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Contents

Preface

The aim of this volume is to present the latest global knowledge of the thyroid in children and adolescents. The book consists of 16 chapters starting with the ontogenesis and anatomy of the hypothalamic-pituitary-thyroid axis and ending with the thyroid and trace elements which affect thyroid function in this age group. Special emphasis has been placed on including novel information regarding specific topics of thyroid function.

Distinguished experts in the fields of pediatric endocrinology, thyroidology and molecular endocrinology review the present knowledge and advances in pediatric thyroidology. Topics ranging from thyroid disease during pregnancy to iodine deficiency and excess in childhood, thyroid autoimmunity in pediatric age, hypothyroidism and hyperthyroidism in pediatric age, thyroid eye disease in childhood, thyroid cancer in pediatric age, and many others are included.

We believe that this book will become one of the main reference sources for pediatricians and endocrinologists and provide the reader with further insights into the pathophysiology, clinical presentation and treatment of thyroid disease.

We wish to thank the whole team at Karger publishers as well as our colleagues and the many authors who did the hard work and from whom we have learned a lot. They all approached their assignments with tremendous enthusiasm, met their short deadlines extremely well, and dealt with suggestions and comments with promptness and restraint. We thank them cordially for their efforts. We hope the reader is equally enthusiastic.

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Ontogenesis and Anatomy of the Hypothalamic-Pituitary-Thyroid Axis

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Historical Note

Aristotle originally stated that the brain was necessary for the maintenance of body integrity by regulating food intake and behavior in relation to body temperature. According to Aristotle, the pituitary was the organ through which one of the four essential humors of the body, the phlegm or pituita, passed from the brain into the body.

Five hundred years later Galen of Pergamon described the anatomy of the third ventricle region, the location of the pituitary gland inside the sella turcica embodied in a vascular network, the rete mirabilis, and observed nerves adjoining the 'soft flesh' in the neck, i.e. the thyroid gland [1]. He first proposed that the energy of the body (the vital spirit) was carried through the arteries at the level of the rete mirabilis, where it was transformed into nerve impulse (the animal spirit), eventually transferred by the nerves to the periphery of the body, 'glands' included, raising implicitly the possibility for a nervous influence over the thyroid activity. The Galenic model remained virtually unaltered up to the beginning of the 14th century, when the anatomist Mondino de' Liuzzi suggested that the thyroid gland interacted with the heat of the blood present in the internal carotid arteries due to their anatomical relation with the thyroid. He proposed that the third ventricle serves as an 'integrator' of body functions [1].

In the 19th century, Rathke studied the development of the pituitary (hypophysis) and showed that it consisted of two parts, the anterior pituitary (or adenohypophysis) and the posterior pituitary (or neurohypophysis). The importance of the hypothalamic-pituitary region influenced the work of some of the most famous Renaissance artists including Leonardo da Vinci, and Michelangelo Buonarroti. Luigi Galvani described that the peripheral nerves

were carrying electrical impulses supporting the Galenic idea that autonomic fibers might influence the secretion of 'humors' from peripheral glands such as thyroid gland. The thyroid gland had not been identified until the Renaissance. The regulation of energy body stores and temperature by the hypothalamic-pituitary-thyroid axis was suggested again by Claude Bernard in the late 1800s. The current term 'hypothalamus', however, was not actually introduced until 1893 by the Swiss anatomist, Wilhelm His. From these observations, Harris developed the concept of the control of the adenohypophysis by humoral factors produced in the hypothalamus. This led to the award of the Nobel Prize to Schally and Guillemin, who independently isolated the structures of some of these so-called 'Releasing hormones'.

The Hypothalamic-Pituitary-Thyroid Axis

Thyroid hormones play an important role in normal growth and development of the maturing human. In the adult, thyroid hormones maintain metabolic homeostasis by regulating oxygen consumption, body weight and intermediate metabolism. Thyroid function is under hypothalamic-pituitary control. Thus, thyroid hormones are produced by the thyroid gland in response to stimulation by thyroid-stimulating hormone (TSH) produced by the anterior pituitary. TSH, in turn is regulated by the hypothalamic peptide, thyrotropin-releasing hormone (TRH). The function of the entire complex is modified by the availability of the thyroid hormones in a typical negative feedback manner leading to the concept of a functional unit, the hypothalamic-pituitary-thyroid (HPT) axis.

