart rate, respiratory rate, blood pressure, level of consciousness (Glasgow coma scale)], details of fluid and electrolyte therapy, amount of administered insulin, and urine output. A key to successful management of diabetic ketoacidosis is meticulous monitoring of the patient's clinical and biochemical response to treatment so that timely adjustments in the treatment regimen can be made when indicated by the patient's clinical or laboratory data. Frequent re-examination of laboratory parameters is required to prevent serious electrolyte imbalance and administration of either insufficient or excessive fluid.
- A heparin-locked intravenous catheter should be placed for convenient and painless repetitive blood sampling.
- A cardiac monitor should be used for continuous electrocardiographic monitoring (Table 21.14).

Table 21.14. Goals of therapy.

- Correct dehydration
- Restore blood glucose to near normal levels
- Correct acidosis and reverse ketosis
- Avoid complications of treatment
- Identify and treat the precipitating event

Fluid and electrolyte therapy

All patients with DKA are dehydrated and suffer total body depletion of sodium, potassium, chloride, phosphate, and magnesium (Table 21.13). The high effective osmolality of the extracellular fluid (ECF) compartment results in a shift of water from the intracellular fluid compartment (ICF) to the ECF and decreases the serum sodium concentration ≈ 1.6 mmol/L per 5.6 mmol/L (100 mg/dL) blood glucose above normal. The presence of hyperlipidemia may also lower the measured serum sodium concentration (depending on the methodology used to measure serum sodium concentration). Therefore, the serum sodium concentration may give a misleading estimate of the degree of sodium loss. The effective osmolality (see formula below) at the time of presentation is frequently in the range 300–350 mOsm/L. Increased serum urea nitrogen and hematocrit are useful markers of severe ECF contraction. At the time of presentation, patients are ECF contracted, and clinical estimates of the deficit in patients with severe DKA are usually in the range of 7–10%. In mild to moderately severe DKA, fluid deficits are more modest, in the range 30–50 mL/kg. Shock with hemodynamic compromise is rare in childhood.

The onset of dehydration is associated with a reduction in glomerular filtration rate (GFR), which results in decreased glucose and ketone clearance. Intravenous fluid administration expands the intravascular volume and increases glomerular filtration, which increases renal excretion of glucose and ketoanions, and results in a prompt decrease in blood glucose concentration. The goals of fluid and salt replacement therapy in DKA are restoration of circulating volume, replacement of sodium and the ECF and ICF water deficit, restoration of GFR with enhanced clearance of glucose and ketones from the blood, and avoidance of cerebral edema. In both animals and humans, intracranial pressure rises as intravenous fluids are given. Although there is no compelling evidence showing superiority of any fluid regimen over another, there are data that suggest that rapid fluid replacement with hypotonic fluid is associated with an increased risk of cerebral edema, and slower fluid deficit correction with isotonic or near-isotonic solutions results in earlier reversal of acidosis. Large amounts of 0.9% saline have also been associated with the development of hyperchloremic metabolic acidosis.

Initial intravenous fluid administration and, when necessary, volume expansion should begin immediately with an isotonic solution (0.9% saline or balanced salt solution such as Ringer's lactate). The volume and rate of administration depends on the patient's circulatory status. When volume expansion is clinically indicated, 10–20 mL/kg is given over 1–2 h and may be repeated if necessary. Subsequent fluid management should be with a solution that has a tonicity ≥ 0.45% saline [0.9% saline or balanced salt solution (Ringer's lactate) or 0.45% saline with added potassium]. The rate of intravenous fluid administration should be calculated to rehydrate the patient at an even rate over 48 h. As the severity of dehydration may be difficult to determine and is often overestimated, the daily volume of fluid should usually not exceed 1.5–2 times the usual daily requirement based on age, weight, or body surface area (Table 21.13). Urinary losses should not be added to the calculation of replacement fluids. The development of hyponatremia or failure to observe a progressive rise in serum sodium concentration with a concomitant decrease in blood glucose concentration during treatment is a risk factor for cerebral edema. The composition of the hydrating fluid should be changed appropriately to increase the serum sodium concentration.

When the blood glucose concentration reaches ≈ 17 mmol/L (300 mg/dL), 5% dextrose is added to the infusion fluid. It may be necessary to use ≥ 10% dextrose to prevent hypoglycemia. Administration of intravenous fluids should be continued until acidosis is corrected and the patient can tolerate fluids and food. Inadequate fluid administration should be evident from examination of the cumulative fluid balance and persistent tachycardia in the absence of a fever.

Insulin

Insulin is essential to restore blood glucose to normal and to suppress lipolysis and ketogenesis. Rehydration alone decreases the blood glucose concentration but does not reverse ketoacidosis. Several routes (subcutaneous, intramuscular, and intravenous) of insulin administration and doses have been used and are effective in managing DKA, but low-dose intravenous insulin administration is the standard of care. At a dose of 0.1 unit/kg/h, intravenous regular (soluble) insulin achieves steady-state serum insulin levels of 50–200 μU/mL within 60 min. These serum insulin levels are adequate to offset the insulin resistance characteristic of DKA. They suppress glucose production, significantly increase peripheral glucose uptake, and inhibit lipolysis and ketogenesis. The dose of insulin should remain at 0.1 unit/kg/h until resolution of ketoacidosis (pH > 7.30 and bicarbonate > 15 mmol/L and/or closure of the anion gap). It should be noted, however, that resolution of ketoacidemia takes longer than restoration of blood glucose concentrations to normal. Therefore, intravenous insulin therapy must not be discontinued until ketoacidosis has resolved, even if the blood glucose concentration is normal or near to normal. To prevent an unduly rapid fall in blood glucose concentration and

development of hypoglycemia, dextrose should be added to the intravenous fluid when the plasma glucose has fallen to ≈ 17 mmol/L (300 mg/dL).

Continuous intravenous insulin should be administered via an infusion pump. Regular insulin is diluted in normal saline (50 units of regular insulin in 50 mL of saline) and is given at a rate of 0.1 unit/kg/h. An intravenous priming dose of 0.1 unit/kg is not necessary but may be used at the start of insulin therapy, particularly if insulin treatment has been delayed. This rate of insulin infusion is sufficient to reverse ketoacidosis in most patients. However, if the response is inadequate (especially if blood glucose level is falling but acidosis is not improving, i.e. anion gap is not decreasing) owing to severe insulin resistance, the rate of insulin infusion should be increased until a satisfactory response is achieved. Rare patients with severe insulin resistance do not respond satisfactorily to low-dose insulin infusion and require two or three times the usual dose. It is essential to monitor the blood glucose, venous (or arterial) pH, and anion gap response to insulin therapy. In addition, one should consider other possible explanations for failure to respond to insulin and especially an error in insulin preparation. When intravenous administration is not possible, the intramuscular or subcutaneous route of insulin administration may be used, and rapid-acting insulin (lispro or aspart) may be preferable to regular insulin in these circumstances. Poor tissue perfusion in a severely dehydrated patient will impair SC absorption of insulin and, initially, insulin should be given intramuscularly.

The half-life of insulin in serum is 5 min so that the serum insulin concentration decreases rapidly if the insulin infusion is stopped. If the infusion were to infiltrate and this was not recognized promptly, inadequate serum insulin levels would ensue. Low-dose intravenous insulin therapy must be carefully supervised.

When ketoacidosis has resolved and the change to subcutaneous insulin is planned, the first SC injection should be given at an appropriate interval before stopping the infusion to allow sufficient time for the subcutaneously injected insulin to begin to be absorbed.

Potassium

Potassium is predominantly lost from the intracellular pool because of hypertonicity, insulin deficiency, and buffering of hydrogen ions within the cell. During acidosis, intracellular potassium enters the extracellular compartment and is lost in urine and vomit. Serum potassium concentrations at the time of presentation may be normal, increased or, infrequently, decreased. Hypokalemia at presentation may be related to prolonged duration of disease and persistent vomiting, whereas hyperkalemia primarily results from impaired renal function.

Adults with DKA have total body potassium deficits of the order of 4–6 mmol/kg and, although data in children are sparse, similar deficits have been described in a few cases. After treatment is started, insulin promotes uptake of glucose and potassium by cells, and correction of acidosis promotes the return of potassium to the intracellular compartment. The serum potassium concentration may decrease abruptly, predisposing the patient to cardiac arrhythmias. Potassium replacement should be started immediately if the patient is hypokalemic. Otherwise, it should be started concurrent with commencing insulin therapy.

If the patient presents with hyperkalemia, potassium administration should be deferred until urine output has been documented and the potassium concentration has decreased to a normal level. The amount of potassium administered should be sufficient to maintain serum potassium levels in the normal range. The usual starting potassium concentration in the infusate should be 40 mmol/L, and potassium administration should continue throughout the period of intravenous fluid therapy. Careful monitoring of the serum level and provision of adequate potassium is essential to prevent hypokalemia and life-threatening arrhythmias. The electrocardiogram can be used as a guide to therapy and is especially valuable while waiting for the serum potassium concentration to be measured. Flattening of the T wave, widening of the QT interval, and the appearance of U waves indicate hypokalemia. Tall, peaked, symmetrical, T waves and shortening of the QT interval are signs of hyperkalemia.

The plasma potassium concentration should be rechecked every hour if the plasma concentration is outside the normal range. Potassium may be given as the chloride, acetate, or phosphate salt. Use of potassium acetate and potassium phosphate reduces the total amount of chloride administered and partially corrects the phosphate deficit.

Phosphate

Depletion of intracellular phosphate occurs in DKA, and phosphate is lost because of osmotic diuresis. In adults, deficits are in the range of 0.5–2.5 mmol/kg, but comparable data in children are sparse. After starting therapy, plasma phosphate levels decrease rapidly because of urinary excretion and because insulin causes phosphate to re-enter cells. Low serum phosphate levels have been associated with a variety of metabolic disturbances, but the effect of hypophosphatemia on 2,3-diphosphoglycerate concentrations and on tissue oxygenation are especially relevant to DKA management. Although phosphate depletion persists for several days after resolution of DKA, prospective studies have failed to show any significant clinical benefit from phosphate replacement. Nevertheless, serum phosphate should be monitored, and severe hypophosphatemia should be treated with potassium phosphate while serum calcium is carefully monitored to avoid phosphate-induced hypocalcemia.

Acidosis and bicarbonate

Severe acidosis is reversible simply by fluid and insulin replacement. Insulin stops further synthesis of ketoacids and promotes ketone utilization. The metabolism of ketoanions results in the regeneration of bicarbonate and correction of acidemia. Treatment of hypovolemia improves tissue perfusion and restores renal function, thus increasing the excretion of organic acids and reversing any lactic acidosis, which may account for up to 25% of the acidemia.

In DKA, the anion gap is increased primarily because of a marked increase in the concentrations of the major ketoanions, β-hydroxybutyrate (βOHB) and acetoacetate. Acetone is formed by spontaneous decarboxylation of acetoacetate. Acetoacetate and acetone, but not β-hydroxybutyrate, are measured by the commonly used clinical reagent strip or tablet methods that employ the sodium nitroprusside reaction. At initial presentation with DKA, the concentration of βOHB is four- to 10-fold higher than that of acetoacetic acid. With insulin therapy and correction of the acidosis, the βOHB is reoxidized to acetoacetate, which is eventually metabolized. Blood ketone meters only measure βOHB.

The indications for bicarbonate therapy in DKA are unclear. Controlled trials of sodium bicarbonate in children and adults have been unable to show clinical benefit or any important difference in the rate of rise in the plasma bicarbonate concentration. There are physiologic reasons not to use bicarbonate. Its use may cause paradoxical central nervous system (CNS) acidosis. Bicarbonate combines with H^+ and then dissociates to CO_2 and H_2O. The HCO_3^- diffuses poorly across the blood–brain barrier, whereas CO_2 diffuses freely into the cerebrospinal fluid. Hence, the use of bicarbonate may worsen acidosis within the CNS while serum acidosis improves. Rapid correction of acidosis causes hypokalemia, may aggravate sodium load, and contributes to serum hypertonicity. It may also impair tissue oxygenation by increasing the affinity of hemoglobin for oxygen (i.e. shift the hemoglobin–oxygen dissociation curve to the left). Alkali therapy may increase hepatic ketone production and thus slow the rate of recovery from ketosis [25]. The use of bicarbonate in children with DKA is associated with an increased risk of cerebral edema [62].

There may be selected patients who may benefit from cautious alkali therapy, including patients with severe acidemia (arterial pH < 6.9), in whom decreased cardiac contractility and peripheral vasodilatation can further impair tissue perfusion, and patients with life-threatening hyperkalemia. Administration of bicarbonate is indicated when acidosis is severe (arterial pH ≤ 6.9) and when hypotension, shock, or an arrhythmia is present. In these circumstances, 1–2 mmol/kg or 40–80 mmol/m^2 sodium bicarbonate is infused over 2 h, and the plasma bicarbonate level is rechecked. Bicarbonate should not be given as a bolus because this may precipitate an acute cardiac arrhythmia (Table 21.15).

Table 21.15. Complications of therapy.

- Inadequate rehydration
- Hypoglycemia
- Hypokalemia
- Hyperchloremic acidosis
- Cerebral edema

Clinical and biochemical monitoring

Initially, plasma glucose should be measured hourly. Thereafter, plasma glucose, serum electrolytes (and calculated sodium), pH, pCO_2, TCO_2, anion gap, calcium, and phosphorus should be measured every 2–4 h for the first 8 h and then every 4 h until they are normal. The data must be recorded carefully on a flow sheet.

Investigating the cause of ketoacidosis

The management of DKA is not complete until its cause has been identified and treated. An intercurrent infection is not the usual cause when the patient is properly educated in diabetes management, is receiving regular follow-up care by a competent physician, and has access to a diabetes treatment team. In previously diagnosed patients on treatment with insulin, omission of insulin, either inadvertently or deliberately, is the most common cause. There is often an important psychosocial reason for insulin omission. This can be an attempt to lose weight in an adolescent girl with an eating disorder, a means of escaping an intolerable or abusive home situation, clinical depression, or other reason for the patient to be unable to manage his or her own diabetes unassisted [25]. A psychiatric social worker or clinical psychologist should be consulted to help to identify the psychosocial reason(s) underlying the development of DKA.

Useful calculations for managing DKA

1 Effective osmolality $2[Na^+ + K^+]$ + glucose (mmol/L)
Effective serum osmolality correlates with mental status abnormalities. Blood or serum urea nitrogen diffuses freely into cells and does not contribute to effective osmolality.
2 Corrected sodium = $[Na^+]$ + {1.6 × [plasma glucose (mmol/L) – 5.6] ÷ 5.6}
Corrected serum sodium assists in estimation of free water deficits.
3 Anion gap = $[Na^+]$–$[Cl^- + HCO_3^-]$
A decreasing anion gap indicates successful therapy of metabolic acidosis.
4 Evaluation for pure metabolic acidosis:
pCO_2 = last two numbers of the pH
$pCO_2 = 1.5$ [serum HCO_3^-] + 8 ± 2
A lower than predicted pCO_2 indicates respiratory alkalosis and may be a clue to sepsis.

Morbidity and mortality from DKA in children

Reported mortality rates from DKA in national population-based studies are reasonably constant in the range of 0.15–0.31%. In areas with sparse medical facilities, the risk of dying from DKA is greater, and children may die before receiving treatment. Cerebral edema accounts for 57–87% of all deaths from DKA. The incidence of cerebral edema has been fairly consistent between national population-based studies, such as 0.46% in Canada to 0.87% in the USA. Mortality rates from cerebral edema in population-based studies are 21–25%. Significant morbidity is evident in 10–26% of survivors. Other causes of DKA-related morbidity and mortality include hypokalemia, hyperkalemia, hypoglycemia, sepsis, and other CNS complications such as thrombosis [25].

Cerebral edema typically occurs 4–12 h after commencement of treatment but can occur before treatment has begun or at any time during treatment. Symptoms and signs are variable and include onset of headache, gradual decrease or deterioration in level of consciousness, inappropriate slowing of the heart rate, and an increase in blood pressure. Cerebral edema is more common in children with severe DKA, new-onset type 1 diabetes, younger age, and longer duration of symptoms [62]. The cause of cerebral edema remains poorly understood.

Treatment of cerebral edema [25]

Treatment should be initiated as soon as the condition is suspected. The rate of fluid administration should be reduced. Intravenous mannitol (0.25–1 g/kg) should be given over 20 min and can be repeated, if necessary, in 2 h if there is no initial response. Hypertonic saline (3%), 5–10 mL/kg over 30 min may be an alternative to mannitol. Intubation may be necessary for the patient with impending respiratory failure, but aggressive hyperventilation (to a $pCO_2 < 22$ mmHg) has been associated with poor outcome and is not recommended (Table 21.16) [63].

Management of sick days: prevention of DKA

Even a relatively minor illness in a child with type 1 diabetes can cause rapid deterioration of metabolic control. The stress of infection, surgery, injury, or severe emotional distress increases counter-regulatory hormone levels, which cause hyperglycemia and stimulate lipolysis and ketogenesis. Even when carbohydrate consumption is reduced by illness, blood glucose levels usually increase. Unchecked, these metabolic disturbances can rapidly progress to DKA. The aim of sick day management is to minimize deterioration of metabolic control and prevent DKA. The principles of sick day management are listed (Table 21.17).

Table 21.16. Factors associated with increased risk of cerebral edema.

- An attenuated rise in measured serum sodium concentration during treatment
- More severe acidosis
- Administration of bicarbonate to correct acidosis
- More profound hypocapnia at presentation
- Increased serum urea nitrogen at presentation reflecting more severe dehydration

Table 21.17. Principles of sick day management.

- Never omit intermediate- or long-acting insulin injections or basal insulin infusion
- Maintain hydration
- Frequently measure blood glucose and blood or urine ketone levels
- Administer supplemental rapid- or short-acting insulin every 2–4 h when indicated (Table 21.18)
- Treat the underlying illness
- Monitor for signs and symptoms that demand urgent medical attention

Failure to administer the child's usual basal doses of insulin can have disastrous consequences. Supplemental injections of rapid- or short-acting insulin are often required to prevent or correct hyperglycemia and/or ketosis (see Table 21.18 for guidelines). The child who has a gastrointestinal illness with nausea, vomiting, or diarrhea and low, normal, or near normal blood glucose levels is a special case because there is an increased risk of hypoglycemia. The dose of rapid- or short- and intermediate-acting insulins may have to be reduced. Ketonuria with blood glucose levels that are normal or near to normal usually signifies starvation ketosis but must be distinguished from "euglycemic ketoacidosis."

Fluid requirements increase as a result of osmotic diuresis and increased insensible fluid losses due to fever; dehydration can develop rapidly if insufficient fluid is consumed. The child should be encouraged to drink at least 1–2 mL per pound of body weight per hour (or a minimum of 1500 mL/m^2/24 h, the usual maintenance fluid requirement). The child should receive fluids that provide sodium, glucose, and potassium to replace urinary losses of these electrolytes that occur with metabolic decompensation. Fluids suitable for sick days are broth or bouillon (high salt content), water, carbonated beverages (Coca-Cola, ginger ale), Gatorade and fruit juices (see Appendix 1). Sugar-free fluids are recommended if the child is able to continue to follow his/her meal plan and/or blood glucose is > 11 mmol/L (200 mg/dL). However, when the child is unable to eat solid foods and the blood glucose is < 11 mmol/L (200 mg/dL), the liquids should contain a source of glucose. Weight loss is a reliable sign of dehydration and, if a bathroom scale is available, the child should be weighed carefully several times each day.

Blood glucose should be measured every 2–4 h throughout the day and night and ketone levels checked each time

Table 21.18. Guidelines for insulin therapy when the child is ill.

Blood glucose (mmol/L)	< 4.5	4.5–11	11–17	17–22	> 22
Short- or rapid-acting insulin	Omit	Usual dose*	Usual dose*	Usual dose*	Usual dose*
Supplemental rapid-acting insulin ("booster dose")	None	None	†K+ 5% †K++ 10%	†K–, trace or + 10% †K++ 15% K+++ 20%	With or without ketones
Intermediate-acting insulin (NPH/lente)	1/2–2/3 of usual dose	Usual dose‡	Usual dose‡	Usual dose‡	Usual dose‡
Long-acting insulin (glargine)	Usual dose	Usual dose	Usual dose	Usual dose	Usual dose
Remeasure glucose	≤ 1 h	2–4 h	2–4 h	2–4 h	2–4 h
Additional action	Notify diabetes team		If no improvement, repeat booster§	If no improvement, repeat booster§	If no improvement, repeat booster§

*Usual dose unless it appears that child is unable or unwilling to eat/drink normally, in which case the dose of rapid-acting insulin should be decreased or postponed until after the child has eaten the meal.
†K–, trace, +, ++, +++, urine ketones absent, trace (0.5 mmol/L), small (1.5 mmol/L), moderate (4 mmol/L), and large (≥ 8 mmol/L) respectively.
The Precision Xtra™ meter measures blood βOHB concentration quantitatively: 0–0.5 mmol/L is normal; 0.6–1.5 mmol/L is moderate ketonemia; 1.6–3.0 mmol/L indicates that the patient is at risk of developing DKA; > 3.0 mmol/L is consistent with DKA.
‡Depends on anticipated ability to eat and drink. If this is in doubt, reduce dose to two-thirds of usual dose.
Supplemental ("booster") insulin doses are calculated as a percentage of the usual (average) total daily dose of insulin (i.e. the sum of all rapid-, short-, intermediate-, and long-acting insulins).
§Notify diabetes team if ketones do not clear after second supplemental dose.

Table 21.19. Signs and symptoms in an ill child with diabetes mellitus requiring urgent medical attention.

- Signs of dehydration: dry mouth or tongue, cracked lips, sunken eyes, dry flushed skin, decreased urine output, weight loss
- Inability to consume the recommended amount of fluid or carbohydrate
- Persistent or recurrent vomiting for more than 4 h
- Symptoms suggestive of DKA: nausea, abdominal pain, vomiting, hyperventilation, drowsiness
- Blood glucose > 14 mmol/L (250 mg/dL) and ketonuria for more than 12 h; for a child using an insulin pump after 6–8 h
- Inability to maintain blood glucose > 4.5 mmol/L (80 mg/dL).

the child urinates. If the blood glucose concentration is < 4.5 mmol/L (80 mg/dL), measurements should be repeated hourly until it is > 4.5 mmol/L (80 mg/dL). Cotton balls placed in the diaper can be used to obtain urine for ketone testing in infants and toddlers. Alternatively, blood β-hydroxybutyrate concentration can be measured directly in capillary blood with a meter (Precision Xtra™). Normal levels are < 0.5 mmol/L. During starvation or illness or when insulin delivery is insufficient for any reason, the blood ketone concentration increases rapidly and, in a person with type 1 diabetes mellitus, a level of ≥ 3.0 mmol/L together with hyperglycemia indicates DKA.

The blood glucose levels and severity of ketonuria or ketonemia are used to guide the administration of supplemental insulin (Table 21.18). Rapid-acting insulin (lispro or insulin aspart) may be given every 2.5–3 h or short-acting (regular) insulin every 3–4 h until blood glucose is less than 11 mmol/L (200 mg/dL) and ketonuria has been reduced to negative or trace or blood βOHB < 0.5 mmol/L.

Evidence that continued management of the child at home may no longer be safe and the child requires urgent medical attention is listed below (Table 21.19). Assiduous attention to these guidelines enables most intercurrent childhood illnesses to be managed successfully at home.

Illness in the child managed with an insulin pump

The child using an insulin pump requires additional specific attention during sick day management. Because nausea and vomiting may be the earliest manifestations of interrupted insulin delivery and impending ketoacidosis, the patient may incorrectly assume that the symptoms are caused by a viral illness and not recognize the insulin-deficient state. Therefore, the patient who experiences nausea or vomiting must immediately check blood or urine for ketones. Ketosis is evidence of a potential impending medical emergency because, when insulin delivery is interrupted, DKA can develop within 4–6 h. Rapid-acting insulin must immediately be given SC with a syringe. The dose may be based on the child's usual "correction factor" to reduce the blood glucose concentration to 6 mmol/L. Alternatively, the guidelines for supplemental insulin in Table 21.18 may be used. The infusion set should be replaced and the pump examined carefully to look for possible causes of failure to deliver insulin. These include battery failure, mechanical failure, an empty insulin reservoir, leakage at the site where the catheter connects to the syringe, occlusion of the

catheter, and withdrawal of the catheter from its SC insertion site on the skin. A temporary increase in the basal rate of insulin infusion may be required during illness. If blood glucose levels exceed the target range, a 4-h trial of a 25% increase in the basal rate may be sufficient to restore the blood glucose to an acceptable level. If the blood glucose level has not decreased after 4 h, the basal rate should be increased by 50%.

Side-effects of treatment

Weight gain

Intensively treated subjects in the DCCT had a considerably increased risk of becoming overweight [64], which was greatest in individuals with higher baseline hemoglobin A1c levels. Weight gain is attributable to reduced glycosuria and, secondarily, to a reduction in daily energy expenditure. Furthermore, frequent symptomatic hypoglycemia necessitating consumption of carbohydrate to restore normoglycemia also contributes to the tendency to gain weight in some intensively managed individuals. It has also been suggested that intermittent hyperinsulinemia and lack of amylin to regulate appetite may underlie the propensity to weight gain in type 1 diabetes.

Longitudinal studies have found a J-shaped curve relating body mass index (BMI) to mortality in type 1 diabetes, with the highest relative all-cause mortality in those with the lowest BMI. It is generally accepted, therefore, that the long-term benefits of intensive glycemic control greatly outweigh the adverse effects of weight gain.

Children and adolescents who adopt basal-bolus insulin therapy may be tempted to eat more liberally and increase their calorie consumption as they are no longer obliged to follow a regimented meal plan. Anticipatory counseling should candidly address this issue. The highly motivated patient can take advantage of the flexibility of a basal-bolus regimen to balance insulin replacement carefully with calorie intake to avoid obesity or even lose weight.

Local effects of insulin

Lipohypertrophy refers to the accumulation of excess adipose tissue at the sites of SC insulin injection. It is the most common cutaneous side-effect of insulin administration, occurring in 25–50% of individuals with type 1 diabetes. Rotation of injection sites, thereby avoiding repeated insulin injections in a single area, prevents lipohypertrophy. In addition to its undesirable cosmetic appearance, it is important to avoid lipohypertrophy because it causes erratic absorption of insulin.

Lipoatrophy and insulin allergy are much less common with use of human insulin preparations. Rotation of the site of insulin injections may also decrease the risk of lipoatrophy. Insulin absorption from lipoatrophic areas may also be erratic. Lipoatrophy may resolve by carefully injecting insulin into the perimeter of the affected area. In severe cases, topical glucocorticoid injection into and around the site may be helpful. If the patient is using animal-source insulin, switching to human insulin may prevent further atrophy and lead to gradual filling in of the atrophic area.

Insulin allergy may result in local or systemic effects, with acute (redness, itching, burning, hives) or chronic reactions. Severe reactions, including generalized urticaria and anaphylaxis, are extremely rare. Switching to synthetic human insulin may prevent further allergic reactions. This should be done after preliminary skin testing in a supervised setting. Insulin delivery by pump has been reported to stop local reactions in some cases. In rare instances, insulin desensitization may be necessary.

Cellulitis or abscess may occur at the injection site but is rare when patients use sterile disposable syringes and needles and employ basic principles of hygiene. Injection through clothing is strongly discouraged. Insulin pump therapy is associated with increased rates of cellulitis, abscess, and local scarring at the sites of subcutaneous catheter insertion. It is essential to replace the catheter every 2–3 days and remove a catheter immediately if the site becomes red or painful.

Hypoglycemia

Hypoglycemia is the most common acute complication of the treatment of diabetes mellitus in children and adolescents, and concern about hypoglycemia is a central issue in treating children with type 1 diabetes. It is the principal factor limiting attempts to achieve near normal glycemic control [65]. Patients, parents, and the diabetes team continuously have to balance the risks of hypoglycemia against those of long-term hyperglycemia. After an episode of severe hypoglycemia, the confidence of the patient and parents is often shaken, and fear of a recurrence may induce the patient or parents to change their diabetes management to prevent a recurrence. Altered patient behaviors may include overeating and/or deliberate selection of inadequate doses of insulin to maintain higher blood glucose levels perceived as being safe, resulting in deterioration of glycemic control [66]. Concern about nocturnal hypoglycemia causes more anxiety for some parents than any other aspect of diabetes, including the fear of long-term complications. Some parents believe that an episode of severe hypoglycemia during the night-time may go undetected or not be treated in a timely fashion and lead to permanent brain damage or death [67].

The glucagon response to hypoglycemia is lost early in the course of the disease, and patients with type 1 diabetes depend on sympathoadrenal responses to prevent or correct hypoglycemia [68]. Mild hypoglycemia itself reduces

epinephrine responses and symptomatic awareness of subsequent episodes of hypoglycemia. Little is known about counter-regulatory responses in preschool-aged children.

Symptoms and signs of hypoglycemia

Symptoms of hypoglycemia are caused by neuronal deprivation of glucose and have been categorized into subgroups: autonomic (sweating, palpitations, shaking, hunger), neuroglycopenic (confusion, drowsiness, odd behavior, speech difficulty, inco-ordination), non-specific malaise (hunger and headache) [69]. The most common signs and symptoms of hypoglycemia in children with diabetes are pallor, weakness, tremor, hunger, fatigue, drowsiness, sweating, and headache. Autonomic symptoms are less common in children less than 6 years old whose symptoms are more often neuroglycopenic or non-specific in nature. Behavioral changes are often the primary manifestation of hypoglycemia in young children, and this difference has important implications for parent education on hypoglycemia. Also, in contrast to adult patients who are usually able to distinguish between autonomic and neuroglycopenic symptoms, children and their parents report that symptoms tend to cluster [70]. The coalescence of autonomic and neuroglycopenic symptoms in children may indicate that both types of symptoms are generated at similar glycemic thresholds.

Hypoglycemia is classified in terms of its severity as mild, moderate, or severe. Most episodes are mild. Cognitive impairment does not usually accompany mild hypoglycemia, and older children are able to treat themselves. Mild symptoms abate within about 15 min after treatment with rapidly absorbed carbohydrate. Moderate hypoglycemia has neuroglycopenic as well as adrenergic symptoms, such as headache, mood changes, irritability, decreased attentiveness, drowsiness, and behavior change. Young children typically require assistance with treatment because they are often confused and have impaired judgment. Weakness and poor co-ordination may make self-treatment difficult. Moderate hypoglycemia causes more protracted symptoms and may require a second treatment with oral carbohydrate. Severe hypoglycemia is characterized by unresponsiveness, unconsciousness, or convulsions and requires emergency treatment with parenteral glucagon or intravenous glucose.

Children who have had diabetes for several years may describe a change in their symptomatology over time. Autonomic symptoms tend to occur less frequently and are more muted, and neuroglycopenic symptoms (drowsiness, difficulty concentrating, lack of co-ordination) are more common. Patients must learn to recognize the change in symptoms to prevent severe episodes. The blood glucose concentration at which symptoms occur varies among patients, and the threshold may vary in the same individual in parallel with antecedent glycemic control. Children with poorly controlled diabetes experience symptoms of hypoglycemia at higher blood glucose concentrations than those with good glycemic control, similar to adults with diabetes.

Impact of hypoglycemia on the child's brain

Numerous studies have documented cognitive impairments in children and adolescents with type 1 diabetes. Global intellectual deficits have been described as well as specific neurocognitive impairments in memory, visuospatial skills, and attention. Neuropsychological complications have been detected within 2 years of onset of diabetes [71]. Children with long-term diabetes, especially those who developed the disease before the age of 6 years, appear to be at greatest risk. However, it is difficult to dissect out the contributions of metabolic disturbances (hyperglycemia and hypoglycemia) and the psychosocial effects of chronic disease in a young child [72]. There is evidence linking hypoglycemia to the neuropsychological defects. Rovet and Ehrlich observed specific defects associated with a positive history of severe hypoglycemic events [73], but others found no evidence of an association with severe episodes and thought that asymptomatic hypoglycemia may be more important. Impaired intellectual development without a clear relationship to experienced hypoglycemia has also been reported [74]. Thus, cognitive impairments in children with early-onset diabetes mellitus may result from a number of factors, the relative important of which is still unclear, including severe hypoglycemia, recurrent asymptomatic hypoglycemia, psychosocial effects of chronic illness, and chronic hyperglycemia [72]. The neurocognitive sequelae of intensive diabetes management in children whose brains are still developing are still largely unknown. Preliminary findings suggest poorer memory skills, presumably the consequence of recurrent and severe hypoglycemia [75].

Even in the absence of typical symptoms, cognitive function deteriorates at low blood glucose levels [76]. Moderate and severe hypoglycemia is disabling, affects school performance, and makes driving a car or operating dangerous machinery hazardous, and the utmost effort should be made to avoid such events. Repeated or prolonged severe hyperinsulinemic hypoglycemia can cause permanent CNS damage especially in very young children. Fortunately, hypoglycemia is a rare cause of death in children with type 1 diabetes [77].

Frequency of hypoglycemia

The true frequency of mild (self-treated) symptomatic hypoglycemia is almost impossible to ascertain because mild episodes are quickly forgotten and/or are not recorded. Tupola *et al*. [78] prospectively examined the frequency of hypoglycemia [blood glucose < 3 mmol/L (< 54 mg/dL)] in 161 children and adolescents predominantly treated with multiple doses of insulin, who were asked to document

hypoglycemia episodes in a 3-month diary. Fifty-two per cent of the clinic population experienced episodes of hypoglycemia (0.6 hypoglycemia events per patient per month), of which 77% were mild.

The literature is replete with reports of the frequency of severe hypoglycemia in children and adolescents with diabetes, but the various methods of collecting data, variability among clinic populations and therapeutic methods, and definitions of severe hypoglycemia make comparisons among the reports and interpretation of the data difficult [71]. For example, in some studies, severe hypoglycemia is defined as loss of consciousness, whereas others include children who required assistance with treatment. In young children, all episodes of hypoglycemia require the assistance of a third party for treatment regardless of the severity of the symptoms. It is not surprising, therefore, that the reported incidence of moderate or severe hypoglycemia in the pediatric diabetes population varies widely. Recent prospective studies with strict definitions of hypoglycemic events and well-described populations continue to show disturbingly high rates of severe hypoglycemia; younger children and patients with tight glycemic control are at greatest risk (Table 21.20) [72].

Many, but not all, studies have found an increased frequency of severe hypoglycemia in younger children and in association with lower hemoglobin A1c concentrations. Other factors associated with a higher risk of moderate and severe hypoglycemia are a prior history of severe hypoglycemia, relatively higher doses of insulin and low C-peptide secretion, longer duration of diabetes, male gender, psychiatric disorders, and lack of health insurance [79].

Causes of hypoglycemia in diabetes mellitus

Patients with type 1 diabetes mellitus are susceptible to hypoglycemia for many reasons (Table 21.21). Patient errors relating to insulin dosage, decreased food intake, or unplanned exercise account for 50–85% of episodes of hypoglycemia in children and adolescents. After years of living with diabetes, some patients and/or their parents conduct their routine diabetes self-care practices without thinking carefully about the intricate interplay among insulin, food, and exercise.

Improved methods of replacing insulin (CSII and multiple-dose regimens with insulin analogs) combined with behavioral educational approaches, such as blood glucose awareness training and intermittent continuous glucose monitoring, may enable patients to achieve improved glycemic control with less risk of severe hypoglycemia than was previously possible. These claims have yet to be confirmed in large prospective studies. Several reports have shown that insulin pump therapy is associated with fewer hypoglycemic events despite improved glycemic control. This may be because CSII permits lower (and adjustable) rates of basal insulin delivery compared with injection

Table 21.20. Incidence of severe hypoglycemia* in children and adolescents.

Study (author year)	Age group (years)	No. of patients	Definition of severe hypoglycemia	Incidence†	Mean or median HbA1c (%)	Methodology
DCCT 1994	13–17	195	Coma, seizure			Prospective randomized clinical trial
intensive therapy				26.7	8.06	
conventional therapy				9.7	9.76	
Nordfeldt 1997	1–18	146	Coma, seizure	15–19	8.1–6.9	Prospective
Mortensen 1997	1–18	2873	Coma, seizure	22	8.6	Cross-sectional international
Rosilio 1998	1–19	2579	Coma, seizure, glucagon	45	8.97	Cross-sectional national
Davis 1998	0–18	709	Coma, seizure	15.6	8.6	Prospective population based
Tupola 1998	1–24	329	Coma, seizure, glucagon	3.6	9.1–9.6	Retrospective
Tupola 1998	1–24	287	Coma, seizure, glucagon	3.1	9.0–9.1	Prospective
Thomsett 1999	1–19	268	Coma, seizure	25	8.6	Retrospective
Nordfeldt 1999	1–18	139	Unconsciousness	17.0	6.9‡	Prospective
Levine 2001	7–16	300	Coma, seizure, glucagon, IV dextrose	8	8.7–8.9	Prospective
Rewers 2002	0–19	1243	Coma, seizure, admission	19	8.8–9.0§	Prospective

*Severe hypoglycemia is variably defined in these studies as coma, seizure, treatment with glucagon, intravenous dextrose, treatment in an emergency department, or admission to hospital.

†Events per 100 patient–years.

‡Median value, normal range 3.6–5.4%.

§Range of median values.

Adapted from data in [79].

Table 21.21. Causes of hypoglycemia in children and adolescents with diabetes mellitus.

Insulin errors (inadvertent or deliberate)
Reversal of morning and evening dose
Reversal of short- or rapid-acting insulin and intermediate-acting insulin
Improper timing of insulin in relation to food
Excessive insulin dosage
Surreptitious insulin administration, suicide gesture or attempt
Erratic or altered absorption
Inadvertent intramuscular injection
More rapid absorption from exercising limbs
Unpredictable absorption from lipohypertrophy at injection sites
More rapid absorption after sauna, hot bath, sunbathing
Diet
Omission or reduced size of meals or snacks
Delayed snacks or meals
Eating disorders
Gastroparesis
Malabsorption, e.g. gluten enteropathy
Exercise
Unplanned physical activity
Prolonged duration and/or increased intensity of physical activity
Failure to reduce the dose of basal insulin to combat the "lag effect" of exercise
Alcohol and/or drugs
Impaired gluconeogenesis from excessive consumption of ethanol
Impaired cognition from use of ethanol, marijuana, cocaine, other recreational drugs
Hypoglycemia-associated autonomic failure
Hypoglycemia unawareness
Defective glucose counter-regulation
Miscellaneous uncommon causes of hypoglycemia
Adrenocortical insufficiency
Hypothyroidism
Growth hormone deficiency
Renal failure
Decreased insulin requirement in first trimester of pregnancy
Insulin antibodies

therapy, especially at night when hypoglycemia is most common. Rapid-acting insulin analogs decrease the frequency of hypoglycemia, and insulin glargine together with premeal insulin lispro decrease the incidence of nocturnal hypoglycemia in adolescents compared with NPH combined with regular insulin [49].

Nocturnal hypoglycemia

Hypoglycemia, often asymptomatic, frequently occurs during sleep. Moderate and severe hypoglycemia is more common during the night and early morning (before breakfast) than during the daytime [80]. In the DCCT, 55% of severe hypoglycemia events occurred during sleep, and 43% occurred between midnight and 08.00 h [80,81].

Children and adolescents with diabetes studied either in hospital or at home with frequent intermittent or continuous blood glucose measurements during the night show a high incidence (14–47%) of asymptomatic hypoglycemia. Episodes of hypoglycemia during sleep often exceed 4 h in duration, and up to half these episodes may be undetected because the subject does not awaken from sleep. The incidence of hypoglycemia on any given night may be affected by numerous factors, including the insulin regimen, the timing and content of meals and snacks, and antecedent physical activity. The highest frequency of asymptomatic nocturnal hypoglycemia occurs in children less than 10 years old. Low blood glucose concentrations in the early morning (before breakfast) are associated with a higher frequency of preceding nocturnal hypoglycemia, and knowledge of this fact is useful in counseling patients to modify the evening insulin regimen and bedtime snack to prevent more severe nocturnal hypoglycemia.

Sleep impairs counter-regulatory hormone responses to hypoglycemia in normal subjects and in patients with diabetes mellitus [82]. Because a rise in plasma epinephrine is normally the main hormonal defense against hypoglycemia in patients with diabetes, impaired counter-regulatory hormone responses to hypoglycemia explain the increased susceptibility to hypoglycemia during sleep. Furthermore, asymptomatic nocturnal hypoglycemia may impair counter-regulatory hormone responses. Thus, impaired defenses against hypoglycemia during sleep may contribute to the vicious cycle of hypoglycemia, impaired counter-regulatory responses, and unawareness of hypoglycemia either awake or asleep. Recurrent asymptomatic nocturnal hypoglycemia is, therefore, an important cause of hypoglycemia unawareness, which, in turn, leads to more frequent and severe hypoglycemia because of failure to experience autonomic warning symptoms before the onset of neuroglycopenia [65].

Treatment

Except in preschool-aged children, most episodes of symptomatic hypoglycemia are self-treated with rapidly absorbed carbohydrate such as glucose tablets, juices, soft drinks, candy, crackers, or milk. Glucose tablets raise blood glucose levels more rapidly than orange juice or milk, and the dosage is easily calibrated. Glucose tablets are the treatment of choice for children old enough to chew and safely swallow large tablets. The recommended dose is 0.3 g of glucose per kg of body weight. Blood glucose should be remeasured 15 min after treatment and, if the blood glucose level does not exceed 3.9–4.4 mmol/L (70–80 mg/dL), treatment should be repeated. The glycemic response to oral glucose usually lasts less than 2 h. Therefore, after treatment with oral glucose,

unless a scheduled meal or snack is due within 1 h, the patient should be given either a snack or a meal containing carbohydrate and protein.

Hypoglycemia frequently occurs when a child with diabetes is unable to consume or absorb oral carbohydrate because of nausea and vomiting associated with an intercurrent illness (e.g. gastroenteritis) or oppositional behavior. To maintain blood glucose concentrations in a safe range, parents either seek emergency medical attention or attempt to force feed oral carbohydrate in an ill child, which often leads to more vomiting. Mini-dose glucagon raises blood glucose by 3.3–5 mmol/L (60–90 mg/dL) within 30 min, and its effect lasts approximately 1 h. This method is effective in managing most situations of impending hypoglycemia at home. Using a U100 insulin syringe and after dissolving 1 mg of glucagon in 1 cc of diluent, children ≤ 2 years receive 2 "units" (20 μg) of glucagon SC, and children older than 2 year, receive 1 unit (10 μg) per year of age up to 15 units (150 μg). If the blood glucose concentration does not increase within 30 min, double the initial dosage should be administered [83].

Severe reactions (unresponsiveness, unconsciousness, or convulsions) require emergency treatment with parenteral glucagon (IM or SC). Glucagon raises blood glucose levels within 5–15 min and usually relieves symptoms of hypoglycemia. Symptoms of experimentally induced hypoglycemia in children with diabetes are relieved within 10 min of giving glucagon by either SC or IM injection. Mean blood glucose and plasma glucagon levels are slightly but not significantly higher after IM than after SC injection. Both 10 μg/kg and 20 μg/kg glucagon relieve clinical signs and symptoms, but the increment in blood glucose concentration after 10 min is less after a dose of 10 μg/kg [1.1 ± 0.3 vs. 1.7 ± 0.7 mmol/L (20 ± 5 vs. 31 ± 13 mg/dL)]. However, after 20 and 30 min, the differences in blood glucose concentrations are not significant. Nausea and/or vomiting occur after the injection in a minority of children who receive a dose of 20 μg/kg but usually does not occur after 10 μg/kg glucagon [84]. Excessively high plasma glucagon levels are more likely to cause nausea and/or vomiting. The recommended dose, therefore, is 15 μg/kg to a maximum of 1.0 mg. In children with diabetes and in healthy adults, there appears to be no important difference between the effects of glucagon injected either SC or IM. The plasma glucagon levels attained are higher than those in peripheral venous or portal blood of healthy adults during insulin-induced hypoglycemia and are probably higher than is necessary for maximal effect. The increase in blood glucose concentration after glucagon administration is sustained for at least 30 min. Therefore, it is unnecessary to repeat the dose or force the child to eat or drink for at least 30 min. Intranasal glucagon has a similar effect but is not available in the USA. In an emergency department or hospital, the preferred treatment is intravenous glucose (0.3 g/kg). Because the glycemic response is transient after bolus administration of glucose, intravenous glucose infusion should be continued until the patient is able to swallow safely.

If severe hypoglycemia was prolonged and the patient had a seizure, complete recovery of mental and neurologic function may take many hours despite restoration of normal blood glucose levels. Permanent hemiparesis or other neurologic sequelae are rare, but the post-ictal period may be complicated by headache, lethargy, nausea, vomiting, and muscle ache.

Driving a motor vehicle

Hypoglycemia increases the rate of driving mishaps among adults with type 1 diabetes. Factors associated with an increased risk of accidents are failure to measure the blood glucose level before driving and a lower blood glucose level at which subjects choose not to drive [85]. Driving is impaired at plasma glucose concentrations of ≤ 3.3 mol/L (60 mg/dL) [86]. The adolescent with diabetes who is learning to drive should be counseled always to measure his blood glucose level before driving and not to drive unless blood glucose is greater than 4 mmol/L (70 mg/dL). Furthermore, patients should be advised to stock the glove compartment with a source of rapidly absorbed carbohydrate and non-perishable snacks, and to pull over as soon as it is safe to do so and stop the car when symptoms of hypoglycemia are detected.

Dead in bed

Sudden unexplained deaths during sleep have been described in adolescents with type 1 diabetes. These events are rare. Young adult males are at highest risk. Lethal cardiac arrhythmias triggered by hypoglycemia may be responsible for some cases, and severe hypoglycemia related to recreational drug abuse may account for others.

Chronic complications of diabetes

Non-vascular complications of diabetes

Cataracts

Cataracts rarely occur in children with diabetes and, when present at the time of diagnosis, may regress after treatment of diabetes has been instituted.

Limited joint mobility (LMJ)

LMJ, also referred to as cheiroarthropathy, is caused by glycosylation of collagen in the connective tissue of skin and tendons. It manifests as inability to extend the fingers and/or wrists normally because of loss of skin elasticity and contraction of tendons. LMJ is a sign of chronic poor glycemic

control and is associated with increased risk of microvascular complications.

Growth

Growth failure in children with diabetes is uncommon even with only "average" glycemic control. Nonetheless, abnormality of the growth hormone (GH)–insulin-like growth factor (IGF)-1 axis is common. With average blood glucose control, GH secretion is increased, and serum levels of IGF-1 and IGF binding protein (BP)-3 tend to be reduced. Delayed puberty and growth failure typically occur only when a child or adolescent experiences chronic very poor glycemic control. Mauriac syndrome is rarely seen in the modern era. This syndrome includes growth failure, delayed puberty, a cushingoid pattern of fat distribution, and hepatosteatosis. It is thought to be due to recurrent cycles of adequate alternating with inadequate insulinemia.

Skin

Necrobiosis lipoidica diabeticorum is an uncommon poorly understood complication that causes unsightly lesions that usually appear in the pretibial area. Treatment is generally unsatisfactory, but intralesional injection of corticosteroids often results in some improvement.

Disordered eating and eating disorders

Adolescent females with type 1 diabetes have a twofold increased risk of developing an eating disorder compared with their peers without diabetes [87]. Eating disorders in adolescents with type 1 diabetes are associated with poor metabolic control and earlier onset and progression of microvascular complications. This problem should be suspected in adolescent females who are unable to achieve and maintain blood glucose targets or who have unexplained weight loss or deterioration of metabolic control [88]. Screening should be conducted by asking non-judgmental questions about weight and shape concerns, dieting, episodes of binge eating, and insulin omission for the purpose of controlling weight [87]. Patients with identified eating disorders or deliberate misuse of insulin should receive intensive multidisciplinary care that includes a mental health professional with expertise in eating disorders. Use of a basal-bolus insulin regimen allows increased meal flexibility.

Vascular complications

The vascular complications of diabetes are classified as either microvascular (retinopathy, nephropathy, and neuropathy) or macrovascular, which includes coronary artery, peripheral, and cerebral vascular disease. The microvascular complications can develop within 5 years of the onset of type 1 diabetes mellitus but rarely develop before the onset of puberty. Clinically significant macrovascular complications are virtually never seen until adulthood.

Intensive glycemic control decreases the risk of microvascular disease, as demonstrated by the marked reductions in the risks of retinopathy, nephropathy, and neuropathy observed in the intensively treated cohort of the DCCT. In addition to hyperglycemia, several other modifiable risk factors contribute to and influence the risk of vascular complications. Use of tobacco considerably increases the risk of onset and progression of retinopathy, nephropathy, and macrovascular disease. Hypertension, likewise, is associated with increased risk and rate of progression of retinopathy, nephropathy, and macrovascular disease. Dyslipidemia (increased ratio of LDL to HDL cholesterol), greatly contributes to the risk of macrovascular disease and may increase retinopathy. A family history of hypertension or nephropathy increases the risk of nephropathy.

Retinopathy

Diabetic retinopathy damages the microvasculature of the retina and is the most common cause of acquired blindness in economically developed countries. Although improvement in glycemic control delays the onset of retinopathy and retards its progression, nearly all individuals with diabetes will eventually develop mild non-proliferative retinopathy. This may progress to moderate or severe non-proliferative retinopathy, characterized by abnormal blood flow in the retinal microvasculature. Proliferative retinopathy, characterized by growth of new vessels, carries a high risk of visual loss due to hemorrhage or retinal detachment. Macula edema may occur at any stage of retinopathy and threaten visual acuity. Screening detects early disease and leads to effective treatment with laser retinal photocoagulation before vision is impaired.

Nephropathy

Diabetic nephropathy is the most common cause of endstage renal disease in western countries and eventually occurs in 30–40% of persons with type 1 diabetes. Improving glycemic control and treatment of hypertension, if present, delays the onset of nephropathy and slows its progression. Microalbuminuria, defined as ≥ 30 mg/day or ≥ 20 μg/min albumin in the urine, is the earliest stage of clinical nephropathy. Sustained microalbuminuria is highly predictive of progression to overt nephropathy (clinical albuminuria), defined as ≥ 300 mg/24 h or ≥ 200 μg/min albumin in the urine, but microalbuminuria may be less predictive in adolescents during the first decade of diabetes. Overt albuminuria is accompanied by systemic hypertension and progressive impairment of glomerular filtration, and typically precedes the development of endstage renal disease by 10 years.

Progression of nephropathy can be delayed by improving glycemic control, controlling hypertension, if present, and by treatment with an angiotensin-converting enzyme (ACE) inhibitor. If an ACE inhibitor is used, it is important to monitor for hyperkalemia.

Neuropathy

Clinically significant neuropathy resulting from diabetes is exceedingly rare in the pediatric age range [89]. Early signs of peripheral neuropathy include loss of ankle reflexes and decreased vibration sense or touch sensation to monofilament in the great toe. Although sensitive cardiovascular testing may detect subtle autonomic abnormalities in some adolescents with diabetes, they tend to be transient and are of unknown clinical importance. As with other microvascular complications, improvements in HbA1c decrease the risk of onset of neuropathy.

Macrovascular

Both men and women whose diabetes commences in childhood are at high risk of macrovascular disease. Women with type 1 diabetes lose the protective effect of their gender. Although the absolute risk is low until shortly before age 30 years, macrovascular events are the most common cause of mortality in persons with type 1 diabetes, and individuals with renal complications have an especially high risk. Other predictors of macrovascular risk and/or progression include dyslipidemia, hypertension, and smoking [90].

It is not yet clear to what extent intensive glycemic control reduces the risk of macrovascular events. No single study, including the DCCT, has been able to answer this question satisfactorily, but a meta-analysis concluded that intensive diabetes therapy does protect against macrovascular events [91]. Long-term progression of carotid artery intimal thickening is slower in the intensively treated cohort of the DCCT [90]. Strategies to reduce lifetime risk of macrovascular disease in children with diabetes include avoiding use of tobacco, early and vigorous treatment of hypertension, treatment of dyslipidemia, and intensive glycemic control.

Screening for long-term complications [89]

Development of diabetic complications is insidious but can usually be detected years before the patient has symptoms or organ function is impaired. Systematic screening can detect abnormality at an early stage when intervention to arrest, reverse, or retard the disease process will have the greatest impact. Diabetic retinopathy is rare before the onset of puberty or in patients who have had type 1 diabetes for less than 5 years. Therefore, an annual dilated retinal examination by an ophthalmologist should begin 3–5 years after diagnosis once the child is age 10 years or older [37]. Temporary rapid progression of retinopathy may occur when metabolic control improves drastically and, in these circumstances, retinal examinations should be performed more frequently.

Similarly, after the child has had diabetes for 5 years, an annual screening measurement of urine albumin and creatinine concentrations should be performed to detect microalbuminuria [37]. Several methods can be used to screen for microalbuminuria. The most convenient is to measure the albumin-to-creatinine ratio in a random spot urine specimen. First-void collections upon arising in the morning avoid the confounding effect of increased albumin excretion induced by upright posture. Timed collections, either 24 h or timed overnight, are more accurate but less convenient than spot samples. Standard hospital laboratory assays for urinary protein are not sufficiently sensitive, and measurement should be performed by an assay that specifically detects microalbuminuria. Albumin excretion is transiently elevated by hyperglycemia, exercise, and febrile illness. Because of marked day-to-day variability in albumin excretion, microalbuminuria should be confirmed in at least two of three collections over a 3- to 6-month period to establish the diagnosis of diabetic nephropathy and before instituting treatment. Circulatory and neurologic complications of diabetes are seldom clinically significant during childhood and adolescence.

In contrast to the above recommendations for type 1 diabetes in children, monitoring lipids, urinary albumin excretion, and screening eye examinations should begin at diagnosis in type 2 diabetes [55].

Conclusion

In 1993, the DCCT recommended that most youth with diabetes should receive intensive therapy. Technological innovations since then, including better pumps and insulin analogs that facilitate more physiologic insulin replacement, have made it possible to achieve tighter blood glucose control with reduced risk of severe hypoglycemia in children and adolescents with diabetes. Increased use of more physiologic and flexible insulin regimens together with frequent blood glucose monitoring, carbohydrate counting, and patient empowerment has made it possible to insure normal growth and development and safely to achieve levels of blood glucose control that were previously unattainable. It is reasonable to expect that the benefits of sustained improvement in glycemic control will prevent or, at least, delay the appearance of the chronic complications of diabetes. Epidemiologic data provide evidence that this is already the case [92]. The arduous and incessant task of controlling blood glucose in a child is difficult and frustrating, and the risk of hypoglycemia is always present. Members of the diabetes team must set realistic and attainable goals for each patient while constantly providing encouragement and support. The resources of a multidisciplinary health care team in collaboration with the

child's primary care physician are essential for the successful management of childhood diabetes. Unfortunately, in the past decade, type 2 diabetes has emerged as a major new challenge for those who provide care for children with diabetes.

Appendix 1 Fluids used for oral hydration with sick day management

Product	Carbohydrate (g/100 mL)	Na^+ (mmol/L)	K^+ (mmol/L)
Coca-Cola®	10.9	4.3	0.1
Ginger ale	9.0	3.5	0.1
Apple juice	11.9	0.4	26
Orange juice	10.4	0.2	49
Gatorade	5.9	21	2.5
Pedialyte	2.5	45	20
Milk	4.9	22	36
Broth/bouillon	0	129–149	≈ 8

Appendix 2 Sources of carbohydrate

The following all have approximately 15 g of carbohydrate

Apple juice 4 oz or 1/2 cup, unsweetened apple sauce 1/2 cup
Grape juice 3 oz or 1/3 cup
Orange juice 4 oz or 1/2 cup
Coca-Cola® and ginger ale 5 oz (≈ 2/3 cup)
Twin popsicle 1
Regular jello 1/2 cup
Regular ice-cream 1/2 cup
Honey 1 tablespoon
Cake frosting 4 teaspoons
Table sugar 1 tablespoon
Glucose tablets 3 (each contains 5 g)
Lifesavers® 6
Saltines 6

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22 Hypoglycemia

Khalid Hussain and Mark J. Dunne

Introduction

Hypoglycemia is a common metabolic and endocrine abnormality in infancy and childhood. Despite its high prevalence, there is controversy about the definition of hypoglycemia, the association between brain function and blood glucose concentrations, and management.

Hypoglycemia results in reduced supply of glucose to vital organs, especially the brain for which a continuous supply of glucose is essential. Recurrent and persistent hypoglycemia can cause significant morbidity and mortality, with the risk of sudden death or long-term neurological damage. Although glucose is obviously first-line treatment, definitive management depends on understanding the cause.

The definition of hypoglycemia

The definition of hypoglycemia, especially in newborns, is confusing and controversial [1] because there is poor correlation between plasma glucose concentrations, the onset of clinical symptoms, and the long-term neurological sequelae. It is difficult to define a blood glucose level that will require intervention (especially in neonates) as there is uncertainty over the level and duration of hypoglycemia that can cause neurological damage.

Four different approaches have been used to define hypoglycemia [1]. It may be defined statistically, with the disadvantage that blood glucose levels below a certain centile are not always associated with the presence or absence of symptoms. It may be defined functionally, relating hypoglycemia to evidence of concurrent physiological counter-regulatory hormone responses or to neurological dysfunction. In this case, there are few data on counter-regulatory hormonal responses to specific blood glucose levels in different subjects. Thirdly, it may be defined by symptoms in relation to a specific blood glucose level, but the disadvantage here is that there is poor correlation between symptoms and blood glucose concentrations. The fourth approach is based on the neurophysiological responses to falling blood glucose concentrations [2].

These data have led to the proposal that hypoglycemia should be defined as a concentration less than 2.6 mmol/L, as measured with a laboratory research method. Hawdon *et al*. [3], however, have shown that around 20% of normal full-term infants have blood glucose concentrations less than this in the first 48 h. These infants show concurrent hyperketonemia, and the assumption, which still needs to be proved, is that the babies will not demonstrate neural dysfunction because of the protective effect of the availability of alternative fuels. In short, hypoglycemia is a continuum, and the blood glucose concentration should be interpreted in the context of the clinical presentation, counter-regulatory hormonal responses, and in relation to the intermediate metabolites.

Importance of diagnosis

Hypoglycemia is of particular importance in childhood since it is a potent cause of neurological damage when it is persistent or recurrent [4]. Even mild hypoglycemia, at levels that were previously thought to be innocuous, may in fact be associated with serious long-term effects in preterm infants [5], so diagnosis and treatment of hypoglycemia are important. Hypoglycemia can be due to many causes (Table 22.1).

When confronted with a child with hypoglycemia, it is critical to obtain a blood sample for detailed investigations before giving enteral feeds or intravenous glucose, because this sample enables the defect in the many metabolic and endocrine pathways involved in the etiology of the hypoglycemia to be identified. A urine sample should be saved at the same time. The substances to be measured are shown in Table 22.2; Table 22.3 lists the more detailed investigations required if there is a clinical or biochemical clue from the preliminary investigations of the cause of the hypoglycemia.

The conditions of collection and storage have an important effect on the measured blood glucose concentration. Whole blood glucose level may be up to 15% lower than that seen in plasma. The methodology used for the measurement of

Table 22.1. Summarizing the different causes of hypoglycemia in the neonatal, infancy, and childhood period.

Hyperinsulinism
Transient: "infant of diabetic mother/perinatal asphyxia/rhesus disease/ intrauterine growth retardation/Beckwith–Weidemann syndrome/ "idiopathic"
Congenital: SUR1/KIR6.2/glucokinase/glutamate dehydrogenase mutations Defects in the metabolism of fatty acids (SCHAD) Carbohydrate-deficient glycoprotein syndrome (CDG) Insulinomas
Hormonal deficiency
Cortisol/growth hormone/ACTH/glucagon/epinephrine
Defects in hepatic glycogen release/storage
Glycogen storage diseases: glucose-6-phosphatase, amylo 1–6 glucosidase deficiency, liver phosphorylase deficiency, hepatic glycogen synthase deficiency
Defects in gluconeogenesis
Fructose-1,6-bisphosphatase deficiency, phosphoenolpyruvate carboxykinase (PEPCK) deficiency, pyruvate carboxylase deficiency
Defects of fatty acid oxidation and carnitine metabolism
Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency
Short-chain acyl-CoA dehydrogenase (SCAD) deficiency
Long-/short-chain-L-3-hydroxy-acyl CoA (L/SCHAD) deficiency
Carnitine deficiency (primary and secondary)
Carnitine palmitoyltransferase deficiency (CPT 1 and 2)
Defects in ketone body synthesis/utilization
HMG CoA-synthase deficiency/HMG CoA-lyase deficiency
Succinyl-CoA: 3-oxoacid CoA-transferase (SCOT) deficiency
Metabolic conditions (relatively common ones)
Organic acidemias (propionic/methylmalonic)
Maple syrup urine disease, galactosemia, fructosemia, tyrosinemia
Hereditary fructose intolerance
Glutaric aciduria type 2
Mitochondrial respiratory chain complex deficiencies
Drug induced
Sulfonylurea/insulin/beta-blocker/salicylates/alcohol
Miscellaneous causes (mechanism may not be so clear)
Idiopathic ketotic hypoglycemia (diagnosis of exclusion)
Infections (sepsis, malaria), congenital heart disease

blood glucose will also give different results. Bedside measurement of blood glucose by reagent strip tests used with or without reflectance meters is notoriously imprecise at low blood glucose concentrations, whereas more sophisticated cuvette-based methods may also have a substantial error limit. Knowledge of the method of assay for blood glucose is essential for interpreting clinical data. For measurement of blood glucose, blood samples are collected into fluoride-containing tubes to inhibit glycolysis.