This chapter focuses on the ontogenesis and functional anatomy of the hypothalamic-pituitary system first, and the thyroid gland itself. Emphasis is placed on the molecular aspects regarding the morphogenesis of the functionally linked endocrine glands constituting the HPT axis.

The Hypothalamic-Pituitary System

Ontogenesis

The components and anatomical organization of the hypothalamus and pituitary are intimately coupled reflecting their close functional relationship [2]. The hypothalamic-pituitary system is derived from two separate ectodermal components. The first is Rathke's pouch, a dorsal outgrowth of the buccal cavity that detaches itself and develops into the anterior pituitary. The second component, the infundibulum, develops as a downgrowth from the ventral diencephalon forming the floor of the third ventricle and developing into the

pituitary stalk and the posterior lobe of the pituitary gland. The remainder of this ventral neuroectoderm forms the median eminence, while the hypothalamic nuclei differentiate in its lateral walls to form the sides of the third ventricle. The hypothalamus emerges from the ventral diencephalon, during the 6th week of gestation in humans and between embryonic (E) days E-11 and E-18 in rats. The anterior pituitary appears at about day E-8.5 and detaches from the ectoderm at E-12.5. Thus, the pituitary gland originates from two embryonic tissues. The anterior lobe (adenohypophysis) is derived from the oral ectoderm and the posterior lobe (neurohypophysis) from the neural ectoderm. However, during this process there is a direct association between the neuroectoderm of the diencephalon and Rathke's pouch. The close apposition of these tissues suggests that cell to cell contact and tissue interactions may be important for their determination and differentiation. Indeed, recent studies indicated that several genes expressed in the ventral diencephalon are involved in the development of Rathke's pouch, providing evidence that the infundibulum has a critical role in pituitary organogenesis [3]. During pituitary organogenesis, signaling molecules and transcription factors are expressed in overlapping but distinct spatial and temporal patterns controlling pituitary development as well as cell determination and specification. As mentioned earlier, the primordium of the anterior pituitary, Rathke's pouch can be identified by the third week of gestation in humans. It forms an upward invagination of the oral ectoderm that comes in contact with the neuroectoderm of the primordium of the ventral hypothalamus. Eventually, cell proliferation within Rathke's pouch and cell differentiation results in the formation of the anterior pituitary lobe that becomes populated by highly differentiated cell types. Ultimately, transcription factors are also involved in the cell-specific expression of the gene products of these cells, the pituitary hormones.

Functional Anatomy

Hypothalamic Nuclei

During development of the hypothalamus, neurosecretory cells are organized into several nuclei, including the paraventricular, supraoptic and arcuate nuclei. Functionally, two different neurosecretory systems are organized in the hypothalamus. One is composed of the supraoptic and paraventricular nuclei formed of magnocellular neurons, whose axons migrate into the posterior lobe of the pituitary gland. The other group, referred to collectively as the hypothalamic-hypophysiotropic nuclei, is formed of parvocellular neurons and synthesizes the hypophysiotropic neuropeptides. The neurons of this region terminate in the median eminence, in close proximity to the capillaries of its primary plexus and release neurosecretory peptides into the hypothalamic-pituitary portal venous system.

TRH-secreting neurons release TRH, a tripeptide which is synthesized as a pre-pro TRH in the hypothalamus. The gene for TRH in humans is on chromosome 3. The TRH neuron bodies are densely innervated by catecholamine and NPY-containing axons, which also regulate the secretion of the pre-pro TRH molecule. Somatostatin-containing axons regulate in a negative manner the secretion of TRH. Regulation of the synthesis and processing of pre-pro TRH appears to be tightly controlled. Apart from its known actions on TSH and PRL secretion, TRH influences cell division and differentiation in the pituitary and may also be critical in development. In fetal rat pituitary cells in vitro, TRH has been shown to influence the differentiation of thyrotrophs, gonadotrophs and lactotrophs [4]. In vivo, TRH has a mitogenic effect on thyrotrophs and somatotrophs.

After its secretion, TRH binds to a specific G-protein coupled receptor in the plasma membrane of the thyrotrophs to induce the synthesis and release of TSH, and in this way the production of thyroid hormones. TRH may also induce the release of prolactin from lactotroph cells in the anterior pituitary.

Anterior Pituitary

In the adult, the pituitary gland lies in a bony cavity, the sella turcica or pituitary fossa, in the sphenoid bone. The human adult pituitary gland weighs about 0.5 g, but this can double during puberty or pregnancy. The anterior pituitary accounts for about three quarters of its weight. The pituitary is connected to the hypothalamus by the pituitary stalk which carries axons for the posterior lobe as well as blood vessels for the anterior lobe. Blood flows from the primary capillary plexus in the median eminence down the portal veins to the sinusoidal vessels in the anterior pituitary.

The secretory cells of the anterior pituitary are arranged in cords separated by the sinusoidal capillaries arising from the hypophyseal portal vessels. Using light-microscopy techniques, the cells of the anterior pituitary are classified as chromophobes (poorly stained) and chromophils (well stained), which are further subdivided into those that stain with acid dyes (acidophilic) and those that stain with basic dyes (basophilic). Using immunocytochemical stains for particular hormones, acidophilics can be divided into two subgroups, the somatotrophs, which secrete GH and the lactotrophs which produce prolactin. The basophilics can be divided into three populations of cells, the gonadotrophs producing LH and FSH, the corticotrophs, producing ACTH and the thyrotrophs producing TSH.

TSH is a 118 amino acid glycoprotein composed of two noncovalently bound α and β subunits. The gene for the α subunit is in chromosome 6 and the gene for the β subunit in chromosome 1 [5]. The α subunit is common among glycoprotein hormones TSH, LH, FSH and hCG, whereas the β subunit is

specific for the TSH molecule being responsible for its biologic and immunologic specificity.

Pituitary Organogenesis – Molecular Aspects

Morphogenic Signals Involved in Early Pituitary Development

The development of the anterior pituitary depends on the competency of the oral ectoderm to respond to inducing signals from the neural epithelium, the ventral diencephalon. One of the early extrinsic signals required for the initial commitment of cells of the oral ectoderm to form the pituitary gland is the bone morphogenic protein (Bmp-4) signal from the ventral diencephalon. Members of the fibroblast growth factor (Fgf) family (Fgf-8 and Fgf-10) and Wnt-5α are also expressed in the ventral diencephalon in distinct overlapping patterns with Bmp-4 to control pituitary proliferation and positional determination of pituitary cell lineage [6].

Fgf signaling plays an instructive role by inducing the gene encoding the LIM homeodomain transcription factor Lhx3/P-Lim which is required for progression of pituitary development beyond the initial invagination of Rathke's pouch [7]. Bmp-4 is also required for continued organ development after pouch formation. These extrinsic ventral diencheplalic signals are required for initial organ commitment, proliferation and progression. Subsequent patterning of Rathke's pouch is determined by intrinsic and ventral mesenchymal signals, including Bmp-2 and Wnt-4 expressed in the developing gland. These, together with sonic-hedgehog (Shh) establish the positional identity and stimulate proliferation of specific ventral cell types.

The ventral → dorsal Bmp-2 signals and the dorsal → ventral Fgf-8 signals appear to create opposing activity gradients that dictate the expression of specific transcription factors underlying cell lineage specification. Thus, the Fgf-8 gradient determines the dorsal cell phenotypes and dorsally expressed transcription factors, whereas Bmp-2 controls the expression of different, ventrally expressed pituitary transcription factors required for terminal differentiation of ventral cell types [8]. However, for progression of terminal differentiation of pituitary cell types, attenuation of Bmp signaling is also required.