Table 22.2. The intermediary metabolites and hormones to be measured in the initial investigations of hypoglycemia.

Blood	Urine
Glucose	Ketones
Insulin	Reducing substances
Cortisol	Organic acids
Growth hormone	
Non-esterified fatty acids	
Acetoacetate	
3β-Hydroxybutyrate	
Carnitine (free and total)	
Blood spot acyl-carnitine	
Ammonia	
Lactate	

Blood glucose homeostasis in the fetus

The fetus receives a constant intravenous supply of glucose across the placenta, fetal glucose uptake being directly related to both the maternal blood glucose concentration and the transplacental gradient. Fetal metabolism is directed toward anabolism, with the formation of glycogen, fat, and protein. Glycogen is stored in the liver during the latter part of gestation and serves as the immediate source of new glucose during the first hours after birth. The enzyme systems for anabolism have been shown to be active with advancing gestational age in fetal liver. As the fetus is constantly supplied with a glucose infusion, it is not dependent upon its own glucose-generating capacity.

Insulin is the main fetal anabolic hormone, and the orderly anatomical and functional development of the hormone-secreting pancreatic islet cell types is of crucial importance for coping with the metabolic crisis faced at birth. Fetal endocrine cells originate from the duct epithelium, the cells migrating to form the functional unit of the endocrine pancreas, the islet of Langerhans. By the 22nd week, well-formed mature islets can be recognized.

The differentiation of the endocrine cell types from a pluripotent stem cell is dependent upon an orderly activation of a sequence of transcription factors that trigger the cell lineage. In addition to anatomical changes in the pancreatic endocrine cells, there are also functional changes. Thus, the fetal β cells show decreased responsiveness to acute changes in glucose and amino acid concentrations *in vivo* and *in vitro* when compared with the adult pancreas. The net effect of the developmental changes is to insure that there is a high insulin–glucagon ratio, which favors anabolism.

Postnatal metabolic and endocrine adaptation

The transition from a transplacental supply of nutrients to intermittent feeding and fasting with the introduction of milk

Table 22.3. More detailed investigations will depend on the possible etiology of the hypoglycemia.

? Hyperinsulinism (or insulin-like action)
C-peptide
Proinsulin, preproinsulin
IGFBP-1 (inverse relation to insulin)
Transferrin isoelectric focusing (especially if hyperinsulinism associated with syndrome)
"Abnormal" IGF-II forms
Insulin autoantibodies
? Hormonal
Growth hormone provocation testing
Glucagon (extremely rare)
Epinephrine/norepinephrine (extremely rare)
? Fatty acid oxidation disorder
Fatty acid flux studies (skin biopsy for fibroblast culture)
? Glycogen storage disease
Cholesterol, triglycerides
Urate, LFTs
? Gluconeogenesis
Leukocyte fructose 1,6 bisphosphatase activity
Liver phosphorylase
? Hepatic glycogen synthesis disease
Pre- and post-prandial blood glucose and lactate profiles
? Ketone body synthesis/utilization disorder
HMG Co-A/succinyl-CoA: 3-oxoacid CoA-transferase (SCOT) mutational analysis
Liver biopsy
? "Metabolic"
Red cell galactose-1-phosphateuridyltransferase activity (galactosemia)
Plasma amino acids (maple syrup urine disease)
Urinary succinylacetone (tyrosinemia)
Transferrin isoelectric focusing (CDG syndromes)
Pyruvate
Acetoacetate
Mitochondrial respiratory chain complex activity
? Unexplained
Urine toxicology (specifically request for the possible offending agent)

feeds into the gut is accomplished by the normal infant at term with little external evidence of the magnitude of the changes taking place. The process of adaptation is, however, incomplete and is compromised when the infant is born prematurely or following intrauterine growth retardation [6].

A normal infant at term shows an immediate postnatal fall in blood glucose concentration during the first 2–4 h from values close to maternal levels to around 2.5 mmol/L, which implies that one or more of the mechanisms required for fasting adaptation are not fully developed at birth [7]. It is now clear that this fall in glucose is necessary to induce the hormonal surges involved in stimulating enzymic activation.

There is a major and abrupt increase in plasma glucagon concentrations within minutes to hours of birth in all mammalian species [8]. This is accompanied by a dramatic surge in catecholamine secretion [9]. Plasma growth hormone (GH) levels are considerably elevated at birth. This changing hormonal milieu is effective in mobilizing glucose from glycogen and substrate delivery through lipolysis and proteolysis. Moreover, the release of free fatty acids into the circulation is followed by an abrupt increase in the production of ketone bodies from the liver. The role of cortisol remains somewhat enigmatic. Severe hypoglycemia occurs if the normal relationship of these hormones is disturbed, but the net effect of the changes is to stabilize the blood glucose concentration at a lower level during the first few hours while milk feeding is initiated. The availability of ketone bodies allows a sparing effect of glucose for brain utilization.

Mechanisms maintaining a normal blood glucose concentration

The maintenance of a normal blood glucose concentration involves a complex interaction between plasma glucose, insulin, and the various counter-regulatory hormones, including glucagon, adrenaline, cortisol, and GH. Glucose metabolism accounts for approximately half of the daily energy needs [10].

Glucose can be stored in the form of glycogen and fat, and it can be used for the synthesis of proteins and structural components such as cell membranes via the recycling of its carbon atom. Normal blood glucose concentration is maintained by a balance between glucose production and glucose utilization, and any factor that alters this equilibrium leads to hypoglycemia. Insulin decreases glucose production and increases glucose utilization, whereas glucagon, adrenaline, cortisol, and GH increase glucose production and decrease glucose utilization (Fig. 22.1) [11].

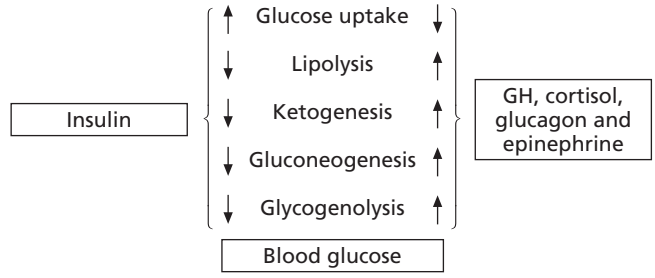


Fig. 22.1. Summarizing the opposing effects of insulin, GH, and cortisol on the regulation of blood glucose. Insulin actions are geared towards lowering blood glucose levels whereas GH/cortisol, glucagons, and epinephrine have the opposite biochemical actions. GH, growth hormone.

Glucose production

In addition to ingestion, glucose can be produced from fat, from protein via gluconeogenesis, and from the release of stored glycogen in the liver. The breakdown of glycogen results in a readily available source of glucose for the brain and other neural tissues, which are obligate glucose users. The liver and, to a small extent, the kidneys are the only two tissues able to release glucose into the circulation. Their ability to do this is dependent upon the fact that they have significant amounts of glucose-6-phosphatase. The liver provides glucose by glycogenolysis (breakdown of stored glycogen) and gluconeogenesis. It is estimated that glycogenolysis accounts for about 30–40% of overall hepatic output of glucose in adults [12]. No data are available for children.

Glycogenolysis occurs as a result of the actions of several enzymes. During fasting, glycogen phosphorylase initiates glycogen breakdown by cleaving glucose-1-phosphate, which is converted to glucose-6-phosphate by a debranching enzyme. Glucose-6-phosphatase then releases free glucose. The metabolism of glycogen is predominantly controlled by the activities of glycogen synthase and phosphorylase, and insulin and glucagon are the major hormones controlling them.

Gluconeogenesis involves the synthesis of glucose from non-carbohydrate sources. The major gluconeogenic precursors after an overnight fast are lactate and alanine (also glutamine, glycerol, and pyruvate). The majority of the lactate and alanine generated after an overnight fast originates from plasma glucose and represents recycling of carbon atoms. The first reaction in gluconeogenesis involves the conversion of pyruvate to oxaloacetate to phosphoenolpyruvate. The second reaction converts fructose-1,6-biphosphate to fructose-6-biphosphate, which is the rate-limiting step for the process of gluconeogenesis. The final step involves the conversion of glucose-6-phosphate to free glucose.

The rate of gluconeogenesis on a body weight basis is greater in children aged 8–9 years than in adolescents aged 14–16 years, but the fraction of glucose production derived from gluconeogenesis is identical between the two groups of subjects [13]. Gluconeogenesis contributes 50% of glucose production in the childhood period.

Glucose utilization

The factors that determine glucose utilization include plasma glucose concentration, the tissue requirement for glucose, the availability of alternative substrates and, in certain tissues, their sensitivity to insulin. Glucose uptake by tissues occurs by facilitated diffusion. The transport of glucose into tissues depends on the specific glucose transporters, GLUT 1–5. GLUT 1 is an insulin-independent transporter found in all cells [14], which is responsible for glucose transport across the blood–brain barrier. GLUT 2 has been represented as the main glucose transporter in liver and pancreatic β cells. GLUT 2 is insulin independent, being a low-affinity transporter not easily saturated even at high plasma glucose concentrations. Tissues that use GLUT 2 as glucose transporter experience a rise in cellular glucose with increases in plasma glucose, which allows the pancreatic β cells and hepatocytes to act as glucose sensors [15]. However, it has also been reported that, in human β cells (as opposed to rodent tissues), GLUT 1 is the major glucose transporter. It is estimated that there is a 100-fold lower abundance of GLUT 2 than GLUT 1 in human vs. rat β cells [16].

GLUT 3 is distributed in the central nervous system and is an insulin-independent glucose transporter that has the highest affinity for glucose [14]. GLUT 4 is an insulin-dependent transporter in muscle and adipose tissue. GLUT 5 is primarily expressed in the jejunal brush border and is mainly a fructose transporter.

Insulin regulates the steady-state concentration of the insulin-dependent transporters by promoting their synthesis, but also causes mobilization of these transporters to the cell membrane when the plasma glucose concentration increases.

Glucose taken up by cells may be stored as glycogen or fat, oxidized to carbon dioxide, or converted to lactate. The proportion of glucose that contributes to these different fates depends upon the degree of fasting, the hormonal milieu, and the presence of alternative energy substrates (Fig. 22.2) [10].

Integration of the changes associated with feeding and fasting

Insulin regulates glucose production and utilization during both the fed and the fasted states. Following the ingestion of a meal, plasma glucose concentration starts to increase within 15 min [17]. This and the stimuli from neurogenic and enteroinsular axes stimulates insulin secretion from the β cells. Levels of plasma glucose peak at around 30–60 min and then decline until absorption is complete, usually about 4–5 h later, with plasma insulin concentrations following a similar time course [17].

After a meal, there is marked suppression of endogenous hepatic glucose production, the magnitude of which is largely determined by the insulin and glucagon responses [18]. Suppression may be up to 50–60%, with about 25 g less glucose being delivered into the systemic circulation.

Post-prandial plasma glucose levels are determined by the balance between the rates of glucose removal from and delivery to the systemic circulation. Lipolysis, ketogenesis, glycogenolysis, and gluconeogenesis are all suppressed postprandially. The tissues mainly responsible for the removal of glucose from the systemic circulation include the liver, small intestine, brain, muscle, and adipose tissue. The magnitude of glucose uptake by the tissues, except for the brain, is determined largely by the plasma insulin concentration. Glucose

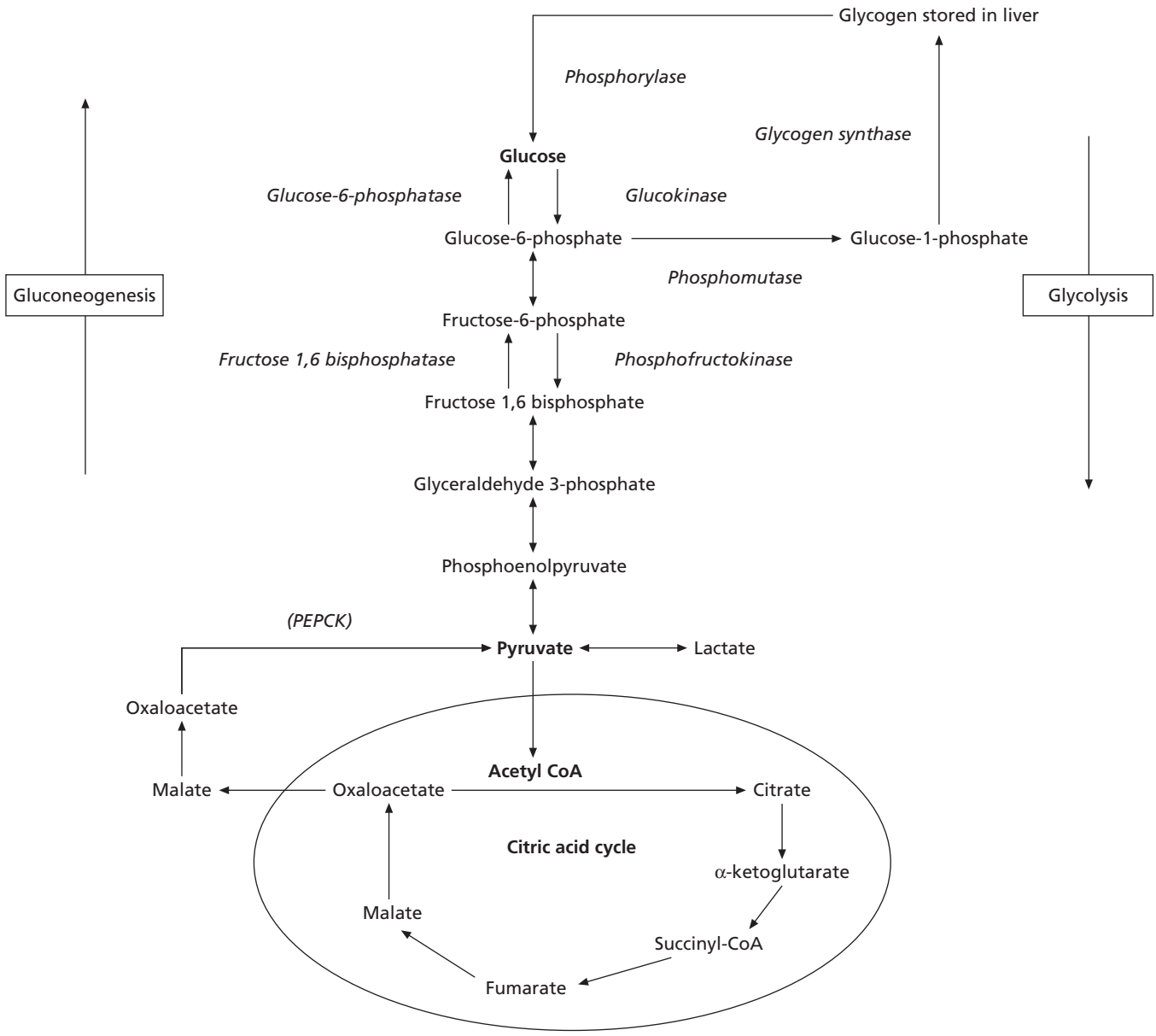


Fig. 22.2. Outline of glucose metabolism. The key gluconeogenic enzymes are shown in gray shaded boxes. Pyruvate plays a pivotal role in intermediary metabolism. PEPCK, phosphoenolpyruvate carboxykinase.

uptake by the brain is determined by the plasma glucose concentration and is independent of insulin (Fig. 22.3).

The 4- to 6-h interval that follows the ingestion of a meal is sometimes referred to as the post-absorptive state. A steady state is reached during it, whereby glucose production equals glucose consumption, and plasma glucose concentrations are maintained within a normal range. Glucose turnover (glucose production and utilization) is then approximately 10 μmol/kg/min [19]. Non-insulin dependent utilization of glucose accounts for 80%, mainly by the brain (which accounts for 50% of the total), red blood cells, kidneys, and the gastrointestinal system [10]. The glucose concentrations are maintained by interactions between insulin and glucagon, cortisol, GH, adrenaline, and noradrenaline. Glucagon allows the controlled release of stored glycogen from the liver; insulin restrains the effects of glucagon by preventing accelerated lipolysis and proteolysis. Cortisol and GH play permissive roles in setting the sensitivity of the peripheral tissues to glucagon and insulin.

As the period of fast lengthens, tissue glucose utilization decreases while utilization of free fatty acids and ketone bodies increases. Hepatic glucose output is reduced by a decrease in glycogenolysis, with an increase in the rate of gluconeogenesis. The increased gluconeogenesis is

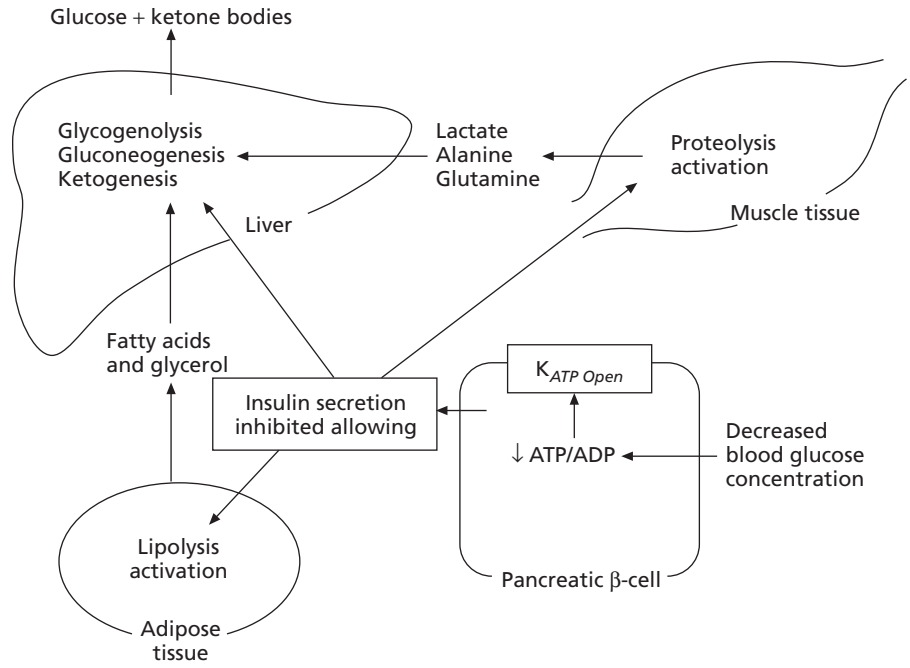


Fig. 22.3. Outline of the biochemical changes that occur during a fast. The decrease in the blood glucose concentration inhibits insulin secretion, which then allows lipolysis, glycogenolysis, gluconeogenesis, ketogenesis, and proteolysis to be activated. These processes generate glucose and ketone bodies.

probably related to the increased secretion of glucagon and other counter-regulatory hormones, as well as the reduction in insulin secretion. Glucagon secretion with reduced insulin allows stored fats to be converted to glycerol and fatty acids and proteins to be converted to amino acids for gluconeogenesis. The liberated free fatty acids are transported to the liver bound to albumin, where they can either undergo β-oxidation in the mitochondria to yield ketone bodies or be re-esterified to triacylglycerols and phospholipids.

Long-chain fatty acids have to combine with a transporter molecule called carnitine forming a fatty acid carnitine ester before they can be utilized in the mitochondria (Fig. 22.4). This allows the fatty acids to diffuse across the outer mitochondrial membrane and be converted to the corresponding acyl-CoA esters in the intermembranous space. Fatty acylcarnitines are formed by the action of carnitine palmitoyltransferase 1 (CPT I) bound to the inner face of the outer mitochondrial membrane [20]. The resultant acylcarnitines then cross the inner mitochondrial membrane in exchange for free carnitine and are converted back to acyl-CoA esters by CPT II [21]. Medium- and short-chain fatty acids can enter the mitochondria without combining with carnitine.

The carnitine–acylcarnitine shuttle is regulated by malonyl-CoA, which modulates the activity of CPT I [22]. In the fed state, the concentration of malonyl-CoA is high, and this inhibits CPT I with flux through the β-oxidation pathway being reduced [21]. Hence, the delivery of fatty acids to the tissues in the fed state is diminished as a result of low rates of lipolysis, and the entry of fatty acids into the mitochondria is also inhibited. In the fasted state, the rate of fatty acid synthesis is low, with low concentrations of malonyl-CoA allowing more efficient transport of fatty acids across the inner mitochondrial membrane.

Once inside the mitochondria, each fatty acid undergoes β-oxidation to shorten progressively the carbon chain. These reactions are catalyzed by intramitochondrial enzymes (acyl-CoA dehydrogenases), each of which acts upon a specific chain length fatty acid, medium, long, and short. β-Oxidation yields acetyl-CoA, which can then either be converted to ketone bodies, acetoacetate, and 3β-hydroxybutyrate via the hydroxymethylglutaryl-CoA (HMG-CoA) pathway or undergo complete oxidation in the tricarboxylic acid cycle (Fig. 22.4).

Muscle and other tissues become progressively more dependent on free fatty acids and ketone bodies for their continued energy requirements as the period of the fast is prolonged. Ketone bodies produced in the liver from the oxidation of fatty acids are exported to peripheral tissues as an energy source. They are particularly important for the brain, which has no other substantial non-glucose-derived energy source. Ketone bodies replace glucose as the predominant fuel for nervous tissue, thereby reducing the obligatory requirement of the brain [23].

Glucose and the brain

Glucose is the major substrate for normal brain function [24]. The entry of glucose into the brain and brain glucose metabolism are not insulin sensitive [25]. The maturation and density of GLUT 1 (located at the blood–brain barrier) and GLUT 3 (at the neuronal membrane) parallel the development of cerebral glucose utilization.

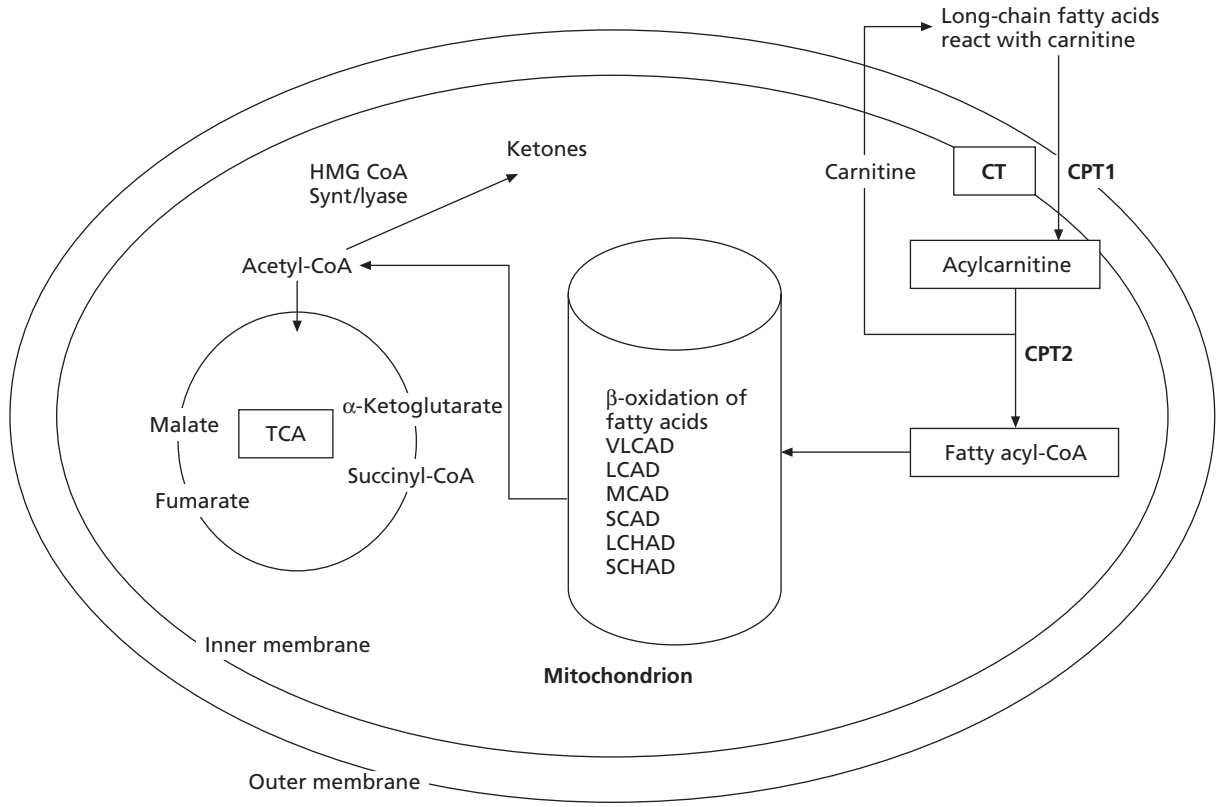


Fig. 22.4. Outline of carnitine and fatty acid metabolism. Long-chain fatty acids require carnitine for transportation into the mitochondria. Once inside the mitochondria, the fatty acyl-CoA undergoes β-oxidation yielding acetyl-CoA. This can then either be converted to ketone bodies or enter the citric acid cycle. CT, carnitine translocase; CPT, carnitine palmitoyltransferase; TCA, tricyclic acid cycle; VLCAD, very-long-chain acyl-CoA dehydrogenase; LCAD, MCAD, SCAD, long-, medium-, and short-chain acyl-CoA enzymes; LCHAD, SCHAD, long- and short-chain L-3-hydroxyacyl-CoA dehydrogenase.

Certain parts of the brain (e.g. the cortex) seem to be more susceptible to hypoglycemic damage than others (e.g. the cerebellum), probably because of regional differences in cerebral metabolic capacity [26]. Positron emission tomography (PET) has shown minute-to-minute regional changes in cerebral glucose consumption and blood flow during a variety of sensory and motor activities in humans. In newborn dogs, the brain can alter regional blood flow to insure that brainstem structures continue to receive adequate glucose compared with other regions [27]. Similar increases in cerebral blood flow have been documented in human preterm infants during hypoglycemia [28].

During moderate acute hypoglycemia in adults, there are no changes in cerebral functional activity; cerebral glucose utilization decreases and blood flow increases only when hypoglycemia is severe (lower than 2 mmol/L) [29]. During chronic hypoglycemia, the brain adapts to the low circulating levels of glucose by increasing the number of glucose transporter sites and decreasing cerebral glucose utilization and function at normal levels while cerebral blood flow is more moderately increased than during acute hypoglycemia.

Neuronal damage due to severe and prolonged hypoglycemia occurs mainly in the cerebral cortex, hippocampus, and caudate putamen as a result of active release of excitatory amino acids. Salient neurochemical changes include an arrest of protein synthesis in many but not all brain regions, a shift of brain redox equilibria toward oxidation, incomplete energy failure, loss of ion homeostasis, cellular calcium influx, intracellular alkalosis, and a release of neuroactive amino acids, especially aspartate, into the extracellular space of the brain [30].

The metabolic release of excitatory amino acids (aspartate and, to a lesser extent, glutamate) into the interstitial space of the brain during profound hypoglycemia allows binding to neuronal dendrites and perikarya, but not to other cell types in the nervous system, thus giving rise to selective neuronal death. The alteration of cerebral mitochondria due to increased free radical production is one of the early events in the pathogenesis of hypoglycemic brain injury [31].

The utilization of alternative substrates may provide another possible mechanism by which the brain protects itself against hypoglycemia. In healthy adults infused with β-hydroxybutyrate during insulin-induced hypoglycemia, the counter-regulatory hormonal response to hypoglycemia is lowered, as is the delay in cognitive dysfunction [32]. These studies are difficult in children, but the human fetus and

neonate can take up and oxidize ketone bodies [33]. The uptake of ketone bodies by the brain is proportional to the circulating concentration, and the uptake is higher in neonates than in adults [33]. Oral supplementation of DL sodium β-hydroxybutyrate has been given to two patients with persistent hyperinsulinemic hypoglycemia, demonstrating effective uptake across the blood–brain barrier [34].

Differences in blood glucose regulation between children and adults [35]

Young children have limited glycogen stores, adequate for a period of starvation of only approximately 12 h, after which the maintenance of a normal blood glucose concentration is dependent on gluconeogenesis. Children fasted for 30 h had the lowest glucose and alanine concentrations compared with adult men and women [36], and children are unable to tolerate prolonged periods of starvation for this reason. In adults, levels of free fatty acids, glycerol, and ketones in the blood gradually increase as the period of starvation is extended [37]. During a brief period of fasting in children, ketosis and ketonuria develop readily, suggesting that children convert more rapidly to a fat-based fuel economy [38].

Children have higher glucose production rates in comparison with adults in order to meet the increased metabolic demands of the brain because the brain relative to body size is much larger than in adults. Brain size is the principal determinant of factors that regulate hepatic glucose output throughout life [39]. Fasting newborn and young children demonstrate a high glucose utilization rate per kg body weight relative to adult requirements [40], which is why they are more susceptible to hypoglycemia than adults.

The diagnostic approach

Clinical history, description of symptoms, physical examination, and a step-by-step approach are the cornerstones of diagnosis. Given the complexity of the metabolic and endocrine adaptations that occur at birth, hypoglycemia occurs more frequently during the first days than at any other time of life. This is transient in the majority of cases, but symptoms may be very non-specific (Table 22.4), so any child with symptoms must have a blood glucose level measured and documented.

The clinical history in the neonatal period should include details of pregnancy and delivery, birthweight, gestational age of the infant, noting in particular any evidence of fetal distress, birth asphyxia, and smallness for dates. The relationship of a hypoglycemic episode to the most recent meal can be important. Hypoglycemia occurring after a short fast (2–3 h) may suggest a glycogen storage disease, but hypoglycemia occurring after a long fast (12–14 h) may suggest a disorder of gluconeogenesis. Post-prandial hypoglycemia may indicate galactosemia, hereditary fructose intolerance, or the dumping syndrome. A family history of sudden infant deaths may be a clue to an unrecognized, inherited metabolic disorder. Any provocation factors such as an upper respiratory tract infection or an episode of gastroenteritis leading to hypoglycemia should be documented. From the history and physical examination, certain groups of infants at risk of transient hypoglycemia who need monitoring can be identified (Table 22.5).

The recognition and diagnosis of hypoglycemia in the neonatal period depends on routine monitoring of blood glucose levels at frequent intervals after birth in asymptomatic infants at risk and in any infant who demonstrates a symptom that might suggest hypoglycemia. It is important to monitor blood glucose in relation to the time of feeds. A blood glucose concentration that increases after a feed is less worrying than one that is persistently low. If low concentrations are obtained during routine monitoring in asymptomatic high-risk infants or at the time of symptoms in symptomatic infants, the result should be confirmed in the laboratory, but intervention does not need to wait for the result. Resolution

Table 22.4. The symptoms of hypoglycemia may be very non-specific.

Any non-specific symptom may indicate hypoglycemia
Feeding poorly
Irritability
Lethargy
Stupor
Apnea, cyanotic spells
Hypothermia
Hypotonia, limpness
Tremor
Seizures
Coma

Table 22.5. Groups at risk from hypoglycemia.

Premature infants
Intrauterine growth-retarded infants
Infants born to diabetic (insulin-dependent and gestational) mothers
Infants subjected to perinatal asphyxia
Infants born with erythroblastosis fetalis
Infants with Beckwith–Wiedemann syndrome
Maternal administration of some drugs such as sulfonylureas/beta-blockers
Macrosomic infants
Any "sick" infant
Polycythemic infants
Hypothermic infants
Infants with congenital heart disease
Infants with "metabolic" conditions
Older children with infections such as malaria

of symptoms after glucose confirms that they were due to hypoglycemia.

The presence or absence of maternal diabetes or rhesus incompatibility are important. Increased birthweight and macrosomia should raise the possibility of neonatal hyperinsulinism. Distinctive physical signs such as transverse ear lobe creases, exomphalos, and macroglossia should raise the possibility of the Beckwith–Wiedemann syndrome, whereas the presence of micropenis and undescended testes might indicate the presence of hypopituitarism. Midline defects, including cleft palate, could indicate congenital hypopituitarism, while ambiguous genitalia could indicate congenital adrenal hyperplasia.

Hepatomegaly should always be sought and is associated with abnormal glycogen metabolism, defects in gluconeogenesis, and galactosemia. Moderate hepatomegaly due to glycogen accumulation may, however, develop in infants with hyperinsulinism who are receiving high infusion rates of glucose to maintain normoglycemia. Particular attention should be paid to the rate of growth, micropenis, undescended testes, skin pigmentation, blood pressure, and weight loss in childhood.

The diagnostic fast

In many conditions, hypoglycemia occurs only in relation to periods of low caloric intake or starvation. Starvation tests are potentially very dangerous and must be conducted only under strictly controlled conditions by staff experienced in their administration, with a secure intravenous infusion available for immediate correction of hypoglycemia [41]. The hazards are greatest in defects in fatty acid oxidation, as the induced hyperfatty acidemia carries a risk of inducing a cardiac arrhythmia. Sequential measurements of intermediary metabolites and glucose are taken throughout the fast, with the crucial blood sample being drawn when hypoglycemia occurs. The urine sample passed then or after restoration of normoglycemia should be deep frozen for measurement of organic acids and other abnormal metabolites.

Other measurements from this specimen obtained at the time of hypoglycemia can help in diagnosis. Cortisol deficiency may be revealed, and further tests to define the integrity of the hypothalamo–pituitary–adrenal axis are thus mandatory. Low GH levels at the time of fasting hypoglycemia do not exclude or confirm deficiency [42] but, with abnormal growth, a validated test, such as glucagon provocation, should be performed [41].

The documentation of abnormal urinary organic acids is particularly helpful when hypoglycemia is due to methylmalonic acidemia, maple syrup urine disease (MSUD), or mitochondrial β-oxidation defects. In the last case, clues to the deficient enzymes are provided by the chain length of the dicarboxylic acids in the urine and the presence of hydroxyl groups or unsaturated bonds. The presence of urinary glycine conjugates may also be diagnostic of fatty acid oxidation defects.

Overview of the different causes of hypoglycemia in childhood

Hypoglycemia in childhood can be due to many causes broadly divided into those resulting from hormonal abnormalities (hyperinsulinism, cortisol, or GH deficiency) or defects of hepatic glycogen release/storage, gluconeogenesis, carnitine metabolism, fatty acid oxidation, and unknown causes, such as idiopathic ketotic hypoglycemia (Table 22.1).

Hormonal abnormalities

Hyperinsulinism of infancy (HI) is the commonest cause of recurrent and severe hypoglycemia at this time of life [43]. It is characterized by excessive and inappropriate secretion of insulin in relation to the prevailing blood glucose concentration and can be transient or persistent.

The transient form is associated with maternal diabetes mellitus, intrauterine growth retardation, perinatal asphyxia [44], erythroblastosis fetalis [45], Beckwith–Wiedemann syndrome, administration of some drugs (e.g. sulfonylureas) to the mother, and after intravenous maternal glucose infusions during labor. It may be idiopathic [46].

A connection between hyperlactatemia and severe transient neonatal hyperinsulinism has also been recognized in non-asphyxiated infants [47], but the mechanism is not clear, and the HI tends to resolve spontaneously. Iatrogenic hyperinsulinism due to a malpositioned umbilical artery catheter was reported in two infants [48]. Repositioning of the catheter to avoid direct infusion into the arterial blood supply to the pancreas resulted in prompt cessation of hyperinsulinemic hypoglycemia. Transient HI is seen most commonly in the infant born to a poorly controlled diabetic mother.

The infant of a diabetic mother (IDM) shows macrosomia and organomegaly attributed to fetal hyperinsulinemia. Glucose crosses the placenta by facilitated diffusion, thereby imposing upon the fetus a carbohydrate surplus to which it responds with increased secretion of insulin. Because insulin is anabolic, the fetal hyperinsulinemia stimulates protein, lipid, and glycogen synthesis, which leads to macrosomia. The underlying mechanism is unclear. Insulin is thought to play a role, but its molecular basis or whether it acts in conjunction with other growth factors is not known. Studies in sheep have demonstrated that fetal hyperinsulinemia regulates the magnitude of the mitogenic cellular responses to growth factors, with insulin acting as a priming factor for insulin growth factors [49].

The relationship between maternal diabetes control and macrosomia is not close [50], and some infants may still be

born with macrosomia after a pregnancy in which maternal blood glucose levels have been apparently well controlled [46]. The physical appearance of these infants is striking. The organomegaly is selective in the liver and the heart; skeletal length is increased in proportion to weight, but brain size is not increased relative to gestational age so the head may appear disproportionately small. The hypertrophic cardiomyopathy of the IDM is transient.

Congenital anomalies in IDM occur two to four times more frequently than in the general population [51]. The problem has become more compelling because the perinatal mortality of IDMs has fallen, and malformations now account for a large proportion of perinatal losses, replacing respiratory distress syndrome as the leading cause of death [52]. The cause of diabetic embryopathy is not understood. It probably arises from the perturbed intrauterine environment during the period of organogenesis. The teratogenic effect of hyperglycemia has been suggested by human and animal studies [53]. Of particular significance is the syndrome of caudal regression, in which agenesis or hypoplasia of the femora occurs in conjunction with agenesis of the lower vertebrae (sacral agenesis). Other anomalies include anencephaly, meningomyelocele, holoprosencephaly, a number of structural abnormalities of the heart, and the small left colon syndrome.

Most IDMs have a transient asymptomatic hypoglycemia before a spontaneous increase in blood glucose levels occurs after the age of 1–4 h. Others have more prolonged and severe symptomatic hypoglycemia, and a minority develop late hypoglycemia after a benign course initially. All regain normal blood glucose control within the first few days after birth. IDMs have hyperinsulinism at birth due to increased placental transfer of glucose and other nutrients submitting increased insulin secretion. The pancreas shows hyperplasia and hypertrophy in the Islets of Langerhans, without evidence of so-called "nesidioblastosis." Some infants fail to develop the normal increase in plasma glucagon at 2–4 h of age [54] although others have demonstrated a substantial counter-regulatory hormone response which may curtail the period of hypoglycemia.

In relation to management, peripheral blood glucose values should be monitored 3- to 4-hourly before feeds for 6–12 h after birth. Transient hypoglycemia may be prevented by giving enteral feeds with milk within 1–2 h after delivery. Sick infants unable to tolerate enteral feeding or those who remain hypoglycemic despite full enteral feeds should receive an intravenous infusion of glucose at a rate of 4–6 mg/kg/min in the first instance to prevent the development of hypoglycemia. Slow withdrawal of glucose support should then be instituted. A single injection of glucagon (0.03–0.1 mg/kg) may have a temporary hyperglycemic effect by releasing glucose from glycogen stores. Reactive hypoglycemia may occur after glucagon or if glucose infusion rates are decreased too quickly.

Beckwith–Wiedemann syndrome (BWS) is a congenital overgrowth syndrome that is clinically and genetically heterogeneous. Phenotypically, BWS is associated with pre- and postnatal overgrowth, organomegaly, hemihypertrophy, omphalocele, ear lobe anomalies, and renal tract abnormalities with predisposition to embryonal tumors. Genetically, BWS is a multigenic disorder caused by dysregulation of imprinted growth regulatory genes within the 11p15 region [55]. At this location, genetic imprinting with loss of maternally expressed tumor and/or growth suppressor genes (p57KIP 2 and H19) or duplications and uniparental disomy of paternally expressed growth promoter genes (IGFII) have been implicated in the pathogenesis of BSW [56]. About 20% of patients with BWS have paternal uniparental disomy for 11p15 [57].

The incidence of hyperinsulinemic hypoglycemia in children with BWS is about 50% [58]. This can be transient or prolonged and is asymptomatic in the majority of infants, resolving within 3 days of life; about 5% of children have hyperinsulinemic hypoglycemia beyond the neonatal period requiring either continuous feeding or a partial pancreatectomy [59]. The milder forms respond to diazoxide and somatostatin analogs [59]. There are few histological studies of the pancreas in patients with BWS, and all suggest that the histology may be similar to the diffuse form of hyperinsulinism of infancy [60,61]. The underlying mechanism(s) leading to persistent hyperinsulinemic hypoglycemia in this syndrome is/are unclear. It is not clear why the hyperinsulinism of this syndrome is usually transient. Children with BWS and severe medically unresponsive hyperinsulinemic hypoglycemia may have defects in pancreatic β-cell ATP-sensitive K^+ channels as a mechanism leading to unregulated insulin secretion [62].

Congenital hyperinsulinism of infancy is by far the most difficult to manage clinically. It is associated with a high incidence (up to 25%) of neurological handicap, which has not changed over the course of the last 20 years. The hyperinsulinism causes hypoglycemia primarily as a result of increased utilization of glucose with a decreased rate of endogenous glucose production. These effects are entirely due to inappropriate secretion of insulin, which has been called a variety of names, including idiopathic hypoglycemia of infancy, leucine-sensitive hypoglycemia, neonatal insulinoma, microadenomatosis, focal hyperplasia, nesidioblastosis, and persistent hyperinsulinemic hypoglycemia of infancy (PHHI) [63].

Sporadic and familial variants of congenital hyperinsulinism of infancy are recognized, with sporadic forms being relatively uncommon (incidence 1 per 40 000 live births) [64] and familial forms being common (as high as 1 in 2500 live births) in communities with high rates of consanguinity [65].

The condition presents in the newborn period or during the first 2–6 months after birth in term and preterm neonates [66]. Many neonates have a characteristic appearance strikingly resembling that of an infant of a diabetic mother. This

suggests that the hyperinsulinism has been present for some time before birth. Very rarely, HI may present in an older child when it is likely to be due to an insulinoma [67].

The characteristic metabolic and endocrine profile of a blood sample drawn at the time of hypoglycemia is hyperinsulinemia, hypoketotic, hypofatty acidemic hypoglycemia with inappropriately raised insulin accompanied by high concentrations of C-peptide levels. High intravenous infusion rates of glucose may be required to maintain a blood glucose concentration above 3 mmol/L. Because of the anabolic effects of insulin, the hypoglycemia occurs despite a liver engorged with glycogen that can be mobilized by administration of glucagon. The glycemia can usually be improved by an infusion of somatostatin that will switch off insulin secretion [68]. Neonates with hyperinsulinemic hypoglycemia fail to generate an adequate serum cortisol counter-regulatory hormonal response, which appears to be related to inappropriately low plasma adrenocorticotrophic hormone (ACTH) concentrations at the time of hypoglycemia [69].

The level of insulin in the blood may not be particularly high, but what is an appropriate insulin concentration for normoglycemia becomes inappropriate in the presence of hypoglycemia [70]. The demonstration of any measurable insulin in a hypoglycemic sample is strong evidence for a failure of basal insulin control.

The immediate imperative of management is to give glucose sufficient to maintain blood glucose concentrations above 3 mmol/L. Infusion rates in excess of 4–6 mg/kg/min, even > 20 mg/kg/min, may be necessary. Having stabilized the blood glucose concentration, it is imperative to determine whether the patient will respond to conventional medical therapy with diazoxide and a thiazide diuretic. Both drugs should be given concurrently to overcome the tendency of diazoxide to cause fluid retention and to capitalize on the fact that the drugs have synergistic effects in increasing blood glucose concentration [71]. A convenient starting dose of diazoxide is 5–10 mg/kg/day in three 8-hourly aliquots, increasing to a maximum of 20 mg/kg/day.

In the light of understanding the defect in the molecular physiology of congenital hyperinsulinism [72], the use of a calcium channel blocking agent, such as nifedipine, has been promoted [73], with some patients showing good response [74]. However, as some patients also have impairments in voltage-gated calcium entry, nifedipine may not always be beneficial. Glucagon given by continuous infusion (starting dose 1.0 μg/kg/h) concurrently with the somatostatin analog octreotide (initial dose 10 μg/kg/day) may confer substantial benefit [71].

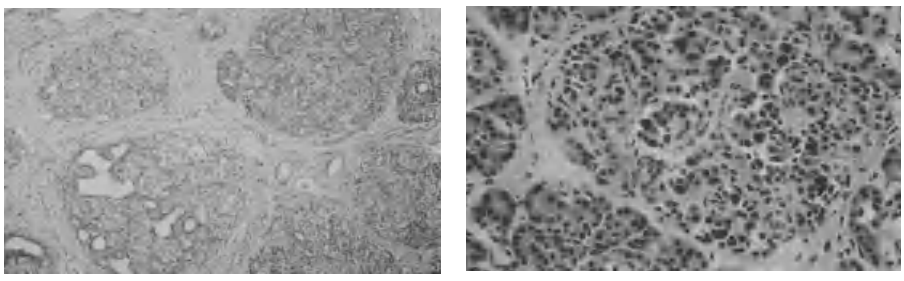
The pediatrician is faced with an important challenge when managing a child who proves to be unresponsive to conventional therapy with diazoxide. The options are either to contemplate the long-term combined continuous subcutaneous infusion of glucagon and somatostatin or to consider surgical resection of the pancreas. Few centers have experience of the first of these treatments, and the practical aspects of the management may be considerable [68].

Partial pancreatectomy is, however, not without risk and not a procedure to be undertaken lightly. The operation most commonly performed is a 95% pancreatectomy in the first instance, but some children still remain hypoglycemic, and a further attempt can then be made to control the procedure with diazoxide. In a minority of cases, total pancreatectomy may be necessary to control the severe hyperinsulinism, which may be exacerbated by regeneration of the pancreatic remnant.

HI has been classified into diffuse and focal disease (Fig. 22.5). The recognition of "focal" disease has led to performing preoperative percutaneous transhepatic pancreatic vein catheterization with the withdrawal of multiple blood samples to identify "hotspots" of insulin secretion. Rapid-frozen sections are used to identify areas of focal hyperplasia at surgery, which are then resected [61]. It has been proposed that the acute insulin responses to intravenous glucose, calcium, and tolbutamide may help to differentiate focal from diffuse disease [75]. These tests are based on the principle that children with diffuse SUR1 defects (see below) fail to show an insulin secretory response to intravenous tolbutamide but show a positive insulin response to intravenous calcium. The results of these tests can be difficult to interpret, and there is poor correlation between phenotype and genotype [76].

^{18}F-fluoro-L-dopa PET has been used successfully to localize the focal domain [77]. This has many advantages over the highly invasive pancreatic venous sampling and intra-arterial calcium stimulation tests.

A number of histological appearances have been proposed to explain the disease, including focal hyperplasia, diffuse



(a) (b)

Fig. 22.5. (a) Normal pancreatic histology with islets showing small nuclei. (b) Typical histology of diffuse hyperinsulinism with islets showing enlarged nuclei.

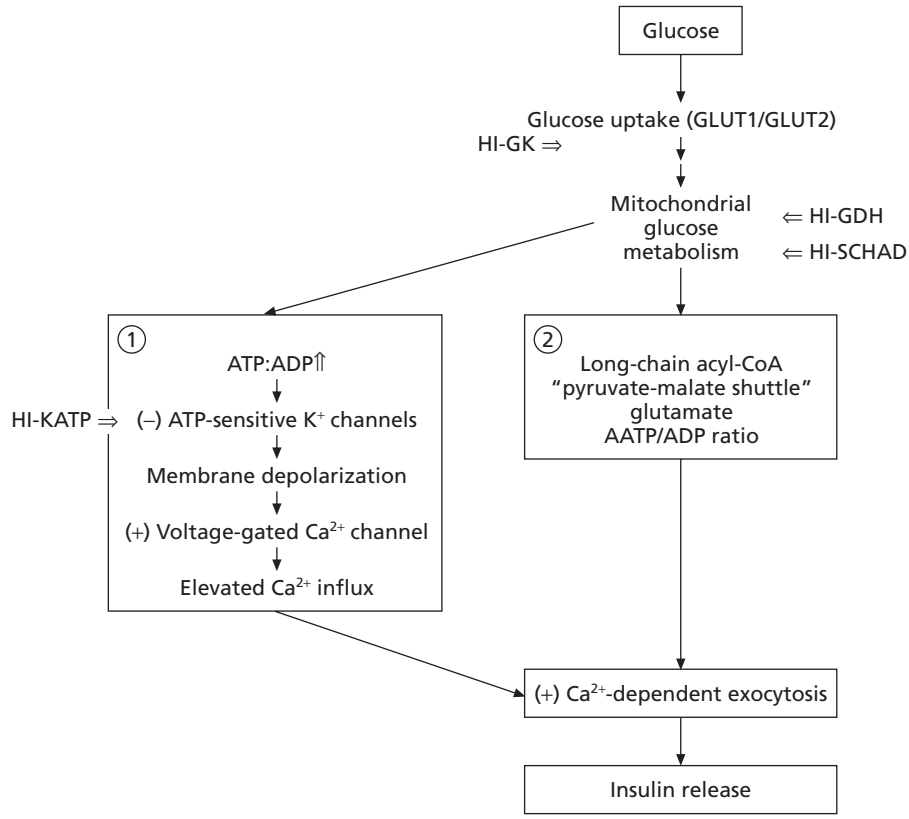


Fig. 22.6. Overview of stimulus secretion coupling in pancreatic β cells. Mitochondrial metabolism is responsible for the generation of signals associated with both first-phase insulin releases – "triggering pathway" (Box 1) and the second-phase responses – "amplification" pathway (Box 2). At the level of the β cells, HI is caused by gene defects in K_{ATP} channels, glucokinase, glutamate dehydrogenase, and SCHAD. SCHAD, short-chain L-3-hydroxyacyl-CoA dehydrogenase; G, glucokinase; GDH, glutamate dehydrogenase.

hyperplasia, ductoinsular proliferation, microadenomatosis, isolated insulinoma, and endocrine dysfunction with no histological abnormality. That the disease is more complicated than a maturation arrest was first emphasized by demonstrating dysregulation of glucose-insulin stimulus secretion coupling *in vitro* in islets obtained from a resected pancreas [70]. Recent studies have now provided an explanation for this finding.

The stimulus "response coupling" event is controlled by potassium channels in the β-cell membrane that are sensitive to intracellular nucleotides, in particular the ratio between ATP and ADP. As the intracellular glucose concentration increases, β-cell glycolysis increases the ratio of ATP to ADP. This closes the potassium ATP-sensitive channel, resulting in depolarization of the β-cell membrane. This phenomenon leads to the influx of calcium through voltage-gated channels, which triggers exocytosis [78]. Thus, the potassium channel functions as a "on/off" switch for triggering insulin secretion (Fig. 22.6).

Potassium ATP channels consist of a heteromultimeric complex of at least two proteins designated SUR1 and Kir6.2 [79]. The functional integrity of these proteins is necessary for potassium channel movement, and the genes responsible for them have been localized very close to each other on the short arm of chromosome 11(11p14–15.1). A number of mutations in the SUR1 and Kir6.2 genes have been defined, particularly in children with the familial forms of HI (HI-K_{ATP}) [80].

The focal form of the disease appears to be associated with a different genetic background, namely genetic imprinting [61]. This is not found in diffuse disease. In this circumstance, there is loss of heterozygosity with paternal imprinting (Fig. 22.7).

Two other discoveries have emphasized the complexity of hyperinsulinism. Abnormal activation of both glucokinase (HI-GK) [81] and glutamate dehydrogenase (HI-GDH) [82] leads to increased intracellular concentrations of ATP, which trigger insulin secretion in the absence of any defect in membrane polarization. It has been proposed that the glutamate dehydrogenase syndrome, which leads to hyperammonemia with hypoglycemia, may be the cause of the so-called "leucine-sensitive" hypoglycemia described in previous years. HI has also been reported in association with defects of fatty acid metabolism (HI-SCHAD) [83].

Hypoglycemia due to hormone deficiency

The counter-regulatory hormonal system insures a continuous supply of glucose to vital organs. Hormones participate either by immediate actions or by chronic (permissive) effects, which may alter the responsiveness of target tissues. Glucagon and adrenaline are the two hormones important for the immediate restoration of the blood glucose concentration; cortisol and GH have permissive roles.

Deficiency of any of these hormones can cause hypoglycemia. Glucagon deficiency is extremely rare, and the initial

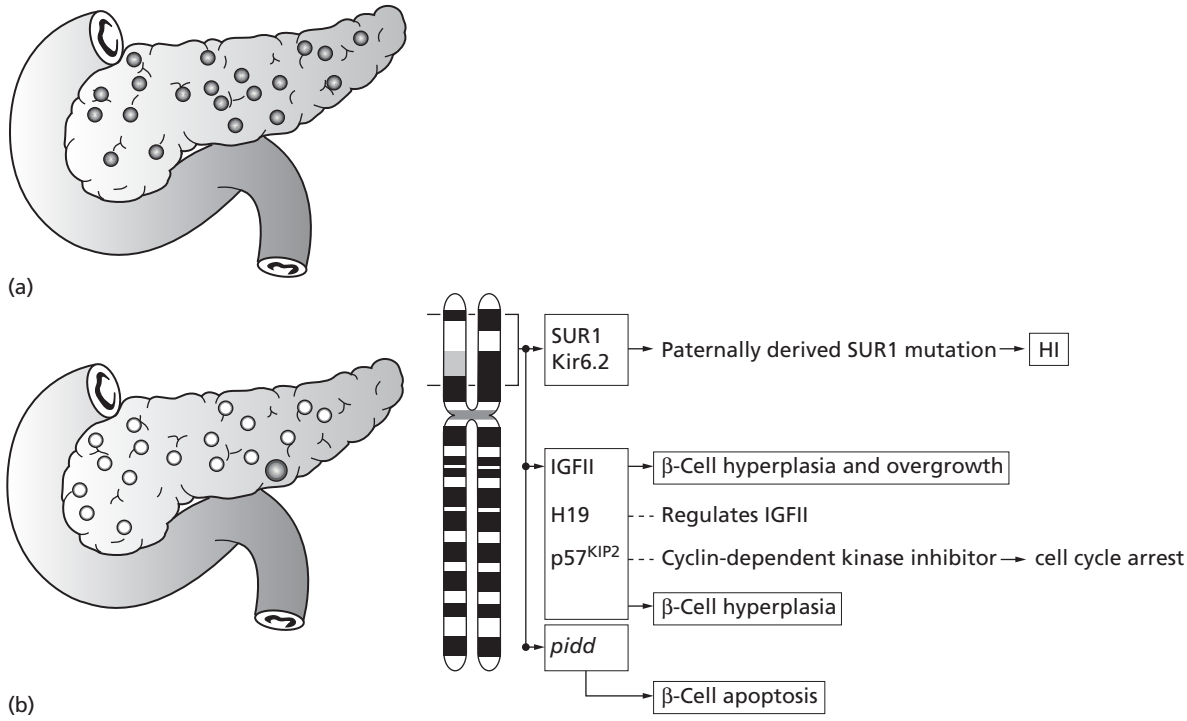


Fig. 22.7. (a) Diffuse disease affecting the whole pancreas. Diffuse disease is most commonly due to autosomal-recessive mutations in genes encoding the components of the K_{ATP} channel. (b) The focal form of the disease is localized to one region of the pancreas. Owing to a somatic event (loss of maternal chromosome 11p), the focal lesion is associated with loss of tumor suppressor genes (p57kip2 and H19) and increased expression of growth-promoting genes (IGF-II). This combination leads to β-cell hyperplasia. The HI is due to a paternally inherited mutation in the SUR1 or KIR6.2 genes.

case report of persistent neonatal hypoglycemia resulting from glucagon deficiency may in fact have been due to hyperinsulinism [84,85]. Although adrenaline deficiency has been reported, it is extremely rare as a cause of hypoglycemia [86].

GH and cortisol have numerous effects on glucose metabolism, including increasing the rates of gluconeogenesis and glycolysis and antagonizing the effects of insulin. The glycemic thresholds for the activation of glucose counter-regulatory hormones such as GH and cortisol in adults lie within or just below the physiological blood glucose concentration and slightly higher than the threshold for symptoms [87]. This implies that GH and cortisol start to rise in response to blood glucose concentrations within the normoglycemic range and that these increases are probably inversely proportional to the nadir in blood glucose [88]. GH and cortisol respond differently to hypoglycemia that is spontaneous compared with that induced by insulin infusion (insulin tolerance test [42]). This may be related to the rate of fall of the blood glucose concentration, which is why a low GH value at the time of spontaneous hypoglycemia may not necessarily indicate GH deficiency.

The etiology of the hypoglycemia due to cortisol and GH deficiency results from a combination of factors that include reduced gluconeogenic substrate availability (decreased mobilization of fats and proteins) and increased glucose utilization due to increased insulin sensitivity of tissues in the absence of these two hormones.

Congenital hypopituitarism may present with life-threatening hypoglycemia, abnormal serum sodium concentrations, shock, microphallus, and later growth failure. Causes include septo-optic dysplasia, other midline syndromes, and mutations of transcription factors involved in pituitary gland development. Children with acquired hypopituitarism typically present with growth failure and may have other complaints depending on the etiology and the extent of missing pituitary hormones. Acquired hypopituitarism may result from tumors (most commonly craniopharyngioma), radiation, infection, hydrocephalus, vascular anomalies, and trauma. The incidence of hypoglycemia due to panhypopituitarism can be as high as 20%, and hypoglycemia associated with hypopituitarism may be a cause of sudden death [89]. Congenital hypothyroidism in association with congenital hypopituitarism may very rarely cause hypoglycemia [90]. Appropriate replacement therapy with hydrocortisone and GH can alleviate hypoglycemia.

Hypoglycemia due to defects in hepatic glycogen release/storage

Glucose is stored as glycogen mainly in the liver but also in the muscle and kidneys. Defects in the storage or release

of hepatic glycogen can cause hypoglycemia. Glucose-6-phosphatase deficiency (glycogen storage disease type I, Von Gierkes disease) is the commonest of the glycogen storage diseases causing hypoglycemia. The deficiency of this enzyme results in the inability to release free glucose from glucose-6-phosphate, with resultant hepatomegaly due to stored glycogen. Children present with recurrent hypoglycemia associated with lactic acidosis, hyperuricemia, and hyperlipidemia. The aim of treatment is to prevent hypoglycemia using a combination of continuous nasogastric drip feeding and cornstarch.

The two other glycogen storage diseases causing hypoglycemia result from deficiencies of the enzymes amylo-1,6-glucosidase (glycogen storage disease type III, GSDIII) and liver phosphorylase (glycogen storage disease VI). The clinical and biochemical features of GSDIII subjects are quite heterogeneous: the clinical manifestations are hepatomegaly, hypoglycemia, hyperlipidemia, short stature and, in a number of subjects, cardiomyopathy and myopathy. Glycogen storage disease type VI (GSDVI) presents with mild clinical manifestations and follows a benign course. Patients have prominent hepatomegaly, growth retardation, and variable but mild episodes of fasting hypoglycemia and hyperketosis during childhood. Hyperlacticacidemia and hyperuricemia are characteristically absent. Patients may demonstrate elevated serum transaminases, hyperlipidemia, hypotonia, and muscle weakness. These clinical features and biochemical abnormalities generally resolve by puberty. Rare variants may have associated proximal renal tubular acidosis, myopathy, or fatal cardiomyopathy.

Glycogen synthase plays an important role in the storage of glycogen in the liver, and deficiency of it is a rare cause of hypoglycemia in childhood [91]. The characteristic features include fasting hypoglycemia with hyperketonemia but with normal lactate. After a meal, the plasma lactate will increase as glucose is channeled along the glycolytic pathway with hyperglycemia. Mutations in the glycogen synthase gene (*GYS2*) localized on chromosome 12p12.2 have been described in some patients.

Hypoglycemia due to defects in gluconeogenesis

Gluconeogenesis, the formation of glucose from lactate/pyruvate, glycerol, glutamine, and alanine, plays an essential role in the maintenance of normoglycemia during fasting. Inborn deficiencies of each of the four enzymes of the glycolytic–gluconeogenic pathway that insure a unidirectional flux from pyruvate to glucose [pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase, and glucose-6-phosphatase] are known [92]. Gluconeogenesis can be viewed as a reversal of glycolysis with differences. Patients with defects in gluconeogenesis present with fasting hypoglycemia and lactic acidosis. Pyruvate carboxylase deficiency may lead to a more widespread clinical presentation with lactic acidosis, severe mental and developmental retardation, and proximal renal tubular acidosis [93].

Hypoglycemia due to disorders of carnitine metabolism and defects of fatty acid oxidation

Primary carnitine deficiency is an autosomal-recessive disorder of fatty acid oxidation that can present at different ages with hypoketotic hypoglycemia and cardiomyopathy and/or skeletal myopathy. This disease is suspected based on reduced levels of carnitine in plasma and confirmed by measurement of carnitine transport in the patient's fibroblasts. Carnitine transport is markedly reduced (usually < 5% of normal) in fibroblasts from patients with primary carnitine deficiency.

The "hepatic" CPT1 isoform is expressed in liver, kidney, and fibroblasts and at low levels in the heart, while the other isoform (muscle) occurs in skeletal muscle and is the predominant form in the heart. Patients with hepatic CPT1 isoform deficiency present with hypoketotic hypoglycemia, hepatomegaly with raised transaminases, renal tubular acidosis, transient hyperlipidemia and, paradoxically, myopathy with elevated creatinine kinase or cardiac involvement and seizures and coma in the neonatal period [94]. The typical biochemical finding in the urine is dicarboxylic acids of chain lengths C6–C10.

CPT2 deficiency has several clinical presentations. The "benign" adult form is characterized by episodes of rhabdomyolysis triggered by prolonged exercise. The infantile-type CPT2 deficiency presents as severe attacks of hypoketotic hypoglycemia, occasionally associated with cardiac damage commonly responsible for sudden death before 1 year of age [95]. In addition to these symptoms, features of brain and kidney dysorganogenesis are frequently seen in neonatal-onset CPT2 deficiency, which is almost always lethal during the first month of life. Treatment is based upon avoidance of fasting and/or exercise and a low-fat diet enriched with medium-chain triglycerides and carnitine ("severe" CPT2 deficiency).

The commonest disorder of fatty acid β-oxidation is medium-chain acyl-CoA dehydrogenase (MCAD). This autosomal-recessive condition is characterized by intolerance to prolonged fasting, recurrent episodes of hypoglycemic coma with medium-chain dicarboxylicaciduria, impaired ketogenesis, and low plasma and tissue carnitine levels [96]. The disorder may be severe and even fatal in young patients. Other defects of β-oxidation (long-chain acyl-CoA dehydrogenase) may present with hypoketotic hypoglycemia associated with neurological (hypotonia) and cardiovascular complications (cardiomyopathy). The pattern

of dicarboxylicaciduria accumulation is characteristic for each enzymatic defect of the β-oxidation spiral.

Hypoglycemia due to defects in ketone body synthesis/utilization

Ketone bodies are an alternative form of fuel to glucose for the brain. Each ketone body is synthesized from the combination of acetyl-CoA and acetoacetyl-CoA to form hydroxymethylglutaryl-CoA (HMG-CoA). This is split by HMG-CoA lyase to yield acetoacetate, which is then converted to β-hydroxybutyrate. Hypoglycemia may occur as a result of defects in either the synthesis or the utilization of ketone bodies. Hereditary deficiency of mitochondrial HMG-CoA synthase can cause episodes of severe hypoketotic hypoglycemia. Typical findings include hypoketosis, elevated free fatty acids, normal acylcarnitines, and specific urinary organic acids during acute episodes. A rare cause of hypoglycemia due to the inability to utilize ketone bodies is deficiency of succinyl-CoA:3-oxoacid CoA-transferase (SCOT) [97], which is characterized by intermittent ketoacidotic crises and persistent ketosis.

Idiopathic ketotic hypoglycemia

Idiopathic ketotic hypoglycemia is common [98]. It usually presents between the ages of 18 months and 5 years and remits spontaneously by the age of 9–10 years. The typical history is of a child who may miss a meal and develop hypoglycemia unpredictably usually following an upper respiratory tract infection. The hypoglycemia is associated with raised ketone bodies and free fatty acids with suppressed insulin levels. Ketotic hypoglycemia is characterized by low levels of plasma alanine, but the precise mechanism responsible for the hypoglycemia is not understood [99]. The hormones such as glucagon and cortisol seem to be appropriately raised, but the role of GH is unclear [42].

Idiopathic ketotic hypoglycemia is a poorly defined term and may include groups of conditions in which there is no clear cause of the hypoglycemia. Conditions such as hepatic glycogen synthase deficiency [100] and acetoacetyl CoA thiolase deficiency have been reported as presenting with ketotic hypoglycemia [101]. Ketotic hypoglycemia is a diagnosis of exclusion.

Miscellaneous causes of hypoglycemia

Metabolic

Hypoglycemia can occur as a result of a number of metabolic conditions including galactosemia, fructosemia, tyrosinemia, organic acidemias, maple syrup urine disease, glutaric aciduria type II, and in mitochondrial respiratory chain defects [102]. Hereditary fructose intolerance, caused by catalytic deficiency of aldolase B (fructose-1,6-bisphosphate aldolase), is a recessively inherited condition in which affected homozygotes develop hypoglycemia and severe abdominal symptoms after taking foods containing fructose and cognate sugars. Continued ingestion of noxious sugars leads to hepatic and renal injury and growth retardation.

Factitious

Hypoglycemia can be induced pharmacologically, intentionally as a diagnostic tool, accidentally as a complication of the treatment of diabetes mellitus, or as a consequence of poisoning either with insulin itself or with drugs such as sulfonylureas [103], which stimulate insulin release. Whenever severe hypoglycemia with documented hyperinsulinism occurs in a previously healthy child, the possibility of malicious administration of insulin or an oral sulfonylurea should always be suspected. The clue in the biochemistry will be a raised insulin level with normal C-peptide in the case of insulin administration.

Abnormal processing of IGF-II

Persistent hypoketotic, hypofatty acidemic hypoinsulinemic hypoglycemia can occur in very rare circumstances as a result of non-islet cell tumor hypoglycemia (NICTH) [104]. In this condition, the neoplastic cells express the gene for insulin-like growth factor (IGF)-II and produce substantial amounts of incompletely processed, high-molecular-weight IGF-II precursor proteins ("big" proIGF-II). Part of this "big" proIGF-II enters the circulation and provokes excessive insulin-like activity in the body [105].

New syndromes

Two new syndromes that lead to hypoglycemia have been described in children. The first is hemihypertrophy and severe persistent hypoketotic, hypofatty acidemic hypoinsulinemic hypoglycemia [106]. No "big" pro-IGF-II forms or circulating insulin receptor antibodies were found. Glucose and protein isotope turnover studies showed marked suppression of hepatic glucose production during fasting. There was no evidence for constitutive autophosphorylation of the insulin or IGF-1 receptor, and no evidence for upregulation of IGF-1 receptor. The precise pathophysiology of this novel case is still unclear and is under investigation.

The first case of a child with a defect in prohormone convertase 1 (PC 1) due to a compound heterozygote for novel missense and nonsense mutations has been identified [107]. This child presented with the phenotypes of obesity, hypoadrenalism, reactive hypoglycemia, and elevated circulating levels of certain prohormones, with neonatal diarrhea, malabsorptive in type.

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23 Endocrine consequences of systemic disease: critical illness

Greet Van den Berghe

Introduction

Critical illness is any condition that requires support of failing vital organ systems. Life-threatening conditions can be caused by trauma, surgery, or medical illnesses. Critical illness may become prolonged when recovery does not set in within a few days. When this happens, organ support may be needed for weeks or even months. Despite feeding, wasting of protein from skeletal muscle and solid organs continues, which impairs vital functions, induces weakness, and hampers recovery. The frustrating clinical problem of feeding-resistant hypercatabolism perpetuates dependency on intensive care and renders the patient susceptible to lethal infections. Mortality is high [1], but the scoring systems for severity of illness in adults, such as the Acute Physiology And Chronic Health Evaluation (APACHE) II score, or children, such as the Pediatric Risk of Mortality (PRISM) score or Paediatric Index of Mortality (PIM) [2], do not predict mortality in individual patients.

The endocrine changes occurring during critical illness were formerly considered to be part of a uniform stress response reflecting adaptation of the body for survival. New data reveal that the acute and chronic phases of critical illness present with distinct endocrine alterations [3,4]. It remains a matter of debate to what extent these biphasic changes are protective or contribute to the metabolic disturbances in the critically ill. The endocrine stress responses are of central and peripheral origin. Furthermore, patients in intensive care may have pre-existing central and/or peripheral endocrine diseases, either previously diagnosed or unknown. The puzzle is thus complex, and endocrine function testing is a challenge in a critically ill patient. Moreover, the inability to define the endocrine changes as either adaptation or pathology renders the issue of treatment even more controversial.

Pathophysiology and treatment options

The growth hormone axis

In healthy individuals, growth hormone (GH) is released in a pulsatile fashion under the interactive control of GH-releasing hormone (GHRH) and somatostatin. Since the 1980s, a series of synthetic GH-releasing peptides (GHRPs) and non-peptide analogs have been developed with potent GH-releasing capacities acting through a specific G-protein-coupled receptor located in the hypothalamus and the pituitary [5,6]. A highly conserved endogenous ligand for this receptor was subsequently discovered and named "ghrelin" [7]. Ghrelin was found to originate in peripheral tissues, such as the stomach, as well as in the hypothalamic arcuate nucleus, and appears to be a third key factor in the regulation of pulsatile GH secretion, which is important for its metabolic effects [1].

Alterations within the GH axis in the acute phase of critical illness

Peak GH levels, interpulse concentrations, and GH pulse frequency are all increased during the first hours and days of critical illness [3,8,9] (Fig. 23.1). It is not clear what causes this. As in starvation, more frequent withdrawal of somatostatin and/or an increased availability of (hypothalamic and/or peripheral) GH-releasing factors could be involved. A drop in serum levels of GH-binding protein (GHBP) precedes a decrease in serum concentrations of insulin-like growth factor-I (IGF-I) and the most abundant GH-dependent binding protein, IGF-binding protein-3 (IGFBP-3) and its acid-labile subunit (ALS) [10]. Low circulating GHBP has been shown to parallel reduced GH receptor expression in peripheral tissues [10]. Serum levels of the small IGF-binding proteins, such as IGFBP-1, IGFBP-2, and IGFBP-6 are elevated

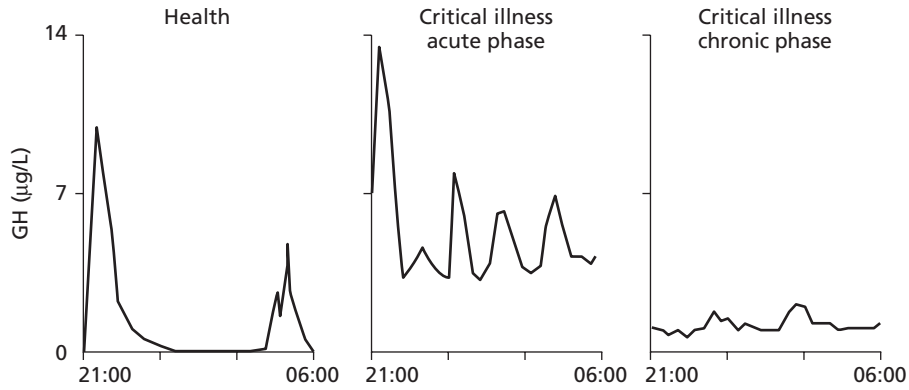

Fig. 23.1. Nocturnal serum concentration profiles of GH illustrating the differences between the acute phase and the chronic phase of critical illness within an intensive care setting. From [79] with permission.

[11,12]. This constellation, which has been confirmed in experimental human and animal models of acute stress and in acutely ill patients, has been interpreted as acquired peripheral GH resistance [8,11]. It has been suggested that the changes are brought about by the effects of cytokines, such as tumor necrosis factor (TNF)-alfa, interleukin (IL)-1, and IL-6. Reduced GH receptor expression and thus low IGF-I levels are presumed to be the primary (cytokine-induced) events that evoke abundant release of GH during acute stress through reduced negative feedback inhibition. This excessive GH may still exert its direct lipolytic, insulin-antagonizing, and immune-stimulating actions, while the indirect IGF-I-mediated effects of GH are attenuated [13,14]. This explanation is plausible in that such changes would prioritize essential substrates such as glucose, free fatty acids (FFA), and amino acids such as glutamine toward survival rather than anabolism. Increased IGFBP-3 protease activity in plasma has also been reported, however, and is thought to result in increased dissociation of IGF-I from the ternary complex, thereby shortening the half-life of circulating IGF-I. The latter could theoretically be an adaptive escape mechanism to secure the availability of free IGF-I at the tissue level [15].

Distinct alterations within the GH axis during prolonged critical illness

In prolonged critical illness, the alterations within the somatotrophic axis change. The pattern of GH secretion first becomes chaotic, and the amount of GH released in pulses is reduced [4,16–18] (Fig. 23.1). Although the non-pulsatile fraction is still somewhat elevated and the number of pulses is still high, mean nocturnal GH serum concentrations are only moderately elevated, if at all [16], compared with the healthy, non-stressed condition, and substantially lower than in the acute phase of stress [3]. When intensive care adults were studied from 7 to 10 days' illness onward in the absence of drugs known to exert profound effects on GH secretion, such as dopamine [19,20], calcium entry blockers, or glucocorticoids, mean nocturnal GH levels were around 1 μg/L [16], trough levels were easily detectable (and thus still elevated), and peak GH levels hardly ever exceeded 2 μg/L [4,16–18]. Although these changes occur in children on a somewhat higher baseline [21], qualitatively they are surprisingly independent of the patient's age, gender, body composition, and type of underlying disease [1,3].

The pulsatile fraction of substantially reduced GH secretion correlates positively with circulating levels of IGF-I, IGFBP-3, and ALS, all of which are low [4,17,18]. Thus the smaller the GH pulses, the lower the circulating GH-dependent IGF-I and ternary complex binding proteins. This clearly no longer exclusively represents GH resistance. Serum levels of GHBP [1], which are assumed to reflect GH receptor expression in peripheral tissues, are increased in patients critically ill for several weeks compared with those measured in a matched control group. This suggests recovery of GH responsiveness with a longer duration of severe illness [1,4]. Low serum levels of GH-dependent IGF-I and binding proteins (IGFBP-3, ALS, and IGFBP-5) parallel biochemical markers of impaired anabolism, such as low serum osteocalcin and leptin, during prolonged critical illness [4].

These findings suggest that relative GH deficiency characterized by reduced pulsatile GH secretion participates in the pathogenesis of the "wasting syndrome," especially in the chronic phase of critical illness. Men show a greater loss of pulsatility and regularity within the GH secretory pattern than women (despite indistinguishable total GH output) and have lower circulating IGF-I and ALS levels [1] (Fig. 23.2). It remains unknown whether this sexual dimorphism within the GH/IGF-I axis and the fact that males seem to be at higher risk of death from prolonged critical illness than females [1] is a casual or causal association.

Pathophysiology of chronic changes within the GH axis

The pathogenesis of the abnormal GH secretory pattern in prolonged critical illness is complex. One possibility is that the pituitary is involved in the multiple organ failure becoming unable to synthesize and secrete GH. Alternatively, lack of pulsatile GH secretion could be due to increased somatostatin tone and/or reduced stimulation by endogenous

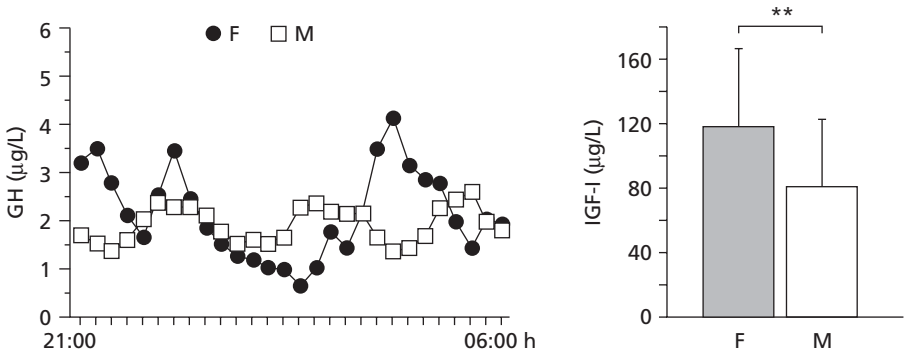

Fig. 23.2. The female pattern of GH secretion (more irregular and less pulsatile GH secretory pattern for an identical mean nocturnal GH level) in prolonged critically ill men compared with women is illustrated by the representative nocturnal (21:00 h–06:00 h) GH serum concentration series (sampling every 20 min) obtained in a male (squares) and a matched female (circles) patient. Concomitantly, protracted critically ill men have lower circulating levels of IGF-I than female patients. IGF-I results are presented as mean ± SD. $^{**}P < 0.01$. From [79] with permission.

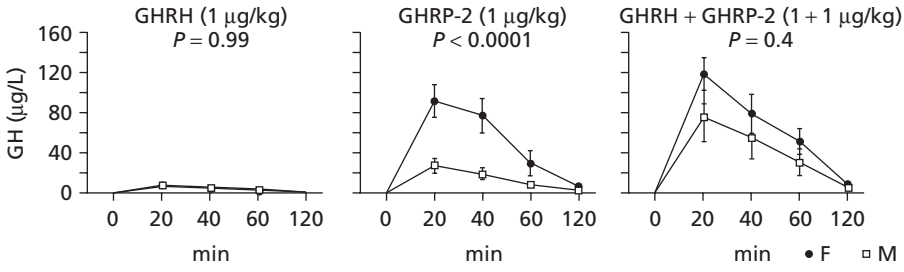

Fig. 23.3. Responses (increments above baseline) of GH obtained 20, 40, 60, and 120 min after intravenous bolus administration of GHRH (1 μg/kg), GHRP-2 (1 μg/kg), and GHRH + GHRP-2 (1 + 1 μg/kg) in matched male and female protracted critically ill patients. Five men and 5 women were randomly allocated to each secretagogue group. Results are presented as mean ± SEM. Circles depict results from female and squares from male patients. *P*-values were obtained using repeated measures ANOVA. From [79] with permission.

releasing factors such as GHRH and/or ghrelin. Studying GH responses to the administration of GH secretagogues (GHRH and GHRP) in supramaximal doses differentiates between a primarily pituitary and a hypothalamic origin of impaired GH release in prolonged critically ill patients. The combined administration of GHRH and GHRP appears to be a most powerful stimulus for pituitary GH release in humans. A low GH response in critical illness would thus confirm pituitary dysfunction and/or a high somatostatin tone, whereas a high GH response would be compatible with reduced endogenous stimulation of the somatotropes.

We found that GH responses to a bolus injection of GHRP were high in prolonged critically ill patients and several-fold higher than the response to GHRH, the latter being normal or often subnormal [22]. GHRH + GHRP evoked a clear synergistic response, producing the highest GH responses ever reported in a human study [22]. The high GH responses to secretagogs exclude the possibility that the blunted GH secretion during protracted critical illness is due to lack of pituitary capacity to synthesize GH or to increased somatostatin suppression of GH release. Instead, a reduced availability of active ghrelin could be inferred.

The combination of low availability or activity of somatostatin and of an endogenous GHRP-like ligand such as ghrelin emerges as a plausible mechanism to explain the reduced GH burst amplitude, the increased frequency of spontaneous GH secretory bursts, and the elevated interpulse levels, as well as the striking responsiveness to GHRP alone or in combination with GHRH when responsiveness to GHRH alone is not increased. Females with prolonged critical illness have a markedly higher response to a bolus of GHRP compared with males, a difference that is abolished when GHRH is injected together with GHRP [1] (Fig. 23.3). Less endogenous GHRH action in prolonged critically ill men (possibly due to the concomitant profound hypoandrogenism [1]) accompanying loss of action of an endogenous GHRP-like ligand with prolonged stress in both genders may explain this finding.

Effects of GH-releasing factors in the chronic phase of critical illness

Continuously infusing GHRP (1 μg/kg/h) and, even more so, GHRH + GHRP (1 + 1 μg/kg/h), for up to 2 days was found to amplify pulsatile GH secretion (> sixfold and > 10-fold respectively) in this condition without altering the relatively high burst frequency [17,18] (Fig. 23.4). Reactivating pulsatile GH secretion evoked a proportionate rise in serum IGF-I (66% and 106%), IGFBP-3 (50% and 56%), and ALS (65% and 97%), indicating peripheral GH responsiveness [17,18] (Fig. 23.4). The presence of responsiveness to reactivated pulsatile GH secretion in these patients marks the pattern present in the chronic as opposed to the acute phase of critical illness, which is thought to be primarily a condition of GH resistance. After 2 days' treatment with GHRP, (near) normal

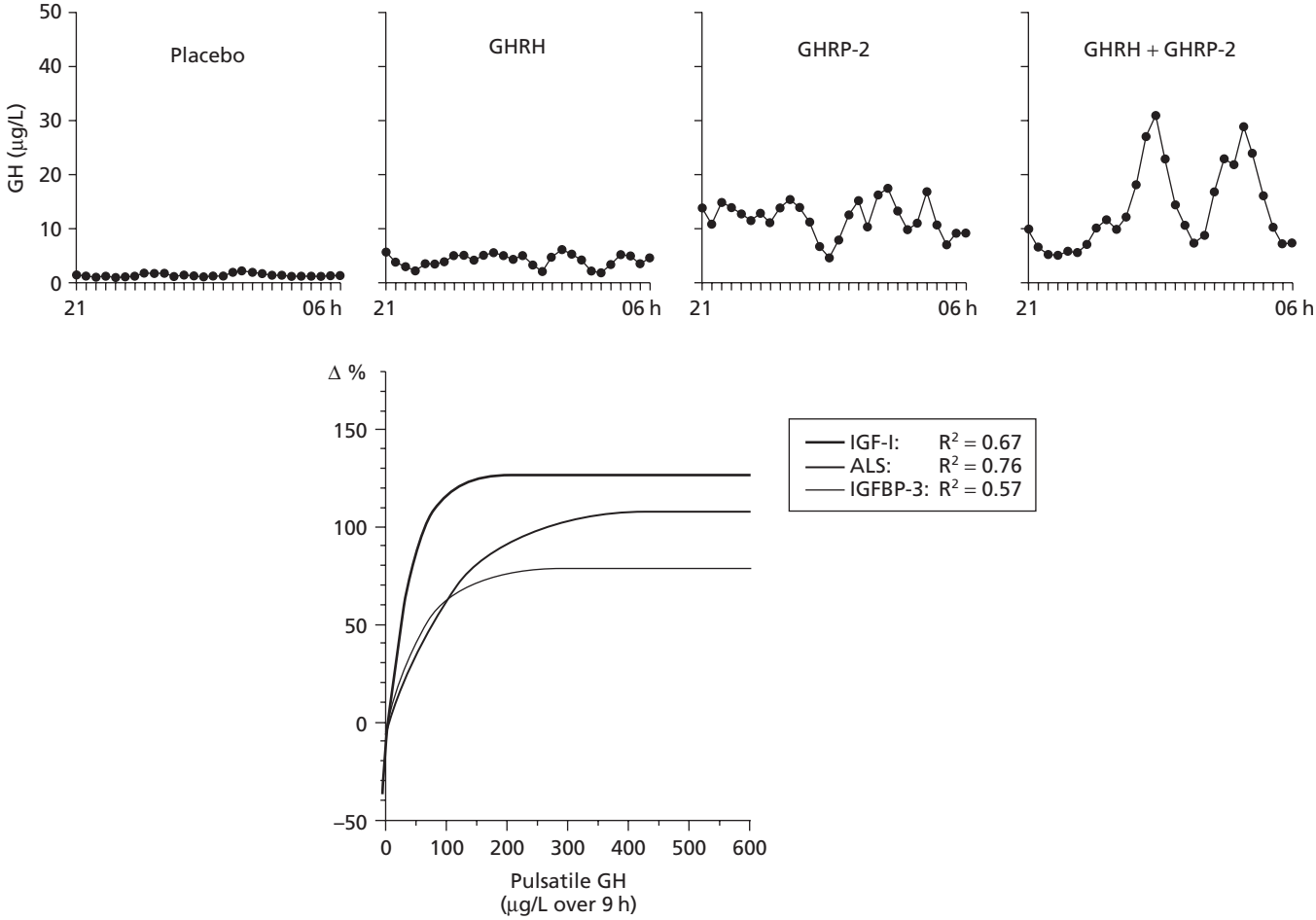

Fig. 23.4. Nocturnal serum GH profiles in the prolonged phase of illness illustrating the effects of continuous infusion of placebo, GHRH (1 μg/kg/h), GHRP-2 (1 μg/kg/h), or GHRH + GHRP-2 (1 + 1 μg/kg/h). Exponential regression lines have been reported between pulsatile GH secretion and the changes in circulating IGF-I, ALS, and IGFBP-3 obtained with 45-h infusion of placebo, GHRP-2, or GHRH + GHRP-2. They indicate that the parameters of GH responsiveness increase in proportion to GH secretion up to a certain point, beyond which further increase in GH secretion apparently has little or no additional effect. The latter point corresponds to a pulsatile GH secretion of approximately 200 μg/L over 9 h, or less, a value that can usually be evoked by the infusion of GHRP-2 alone. In chronic critical illness, GH sensitivity is clearly present, in contrast to the acute phase of illness, which is thought primarily to be a condition of GH resistance. Adapted with permission from [79].

levels of IGF-I, IGFBP-3, IGFBP-5, and ALS were reached and maintained for at least 5 days [4] (Fig. 23.5). Concomitantly, GH secretion after 5 days' treatment with GH secretagogs was found to be lower than after 2 days, suggesting active feedback inhibition loops, which probably prevented overtreatment [4,18].

When GHRP was infused together with thyrotrophin-releasing hormone (TRH) for 5 days, the self-limiting endocrine responses induced anabolism in several peripheral tissues, as indicated by a rise in serum levels of osteocalcin, insulin, and leptin and a decrease in urea production [4]. Infusion of GHRP without GHRH usually reactivates pulsatile GH secretion and elicits the IGF-I and IGFBP responses in prolonged critical illness but, in critically ill men, especially those with a very long intensive care stay, it may be necessary to add a low dose of GHRH (0.1 μg/kg/h suffices) because of the lack of endogenous GHRH activity accompanying the reduced availability of the GHRP-like ligand [1].

Treatment with GH during critical illness

In view of the anabolic properties of GH and IGF-I, a large multicenter study investigated the effects of high-dose GH treatment in patients requiring prolonged intensive care [23]. Instead of improving outcome, this intervention doubled mortality and worsened morbidity. Although the authors did not provide an explanation for this unexpected outcome, the difference between the acute and the chronic stress response may be of importance. The rationale for the use of high GH doses was presumably that stress-associated hypercatabolism and the catabolic state of prolonged critical illness are caused by GH resistance in the presence of normal or

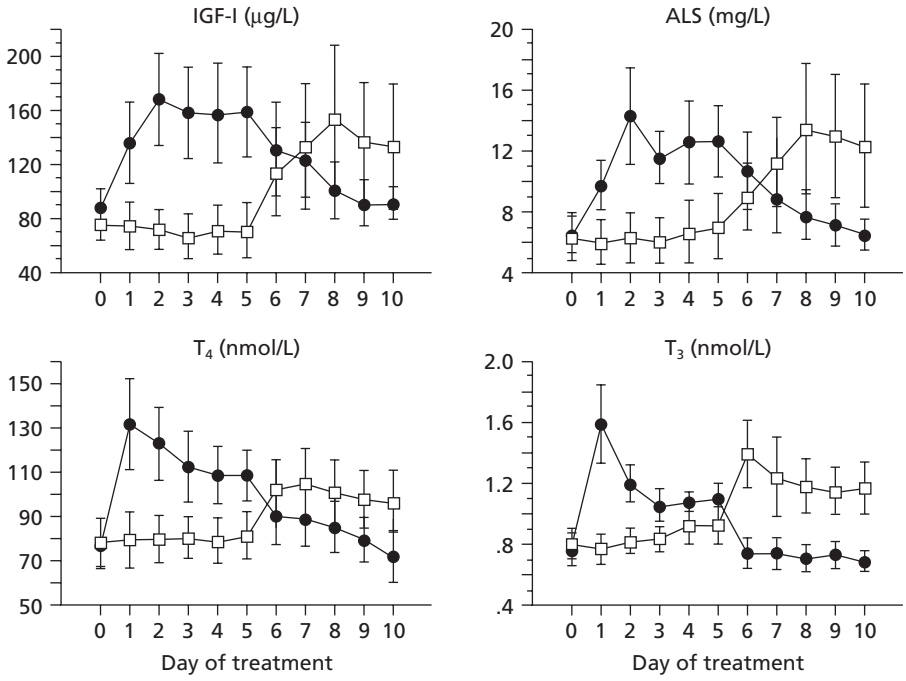

Fig. 23.5. Serum concentrations (mean ± SEM) of IGF-I, ALS, T_4, and T_3 in response to a randomized treatment with either 5 days of GHRP-2 + TRH infusion (1 + 1 μg/kg/h) followed by 5 days of placebo (filled symbols) or 5 days of placebo followed by 5 days of GHRP-2 + TRH infusion (1 + 1 μg/kg/h) (open symbols) in a group of 10 male and four female critically ill ventilated ICU patients. All $P < 0.0001$ with ANOVA. The mean age of the patients was 68 years. The mean intensive care stay at the time of study start was 40 days. From [79] with permission.

adaptively altered pituitary function and that the induction of anabolism would thus require very high GH doses. The knowledge now available on the different states of the somatotrophic axis in acute and prolonged critical illness clarifies, at least partly, why the administration of high GH doses to sick, but often GH-responsive, patients may have had a bad outcome. High doses of GH administered in the chronic phase of critical illness can induce IGF-I levels in the acromegalic range leading to excessive fluid retention (up to 20% of body weight), hypercalcemia, and pronounced insulin resistance with hyperglycemia [24]. In view of the broad spectrum of GH target tissues and taking into account pre-existing impairment of vital organ functions in the critically ill, excessive doses of GH may have further impaired the function of multiple organs.

A clinically relevant question that arises from the results of this trial is what intensive care physicians should do when patients who are GH deficient and on GH treatment become critically ill and admitted to the intensive care unit (ICU). Should GH therapy be continued? A consensus statement from the GH Research Society [25] advises that it should in view of the lack of evidence for any harmful effect of low GH doses used for replacement treatment.

The thyroid axis (Fig. 23.6)

Changes in the acute phase of critical illness

Within 2 h of surgery or trauma, serum levels of T_3 decrease, whereas T_4 and thyroid-stimulating hormone (TSH) rise briefly [26]. Low T_3 levels at that stage are caused mainly by

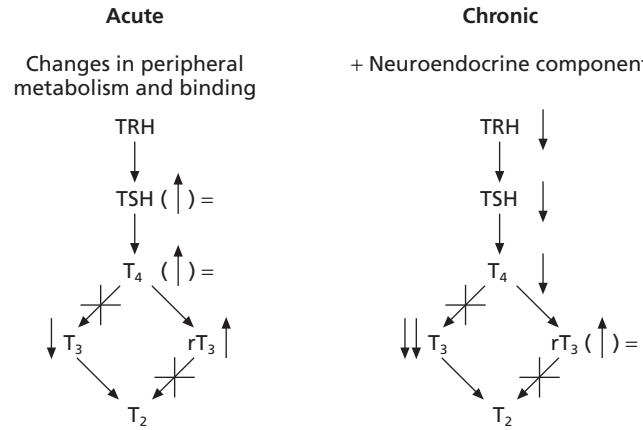

Fig. 23.6. Simplified overview of the major changes occurring within the thyroid axis during the acute and the chronic phase of critical illness. From [79] with permission.

decreased peripheral conversion of T_4 to T_3 [27]. Subsequently, circulating TSH and T_4 levels often return to normal, whereas T_3 levels remain low. Although mean serum TSH concentrations are indistinguishable from normal at that time, the nocturnal TSH surge is absent [28,29]. The magnitude of the T_3 drop within 24 h reflects the severity of illness [30,31].

TNF-alfa, IL-1, and IL-6 have been investigated as putative mediators of the acute low T_3 syndrome but, although they are capable of mimicking the acute stress-induced alterations in thyroid status, cytokine antagonism in a human model failed to restore normal thyroid function. Low concentrations of binding proteins and inhibition of hormone binding, transport, and metabolism by elevated levels of FFA and bilirubin

have been proposed as factors contributing to the low T_3 concentration at tissue level.

Teleologically, the acute changes in the thyroid axis may reflect an attempt to reduce energy expenditure, as happens during starvation [32], which would be an appropriate response that does not warrant intervention. Data to support or refute this statement are lacking. Although short-term intravenous administration of T_3 to patients after cross-clamp removal during elective coronary bypass grafting has been shown to improve post-operative cardiac function [33,34], the pharmacological doses of T_3 that resulted in supranormal serum T_3 levels and the absence of an effect on mortality do not negate an adaptive purpose of the acute low T_3 syndrome.

Changes in prolonged critical illness

Patients in ICUs for several weeks present a different set of changes within the thyroid axis. A single sample usually reveals low or low–normal TSH values and low T_4 and T_3 serum concentrations [35], but repeated sampling overnight revealed that the pulsatility in the TSH secretory pattern is dramatically reduced and that, as for the GH axis, it is the loss of TSH pulse amplitude that is related to low serum levels of thyroid hormone [35]. When death follows chronic severe illness, the expression of the TRH gene in hypothalamic paraventricular nuclei is reduced at post-mortem examination, whereas this is not the case after death from acute insults such as a road accident [36]. There is a positive correlation between TRH mRNA in the paraventricular nuclei and blood levels of TSH and T_3. These findings indicate that production and/or release of thyroid hormones is reduced in the chronic phase of critical illness as a result of reduced hypothalamic stimulation of the thyrotropes leading to reduced stimulation of the thyroid gland. In line with this concept is a rise in TSH that marks the onset of recovery from severe illness [37]. The exact mechanisms underlying the neuroendocrine pathogenesis of the low thyroid hormone levels in prolonged critical illness are unknown. As circulating cytokine levels are usually lower at that stage, other mechanisms operating within the central nervous system (CNS) are presumably involved. Endogenous dopamine and prolonged hypercortisolism may each play a role as exogenous dopamine and glucocorticoids provoke or severely aggravate hypothyroidism in critical illness [38].

Recent data indicate that, as well as resetting hypothalamic control, activity of type I deiodinase (D1) is suppressed and of type III deiodinase (D3) is increased in the livers of prolonged critically ill patients. These alterations in enzyme activity were found to determine the ratio of active to inactive thyroid hormone (T_3/reverse T_3), indicating that changes in thyroid hormone metabolism are contributing to the picture of low T_3 syndrome in the chronic phase of critical illness [39]. In a rabbit model, the downregulation of D1 and upregulation of D3 were reversed by the simultaneous infusion of TRH and the GH-releasing peptide-2 (GHRP-2) [40].

Alternative splicing gives rise to two receptor isoforms for thyroid hormone, TR alfa-1 and TR alfa-2. The TR alfa-1 isoform is a bona fide T_3 receptor, whereas the TR alfa-2 acts as a dominant-negative isoform. The ratio of these splice variants could therefore have a marked influence on T_3-regulated gene expression, especially in view of the changing metabolism of thyroid hormone during illness. An inverse correlation between the T_3/reverse T_3 ratio and the TR alfa-1/TR alfa-2 ratio in liver biopsies of prolonged critically ill patients has been observed, and higher TR alfa-1/TR alfa-2 ratios were present in sicker and older patients compared with the less sick and younger ones [41]. Hence, critically ill patients appear to adapt to decreasing thyroid hormone levels by increasing the expression of the active form of the thyroid hormone receptor gene and thereby possibly increasing cellular sensitivity to the hormone. This does not support an adaptive nature of the low T_3 syndrome in prolonged critically ill patients.

Low thyroid hormone levels in protracted critical illness correlate inversely with urea production and bone degradation, which could again reflect either an adaptive, protective mechanism against hypercatabolism or a causal relation [4]. Restoring physiological levels of thyroid hormones by continuously infusing TRH together with GHRP-2 (Fig. 23.5) was found to reduce rather than increase hypercatabolism [4], an effect that was related only to thyroid hormone changes. During TRH infusion in prolonged critical illness, the negative feedback exerted by thyroid hormones upon the thyrotropes was maintained, thus precluding overstimulation of the thyroid axis [16,18]. This regulation may be important during critical illness to avoid hyperthyroidism, which would aggravate catabolism. The co-infusion of TRH and GH-releasing factors appears to be a better strategy than the infusion of TRH alone, as the combination, but not TRH alone, avoids a rise in circulating reverse T_3 [4,16]. The latter is in line with the effects that TRH and GHRP-2 exert on the activity of type I and type III deiodinases, and probably on other important interactions among different anterior pituitary axes for optimal peripheral responses [42].

Treatment with thyroid hormone or releasing factors during prolonged critical illness

It remains controversial whether correction of illness-associated low serum and tissue concentrations of T_3 by either T_4 or T_3 administration improves outcome. T_4 administration has no clinical benefit in an intensive care setting but, in view of the impaired conversion of T_4 to T_3, this is not surprising [43–45]. Giving T_3 to dopamine-treated pediatric patients after correction of a congenital cardiac anomaly improved post-operative cardiac function [46]. However dopamine-induced hypothyroidism is an iatrogenic thyroid

dysfunction, and the results of this trial do not necessarily apply to non-iatrogenic, illness-induced, low T_3 levels. In view of the potentially permanent consequences for CNS development of hypothyroidism in newborns, dopamine should be avoided in this age group of ICU patients [21].

TRH may be safer than T_3 because infusing TRH allows peripheral shifts in thyroid hormone metabolism during intercurrent events to induce appropriate concentrations of thyroid hormones in the circulation and at tissue level [18]. The peripheral tissue responses to the normalization of serum concentrations of IGF-I and binding proteins evoked by GHRP infusion seem to depend on the co-infusion of TRH and the concomitant normalization of the thyroid axis. GHRP-2 infused alone provokes identical increments in serum concentrations of IGF-I, IGFBP-3, and ALS, but is devoid of the anabolic tissue responses that are present with the combined infusion of GHRP and TRH [16]. Outcome benefit of TRH infusion alone or in combination with GH secretagogues in prolonged critical illness has yet to be studied.

The diagnosis of pre-existing thyroid disease and its management during critical illness can be extremely difficult. In view of the hypothalamo-pituitary suppression occurring in the chronic phase of critical illness, it is impossible at that time to diagnose pre-existing central hypothyroidism in patients with or without previous endocrine disease. Patients with pre-existing primary hypothyroidism, myxedema coma being the extreme presentation, are expected to have low serum levels of T_4 and T_3 in combination with very high TSH concentrations but, when primary hypothyroidism and severe non-thyroidal critical illness coincide, the TSH rise may be absent. A decrease in serum T_3 and an increase in reverse T_3 are the most common changes in acute non-thyroidal critical illness, but serum T_3 may be undetectable and T_4 dramatically reduced in patients with protracted non-thyroidal critical illness. Therefore, in patients with myxedema coma and severe co-morbidity (pneumonia, sepsis), serum T_3 and T_4 are very low but could be indistinguishable from values observed in prolonged non-thyroidal critical illness. Whereas serum TSH is increased markedly in uncomplicated primary hypothyroidism, it is normal or even-decreased in severely ill patients. TSH may thus be lower than anticipated from the severe hypothyroid condition of the patient with myxedema coma and concomitant illness. A high serum TSH concentration confirms primary hypothyroidism, but a normal or low TSH does not exclude it.

Iatrogenic factors causing hypothyroidism in a surgical ICU include iodine wound dressings, iodine-containing contrast agents used for radiological imaging, and drugs such as somatostatin and amiodarone as well as high-dose corticosteroids and/or dopamine. The finding of a high ratio of T_3 to T_4 in serum, a low thyroid hormone-binding ratio, and a low serum reverse T_3 may favor the presence of primary hypothyroidism: opposite changes occur in non-thyroidal critical illness. The accuracy of any measurements is limited and, in many patients, only history, physical examination, and the possible presence of thyroid autoantibodies may give clues to the presence or absence of thyroid disease. Repeated thyroid function testing after improvement of non-thyroidal illness is required to confirm the diagnosis.

When and how to treat primary hypothyroidism during the course of an intercurrent non-thyroidal critical illness is controversial. One exception is myxedema coma, for which there is general agreement that patients should be treated with a parenteral form of thyroid hormone. The initiation of thyroid hormone replacement therapy is controversial as studies on the optimal treatment regimen are lacking. The first uncertainty relates to the type of thyroid hormone to be given: should it be T_4 alone, T_3 alone, or the combination of both? The second uncertainty is the optimal initial dosage of any thyroid hormone replacement regimen. Many clinicians prefer a loading dose of intravenous T_4 (up to 300–500 μg in an adult) in order to restore circulating levels of T_4 quickly to approximately 50% of the euthyroid value, followed by lesser amounts (50–100 μg) of intravenous T_4 daily until oral medication can be given. Higher doses do not seem to be beneficial, although there is no increased cardiovascular risk in severely ill hypothyroid patients [47].

Some authors have advocated the use of T_3 in addition to T_4 because T_3 does not require conversion by 5′-deiodinase enzymes to a biologically active form. In an animal study, replacement with T_4 alone did not insure euthyroidism in all tissues, and a subsequent study showed that only combined treatment with T_4 and T_3 induced euthyroidism in all tissues [48]. Tissue-specific deiodinase activities acting as local regulatory mechanisms may explain these findings. In a more recent study, it appeared that partial substitution of T_3 for T_4 may improve mood and neuropsychological function in hypothyroid patients, possibly by increasing bioavailability of T_3 in the CNS [49]. While these results await confirmation, replacement therapy with a combination of T_4 and T_3 in compensated hypothyroidism remains experimental.

In my unit, we treat presumed hypothyroidism, either pre-existing or iatrogenically induced when reversal of the iatrogenic cause appears impossible, with a dose of 50–200 μg of T_4 intravenous (IV) bolus per 24 h for adults combined with T_3 0.6 μg/kg ideal body weight per 24 h in continuous IV infusion [50]. Higher doses are used for children and newborns. For newborns up to 4 weeks of age, the IV T_4 dose is 15–20 μg/kg body weight as a bolus injection once every 24 h combined with T_3 in continuous IV infusion at a dose of 1.5 μg/kg/24 h [50]. For babies older than 4 weeks, the IV T_4 dose is one 25–50 μg bolus injection combined with a continuous IV infusion of T_3 at 0.8–1.0 μg/kg/24 h [50]. Low–normal circulating levels of thyroid hormones are targeted. As the dose requirements decrease rapidly once critical illness improves, frequent follow-up of serum concentrations of T_4, T_3, and thyroxine-binding globulin (TBG) are required for timely tapering and stopping the treatment.

Prolactin

Prolactin responses to acute and prolonged critical illness

It has been suggested that changes in prolactin secretion in response to stress may contribute to altered immune function during the course of critical illness. The evidence for this includes the presence of prolactin receptors on human T and B lymphocytes and the prolactin dependency of T lymphocytes for maintaining immune competence [51]. Inhibition of prolactin release in mice results in impaired lymphocyte function, depressed lymphokine-dependent macrophage activation, and death from a normally non-lethal exposure to bacteria. Cyclosporine competes with prolactin for a common binding site on T cells, which may partly explain its effects [52]. Bromocriptine has been shown to be an adjuvant immunosuppressant in humans after heart transplantation [52]. Prolactin was among the first hormones known to have increased serum concentrations in response to acute physical or psychological stress [53], a rise that may be mediated by vasoactive intestinal peptide (VIP), oxytocin, dopaminergic pathways, and/or other still uncharacterized factors. Cytokines may play a signaling role. Whether hyperprolactinemia during the initial phase of critical illness contributes to the vital activation of the immune cascade remains speculative.

In chronic critical illness, serum prolactin levels are no longer as high as in the acute phase, and the secretory pattern is characterized by a reduced pulsatile fraction [18,32]. A role for endogenous dopamine has been suggested [54]. It is unknown whether the blunted prolactin secretion in the chronic phase plays a role in the anergic immune dysfunction or in the increased susceptibility to infections characterizing the chronically ill. However, exogenous dopamine, often infused as an inotrophic drug in intensive care patients, suppresses prolactin secretion, aggravates T-lymphocyte dysfunction, and impairs neutrophyl chemotaxis [54,55].

Gonadal axis

Changes in luteinizing hormone (LH) and testosterone in acute and prolonged critical illness

LH pulsatility is important for its bioactivity. As testosterone is the most important endogenous anabolic steroid, changes within the LH–testosterone axis in the male could be relevant for the catabolic state of critical illness. A variety of catabolic states are accompanied by low serum testosterone levels in men. These conditions include starvation, the post-operative phase [56], myocardial infarction [57], burn injury [58,59], psychological and physical stress [60,61], and chronic critical illness [62].

Low serum testosterone concentrations and elevated LH levels observed during the acute stress of surgery or myocardial infarction [56,57,63] suggest immediate Leydig cell suppression, the exact cause of which remains obscure. Inflammatory cytokines (IL-1 and IL-2) may play a role. It may be appropriate that the secretion of anabolic androgens be switched off in circumstances of acute stress in order to conserve energy and metabolic substrates.

When critical illness becomes prolonged, hypogonadotrophism develops [58,64], and circulating levels of testosterone become extremely low (often undetectable) in men, whereas free estradiol concentrations remain normal, suggesting increased aromatization of adrenal androgens [1]. The progressive decrease in serum gonadotropin levels lags behind the rapid decline in serum testosterone [57,63,65]. In prolonged critically ill men, a high LH pulse frequency with an abnormally low LH pulse amplitude has been observed [62], which was interpreted as an impaired compensatory LH hypersecretion in response to the very low serum testosterone levels. Thus, again, it seems to be mainly impairment of the pulsatile component of LH secretion that occurs in response to the sustained stress of prolonged critical illness [62].

Endogenous dopamine, opiates, and the preserved levels of circulating estradiol [1] may be involved in the pathogenesis of hypogonadotrophism, as all of these may blunt LH secretion [62,66]. Animal data suggest that prolonged exposure of the brain to IL-1 may also play a role through the suppression of LH-releasing hormone (LHRH) synthesis. Androgen treatment in prolonged critical illness failed to confer clinical benefit [67]. In view of the secretory characteristics of the other anterior pituitary hormones, we investigated the therapeutic potential of LHRH pulses in prolonged critically ill men, alone or together with GHRP-2 and TRH. LHRH alone appeared to be only partially and transiently effective [68] but, when LHRH pulses were given with GHRP-2 and TRH infusion, target organ responses and anabolic effects followed [16]. These data underline the importance of correcting all the hypothalamic/pituitary defects instead of applying a single hormone treatment.

Adrenal axis

Pituitary–adrenal responses to acute and prolonged critical illness

The pituitary–adrenal axis also responds differently to acute and prolonged critical illness. Stress-induced hypercortisolism is associated with augmented adrenocorticotrophic hormone (ACTH) release, which is presumably driven by corticotrophin-releasing hormone (CRH), cytokines, and the noradrenergic system. Circulating aldosterone also rises markedly, probably under the control of an activated renin–angiotensin system [69]. Hypercortisolism acutely shifts carbohydrate, fat, and protein metabolism, so that energy is instantly and selectively available to vital organs such as the

brain, and anabolism is delayed. Intravascular fluid retention and the enhanced inotrophic and vasopressor response to catecholamines and angiotensin II, respectively, offer hemodynamic advantages to the "fight and flight" reflex. In addition, hypercortisolism elicited by acute disease or trauma can be interpreted as an attempt of the organism to mute its own inflammatory cascade, thus protecting itself against over-responses.

In chronic critical illness, serum ACTH was found to be low whereas cortisol concentrations remained elevated, indicating that cortisol release may be driven through an alternative pathway possibly involving endothelin [70]. Why ACTH levels are low in chronic critical illness is unclear; a role for atrial natriuretic peptide or substance P has been suggested [70]. In contrast to serum cortisol, circulating levels of adrenal androgens such as dehydroepiandrosterone sulfate (DHEAS), which has immunostimulatory properties on Th1 helper cells, are low during chronic critical illness [71,72]. Moreover, despite increased plasma renin activity, paradoxically decreased concentrations of aldosterone are found in protracted critical illness [73]. This constellation suggests a shift of pregnenolone metabolism away from both mineralocorticoid and adrenal androgen pathways toward the glucocorticoid pathway orchestrated by an unknown peripheral drive. The latter mechanism may fail, as indicated by a 20-fold higher incidence of adrenal insufficiency in critically ill patients over the age of 50 years being treated in the ICU for more than 14 days [74]. The fact that this type of relative adrenal failure coincides with adverse outcome suggests that high levels of glucocorticoids remain essential for hemodynamic stability. Whether hypercortisolism in the chronic phase of critical illness is exclusively beneficial remains uncertain. Sustained hypercortisolism in the presence of low levels of DHEAS and prolactin could theoretically cause imbalance between immunosuppressive and immunostimulatory pathways, and thus could be seen as participating in the increased susceptibility to infectious complications. Other conceivable though unproven drawbacks of prolonged hypercortisolism include impaired wound healing and myopathy, complications that are often observed during prolonged critical illness.

Treatment of adrenal failure during critical illness

In a patient with previously diagnosed primary or central adrenal insufficiency and in patients previously treated with systemic glucocorticoids, treatment should be continued and additional cover should be provided for the stress of critical illness. An Addisonian crisis needs urgent treatment with hydrocortisone 100 mg followed by 50–100 mg every 6 h on the first day, 50 mg every 6 h on the second day, and 25 mg every 6 h on the third day, tapering to a maintenance dose by the fourth to fifth day. In prolonged critical conditions, the maintenance dose should be kept at two to three times the basal need. Special attention should be given to patients with concomitant diabetes insipidus, as lack of cortisol may prevent polyuria because cortisol is needed for free water clearance. Glucocorticoid therapy in these patients may induce or aggravate diabetes insipidus. The post-hypophysectomy phase for Cushing disease is characterized by a high vulnerability to Addisonian-like crisis. Drugs such as phenytoin, barbiturates, rifampicin, and thyroid hormone can accelerate glucocorticoid metabolism by induction of microsomal enzyme activity and can increase the glucocorticoid replacement dose requirements. If this increased requirement is not met, adrenal crisis may occur.

The concept of relative hyopthalamo-pituitary–adrenal insufficiency in patients with sepsis or septic shock has been launched recently [75,76]. This concept advocates short-term treatment with stress doses of glucocortioids for septic patients without full-blown adrenal failure [77,78]. The controversy is partly explained by a problem in making the diagnosis because normal values for baseline cortisol levels in this type of stress, as well as normal reference values for cortisol responses to a short ACTH test, not to mention the option to use a low-dose or high-dose ACTH test, are not available. Another area of controversy is the dose and the duration of treatment once it has been initiated. Treating septic patients with glucocorticoids in too high a dose and for too long a time will aggravate the loss of lean tissue, increase the risk of polyneuropathy and myopathy, prolong ICU dependency, and increase the susceptibility for potentially lethal complications.

Insulin

A high serum concentration of IGFBP-1 predicts adverse outcome: a link to insulin?

A high serum cortisol and/or low T_3 indicate a poor prognosis in the acute phase of critical illness [30]. These markers lack sensitivity in the prolonged critically ill patient. A high serum concentration of IGFBP-1 predicts the outcome of chronic critical illness better [1,4,79] (Fig. 23.7). IGFBP-1 is a small IGF-binding protein produced almost exclusively by the liver (except in pregnancy). It is distinct among the members of the IGFBP family in being acutely regulated by metabolic stimuli. Studies with cultured human liver explants suggest that the major regulatory influences on IGFBP-1 production are insulin, which is inhibitory, and hepatic substrate deprivation, which is stimulatory, acting through a cyclic AMP-dependent mechanism. An inverse correlation of IGFBP-1 with IGF-I and the GH-dependent proteins ALS and IGFBP-3 during critical illness is consistent with its inverse regulation by GH [80]. Higher IGFBP-1 levels observed in prolonged critically ill patients who did not survive coincided with lower insulin concentrations compared with survivors, for the same range of blood glucose level, a surprising finding considering that these patients are thought

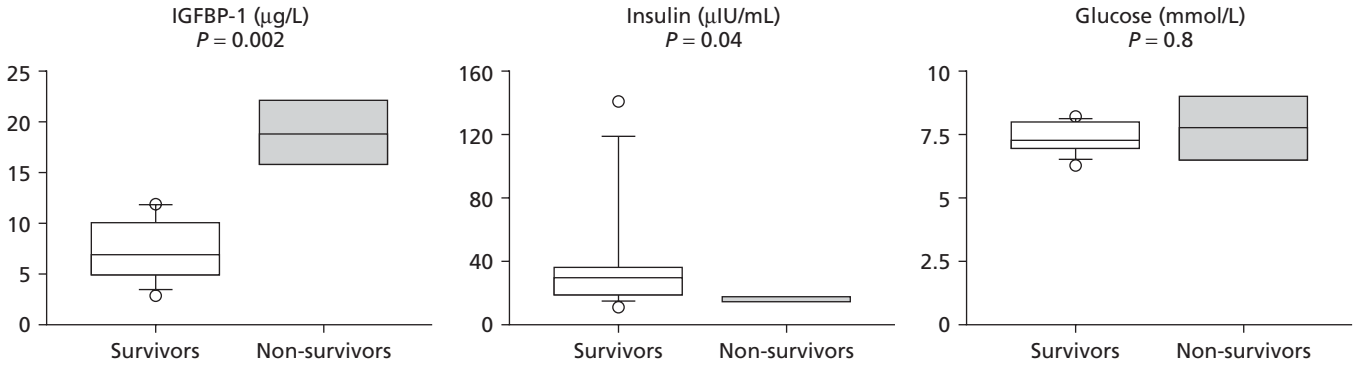

Fig. 23.7. Serum IGFBP-1 concentration is higher in non-survivors compared with survivors in prolonged critical illness. Concomitantly, non-survivors revealed lower serum insulin levels for the same blood glucose level. Box plots represent medians; P25–P75, P10–P90, and circles represent the absolute values for outliers. From [79] with permission.

to be insulin resistant (Fig. 23.7). Whether or not this indicates that insulin secretion is also becoming impaired in prolonged critically ill patients remains unclear. It is clear, however, that the hepatocyte alters its production of IGF-regulatory proteins in unfavorable metabolic conditions. The trigger for this might be reduced hepatocyte substrate availability caused by hepatic hypoperfusion, hypoxia, hypoglycemia, relative insulin deficiency, or hepatic insulin resistance leading to increased cyclic AMP production, which would both suppress IGF-I and ALS and stimulate IGFBP-1. It is unclear to what extent loss of GH pulsatility may contribute to this switch, but recent data suggest that activation of hepatic IGF-I and ALS expression may require pulsatile GH [4]. Animal studies similarly suggest that suppression of hepatic IGFBP-1 expression by insulin requires acute, rather than prolonged or non-pulsatile, GH action.

Why some prolonged critically ill patients fail to recover and eventually die in spite of optimal intensive care is not understood. High serum IGFBP-1 levels, relatively low circulating insulin levels, and adverse outcome of prolonged critical illness suggested that certain effects of insulin, specifically in the liver, may be crucial for recovery and survival. This novel concept generated the hypothesis that treatment with insulin may offer therapeutic potential to improve the outcome of critical illness.

Intensive insulin therapy reduces morbidity and mortality of critical illness

Hyperglycemia is well known in trauma, burns, and critical illness. It results from the combined action of counter-regulatory hormones, cytokines, and nervous signals on glucose metabolic pathways. Insulin resistance, manifested by increased serum insulin levels, impaired peripheral glucose uptake, and elevated hepatic glucose production, develops during critical illness. Strict maintenance of normoglycemia (below 110 mg/dL) with intensive insulin therapy markedly reduced the mortality of adult intensive care patients, particularly of those with prolonged critical illness (mortality reduced by half) [81] (Fig. 23.8). Intensive insulin therapy was also protective against acute renal failure, with a 42% reduction in the need for dialysis, and prevented the occurrence

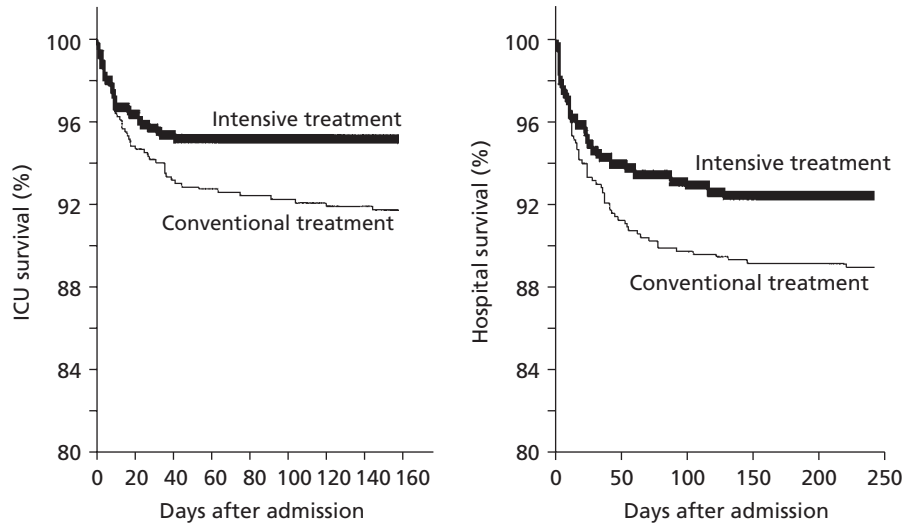

Fig. 23.8. Kaplan–Meier cumulative survival plots for intensive care and in-hospital survival, showing the effect of intensive insulin treatment in a study of 1548 critically ill patients. Patients discharged alive from intensive care (left) and hospital (right) were considered survivors. *P*-values were obtained by log rank (Mantel–Cox) significance testing. The difference between the intensive insulin group and the conventional group was significant for intensive care survival (unadjusted $P = 0.005$; adjusted $P < 0.04$) and for hospital survival (unadjusted $P = 0.01$). Reproduced with permission from [81].

of polyneuropathy. Controlling hyperglycemia with insulin restores impaired immunity by preventing the disturbed phagocytotic capacity of monocytes [82]. Hyperglycemia in the critically ill is also associated with a deranged serum lipid profile, which can be partially normalized by intensive insulin therapy, totally eliminating hypertriglyceridemia and substantially increasing the low serum levels of high- and low-density lipoproteins (HDL and LDL) [83]. In addition, intensive insulin therapy exerted an anti-inflammatory effect [84] in critically ill patients.

From these findings, it is clear that blood glucose levels should be tightly controlled below 110 mg/dL by continuous insulin infusion, in both diabetic and non-diabetic critically ill adult patients. Whether this also applies to critically ill children is the subject of an ongoing clinical trial.

Implications for clinical practice

The difference between the acute and chronic endocrine stress response may not be trivial in relation to outcome of critical illness. It was the (inappropriate) assumption that acute stress responses, such as GH resistance, persist throughout the course of critical illness, which (inappropriately) justified administration of high doses of GH to long-stay intensive care patients to induce anabolism [23]. The concomitant endocrine changes in chronic critical illness may have predisposed to severe side-effects of high doses of GH. In view of the significant benefits of strict glycemia [81], GH-induced insulin resistance and hyperglycemia may have played a role.

Although it has been shown that anabolism can be reinitiated when GH secretagogues, TRH, and gonadotropin-releasing hormone (GnRH) are co-infused in critically ill patients, the effect on survival remains unknown. Hence, because of lack of appropriately designed and powered clinical trials, these and other endocrine interventions in the critically ill should still be considered experimental. One notable exception in adult ICU is the maintenance of normoglycemia with intensive insulin therapy, a strategy that substantially improved the outcome of intensive care patients.

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24 Consequences of radiotherapy, chemotherapy, and bone marrow transplant

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Introduction and overview

One in 650 children develop cancer by the age of 15 years, leukemia and brain tumors representing about 50% (Fig. 24.1). Improvements in survival mean that current overall survival rates for all childhood cancers are approximately 70%. For a child with standard risk leukemia, the cure rate is about 85%. One in 900 young adults in the UK is a survivor (more than 5 years from end of therapy and disease free) of childhood cancer; by 2010, this will have risen to 1 in 250.

The improvement in survival is a therapeutic achievement, but it is not without cost. The majority of children who survive childhood cancer will have a late effect of treatment [1–4], cardiac, neuropsychological, fertility, and endocrine problems being particularly common. Endocrine problems occur more commonly than other late effects. Of 650 long-term survivors attending a late effects clinic in the US, 40% had endocrine sequelae, with growth hormone deficiency, primary hypothyroidism, and primary ovarian failure being the most common disorders [2].