Transcription Factors Controlling Early Pituitary Development and Pituitary Cell Type Determination

The transient signaling gradients result in the induction of expression of transcription factors in spatially overlapping patterns, which are thought to be cell-autonomous determinants of pituitary cell fate. These factors may act as molecular memory of prior signals in the positional determination of specific cell types. They include members of the LIM homeodomain family of transcription

factors expressed in Rathke's pouch such as Lhx-3, Lhx-4 and Isl-1. These factors appear to control the earliest phases of pituitary development [9].

Two pituitary homeobox (Pitx) genes are expressed throughout the pituitary, with distinct overlapping patterns of expression. Pitx-1 interacts with the pituitary-specific POU domain protein Pit-1 and is expressed in the early stages of pituitary organogenesis in the oral ectoderm. Targeted disruption of Pitx-1 leads to decreased expression of terminal differentiation markers of gonadotrophs and thyrotrophs [10]. The Pitx-2 gene appears to collaborate with Lhx-3 to regulate the same pituitary specific genes. Both factors act synergistically to activate the expression of the α subunit gene. Thus, the induction of Lhx-3 expression in response to infundibular Fgf signals is a critical step in the selection of oral ectoderm for development into the pituitary gland and it acts synergistically with Pitx-2 to direct the expression of pituitary-specific genes.

The paired homeodomain factor Prop-1 (prophet of Pit-1) and Rpx (Rathke's pouch homeobox) expressed in an overlapping spatial and temporal pattern are required for Rathke's pouch cell types to produce the anterior lobe of the pituitary. The expression of Prop-1 is coincident with the closure of Rathke's pouch and it is down regulated at the time of terminal differentiation of the pituitary specific cells. Prop-1 appears to be important for the expression of all pituitary cell lineages. Mutations in the Prop-1 gene can be the cause of combined pituitary hormone deficiency in humans [11].

The expression of Rpx is restricted to the oral ectoderm and Rathke's pouch and down-regulation of this gene is required for the progression of pituitary development and the appearance of terminal differentiation markers for anterior pituitary cell types. The Rpx gene can dimerise with Prop-1 to inhibit Prop-1 activity, suggesting that Rpx acts to antagonize Prop-1 function [12].

An additional paired-domain factor important in the early development of Rathke's pouch is Pax-6. This gene is transiently expressed in the dorsal part of the pouch and is down-regulated when cell-type differentiation starts. In the absence of Pax-6, the ventral lineages, particularly thyrotrophs become dorsally extended at the expense of somatotroph and lactotroph cell-types and *Pax-6* null mice are GH and prolactin deficient. Thus, Pax-6 is required for delineating the dorsal/ventral boundaries between the thyrotroph/gonadotroph and the somatotroph/lactotroph progenitor regions of the pituitary gland.

Transcription Factors Controlling Terminal Differentiation of Specific Cell Types

Anterior pituitary cell types are initially positionally determined as they emerge from proliferation zones, with the somatotroph cells arising caudomedially, gonadotrophs more ventrally and corticotrophs ventrally. For each cell type to progress beyond initial patterns, by transient signaling gradient, induction of

additional specific transcription factors is required. These transcription factors include Pit-1 (somatotrophs, lactotrophs, thyrotrophs); the orphan receptor SF-1 and Egr-1 (gonadotrophs); and T-pit and possibly STAT-3 (corticotrophs).

Pit-1, a member of the family of POU domain-containing transcription factors was originally identified through analysis of the nuclear proteins regulating the transcription of GH and prolactin. Later, Pit-1 was found to be required for generation and cell- type specification of three pituitary cell- lineages: somatotrophs, lactotrophs and thyrotrophs [13]. Pit-1 binds to the promoter region of the genes for GH, prolactin, the β subunit of TSH, the receptor of GHRH, the type-1 somatostatin receptor 1 and the TRH receptor, interacting with other transcription factors to form functionally active heterodimers [14]. Pit-1 also interacts with members of the nuclear-receptor family including thyroid hormone receptors (TRs) and retinoid acid receptors (RARs).

Finally, the interaction between Pit-1 and the zinc finger protein GATA-2 is a critical determinant of the development of both thyrotrophs and gonadotrophs. In the thyrotrophs, this interaction leads to synergistic activation of thyrotroph-specific genes such as the genes for the β subunit of TSH. In the absence of Pit-1, GATA-2 expression appears sufficient to induce the entire set of transcription factors that are required for gonadotroph cell type specification. Conversely, the absence of GATA-2 dorsally is critical for differentiation of Pit-1-positive cells to somatotroph/lactotroph phenotypes [15].

In conclusion, coordination between signal molecules and transcription factors is necessary for the early pattering, proliferation and specification of pituitary cell types including the thyrotrophs.

The Thyroid Gland

Ontogenesis

In the human embryo, the thyroid gland is the first endocrine gland to develop. The thyroid gland consists of two distinct cell types, the thyroid follicular cells (TFCs) and the parafollicullar or C cells responsible for the dual endocrine function of the gland, the production of thyroid hormones and calcitonin, respectively. The TFCs, the most abundant cell type form the thyroid follicles, whereas the C cells are scattered in the interfollicullar space, mostly in a parafollicular position.

The two cell types originate from two separate embryological structures: the TFCs originate from the thyroid anlagen, whereas the C cells from the ultimobranchial bodies. The thyroid anlagen is a thickening consisting of embryonic endodermal cells in the floor of the primitive pharynx. The ultimobranchial bodies are a pair of embryonic structures derived from the fourth pharyngeal

pouch and located on the sites of the developing neck. Precursors of C cells migrate from the neural crest and colonize the ultimobranchial bodies. The thyroid anlagen appears as a visible bud on embryonic days 16–17 in humans. Subsequently, the bud expands ventrally as a diverticulum, with rapid proliferation of cells but it remains attached to the pharyngeal floor by a tubular stalk the thyroglossal duct. The progenitor thyroid cells continue to proliferate distally and then laterally, leading to the formation of a bilobed structure connected by an isthmus. This caudal migration occurs from E-24 to E-32 in humans and is accompanied by elongation and eventually degeneration of the thyroglossal duct. The thyroid reaches its final position in the base of the neck at about E-40 to E-50 and, at this time, it merges with the two lateral anlagen, the ultimobranchial bodies, resulting in the incorporation of C cells in the thyroid parenchyma. In the adult thyroid, the C cells disperse either singly or in small groups in the interfollicullar space and their contribution to thyroid mass is minimal (10%). The merging of the two populations is complete at about E-50 in humans, at which time the thyroid gland exhibits the definitive external form with an isthmus connecting the two lateral lobes. The foramen cecum of the base of the tongue is a remnant of the origin of the thyroid gland in the floor of the primitive pharynx. The pyramidal lobe, a vestige of the embryonic thryoglossal tract, is a narrow projection of thyroid tissue extending upward from the isthmus and lying on the surface of the thyroid cartilage. The timing of events during human thyroid development is shown in table 1.

It appears that most of the critical events in thyroid morphogenesis take place in the first 60 days of gestation in humans. For this reason, morphogenic errors during this period result in developmental thyroid abnormalities. These may cause displacement of cells derived from the thyroid anlagen leading to abnormal thyroid migration and ectopic thyroid tissue. Mutations in thyroid transcription factors may also lead to abnormalities in thyroid development resulting in congenital hypothyroidism [16, 19, 36]. Also, the thryroglossal duct may not degenerate but persist as a fistulous tract containing some thyroid follicular cells from which thyroglossal cysts or rarely thyroid carcinoma may arise.

Functional Anatomy and Ultrastructure of the Thyroid Gland

The gross anatomy of the thyroid gland is well defined. The gland is located in the neck region just caudal to the larynx and adherent to the front of the trachea. Its name derives from the Greek word ‘thyreos’ meaning shield and was proposed by Thomas Warton in 1656. It is believed that the name was given because it describes its gross morphology resembling an ancient Greek shield or because of its topographic association with the laryngeal thyroid cartilage

Table 1. Timing of events during human thyroid development [18]

Developmental stage according to Carnegie staging (CS) and the anatomical or morphological events in thyroid development. Estimated age in parentheses.