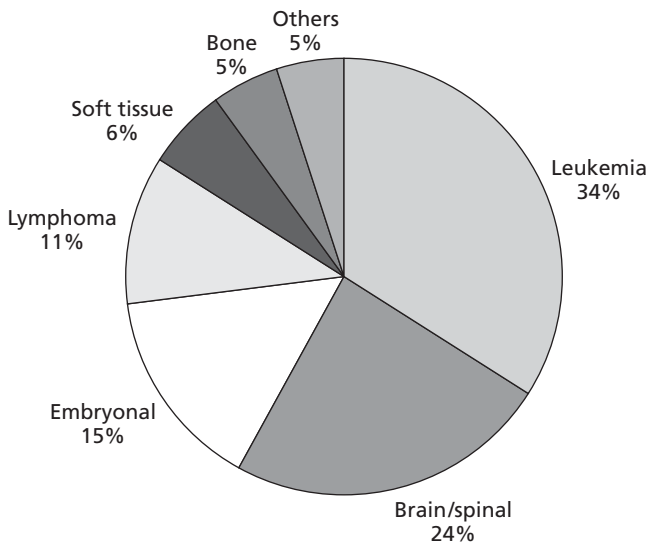

Fig. 24.1. Distribution of childhood cancers.

Endocrine surveillance for this population is essential and should be tailored to each patient's needs, based on the nature of the treatment and risk factors, such as age at treatment, gender, and the time elapsed since treatment.

As the number of survivors increases and more is known about the late effects, treatment is modified where possible to minimize them. Cure must remain the first consideration, but this should be achieved with minimal toxicity.

There are few longitudinal prospective studies, with published work being mostly retrospective and cross-sectional. Many study groups comprise heterogeneous groups of patients who have received a range of treatment modalities, making it difficult to determine the etiology of damage observed. The continually changing nature of therapy and the evolutionary nature of endocrine damage mean that evaluation of a treatment regimen is not possible until sufficient children are several years (or more for evaluation of effects in adulthood) from the end of therapy. By this time, treatment for the same condition may well have been modified. Despite these problems, a considerable body of evidence can be used to inform prognostic discussion with parents and children, screening for endocrinopathy and therapeutic intervention.

Principles of treatment for childhood cancer

Treatments for childhood cancer include surgery, radiotherapy, and chemotherapy. Increasing intensity of treatment has contributed to improved survival rates but is likely to result in increased incidence and severity of both early and late side-effects. Increased recognition of late morbidity has led to treatment modifications aimed at reducing late effects. For example, whereas all children previously received prophylactic cranial radiotherapy (18–24 Gy) as central nervous

system (CNS) prophylaxis in leukemia, this is now reserved for those with CNS disease and has not been routine therapy since 1990 in the UK and earlier in other parts of the world.

Children treated with surgery alone are least likely to suffer long-term side-effects, apart from those directly attributable to surgery. Radiotherapy and chemotherapy have the potential to cause late endocrine problems. In general, the more treatment, the younger the age, and the longer the time from treatment, the greater is the risk of endocrine problems. Where radiotherapy and chemotherapy are given together, there is a tendency for one to potentiate the toxicity of the other.

Radiobiology

The radiosensitivity of a tissue is directly proportional to its mitotic activity and inversely proportional to its differentiation: poorly differentiated, rapidly dividing tumors are more likely to be radiosensitive than slowly growing, well-differentiated ones. In terms of normal tissue, this means that rapidly dividing, highly active tissues, such as skin and bone marrow, are "radiosensitive," whereas more specialized, mature, quiescent cells, such as brain or bone, are relatively radioresistant; the quiescent oocyte is an important exception [5]. In spite of relative radioresistance by more mature cell types, all cells are damaged by radiotherapy and, once a quiescent cell is stimulated to divide, the radiation dose required to cause chromosomal damage, mitotic delay, and inhibition of DNA synthesis is similar in all cells. This means that all organ systems will be damaged by radiation but explains why effects on slowly or non-proliferating cell populations may become obvious only over time.

For any given tissue, the shape of the radiation dose–effect curve for a given effect that most accurately fits *in vitro*, *in vivo*, and clinical data can be described by the linear quadratic model [6]. According to this model, the effect of radiation on a given tissue is determined by the number of fractions (n), the dose of each fraction (d), and specific characteristics of the tissue in question, the α and β exponents. α refers to a "single-hit" (linear) component. The β exponent refers to the "multi-hit" (quadratic) component. This consists of the cumulative effect of sublethal injuries and/or the progressive destruction of the cell's ability to repair itself resulting in cell depletion. Early-reacting tissues, such as bone marrow, mucosa, and most tumors, are susceptible to single-hit injury and have a high α/β ratio, (typically $\alpha/\beta = 10$). In contrast, late-reacting tissue, such as neural tissue, is particularly susceptible to multihit injury and has a low α/β ratio (2 assumed for CNS). The relationship between dose and response for a range of fractionation regimens is given by the equation:

$$\text{Radiation effect} = n(\alpha d + \beta d^2)$$

The effect of this equation is that reducing the fraction size (dose) has relatively greater effect on late-reacting (neural) tissue response than on early-responding (tumor) tissue. Hyperfractionation of the dose (dividing the total dose into smaller fractions) over the same time may reduce late effects without compromising tumor control. Hyperfractionation has the potential preferentially to increase the antitumor effect without an equivalent increase in CNS late effects. A randomized trial of treatment for medulloblastoma in Europe (PNET4) comparing standard fractionation with hyperfractionation has been instituted.

Volume of brain irradiated, fraction size, time interval between fractions, age at treatment, total dose delivered, and time elapsed since treatment are all important factors in determining late neuroendocrine toxicity with conventional external beam radiotherapy. The highest estimated hypothalamo-pituitary doses (up to 70 Gy) occur following treatment for nasopharyngeal tumors with a consequently high incidence of hypothalamo-pituitary deficits [7]. Newer radiation techniques, particularly stereotactic radiosurgery, may have potential for disease control with less toxicity because of the highly focused nature of the irradiation, but experience in children is limited to date.

Chemotherapy

A number of mechanisms have been proposed for the pathogenesis of chemotherapy-induced endocrine damage [8]. Cytotoxic drugs can induce cell death or injury (including endocrine cell death/injury) through effects on DNA replication, protein synthesis, transcription, or microtubule function. They can disturb the synthesis or processing of a hormone at the transcriptional, translational, or post-translational levels. Chemotherapy can enhance or inhibit the secretion of hormones through effects on receptors or messenger metabolism. Effects on signal transduction pathways are a further mechanism by which chemotherapy may affect hormone action.

Radiotherapy and chemotherapy combined

Cumulative damage from treatment with both chemotherapy and radiotherapy may occur because of additive effects, with insufficient time for regeneration between treatments. Where adjuvant chemotherapy is combined with radiotherapy, increased risk of damage to the hypothalamo-pituitary axis, height prognosis, thyroid function, bone health, and gonadal function have all been reported [9–12].

Acute effects of therapy

In addition to the well-described late endocrine effects of radiation and chemotherapy, a number of chemotherapeutic agents cause acute endocrine disturbance. An awareness of these is helpful for the pediatric endocrinologist working with colleagues in oncology/hematology.

Disorders of fluid balance

The syndrome of inappropriate antidiuresis (SIAD) is well described in association with vincristine (VCR) and vinblastine [8]. During maintenance therapy for acute lymphoblastic leukemia (ALL), VCR is given 4-weekly. The occurrence of SIAD is idiosyncratic rather than dose related. Paired urine and plasma electrolytes and osmolalities will confirm the diagnosis and should be measured in any child who has received VCR and is hyponatremic. Management consists of fluid restriction while the SIAD resolves. Other chemotherapy agents that have been associated with SIAD include chlorambucil, cyclophosphamide, cisplatin, and carboplatin.

Nephrogenic diabetes insipidus (DI) may occur as a result of tubular toxicity, particularly from ifosfamide. Proximal renal tubular defects are much more common than distal, and nephrogenic DI is rare. Hypomagnesemia, which may be severe, is common in association with ifosfamide tubulopathy.

Disorders of glucose metabolism

L-Asparaginase, a drug used in the treatment of leukemia, may cause hyperglycemia and glycosuria without ketonuria. Frequency is estimated at 1–14%. Insulin may be required during treatment with asparaginase, but the hyperglycemia is reversible once treatment with asparaginase is discontinued. Asparaginase may also cause pancreatitis, and diabetes, which may be transient or permanent, may be associated with this.

Treatment for ALL and some lymphomas includes 4-weekly pulses of steroids. Historically, these were mainly given as prednisolone, but there is a survival advantage in using dexamethasone, so standard therapy for ALL now includes dexamethasone as the glucocorticoid. Five-day courses of 6 mg/m^2/day are given 4-weekly during the maintenance phase of therapy (with VCR on day 1 of the 5-day course). In a small number of susceptible children, this produces glucose intolerance, and insulin therapy may be required.

Recombinant interferon has also been associated with hyperglycemia in non-diabetic patients and worsening of existing diabetes in those who were previously diabetic.

Late effects on growth and endocrine function

Treatment of childhood brain tumors

Brain tumors are the second commonest childhood cancer. CNS tumors are diverse, representing many histological types and arising in a variety of anatomical sites. The overall 5-year survival rate for all pediatric CNS tumors is 67% but varies widely depending on histological type. Therapy depends on a range of factors, including histological type, position of the tumor, and age of the child. Cranial irradiation is widely used for the treatment of malignant brain tumors, increasingly in combination with adjuvant chemotherapy. In young children, where toxicity of radiotherapy is greatest, chemotherapy is increasingly used to delay or, if possible, avoid radiation.

Medulloblastoma is the commonest malignant tumor, accounting for between 15% and 20% of all childhood primary malignant CNS tumors. Standard therapy consists of surgery, craniospinal irradiation (CSI), and adjuvant chemotherapy. These combined modalities achieve an average survival rate of 70% [13]. Significant long-term morbidity is almost universal, with endocrine and neurocognitive consequences being particularly common [9,14–16].

Brain tumors and endocrine dysfunction

Children treated with cranial irradiation have a high risk of damage to the hypothalamo-pituitary axis (HPA) [7,17–23]. While it is clear that endocrinopathy is common, whether this is attributable to the irradiation alone remains debatable. Other factors may be involved in the etiology of the endocrinopathy, both surgery and the site of the tumor itself being potential influences. Study of the evolution of the endocrinopathy in a group of 165 adults with pituitary tumors who received post-operative local irradiation (20–45 Gy) demonstrated the effect of irradiation alone, as all subjects had normal pituitary function post-operatively [24]. Hierarchical loss of hormones was demonstrated with growth hormone (GH) being the most radiosensitive, followed by gonadotropins and adrenocorticotrophic hormone (ACTH). Thyroid-stimulating hormone (TSH) was the most radioresistant (Fig. 24.2).

Similar hierarchical loss has been demonstrated after irradiation for nasopharyngeal tumors. Over 5 years, of 31 adults treated for nasopharyngeal carcinoma with estimated doses to the hypothalamus and pituitary of 40–62 Gy, 63.5% were found to be GH deficient, and 30.7%, 26.7%, and 14.9% of the patients were deficient in gonadotropins, ACTH, and thyrotrophin respectively [25]. Children treated for head and neck rhabdomyosarcomas also have a high incidence of endocrinopathy [26].

Other evidence to support an important causative role for irradiation in the endocrine dysfunction observed in brain tumor survivors comes from studies of growth and neuroendocrine function in children receiving low-dose irradiation (18–24 Gy) as CNS prophylaxis for leukemia [27,28]. Animal work also demonstrates neuroendocrine damage after fractionated cranial irradiation [29,30]. Although there is differential radiosensitivity of hypothalamo-pituitary function in the young adult rat and GH is the earliest and most severely affected, the relative radioresistance of TSH observed in humans is not seen, whereas ACTH and gonadotropins were relatively well preserved [29].

The likelihood of pituitary damage increases with increasing total doses of radiotherapy [19,20], and fractionation

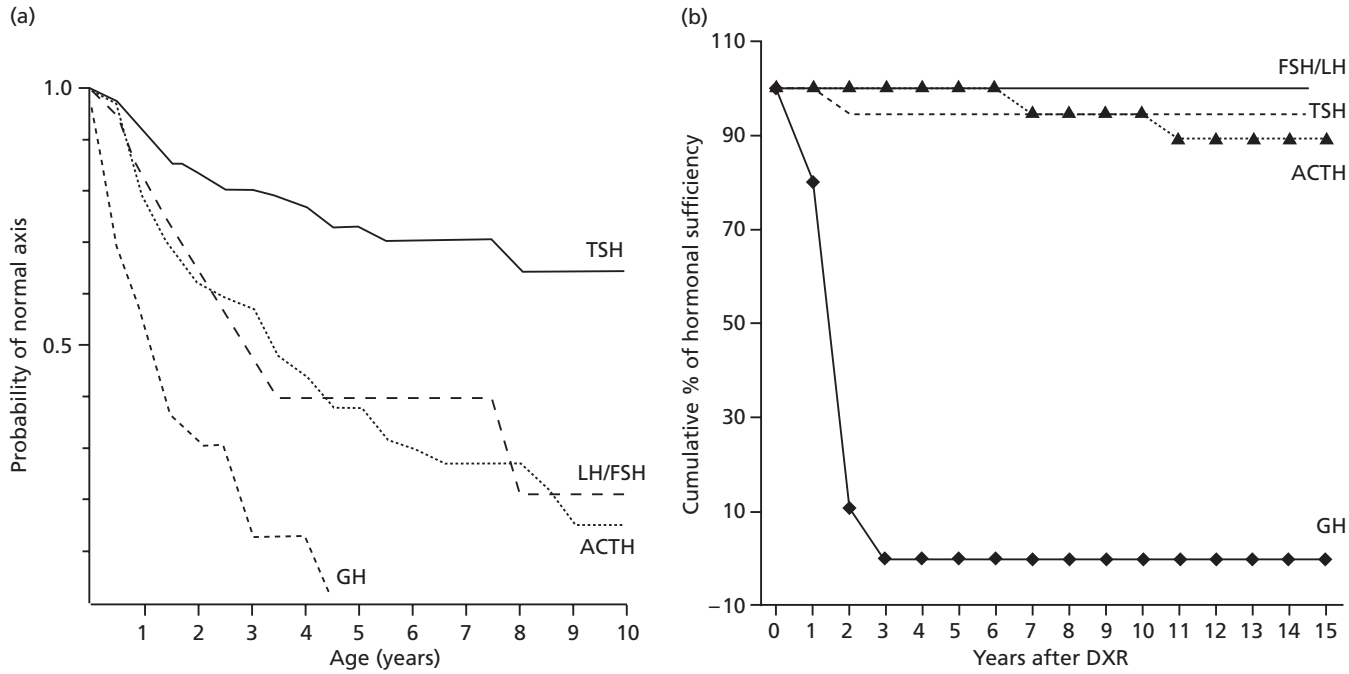

Fig. 24.2. (a) Ten-year life table analysis, in 37 of 165 adults with intrasellar or anatomically adjacent tumors, and normal post-operative hypothalamo-pituitary function, indicating the likelihood of an evolving endocrinopathy after 37.5–42.4 Gy pituitary irradiation in 15 fractions over 20–22 days (redrawn from [24]). (b) Ten-year probability of an evolving endocrinopathy occurring after a median estimated hypothalamo-pituitary irradiation dose (DXR) of at least 40 Gy in 1.8-Gy fractions, in 20 young adult survivors of resected posterior fossa tumors tested twice at the onset of growth failure and at completion of growth, who were otherwise asymptomatic.

schedule is also important. The same total dose will have greater impact if delivered in fewer fractions, each consisting of a larger individual dose. In a population-based study examining the correlation between biological effective dose (BED) of radiation and the incidence of growth hormone deficiency (GHD), a correlation between BED and incidence of GHD was shown [20]. BED is a means of expressing the biological effect of various treatment schedules in a uniform way. Patients with GHD (80% of the study population) had received a median BED of 77.5 Gy, whereas those without GHD had received a median BED of 54.5 Gy.

In spite of the large body of evidence supporting the role of irradiation, other factors are important. In a study of preoperative endocrine function in children with brain tumors, a high proportion had evidence of endocrinopathy before radiotherapy [31]. Sixty-seven patients with a range of tumor types were evaluated; 66% had evidence of endocrinopathy before radiation, including 47% of patients with posterior fossa tumors. These findings raise the question of whether routine evaluation of the HPA should be undertaken prior to radiation.

Slowly replicating neural cells may not at first demonstrate damage and, even where preoperative neuroendocrine function is normal, delayed tumor- or surgery-induced injury may be an important factor independently of additional radiation- and chemotherapy-induced damage.

Table 24.1. Likely endocrine deficit according to cranial irradiation dose (brain tumors distant from the pituitary).

Dose	Endocrinopathy
> 55–70 Gy	Hypopituitarsim, hyperprolactinemia, adult GHD
30–55 Gy	GHD, evolving endocrinopathy, pubertal GH insufficiency, early puberty, adult GHD
18–24 Gy	GH neurosecretory disturbance, adult GHD, early puberty (girls)
10–15 Gy	GH neurosecretory disturbance, adult GHD (uncommon)

Children who are younger at diagnosis are more susceptible to irradiation damage. The risk of growth impairment, GHD, and early or precocious puberty is increased by younger age at diagnosis [17,21,32], girls being more susceptible than boys to endocrinopathy, particularly with respect to early or precocious puberty [33]. Table 24.1 summarizes the likely pituitary deficit according to cranial irradiation dose.

Nature and site of the neuroendocrine defect

There remains considerable debate over whether the post-irradiation endocrinopathy is neural or vascular, hypothalamic or pituitary in origin. This has diagnostic and therapeutic implications.

Intermittent pulsatile subcutaneous gondatrophin-releasing hormone (GnRH) therapy can successfully induce puberty in

both sexes, with resultant ovulatory cycles in girls and fertility in hypogonadotrophic females. Continuous subcutaneous growth hormone-releasing hormone (GHRH) therapy promotes growth in children with GH deficiency of presumed hypothalamic origin after irradiation for periods up to 1 year. [34]. The response depends on the integrity of somatostatin (SS) secretion and is generally less than that seen with GH alone. However, if depot preparations of GHRH or the related orally active GH-releasing peptides were to become available, they might prove a more physiological, cost-effective, and user-friendly therapeutic option than GH therapy. Significant pituitary damage would limit the usefulness of such therapy.

From a diagnostic perspective, stimuli used for provocative GH testing work through hypothalamic or pituitary pathways depending on the test. Tests that act through pituitary pathways may underestimate the incidence of GH secretion, and insulin hypoglycemia has been recommended in this context for this reason [7].

Evidence to support hypothalamic damage includes discordant suppression of insulin-mediated and spontaneous GH release in irradiated monkeys [30]. Elevations of prolactin after irradiation of nasopharyngeal or pituitary tumors, decrease in TSH periodicity and (hypothalamically induced) nocturnal TSH surge, and preserved response to GHRH in the presence of suboptimal responses to other provocative agents are confirmatory of damage at the hypothalamic level.

Diabetes insipidus, a typical hypothalamic disorder, is rare. In addition, hyperprolactinemia is not seen after treatment for extrasellar tumors, and GH secretion and pituitary GHRH responses decline with time, suggesting evolving pituitary damage.

Spoudeas *et al*. have suggested that the disease and/or its surgery disrupt afferent or efferent GH chemoreceptor responses to hypoglycemia and suppress hypothalamic SS secretory tone, which is important in regulating GH pulsatility [22]. They also propose that superimposing cranial irradiation suppresses physiological GH peak generation, suggesting eventual hypothalamic GHRH deficiency and possible dysfunction or atrophy of the pituitary somatotroph as a result. Chemotherapy may play a role in this evolving neural dysregulation of the HPA.

Overall, current evidence suggests that the main site of radiation damage is the hypothalamus rather than the pituitary. The pituitary may be affected, particularly with increasing time from treatment [7].

Growth

Survivors of malignant childhood brain tumors are at high risk of adult short stature with a final height (FH) less than the 10th centile [17,32,35]. The US Childhood Cancer Survivor Study (CCSS) examined reported FH and body mass index (BMI) in 921 adult survivors of childhood brain cancer [32]. Nearly 40% were below the 10th centile for height. Young age at diagnosis and treatment with radiation involving the HPA were the strongest risk factors for short stature. BMI distribution in survivors did not differ significantly from population norms, although female sex, young age at diagnosis, and dose of HPA radiation were associated with obesity (Fig. 24.3).

Growth impairment is evident within the first year of treatment for brain tumors, and there does not appear to be a difference in growth between those who are GH deficient and those who are not in the first year of therapy. This early growth deceleration is not usually followed by catch-up, in contrast to the situation in leukemia.

Spinal radiation is an essential component in treatment of brain tumors with a propensity to metastasize to the spine, such as medulloblastoma and ependymoma. The impact of this on growth is considerable in terms of both FH and disproportion. The loss of height attributable to spinal radiation has been estimated as 9 cm if irradiation was given at 1 year of age, 7 cm if given at 5 years of age, and 5.5 cm if given at 10 years of age [36].

Adjuvant chemotherapy increases the risk of growth disturbance and endocrinopathy [9,11,35], but the US study, while demonstrating a final height below the 10th percentile in 40% of the survivors, did not show an independent effect of adjuvant chemotherapy once radiation dose was taken into the analysis. [32]. Nevertheless, a significantly increased risk of endocrine (and cardiovascular) late effects after treatment was shown with adjuvant chemotherapy in addition to radiation in childhood brain cancer survivors [9].

Growth hormone

Growth hormone deficiency is the commonest endocrinopathy after radiotherapy with 60–100% of children who have received whole brain irradiation having demonstrable GHD 2–5 years from the end of therapy [7,18,20]. The risk is highest in children treated at a young age and increases with time from treatment. There is unlikely to be a "safe" dose. This is in keeping with the radiobiology of damage to neuroendocrine cells and means that lifelong surveillance for pituitary deficiencies is necessary, even in those individuals who do not develop hormone deficiencies in the first few years after completion of therapy.

Growth hormone neurosecretory dysfunction (GHNSD) is well described after radiation and is characterized by reduced physiological GH secretion with preserved responses to pharmacological stimuli [7]. The few longitudinal studies of GH secretion in children treated with radiotherapy, whether total-body irradiation (TBI) (10 Gy), cranial irradiation for leukemia or brain tumors distant from the HPA, all suggest an evolving neurosecretory disturbance, the first abnormality being a reduction in GH pulse frequency. Abnormalities of GH pulsatility after lower doses of radiotherapy are particularly evident during puberty [37]. At a later

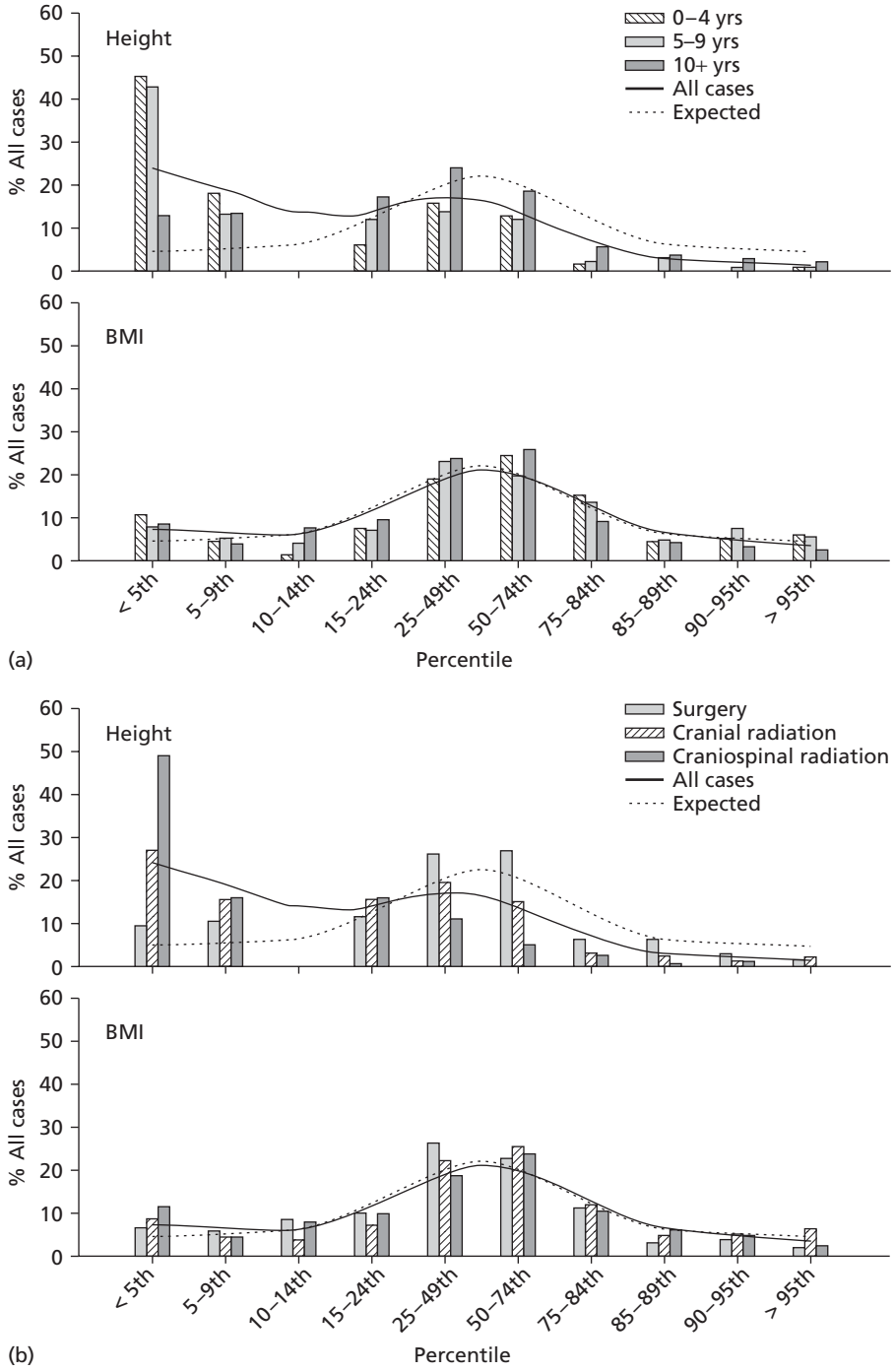

Fig. 24.3. Age- and sex-specific percentiles for height and BMI comparing normative data from the NHIS with the distribution among surviving brain tumor patients by age at diagnosis (a) and by treatment modality (b). From [32] with permission.

stage, reduction in pulse amplitude is observed, and classical GHD as defined by standard provocation testing is evident.

Any child who has received treatment for a brain tumor that includes cranial or craniospinal irradiation is at high risk of subsequent GHD. This evolves over time and once present is permanent. Thus, close monitoring of growth is essential. Auxology must include sitting height and calculation of BMI, as well as standing height. Where there has been spinal irradiation, leg length growth is a better indicator of height velocity than spinal growth because of the direct effect of radiation on spinal epiphyses.

All children with suboptimal growth should have their GH status formally evaluated. There is an argument for evaluating the HPA at 2–3 years from treatment even if growth is normal, as it has been shown that apparently normal growth may reflect early puberty and/or insulin-driven growth in

association with obesity. A hypothalamically mediated test of GH secretion should be used, and insulin tolerance test (ITT) and clonidine, the two most widely used in childhood, are both hypothalamically mediated.

The role of GH-dependent markers is uncertain. Reduced insulin-like growth factor (IGF)-1 and IGF binding protein (IGFBP-3) are consistent with GHD but are also influenced by other factors such as nutrition, liver function, and diabetes. Normal IGF-1 and IGFBP-3 levels have been documented in survivors with documented GHD, thus limiting their usefulness in this setting, but IGF-1 levels < –2 SDS in 95% of GHD cancer survivors have been reported [19]. A high proportion of the patients studied were adults with longstanding severe GHD, which may have influenced the findings.

Given the high incidence of GHD at 2 years from treatment, a reasonable argument could be made for initiation of GH therapy in all children treated with standard radiation schedules in doses above 30 Gy. In practice, most centers do undertake pharmacological testing. Given the high incidence of GHNSD and debate over the site of radiation damage, false-negative tests are probably relatively common, and a trial of GH may be merited if there is persistently poor growth, even with an apparently normal pharmacological test of GH secretion.

Growth hormone response is better if treatment is instituted early, and there is no evidence to suggest that treatment with GH increases the risk of relapse or second malignancy. Despite the paucity of evidence to support an increase in recurrence of second malignant neoplasms (SMNs) as a result of GH therapy, the risk of relapse is greatest within the first 2 years of treatment, and it is common to delay initiation of GH therapy until 2 years after treatment.

In contrast to children with classical GHD, children with radiation-induced GHD do not demonstrate catch-up growth in response to GH replacement, although it does prevent further loss of stature [7]. It is important to insure an adequate dose of GH, and doses higher than conventional GH replacement may be beneficial. GH replacement, while it improves FH, will exaggerate disproportion following CSI because of the poor response of the irradiated spine to GH.

Growth hormone and relapse/recurrence

Untreated GHD originating in childhood will cause suboptimal linear growth and markedly reduced FH; young adults may develop a range of metabolic disturbances, reduced bone density, raised plasma lipids, obesity, and impaired quality of life. A number of factors have raised concern that GH therapy may increase the risk of relapse or second malignancy in patients previously treated for cancer. These include the association between acromegaly and risk of colon cancer, and the correlation between high plasma IGF-1 and an increased risk of common cancers of adulthood, such as breast, prostate, colon, and lung. *In vitro* studies demonstrate that IGF-1 can act as both a mitogen and an anti-apoptotic agent in a variety of cancers, and there are reports of leukemia in GH-treated children. Children treated for cancer have a small but significant risk SMNs.

Despite these concerns, evidence to date is reassuring, although there are a number of methodological concerns in relation to the evidence available. All the studies comparing risk of tumor relapse or SMNs in childhood cancer survivors treated or not with GH are retrospective. Most studies are small, and many focus only on subjects previously treated for intracranial tumors.

The largest UK study compared recurrence rate in 180 GH-treated and 891 GH-naive survivors of childhood brain tumors and did not demonstrate any difference in recurrence rates between the two groups [38]. The US CCSS, which is a survey-based follow-up of 14 000 pediatric cancer survivors, reported its findings in relation to GH therapy and SMNs [39]. The study group included 361 children treated with GH. GH therapy did not appear to increase the risk of disease recurrence or death in survivors. However, there did appear to be a small excess of SMNs (relative risk 3.21) in GH-treated survivors, mainly due to an increased risk of SMNs in survivors of acute leukemia.

The authors suggest that the data should be interpreted with caution as the number of events (15 SMNs) was small and the confidence limits wide. In addition, the study is retrospective and methodologically less than ideal. Even if the results were correct, the absolute number of excess tumors that would occur as a result of GH therapy is only three or four per 1000 person-years at 15 years from diagnosis. Small risk needs to be balanced against potential benefits of GH, which may be considerable. GH doses in the US are generally higher than those used in Europe [40].

Although the evidence to date is generally reassuring, continued vigilance is clearly required. The UK version of the CCSS is currently collecting data, and an equivalent analysis will be undertaken once data collection is complete. Ideally, a randomized controlled study of GH therapy in GHD children previously treated for childhood malignancy should be undertaken but, given the known benefits of GH, would not be ethically justifiable.

Combination therapy with GnRH analogs

Early or precocious puberty is also common after cranial irradiation. The combination of precocious puberty and GHD carries a particularly poor height prognosis, especially as many of the children will have compromised spinal growth secondary to CSI. There is an argument for combining treatment with a gonadotropin analog and GH to maximize growth potential and some evidence to suggest that this is beneficial [41]. The possibility of slowing down puberty in children treated with CSI in whom puberty is occurring at a normal age but progressing rapidly and who have a particularly poor height prognosis is open to debate but may be worth consideration on an individual basis.

Reduction in bone mineral density (BMD) is a recognized side-effect of treatment with GnRH analog and, in a population who are already known to be at risk of reduced BMD, this is clearly a concern. Published studies examining effect on BMD are few but do not suggest a sustained effect on BMD by combination therapy with GnRH analog and GH [42].

Adult GH replacement

Established GHD after treatment of brain tumors persists into adult life [18]. There is a particularly strong argument for the use of GH in the adult childhood cancer survivor population; GH has potential benefit for bone mass and cardiovascular risk profile, and survivors of childhood brain cancers have been shown to be at risk of both [9,43–46]. However, studies to date have not demonstrated major improvements in these outcome measures in response to GH replacement therapy in adulthood in childhood cancer survivors, although benefit in quality of life has been demonstrated [43].

Disorders of gonadotropin secretion

Gonadotropins are the next most commonly affected hormone after GH, and damage to gonadotropin-secreting cells by radiation may be manifest either by early activation and precocious or early puberty or by gonadotropin deficiency. Both may occur at different times in the same patient, early puberty being followed by central gonadal failure. Gonadotropin deficiency is most likely after high-dose irradiation of pituitary or closely located tumors. Early puberty is common, and younger age at diagnosis is associated with increased risk. In a study of 46 children previously irradiated for brain tumors, all of whom had GHD, onset of puberty occurred at 8.51 years in girls and 9.21 years in boys [47]. The likely mechanism for early puberty is disinhibition of cortical influences on the hypothalamus, resulting in increased amplitude and frequency of GnRH pulsatile secretion.

Gonadal function

Gonadal function may be directly affected as a consequence of CSI or gonadotoxic chemotherapy. The combination of these factors on the hypothalamo-pituitary–gonadal axis can make interpretation of data difficult.

Alkylating agents are used in a number of chemotherapeutic regimens for childhood brain tumors and are particularly likely to induce gonadal failure. The current standard chemotherapy for medulloblastoma contains cisplatin and the alkylating agent, lomustine (CCNU), both of which have been implicated in gonadal failure.

CSI is more likely to affect ovarian than testicular function, given the proximity of the ovaries to the radiation field, especially in young girls. The mobility of the ovaries in young girls makes estimates of scatter dose difficult, but it is likely to be in the region of 0.9–2.5 Gy, compared with an estimated dose to the scrotum of 0.4–1.2 Gy. The effects of CSI and gonadotoxic chemotherapy are additive, and ovarian failure is more common than testicular failure. Many patients undergo spontaneous puberty, despite raised follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, but some will require induction of puberty or later hormone replacement therapy for pubertal arrest or later gonadal failure. The relative risk of "need for hormones to induce puberty" compared with siblings was 86.1 (CI 31.1–238.2) in the CCSS survey-based study of endocrine and cardiovascular outcomes in 1607 survivors of childhood brain tumors [9]. Early menopause in women who undergo spontaneous puberty and subsequent regular menstruation is a concern, but there is a paucity of data in this area.

Thyroid function

Where children receive CSI, the thyroid is inevitably included in the radiation field. Cranial irradiation (CI) may result in minor scattered radiation to the thyroid gland, the effect of which is less well documented than that of CSI. Estimates of the frequency of primary hypothyroidism following craniospinal radiation vary from 24% to 68% [48,49]. It is suggested that the incidence of primary hypothyroidism is reduced by hyperfractionation regimens [50].

Of patients with primary hypothyroidism, compensated hypothyroidism (elevated TSH with normal T_4 and T_3) has been observed in 73% and overt hypothyroidism in 27% [49]. There is a theoretical argument for treating the elevated TSH in this setting; there is a small but significantly increased risk of secondary thyroid malignancy in an irradiated thyroid [51,52]. There is concern that hyperstimulation of an "at risk" gland may further increase this risk.

Central hypothyroidism is much less common after treatment for brain tumors than primary hypothyroidism. Estimates of the frequency of hypothalamo-pituitary–thyroid (HPT) dysfunction range from 4% to 6% [11,18,49]. This may well increase over time and is likely to be an underestimate in the very long-term survivors in the adult population treated many years earlier for a childhood brain tumor. Because thyroid function is commonly evaluated using basal TSH and free T_4 concentrations, subtle deficits of HPT function may be missed.

In a study of 208 childhood cancer survivors with a range of primary diagnoses, central or mixed hypothyroidism was found in 32% of the cohort by 10 years from diagnosis [53]. The incidence of central hypothyroidism was highest in survivors of suprasellar or nasopharyngeal tumors (50%), patients who had posterior fossa or supratentorial tumors (35%), and after bone marrow transplant (BMT) (35%). A blunted nocturnal TSH surge was a particularly common finding, and the consequent reduction in daily thyroid hormone production may be sufficient to slow the growth of some children.

The effect of adjuvant chemotherapy on thyroid function is less clearcut than its effect on growth and GH secretion, with studies showing conflicting results. In a study of 119 brain

tumor survivors, Livesey and Brook found that the risk of primary hypothyroidism was 69% in children treated with CSI and adjuvant chemotherapy compared with only 23% of those treated with CSI alone [11]. Other authors have not demonstrated an increase in the frequency of thyroid dysfunction attributable to adjuvant chemotherapy [49,54].

Annual thyroid screening should be undertaken. Evaluation of basal free T_4 and TSH may miss central hypothyroidism. Because of the concern that a persistently elevated TSH will increase the risk of malignant transformation in a gland that is already at risk, persistently elevated TSH should be treated with thyroxine replacement even where the free T_4 remains in the normal range.

Regular palpation of the neck is essential given the risk of secondary thyroid neoplasms, and there should be a low threshold for undertaking ultrasound scanning and, where appropriate, fine-needle aspiration. Thyroid function screening using thyrotrophin-releasing hormone (TRH) tests may improve the sensitivity with which thyroid dysfunction is diagnosed and thus optimize replacement therapy.

Adrenal axis

A very low incidence of HP–adrenal axis dysfunction is found following treatment for brain tumors [11,18]. Of 20 survivors of posterior fossa tumors treated with cranial irradiation, all but two demonstrated adequate cortisol responses to hypoglycemia 10 years after completion of therapy [18]. In contrast, a study of 73 children treated with radiotherapy and chemotherapy demonstrated a suboptimal response to insulin hypoglycemia or standard dose ACTH test in 14 (19%) of the patients using the same criteria (peak above 500 nmol/L) [55]. A peak cortisol of 500 nmol/L or more was reached in the rest of the cohort, but they still had significantly lower peak cortisol levels than control subjects. Median follow-up in the latter study was longer (15 years) than in the former (11 years), suggesting evolving defects over time in line with other abnormalities of the HPA. Dose of radiation and time from treatment were identified as risk factors for adrenal dysfunction, with no evidence of an additive effect of adjuvant chemotherapy.

Although adrenal dysfunction is rare, it is potentially life-threatening. If dynamic testing is undertaken, this should include a test that can evaluate the hypothalamo-pituitary–adrenal axis. The best test for this is a source of debate because some patients have an adequate response to a standard ACTH test but suboptimal response to ITT. Where adrenal insufficiency is demonstrated, replacement therapy should be instituted. It may also be necessary to provide cover just for illness and surgery in patients with subtle adrenal dysfunction.

Bone mineral density

Reduced bone mineral density following treatment for childhood brain tumors is well described and likely to be multifactorial [44]. Endocrinopathies, direct effects of radiation on the growing skeleton, effects of chemotherapy, illness, and persisting immobility are all possible etiological factors.

Optimal screening programs and the possible role of preventative therapies remain to be determined. Influence of size on the interpretation of DEXA (dual-energy X-ray absorptiometry) scanning is of particular relevance in this population. DEXA requires correction for body and bone size. Quantitative computed tomography (QCT) is independent of size and provides both cortical and trabecular bone mass but delivers more radiation. Dietary and lifestyle advice and supplementation of diet to insure calcium intake is adequate should be undertaken. Estimation of vitamin D may be useful to establish dietary adequacy.

Body mass index

Increased body mass index after childhood cancer is common. Of 148 survivors of childhood brain tumor, age at diagnosis, radiation dose to the hypothalamus (51–72 Gy), and presence of any endocrinopathy were identified as risk factors [56]. Additional factors when BMI was compared with the slope for the general American population included tumor location (hypothalamic, $P = 0.001$), histology (craniopharyngioma, $P = 0.009$; pilocytic astrocytoma, $P = 0.043$; medulloblastoma, $P = 0.039$), and extent of surgery (biopsy, $P = 0.03$; subtotal resection, $P = 0.018$). Hypothalamic damage due to the surgery or following radiation were the prime factors in the etiology of obesity. In females, the risk of obesity was associated with younger age at diagnosis (less than 10 years) and increased radiation dose (Fig. 24.3).

Body mass index should be monitored and early dietary intervention provided, especially in patients known to be at high risk. The role of pharmacological intervention has not been established but, where there is severe obesity and likely hypothalamic damage, pancreatic lipase inhibitors may be worth considering. Care should be taken that doses of endocrine replacement therapy are optimized.

Summary and conclusions

The risk of endocrinopathy after radiation for brain tumors in childhood is very high. Accurate auxology including sitting height is essential, as is early involvement of a pediatric endocrinologist. Early diagnosis and treatment of GHD is desirable to optimize response, and care should be taken that adequate doses of GH are being administered. Combined GnRH and GH therapy where puberty occurs early or is progressing rapidly with suboptimal growth is recommended. Evidence to date in relation to GH therapy and risk of relapse or recurrence is reassuring.

Primary hypothyroidism secondary to CSI is also relatively common, and T_4 replacement is recommended where there is an elevated TSH even where T_4 and T_3 are normal because of the known increased risk of secondary thyroid malignancy.

Table 24.2. Endocrinopathy after treatment for childhood brain tumor.

Endocrinopathy	Risk factors
Short stature	Increasing dose of radiation Craniospinal radiation (CSI) Younger age at treatment Precocious puberty GHD Adjuvant chemotherapy
Panhypopituitarism	Radiation > 55–70 Gy Hypothalamic/pituitary tumors Nasopharyngeal tumors Increasing time from treatment
GH insufficiency	Increasing dose of radiation – nearly universal with doses above 30 Gy Increasing time from treatment Younger age at treatment Adjuvant chemotherapy
Thyroid dysfunction	CSI Role of adjuvant chemotherapy unclear Increasing time from treatment Central hypothyroidism; radiation dose ≥ 30 Gy
Precocious puberty	Female gender Younger age at treatment
Gonadal dysfunction	Central hypogonadism; radiation dose ≥ 30 Gy Female sex CSI (girls) Gonadotoxic chemotherapy especially alkylating agents Increasing time from treatment
Adrenal insufficiency	Increasing time from treatment Central adrenal insufficiency; radiation dose ≥ 30 Gy Rare compared with other endocrinopathies
Overweight/obesity	Female gender Younger age at treatment Hypothalamic damage; tumor, surgery, radiation Hypothalamic dose ≥ 51 Gy Physical inactivity (neurological impairment) Endocrinopathy

Table 24. 3. Suggested endocrine follow-up for childhood brain tumor survivors treated with radiotherapy and/or chemotherapy.

Auxology including sitting height	3- to 6-monthly Plot BMI
Bone age	Annually
Special points on examination	Pubertal assessment at each visit Palpation of thyroid
GH status	Evaluate at 2 years from diagnosis If normal, repeat if concern re. growth, and consider retesting at 2-yearly intervals even if growth normal Hypothalamically mediated test of GH secretion preferable Full APFTs usually undertaken to evaluate other HP hormones at same time as GH test GH therapy – monitor IGF-1 Consider trial of GH if poor growth without confirmed GHD Combined therapy with GnRH analog for GHD with early puberty or poor pubertal growth (even with normal timing of puberty)
Thyroid function	T_4 and TSH annually Consider TRH/TSH surge test Replace thyroxine if TSH persistently elevated
Gonadotropins	Evaluate if: Possible early/precocious puberty Pubertal delay or arrest, secondary amenorrhea or concern re gonadal failure
Evaluation of BMD	Annual PTH and vitamin D Role of DEXA scanning unclear – must correct for size QCT not widely available

Other endocrinopathies are less common but where they occur should be treated with appropriate replacement therapy. Tables 24.2 and 24.3 summarize risk factors and follow-up recommendations.

Endocrine dysfunction and treatment for childhood leukemia

Acute lymphoblastic leukemia (ALL) is the commonest childhood malignancy and has an impressively high cure rate. Treatment consists of combination chemotherapy and some form of cranial prophylaxis to prevent CNS recurrence of leukemia. Prior to 1990, this prophylaxis was given as low-dose (18–24 Gy) CI. Because of increasing concern about neuropsychological and, to a lesser extent, endocrine late effects, CI was discontinued, and CNS prophylaxis is provided by intrathecal therapy, radiotherapy being reserved for children with proven CNS disease.

Chemotherapy alone has important late effects, particularly on bone density and body composition. Children with leukemia who require a BMT as part of their therapy are at high risk of subsequent endocrinopathy. Standard treatment for ALL does not contain significant doses of gonadotoxic chemotherapy.

Growth and endocrine function after treatment for childhood leukemia

Growth and GHD

Growth during therapy for ALL has been studied extensively [4,7,57]. It is impaired during treatment but, in contrast to

children treated for brain tumors, catch-up may be observed after completion of therapy. A reduction in final height has also been observed in a large number of studies, but the etiology of this remains the subject of debate [58,59]. Cranial irradiation and subsequent GHD was clearly a factor but is no longer.

The role of chemotherapy is controversial, but it and steroids both adversely influence growth by influencing physiological bone turnover, especially osteoclast activity. Final height studies of children treated for leukemia with chemotherapy alone are conflicting, some demonstrating a significant reduction in FH [60] while others do not [61]. Determining which of the chemotherapeutic agents are responsible for any observed reduction in FH is difficult, but it is likely that steroids and methotrexate play a role, as both have demonstrable effects on bone.

Auxology, including sitting height and BMI calculation, is essential. Pubertal staging should be undertaken at 3- to 6-monthly intervals until completion of growth and puberty. Where GHD is diagnosed, replacement therapy is appropriate and thought to be safe. Where patients have received previous anthracycline therapy (part of standard ALL protocols), care should be taken when instituting GH therapy because the resulting growth spurt may exacerbate anthracycline cardiomyopathy. Cumulative doses of anthracyclines are relatively low in standard ALL therapy, reducing the risk of significant cardiomyopathy.

Thyroid dysfunction

Thyroid dysfunction is rare after treatment for leukemia that does not include BMT.

Adrenal function

Although adrenal insufficiency is rare after treatment for childhood leukemia, a small number of children treated for ALL show persistent suppression of endogenous adrenal function as a consequence of steroid therapy. While this usually recovers over time, it may rarely be permanent.

Bone density

Chemotherapy (particularly methotrexate and steroids), the disease itself, reduced activity, GHD, and poor nutrition are all risk factors for reduction in BMD. Reduced BMD has been observed in a number of studies although the relative contribution of CI and chemotherapy/steroids remains a source of debate [62–67].

Education of childhood leukemia survivors to the potential risk is important, and dietary and lifestyle measures to improve BMD should be given. Consideration should be given to measurement of calcium and vitamin D and supplementation if necessary. Whether or not the reductions in BMD are associated with an increased fracture risk later is unclear.

Body composition

Elevated BMI after therapy for childhood ALL has been well described [57]. BMI increases during therapy and remains elevated afterwards. The etiology of this is uncertain and likely to be multifactorial. CI was one important etiological factor, but increased BMI in ALL survivors treated with chemotherapy alone suggests that others play a role. Steroids may be important, and the cumulative dose of dexamethasone might well be relevant [68]. If this is the case, universal treatment with dexamethasone on the current ALL protocols is likely to magnify the problem in subsequent cohorts of survivors. In addition to the possible effects of chemotherapy and steroids, there is also some evidence to suggest that reduced exercise plays a role [69].

Regular monitoring of weight and BMI is essential. Whatever the etiology of obesity, early intervention with advice about dietary intake and the importance of exercise is likely to improve outcome. Weight-bearing exercise will be of benefit in terms of bone health as well as body composition.

Bone marrow transplantation

Bone marrow transplantation (BMT) may be undertaken for resistant or high-risk leukemia, and less commonly for other malignant disease of childhood particularly lymphoma. BMT may also be undertaken for the treatment of non-malignant hematological disorders, particularly aplastic anemia and thalassemia. A total of 14 309 patients, who were under 21 years at the time, are currently alive after undergoing allogeneic BMT between 1968 and 2002 [70].

Conditioning for BMT is undertaken with either TBI with chemotherapy (usually including cyclophosphamide) or chemotherapy alone (most commonly busulphan and cyclophosphamide, Bu/Cy). BMT, particularly where TBI is used as part of the conditioning regimen, is associated with significant endocrine morbidity. Fractionation of TBI attempts to reduce the late sequelae of TBI. Conditioning for BMT formerly employed 10 Gy in a single fraction. Current practice is to give 1440 cGy as eight fractions over 3 days. Reduced intensity conditioning is used where possible, which may have less long-term morbidity.

Some patients with ALL will have received prior CI, especially those treated for ALL in the 1970s and 1980s. Patients being transplanted for leukemia/lymphoma will usually have been pretreated with a number of chemotherapeutic agents.

Following BMT, immunosuppression is required as prophylaxis against graft-versus-host disease (GvHD), usually for about 6 months. Chronic GvHD and its treatment with immunosuppression, usually including steroids, results in growth impairment.

Growth and growth hormone deficiency

Impaired growth during and after BMT is well described [71–75]. The mechanism of growth impairment is multifactorial with disturbed puberty, direct action of cytostatic agents and TBI on growth plates, GHD, GvHD and its treatment, and nutritional factors all playing a role. BMT may follow therapy for primary or relapsed malignancy, which has itself resulted in disturbed growth or pubertal development. The relative importance of TBI and chemotherapy in the growth impairment is disputed, in keeping with similar debates in relation to growth and treatment for ALL that does not include CI.

Chemotherapy

Studies of children conditioned with Bu/Cy have reported conflicting results. One study of 80 children for up to 5 years after BMT found no significant growth impairment, but others have demonstrated impaired growth after Bu/Cy and GHD in children conditioned with Bu/Cy alone.

Comparison between a small group of neuroblastoma survivors intensively treated pre-BMT with a group treated for leukemia showed the former to be more severely affected, suggesting a role for pretreatment chemotherapy in subsequent growth impairment [76]. A study of thalassemic patients who underwent BMT with Bu/Cy found short stature (below –2 SDS) in 17% of the patients, with the risk being increased in children transplanted after the age of 7 years. Possible factors included differential metabolism of Bu in different age groups, impaired pubertal growth, desferrioxamine-induced growth impairment, and iron overload. These additional factors make extrapolation of these results to the leukemic population transplanted without TBI difficult. Although GHD is reported in thalassemic patients, none of the patients in this study was found to have GHD. Firm conclusions about the impact of Bu/Cy conditioning on FH await more FH data.

Total-body irradiation (TBI)

A number of studies have demonstrated a reduction in FH following TBI and BMT, which is worse after single-fraction than fractionated TBI [77]. This is less clear in larger studies, although there are few FH data. Cranial irradiation prior to BMT increases the FH loss, and post-transplant complications, particularly chronic GvHD, also increase the risk of growth impairment [78]. Radiation-induced skeletal dysplasia is also a factor and probably contributes to the observed disproportion. TBI may also limit epiphyseal responsiveness to GH in GH-deficient patients.

Some 20–70% of patients have GHD after TBI, the risk being greatest for children who received previous CI [7,77]. The majority of these studies have demonstrated reduced GH responsiveness to provocation studies, although a few have examined spontaneous GH secretion. There is some evidence to suggest that the threshold dose for TBI causing GHD is between 8 and 10 Gy, while doses of 10–12 Gy clearly induce GHD. It is possible that 7–8 Gy may induce GHD but not until many years after therapy.

There are few data about response to GH therapy following TBI, particularly in relation to pubertal growth and FH. Short-term studies (1–3 years) demonstrate variable but improved height velocity in response to GH, with the magnitude of the improvement varying between studies, but most contained small numbers of patients who were not followed to FH.

Regular auxology, including sitting height, is essential. Where there is a persistent reduction in height velocity (despite appropriate thyroid and/or sex steroid replacement), provocative GH testing should be undertaken. Optimization of nutrition is also important. Steatorrhea and malabsorption have been reported after BMT [79], with an increased risk in patients with chronic GvHD, and their possible role should be considered.

If GHD is confirmed, replacement therapy is reasonable in spite of a paucity of clear evidence of benefit for FH. A randomized trial would be required to evaluate its benefit but will never be undertaken for ethical reasons. GH replacement should therefore be considered on an individual basis. Available evidence suggests that effects on relapse or secondary malignant neoplasm are very small [39]. When present, GHD is not usually severe, unless previous CI has been given, so patients are therefore unlikely to meet the criteria necessary to justify continuation of therapy into adulthood.

Puberty, gonadal function, and fertility

Gonadal damage following BMT with either cytostatic or TBI-based conditioning regimens is well described. Age at transplant, conditioning schedule, and gender are all important risk factors.

The risk of gonadal dysfunction in females after BMT is high following both TBI and Bu/Cy conditioning [80–83]. Cyclophosphamide alone, in the doses used for BMT conditioning, generally does not impair gonadal function. Most girls who have undergone BMT develop ovarian failure, but ovarian recovery is well documented and fertility is possible. Older age at transplant increases the risk of ovarian failure and reduces the likelihood of ovarian recovery. Girls with ovarian failure had a median age at BMT of 8.6 years compared with 6.1 years for those with intact ovarian function [84]. In the same study, 21 of 32 girls achieved menarche.

Busulphan carries a high risk of subsequent ovarian failure, and the protective effect of young age at BMT is less clear. Where ovarian failure is established, the possibility of recovery exists, with the likelihood of this being inversely proportional to age and increased by fractionation of TBI.

In women with preserved or recovered ovarian function, regular menses and pregnancy may occur, but uterine and ovarian volumes are reduced after TBI [85]. Impaired uterine blood flow and reduced endometrial thickness have also been observed, but sex steroid replacement in physiological doses significantly increases uterine size and endometrial thickness and re-establishes uterine blood flow [85]. These observations have important clinical significance and suggest that standard estrogen replacement is probably suboptimal.

Where pregnancy is achieved, spontaneous abortion is common, and there is a high risk of low birthweight and preterm delivery.

The germinal epithelium of the testes is much more radio- and chemosensitive than Leydig cells. This means that oligo- or azoospermia and infertility is common after BMT, although Leydig cell function and testosterone secretion may be preserved despite elevated FSH and LH [83,86]. Testosterone replacement is required infrequently.

Radiation doses to the testes greater than 4 Gy are associated with almost universal failure of spermatogenesis so that the majority of males will be infertile after TBI. There is also a high incidence of germinal damage and associated infertility after Bu/Cy. The gold standard for evaluating tubular function is semen analysis, but this is not always possible, especially in younger males. It has been suggested in younger patients and those who do not wish to undergo semen analysis that serum inhibin can be utilized as a marker of fertility. Low serum inhibin concentrations have been demonstrated, but further work is needed before semen analysis can be replaced by measurement of serum inhibin [87]. Recovery of spermatogenesis after BMT has been documented but is rare [77]. Erectile dysfunction may be observed after TBI, and it has been suggested that this is due to cavernosal arterial insufficiency [88].

The relationship between age and risk of gonadal failure is less clearcut in males than in females with studies having conflicting results.

Pubertal staging should be undertaken at 3- to 6-monthly intervals until completion of growth and puberty. Testes are inappropriately small and soft for the stage of pubertal development so testicular enlargement cannot be used to monitor pubertal progression in this population. FSH, LH, and estradiol/testosterone should be monitored annually in both males and females. In units where inhibin measurement is easily available, this may be helpful in advising patients about the likelihood of infertility in males.

Ovarian failure requires estrogen replacement in doses appropriate for pubertal stage. Given the evidence that there is impaired uterine growth, reduced endometrial thickness, and abnormal uterine blood flow and that these respond to increased sex steroid replacement, it may be appropriate to monitor these parameters using ultrasound to ensure that optimal replacement is provided. As recovery of ovarian function is well documented, estrogen replacement should be discontinued for 6–8 weeks every 2 years to determine whether ovarian recovery has occurred.

Referral to infertility services is appropriate for both males and females.

Thyroid dysfunction

Because TBI includes the neck, it carries a risk of both thyroid dysfunction and subsequent thyroid malignancy. Young age at TBI increases the risk of subsequent neoplasm. The risk of thyroid dysfunction is reduced by fractionation of TBI. Chemotherapy conditioning regimens carry a risk of thyroid dysfunction, with an 11% incidence after Bu/Cy reported in a study of 270 adults after BMT compared with 16.9% following 12 Gy (fractionated) TBI [89]. Typical findings after BMT (TBI 10–12 Gy) are a mildly elevated TSH with a normal serum thyroxine, but a range of other thyroid abnormalities have been observed, including hyperthyroidism 12–18 months after BMT [90]. This has been well described after neck irradiation for Hodgkin's disease [91]. In a longitudinal study of thyroid function peri- and post-transplant, without the use of preparative TBI, a sick euthyroid syndrome (ETS) was found in 29 of 61 evaluable patients. It was associated with a poor outcome [92]. Hypothyroidism was observed in 14% of patients. Thus, it is clear that all survivors of BMT are at risk of thyroid dysfunction, although the risk is greatest in those treated with TBI.

Annual thyroid function and neck palpation should be undertaken in all survivors of BMT regardless of conditioning regimen. Concern that a persistently elevated TSH may increase the risk of neoplasm in a predisposed thyroid means that thyroxine replacement should be instituted once TSH is persistently elevated.

Adrenal function

Although there was initial concern that adrenal function would be adversely affected after TBI and BMT, this has not been observed in the majority of studies [77]. The adrenal gland is relatively radioresistant, and impaired ACTH secretion is not observed after TBI, even when preceding CI has been administered. Patients who require prolonged steroid therapy for GvHD are at risk of endogenous adrenal suppression and, although recovery is usual, it is not universal. Evaluation of the adrenal axis should be undertaken after discontinuation of steroids in this setting and replacement therapy administered if necessary.

Metabolic syndrome

Of 23 survivors of BMT, 52% had insulin resistance with impaired glucose tolerance in six and type 2 diabetes in four [93]. The triad of features that make up the metabolic syndrome (insulin resistance, hypertension, and dyslipidemia)

was observed in nine (39%) survivors. GH status was not evaluated, and GHD may have contributed to the abnormalities observed. However, the findings are a cause for concern, and frank diabetes in six of a total of 74 children who received BMT for leukemia or non-Hodgkin's lymphoma (NHL) has been reported [94]. Plasma lipids were not reported, but only one patient was overweight. Irradiation, endocrine replacement, and corticosteroid therapy all play a role in pancreatic dysfunction.

Annual evaluation of fasting glucose insulin level and lipids is probably indicated in survivors of BMT. Those with abnormal insulin glucose levels should undergo an oral glucose tolerance test.

Bone density and osteonecrosis

BMT carries a number of potential risk factors for reduced BMD, including disease itself, chemotherapy, and/or TBI, poor nutrition, gonadal dysfunction, GvHD and its treatment, and reduced physical activity. Osteoporosis and osteopenia have been reported following BMT in adults. Studies in children are limited and have been less clearcut [94,95]. A prospective study of bone mass and turnover in adults after BMT demonstrated that the sharpest decline in BMD occurred in the first 6 months [96]. Assessment of BMD in children has largely been undertaken using DEXA scanning with its associated methodological difficulties. However, a recent study evaluated bone mineral density in 48 survivors of pediatric BMT using QCT [70], and the incidence of osteonecrosis was determined using magnetic resonance imaging (MRI). QCT *z*-scores demonstrated that 21% of the study population had osteoporosis (*z*-score < –2) and 26% osteopenia (*z*-score between –1 and –2). Ten of 43 patients who underwent MRI had evidence of osteonecrosis, a much greater frequency than previous studies. Eight of these were clinically significant. Approximately 15% of the study population had evidence of both reduced BMD and osteonecrosis.

All patients should be counseled about bone health and given appropriate dietary and lifestyle advice. An awareness of the possibility of osteonecrosis should prompt early investigation of persistent joint pain, particularly in the hips or knees. MRI is the investigation of choice for this.

Tables 24.4 and 24.5 summarize risk factors and follow-up recommendations.

Table 24.4. Endocrinopathy after BMT.

Endocrinopathy	Risk factors
Short stature	TBI Previous cranial radiation GHD Chronic GvHD Poor nutrition/malabsorption
GH insufficiency	TBI Previous cranial radiation
Thyroid dysfunction	Hypothyroidism Female gender TBI Bu/Cy conditioning (lower risk than TBI) Thyroid nodules Female gender Younger age at treatment Hyperthyroidism (rare) TBI Thyroid cancer Increased time from treatment Younger age at treatment Female gender
Gonadal dysfunction	Female Older age at treatment TBI Busulfan Male Radiation ≥ 4 Gy – azoospermia very likely Radiation ≥ 20 Gy Leydig cell failure likely (testicular boost for testicular relapse is 24 Gy) Busulfan Effect of age unclear
Adrenal insufficiency	Chronic GvHD
Reduced BMD	Hypogonadism Chronic GvHD Inactivity Poor nutrition
Metabolic syndrome	Risk factors unknown, TBI probably important ?Role of GHD

Summary and conclusions

Impaired growth and endocrine dysfunction are common after treatment for childhood cancer, particularly after BMT and treatment for malignant brain tumors. Active surveillance tailored to an individual child's treatment regimen should be undertaken. Dietary and lifestyle advice is also important because of the well-described risk of increased BMI and reduced BMD after treatment for childhood cancer, particularly brain tumors, ALL, and BMT.

Most children treated with radiation for a malignant brain tumor will be GH deficient. GH therapy has been widely used in GHD childhood cancer survivors. The benefit is probably less than that observed in children with classical GHD, especially in brain tumor survivors. Early diagnosis and treatment and optimization of GH dose is essential. GH treatment appears to be safe in this context.

Neck irradiation carries significant risk of both thyroid dysfunction and subsequent thyroid neoplasm. Therapy of persistently elevated TSH even where free T_4 is still normal is recommended.

Table 24.5. Suggested endocrine follow-up for BMT survivors.

Auxology including sitting height	3- to 6-monthly Plot BMI
Bone age	Annually
Special points on examination	Pubertal assessment at each visit Testicular volume not a useful indicator of pubertal progression Palpation of thyroid BP
GH status	Evaluate if persistent poor growth
Thyroid function	T_4 and TSH annually Replace thyroxine if TSH persistently elevated
Gonadotropins	LH, FSH, estradiol/testosterone annually Consider pelvic USS in females; uterine size, endometrial thickness, Doppler studies Semen studies in males as requested by patient Ovarian failure may be reversible – trial off estrogen for 6–8 weeks every 2 years recommended
Glucose:insulin ratio	Annually GTT if abnormal
Lipid	Fasting lipids annually
Evaluation of BMD	Annual PTH and vitamin D Role of DEXA scanning unclear – must correct for size QCT not widely available

Gonadal failure is common, especially after BMT. There is evidence to suggest that current strategies do not provide adequate estrogen replacement and that uterine/ovarian sonography may be a useful means of monitoring the adequacy of estrogen replacement.

While there is clear evidence of an effect of treatment for brain tumors and leukemia (including BMT) on bone health, optimal screening strategies remain to be determined, not least because of variation in access to DEXA and QCT across units.

Improved survival after childhood cancer is one of the success stories of the twentieth century. It is essential that these children receive optimal follow-up to enable them to achieve their potential.

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25 Tests and normal values in pediatric endocrinology

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Laboratory tests are essential to the diagnostic process in endocrinology. A variety of routine biochemical, hematological, and immunological tests may be indicated for the investigation of disease together with the measurement of hormones. The efficacy of endocrine tests depends on the choice of tests, the preparation of the patients, the integrity of the specimens, the quality of the measurements, and the validity of the reference data [1]. The measurement of basal hormone concentrations is generally of limited use because the secretion of hormones is a complicated process in which the hypothalamo-pituitary–endorgan axis is subject to positive and negative feedback mechanisms. Feedback control may be over-ridden by external factors, such as stress, circadian rhythm, and a variety of drugs. The investigation of endocrine disorders therefore frequently involves the use of dynamic tests to check the ability of individual axes to respond to suppressive or stimulatory effects of a variety of provocative agents. The performance of such tests is complicated and invariably involves some discomfort and, in certain cases, risk to the patient.

The time invested in performing the tests, including preparation of the patient and collection, transportation, and analysis of the samples, is considerable. The volumes of blood required, particularly where combined tests need to be performed in a neonate, are significant. It is crucial therefore that these investigations are targeted to inform clinical decisions. This requires meticulous planning and co-ordination with involvement and communication with the laboratory at all times.

General principles for all endocrine tests

Safety considerations

Precautions and contraindications are described in the protocols for each test and should be considered carefully before embarking on the test. Measures have been recommended [2,3] as a prerequisite for undertaking endocrine/metabolic tests in children to minimize adverse effects.

- Performance in or supervision by specialized pediatric endocrine centers.
- Detailed knowledge of the particular test protocol and provocative agents.
- Specialized nursing staff familiar with the tests.
- An environment where full pediatric emergency resuscitation facilities are available.
- Adjustment of protocols for particular individuals or circumstances. It should not be assumed that the same standard protocol can automatically be safely applied to all patients.
- Appropriate laboratory back-up, particularly for tests involving fasting, induction of hypoglycemia, or water deprivation.
- Glucose meters with acceptable accuracy/precision in the hypoglycemic range for immediate glucose monitoring in the testing area.
- Medical officer readily available (for certain tests, e.g. insulin hypoglycemia test, immediately available).
- Experienced personnel to site intravenous cannulae.

Patient preparation

1 Confirm (by discussion with a clinical pediatric endocrinologist if appropriate) that the test to be undertaken is appropriate. Insure that all relevant baseline tests (endocrine and non-endocrine) have been performed (e.g. renal function tests to exclude renal failure, electrolytes and glucose in suspected adrenal or pituitary disease).
2 Insure that up-to-date protocols are available in the clinical area, that medical and nursing staff are competent in the performance of the test(s) and briefed on sample collection and transportation to the laboratory.
3 Insure that liaison has occurred with the laboratory, which is thereby prepared for urgent processing/analysis of samples.
4 Confirm that the patient and parent understand the reasons for the test, the test protocol, and that the patient has been suitably prepared (e.g. fasted where this is a requirement of the procedure).

5 Measure the height and weight of the child (essential that this is done on the day of the test) and calculate the correct doses of provocative agents. Surface area can be calculated using the formula: surface area (m^2) = $\sqrt{}$ {[height (cm) × weight (kg)]/3600}.
6 Confirm that the dose of the provocative agent has been calculated correctly and checked by another member of staff. Also confirm that the agent has been prepared correctly and that this too has been checked by another member of staff.

Sample collection

Blood

1 Most tests require the insertion of an intravenous (IV) cannula for serial blood sampling [2]. Apply local anesthetic cream (EMLA cream or patch) for a minimum of 1 h, clean the site with sterile water, and insert the cannula. Maintain patient with a heparin/saline solution (1 U/mL).
2 Collect basal samples, including a sample taken at the time of cannulation (e.g. $t = -60$ min). This is essential as the stress generated by the procedure may be sufficient to stimulate hormone production, which may be followed by a period of quiescence. This has the potential to generate false-positive results if sampling only commences at $t = 0$.
3 Insure that the correct sample tubes are available and labeled appropriately. The local laboratory will provide a policy indicating minimum requirements for sample labeling, which will include patient ID, ward/clinic, date of test, and sample test time, e.g. 0, 30, 60 min.
4 Check the blood volumes required at each time. Additional samples are frequently required at $t = 0$, and volumes may vary at different times throughout the test (e.g. combined pituitary function tests).
5 Complete the request form supplied by your local laboratory legibly and fully, providing information on patient ID, gender, ward/clinic, times of samples, tests requested at each time, and clinical details stating indication for tests. Give contact details of the doctor to be contacted in the event of any problem with the samples collected.

Urine

1 Provide written instructions for the patient and parents on how to collect a timed (usually 24-h or overnight) urine sample and insure that these written instructions are explained verbally and understood.
2 Insure that the appropriate container (plain or with the correct preservative) is obtained from the local laboratory and supplied to the patient.
3 Insure that the container is correctly labeled and the accompanying request form is completed in full.

Table 25.1. Hormones of the anterior pituitary gland and their control.

Cell type	Hormone	Hypothalamic hormone
Thyrotroph	TSH	TRH (+) Somatostatin (–)
Corticotroph	ACTH	CRH (+)
Gonadotroph	LH/FSH	GnRH (+)
Somatotroph	GH	GHRH (+) Somatostatin (–)
Lactotroph	Prolactin	Dopamine (–) TRH (+)

(+) stimulatory, (–) inhibitory.

Pituitary

The anterior pituitary gland secretes six known hormones: growth hormone (GH), adrenocorticotrophic hormone (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and prolactin. Each of these is regulated by releasing or inhibitory hormones from the hypothalamus. A summary of the anterior pituitary hormones and their control is provided in Table 25.1.

The posterior pituitary is responsible for the synthesis and secretion of oxytocin and arginine vasopressin (AVP). The principal stimulus for AVP secretion is an increase in plasma osmolality.

Indications for testing pituitary function

There are three broad groups of pediatric patients for whom pituitary function tests are required.
1 Patients with short stature and/or abnormal growth velocity in whom other causes of growth failure (e.g. hypothyroidism, chronic systemic disease, Turner syndrome, skeletal disorders) have been excluded.
2 New patients with suspected hypopituitarism. This includes patients with target organ failure not associated with appropriate elevation of the relevant pituitary trophic hormone, patients with suspected diabetes insipidus, patients presenting with clinical features of hypothalamo-pituitary tumors (e.g. headaches, visual failure), patients with optic nerve hypoplasia or septo-optic dysplasia and patients in whom a hypothalamic-pituitary mass is found incidentally during the course of radiological investigations.
3 Patients with known hypothalamo-pituitary disease in whom an evolving endocrine deficit is anticipated. This group mainly comprises patients who have received radiotherapy for a pituitary tumor.
The last two groups are equally applicable to adult patients [4].

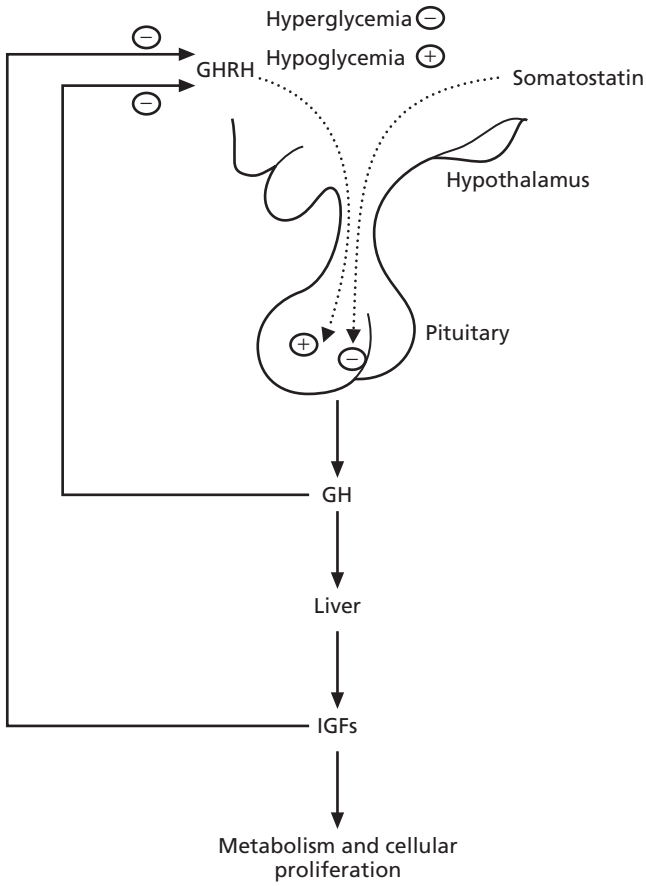

Fig. 25.1. Components of the GH–IGF axis.

Anterior pituitary

Growth hormone deficiency

The diagnosis of growth hormone deficiency (GHD) in childhood requires clinical and auxological assessment, combined with biochemical tests of the GH insulin-like growth factor (IGF) axis and radiological evaluation [5]. The components of the GH–IGF axis are depicted in Figure 25.1. GHD may present as an isolated problem or in combination with multiple pituitary hormone deficiency (MPHD). Criteria to initiate immediate investigation for GHD have been defined [6] and include:

1 Severe short stature, defined as a height more than 3 SD below the mean.

2 Height more than 1.5 SD below mid-parental height.

3 Height more than 2 SD below the mean and a height velocity over 1 year more than 1 SD below the mean for chronological age, or a decrease in height SD of more than 0.5 over 1 year in children over 2 years of age.

4 In the absence of short stature, a height velocity more than 2 SD below the mean over 1 year or more than 1.5 SD sustained over 2 years.

5 Signs of an intracranial lesion.

6 Signs of MPHD.

7 Neonatal symptoms and signs of GHD (e.g. hypoglycemia, prolonged jaundice).

As normal GH secretion is pulsatile, with four to six pulses per 24 h, random single GH estimations are rarely helpful in diagnosing or excluding GHD. Hence, a variety of provocation tests have been used. However, it has been argued that pharmacological testing is non-physiological and may bear no resemblance to endogenous secretion. Tests have numerous limitations [7], and there is no "gold standard" test against which biochemical tests can be evaluated. Growth hormone status is a continuum from normality to complete deficiency [8] so defining the point at which mild GH insufficiency becomes normality is not possible. Arbitrary cutoffs have been used, which define inadequate GH secretion as a peak GH concentration during a stimulation test of less than 15 or 20 mU/L.

Growth hormone concentrations are highly assay dependent. The number of GH variants, isoforms, and molecular states in human plasma is considerable. Ability to quantify each of these variants depends on assay design, antibody specificity, and the composition of the standard used [9]. Competitive immunoassays employing polyclonal antibodies are better at averaging or integrating the multiple epitopes involved, but the precision of such assays is poor and sensitivity inadequate. They have now been largely superseded by non-competitive two-site assays employing monoclonal antibodies specific for 22-kDa GH. Continued use of pituitary GH standard (80/505), a heterogeneous material, rather than recombinant GH standard (88/624) inhibits progress toward agreement between laboratories. Immunoassays show poor comparability, with up to threefold differences and poor correlation, despite improvement in technology [10].

All provocation tests have poor specificity (50–80%) with a high incidence of false positives [7]. In an attempt to overcome this problem, it has been common practice to carry out two tests either separately or sequentially, but the outcome of this is merely to compound the errors. Alternative approaches to assessing GH secretion have proved either too demanding (e.g. 12- or 24-h GH profiles) or to offer no advantage in terms of predictive value (e.g. urinary GH). It has been argued that provocative tests are reliable only in patients in whom the clinical signs and symptoms are clear and that they should be abandoned [7]. However, guidelines on the use of human GH in children with growth failure recommend the performance of provocation tests to support the clinical diagnosis of GH deficiency [11]. Stimulation tests may be combined with one or more other measures of the GH axis, namely IGF-I and/or IGF binding protein-3 (IGFBP-3) to improve diagnostic efficiency [8].

Sex steroid priming

In pre- and peripubertal children who have a subnormal response to provocative testing, sex steroid priming may

Table 25.2. Sampling for insulin tolerance test.

Time (min)	Meter glucose	Lab glucose (fluoride oxalate tube)	GH (plain tube)	Cortisol (plain tube)
–30 (before insulin)	+	+	+	+
0 (before insulin)	+	+	+	+
30	+	+	+	+
45	+	+	+	+
60	+	+	+	+
90	+	+	+	+
120	+	+	+	+

increase the response to that seen in late puberty and should be considered. The rationale for this is the estrogen-induced rise in GH during puberty. There is no consensus on the appropriate age cutoff for priming but, as a guideline, it should be considered in patients with a bone age greater than 10 years, and possibly younger in obese children. Oral ethinylestradiol should be given (20 μg in the evening) to girls and boys less than 11 years daily for 3 days and the test carried out on day 4. Be aware that ethinylestradiol will almost certainly cause nausea. In boys older than 11 years, give Sustanon (100 mg intramuscularly) as a single injection 5–7 days before the test.

Insulin tolerance test (ITT)

Background

Insulin-induced hypoglycemia suppresses the somatostatin tone and stimulates the α-adrenergic receptors. It induces not only GH but also ACTH release and a rise in serum cortisol concentrations. The advantage of the insulin tolerance test is that, in addition to stimulating GH secretion, it also tests the integrity of the entire hypothalamo-pituitary–adrenal axis. This test has been associated with morbidity and mortality, mainly because of the use of inappropriate amounts of hyperosmolar fluid to correct hypoglycemia. The decision to undertake an ITT should be considered carefully and carried out only in a specialized pediatric endocrine investigation unit.

Precautions

1 The test is contraindicated in children with diagnosed epilepsy or a history of unexplained blackouts and in children < 2 years of age, for whom a glucagon provocation test may be more appropriate.

2 Hypothyroidism impairs the GH and cortisol response. Patients with adrenal and thyroid insufficiency should have corticosteroid replacement commenced before thyroxine, as thyroxine may precipitate an adrenal crisis. The insulin provocation test may need to be repeated after 3 months of thyroxine therapy in patients with confirmed thyroid or dual insufficiency.

3 Special precautions are required in children suspected of having panhypopituitarism.

4 Intravenous 10% dextrose (never 50%) must be immediately available.

5 A glucose meter with acceptable performance in the hypoglycemic range must be available, and staff performing the test must be certified competent to use it.

Preparation

1 Check that thyroid function tests are normal.

2 Prime with sex steroids if indicated.

3 The child should be fasted for 8 h before the test, only water is allowed.

4 The child should be weighed prior to the test, in order to calculate accurately the dose of insulin to be administered.

5 Insert IV cannula (see section on blood collection) and maintain patent with heparinized normal saline. The stress of cannulation can cause an increase in GH, making interpretation of the test difficult. After cannulation, wait for 30–60 min before commencing the test and take a blood sample at –30 min to aid interpretation if the basal sample is found to be elevated.

Protocol

1 t = –30 min; take blood (plain tube) for GH and cortisol estimation.

2 t = 0 min; give soluble insulin 0.10–0.15 U/kg IV, using the lower dose if there is a strong suspicion of panhypopituitarism or if the child has had previous cranial surgery or radiotherapy. The dose may need to be increased in patients with diabetes mellitus, insulin resistance, or obesity.

3 Take samples as shown in Table 25.2. Measure glucose concentrations on the glucose meter but also send a sample to the laboratory for urgent analysis.

4 Observe child closely for clinical signs and symptoms of hypoglycemia (e.g. sweating and drowsiness).

5 The results can be interpreted only if adequate hypoglycemia has been achieved. This is defined as a laboratory glucose half the fasting value or < 2.2 mmol/L. If there have been no clinical signs of hypoglycemia by 45 min, the dose of insulin should be repeated and the test continued with blood samples timed again from 0 min.

6 Once hypoglycemia has occurred, the child should be given glucose drinks. If the child remains persistently hypoglycemic

or loses consciousness or fits, he/she should be treated with an IV bolus of 200 mg/kg glucose (2 mL/kg 10% dextrose) over 3 min followed by an IV infusion using 10% dextrose at 2.4–4.8 mL/kg/h (4–8 mg/kg/min glucose). Check glucose concentrations on the glucose meter after 4–5 min and adjust dextrose infusion to maintain blood glucose at 5–8 mmol/L. If there is no improvement in conscious concentration after normal glucose concentration is restored, an alternative explanation should be sought. Do not stop sampling.

7 If panhypopituitarism is suspected, give 100 mg of hydrocortisone IV at the end of the test or earlier if recovery from hypoglycemia is slow.

8 The child must not be sent home until an adequate high-carbohydrate meal has been eaten without vomiting and the blood glucose has been maintained at 4 mmol/L for a minimum of 2 h.

9 This test may be conducted as part of a combined ITT/thyrotrophin-releasing hormone (TRH)/gonadotropin-releasing hormone (GnRH) pituitary function test, in which case the sampling protocol is shown in Table 25.7.

Interpretation

Interpretation is not possible unless adequate hypoglycemia (glucose < 2.2 mmol/L) has been achieved.

A GH concentration of > 20 mU/L excludes GH deficiency. However, the precise cutoff applied varies between centers and is dependent on the bias of the assay used by the local laboratory. Biochemical data must be interpreted in conjunction with clinical and auxological data in order to make decisions about GH treatment in an individual patient. Combining the test with measurement of IGF-I and its binding proteins is one approach that has been recommended to aid the decision in patients with peak GH concentrations in the partially deficient range (> 7.5 but < 15 mU/L) [5]. GH concentrations are increasingly being expressed in ng/mL or μg/L, and mass units may replace the current international unit.

Interpretation of the cortisol response is possible only if hypothyroidism has been excluded. An adequate cortisol response is defined as a peak concentration of > 550 nmol/L. Patients with peak concentrations < 550 but > 400 nmol/L may only need steroid cover for major illnesses and stresses.

Glucagon stimulation test

Background

Glucagon stimulates the release of GH and ACTH by a hypothalamic mechanism and therefore indirectly stimulates cortisol secretion [12]. The precise mechanism of the stimulation is unclear, particularly in cases where rebound hypoglycemia does not occur [13]. The test is particularly useful for the assessment of GH and cortisol reserve in children < 2 years and others in whom the insulin tolerance test is contraindicated. The timing of the peak GH response depends on whether the glucagon is injected intravenously or intramuscularly. Glucagon has also been administered subcutaneously, but this is not recommended as absorption is unreliable [2].

Table 25.3. Sampling for glucagon stimulation test.

Time (min)	Meter glucose	GH (plain tube)	Cortisol (plain tube)
–30 (before glucagon)	+	+	+
0 (before glucagon)	+	+	+
60	+	+	+
90	+	+	+
120	+	+	+
150	+	+	+
180	+	+	+

Precautions

1 The test is contraindicated in patients suspected of having pheochromocytoma or hyperinsulinism and is unreliable in patients with diabetes mellitus.

2 As for the insulin tolerance test, hypothyroidism impairs the GH and cortisol response.

3 Glucagon may cause nausea, vomiting, and abdominal pain.

Preparation

As for the insulin tolerance test.

Protocol

1 t = –30 min; take blood (plain tube) for GH and cortisol estimation.

2 t = 0 min; give glucagon IV or IM 20 μg/kg up to a maximum of 1 mg.

3 Take samples as shown in Table 25.3.

4 In children with suspected hypopituitarism, prolonged fasting may induce hypoglycemia. Blood glucose should be checked using a glucose meter in these patients whenever a sample is taken for GH/cortisol. If the patient shows signs/symptoms of hypoglycemia, send an urgent sample to the laboratory for glucose analysis.

5 If hypoglycemia is confirmed, treatment should be instigated as described for the insulin tolerance test.

6 This test may be performed as a combined glucagon/TRH/GnRH pituitary function test. The sampling protocol is shown in Table 25.8.

Interpretation

For interpretation of GH and cortisol concentrations, see insulin tolerance test. There are conflicting reports in the literature [12,14,15] regarding the sensitivity of this test relative to the insulin tolerance test.

Table 25.4. Sampling for arginine, clonidine, and L-dopa stimulation tests.

Time (min)	Arginine	Clonidine	L-Dopa
–30	+ (before infusion)		
0	+ (at end of infusion)	+ (before clonidine)	+ (before L-dopa)
15	+		
30	+	+	+
45	+		
60	+	+	+
90	+	+	+
120	+	+	+
150		+	

Arginine stimulation test

Background

The injection of various amino acids (e.g. ornithine, arginine) is followed by an increase in GH concentrations in blood. Arginine stimulates GH secretion by reducing somatostatin tone and possibly by stimulation of α-adrenergic receptors with GHRH release.

Precautions

Hypothyroidism impairs the GH response.
Arginine may cause nausea and some irritation at the infusion site.
Vomiting has been described in a few patients.

Preparation

As for the insulin tolerance test.

Protocol

1 $t = -30$ min: take blood (plain tube) for GH estimation.
2 $t = 0$ min; give arginine 0.5 g/kg up to a maximum of 30 g. This is given by infusion IV of a 10% solution of arginine monochloride in 0.9% NaCl at a constant rate over 30 min.
3 Take samples as shown in Table 25.4.
4 In children with suspected hypopituitarism, blood glucose should be checked as described in the glucagon stimulation test protocol.
5 This test may be performed as a combined arginine/TRH/GnRH/synacthen dynamic function test. The sampling protocol is shown in Table 25.9.

Interpretation

For interpretation of peak GH concentrations, see insulin tolerance test. Usually, the peak GH concentration is reached about 60 min after staring the arginine infusion.

Clonidine test

Background

Clonidine, a selective α-receptor agonist, causes GH release via GHRH secretion [15].

Precautions

Clonidine causes hypotension and drowsiness, although the former is rarely severe enough to require treatment.

Preparation

1 Check thyroid function tests.
2 Prime with sex steroids if indicated.
3 Fast child for 8 h prior to the test (only water is allowed).
4 Measure height and weight of child and calculate surface area (see patient preparation section).

Protocol

1 Give clonidine, 0.15 mg/m^2 orally.
2 Take samples as shown in Table 25.4.
3 Measure blood pressure every 30 min until 1 h after the test.
4 In children with suspected hypopituitarism, blood glucose should be checked as described in the glucagon stimulation test protocol.
5 Child may be safely discharged 1 h after the test if fully awake.

Interpretation

For interpretation of peak GH concentrations, see insulin tolerance test. Studies in normal children [16] have indicated a higher GH response to clonidine compared with other provocative agents.

L-Dopa stimulation test

Background

L-Dopa increases GH secretion through dopaminergic and α-adrenergic pathways.

Precautions

L-Dopa may cause nausea and occasionally vomiting, vertigo, fatigue, and headache.

Preparation

1 Check thyroid function tests.
2 Prime with sex steroids if indicated.
3 Fast child for 8 h prior to the test (only water is allowed).
4 Weigh child and select appropriate dose of L-dopa.

Protocol

1 Give L-dopa orally: < 15 kg body weight, 125 mg; < 35 kg body weight, 250 mg; > 35 kg body weight, 500 mg.
2 Take samples as shown in Table 25.4.

3 In children with suspected hypopituitarism, blood glucose should be checked as described in the glucagon stimulation test protocol.

Interpretation
For interpretation of peak GH concentrations, see insulin tolerance test. The timing of peak response to L-dopa varies widely.

Growth hormone-releasing hormone (GHRH) stimulation test

Indication
The GHRH stimulation test is not a frontline diagnostic test. It can be used to distinguish hypothalamic from pituitary causes of GH deficiency in patients who have previously demonstrated a subnormal response to the standard provocation tests. Hypothalamic dysfunction is a common occurrence in isolated GH deficiency.

In adults, GHRH (1 μg/kg IV) has been used in combination with somatostatin antagonists arginine (0.5 g/kg IV) or hexarelin (0.25 μg/kg). Such combinations provide a potent and reproducible test of pituitary GH secretion without side-effects, which may eventually replace traditional provocative agents. It has been suggested that these combination tests directly explore the pituitary GH-releasable pool, while testing with GHRH alone explores more the integrity of hypothalamic mechanisms involved in the control of somatotroph function [17]. The use of these tests has not however been studied extensively in children.

Precautions
GHRH commonly causes facial flushing, but there are no other side-effects.

Patient preparation
1 Check thyroid function tests.
2 Prime with sex steroids if indicated.
3 Fast child for 8 h prior to the test (only water is allowed).
4 Weigh child and select appropriate dose of GHRH.
5 Insert IV cannula (see section on blood collection) and maintain patent with heparinized normal saline. After cannulation, wait for 30 min before commencing the test and take a blood sample at –15 min to aid interpretation if the basal sample is found to be elevated.

Protocol
1 $t = -15$ min: take blood (plain tube) for GH estimation.
2 $t = 0$ min; give IV bolus of GHRH (1 μg/kg diluted in 10 mL of normal saline).
3 Take further samples at 0 (before GHRH), 5, 15, 30, 60, 90, 120 min.
4 In children with suspected hypopituitarism, blood glucose should be checked as described in the glucagon stimulation test protocol.

Interpretation
A good response to GHRH (but not to ITT or glucagon) suggests a hypothalamic cause of GHD. Failure to respond suggests an abnormality in the pituitary gland or in the GHRH receptor. However, because GHRH is required for both the synthesis and the release of GH, a single bolus of GHRH may also fail to elicit a response in hypothalamic disease. The pituitary somatotroph cells may be sensitized by priming the pituitary gland with daily GHRH injections over several days, after which time the GHRH test should be repeated.

Measurement of IGF-I and IGF binding proteins

GH action is mediated via the production of IGF-I and -II. Hepatic synthesis of these growth factors is mainly regulated by GH, and severe GHD is associated with a reduction in their concentrations. IGF-I circulates bound to IGFBP-3, which then forms a ternary complex with acid-labile subunit (ALS).

IGF-I and IGFBP-3 concentrations in the circulation are valuable markers of GH insufficiency and are frequently used as an adjunct to provocative testing. Their concentrations are more stable and their circulating half-lives are much longer than GH itself, although concentrations are affected by nutritional status, liver and renal disease, hypothyroidism, diabetes mellitus, and sex steroids. Studies have shown the markers to have high specificity but low sensitivity [18]. Hence, low concentrations are highly indicative of GHD, but normal concentrations do not necessarily exclude the diagnosis. In cases of congenital (Laron syndrome) and acquired GH insensitivity, GH concentrations are elevated and IGF-I and IGFBP-3 concentrations very low [19]. IGF-I and IGFBP-3 concentrations are highly method dependent and are affected by age, sex, and pubertal status. Comprehensive reference ranges must be developed locally to enable data to be interpreted (Table 25.5).

IGF-I generation test

Indication
Diagnosis of congenital or acquired GH insensitivity. The former is usually due to a defect in the GH receptor but may also be the result of defective intracellular GH signaling. Conditions that may result in acquired GH insensitivity include malnutrition and liver disease.

Patient preparation
Ensure that nutritional intake is adequate prior to and during the performance of this test.

Protocol
1 Day 1: take blood for IGF-I and IGFBP-3 estimation.
2 Days 1–4: give SC GH (0.1 IU/kg/day = 33 μg/kg/day).
3 Day 5: take blood for IGF-I and IGFBP-3 estimation.

Table 25.5. Possible approach to the interpretation of the results of a single growth hormone stimulation test (abnormal defined as a peak GH concentration less than 15 mU/L) and an IGF-I concentration (abnormal defined as less than –2 SD from the mean of an age- and sex-matched control group).

GH stimulation test	IGF-I concentration	Interpretation	Action
Normal	Normal	Normal GH–IGF-I axis	Nil
Low	Normal	Possible GH insufficiency	Perform second GH stimulation test. If low, GH insufficiency confirmed
Normal/high	Low	Possible GH insensitivity	Perform IGF-I generation test
Low	Low	GH insufficiency confirmed	Central nervous system imaging of the hypothalamic-pituitary axis

Interpretation

An incremental increase in IGF-I of > 20 μg/L and IGFBP-3 of > 0.4 mg/L above the baseline excludes GH insensitivity.

Radiological investigations in growth failure

Radiological investigations are indicated when clinical symptoms suggestive of a pituitary lesion are present, e.g. persistent early morning headaches, visual disturbance and/or field defect, or overt hypopituitarism without explanation [8]. High-resolution computed tomography (CT) or magnetic resonance (MR) scanning can be used. Midline calcification on CT scan is highly suggestive of craniopharyngioma. Structural abnormalities within the midline, as demonstrated by MR scan, are most commonly due to septo-optic dysplasia, a condition frequently associated with pituitary dysfunction. Reduced anterior pituitary height, an attenuated or interrupted pituitary stalk, and/or an ectopically positioned posterior pituitary are all associated with pituitary dysfunction. Germinomas may occur in the hypothalamic region – tumor marker concentrations (α-fetoprotein and β-hCG) are key to the diagnosis of these tumors.

Growth hormone excess

GH excess presenting as pituitary gigantism or juvenile acromegaly is rare and is caused by a GH-secreting pituitary adenoma in most instances. Most tall children are not suffering from a pathological condition, but all children with height velocity > 97th centile over 1 year or > 75th centile over 2 years require investigation [20]. It is important to assess whether growth is disproportionate, as this is more commonly associated with an underlying genetic syndrome. The differential diagnosis of tall stature includes constitutional tall stature, primary growth disorders, which can be divided into those of prenatal onset (e.g. Sotos syndrome, Beckwith–Wiedemann syndrome) and those of postnatal onset (e.g. Marfan syndrome, Klinefelter syndrome), and secondary growth disorders, which encompass hyperthyroidism, obesity, and precocious puberty in addition to GH excess.

Single random GH measurements are not a reliable diagnostic indicator of GH excess, as the sporadic nature of GH secretion and its increase in response to stress may result in an abnormally high random GH concentration in a normal patient. IGF-I and IGFBP-3 concentrations are elevated in GH excess. IGF-I concentrations are less variable than GH concentrations and are a function of the integrated 24-h serum GH concentration As a result, it has been proposed that they should be used as a screening test for GH hypersecretion [21]. However, confirmation of the diagnosis must be made on the basis of failure of GH to suppress during an oral glucose tolerance test (GTT).

Glucose suppression test (GTT) for growth hormone

Indication

Suspected pituitary gigantism or juvenile acromegaly.

Precautions

1 Test is unnecessary in diabetic patients who demonstrate a suppressed GH in the presence of hyperglycemia [2].

2 Patients may feel nauseous, although the incidence is reduced if Lucozade or polycal liquid (a flavored drink based on glucose polymers) are used in place of glucose solution.

Preparation

The diet for the 3 days preceding the test should contain adequate carbohydrate (approximately 60% of calories).

The patient should be fasted overnight for 10–14 h (plain water allowed) and should rest throughout the test.

Prepare the glucose load which may be:

- 1.75 g/kg anhydrous glucose (maximum 75 g) or 1.92 g/kg glucose monohydrate (maximum 82.5 g) dissolved in 100–200 mL of water or
- 9.2 mL/kg Lucozade (maximum 394 mL) using the current formulation of 73 kcal carbohydrate/100 mL.
- 2.64 mL/kg polycal liquid (maximum 113 mL) diluted with water to produce a glucose concentration of no greater than 25 g per 100 mL (1.5 mL of polycal = 1 g of glucose).

Insert IV cannula (see section on blood collection) and maintain patent with heparinized normal saline.

Protocol

1 0 min: take blood samples for GH (plain tube) and glucose (fluoride oxalate tube) estimation.

2 The child should drink the glucose load within 5 min.

3 Take further samples for GH and glucose at 30, 60, 90, and 120 min.

Table 25.6. Guidelines for interpretation of serum prolactin concentrations.

Prolactin concentration (mU/L)	Interpretation
< 425	Normal
425–1000	Suggest repeat sample Does not normally indicate serious pathology
1000–2000	Suggest repeat sample The raised prolactin concentration may be secondary to stress, drugs, PCO, hypothalamic disorders, GH hypersecretion, primary hypothyroidism, or chronic renal failure Patients with "non-functioning" pituitary tumors often have a serum prolactin in this range
2000–4000	Suggestive of microprolactinoma or a hypothalamic disorder Drug treatment is less likely to give this degree of prolactin elevation but is possible
4000–6000	Likely to be a prolactinoma or possibly a hypothalamic disorder
> 6000	Almost always indicates the presence of a macroprolactinoma

Interpretation

GH suppresses to < 2 mU/L in normal individuals. Failure to suppress and sometimes a paradoxical rise in GH concentrations is characteristic of GH hypersecretion.

Hyperprolactinemia

Any process that interferes with dopamine synthesis, disrupts the hypothalamo-pituitary connection, or prevents dopamine binding to its receptors can cause hyperprolactinemia. The etiology of hyperprolactinemia is diverse and includes drugs, stress, pituitary tumors, polycystic ovary syndrome, and primary hypothyroidism. Drugs include tricyclic antidepressants, phenothiazines, metaclopramide, methyldopa, and reserpine. Pituitary tumors are an important cause of hyperprolactinemia – they may be prolactin-secreting tumors or non-functioning pituitary tumors that secondarily increase prolactin concentrations by interfering with the transport of dopamine.

Radiological evaluation is necessary in all cases of hyperprolactinemia. Skull radiographs may be abnormal because of an expanded fossa associated with the empty sella syndrome, and high-resolution CT should be performed in all patients with abnormal plain skull radiographs and elevated serum prolactin concentrations [22].

Secretion of prolactin is pulsatile, and at least three measurements are required to make the diagnosis. Serum thyroxine and TSH concentrations should be measured to exclude hypothyroidism. Hyperprolactinemia may be the result of macroprolactin, a complex of monomeric prolactin and immunoglobulin G that is thought to have limited biological activity. Recovery of prolactin after precipitation with polyethylene glycol (PEG 6000) can be used as a screening test for macroprolactinemia [23]. Macroprolactinemia may co-exist with a pituitary microadenoma; hence, it is very important that all pathological causes of hyperprolactinemia are excluded before the cause of a raised serum prolactin is attributed solely to macroprolactinemia [24]. Interpretation of raised concentrations of prolactin is difficult, but some guidelines are defined in Table 25.6. In the case of prolactinomas, the serum prolactin concentration generally correlates with the size of tumor. If the CT scan shows a large pituitary tumor but the serum prolactin concentration is only mildly elevated, this suggests that the tumor is non-secretory. Provocation tests have been used in an attempt to differentiate hypothalamic and pituitary causes of hyperprolactinemia but were unreliable and are no longer used.

Other releasing hormone tests

Thyrotrophin-releasing hormone (TRH) test

Indications

Investigation of secondary hypothyroidism.

Differentiation of TSH-secreting pituitary tumors and the pituitary variant of resistance to thyroid hormones (TSH and thyroxine are elevated in both these conditions).

Precautions

1 TRH may cause minor side-effects including flushing, a desire to micturate, headache, abdominal and chest discomfort, nausea, and a metallic taste in the mouth.

2 TRH can also cause smooth muscle spasms, so caution must be exercised in patients with asthma or ischemic heart disease [2].

Preparation

Thyroxine and triiodothyronine therapy must be stopped for 3 weeks prior to the test. The patient does not need to be fasted (unless TRH combined with a test of GH secretion).

Protocol

1 $t = 0$ min: insert a reliable cannula and take blood samples for TSH and free thyroxine (plain bottle).

2 Give a bolus dose of TRH, 5 μg/kg IV (up to a maximum of 200 μg).

3 $t = 20, 60$ min: take blood samples for TSH and free thyroxine.

Table 25.7. Sampling for combined ITT/TRH/GnRH test.

	Glucose	Cortisol	GH	LH	FSH	TSH
–30	+	+	+			
0	+	+	+	+	+	+
Give soluble insulin 0.10–0.35 U/kg, GnRH 2.5 μg/kg, TRH 5 μg/kg IV						
30	+	+	+	+	+	+
45	+	+	+			
60	+	+	+	+	+	+
90	+	+	+			
120	+	+	+			

Additional samples at 0 min: prolactin, free thyroxine, testosterone, or estradiol.

Table 25.8. Sampling for combined glucagon/TRH/GnRH test.

Time (min)	Meter glucose	Cortisol	GH	LH	FSH	TSH
–30	+	+	+			
0	+	+	+	+	+	+
Give glucagon 20 μg/kg, GnRH 2.5 μg/kg, TRH 5 μg/kg IV						
20				+	+	+
60	+	+	+	+	+	+
90	+	+	+			
120	+	+	+			
150	+	+	+			
180	+	+	+			

Additional samples at 0 min: as for combined ITT/TRH/GnRH test.

Table 25.9. Sampling for combined arginine/TRH/GnRH/synacthen test.

Time (min)	Meter glucose	Cortisol	GH	LH	FSH	TSH
–30	+		+			
Give arginine 0.5 g/kg by IV infusion over 30 min						
0	+	+	+	+	+	+
Give GnRH 2.5 μg/kg, TRH 5 μg/kg IV, synacthen 36 μg/kg IV						
30	+	+	+	+	+	+
45	+		+			
60	+	+	+	+	+	+
90	+		+			
120	+		+			

Additional samples at 0 min: as for combined ITT/TRH/GnRH test.

4 This test may be performed as combined ITT/TRH/GnRH, glucagon/TRH/GnRH, and arginine/TRH/GnRH/synacthen dynamic function tests. The sampling protocols are shown in Tables 25.7, 25.8, and 25.9.

Interpretation

In normal individuals, a rise in TSH concentration at 20 min with a fall at 60 min is observed.

Basal TSH: 0.5–5.0 mU/L.
Increment: 5–25 mU/L.
A blunted response is seen in hypopituitary disease.
A slightly increased basal concentration with a delayed and exaggerated response (60 min value higher than 30 min) is suggestive but not conclusive of hypothalamic hypothyroidism.
In TSH-secreting pituitary tumors, the response is flat whereas a brisk response is obtained in thyroid hormone resistance.

Gonadotropin-releasing hormone (GnRH) test

Indications

Investigation of hypogonadotrophic hypogonadism suspected prepubertally.
Investigation of precocious puberty.
Monitoring of children with precocious puberty treated with GnRH analogs.

Precautions

GnRH may rarely cause nausea, headache, and abdominal pain.

Preparation

No specific patient preparation is required. Patient does not need to be fasted (unless GnRH is combined with a test of GH secretion).

Protocol

1 $t = 0$ min: insert a reliable cannula and take blood samples for LH, FSH, testosterone or estradiol, and sex hormone binding globulin (plain bottles).
2 Give a bolus dose of GnRH, 2.5 μg/kg IV (up to a maximum of 100 μg).
3 $t = 20, 60$ min: take blood samples for LH/FSH.
4 This test may be performed as combined ITT/TRH/GnRH, glucagon/TRH/GnRH, and arginine/TRH/GnRH/synacthen dynamic function tests. The sampling protocols are shown in Tables 25.7, 25.8, and 25.9.

Interpretation

In normal prepubertal children, there is an incremental rise in LH of 3–4 U/L and in FSH of 2–3 U/L above the basal concentration.

In peripubertal children, higher increments, especially if LH dominant, provide evidence of a pubertal pattern of gonadotropin response.

Pubertal delay and pubertal failure

In children with suspected hypogonadotrophic hypogonadism, lack of response supports the diagnosis. A low response has limited predictive value. In gonadal failure, the basal LH/FSH is elevated and the response to GnRH exaggerated.

Table 25.10. Sampling for combined GHRH/TRH/GnRH/CRH test.

Time (min)	Meter glucose	Cortisol	ACTH	GH	LH	FSH	TSH
–15	+	+	+	+			
0	+	+	+	+	+	+	+
Give sequential IV bolus injections of GHRH (1 µg/kg), TRH (5 µg/kg), GnRH (2.5 µg/kg), and CRH (1 µg/kg)							
5	+			+			
15	+	+	+	+			
30	+	+	+	+	+	+	+
45	+	+	+	+			
60	+	+	+	+	+	+	+
90	+	+	+	+			
120	+	+	+	+			

Precocious puberty
In gonadotropin-independent precocious puberty, spontaneous gonadotropin secretion is suppressed by the autonomous sex steroid secretion, basal LH/FSH concentrations are low, and response to GnRH flat.

In gonadotropin-dependent precocious puberty, basal LH/FSH concentrations are elevated and response to GnRH exaggerated.

Precocious puberty (treated)
Suppressed basal LH/FSH and a flat response to GnRH indicate adequate treatment with GnRH analogs.

Corticotrophin-releasing hormone (CRH) test

Indications
Differentiation of hypothalamic and pituitary causes of secondary adrenal insufficiency.
Differentiation of Cushing syndrome (pituitary versus ectopic).
In conjunction with petrosal sinus sampling, to confirm pituitary ACTH-dependent Cushing disease.

Precautions
CRH may cause mild facial flushing, marked transient hypotension, and occasional allergy.

Preparation
The patient should not be on steroid therapy. Prednisolone must be discontinued for 3 days and hydrocortisone for 24 h prior to the test. If steroid cover is essential, switch to dexamethasone therapy, which does not interfere with the test.
The patient should be fasted from midnight.
If both CRH and high-dose dexamethasone test are to be performed, the CRH test should be completed first.

Protocol
1 An in-dwelling cannula should be inserted 30 min before the test.
2 t = –15 min (before administration of CRH): take blood samples for cortisol (plain tube) and ACTH. ACTH samples must be collected into a plastic lithium heparin tube and sent to the laboratory immediately on ice.
3 t = 0 min (before administration of CRH): take blood samples for cortisol and ACTH. Give CRH IV 1 µg/kg (up to a maximum of 100 µg) over 30 s.
4 Take further blood samples for cortisol and ACTH at + 15, + 30, + 45, + 60, + 90, + 120 min after CRH.
5 This test may be performed as combined GHRH/TRH/GnRH/CRH stimulation test. The sampling protocols are shown in Table 25.10.

Interpretation
ACTH peaks at about 30 min and cortisol at 45–60 min after CRH administration. Reported normal responses vary and should therefore be established locally.

Secondary adrenal insufficiency
A flat ACTH and cortisol response to CRH is consistent with a pituitary cause whereas, in hypothalamic disease, the ACTH response is delayed and exaggerated [25].

Cushing syndrome
Patients with pituitary Cushing disease typically show an exaggerated response with an increase in ACTH > 50% above the basal concentration and an increase in cortisol concentration > 20% above the basal concentration [26]. In ectopic causes of Cushing syndrome, the basal ACTH is high, but there is no response to CRH. The false-negative rate of the test (i.e. patients with pituitary Cushing disease who do not show the typical response) is 10–15%. It should be noted that, because of the overlap between the response in control subjects and patients with Cushing disease, the CRH test must not be used for initial diagnosis of Cushing syndrome.

Petrosal sinus sampling combined with CRH
A central–peripheral ACTH ratio of > 2 basally and > 3 after CRH stimulation is necessary to diagnose Cushing disease with confidence [27].

Combined tests of anterior pituitary function

Indications
To assess GH, cortisol, gonadotropin, and TSH secretion in patients with known or suspected multiple pituitary hormone disease (e.g. patients who have had surgery, radiotherapy, or trauma to the hypothalamic–pituitary area).

Precautions
GnRH cannot be performed as part of a combined test if the patient has been primed with sex steroids to stimulate the GH response. For other precautions, see individual test protocols.

Patient preparation
See individual test protocols.

Note that patients on hydrocortisone should have the morning dose withheld to allow meaningful interpretation of plasma cortisol concentrations.

Protocols
Refer to Tables 25.7, 25.8, and 25.9.

Combined test of hypothalamic/pituitary function – GHRH/TRH/GnRH/CRH stimulation test

Indications
To investigate patients with known or suspected multiple pituitary hormone disease and specifically to distinguish between hypothalamic and pituitary disease.

Precautions and preparation
See individual test protocols.

Protocol
See Table 25.10.

Posterior pituitary

Diabetes insipidus

A patient with a differential diagnosis that includes diabetes insipidus (DI) will by definition complain of polyuria and polydipsia. The first step should be to confirm polyuria. An early morning urine osmolality should be checked to confirm a lack of concentrating ability. It is also important to exclude other causes of polyuria (e.g. diabetes mellitus or renal failure). Plasma urea and electrolytes, glucose, and calcium should be measured, which may reveal hypokalemia, hypercalcemia, hyperglycemia, or renal impairment. Plasma sodium concentrations towards the upper limit of the reference range suggest polydipsia and DI, whereas concentrations towards the lower limit may indicate primary polydipsia. However, overlap of plasma sodium between diagnostic groups means that a water deprivation test is frequently necessary [28].

Water deprivation/DDAVP test
Background/indication
To determine the urine-concentrating ability in patients with polydipsia and polyuria.
To investigate suspected DI and differentiate from compulsive water drinking (psychogenic polydipsia).
The water deprivation test measures urine-concentrating ability, which is lost in patients with DI but maintained in compulsive water drinking (CWD). Pituitary and nephrogenic DI may be differentiated by giving a test dose of desamino-D-arginine vasopressin (DDAVP).

Precautions
1 This test must be arranged in advance with the local laboratory as it requires their close collaboration. Osmolality values are required urgently on all specimens collected.
2 Care must be taken in patients in whom the likelihood of DI is very high. Tests on these patients must be started later.
3 Thyroid and adrenal reserve must be normal or adequately replaced.
4 The patient must be kept under close surveillance throughout the test to avoid surreptitious water drinking and monitored for any signs of dehydration. If 5% weight loss or extreme distress occurs, the test must be terminated and DDAVP (5 μg intranasally or 0.3 μg IM) and free fluids given immediately.

Preparation
The night before the test, take blood for urea, electrolytes, and osmolality. If the osmolality is > 300 mosmol/kg, the water deprivation test must not be undertaken because the diagnosis has already been made.

If the test is to proceed, weigh the patient undressed, record the weight, and insert a reliable IV cannula.

Protocol
1 Stop all fluid intake at 24.00 h (or later in infants or in patients who are polyuric or borderline hyperosmolar).
2 If there is a high index of suspicion of DI in a child < 2 years, fluid restriction should commence in the morning.
3 At 09.00 h, weigh the child again undressed and record the weight. Collect blood and urine for osmolality. Send specimens to the laboratory immediately.
4 Continue to weigh the child hourly. Insure that the child is undressed on each occasion.
5 Collect blood and urine samples for osmolality if possible each time the child is weighed. Liaise with the laboratory throughout.
6 The test is normally continued until midday or until: –
- The urine osmolality exceeds 600 mosmol/kg (or 500 mosmol/kg in infants).
- 5% of initial weight is lost.
- Plasma osmolality exceeds 300 mosmol/kg.

It may be necessary to prolong the test in CWD, especially if the child has been drinking excessively prior to the start.
7 At 12.00 h or when the test is terminated, take blood samples for urea, electrolytes, and osmolality. It may also be useful to collect samples of blood and urine for AVP. These can be processed if the test results are equivocal. AVP in blood is extremely labile. Samples must be collected into prechilled heparin tubes, transported on ice to the laboratory immediately, centrifuged rapidly at 4°C, and the plasma stored at a maximum of –20°C (preferably –70°C) [28]. Time from collection to storage should not exceed 20 min. Urine AVP is stable, and there are no special conditions associated with its collection, transportation, or storage.

8 If urine osmolality remains below 600 mosmol/kg, proceed with the DDAVP test.

Protocol DDAVP test
1 Allow the patient to drink but not excessively or a dilutional hyponatremia may ensue. Fluid intake should be no more than twice the volume of urine passed during fluid restriction.
2 Give DDAVP 5 μg intranasally or 0.3 μg IM.
3 Collect blood and urine samples for osmolality hourly (if possible) for the next 4 h.

Interpretation
In normal subjects, the plasma osmolality does not exceed 295 mosmol/kg, and the urine osmolality rises to above 600 mosmol/kg (urine:plasma osmolality > 2:1).
Central (*cranial*) *DI*: plasma osmolality > 295 mosmol/kg with inappropriately dilute urine (< 300 mosmol/kg). DDAVP produces a normally concentrated urine.

Nephrogenic DI. As for central DI, but DDAVP fails to increase urine osmolality more than 100 mosmol/kg.

Partial DI. Patients have moderate elevation of plasma osmolality and urine osmolality typically between 300 and 600 mosmol/kg.

It is important to exercise caution in interpretation of the test. Any patient with a prolonged polyuria from any cause will have some impairment of urine-concentrating ability because of medullary washout [28]. The majority of patients under investigation will have either central DI or CWD. Patients with CWD often have low serum osmolality at the start of the test, but otherwise may behave similarly to patients with partial cranial DI. In difficult cases, the plasma and urine AVP measurements on samples taken at the end of the water deprivation may aid diagnosis. However, a hypertonic saline infusion test with serial plasma AVP measurement may be necessary to differentiate the two conditions.

Hypertonic saline infusion test
Background
The hypertonic saline infusion produces an acute elevation of plasma sodium (≈ 10 mmol/L), which is sufficient to induce significant increases in plasma AVP. The main purpose of the test is to differentiate partial central DI from CWD and partial nephrogenic DI. It may be used in patients in whom the water deprivation test is equivocal or impractical.

Precautions
The test is potentially dangerous and should be undertaken with great care and only in a specialist pediatric endocrine investigation unit. Patients who are unable to conserve water may rapidly become hypertonic. However, providing the protocol is rigidly followed and patients closely observed throughout, the test has been shown to be well tolerated in children, easy to perform, and to have a high diagnostic efficacy [29].

Preparation
The test requires the active co-operation of the local laboratory with whom arrangements must be made in advance. Osmolality measurement must be available urgently.
Exclude hypercalcemia, hypokalemia, and glycosuria (alternative causes of solute diuresis) before carrying out the test.
Free access to food and fluid, to maintain adequate hydration, is allowed until the morning of the test.
Patient should remain supine from 30 min before and throughout the test.

Protocol
1 No fluid should be consumed during the test (includes ice cubes, mouthwashes, etc.) as this may stimulate AVP.
2 Collect urine for osmolality measurement.
3 Weigh patient.
4 Insert reliable IV cannulae into antecubital veins of both arms and keep patent with heparinized saline.
5 Rest patient for a minimum of 30 min.
6 Collect blood samples for electrolytes, osmolality, and AVP. Refer to water deprivation/DDAVP test protocol for details of special collection procedure for AVP samples.
7 Infuse 5% saline at 0.04 mL/kg/min for 2 h or until a plasma osmolality of 300 mosmol/kg is achieved.
8 Collect blood samples for electrolytes, osmolality, and AVP at 30-min intervals.
9 Measure osmolality of all urine passed.
10 Record thirst behavior and blood pressure at 30-min intervals throughout the test.

Interpretation
The plasma AVP measurements are plotted against serum osmolality. The normal incremental change in AVP in response to increasing serum osmolality is shown in Figure 25.2. Patients with CWD or nephrogenic DI show normal AVP release. In central DI, the slope of the relationship between plasma osmolality and AVP is reduced. Patients with nephrogenic DI have high plasma AVP with an inability to concentrate the urine.

Adrenal axis

Adrenal cortex

The components of the hypothalamic–pituitary–adrenocortical axis are depicted in Figure 25.3. Production of glucocorticoids is controlled by a negative feedback loop, in which the glucocorticoids stimulate or inhibit CRH (and AVP) release from the parvicellular neurons of the hypothalamus. AVP is also released from magnocellular neurons, and this is controlled by serum osmolality and blood volume. The primary

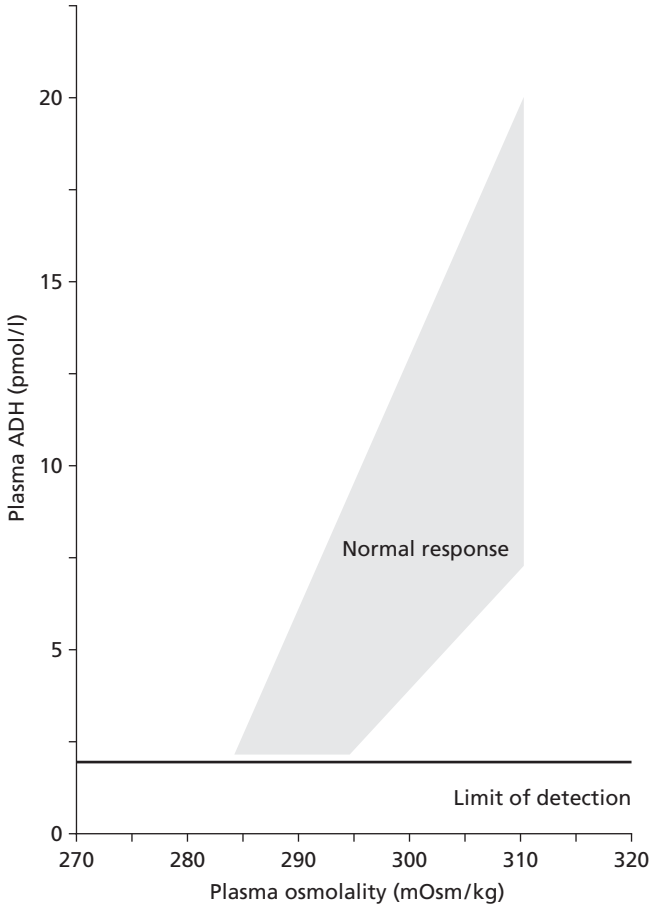

Fig. 25.2. Incremental change in AVP in response to increasing serum osmolality. From Baylis PH, Robertson GL. Plasma vasopressin response to hypertonic saline infusion to assess posterior pituitary function. *J Royal Soc Med* 1980; 73: 255–60, with permission. Reproduced with the kind permission of Professor P.H. Baylis.

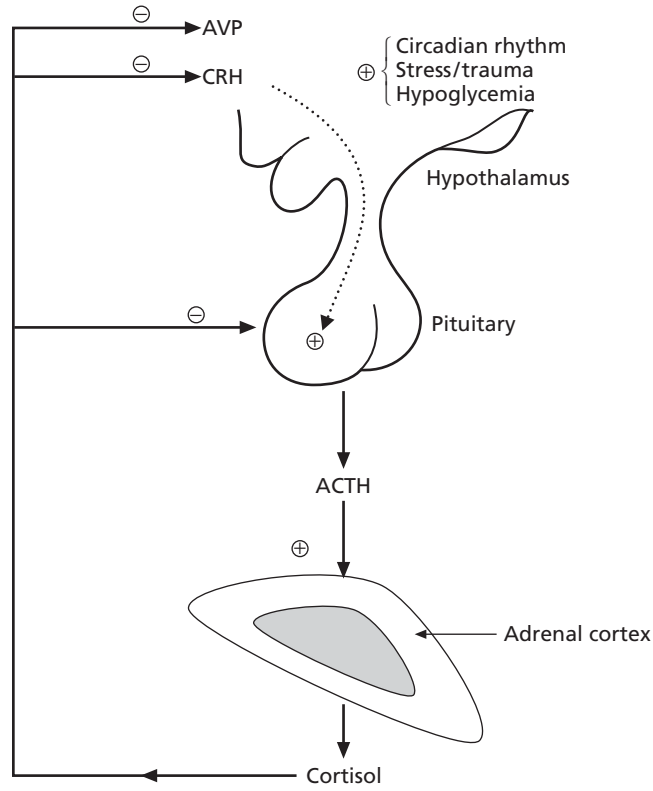

Fig. 25.3. Components of the hypothalamic–pituitary–adrenocortical axis.

stimulus for aldosterone secretion is via the renin–angiotensin system. The control mechanism for adrenal androgen secretion remains unclear.

Adrenal insufficiency

The most common etiology of primary adrenal insufficiency in the last century was tuberculosis, but autoimmune disease now accounts for most of the cases presenting outside the newborn period. Both these diseases affect the whole of the adrenal cortex, resulting in deficiency of glucocorticoid, mineralocorticoid, and androgens. Other conditions resulting in destruction of the adrenal gland include adrenoleukodystrophy, adrenal hemorrhage, and adrenal metastases. Adrenal dysgenesis similarly affects all adrenal hormones and may be the result of congenital adrenal hypoplasia (AHC) or mutations of steroidogenic factor (SF-1). Glucocorticoid deficiency can be the result of an inborn error of steroidogenesis (congenital adrenal hyperplasia).

Secondary adrenal insufficiency results from inadequate ACTH release by the pituitary, most commonly due to suppression of the hypothalamic–pituitary–adrenocortical axis by long-term treatment with exogenous steroid hormones. Other causes of secondary adrenal insufficiency, such as pituitary or suprasellar tumors or pituitary surgery or irradiation, are frequently associated with deficiencies of other pituitary hormones.

There are a number of abnormalities of routine laboratory tests that provide clues to the diagnosis of adrenal insufficiency. In primary adrenal insufficiency, hyponatremia, hyperkalemia, and acidosis are common consequences of aldosterone deficiency. Hyponatremia also occurs in secondary adrenal insufficiency but is due, in this case, to cortisol deficiency, increased AVP secretion, and water retention [30].

Other laboratory abnormalities associated with adrenal insufficiency include hypoglycemia, hypercalcemia (rare), mild normocytic anemia, lymphocytosis, and mild eosinophilia. Basal early morning cortisol concentrations (between 08.00 h and 09.00 h) may be useful in establishing a diagnosis. Morning plasma cortisol concentrations of < 138 nmol/L are highly suggestive of adrenal insufficiency, whereas concentrations > 525 nmol/L rule out the disorder [31,32]. Random cortisol concentrations are of no value except in a patient suffering from acute illness requiring intensive care when a concentration of > 700 nmol/L probably rules out adrenal insufficiency [33].

Simultaneous measurement of cortisol and ACTH identifies most patients with primary adrenal insufficiency – ACTH concentrations are high while cortisol concentrations are low. Normal plasma ACTH rules out primary, but not mild secondary, adrenal insufficiency.

Adrenal cortex antibodies should be measured in all cases of biochemically confirmed primary adrenal insufficiency. In boys who are antibody negative, serum concentrations of very long-chain fatty acids should be measured to exclude adrenoleukodystrophy.

Short synacthen test

Indications

Screening test for suspected adrenal insufficiency.

Investigation of non-classical congenital adrenal hyperplasia (CAH).

Precautions

1 Severe allergic reactions to synacthen have been described, particularly in children with a history of allergic disorders, but are very rare.

2 The dose of synacthen used is excessive and only of value in assessing severe adrenal insufficiency. The standard short synacthen test is insensitive to minor degrees of adrenal suppression (e.g. in children with asthma on inhaled steroid).

3 The test is unreliable if performed within 4 weeks of pituitary surgery as ACTH deficiency may not have been sufficiently prolonged to result in adrenal atrophy [34].

Preparation

The patient does not need to be fasted.