CS10 (22 days)	thickening of the floor of the primitive pharynx between the diverging aorta
CS12 (26 days)	outgrowth and budding of the median thyroid primordium from the floor of the primitive pharynx. The inferior part of the fourth pharyngeal pouch forms the ultimobranchial body
CS13 (28 days)	the median primordium grows caudally and appears bilobed. It is connected to the primitive pharynx by the thyroglossal duct
CS14 (32 days)	migration of the median primordium, still connected to the epithelium of the primitive pharynx
CS15 (33 days)	the thyroglossal duct starts to break down
CS16 (37 days)	the median primordium consists of two lobes, an isthmus and a pedicle remnant. the continuity with the primitive pharynx is lost
CS18 (44 days)	median primordium fuses with the lateral components derived from the ultimobranchial bodies
CS19 (48 days)	the thyroid reaches its final position in front of the trachea just inferior to the cricoid cartilage; it begins to form follicles
10–12 weeks	follicles containing colloid become visible; the thyroid is able to incorporate iodine into thyroid hormones

whose shape resembles a shield. The thyroid is the largest endocrine gland in humans weighing 1–2 g after birth and 10–20 g in adulthood.

The structure of the thyroid gland is unique in that it is the only endocrine gland in which the hormone products are stored in an extracellular location [2, 20]. The functional unit of the thyroid gland is the thyroid follicle, a spherical structure of varying size that consists of an outer layer of thyroid follicular cells (TFCs) which enclose a lumen that contains thyroglobulin-rich colloid. The follicular organization and the polarity of the thyrocytes are essential for the specialized function of the organ. The follicular cells are surrounded by a basement membrane and the colloid lumen is sealed by various cell to cell junctions which are linked to the cytoskeleton. The extracellular matrix plays a role in the adhesion, proliferation, differentiation and migration of thyroid follicular cells and the molecules involved in these processes include type I and IV collagen, fibronectin, laminin and cadherin.

The TFCs have the machinery for thyroglobulin (TG) biosynthesis, transport and storage as well as iodide uptake, organification and thyroid hormonogenesis. Thus, TFCs have long profiles of rough endoplasmatic reticulum and a large Golgi apparatus in their cytoplasm for the synthesis, packaging and transport of

TG into the colloid lumen. The cytoplasm also contains lysosomal bodies, which are important in the secretion of thyroid hormones. The surface characteristics of the apical and basolateral surfaces are different according to their particular role in thyroid hormonogenesis. Thus, the apical surfaces have numerous microvilli that protrude into the follicular lumen increasing the surface area in contact with the colloid. The base of the cell abuts on a capillary and is separated from it by a two layer basement membrane. There are pores in the endothelial lining of the capillaries that may allow plasma to come in direct contact with the basement membrane. There is an extensive network of interfollicullar capillaries providing the follicular cells with an abundant blood supply. The stroma also contains nerve fibers, most of which are sympathetic and some parasympathetic.

The height of the follicular cells varies, depending on the degree of stimulation by TSH, ranging from cuboidal to tall columnar. When TSH secretion is high, the first response is the formation of numerous pseudopodes resulting in increased endocytosis of TG-rich colloid from the follicular lumen. If the TSH secretion is sustained, TFCs become more columnar and the lumen of the follicles become smaller because of the increase in endocytosis of the colloid. A sustained increase in TSH secretion, whether due to iodine deficiency or due to goitrogens results in thyroid cell hyperplasia and enlargement of the entire thyroid gland. The opposite changes occur when TSH secretion is inhibited. The thyroid cells become flat, their microvilli disappear and the follicular lumen increases due to the accumulation of colloid.