All steroid therapy (other than dexamethasone or betamethasone) interferes with the assay of cortisol. If the patient is on prednisolone therapy, this must be discontinued for 3 days prior to the test; hydrocortisone must be discontinued for 24 h. If steroid cover is essential, switch to a maintenance dose of dexamethasone.

Insert a reliable cannula and rest patient for 30 min.

Protocol

1 t = 0 min: take basal blood samples for cortisol and 17-hydroxyprogesterone (if test for investigation of non-classical CAH).

2 Give synacthen (ACTH 1–24) IV 250 μg (or 36 μg/kg for children less than 1 year old).

3 Take further blood samples for cortisol and 17-OH progesterone (if indicated) at + 30, + 60 min after administration of synacthen.

Interpretation

1 Plasma cortisol concentration at 30 min should be > 550 nmol/L. It is extremely important that the 30-min concentration is used for interpretation. There is a significant difference between cortisol responses at 30 min compared with 60 min. Only the 30-min value has been validated against the ITT, and use of the 60-min value can give misleading interpretation [35]. It should also be noted that the use of a cutoff of 550 nmol/L is somewhat arbitrary and was established using earlier studies in which cortisol was measured by a fluorimetric method. Cortisol values are highly method dependent, and bias differences between methods do not show consistency at different time points [36,37]. There are greater method-related differences in specimens taken after synacthen, probably due to release of steroids (other than cortisol), which cross-react to different degrees in different assays. Where possible, locally derived decision concentrations should be defined, which should be reassessed with any change in methodology.

2 An impaired response does not distinguish between adrenal and pituitary failure, as the adrenal glands may be atrophied secondary to ACTH deficiency. Traditionally, the long synacthen test has been used to distinguish between primary and secondary adrenal failure but, with the improved availability and reliability of ACTH assays, this test has become redundant.

3 Patients with pituitary-dependent cortisol insufficiency require dynamic testing of the pituitary gland. Use of the CRH test may allow differentiation of hypothalamic and pituitary causes of secondary adrenal insufficiency.

4 A normal 17-hydroxyprogesterone response to synacthen is an incremental increase of < 10 nmol/L above the basal concentration at 60 min. Patients with late-onset or non-classical CAH show an incremental increase of > 20 nmol/L, while heterozygotes have an intermediate response with considerable overlap into the normal range [38].

Low-dose (1 μg) synacthen test

Background/indications

This is a modified version of the short synacthen test, which uses a physiological rather than a pharmacological dose of synacthen [39]. It may be indicated in children who have a normal response to the standard short synacthen test, but a clinical history (e.g. chronic steroid therapy) or symptoms (e.g. hypoglycemia) suggesting adrenocortical insufficiency [40].

Preparation

The patient does not need to be fasted.

All steroid therapy (other than dexamethasone or betamethasone) interferes with the assay of cortisol. If the patient is on prednisolone therapy, this must be discontinued for 3 days prior to the test; hydrocortisone must be discontinued for 24 h. If steroid cover is essential, switch to a maintenance dose of dexamethasone.

Insert a reliable cannula and rest patient for 30 min.

Protocol

1 Prepare 1 μg solution of synacthen from 250-μg vial as follows:

- Dilute 1 mL to 50 mL with normal saline giving 250 μg in 50 mL.
- Take 1 mL of above solution and dilute with 9 mL of saline giving 5 μg in 10 mL.

2 $t = 0$ min: take basal blood samples for cortisol.

3 Administer 2 mL of above solution (1 μg of synacthen) to patient IV.

4 Flush the line with 5 mL of saline to insure that the whole dose has been administered.

5 Take further blood samples for cortisol at + 20, + 30, and + 40 min after administration of synacthen.

Interpretation

1 Normal response is a peak cortisol concentration of > 550 nmol/L, which may occur at 20, 30, or 40 min. The definition of this cutoff may vary locally and is influenced by the particular cortisol assay as for the standard short synacthen test [41].

2 Peak concentrations below the defined cutoff indicate a degree of adrenal insufficiency.

Assessment of glucocorticoid replacement therapy

Adequate assessment of patients on glucocorticoid replacement therapy is important to avoid the consequences of undertreatment (e.g. poor response to stress, electrolyte disturbances) or overtreatment (e.g. glucose intolerance, hypertension, osteoporosis). Twenty-four-hour urine free cortisol (UFC) concentrations can be used as an initial screen to detect over-replacement, but a full detailed assessment of therapy requires a day curve [42]. Day curves are often performed in children with CAH when 17-hydroxyprogesterone concentrations are also measured.

Hydrocortisone day curve

Preparation

Omit the morning dose of hydrocortisone until the first sample has been taken.

There is no requirement for the patient to be fasted.

Insert an IV cannula at 08.00–08.30 h.

Protocol

1 At 09.00 h, collect blood sample for cortisol estimation.

2 Administer morning dose of hydrocortisone.

3 Collect blood sample at 10.00 h and then at 2-hourly intervals until 24.00 h for cortisol estimation. Administer hydrocortisone therapy according to the patient's normal regime.

Interpretation

The aim is to achieve adequate concentrations throughout the day, avoiding excessive peaks after each dose. The trough concentrations before each dose should not be below 100 nmol/L.

Laboratory tests for detection and monitoring of CAH

Diagnosis of CAH relies mainly on the measurement of plasma concentrations of 17-hydroxyprogesterone (17-OHP). Most pediatric cases of 21-hydroxylase deficiency have grossly elevated concentrations (typically 300–800 nmol/L): unaffected neonatal concentrations are less than 15 nmol/L [43]. In late-onset or non-classical CAH, concentrations may be only marginally elevated, and it may be necessary to measure the response of 17-OHP to synacthen stimulation in order to make a diagnosis. Modest increases in 17-OHP can also occur in deficiencies of 11β-hydroxylase and 3β-hydroxysteroid dehydrogenase. Stressed normal newborn babies may have 17-OHP concentrations as high as 100 nmol/L, and this, together with interference in the assay by fetal adrenal zone steroid sulfates, can lead to diagnostic confusion if 17-OHP is measured in the first few days after birth. Assays for plasma/serum 17-OHP exhibit large and consistent differences in bias; it is essential therefore that laboratories employ reference ranges appropriate to their assay. Elevated plasma 11-deoxycortisol concentrations (> 60 nmol/L) are found in 11β-hydroxyslase deficiency, and androstenedione is raised in both 21-hydroxylase and 11β-hydroxylase deficiencies. CAH may be confirmed by urinary steroid metabolite profile, which will also identify the site of the block from the pattern of metabolites. Biochemical monitoring of CAH patients on treatment should include blood spot 17-OHP to assess glucocorticoid replacement and plasma renin or plasma renin activity to assess mineralocorticoid replacement.

Urinary steroid profile

Background/indication

A urine steroid profile examines many steroid metabolites simultaneously and provides specific diagnostic information. It is useful for investigating adrenal and gonadal tumors and as an aid in the diagnosis of children with ambiguous genitalia, precocious puberty, premature adrenarche, abnormal virilization, and salt-losing states. It can also assist in the differential diagnosis of Cushing syndrome, hypertension, and adrenal suppression.

Protocol

For quantification of urinary steroid excretion rates, a 24-h collection is required. Where this is difficult, a spot or random urine collection will in most cases still be helpful as the finding of abnormal proportions of specific metabolites may allow biosynthetic defects to be identified. A sample of 20 mL of urine should be collected into a plain tube for analysis.

If the child is on hydrocortisone replacement, switch to dexamethasone and inject a depot preparation of synacthen

before urine collection. In this way, the patient can be controlled while stimulating the adrenal to secrete large amounts of steroids, which are excreted in urine [43].

In children with suspected 5α-reductase deficiency, the ratio of 5α to 5β metabolites can be measured in urine after stimulation of androgen by human chorionic gonadotropin (hCG).

Interpretation

1 Congenital adrenal hyperplasia

• 21-Hydroxylase deficiency – in newborn urine, many unusual derivatives of 17-OHP are found, several products being hydroxylated at C-15. In patients > 6 months of age, there are three major peaks (17-hydroxypregnenolone, pregnanetriol, and 11-oxo-pregnanetriol).

• 11β-Hydroxylase deficiency – elevated tetrahydro-11-deoxycortisol (THS) and 6-hydroxy-THS.

• 3β-Hydroxysteroid dehydrogenase deficiency – excess dehydroepiandrosterone (DHEA) and pregnenolone; cortisol metabolites very low or absent.

• 17α-Hydroxylase deficiency (rarely presents in childhood) – corticosterone metabolites elevated; adrenal androgen and cortisol metabolites absent.

• Lipoid adrenal hyperplasia – no steroids in urinary steroid profile.

2 Tumors of the adrenal gland

• Excess of adrenal androgen and/or cortisol metabolites.

3 Defects in testosterone biosynthesis or action

• 5α-Reductase deficiency – low 5α:5β ratio.

• 17-Ketosteroid reductase deficiency – high ratio androsterone:etiocholanolone.

4 Hypertension

• Congenital adrenal hyperplasia – 11β-hydroxylase deficiency, 17α-hydroxylase deficiency.

• 11β-Hydroxysteroid dehydrogenase deficiency (converts cortisol to inactive cortisone) – excess of tetrahydrocortisol: tetrahydrocortisone.

• Dexamethasone-suppressible hyperaldosteronism – high excretion of 18-hydroxycortisol and 18-oxo-cortisol.

5 Defects of aldosterone synthesis or action

• Aldosterone biosynthetic defects.

• 18-Hydroxylase deficiency – high concentrations of corticosterone.

• 18-Oxidation defects – high concentration of 18-hydroxycorticosterone with low plasma aldosterone concentrations.

• Defects of aldosterone action.

• High aldosterone and 18-hydroxycorticosterone concentrations.

6 Adrenal suppression

• From a 24-h urine collection the sum of individual cortisol metabolites in the urine can be calculated. This can give a better indication of adrenal suppression in patients on inhaled steroids in whom plasma cortisol concentrations are equivocal.

7 Cushing syndrome

• Due to adrenal tumors – excess adrenal androgen metabolites will be detected. Steroid pattern can be used to detect recurrence following resection.

• Due to ectopic ACTH-secreting tumor – total steroid output much higher than in Cushing disease or adrenal tumor. Increased cortisol:cortisone metabolites.

Adrenal hyperfunction

Cushing syndrome

Cushing syndrome is a difficult diagnosis to establish. It is rare in childhood, and the symptoms may vary, but should be considered in any child with weight gain and growth failure [44]. Investigation requires a meticulously planned protocol – Figure 25.4 outlines a possible protocol. It is essential that the diagnosis of Cushing syndrome is established before attempting to establish the etiology. In children over the age of 10 years, 75% of cases are due to Cushing disease, while in those under 10 years, the proportion is 50% [45,46]. The ectopic ACTH syndrome is very rare in children. Routine laboratory investigations may be of value in both the diagnosis and the differential diagnosis of Cushing syndrome. These should include full blood count and plasma/serum urea/electrolytes and glucose. Hypokalemia and impaired glucose tolerance are more common in the ectopic ACTH syndrome but do occur in other types of Cushing syndrome.

Confirmation of Cushing syndrome

Examination of the circadian rhythm of cortisol secretion (24.00 h and 08.00 h) and 24-h UFC estimations are useful preliminary investigations prior to provocative tests. A single sleeping midnight cortisol, 48 h after admission to hospital and asleep prior to venipuncture, of < 50 nmol/L excludes a diagnosis of Cushing syndrome [47]. UFC measurements have the advantage of providing an integrated measure of cortisol secretion. The test has a sensitivity of 95% [47] but, in order to attain this, requires multiple collections (ideally four but a minimum of two) from each patient. Many assays lack specificity, with a large number of urinary metabolites exhibiting significant cross-reactivity; it is essential to ascertain that the local laboratory employs an extraction step prior to measurement of urinary cortisol.

Dexamethasone suppression tests

Dexamethasone is a synthetic glucocorticoid many times more potent than cortisol. It suppresses ACTH by negative feedback; consequently, cortisol production falls to very low concentrations. Patients with Cushing syndrome lose the normal negative feedback control and cortisol fails to suppress.

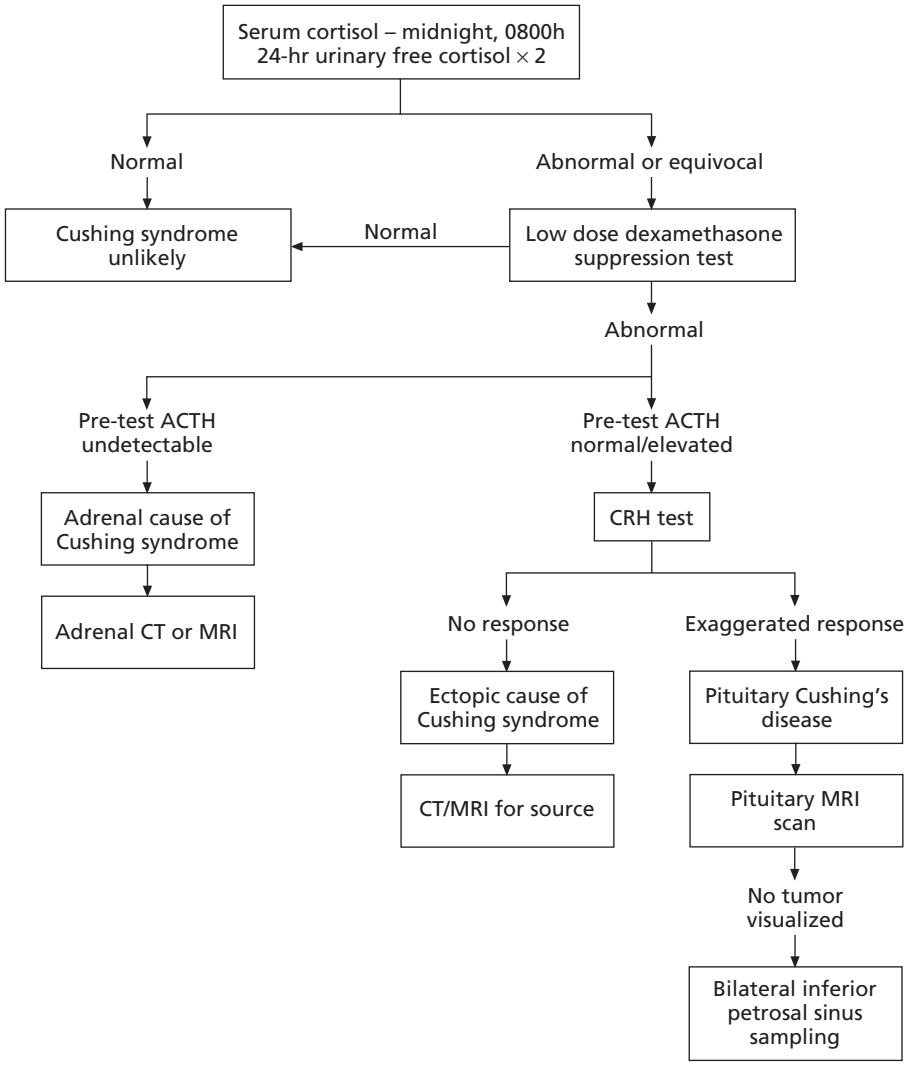

Fig. 25.4. Flow chart for the investigation of Cushing syndrome.

Overnight dexamethasone suppression test

Background/indications

The test can be performed as an outpatient investigation and, as a result, is widely used as a screening test. It has good diagnostic sensitivity but poor specificity; hence, all patients who fail to suppress will require a formal low-dose dexamethasone suppression test.

Precautions

1 The patient should not be on steroid therapy or suffering from major infection or psychological stress.

2 Patients on enzyme-inducing drugs, e.g. anticonvulsants and rifampicin, may rapidly metabolize dexamethasone and give a false-positive result, i.e. no suppression. Ideally, these drugs should be stopped for several weeks prior to investigation.

Preparation

None required.

Protocol

1 Patient takes dexamethasone (15 μg/kg) orally at 23.00 h.

2 At 09.00 h the following morning, a sample is collected for plasma/serum cortisol. Plasma cortisol normally falls after 09.00 h, and false-positive tests may occur if blood sampling is delayed [2].

Interpretation

A normal response is suppression of 0.900 h cortisol to < 50 nmol/L, which excludes Cushing syndrome.

All patients who fail to suppress should undergo a formal low-dose dexamethasone suppression test.

Low-dose dexamethasone suppression test (LDDST)

Indications

Diagnosis of Cushing syndrome.

Precautions/preparation

As for overnight dexamethasone suppression test.

Protocol

1 Day 1: take blood samples for cortisol and ACTH at 09.00 h and 24.00 h. Note that ACTH is unstable, should be collected in plastic tubes (it adheres to glass), and normally requires immediate transportation to the laboratory on ice. Contact the local laboratory for precise details regarding specimen collection and transportation.
2 Days 2 and 3: give oral dexamethasone 0.5 mg (or 10 μg/kg/dose in children < 10 years) strictly 6-hourly.
3 Day 4: collect blood at 09.00 h for cortisol estimation.

Interpretation

In normal individuals, cortisol concentrations suppress to < 50 nmol/L.

Patients with Cushing syndrome, from whatever cause, exhibit detectable plasma cortisol concentrations after dexamethasone administration. Very rarely, patients with Cushing syndrome show normal suppression and, if there is a high index of suspicion in such cases, the patient should be investigated further.

In patients who fail to suppress, a pretest ACTH of < 5 ng/L is highly suggestive of an adrenal cause of Cushing syndrome.

Differential diagnosis of Cushing syndrome

Plasma ACTH concentrations

ACTH measurement is the first step in the differential diagnosis of Cushing syndrome. Patients with adrenal tumors or non-ACTH bilateral adrenal hyperplasia (very rare) will have undetectable plasma ACTH. In these patients, proceed directly to CT or MRI scanning of the abdomen to localize the lesion.

High-dose dexamethasone suppression test (HDDST)

Indications

To differentiate pituitary-dependent and ectopic causes of Cushing syndrome.

Precautions/preparation

As for overnight and low-dose dexamethasone suppression tests.

Protocol

1 Day 1: take blood samples for cortisol and ACTH/ACTH precursors at 09.00 h and 24.00 h.
2 Days 2 and 3: give oral dexamethasone 2 mg (or 40 μg/kg/dose in children < 10 years) 6-hourly.
3 Day 4: take blood at 09.00 h for cortisol measurement.
4 Low- and high-dose dexamethasone suppression tests may be performed sequentially if desired.

Interpretation

Plasma cortisol suppresses to 50% or less of the basal value in pituitary-dependent Cushing syndrome (Cushing disease) but not in adrenal tumors. However, approximately 10% of patients with Cushing disease fail to suppress, whereas 10% of those with ectopic ACTH secretion will suppress [48].

ACTH precursor concentrations may be helpful in the diagnosis: elevated concentrations in patients previously diagnosed with Cushing syndrome suggest ectopic secretion.

Corticotrophin-releasing hormone (CRH) test

See anterior pituitary.

Use of HDDST and CRH test in combination

It has been suggested that combining the CRH test with the high-dose dexamethasone test using the criteria of either an exaggerated response to CRH or a > 50% suppression of cortisol to high-dose dexamethasone improves diagnostic efficacy [49]. However, recent studies [50] suggest that this may not be the case and that the use of this combination may actually impair the outcome of the CRH test. It may be that the CRH test, plus bilateral inferior petrosal sinus sampling in cases in which imaging and the CRH test are discordant, represents the best way forward in terms of obtaining a secure diagnosis of pituitary-dependent Cushing syndrome.

Radiology

Very small tumors can now be visualized using CT scanning and indium-labeled octreotide for bronchial tumors and MRI scanning for pituitary tumors. However, false positives occur frequently, and imaging is recommended as a confirmatory test after clinical evaluation and biochemical tests have been performed [44].

Mineralocorticoid deficiency and excess

Background

The adrenal cortex is also concerned with the maintenance of normal sodium homeostasis via the renin–angiotensin–aldosterone axis. The major disorders that are associated with abnormalities of this axis are summarized in Table 25.11.

Table 25.11. Disorders associated with the renin–angiotensin–aldosterone axis.

Disorder	Renin	Aldosterone
Conn syndrome	Lowered	Raised
Renal artery stenosis	Raised	Raised
Bartter syndrome	Raised	Raised
Renin-secreting tumors	Raised	Raised
Pseudohypoaldosteronism	Raised	Raised
Primary aldosterone deficiency	Raised	Lowered
Secondary aldosterone deficiency	Lowered	Lowered
Congenital adrenal hyperplasia	Raised	Lowered

Investigation of the axis is indicated:

- When a patient presents with hypertension and hypernatremia.
- When a patient presents with salt loss.
- To assess the control of a disease associated with salt loss, e.g. congenital adrenal hypo- or hyperplasia, isolated mineralocorticoid deficiency, Addison disease.

Protocol

Baseline tests include plasma sodium, potassium, creatinine, bicarbonate, and pH, together with the measurement of sodium, potassium, and creatinine concentrations in a 24-h urine. Ambulant and recumbent plasma renin activity (PRA) and aldosterone should be measured. The tests should ideally be performed before and after a 3–5 day low-sodium diet (10–20 mmol/day).

Interpretation

In mineralocorticoid deficiency due to Addison disease, congenital adrenal hyperplasia, or congenital adrenal hypoplasia, the plasma aldosterone concentration is low with an elevated PRA. Pseudohypoaldosteronism is associated with high PRA and high plasma aldosterone concentration. If a low-sodium diet fails to reduce urinary sodium excretion to less than 20 mmol/day, a trial of fludrocortisone should be given and sodium excretion reassessed. Fludrocortisone will have no effect in the case of pseudohypoaldosteronism but will reduce sodium excretion in all cases of true mineralocorticoid deficiency.

In primary hyperaldosteronism (Conn syndrome), hypernatremia, hypokalemia, hyperkaluria, and alkalosis are associated with an elevated plasma aldosterone and a suppressed PRA.

Adrenal medulla

The chromaffin cells of the adrenal medulla are responsible for the synthesis and secretion of catecholamines. Catecholamines are synthesized from tyrosine, derived from ingested food, or synthesized from phenylalanine in the liver [51]. Other major sites of catecholamine production are the brain and sympathetic neurons. Measurement of catecholamines is important in the diagnosis of the catecholamine-secreting tumors – pheochromocytomas and neuroblastomas.

Pheochromocytoma

Pheochromocytomas are rare (incidence is approximately 1 per million per annum), and only 10% occur in childhood. Diagnosis depends on biochemical confirmation of excess catecholamine secretion. Diagnostic tests should include the measurement of catecholamines and metadrenalines (the O-methylated metabolites of catecholamines) – because of the intermittent nature of tumor secretion, catecholamines alone are insufficiently sensitive [52]. Methods for total urinary metadrenalines have now been superseded by measurement of fractionated metabolites. Measurement of plasma free metadrenalines has been shown to exhibit greater diagnostic sensitivity for detection of pheochromocytoma [53]. However, plasma assays are not widely available, and single measurements may miss pheochromocytomas with episodic secretion.

Additionally, there are difficulties with sample collection – patients need to be recumbent for at least 30 min prior to sampling, and samples need to be transported to the laboratory on ice, centrifuged at 4°C within 30 min, and stored at −80°C. Currently, the recommended approach is to measure normetadrenaline, metadrenaline, and catecholamine in a timed overnight urine collection [54]. The pH of the urine collection must be reduced to less than 3.5. Hydrochloric, acetic, or sulfuric acid may be added to the collection container – the choice of stabilizing agent is dependent on the methodology used for measurement of catecholamines and metabolites, and the appropriate container must be obtained from the local laboratory. Abnormal results from overnight urine samples can be followed up by collection of 24-h samples if required for confirmation. For localization of suspected tumors, CT scanning can visualize most pheochromocytomas.

Neuroblastomas

Neuroblastoma is one of the most common malignant tumors of childhood with an incidence of between 1 in 6000 and 1 in 10 000. Neuroblastomas arise in the adrenal gland or in various extra-adrenal sites along the sympathetic chain. Some 90–95% of neuroblastomas are associated with excessive production of catecholamines and metabolites [54]. Urinary concentrations of dopamine, homovanillic acid (HVA), and hydroxymethylmandelic acid (HMMA) are used as tumor markers for neuroblastoma. There is no evidence of diurnal rhythm in the excretion of these markers in patients with neuroblastoma [55], and untimed urine samples can be used, with results reported as mmol of each marker/mol creatinine, provided that appropriate age-related reference ranges are applied. Urine samples should be collected into acidified containers (containing 1.0 mL of 4 M hydrochloric acid) during the morning, following a light breakfast only, to minimize the effects of variation in creatinine excretion [56]. All urines should be tested for adequate acidification and for the presence of ketones, which interfere in the measurement of creatinine.

Gonadal axis

Testis

Clinical examination and basal endocrine investigations may be sufficient to diagnose disorders of testicular function,

obviating the need for provocative testing. Interpretation may be difficult because values are influenced by the stage of pubertal development, and robust, method-related reference ranges for the different stages of puberty are frequently unavailable. Basal testosterone concentrations coinciding with the neonatal surge (occurring at 12–13 weeks) are helpful in the diagnosis of pseudohermaphroditism. A low testosterone will indicate either impaired synthesis or testicular dysgenesis, whereas a normal or elevated concentration will indicate a defect of peripheral androgen action [57]. In inborn errors of testosterone biosynthesis, demonstration of precursor hormones immediately prior to the block is key to making a diagnosis. In 5α-reductase deficiency, the ratio of testosterone to dihydrotestosterone is elevated, in 17β-hydroxysteroid dehydrogenase, there is an increase in androstenedione: testosterone, and in 3β-hydroxysteroid dehydrogenase deficiency, the concentration of DHEA is elevated. The measurement of basal testosterone is also of value in the investigation of hypogonadism in late adolescence when subnormal concentrations indicate a need for further investigation. In other situations in the pediatric patient, basal gonadal steroids may be undetectable, and further investigation requires hCG stimulation.

Human chorionic gonadotropin (hCG) stimulation test

Indications

To detect functioning testicular tissue (e.g. in undescended testes or cryptorchidism).

To define enzyme blocks in testosterone biosynthesis.

Precautions/preparation

None.

Protocol

1 Between 08.00 h and 09.00 h (day 1), collect baseline samples for testosterone. Collect samples for androstenedione and dihydrotestosterone if a biosynthetic defect is suspected.

2 Immediately following collection of baseline blood samples, give hCG IM as follows: 500 IU if weight less than 5 kg; 1000 IU if weight between 5 and 10 kg; 1500 IU if weight between 10 and 15 kg; 3000 IU if weight above 15 kg.

3 Repeat blood samples 72 h after hCG injection (day 4) for testosterone (plus androstenedione and dihydrotestosterone if indicated).

4 Collection of 24-h urine samples before and after hCG stimulation for measurement of steroid metabolites may be useful in investigation of defects in testosterone biosynthesis.

Interpretation

The normal testosterone response depends on the age of the patient [57]. In infancy, a normal testosterone increment may vary from twofold to 10- or even 20-fold. During childhood, the increment is between five- and 10-fold. During puberty, as the basal concentration is higher, the increment is less, i.e. two- to threefold.

Testosterone will respond normally to hCG in cases of complete or partial androgen insensitivity or in 5α-reductase deficiency. In 5α-reductase deficiency, the ratio of testosterone(T) to dihydrotestosterone (DHT) is elevated following hCG stimulation, and this may be helpful in cases in which basal T/DHT ratios are not diagnostic.

A T/DHT ratio > 27 after hCG stimulation suggests 5α-reductase deficiency [2].

An absent response with an exaggerated LH/FSH response to LHRH stimulation indicates primary gonadal failure or anorchia. If there is a defect in testosterone biosynthesis, there will be an increase in precursor steroid secretion following hCG stimulation.

Prolonged hCG stimulation test

If there is no response to the short hCG test, a more prolonged test may be performed. hCG (2000 IU) is administered twice-weekly for 3 weeks. A five- to 10-fold increment from the basal testosterone constitutes a normal response. The size of the phallus should be recorded at the start and on completion of the test to assess whether there has been an increase.

Ovary

Unfortunately, current estradiol assays are insufficiently sensitive to enable the use of basal estradiol to detect failure of the ovary to produce the hormone in childhood. There is also no good or reliable dynamic test comparable to the hCG stimulation test for testicular endocrine function in boys. Laboratory tests are of value in polycystic ovarian disease in which ovarian insufficiency occurs together with hyperandrogenism.

Polycystic ovarian disease

Ovarian insufficiency with hyperandrogenism is not uncommon in children, and adolescents may present with the full gamete of clinical features of polycystic ovarian disease. Insulin resistance has been demonstrated in girls with premature adrenarche, and it has been suggested that this condition may be an antecedent for polycystic ovarian syndrome (PCOS) [58]. The PCOS phenotype may include three major components – anovulation, hyperandrogenism, and hyperinsulinemia. However, the clinical presentation is variable, and not all components are necessarily present. Insulin resistance is a common finding in PCOS independent of obesity: insulin resistance in the obese PCOS patient is postulated to be composed of two components, one unique to PCOS and the other obesity specific [59]. Controversy persists regarding the criteria used for the diagnosis of PCOS, with polycystic ovarian

Table 25.12. Key endocrine investigations and changes in polycystic ovarian syndrome (PCOS).

Endocrine test (serum/plasma)	Nature of abnormality in PCOS
LH/FSH	Increased LH concentrations, LH/FSH ratio > 2
Androgens	Increased testosterone and androstenedione
SHBG	Decreased
Free androgen index	Increased
Estradiol and estrone	Increased
Prolactin	Often moderately increased
Fasting insulin	Increased
Insulin response to oral glucose tolerance test (OGTT)	Increased

morphology being deemed consistent with, but not essential for, the diagnosis of the syndrome [60]. Hormonal tests are an essential component of the workup for a patient suspected of having the syndrome. Table 25.12 summarizes the key endocrine investigations and changes that are consistent with PCOS.

Disorders of puberty

Delayed puberty

Delayed puberty may be due to a pituitary cause (hypogonadotrophic hypogonadism), a gonadal cause (hypergonadotrophic hypogonadism), or constitutional delay (a variant of normal puberty). Basal plasma gonadotropins will differentiate between hypogonadotrophic and hypergonadotrophic conditions. In hypogonadotrophic hypogonadism, further investigation may include a GnRH test, although a low response to this test has limited predictive value (see anterior pituitary section). It is also important to be aware that, between the ages of 5 and 11 years, both basal gonadotropins and the responses to the GnRH test in children with gonadal failure may be within the normal range [61].

In boys, an hCG stimulation test may be indicated to detect functioning testicular tissue and, in girls, ultrasonography of the pelvis to assess development of the uterus and ovaries is key. Hypogonadotrophic hypogonadism and constitutional delay of puberty are difficult to differentiate, and the latter diagnosis is normally made after exclusion of other underlying causes of hypogonadism.

Precocious puberty

Central precocious puberty (CPP) results from premature activation of the hypothalamo-pituitary–gonadal axis, and the pattern of endocrine secretion is the same as in normal puberty. The role of diagnostic testing is to differentiate CPP from gonadotropin-independent precocious puberty (GIPP), which includes gonadal (e.g. McCune–Albright syndrome in girls and rarely boys, testoxicosis in boys, gonadal tumors) and adrenal (e.g. adrenarche, congenital adrenal hyperplasia, adrenal tumors) causes of sexual precocity. It can also assist in the elucidation of premature thelarche (isolated breast development) and thelarche variant, which may be regarded as part of a spectrum that includes CPP.

The GnRH test is the key investigation in identifying and differentiating CPP and GIPP (see anterior pituitary section). The response to GnRH in precocious adrenarche and premature thelarche is prepubertal, while in thelarche variant, the response is intermediate with FSH dominating [62]. The problem is, however, that the pubertal gonadotropin response to GnRH is not well defined and is highly dependent on antibody specificity and standardization of gonadotropin assays used [63].

Rarely, hCG-secreting tumors (e.g. pineal germ cell tumors and teratomas) may cause CPP – these may be identified by the finding of a raised serum hCG concentration. The GnRH response in these cases is prepubertal as normal gonadotrophin secretion is blocked by the sex steroids secreted by the gonads under the influence of tumoral hCG.

If an adrenal cause of sexual precocity is suspected, plasma 17-OH progesterone response to synacthen stimulation (to identify non-classical CAH) and/or serum testosterone, dihydrotestosterone, DHEAS, and androstenedione (all of which may be elevated in adrenal tumors) may be indicated. Urinary steroid profiling is also of great value in the elucidation of adrenal causes of premature sexual maturation. Gonadal tumors may be identified by the presence of very high serum concentrations of sex steroids. Primary hypothyroidism may be associated with premature thelarche in girls and testicular enlargement in boys – plasma total or free thyroxine and TSH may be indicated in order to exclude this condition. Laboratory testing is also of value in monitoring patients with precocious puberty on treatment. Successful treatment of CPP requires suppression of the pubertal LH/FSH response. Regular monitoring of patients with GnRH testing at appropriate intervals in relation to treatment protocols is therefore indicated.

Thyroid axis

The concentration of thyroid hormones in the circulation is regulated by a homeostatic feedback loop involving the hypothalamic-pituitary axis (Fig. 25.5). The main effect of the thyroid hormones is to reduce the response of the pituitary thyrotrophs to TRH rather than altering the secretion rate of TRH from the hypothalamus. Measurement of thyroid hormones and TSH, using sensitive assays that are now

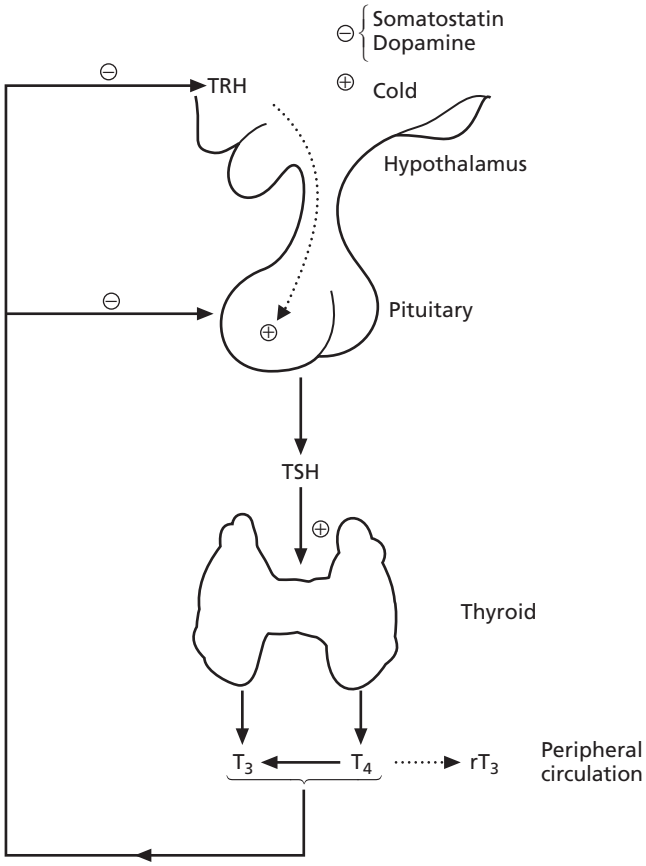

Fig. 25.5. Components of the hypothalamic–pituitary–thyroid axis.

universally available, is usually sufficient for diagnosis of the cause of hypo- or hyperthyroidism, and there are few indications for provocative testing. Total thyroxine (tT_4) methods have largely been superseded by measurement of free thyroxine (fT_4) as methods for the latter are more readily available on automated immunoassay analyzers. However, it should be borne in mind that, although theoretically measurement of the free, physiologically active hormone is preferable, in practice, methods for fT_4 are fraught with interference problems (e.g. by abnormal concentrations and forms of binding proteins, autoantibodies, and endogenous factors associated with non-thyroidal illness) [64,65]. Free triiodothyronine (fT_3) assays are even less reliable than fT_4 assays, and many laboratories continue to measure total triiodothyronine (tT_3). Third-generation, two-site immunometric assays for TSH are generally more robust, but the presence of heterophilic antibodies may give rise to falsely elevated concentrations. It is important to liaise with the local laboratory if laboratory tests do not reflect the clinical picture in an individual patient in order that the samples can be checked for the presence of interfering substances and hormone concentrations measured, if necessary, by an alternative methodology.

Hypothyroidism

It is important to consider screening newborn babies for congenital hypothyroidism separately from the investigation of infants and older children with suspected hypothyroidism. Newborn screening programs for congenital hypothyroidism are based on the measurement of TSH in Europe and on the measurement of T_4 in North America in blood spot samples. Positive screening tests are confirmed by the measurement of plasma fT_4 or total T_4 and TSH. Thyroid ultrasonography can be used to provide information on the location, size, structure, and vascularity of the thyroid gland. Further investigations should be undertaken if a defect of thyroid hormone biosynthesis is suspected. Figure 25.6 outlines a proposed investigation algorithm for such cases. The perchlorate discharge test allows identification of organification defects. ^{123}I iodide is administered [0.1 MBq (2.7 μCi) in infants or a proportion of the adult dose (1 MBq) based on body weight in older children], and this is followed by oral potassium perchlorate (dose range 100–400 mg depending on the age of the child) 1 h later. Discharge of the accumulated radioactivity within 1 h is not normally greater than 10% – more than 60% discharge is consistent with a complete organification defect. Tests for other thyroid hormone biosynthetic defects (e.g. salivary–serum^{123} I ratios for iodine uptake defects and urinary mono- and diiodotyrosine concentrations) are not generally available, and investigation of these defects requires referral to a specialist center.

Investigation of infants and older children with suspected hypothyroidism requires measurement of plasma fT_4 or total T_4 and TSH. A reduction in T_4 with a rise in TSH indicates primary hypothyroidism. Elevated TSH with a normal T_4 is defined as "subclinical" hypothyroidism. Measurement of T_3 is not generally helpful as T_3 may be only slightly reduced in patients with hypothyroidism because of increased peripheral conversion of T_4 to T_3. "Non-thyroidal illness" also commonly causes a reduction in the concentration of T_3.

Secondary hypothyroidism (caused by hypothalamo/pituitary disease) is indicated by a low T_4 and a normal or low TSH, although this picture is also frequently seen in patients with "non-thyroidal illness" or in those on corticosteroid or anticonvulsant drug therapy. In patients with suspected secondary hypothyroidism, a TRH test will confirm the diagnosis and is helpful in distinguishing hypothalamic and hypopituitary causes (see anterior pituitary section).

Some laboratories have a strategy of measuring only TSH as a first-line test in all samples from patients under investigation for thyroid disorders. It is important to be aware that this strategy will fail to recognize secondary hypothyroidism and, if this is to be excluded, a T_4 concentration should be specifically requested. TSH measurements should be used for monitoring patients with hypothyroidism on T_4 replacement therapy – a TSH at the lower end of the reference range usually coincides with an optimal symptomatic response.

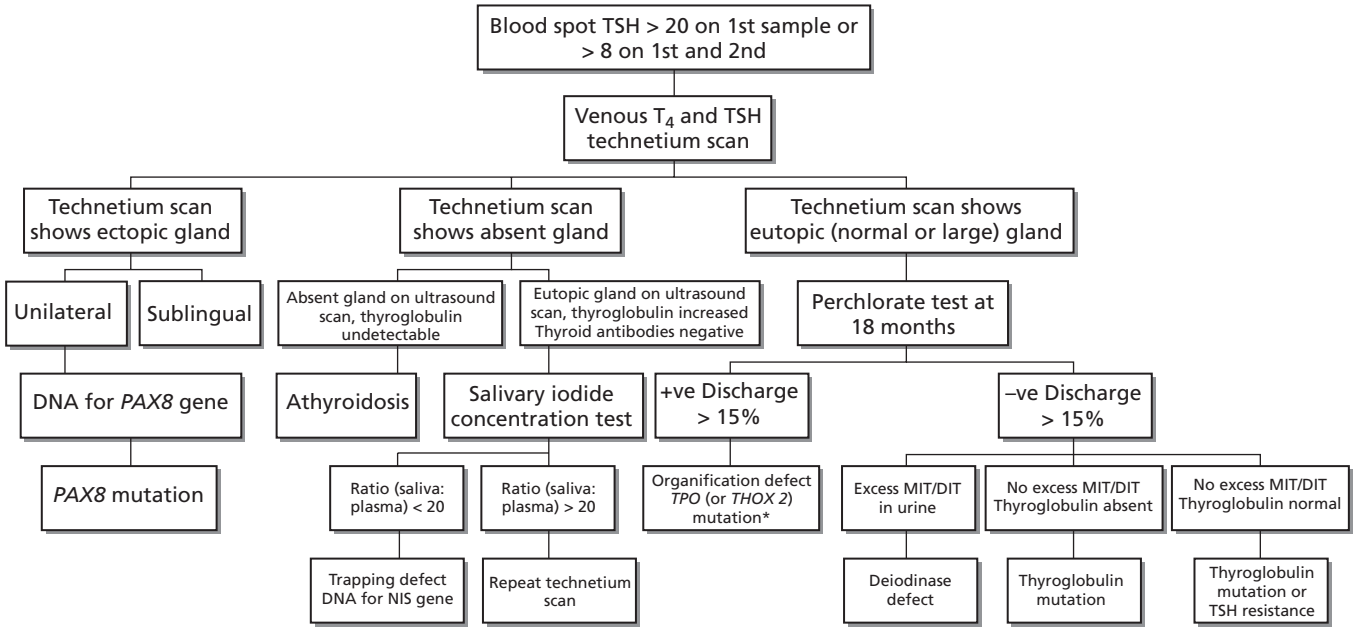

Fig. 25.6. Algorithm for diagnosis of type of congenital hypothyroidism. MIT, mono-iodotyrosine; DIT, di-iodotyrosine. * If there is a hearing impairment, consider defects in the pendrin gene.

Suppressed concentrations are consistent with over-replacement with associated potential deleterious effects on bone [66]. Measurement of antithyroid antibodies is helpful in establishing the etiology of hypothyroidism. Antibodies to thyroglobulin or thyroid peroxidase (microsomal antibodies) are typically strongly positive in Hashimoto's thyroiditis.

Hyperthyroidism

TSH is the single most useful test in confirming the diagnosis of hyperthyroidism. Plasma TSH concentrations are suppressed (< 0.1 mU/L) except in cases of a TSH-secreting pituitary tumor or thyroid hormone resistance. The TRH test is useful for distinguishing pituitary tumors from thyroid hormone resistance (see anterior pituitary section). Measurement of serum TSH α–subunit may also be of value as concentrations are increased in most patients with TSH-secreting pituitary tumors [67]. It is important to be aware that low but detectable concentrations of TSH may be found in "subclinical hyperthyroidism," and suppressed (and sometimes undetectable) TSH concentrations can also be found in "non-thyroidal illness." In most cases of suspected hyperthyroidism, a suppressed TSH in combination with a raised tT_4 or fT_4 confirms the diagnosis, and T_3 measurement is superfluous. T_3 measurement is however indicated when TSH is suppressed in the presence of a normal T_4, in order to diagnose T_3 toxicosis, a condition that is rare except in iodine-deficient countries [68]. T_3 measurements are valuable in monitoring treatment of hyperthyroidism, as elevated concentrations indicate persistent thyroid hormone excess, despite normal or even subnormal T_4 concentrations.

Detection of TSH receptor antibodies (TRAb) in the serum of patients with hyperthyroidism is useful for establishing a diagnosis of Graves' disease in those patients in whom the clinical picture is unclear. Unfortunately, most assays are unable to distinguish antibodies with stimulating activity (found in Graves' disease) from those with blocking activity. Radioiodine uptake used to be a first-line procedure in the investigation of hyperthyroidism. It is no longer performed universally in all patients but may still be of value for the identification of "hot" nodules.

Thyroid cancer

Serum concentrations of thyroglobulin are useful for long-term follow-up of patients treated for differentiated thyroid cancer. Thyroglobulin concentrations should be undetectable in patients who have had total thyroid ablation, and detectable concentrations indicate persistent or recurrent disease. It is important that samples analyzed for thyroglobulin are also screened for the presence of antithyroglobulin antibodies because, if present, these will interfere with thyroglobulin quantitation, most commonly leading to falsely low results.

Calcitonin measurement is increased in medullary thyroid cancer and, thus, serum calcitonin should be measured in patients with a family history of this condition (see multiple endocrine neoplasia).

Calcium, parathyroid, and vitamin D

Hypocalcemia

Reference ranges for plasma/serum calcium vary between different laboratories, and lower concentrations (as low as 1.8 mmol/L) are frequently asymptomatic in the neonate. Most laboratories measure total (free + protein-bound) calcium, which can be low with a simultaneously normal free (ionized) fraction in hypoalbuminemia or acidosis. A number of different formulae are applied by laboratories to correct serum calcium for the serum albumin concentration – as a simple guide, total calcium concentrations rise or fall by approximately 0.2 mmol/L per 1 g/L albumin. Ionized calcium measurements are not practicable for all patients but will detect subtle abnormalities in calcium homeostasis and may be useful in acidotic patients.

There is an inverse relationship between free calcium and pH and, to provide meaningful results, samples must be analyzed at the patient's *in vivo* blood pH. Specimens must be collected and handled anaerobically (i.e. syringes filled completely and sealed in order to prevent loss of CO_2) and transported to the laboratory on ice. Initial investigations in a child with hypocalcemia should include measurement of plasma phosphate, magnesium, alkaline phosphatase, albumin, and creatinine, and assessment of acid–base status. The child (and frequently the mother as well) should be investigated for vitamin D deficiency by measurement of serum 25-hydroxyvitamin D.

Current definitions of vitamin D deficiency describe three cutoffs associated with suboptimal concentrations of the hormone: severe deficiency (< 15 nmol/L), deficiency (< 40 nmol/L), and insufficiency (< 50 nmol/L) [69]. Serum 1,25 dihydroxyvitamin D is not advocated for the investigation of vitamin D deficiency as concentrations may be low, normal, or high. Secondary hyperparathyroidism is an early feature of vitamin D deficiency. PTH concentrations are a good surrogate marker of deficiency. A parathyroid hormone (PTH) concentration > 5 pmol/L is associated with a strong likelihood of subnormal vitamin D concentrations.

PTH concentrations are also essential for the diagnosis and differential diagnosis of hypoparathyroidism (PTH deficiency) and pseudohypoparathyroidism (PTH resistance). The rise in urinary phosphate and cyclic AMP excretion after an infusion of PTH has been used to differentiate type I and type II pseudohypoparathyroidism and pseudopseudohypoparathyroidism. Patients with type I pseudohypoparathyroidism showed blunted phosphaturic and cAMP responses to PTH, patients with type II showed a subnormal phosphaturic but normal cAMP response and, in patients with pseudopseudohypoparathyroidism, both responses were normal. Owing to the unavailability of PTH for infusion, this test is no longer used. In patients with clinical and biochemical features of type I pseudohypoparathyroidism (Albright's hereditary osteodystrophy, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism), samples should be sent for DNA analysis for inactivating mutations of the $G_{s\alpha}$ gene. Such mutations are found in 60% patients with type I pseudohypoparathyroidism, almost all of whom also exhibit the typical clinical features. Maternal PTH concentrations should be measured in persistent neonatal hypocalcemia in order to exclude undiagnosed maternal hyperparathyroidism as a cause of the hypocalcemia.

Hypercalcemia

The demonstration of an albumin-corrected calcium above the reference range on more than one occasion should prompt further investigations to establish the etiology of the hypercalcemia. Measurement of serum PTH is a first-line test as the finding of elevated (or normal) concentrations in a hypercalcemic patient is practically diagnostic of primary hyperparathyroidism, because the normal physiological response to hypercalcemia is suppression of PTH. A rare exception is familial hypocalciuric hypercalcemia (FHH), which is associated with a low fractional calcium excretion (calcium: creatinine ratio < 0.01) and hypermagnesemia.

Other first-line laboratory investigations should include a full blood count and erythrocyte sedimentation rate (ESR), and plasma phosphate and alkaline phosphatase concentrations. The precise diagnostic workup will be influenced by the history and clinical presentation. Elevated parathyroid hormone-related peptide (PTHrp) concentrations confirm a malignant cause of hypercalcemia, although assays are not yet widely available. Vitamin D metabolite concentrations are rarely helpful in making a diagnosis except for measurement of 25-hydroxyvitamin D to exclude intoxication with vitamin D.

Endocrine pancreas

Hypoglycemia

Definition

The definition of hypoglycemia is still far from clear, and this is particularly the case with regard to the neonatal period when traditionally lower cutoffs have been applied. Studies based on outcome in relation to glucose concentration [70] have led to the adoption of a cutoff of 2.6 mmol/L. The symptoms and signs of hypoglycemia in childhood are wide-ranging and non-specific and are frequently absent altogether in neonates. The application of a universal definition of hypoglycemia (in all infants and children) as a laboratory glucose below 2.6 mmol/L with or without symptoms will insure early diagnosis and treatment of this common metabolic problem.

Table 25.13. Intermediary metabolites and hormones to be measured at the time of hypoglycemia.

Blood	Urine
Glucose (laboratory measurement)	Ketones (acetest or ketostix)
Ammonia	Reducing substances
Insulin	Organic acids
C-peptide	
Growth hormone	
Cortisol	
β-Hydroxybutyrate	
Free fatty acids	
Lactate	
Amino acids	
Total/free carnitine*	
Acyl carnitine profile*	

*Total/free carnitine measured in plasma and acyl carnitine on blood spots from Guthrie card.

Measurement of blood glucose

Glucose measurements are frequently performed at the point of care on whole blood using a variety of different devices. It is important to be aware that whole-blood glucose is approximately 15% lower than plasma glucose, the exact figure depending on hematocrit. Point of care devices differ significantly in their reliability, and it is essential that the device used is assessed for accuracy at low concentrations and effect of hematocrit [71]. Hypoglycemia detected using such devices *must always* be confirmed by sending a sample collected into a fluoride oxalate tube to the laboratory for an accurate glucose measurement, although it is important not to delay instigation of treatment. Definitions of hypoglycemia are based on laboratory plasma glucose measurements.

Further investigations

It is of paramount clinical importance to take specimens of blood and urine *at the time of hypoglycemia*. These should be sent to the laboratory where they can be stored at –20°C for analysis later if required. Most cases of neonatal hypoglycemia are transient and do not require extensive metabolic and endocrine investigations. If hypoglycemia persists or if a glucose infusion in excess of 10 mg/kg/min is required to maintain a normal plasma glucose concentration, investigations for hyperinsulinism, metabolic, and endocrine disorders should be undertaken [71]. Table 25.13 provides details of the major investigations – specimen requirements should be checked with the local laboratory.

Interpretation

In normal individuals, at the time of hypoglycemia, insulin secretion is switched off, GH and cortisol are increased, and β-hydroxybutyrate and free fatty acids (FFA) are elevated. Insulin suppresses lipolysis so, in hyperinsulinism, insulin concentrations are inappropriately elevated (> 2 mU/L), but FFA and β-hydroxybutyrate are low. The finding of a suppressed C-peptide in the presence of insulin is indicative of surreptitious exogenous insulin administration. Measurement of ammonia is important to identify hyperinsulinism due to glutamate dehydrogenase activation. Deficiency of the counter-regulatory hormones GH and cortisol is another important endocrine cause of hypoglycemia. The finding of low concentrations of GH or cortisol requires formal assessment of anterior pituitary function and/or the hypothalamic-pituitary–adrenal axis as appropriate.

FFA and β-hydroxybutyrate can also aid in the diagnosis of inherited defects of fatty acid oxidation. In these patients, the concentration of FFA is increased, whereas β-hydroxybutyrate concentration is low. Urinary organic acids and blood acylcarnitine concentrations may be diagnostic. Raised lactate concentrations are found in defects of gluconeogenesis.

Other tests for diagnosis and differential diagnosis of hyperinsulinemia

A key component of the diagnostic criteria for hyperinsulinism is the demonstration of a glucose requirement of > 10mg/kg/min to maintain blood glucose above 2.6–3.0 mmol/L. In order to insure that the need for such an infusion rate is clearly demonstrated, infusion should commence at 4–6 mg/kg/min (the normal neonatal hepatic production rate of glucose). It should then be titrated upwards as necessary to maintain the blood glucose concentrations above 2.6–3.0 mmol/L [72].

The response of infants with diagnosed hyperinsulinism to treatment with diazoxide is useful in helping to identify the specific mutation causing the condition. Mutations that decrease or destroy K_{ATP} channel activity typically do not respond, whereas those associated with upregulation of glutamate dehydrogenase (hyperammonemic hyperinsulinism) or glucokinase will respond [73].

Fasted/fed profile of metabolites

If hypoglycemia relates specifically to fasting or feeding, it may be useful to measure metabolites in the fasted or fed state. Metabolites that it can be useful to measure include plasma lactate, β-hydroxybutyrate, FFA, and alanine in addition to glucose [71]. Starvation tests are extremely dangerous and should never be undertaken without consultation with a unit specializing in inborn errors of metabolism who will provide a clear written protocol.

Hyperglycemia

Type 1 diabetes (due to insulin deficiency) was assumed until recently to be the diagnosis in almost all children presenting

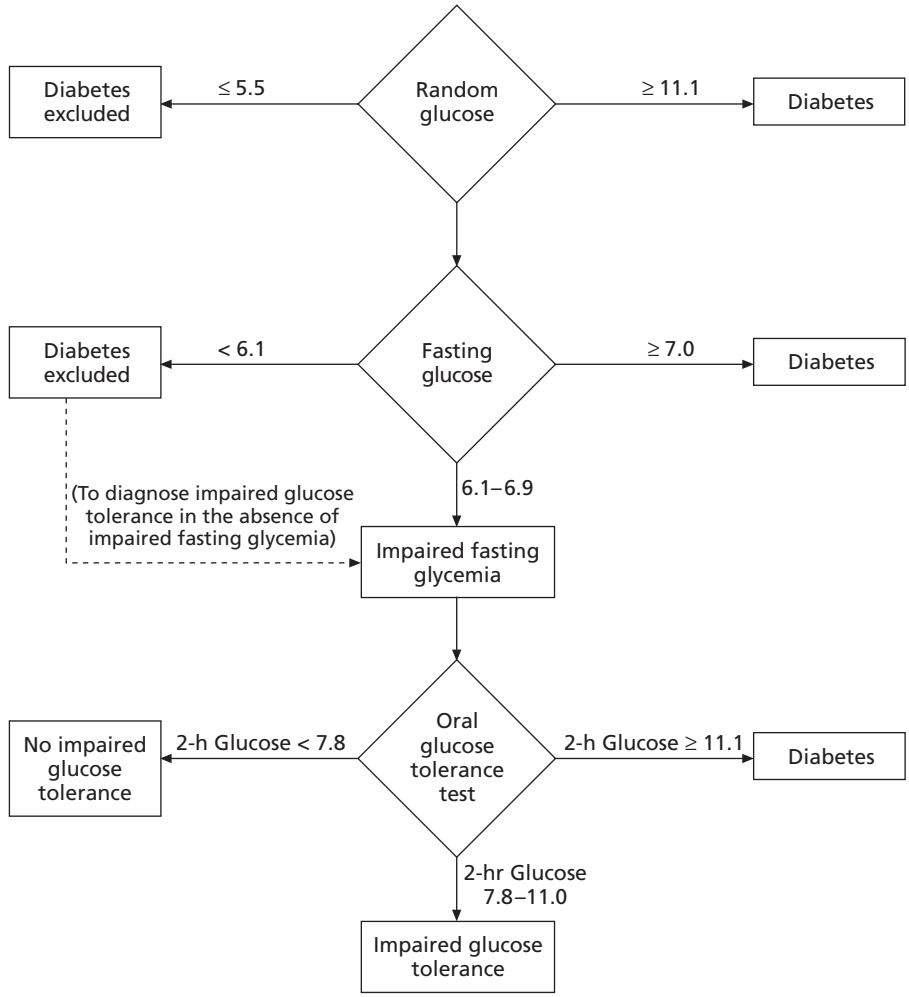

Fig. 25.7. Diagnostic algorithm for diabetes mellitus. Adapted from Lamb E, Day A. New diagnostic criteria for diabetes mellitus: are we any further forward? *Ann Clin Biochem* 2000; 41: 10–16 [75], with permission.

with glucose intolerance. The only alternative diagnosis was that of maturity-onset diabetes in the young (MODY), a heterogeneous condition comprising a group of genetic disorders of pancreatic β-cell function associated with normal insulin sensitivity. More recently, type 2 diabetes has been described in children, in which the predominant defect is insulin resistance. Affected children are often overweight or obese and may have a family history of type 2 diabetes. Investigations need to be able not only to diagnose diabetes, but to distinguish type 1 and type 2 conditions, as treatment for the two conditions is different. This provides a challenge for the pediatrician and the laboratory because many of the clinical and biochemical signs overlap.

Diagnosis of diabetes mellitus

The World Health Organization (WHO) diagnostic criteria for the diagnosis of diabetes are a fasting plasma glucose value greater than or equal to 7.0 mmol/L or a value greater than or equal to 11.1 mmol/L 2 h after a glucose load [74]. The WHO guidelines (which have been endorsed by the British Diabetic Association) recommend an oral glucose tolerance text (OGTT) when either a random glucose is between 5.5 and 11.1 mmol/L or fasting plasma glucose is < 7.0 mmol/L. They also clearly indicate that protocols for the OGTT should stipulate glucose measurement on fasting and 2-h samples only. However, the guidelines fail to give clear recommendations as to whether a random or fasting sample or OGTT should be the first-line investigation for diabetes. The International Diabetes Federation (IDF) have produced clearer strategic guidelines, and Lamb and Day (75) have produced a simple practical diagnostic algorithm (Fig. 25.7) based on these guidelines, which is in keeping with the WHO framework.

OGTT for diabetes mellitus

Indication

For the diagnosis of diabetes mellitus. Specifically, in those patients in whom random and fasting concentrations of glucose are not diagnostic (see Fig. 25.7).

	Glucose concentration (mmol/L)			
	Whole blood		Plasma	
	Venous	Capillary	Venous	Capillary
Diabetes mellitus				
Fasting	≥ 6.1	≥ 6.1	≥ 7.0	≥ 7.1
2 h post glucose	≥ 10.0	≥ 11.1	≥ 11.1	≥ 12.2
Impaired glucose tolerance (IGT)				
2 h post glucose	≥ 6.7 and < 10.0	≥ 7.8 and < 11.1	≥ 7.8 and < 11.1	≥ 8.9 and < 12.2
Impaired fasting glycemia (IFG)				
Fasting	≥ 5.6 and < 6.1	≥ 5.6 and < 6.1	≥ 6.1 and < 7.0	≥ 6.1 and < 7.0

Table 25.14. Values for diagnosis of diabetes using different sample types according to World Health Organization guidelines.

Precautions

Patients may feel nauseous although the incidence is reduced if Lucozade or polycal liquid (a flavored drink based on glucose polymers) is used in place of glucose solution.

Preparation

The diet for the 3 days preceding the test should contain adequate carbohydrate (approximately 60% of calories).

The patient should be fasted overnight for 10–14 h (plain water allowed) and should rest throughout the test. Small children should be encouraged to have a late snack during the evening before the test.

Prepare the glucose load which may be:

- 1.75 g/kg anhydrous glucose (maximum 75 g) or 1.92 g/kg glucose monohydrate (maximum 82.5 g) dissolved in 100–200 mL of water. In teenage patients on the maximum dose, the volume may be increased to 300 mL or
- a proprietary equivalent [e.g. 9.2 mL/kg Lucozade (maximum 394 mL) using the current formulation of 73 kcal carbohydrate/100 mL or 2.64 mL/kg polycal liquid (maximum 113 mL) diluted with water to produce a glucose concentration of no greater than 25 g per 100 mL (1.5 mL of polycal = 1 g of glucose)].

Insert IV cannula (see section on blood collection) and maintain patent with heparinized normal saline.

Protocol

1 0 min: take blood samples for glucose estimation.

2 The child should drink the glucose load within 5 min.

3 Take a further sample for glucose 2 h after finishing the glucose drink.

4 Venous blood (collected into a fluoride oxalate tube) is the preferred sample and the one that relates to the figures shown in the algorithm. Equivalent values for venous and capillary whole blood and for capillary plasma are given in Table 25.14. If it is impossible to collect a venous sample, then 0.2 mL (minimum) of capillary blood in a fluoride tube may be substituted. It is important to indicate clearly the type of sample on the request form. Samples collected at 0 and 120 min must always be of the same type.

Interpretation

Venous plasma

A fasting glucose concentration of ≥ 7.0 mmol/L or a concentration of ≥ 11.1 mmol/L 2 h after glucose load confirms a diagnosis of diabetes mellitus.

Levels between 7.8 and 11.0 mmol/L 2 h after glucose load indicate impaired glucose tolerance.

Equivalent glucose concentrations for alternative blood sample types are given in Table 25.14.

The diagnosis of diabetes should always be confirmed by repeating the test on another day unless there is unequivocal hyperglycemia with acute metabolic decompensation or obvious symptoms.

Differential diagnosis

Family history or clinical presentation may provide key pointers to the differential diagnosis of diabetes mellitus. An autosomal-dominant family history suggests MODY. Genetic tests are also available for the common genetic subtypes of MODY. Family history of type 2 diabetes, obesity, and evidence of insulin resistance with acanthosis nigricans or hyperinsulinemia suggest type 2 diabetes. Ketoacidosis is more commonly a feature of type 1 diabetes but may occur in type 2.

Laboratory assessment of insulin resistance is now becoming a vital tool in making an accurate early differential diagnosis. The gold standard test is the euglycemic-hyperinsulinemic clamp technique. Another common method is to use frequent-sample IV glucose tolerance test. However, the complexity, cost, and invasiveness of both these tests limit their clinical application, and simpler tests are required for clinical use. Paired fasting insulin and glucose measurements are useful investigations to determine whether there is insulin deficiency or insulin resistance. A fasting insulin > 20 mU/L together with a normal glucose concentration indicates insulin resistance; concentrations between 15 and 20 mU/L are borderline. The homeostatic model assessment (HOMA) has also been utilized to provide a measure of basal insulin resistance [HOMA-IR = fasting insulin (mU/L) × fasting glucose (mmol/L) ÷ 22.5]. Lower HOMA-IR values indicate greater

insulin sensitivity, whereas higher HOMA-IR values indicate lower insulin sensitivity (insulin resistance). Although the HOMA-IR is simple and practical, it suffers from the major disadvantage that it is based on measurements of basal glucose and insulin, whereas in a significant proportion of individuals with insulin resistance, the fasting insulin concentrations are normal but stimulated insulin concentrations are raised. A simple approach to assessing stimulated insulin secretion is to undertake insulin measurements at 0 and 30 min in a standard 75-g oral glucose tolerance test and calculate the ratio of the 30-min increment in insulin concentration to the 30-min increment in glucose concentration [76]. This has been shown to correlate with the first-phase insulin response in an IV glucose tolerance test. An alternative is to utilize the whole-body insulin sensitivity index (WBISI), which is also derived from parameters obtained from a 75-g oral glucose tolerance test:

$$\frac{10\,000}{\sqrt{(\text{fasting glucose} \times \text{fasting insulin}) \times (\text{mean glucose} \times \text{mean insulin})}}$$

In order to calculate WBISI, it is necessary to make glucose and insulin measurements at 30-min intervals up to 180 min following ingestion of the oral glucose load. The WBISI has been assessed in obese children and found to give significantly better correlation with insulin sensitivity based on the euglycemic-hyperinsulinemic clamp than HOMA-IR [77]. In order to quantify insulin resistance, it may therefore be necessary to use an extended version of the standard OGTT with more frequent sampling.

Measurements of islet cell antibodies and glutamic acid decarboxylase antibodies may also be helpful in children in whom it has otherwise proved impossible to establish a differential diagnosis. In children presenting with type 1 diabetes, the majority are positive for islet cell antibodies, and 85% are positive for glutamic acid decarboxylase antibodies [76]. Autoantibodies are rare in type 2 diabetes. In children or young people without autoantibodies, consideration may therefore be given to a trial of oral agents and lifestyle changes, whereas the presence of autoantibodies suggests that insulin therapy is the most appropriate option [78].

Obesity

Investigation of the obese child

As the prevalence of childhood obesity is increasing, there is a need to identify laboratory tests that aid in differentiating those children in whom there is a medical cause and those who may be said to have so-called simple obesity. It is important to take a full family and dietary history and to document calorie consumption (an inpatient admission may be necessary to obtain accurate documentation). Physical examination is important, as is assessment of growth velocity and bone age.

Endocrine causes of obesity (with the exception of hyperinsulinism) are all associated with slow growth velocity and therefore eventually short stature. Provocative tests of GH secretion (see anterior pituitary section) and basal serum IGF-I will exclude GH deficiency. Other endocrine investigations include thyroid function tests to exclude hypothyroidism and the measurement of circadian plasma cortisol concentrations and UFC measurements if Cushing syndrome is suspected. Measurement of calcium, phosphate, and PTH should be performed to exclude a diagnosis of pseudohypoparathyroidism. If a child with short stature or slow growth velocity has dysmorphic features and/or learning difficulties, this points to a possible syndromic cause for their obesity (e.g. Prader–Willi, Alstrom, DIDMOAD). Chromosomal analysis is indicated if the child has additional features suggesting Prader–Willi (hyperphagia, muscular hypotonia, hypogonadotrophic hypogonadism) or DIDMOAD (diabetes mellitus, diabetes insipidus, optic atrophy, sensorineural deafness).

In children with normal or increased growth velocity and a normal family history, obesity may be due to insulin resistance. Measurement of fasting glucose and insulin concentrations should be undertaken, followed by the sequential measurement of these parameters during an OGTT (see endocrine pancreas section). If obesity is gross and there is a family history in a child with an abnormal eating pattern, then a mutation in one of the genes associated with human obesity should be considered. Mutations of leptin (an adipocyte-derived signaling molecule that limits food intake and increases energy expenditure) and its receptor have been identified in association with obesity. However, it is now clear that such defects in humans are extremely rare with only three families identified to date [79]. Although subtle changes in circulating leptin concentrations have been reported in a number of conditions including diabetes, thyroid disease, Cushing syndrome, and polycystic ovarian disease, measurement of leptin has no proven diagnostic value and should be confined to research studies. Mutations in other genes – POMC, PC-1, and the MC-4 receptor – are also associated with human obesity, although these too are extremely rare. Not all congenital causes of obesity are genetic, and it has been suggested that it may result from the intrauterine environment. It should be noted that, in most cases of childhood obesity, biochemical and genetic tests are normal, and extensive investigation therefore has a poor return in terms of clinical benefit.

Multiple endocrine neoplasia

Multiple endocrine neoplasia (MEN) syndromes are characterized by the presence of tumors involving two or more endocrine glands. They may be inherited in an autosomal-

Table 25.15. Characteristic tumors in MEN syndromes.

Type	Characteristic tumors
MEN1	Parathyroid glands Pituitary Gland prolactinomas (most common) GH secreting (much less common) Pancreatic islets Insulinoma Glucagonoma Gastrinoma VIPoma PPoma Other associated tumors Adrenal cortical Carcinoid Lipomas Thyroid adenomas
MEN2A	Medullary thyroid carcinoma Pheochromocytoma Parathyroid
MEN2B	Medullary thyroid carcinoma Pheochromocytoma Associated abnormalities ganglioneuromatosis Marfanoid habitus
Familial MTC	Medullary thyroid carcinoma

dominant fashion or may occur sporadically as a result of somatic mutations. There are two major forms of MEN – type 1 and type 2 (which is further subdivided into types 2A and 2B). Each type is characterized by the occurrence of tumors in specific endocrine glands (Table 25.15), although "overlap" syndromes may occur, which feature a combination of tumors from both types. Familial medullary thyroid carcinoma (MTC) may also occur without any additional endocrinopathy. The MEN syndromes commonly present clinically in early adulthood or later, but may cause significant disease in childhood. Genetic and biochemical testing has a key role in these conditions – it identifies those children in affected families who are at risk of developing the disorder and allows close monitoring and, where appropriate, early surgical intervention in those at-risk individuals.

Multiple endocrine neoplasia type 1

Genetic testing

The MEN1 gene, located on chromosome band 11q13, is thought be a tumor suppressor gene and encodes for the nuclear protein MENIN, which acts via the transcriptional regulation pathway to control cell proliferation. There is great diversity of MEN1 mutations, and no evidence currently for any genotype–phenotype correlation. Additionally, genetic testing has in some cases failed to detect mutations with clear clinical diagnosis of MEN1 and, even if at-risk patients are correctly identified, there are no clinical prophylactic measures that can be taken [80]. Notwithstanding these limitations, it has been proposed that all children of a MEN1 patient should be screened to ascertain whether they carry the same genetic mutation [81]. Children identified as carriers, and therefore at risk of developing the disease, should be carefully monitored on an annual basis. Children whose parents have MEN1 that is not linked to an identified gene mutation should be similarly monitored.

Biochemical monitoring

Biochemical monitoring should be commenced between 10 and 15 years of age, or younger if there is a family history of early onset of disease, and all tests should be performed annually. To screen for hyperparathyroidism, serum calcium should be measured and followed up with a serum PTH if the calcium is elevated. Measurement of plasma gut hormones (insulin, glucagon, gastrin, VIP, and pancreatic polypeptide) combined with pancreatic imaging should be performed to screen for islet cell tumors. It is important to note that all gut hormones are extremely unstable in blood, and liaison with the local laboratory prior to collection is essential. Samples should be collected into lithium heparin tubes containing 200 μL of sterile trasylol (10 000 kIU aprotinin/mL) and transported to the laboratory immediately on ice for centrifugation and storage at –20°C. Serum prolactin and IGF-I are the appropriate screening tests for pituitary adenomas commonly found in association with MEN1 – these should be combined with a pituitary MRI scan every 5 years. If IGF-I is found to be elevated, this should be followed up with a five-point serum GH day curve and an OGTT for growth hormone to confirm GH oversecretion.

Multiple endocrine neoplasia type 2

Genetic testing

MEN2 disorders arise as a result of activating mutations in the RET proto-oncogene, which codes for a tyrosine kinase receptor. The tyrosine kinase receptor is a key component of a larger receptor system that is important in the normal embryonic development of the kidney, gastrointestinal neuronal system, and certain components of the sympathetic nervous system. The penetrance of MEN varies between the three different syndromes with MEN2B being the most penetrant and familial MTC being the least. In patients with MEN2B, MTC has been detected shortly after birth and, in MEN2A, C-cell hyperplasia has been reported at 3 years of age. Genetic testing of children with parents with MEN2B should therefore be performed in the first year of life and those with parents with MEN2A or familial MTC before 5 years of age. In the majority of patients with identified

RET mutations, a prophylactic total thyroidectomy should be performed – for these patients, therefore, genetic testing has totally replaced traditional biochemical screening, which is both less sensitive and less specific. In a small subset of patients with the lowest risk mutations (codon 609, 768, 790, 791, and 804 mutations), management is more controversial, and surgery may be delayed until biochemical tests become abnormal [82]. Regular clinical and biochemical monitoring should be performed in all children with an identified RET mutation. All children from a MEN2 family in which the mutation has not yet been identified also require regular biochemical screening and monitoring. In families in which there is an index case of apparently sporadic MTC, genetic screening is indicated in the index case [81]. If a mutation is found, it can be used to screen family members – if no mutation is found, then the probability of familial MEN is low and, in the absence of clinical signs and symptoms, further investigation is not warranted.

Biochemical monitoring

Children from families with MEN2 were previously screened for MTC using the pentagastrin stimulation test. However, this test has, for the most part, been superseded by genetic testing. The pentagastrin test, however, remains useful for screening families in which the mutation is not yet known and for post-operative follow-up of patients with C-cell hyperplasia or MTC. It may also be used to monitor children with the lowest risk mutations in whom a decision has been made to delay thyroidectomy.

Biochemical screening for pheochromocytoma in MEN2 patients is important as these tumors are associated with a risk of sudden death. In MEN2B, pheochromocytoma can occur early in childhood and, in MEN2A, the earliest presentation is at 10 years of age [81]. Timed overnight urine collections should be analyzed annually for normetadrenaline, adrenaline, and catecholamines (see adrenal medulla section) from 5 years of age in the case of MEN2A and as soon as is practicable in the case of MEN2B. MEN2-related pheochromocytomas have a characteristic pattern of catecholamine production resulting in high adrenaline to noradrenaline ratios in urine [82]. This is in contrast to sporadic pheochromocytoma in which noradrenaline is the major product. The relative excess of adrenaline leads to tachycardia, arrhythmias, and tremulousness – hypertension occurs later in association with larger tumors. In children with symptoms consistent with pheochromocytoma or abnormal catecholamine/metadrenaline secretion, adrenal imaging should be performed. Fine-cut CT is very sensitive for the detection of small tumors, although MRI offers greater specificity. Bilateral tumors are more common in MEN syndromes – selective venous catheterization may be useful in localizing adrenal (unilateral and bilateral) and extra-adrenal pheochromocytomas.

To screen for hyperparathyroidism in patients at risk of MEN2A, serum calcium should be measured annually from the age of 10 years. This should be followed up with a serum PTH if the calcium is elevated.

Pentagastrin stimulation test

Background

Medullary carcinoma of the thyroid secretes excess calcitonin, the hormone normally secreted by thyroid parafollicular (C) cells to lower plasma calcium. Sometimes, patients with C-cell disease may have a normal basal plasma calcitonin, and basal calcitonin concentration may be increased in patients other than those with MTC. Therefore, calcitonin secretion following pentagastrin stimulation has been used as a diagnostic test of calcitonin hypersecretion.

Indication

For screening families with clinical symptoms of MEN2 in whom no RET mutation has been identified.
Post-operative follow-up of patients with C-cell hyperplasia or MTC.
To monitor patients with lowest risk mutations in whom thyroidectomy has been postponed.

Precautions

Pentagastrin may cause chest tightness, abdominal cramping, and nausea.

Preparation

The fasting plasma calcium should be measured to exclude hypocalcemia. The patient should be fasted and an intravenous cannula inserted.

Protocol

1 0 min: collect plasma sample for baseline calcitonin estimation.
2 Inject pentagastrin 0.5 μg/kg in 2 mL of saline IV over 10–20 s.
3 Take a further sample for pentagastrin at 3, 5, 10, 15, and 20 min after administration of pentagastrin.

Interpretation

Assay characteristics and reference ranges of laboratories measuring calcitonin are different and should not be compared. It is important to liaise with the laboratory regarding appropriate local values and test interpretation. An exaggerated response suggests a diagnosis of medullary carcinoma of the thyroid, although both false positives and false negatives may occur. Following total thyroidectomy, basal and stimulated calcitonin concentrations should be maintained within the reference range. A high basal concentration or an exaggerated response to stimulation suggests recurrence of the disease.

Table 25.16. Molecular genetics of endocrine disorders.

Disorder	Defective gene
Transcription factors in endocrine development	
Septo-optic dysplasia/pituitary hypoplasia	*HESX1*
Pituitary hypoplasia with GH, prolactin, TSH, and gonadotropin deficiency	*PROP-1*
Pituitary hypoplasia ± GH, prolactin, and TSH deficiency	*POU1F1* (*PIT1*)
Congenital adrenal hypoplasia	*DAX1*
XY sex reversal	*SRY*, *WT1*, *SF-1*, *SOX9*
X-linked Kallmann syndrome	*KALIG-1*
Congenital hypothyroidism	*TTF1*
Defects in hormone biosynthesis	
Isolated GH deficiency	*GH-1*
IUGR with poor postnatal growth	*IGF-I*
Cranial diabetes insipidus	*Prepro-AVP-NPII*
Congenital adrenal hyperplasia	*CYP21A2*, *CYP11A1*, *CYP11B1*, *CYP11B2*, *StAR*, *3β-HSD2*, *CYP17*
Ambiguous genitalia	17,20-lyase, 17β-hydroxysteroid dehydrogenase, 5α-reductase, LH receptor
Defects associated with abnormalities of hormone secretion	
Tall stature	Aromatase, estrogen receptor
Persistent hyperinsulinemic hypoglycemia of infancy	*SUR-1*, *Kir 6.2*, glucokinase, glutamate dehydrogenase Hepatic nuclear factor-4α, glucokinase,
MODY	Hepatic nuclear factor-1α, insulin promoter factor 1, hepatocyte nuclear factor-1β
Hypoparathyroidism	*PTH*
Familial hypocalciuric hypercalcemia	Calcium-sensing receptor
Beckwith–Wiedemann	Loss or imprinting of 11p15 region (*H19*, *p57KIP2* and *IGF-II genes*)
Li–Fraumeni	*p53*
MEN1	*MENIN*
MEN2	*RET* proto-oncogene
Obesity	*POMC*, *PC1*, *LEPTIN*
Prader–Willi syndrome	70% paternal 15q11–q13 deletion, 25% maternal uniparental disomy, 5% imprinting defect
Vitamin D-resistant rickets type 1	25-Hydroxylase, 1α-hydroxylase
Polyglandular autoimmune syndrome	*APECED*
Defects in hormone receptor	
GH resistance (Laron-type dwarfism)	GH receptor
GHD with pituitary hypoplasia	GHRH receptor
Hypogonadotrophic hypogonadism	GnRH receptor
Delayed puberty	FSH-β, LH-β
Thyroid hormone resistance	TR-βreceptor
Nephrogenic diabetes insipidus	V_2 receptor, aquaporin 2 gene
Androgen insensitivity	Androgen receptor
McCune–Albright syndrome	*Gsα*
Familial male precocious puberty	LH receptor
Insulin resistance	Insulin receptor
Familial glucocorticoid deficiency	ACTH receptor
Obesity	Leptin receptor, *MC4R*
Pseudohypoparathyroidism type 1a	*Gsα*

Molecular genetic analysis

Following the development of recombinant DNA technology in the 1970s, there has been dramatic advancement in the understanding of disease processes at the molecular concentration. For endocrinology, this has not only improved our understanding of the mechanisms of hormone action, but has also had considerable impact on the diagnosis and management of endocrine disorders. Genetic defects at several concentrations may be responsible for the development of endocrine disease. Defects may occur in genes responsible for:

1 the development of endocrine cells, tissues, or organs.
2 the biosynthesis of hormones themselves.
3 the development of receptors that bind those hormones and thus initiate signal propagation and a biological response.

DNA analysis is now an integral component of the investigative and diagnostic process for many pediatric endocrine disorders and may be vital for counseling families at risk. Table 25.16 lists some of the disorders in which DNA analysis could be applied. However, this list is by no means exhaustive and is growing rapidly.

Appendix Normal values

All reference values for endocrine tests are highly method dependent. Most endocrine units have established their own normal values in conjunction with their local laboratory. The following is by no means a comprehensive guide but may serve as a useful, quick reference. More extensive age- and sex-related values are available in the literature [83,84]. However, it is important to seek the advice of the local laboratory for the interpretation of all tests for which method-specific normal values have not been derived.

Pituitary

Hormone	Traditional units	Conversion factor	SI units
GH			
Basal	Low, often undetectable		
Peak (after appropriate stimulation)	>6 ng/mL	Dependent on standard used – approx. 2.6 for IS 80/505	>15 mU/L (assay dependent)
Prolactin (PRL)	<20 ng/mL	Approx. 21 for IS 84/500	<425 mU/L
TSH			
Basal			
<1 month	Up to 10 μU/mL	1	Up to 10 mU/L
Child and adult	0.5–5.0 μU/mL		0.5–5.0 mU/L
Stimulated	5.5–30 μU/mL		5.5–35 mU/L
FSH (highly method dependent)			
Basal			
Female			
Prepubertal	0.4–3.0 mIU/mL	1	0.4–3.0 IU/L
Tanner stage II	1.6–7.0 mIU/mL		1.6–7.0 IU/L
Tanner stage III	4.0–7.0 mIU/mL		4.0–7.0 IU/L
Tanner stage IV	3.0–8.0 mIU/mL		3.0–8.0 IU/L
Adult (follicular phase)	2.0–6.6 mIU/mL		2.0–6.6 IU/L
Adult (luteal phase)	1.6–5.7 mIU/mL		1.6–5.7 IU/L
Male			
Prepubertal	0.4–1.6 mIU/mL		0.4–1.6 IU/L
Tanner stage II	0.5–4.0 mIU/mL		0.5–4.0 IU/L
Tanner stage III	2.5–4.5 mIU/mL		2.5–4.5 IU/L
Tanner stage IV	3.0–5.5 mIU/mL		3.0–5.5 IU/L
Adult	1–7 mIU/mL		1–7 IU/L
Incremental rise following GnRH stimulation			
Prepubertal	2–3 mIU/mL		2–3 IU/L
Post-pubertal	>3 mIU/mL		>3 IU/L
LH (highly method dependent)			
Basal			
Female			
Prepubertal	<0.5 mIU/mL	1	<0.5 IU/L
Tanner stage II	1–7 mIU/mL		1–7 IU/L
Tanner stage IV	2–8 mIU/mL		2–8 IU/L
Adult (follicular phase)	2.0–6.6 mIU/mL		2.0–6.6 IU/L
Adult (mid-cycle)			
Adult (luteal phase)	14–72 mIU/mL	14–72 IU/L	
Male	1.6–5.7 mIU/mL	1.6–5.7 IU/L	
Prepubertal	<0.5 mIU/mL	<0.5 IU/L	
Tanner stage II	1–4 mIU/mL	1–4 IU/L	

Pituitary (*continued*)

Hormone	Traditional units	Conversion factor	SI units
Tanner stage IV	2–8 mIU/mL	2–8 IU/L	
Adult	1–10 mIU/mL	1–10 IU/L	
Incremental rise following GnRH stimulation			
Prepubertal	3–4 mIU/mL	3–4 IU/L	
Post-pubertal	>4 mIU/mL	>4 IU/L	
ACTH	(LH dominant)	(LH dominant)	
At 09.00 h			
RIA	<80 pg/mL	0.22	<18 pmol/L
IRMA	<50 pg/mL		<11 pmol/L
ADH (not routinely available)			
Basal	1–6 pg/mL	0.92	1–5 pmol/L
Plasma osmolality	275–295 mosmol/kg	1	275–295 mmol/kg

IS, International Standard.

Growth factors

Hormone	
IGF-I	Normal ranges are dependent on age, sex, and stage of puberty. Information must be provided with the request. Consult reference laboratory for details

Adrenal cortex

Hormone	Traditional units	Conversion factor	
Cortisol			
Basal			
09.00	7–25 µg/dL	27.6	200–700 nmol/L
24.00 (sleeping)	<1.8 µg/dL		<50 nmol/L
Following ACTH stimulation	Peak concentration (at 30 min) > 20 µg/dL or increment of at least 7.2 µg/dL		Peak concentration (at 30 min) > 550 nmol/L or increment of at least 200 nmol/L
Urinary free cortisol	<87 µg/24 h	2.76	<240 nmol/24 h
17-OHP (highly assay dependent)			
Basal	<5 µg/L	3.03	<15 nmol/L
Stressed newborn	Up to 33 µg/L		Up to 100 nmol/L
Following ACTH stimulation	Incremental increase < 3.3 µg/L at 60 min		Incremental increase < 10 nmol/L at 60 min
11-Deoxycortisol	<1 µg/dL	29	<30 nmol/L
DHEAS			
Female			
<8 years	<222 µg/L	0.0027	<0.6 µmol/L
8–10 years	111–593 µg/L		0.3–1.6 µmol/L
10–12 years	296–1185 µg/L		0.8–3.2 µmol/L
12–14 years	370–1852 µg/L		1–5 µmol/L
Adult	1100–4400 µg/L		3–12 µmol/L

Adrenal cortex (*continued*)

Hormone	Traditional units	Conversion factor	
Male			
<8 years	<222 µg/L		<0.6 µmol/L
8–10 years	74–1037 µg/L		0.2–2.8 µmol/L
10–12 years	333–1407 µg/L		0.9–3.8 µmol/L
Adult	750–3700 µg/L		2–10 µmol/L
Androstenedione			
Prepubertal	8.6–52 ng/dL	0.0349	0.3–1.8 nmol/L
Adult	57–230 ng/dL		2–8 nmol/L
Aldosterone (should be interpreted in conjunction with PRA)			
Up to 1 week	Up to 181 ng/dL	27.7	Up to 5000 pmol/L
1 week–1 year	11–54 ng/dL		300–1500 pmol/L
1–2 years	7–54 ng/dL		200–1500 pmol/L
2–10 years	4–29 ng/dL		100–800 pmol/L
10–15 years	2–22 ng/dL		60–600 pmol/L
Adult			
Normal sodium diet			
Upright (4 h)	7–30 ng/dL		190–830 pmol/L
Supine (30 min)	3–16 ng/dL		80–444 pmol/L
Low-sodium diet	Levels increase two- to fivefold		
PRA			
Up to 1 week	Up to 26 ng/h/mL	0.77	Up to 20 pmol/h/mL
1 week–1 year	2.6–9.1 ng/h/mL		2–7 pmol/h/mL
1–2 years	2.6–7.8 ng/h/mL		2–6 pmol/h/mL
2–10 years	1.9–5.2 ng/h/mL		1.5–4 pmol/h/mL
10–15 years	1.0–2.6 ng/h/mL		0.8–2 pmol/h/mL
Adult			
Normal sodium diet			
Upright (4 h)	1.6–7.4 ng/h/mL		1.2–5.7 pmol/h/mL
Supine (30 min)	0.1–3.1 ng/h/mL		0.1–2.4 pmol/h/mL
Low-sodium diet			
Upright (4 h)	5.6–14.2 ng/h/mL		4.3–10.9 pmol/h/mL
Supine (30 min)	2.1–5.4 ng/h/mL		1.6–4.2 pmol/h/mL

DHEAS, dehydroepiandrosterone sulfate; PRA, plasma renin activity.

Adrenal medulla

Hormone	Traditional units	Conversion factor	SI units
Urinary adrenaline			
<2 years	<75 µg/g creatinine	0.000613	<0.046 mmol/mol creatinine
2–4 years	<57 µg/g creatinine		<0.035 mmol/mol creatinine
5–9 years	<35 µg/g creatinine		<0.022 mmol/mol creatinine
10–19 years	<34 µg/g creatinine		<0.021 mmol/mol creatinine
Urinary noradrenaline			
<2 years	<420 µg/g creatinine	0.000667	<0.280 mmol/mol creatinine
2–4 years	<120 µg/g creatinine		<0.080 mmol/mol creatinine
5–9 years	<89 µg/g creatinine		<0.059 mmol/mol creatinine
10–19 years	<82 µg/g creatinine		<0.055 mmol/mol creatinine

Adrenal medulla (*continued*)

Hormone	Traditional units	Conversion factor	
Urinary metadrenaline			
3–8 years	47–240 µg/g creatinine	0.000574	0.023–0.138 mmol/mol creatinine
9–12 years	40–220 µg/g creatinine		0.023–0.126 mmol/mol creatinine
13–17 years	33–145 µg/g creatinine		0.019–0.083 mmol/mol creatinine
Adults	31–140 µg/g creatinine		0.018–0.08 mmol/mol creatinine
Urinary normetadrenaline			
3–8 years	62–705 µg/g creatinine	0.000617	0.038–0.43 mmol/mol creatinine
9–12 years	81–583 µg/g creatinine		0.050–0.36 mmol/mol creatinine
13–17 years	95–375 µg/g creatinine		0.059–0.23 mmol/mol creatinine
Adults	47–310 µg/g creatinine		0.029–0.19 mmol/mol creatinine
Dopamine			
<2 years	<3000 µg/g creatinine	0.000738	<2.216 mmol/mol creatinine
2–4 years	<1533 µg/g creatinine		<1.132 mmol/mol creatinine
5–9 years	<1048 µg/g creatinine		<0.774 mmol/mol creatinine
10–19 years	<545 µg/g creatinine		<0.403 mmol/mol creatinine
HVA			
<1 year	<32.6 mg/g creatinine	0.6196	<20.2 mmol/mol creatinine
2–4 years	<22.0 mg/g creatinine		<13.6 mmol/mol creatinine
5–9 years	<15.1 mg/g creatinine		<9.4 mmol/mol creatinine
10–19 years	<12.8 mg/g creatinine		<7.9 mmol/mol creatinine
Adult	<7.6 mg/g creatinine		<4.7 mmol/mol creatinine
HMMA			
<1 year	<29.1 mg/g creatinine	0.5736	<16.7 mmol/mol creatinine
2–4 years	<16.2 mg/g creatinine		<9.3 mmol/mol creatinine
5–9 years	<11.3 mg/g creatinine		<6.5 mmol/mol creatinine
10–19 years	<9.1 mg/g creatinine		<5.2 mmol/mol creatinine
Adult	<5.9 mg/g creatinine		<3.4 mmol/mol creatinine

HVA, homovanillic acid; HMMA, 4-hydroxy-3-methoxymandelic acid.

Gonads

Hormones	Traditional units	Conversion factor	SI units
Estradiol			
Female			
<12 months	<80 pg/mL	3.67	<300 pmol/L
Prepubertal	<16 pg/mL		<60 pmol/L
Adult			
Follicular	20–70 pg/mL		70–260 pmol/L
Mid-cycle	95–410 pg/mL		350–1500 pmol/L
Luteal	50–300 pg/mL		180–1100 pmol/L
Adult male	<40 pg/mL		<150 pmol/L
Progesterone			
Female			
Prepubertal	<60 ng/dL	0.0318	<2 nmol/L
Follicular	<315 ng/dL		<10 nmol/L
Mid-luteal	940–2515 ng/dL		30–80 nmol/L
Male (all ages)	<60 ng/dL		<2 nmol/L

Gonads (*continued*)

Hormone	Traditional units	Conversion factor	
Testosterone			
Male			
Birth	115–400 ng/dL	0.03467	4–14 nmol/L
First week	falls to		falls to
	10–35 ng/dL		0.5–1.5 nmol/L
15–60 days	115–230 ng/dL		4–10 nmol/L
Prepubertal (from 7 months)	<10 ng/dL		<0.5 nmol/L
Adult male	230–865 ng/dL		10–30 nmol/L
Adult female	10–75 ng/dL		0.5–2.5 nmol/L
Dihydrotestosterone			
Male			
Birth	5–60 ng/dL	34.4	172–2064 pmol/L
First week	falls rapidly		falls rapidly
15–60 days	12–85 ng/dL		413–2924 pmol/L
Prepubertal (from 7 months)	<3 ng/dL		<103 pmol/L
Adult male	30–85 ng/dL		1032–2924 pmol/L
Adult female	4–22 ng/dL		138–757 pmol/L

Thyroid

Hormone	Traditional units	Conversion factor	SI units
FT_4			
<1 month	1.17–2.64 ng/dL	12.87	15–34 pmol/L
<1 year	0.76–2 ng/dL		10–26 pmol/L
Adult	0.7–1.55 ng/dL		9–20 pmol/L
FT_3			
<1 month	1.4–5.5 pg/mL	1.54	2.2–8.5 pmol/L
<1 year	2.0–6.9 pg/ml		3.1–10.6 pmol/L
Adult	2.3–5.5 pg/ml		3.5–8.5 pmol/L
Total T_4			
<1 month	5.9–21.5 μg/dL	12.87	76–276 nmol/L
<1 year	4.9–13.9 μg/dL		63–179 nmol/L
Adult	4.7–12.4 μg/dL		60–160 nmol/L
Total T_3			
<1 month	15–210 ng/dL	0.0154	0.2–3.2 nmol/L
<1 year	50–275 ng/dL		0.8–4.2 nmol/L
Adult	70–180 ng/dL		1.1–2.8 nmol/L
TBG	14–30 μg/mL	1	14–30 mg/L
Perchlorate discharge	<10% at 1 h		
Calcitonin			
Basal			
Hammersmith	<30 pg/mL	1	<30 ng/L
Newcastle	<100 pg/mL		<100 ng/L
After pentagastrin stimulation			
Hammersmith	<80 pg/mL		<80 ng/L
Newcastle	<200 pg/mL		<200 ng/l

Electrolytes, parathyroid, and vitamin D

Hormone/analyte	Traditional units	Conversion factor	SI units
Sodium			
Up to 1 month			130–145 mmol/L
>1 month			135–145 mmol/L
Potassium			
Up to 1 month			3.5–6.0 mmol/L
>1 month			3.4–5.0 mmol/L
Chloride			98–110 mmol/L
Bicarbonate			
1–4 years			17–25 mmol/L
4–8 years			19–27 mmol/L
>8 years			21–29 mmol/L
Urea			2.5–7.5 mmol/L
Creatinine			
1 week			40–125 μmol/L
2 weeks			35–105 μmol/L
3 weeks			25–90 μmol/L
4 weeks			20–80 μmol/L
6 months			20–50 μmol/L
2 years			25–60 μmol/L
6 years			30–70 μmol/L
10 years			30–80 μmol/L
Adult male			65–120 μmol/L
Adult female			50–110 μmol/L
Total protein			63–83 g/L
Albumin			30–51 g/L
Total calcium			
Premature			1.50–2.50 mmol/L
Up to 2 weeks			1.90–2.80 mmol/L
Child			2.20–2.70 mmol/L
Ionized calcium			1.13–1.18 mmol/L
Phosphate			
1 month			1.4–2.8 mmol/L
1 year			1.2–2.2 mmol/L
3 years			1.1–2.0 mmol/L
12 years			1.0–1.8 mmol/L
15 years			0.95–1.50 mmol/L
Adult			0.8–1.4 mmol/L
Magnesium			0.7–1.2 mmol/L
ALP (highly method dependent)			
IFCC method			
Up to 1 month			48–406 U/L
1 month–2 years			82–383 U/L
2–8 years			69–325 U/L
Puberty			74–390 U/L
Adult			28–94 U/L
PTH (should be interpreted in conjunction with calcium concentration)	9–54 pg/mL	0.1053	<5 pmol/L
25-OH vitamin D	2 –50 ng/mL	2.599	50–130 nmol/L (seasonal)
Urine calcium/creatinine			<0.56 mol/mmol

ALP, alkaline phosphatase.

Endocrine, pancreas, and gut hormones

Hormone/analyte	Traditional units	Conversion factor	SI units
Glucose (fasting)			
Up to 1 month			2.5–5.5 mmol/L
Child			3.0–6.1 mmol/L
Insulin			
Fasting	2.3–26 mU/L	Dependent on standard used – approx. 6.0 for IS 83/500	14–156 pmol/L
In presence of hypoglycemia (blood glucose < 2.6 mmol/L)	<2 mU/L		<12 pmol/L
C-peptide			
Fasting	0.36–3.6 ng/ml	333	119–1189 pmol/L
In presence of hypoglycemia	<0.6 ng/ml		<200 pmol/L
Glucagon	<175 pg/ml	0.28	<50 pmol/L
Gastrin	<120 pg/mL	0.45	<55 pmol/L
Vasoactive intestinal peptide	<72 pg/mL	0.42	<30 pmol/L
Pancreatic polypeptide	<1260 pg/mL	0.24	<300 pmol/L

Miscellaneous

Hormone/analyte	Traditional units	Conversion factor	SI units
Alfa-fetoprotein	Mean ± SD (ng/mL)	0.83	Mean ± SD (kU/L)
Premature	134 734 ± 41 444		111 829 ± 34 399
Newborn	48 406 ± 34 718		40 177 ± 28 816
Newborn– 2 weeks	33 113 ± 32 503		27 484 ± 26 977
2 weeks–1 month	9452 ± 12 610		7845 ± 10 466
1 month	2654 ± 3080		2203 ± 2556
2 months	323 ± 278		268 ± 231
3 months	88 ± 87		73 ± 72
4 months	74 ± 56		61 ± 46
5 months	46.5 ± 19		39 ± 16
6 months	12.5 ± 9.8		10.4 ± 8.1
7 months	9.7 ± 7.1		8.1 ± 5.9
8 months	8.5 ± 5.5		7.1 ± 4.6
Adult	<6		<5
β-hCG			<5 IU/L
Ammonia			
Newborn			<100 μmol/L
>1 month			15–40 μmol/L
β-Hydroxybutyrate			0.25–3.05 mg/dL
Free fatty acids			
Fasting			100–600 μmol/L
Lactate			
Fasting			0.5–2.5 mmol/L
Total carnitine			23–60 μmol/L
Free carnitine			15–53 μmol/L

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Appendix Syndrome-specific growth charts

Growth charts for specific growth disorders are depicted here. For further details, please refer to the text of Chapter 6 and Table 6.3.

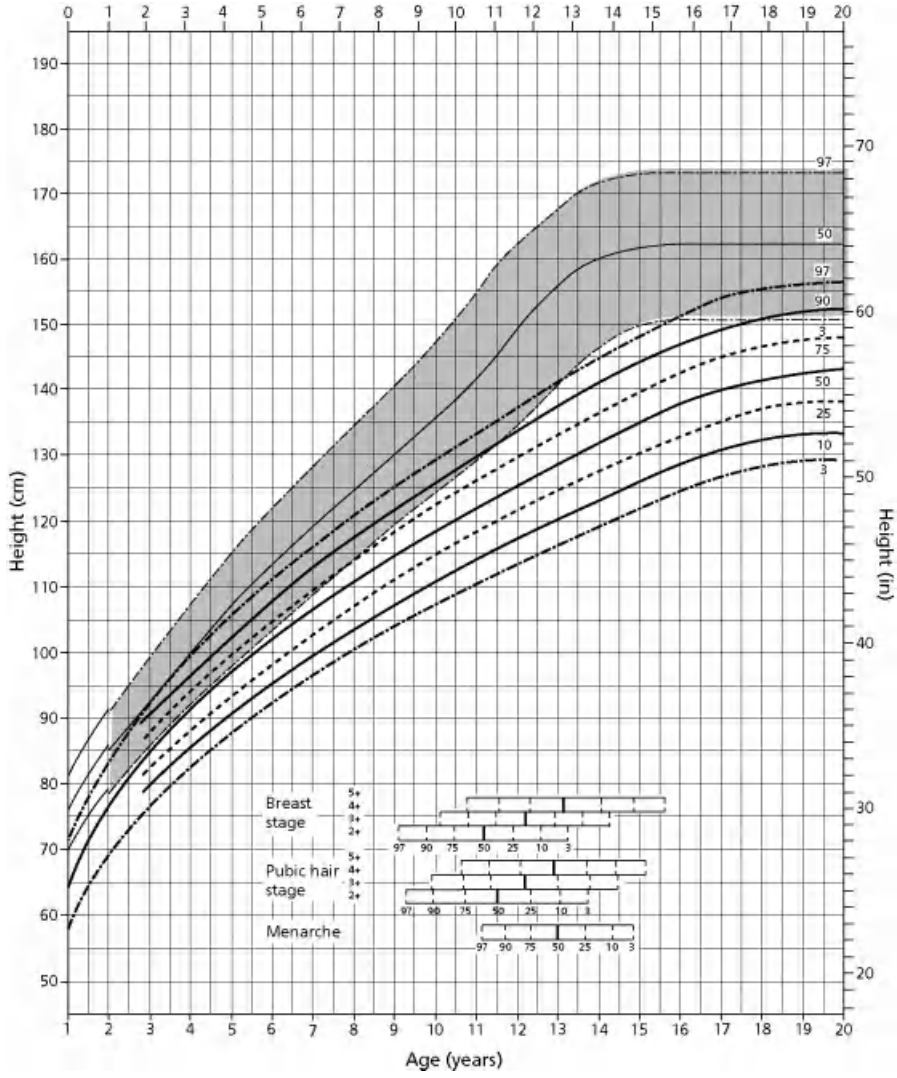

Fig. A.1. Height centiles for girls with untreated Turner syndrome aged 1–20 years. The gray-shaded area represents the 3rd to 97th centiles for normal girls. Pubertal staging is for normal girls. Adapted from Lyon A, Preece M, Grant D. Growth curves for girls with Turner syndrome. *Arch Dis Child* 1985; 60: 932–5.

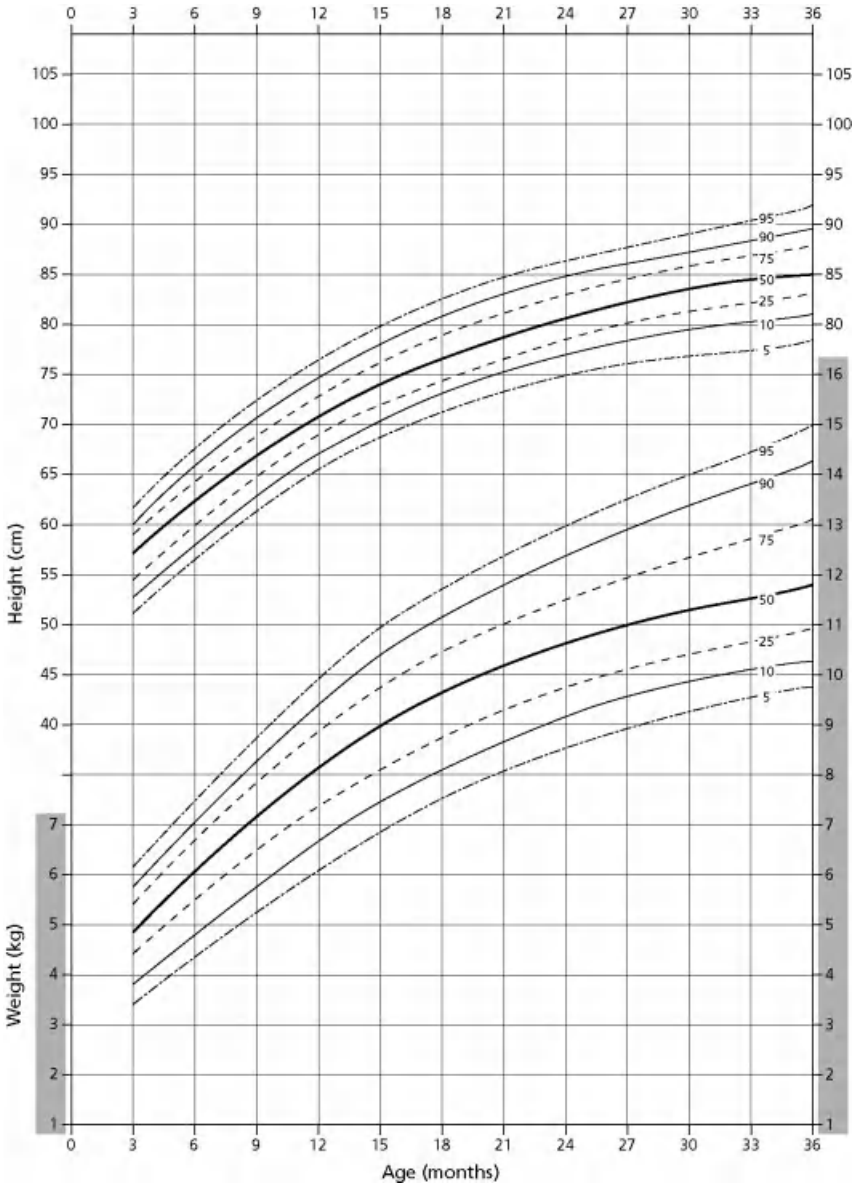

Fig. A.2. Height and weight centiles for boys with trisomy 21 syndrome aged 3–36 months. Adapted from Cronk C, Crocker A, Peuschel S *et al*. Growth charts for children with Down syndrome. *Pediatrics* 1988; 81: 102–10.

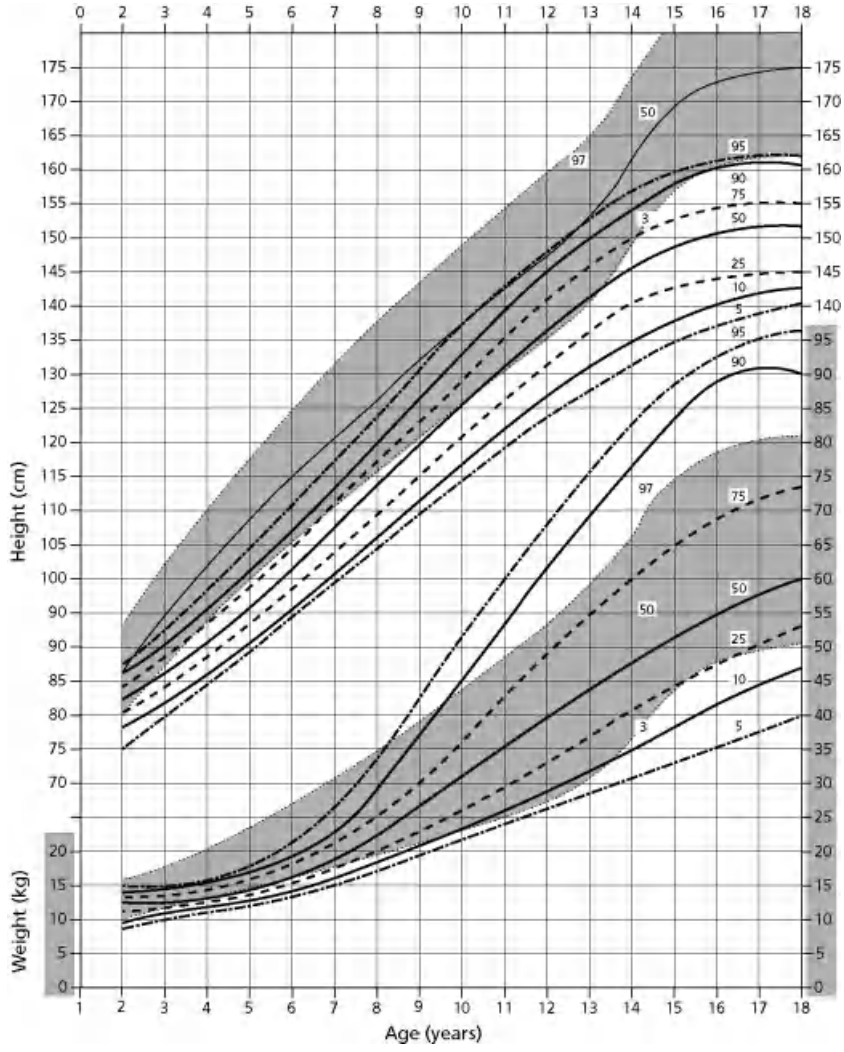

Fig. A.3. Height and weight centiles for boys with trisomy 21 syndrome aged 2–18 years. The gray-shaded areas represent the comparable values for the 3rd to 97th centiles for normal children. Adapted from Cronk C, Crocker A, Peuschel S *et al*. Growth charts for children with Down syndrome. *Pediatrics* 1988; 81: 102–10.

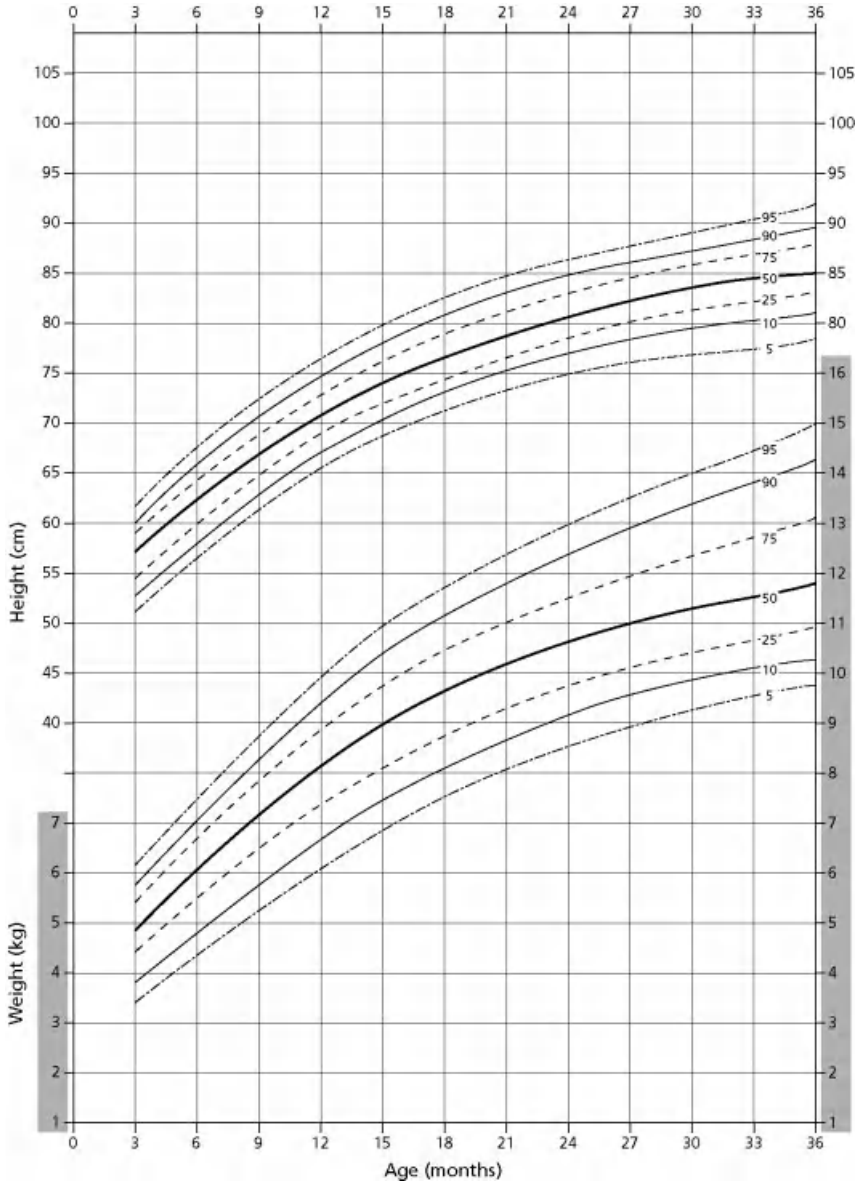

Fig. A.4. Height and weight centiles for girls with trisomy 21 syndrome aged 3–36 months. Adapted from Cronk C, Crocker A, Peuschel S *et al*. Growth charts for children with Down syndrome. *Pediatrics* 1988; 81: 102–10.

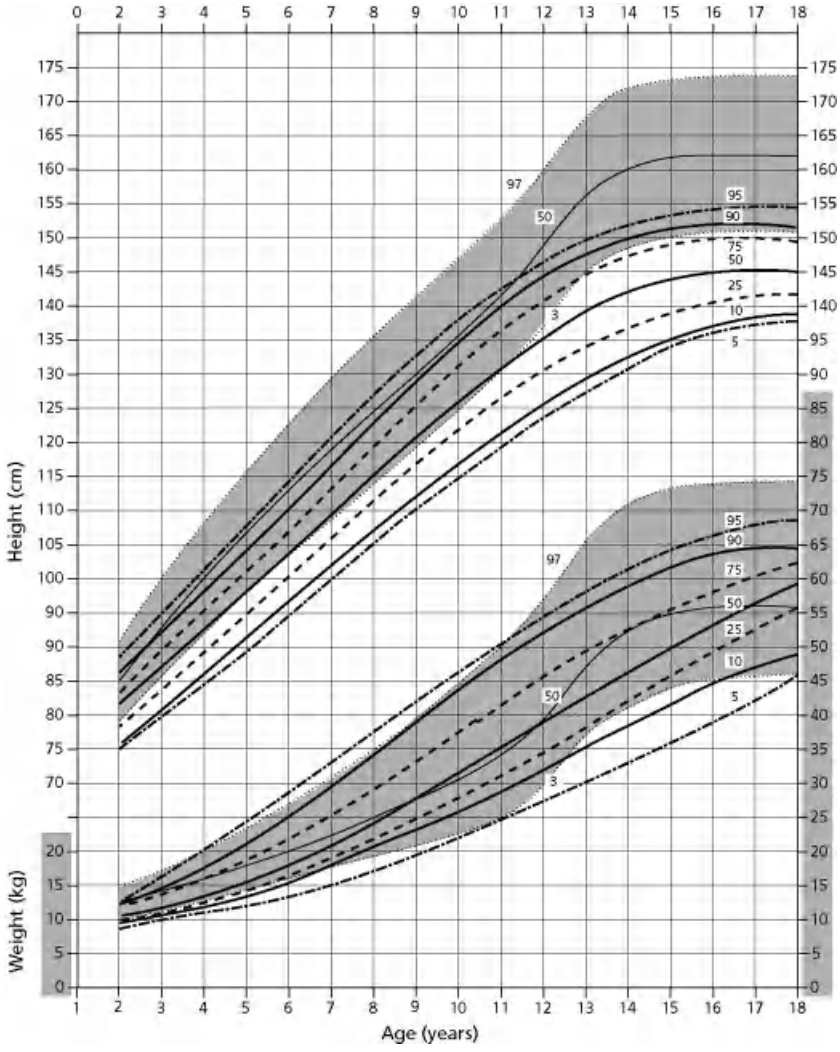

Fig. A.5. Height and weight centiles for girls with trisomy 21 syndrome aged 2–18 years. The gray-shaded areas represent the comparable values for the 3rd to 97th centiles for normal children. Adapted from Cronk C, Crocker A, Peuschel S *et al*. Growth charts for children with Down syndrome. *Pediatrics* 1988; 81: 102–10.

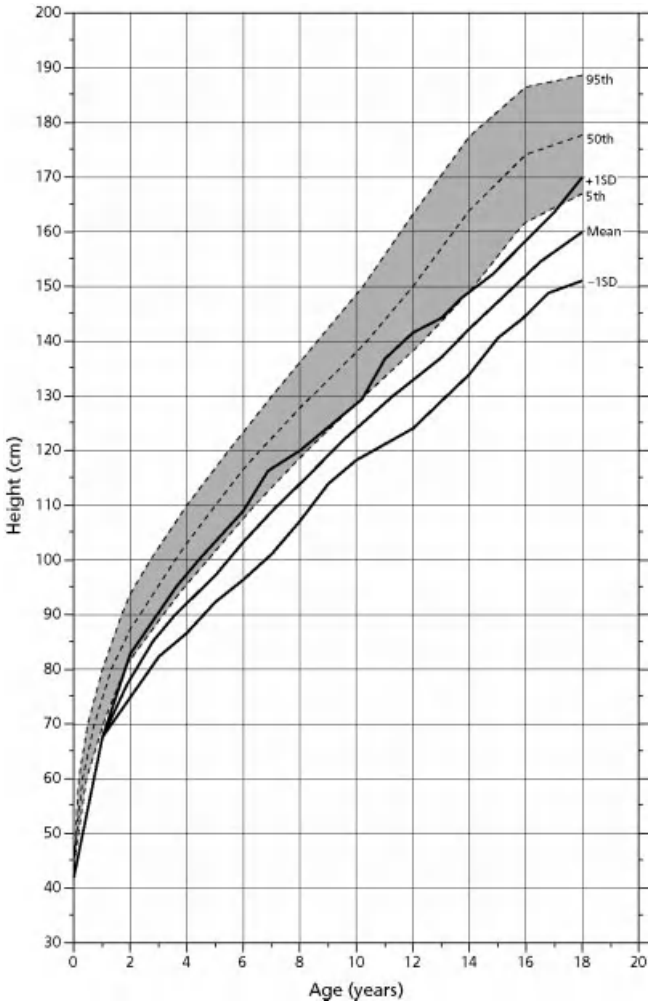

Fig. A.6. Height centiles for boys with Noonan syndrome aged 0–18 years compared with normal values (dashed lines). The data were obtained from 64 Noonan syndrome males in a collaborative retrospective review. Adapted from Witt D, Keena B, Hall J *et al*. Growth curves for height in Noonan syndrome. *Clin Genet* 1986; 30: 150–3.

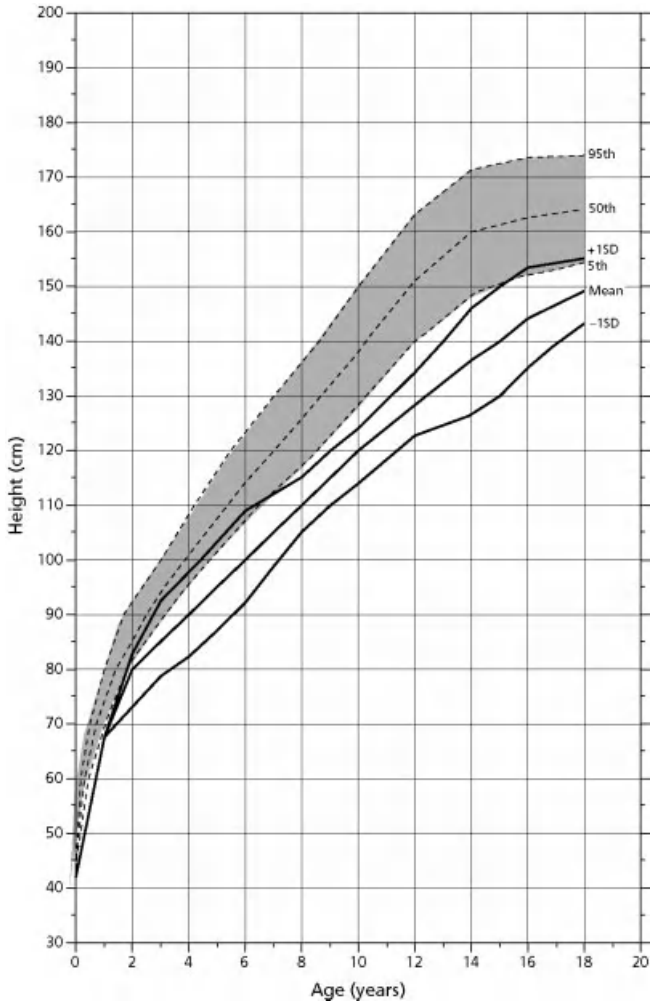

Fig. A.7. Height centiles for girls with Noonan syndrome aged 0–18 years compared with normal values (dashed lines). The data were obtained from 48 Noonan syndrome females in a collaborative retrospective review. Adapted from Witt D, Keena B, Hall J *et al*. Growth curves for height in Noonan syndrome. *Clin Genet* 1986; 30: 150–3.

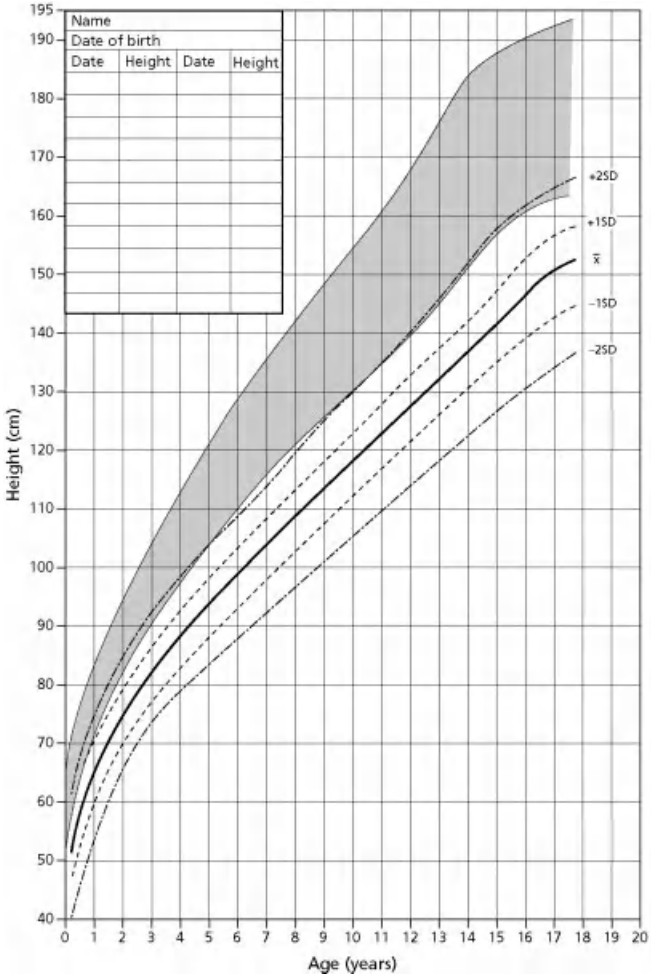

Fig. A.8. Height centiles for boys with Silver–Russell syndrome. The gray-shaded area indicates normal boys ± 2 standard deviations (SD). Adapted from Wollman H, Kirchner T, Enders H *et al*. Growth and symptoms in Silver–Russell syndrome: review on the basis of 386 patients. *Eur J Pediatr* 1995; 154: 958–68.

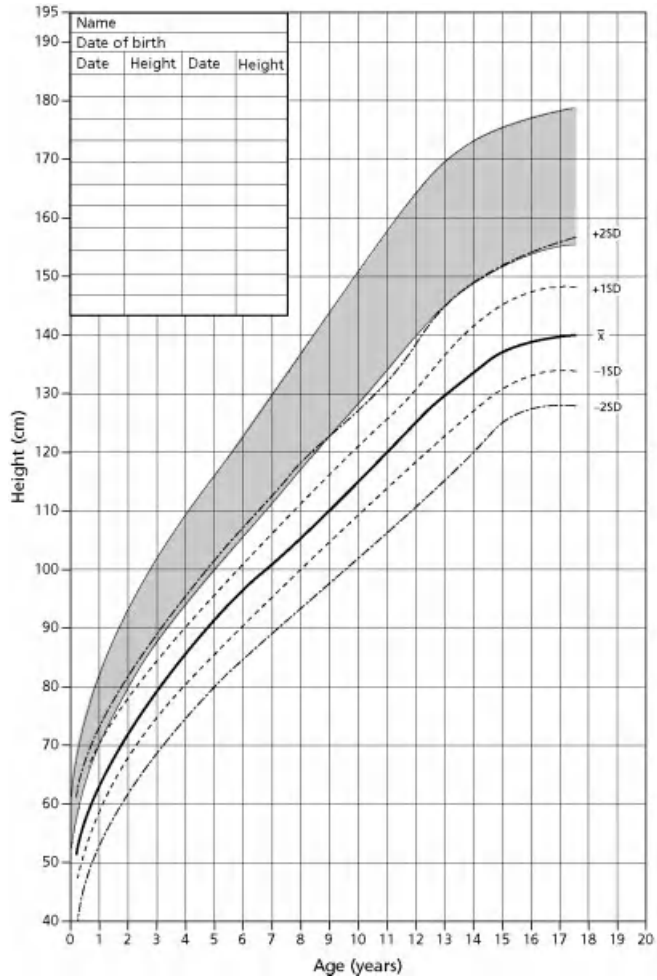

Fig. A.9. Height centiles for girls with Silver–Russell syndrome. The gray-shaded area indicates normal girls ± 2 standard deviations (SD). Adapted from Wollman H, Kirchner T, Enders H *et al*. Growth and symptoms in Silver–Russell syndrome: review on the basis of 386 patients. *Eur J Pediatr* 1995; 154: 958–68.

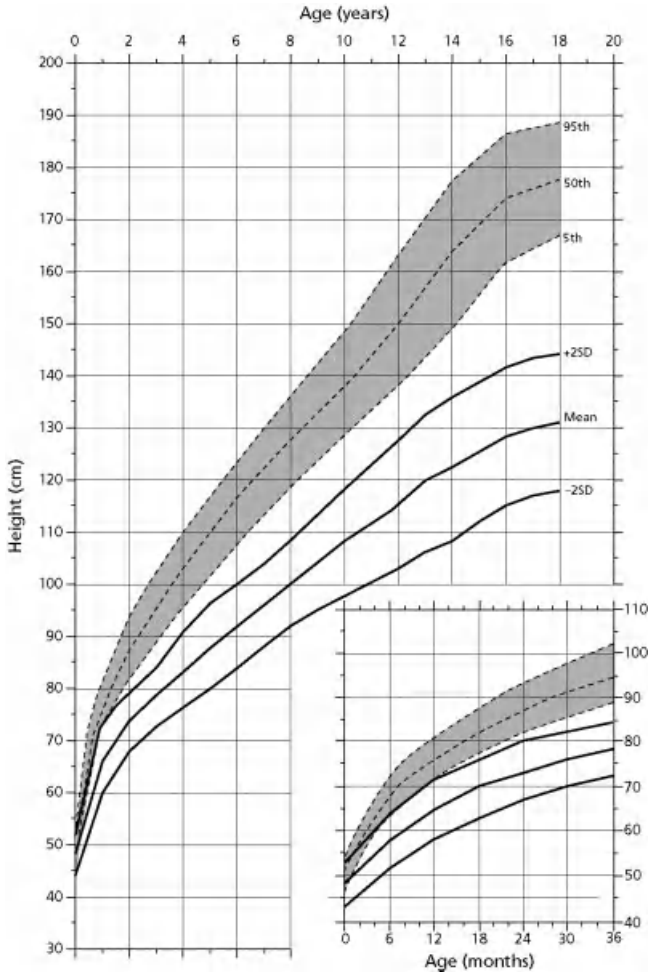

Fig. A.10. Height centiles for boys with achondroplasia (mean ± 2 SD) compared with normal standard curves (dashed lines). Data derived from 189 males. Adapted from Horton W, Rotter J, Rimoin D *et al*. Standard growth curves for achondroplasia. *J Pediatr* 1978; 93: 435–8.

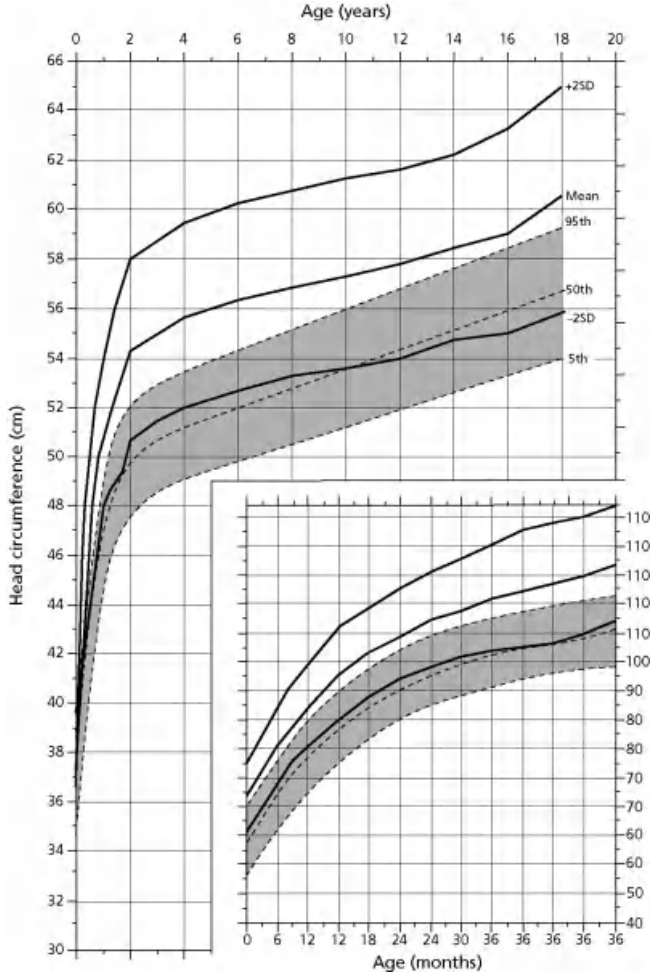

Fig. A.11. Head circumference centiles for boys with achondroplasia compared with normal curves (dashed lines). Data derived from 114 males. Adapted from Horton W, Rotter J, Rimoin D *et al*. Standard growth curves for achondroplasia. *J Pediatr* 1978; 93: 435–8.

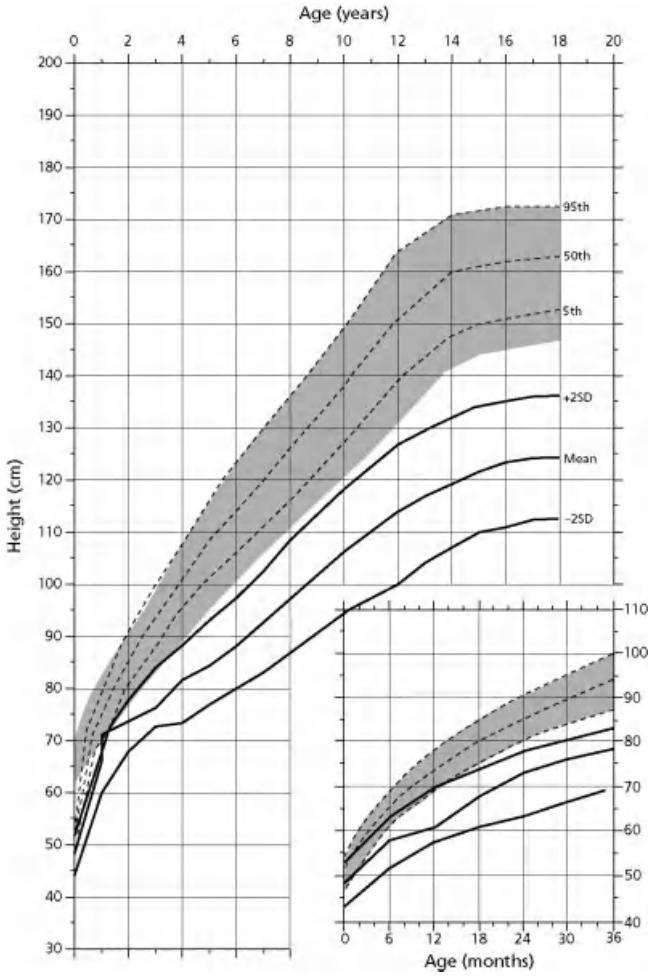

Fig. A.12. Height centiles for girls with achondroplasia (mean ± 2 SD) compared with normal standard curves (dashed lines). Data derived from 214 females. Adapted from Horton W, Rotter J, Rimoin D *et al*. Standard growth curves for achondroplasia. *J Pediatr* 1978; 93: 435–8.

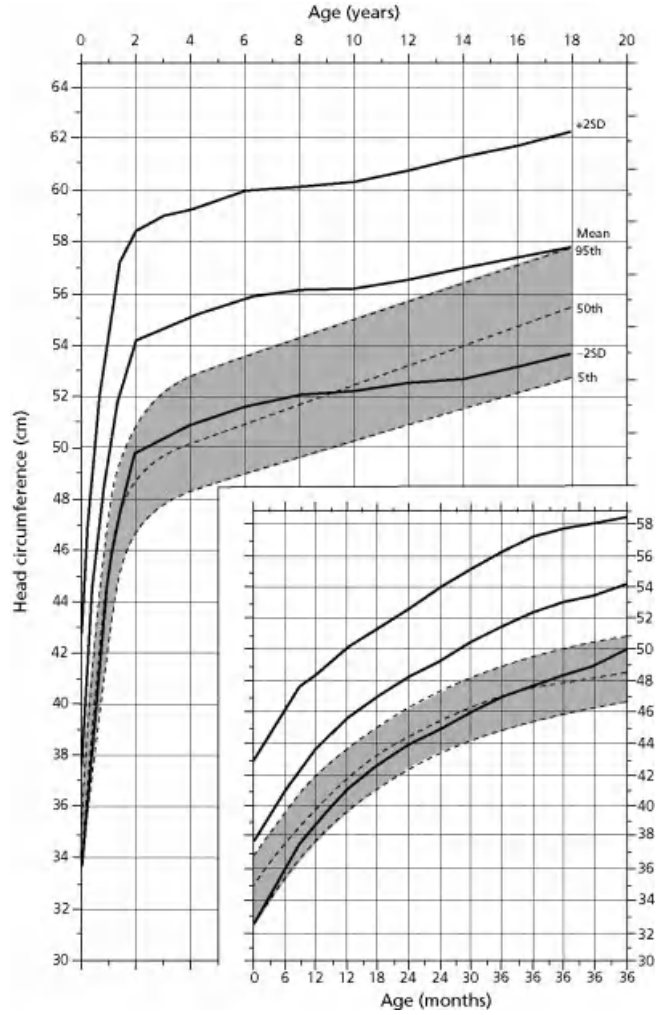

Fig. A.13. Head circumference centiles for girls with achondroplasia compared with normal curves (dashed lines). Data derived from 145 females. Adapted from Horton W, Rotter J, Rimoin D *et al*. Standard growth curves for achondroplasia. *J Pediatr* 1978; 93: 435–8.

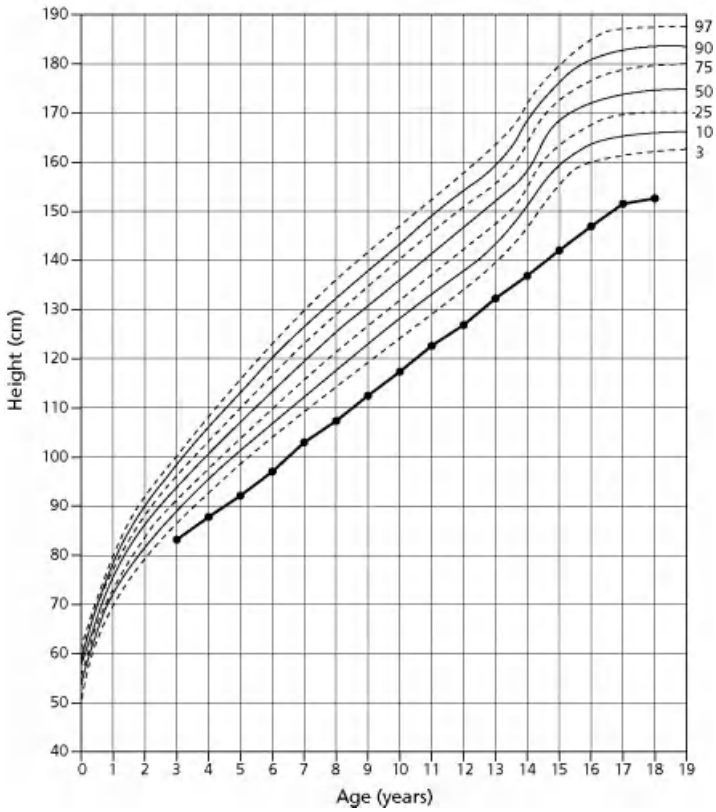

Fig. A.14. Linear growth in hypocondroplasic boys (solid line). Adapted from Appan S, Laurent S, Chapman M *et al*. Growth and growth hormone therapy in hypochondroplasia. *Acta Paediatr Scand* 1990; 79: 796–803.

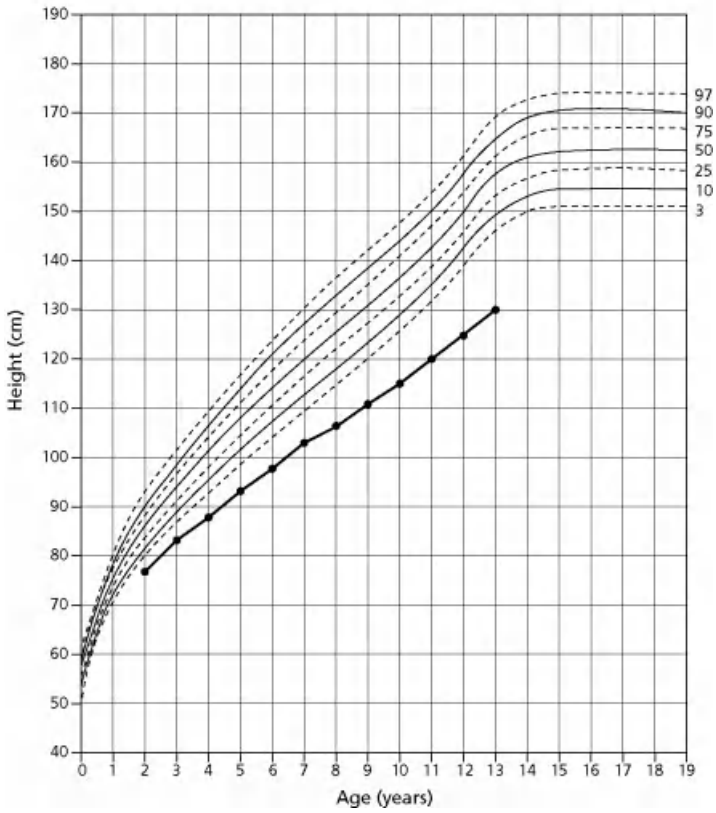

Fig. A.15. Linear growth in hypocondroplasic girls (solid line). Adapted from Appan S, Laurent S, Chapman M *et al*. Growth and growth hormone therapy in hypochondroplasia. *Acta Paediatr Scand* 1990; 79: 796–803.

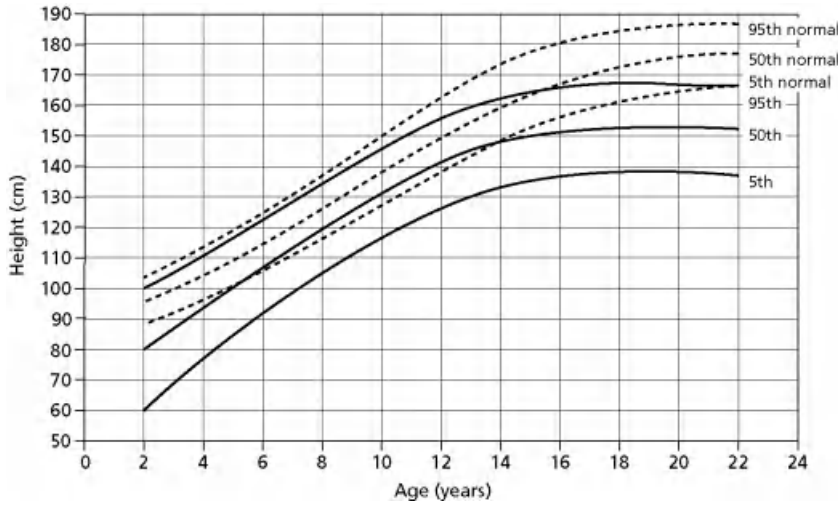

Fig. A.16. Standardized curves for height in Prader–Willi syndrome (PWS) in male patients (solid line) and healthy individuals (broken line). Adapted from Butler MG, Brunschwig A, Miller LK *et al*. Standards for selected anthropometric measurements in Prader–Willi syndrome. *Pediatrics* 1991; 88: 853–60.

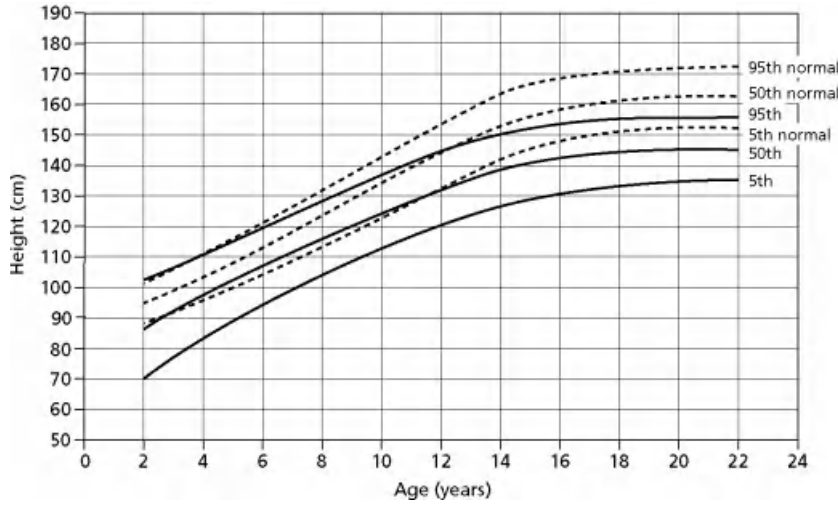

Fig. A.17. Standardized curves for height in Prader–Willi syndrome (PWS) in female patients (solid line) and healthy individuals (broken line). Adapted from Butler MG, Brunschwig A, Miller LK *et al*. Standards for selected anthropometric measurements in Prader–Willi syndrome. *Pediatrics* 1991; 88: 853–60.

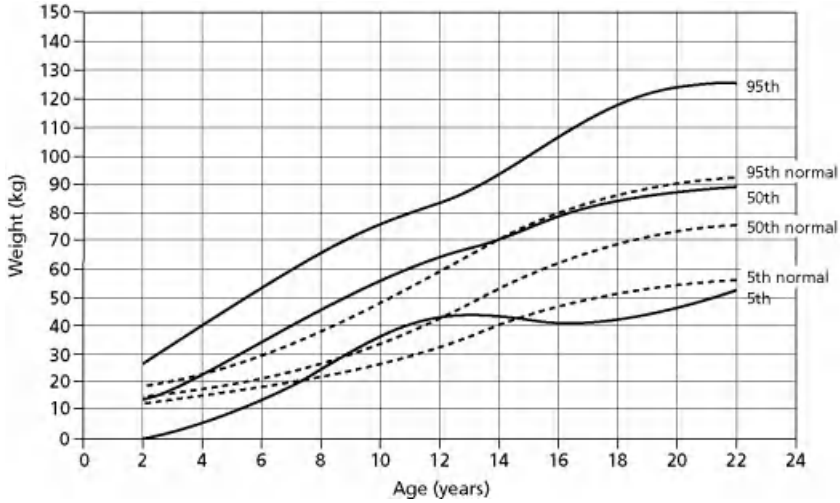

Fig. A.18. Standardized curves for weight in Prader–Willi syndrome (PWS) in male patients (solid line) and healthy individuals (broken line). Adapted from Butler MG, Brunschwig A, Miller LK *et al*. Standards for selected anthropometric measurements in Prader–Willi syndrome. *Pediatrics* 1991; 88: 853–60.

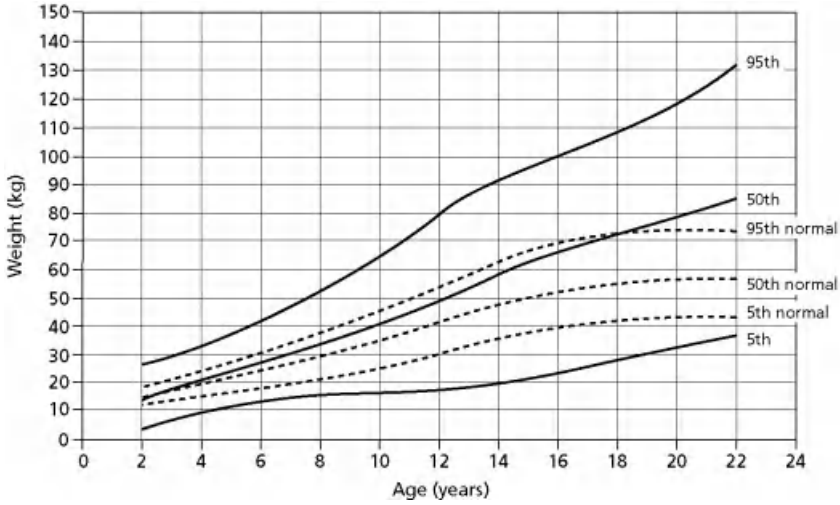

Fig. A.19. Standardized curves for weight in Prader–Willi syndrome (PWS) in female patients (solid line) and healthy individuals (broken line). Adapted from Butler MG, Brunschwig A, Miller LK *et al*. Standards for selected anthropometric measurements in Prader–Willi syndrome. *Pediatrics* 1991; 88: 853–60.

Clinical Pediatric Endocrinology, Fifth Edition
Edited by Charles G.D. Brook, Peter E. Clayton, Rosalind S. Brown

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Note: page numbers in *italics* refer to figures, those in **bold** refer to tables.

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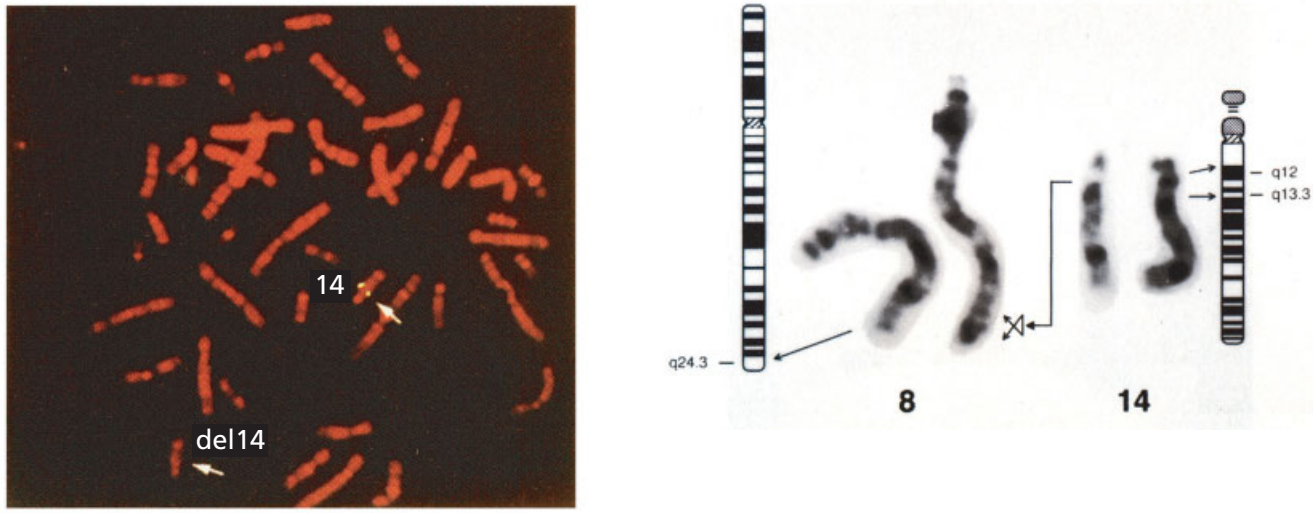

Plate 1 *Left:* FISH analysis on a metaphase spread documenting a heterozygous chromosomal deletion of chromosome 14 containing the transcription factor TTF1 (NKX2.1) in a child presenting with congenital hypothyroidism and respiratory failure. A signal with the specific probe was only detected on one chromosome 14 (yellow), but not on the second one (del14). *Right:* Karyotype analysis of the mother of the affected child revealed a balanced, inverted translocation of a fragment of chromosome 14 into the distal arm of chromosome 8 [46XX, inv ins (8;14)]. The affected child has inherited the maternal chromosome 14 harboring the deletion, together with a normal paternal chromosome 14. (Modified with permission from Elsevier: from Iwatani N *et al*. *J Pediatr* 2000; 137: 272–6.)

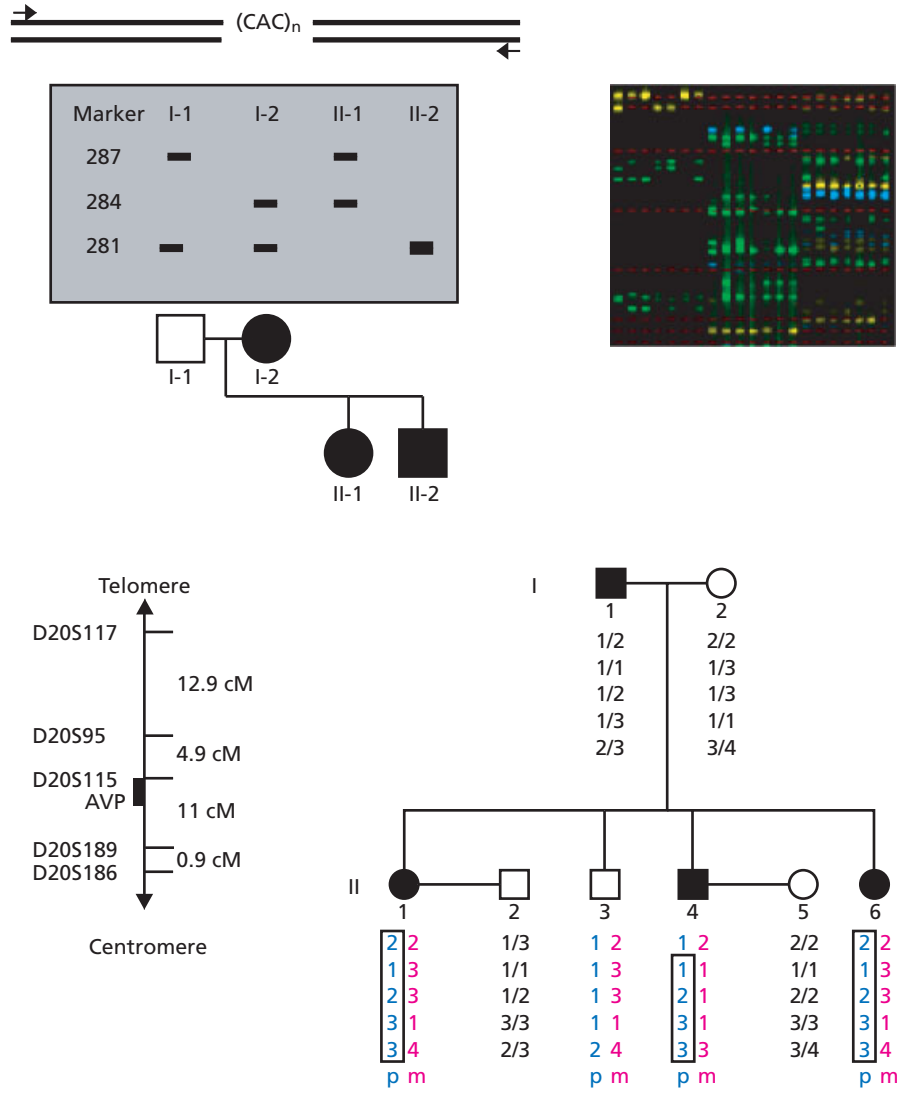

Plate 2 Analysis of polymorphic microsatellite markers and linkage analysis. *Top:* The example depicts a CAC trinucleotide repeat with three alleles in a nuclear family. PCR with primers flanking the polymorphic region results in products of variable length, depending on the number of CAC repeats. After characterization of the alleles in the parents, transmission of the paternal and maternal alleles can be determined in the offspring. The gel on the right shows the concomitant analysis of multiple microsatellites. The PCR products reflecting the different alleles can be distinguished by differences in length and fluorescent labels. The red marker included in every lane is a size standard. *Bottom:* Determination of polymorphic microsatellite markers flanking the AVP gene located on chromosome 20p13 in a family with autosomal-dominant neurohypophyseal diabetes insipidus. The parental origin of the alleles can be determined in generation II (p, paternally inherited; m, maternally inherited). The three affected individuals, II-1, II-4, II-6, share the same alleles for the markers D20S95/115/189/186. In individual II-4, a recombination has occurred between markers D20S117 and 95 of the paternal chromosome. The unaffected individual II-3 has inherited the alternate paternal alleles. Although individual II-5 is homozygous for the alleles segregating with the phenotype in this family, she is not related to I-1 and does not have diabetes insipidus. The haplotype of these markers is only associated with the phenotype in the original family, but not in the general population.

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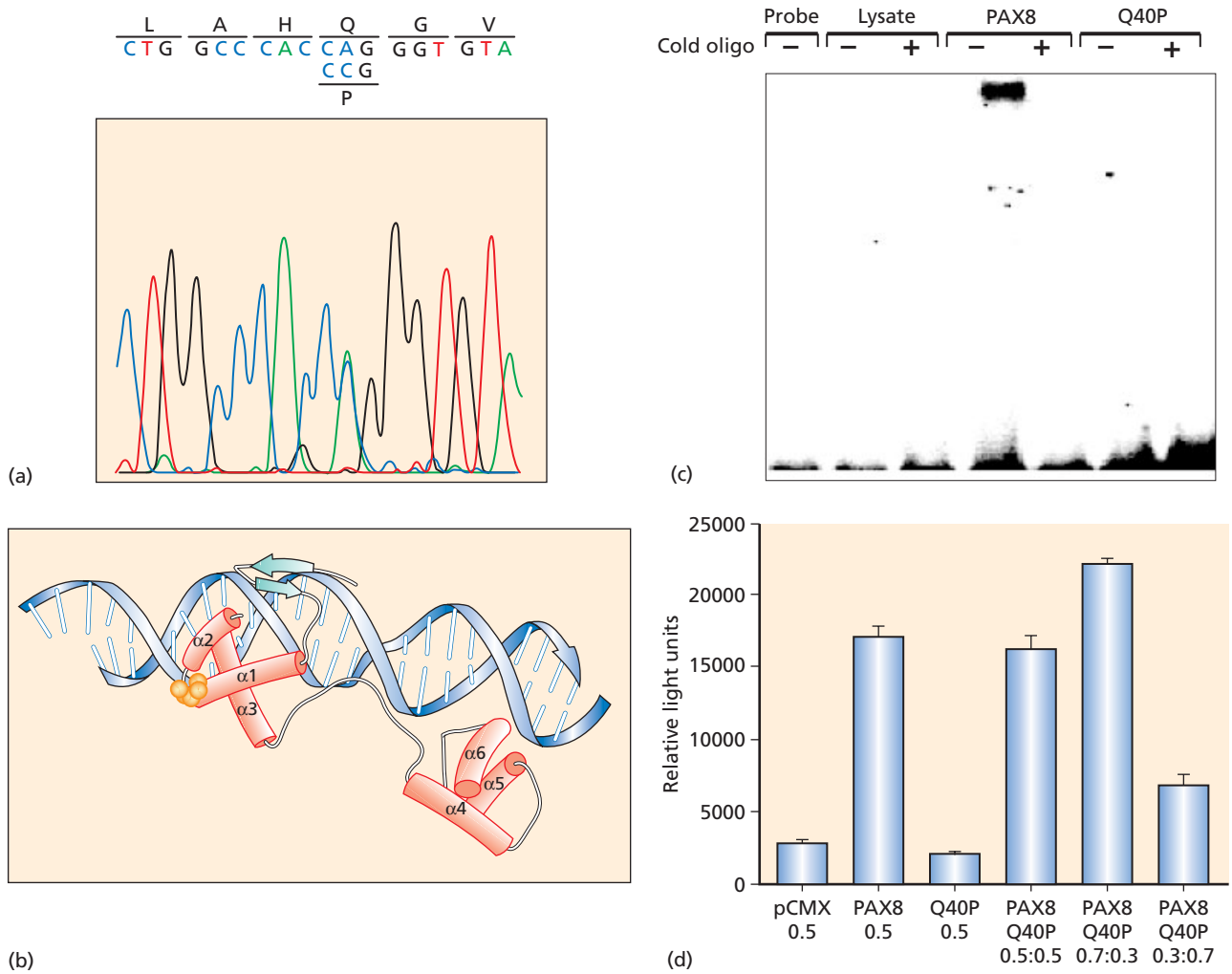


Plate 3 Functional analysis of a mutation in the transcription factor PAX8 found in a child with thyroid hypoplasia and congenital hypothyroidism. (a and b) The point mutation 119A>C leads to a substitution of glutamine 40 by proline (Q40P) in the DNA-binding domain of the transcription factor. (c) Gel shift experiment. A radiolabeled DNA response element migrates very fast through the gel in the absence of a protein–DNA interaction (lanes 1–3). The wild-type PAX8 protein binds to the response element, and this leads to an electromobility shift (lane 4). Cold oligo in excess can compete for the labeled oligo documenting that the interaction is specific (lane 5). The mutated protein is unable to bind to this response element (lane 6). (d) Plasmid vectors encoding wild-type or mutated PAX8 were transfected into embryonic kidney cells together with a luciferase reporter gene. The reporter gene consists of a plasmid containing a PAX8 response element upstream of the coding sequence for luciferase. The transcriptional stimulation of the luciferase gene can be determined by measuring the light emission of cell lysates incubated with the substrate luciferin. The example shows that the wild-type protein stimulates transcription of the luciferase reporter gene (pCMX, control vector). In contrast, there is no significant induction by the mutant. Co-transfection of wild-type and mutant plasmids in different ratios shows that the mutant does not have a dominant-negative effect. (Modified with permission from Congdon T *et al*. *J Clin Endocrinol Metab* 2001; 86: 3962–7, copyright 2001, The Endocrine Society.)

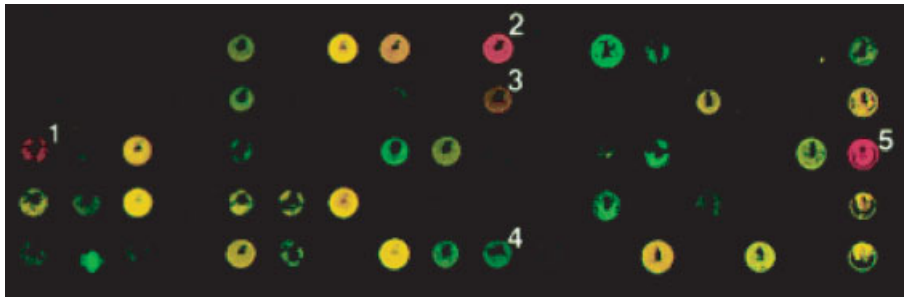


Plate 4 A microarray was hybridized to total RNA from hypothyroid mouse liver (*green* fluorochrome) and hyperthyroid mouse liver after 6 h treatment with thyroid hormone (*red* fluorochrome). The two cDNA probes were mixed and then simultaneously hybridized to the microarray. mRNAs that were more abundant in hypothyroid mouse liver (suppressed by thyroid hormone) were detected as *green* spots, whereas mRNAs that were more abundant in hyperthyroid mouse liver (stimulated by thyroid hormone) were detected as *red* spots. *Yellow* spots represented genes with expression that did not vary substantially between the two samples. The *numbers* indicate spots representing the following genes: 1, Bcl-3; 2, carbonyl reductase; 3, B61; 4, membrane-type matrix metalloproteinase; 5, spot 14. (Modified with permission from Feng X *et al*. *Mol Endocrinol* 2000; 14: 947–55, copyright 2000, The Endocrine Society.)

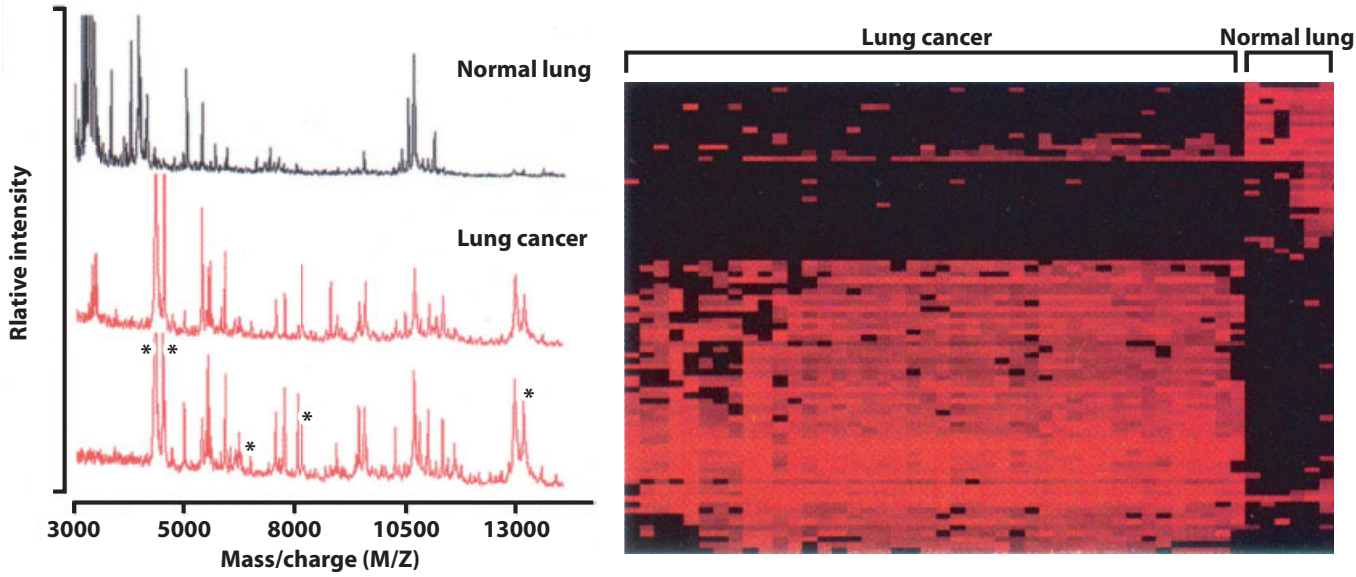


Plate 5 Proteomic fingerprint from normal lung and lung cancer tissue. *Left:* MALDI-TOF MS (matrix-assisted laser desorption ionizaton–time-of-flight mass spectrometry) spectra obtained from tumor and normal lung tissue samples. *Right:* Hierarchical cluster analysis of 42 lung tumors and eight normal lung tissues. Each row represents an individual proteomic signal, and each column represents an individual sample. (Modified with permission from Elsevier: from Yanagisawa K *et al*. *Lancet* 2003; 362: 433–9.)

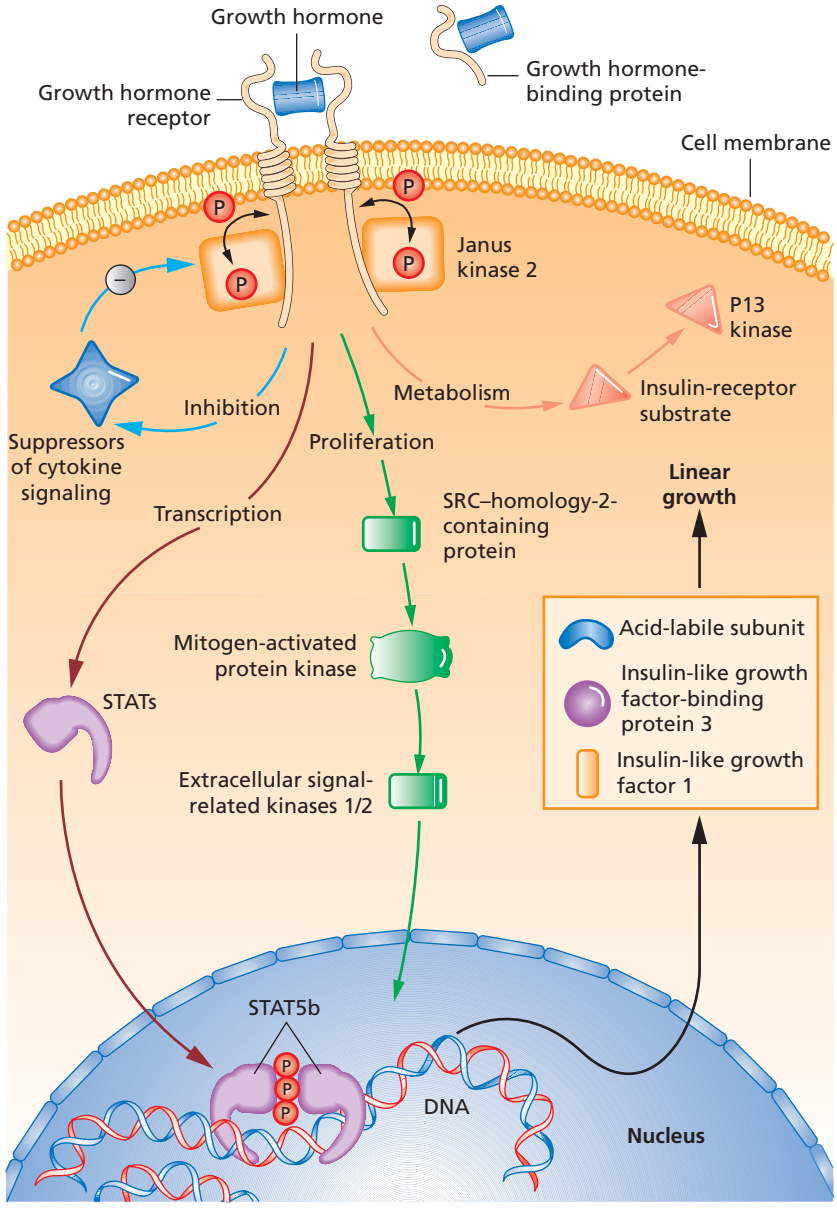
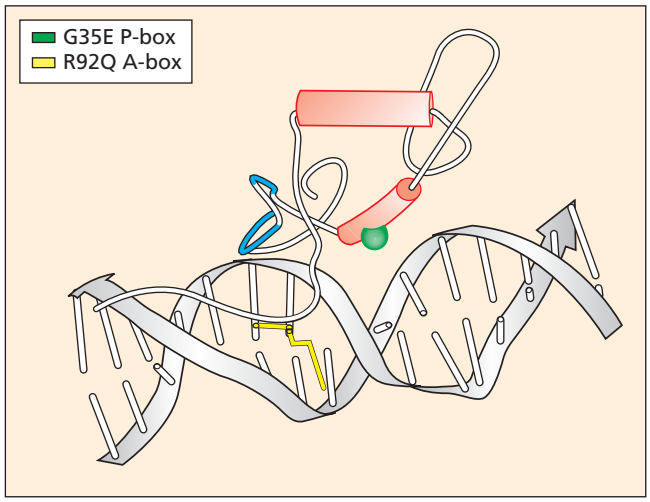
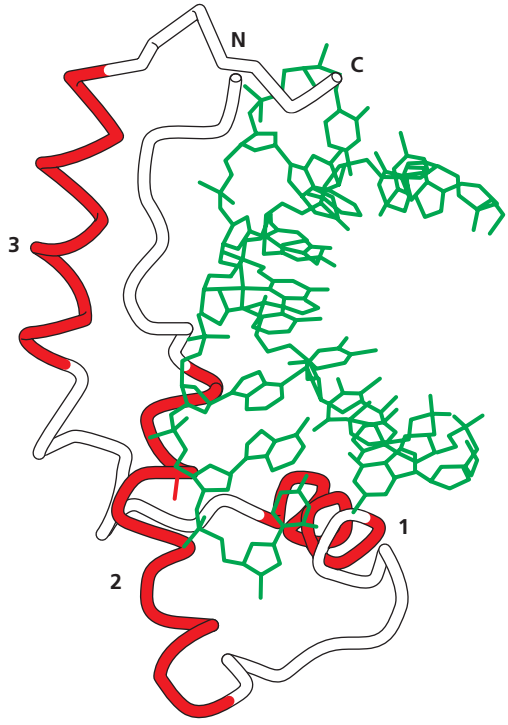


Plate 6 Growth hormone-activated intracellular signaling. Phosphorylation of the growth hormone receptor is followed by the activation of metabolic, proliferative, and transcriptional pathways. STAT5b stimulates the transcription of factors (shown in the box) that are critical for normal linear growth. P, phosphorylation; STAT, signal transducer and activator of transcription (from Eugster EA, Pescovitz OH. *N Engl J Med* 2003; 349: 1110–12.)



(a)



(b)

Plate 7 Models of key transcription factors bound to DNA. (a) The nuclear receptor steroidogenic factor 1 (SF1) regulates an array of genes involved in gonadal and reproductive development and binds as a monomer to extended DNA response elements (PyCA AGGTCA) in the promoters of target genes. A heterozygous mutation (G35E, shown in green) in the primary DNA-binding region ("P-box") of SF1 is associated with a severe clinical phenotype, whereas a homozygous change (R92Q, shown in yellow) in a secondary DNA-binding structure ("A-box") is necessary for expression of clinical features. (b) SRY binds to an AACAAT/A DNA response element and may influence transcription by inducing a structural "bend" in target DNA sequences. These conformational changes affect chromatin remodeling and influence target gene expression. Reproduced with permission from [17] and [10]. Copyright 2002, 2003, The Endocrine Society.

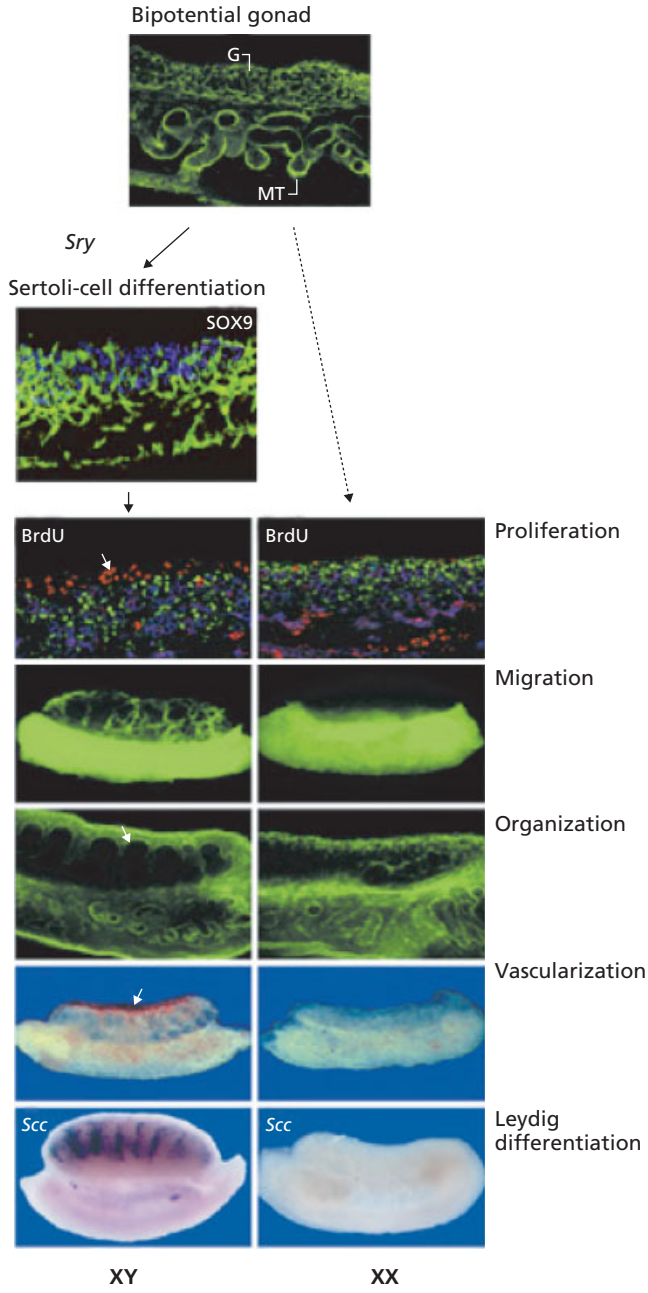


Plate 8 Morphological changes in gonadal development in male (left) and female (right) mice. No morphological differences between XY and XX gonads are seen during the bipotential gonad stage [10.5–11.5 days post coitum (dpc)]. In XY gonads, Sry upregulates nuclear Sox9 (blue) in pre-Sertoli cells and initiates Sertoli cell differentiation by 11.5 dpc (vasculature and germ cells are labeled with platelet endothelial cell adhesion molecule and appear green). Between 11.5 and 12.5 dpc, distinct changes occur in the XY gonad, which are not seen in the XX gonad. These changes include: proliferation of celomic epithelial cells; migration of cells from the mesonephros; structural organization of testis cords; male-specific vascularization; and Leydig cell differentiation. Modified with permission from [26]. Copyright 2004, Nature Publishing Group.

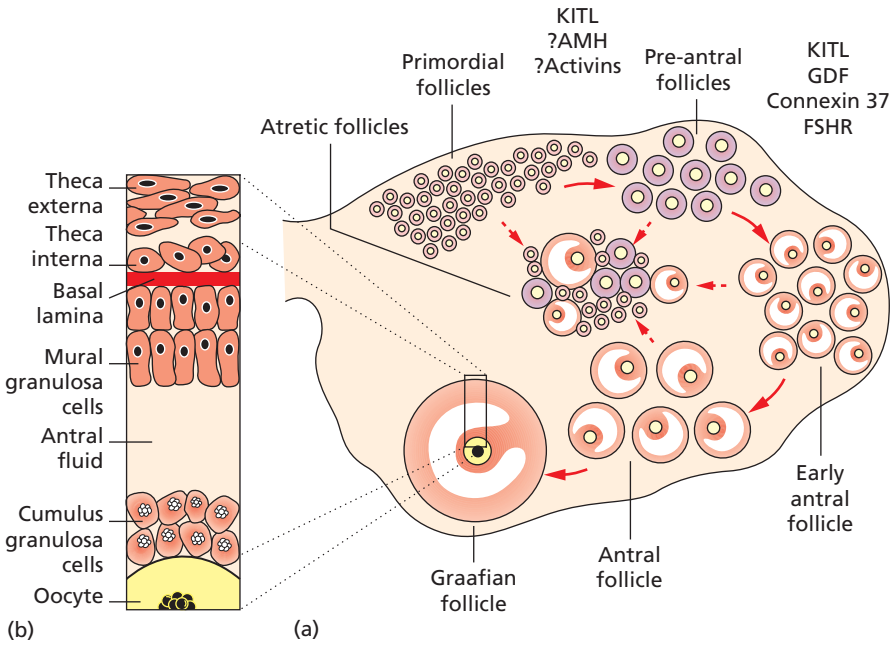
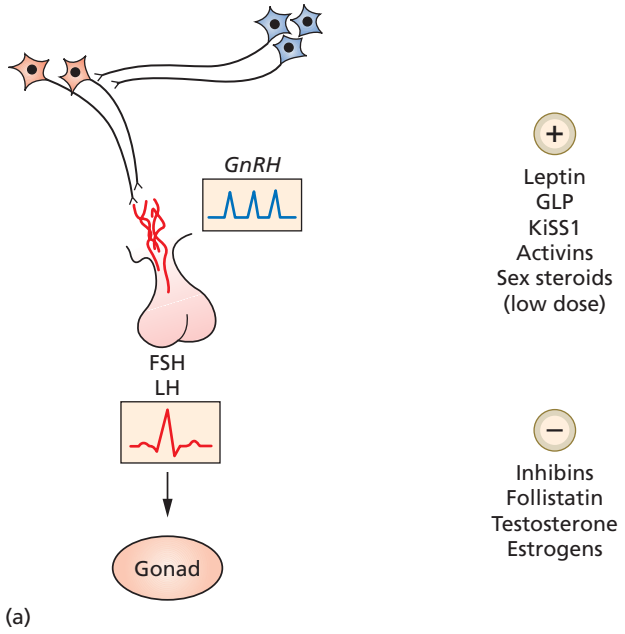


Plate 9 Folliculogenesis in the ovary. (a) Primordial follicles can develop following meiotic division of oogonia, but most primordial germ cells are destined for apoptosis. Different stages of follicular development require the presence of specific factors such as GDF9 and the FSH receptor. (b) Although Graafian follicles containing theca and granulosa cells (inset) can develop in the third trimester, co-ordinated ovulation does not become established until the time of puberty. Modified with permission from [39]. Copyright 2003, Chapterhouse Codex.



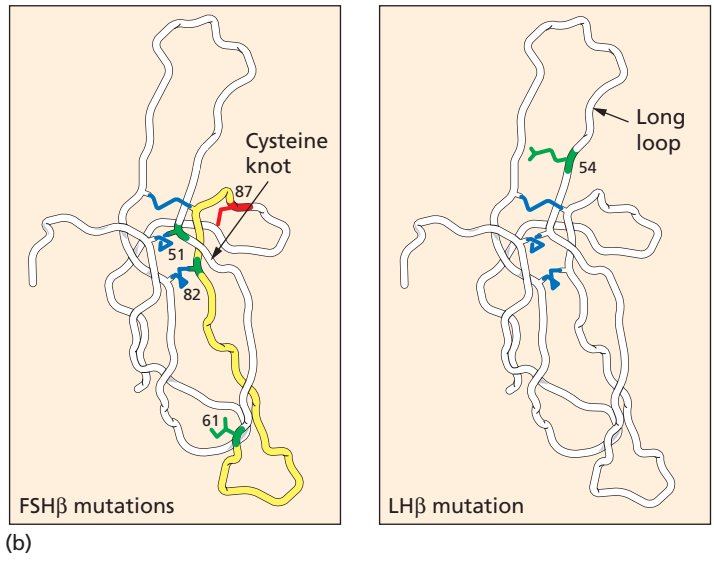


Plate 10 (a) Overview of the hypothalamic–pituitary (gonadotrope)–gonad axis. Several factors can stimulate or inhibit gonadotropin release at the level of the hypothalamus or pituitary (GLP, galanin-like peptide). (b) Locations of reported mutations in the FSH and LH β-subunits mapped on to the crystal structure of human chorionic gonadotropin (hCGβ). Point or deletion mutations in FSHβ (left) interfere with the "cysteine knot" motif and impair dimer stability. In contrast, the single point mutation in LHβ (right) affects the long loop, a region implicated in receptor binding (reproduced with permission from [55] and [56]). Copyright 2002, The Endocrine Society.

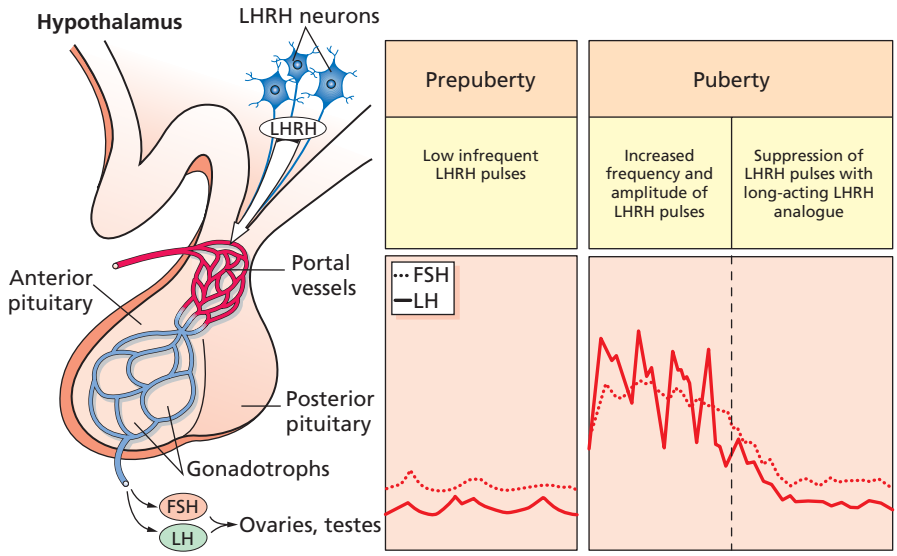


Plate 11 Anatomical arrangement of the gonadotropin system and the secretory pattern of luteinizing and follicle-stimulating hormones during puberty and prepuberty.

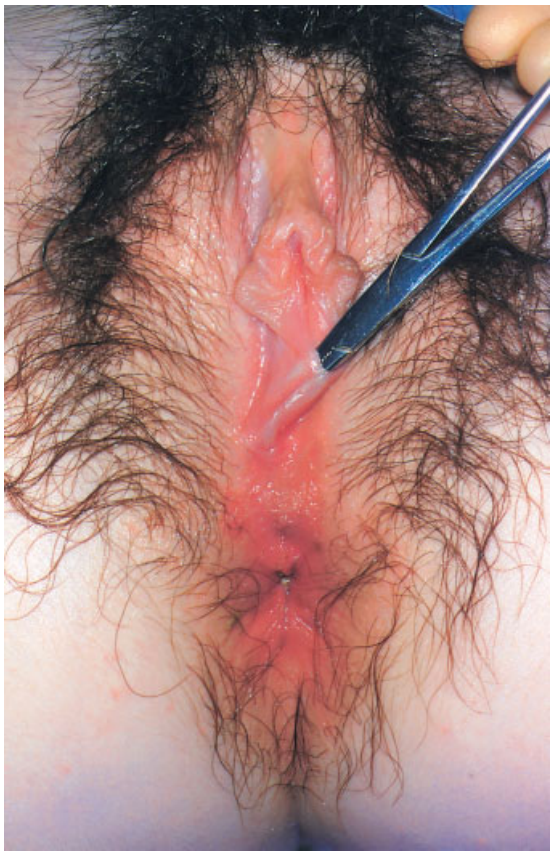


Plate 12 Labial adhesions in a pubertal girl with two previous surgical separations.

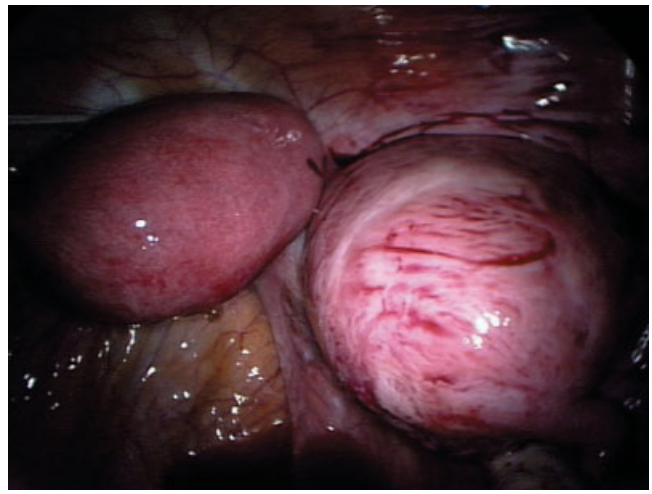


Plate 13 Laparoscopic picture of an obstructed uterine horn.

Plate 14 Amielle vaginal dilators.

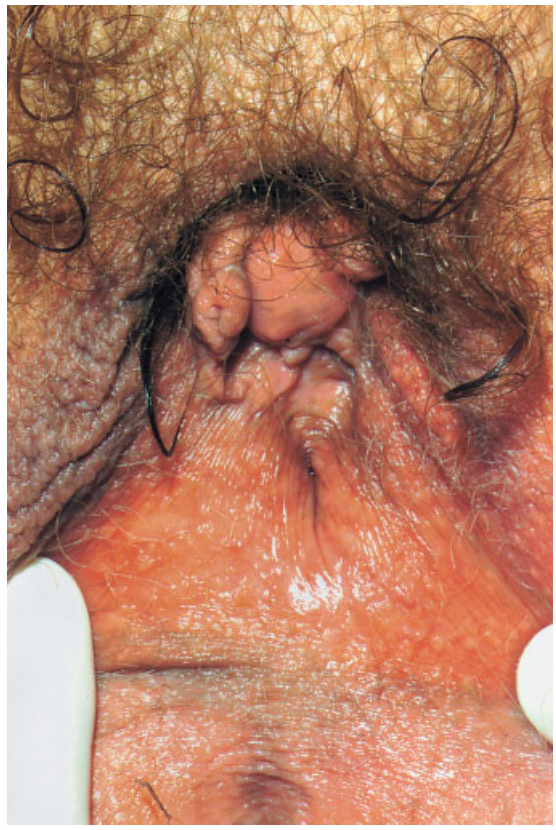


Plate 15 Vaginal stenosis in a teenage girl with congenital adrenal hyperplasia following childhood feminizing genitoplasty.