In addition to TG, a number of other proteins are involved in the synthesis and secretion of the thyroid hormones, T_4 and T_3, by the thyroid follicles. Important among these are the sodium/iodide symporter (NIS) located at the basolateral membrane of the cells, which actively transports iodide into the cells against a steep iodide concentration gradient [21]. From inside the cell, the iodide is transported through the apical membrane into the follicular lumen by anion transporter proteins, among which is pendrin. Subsequent iodide oxidation and binding to the tyrosine residues of TG, as well as the coupling of iodotyrosines to form T_3 and T_4 are catalyzed by the enzyme thyroid peroxidase (TPO) in the presence of hydrogen peroxide (H_2O_2). The latter is generated by a membrane system composed of at least two NADPH-thyroid oxidases, THOX-1 and THOX-2 localized in the apical membrane. Finally, for the production of T_4 and T_3, TG from the follicullar lumen is absorbed across the apical surface by endocytosis in the form of colloid droplets. These fuse with lysosomes, where most of the TG-thyroid hormone complex is hydrolyzed by proteolytic enzymes, freeing T_4 and T_3 molecules that are released into the bloodstream. The same process of proteolysis also releases mono- and diiodotyrosine (MIT and DIT) molecules which are deiodinated by a dehalogenase. The iodide released locally is used for a new cycle of thyroid hormonogenesis.

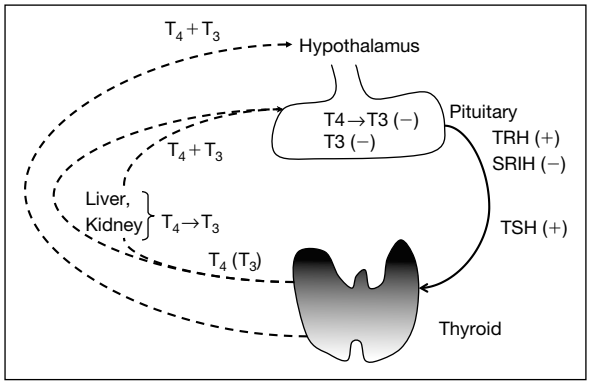

Fig. 1. Regulation of thyroid function. TRH is synthesized in the hypothalamus, reaches the thyrotrophs of in the anterior pituitary via the hypothalamic-hypophysial-portal system and stimulates TSH synthesis and release. TSH binds to its receptor in the thyroid gland, stimulating the synthesis and release of thyroxine (T_4) and triothyronine (T_3). The thyroid gland secrets predominantly T_4. The peripheral deiodination of T_4 to T_3 in the liver and kidney supply roughly 80% of the circulating T_3. Both circulating T_3 and T_4 directly inhibit TSH synthesis and release independently; T_4 via its rapid conversion to T_3 (although a direct negative effect of T_4 has been recently reported on TSH-β gene expression) and T_3 via binding to the thyrotroph nuclear T_3 receptor. Thyroid hormones also inhibit indirectly TSH synthesis via their negative effects on the synthesis of TRH. SRIH = Somatostatin.

The main regulator of all steps in thyroid hormonogenesis is TSH acting through its specific G-protein coupled receptor on the plasma membrane of thyroid cells. TSH action is largely mediated by an increase in intracellular cAMP, which not only regulates thyroid hormone synthesis and secretion but also contributes to thyroid cell differentiation and proliferation.

The genes encoding the above enzymes and proteins are expressed either specifically in thyroid cells for example, TG and TPO, or in a very limited number of tissues, such as NIS and the TSH receptor. These genes become expressed in a coordinate way during thyroid hormonogenesis and are all present in the fully differentiated thyroid cells. The regulation of thyroid function by the hypothalamic-pituitary system is depicted in figure 1.

Molecular Aspects of Thyroid Development – Thyroid-Specific Transcription Factors

The development of the embryonic thyroid gland and its normal migration is dependent on the interplay between several transcription factors. The transcription factors *Titf-1/Nkx2-1*, *Foxe1*, *Pax8* and *Hhex* are expressed simultaneously in the cells of the primitive pharynx that will become TFCs. The

Table 2. Chromosomal localization of genes expressed during thyroid development and molecular features of the corresponding product

Gene	Chromosome		Features of the gene product
	mouse	human	
Titf-1/Nkx2-1	12 C1–C3	14q13	homeodomain transcription factor
Pax-8	2	2q12–14	paired domain transcription factor
Foxe1	4	9q22	forkhead domain transcription factor
Hhex	19	10	homeodomain transcription factor
Tshr	12	14q31	G-protein coupled receptor
Fgfr2	7	10q26	tyrosine kinase receptor
Nkx2-5	17	5q34	homeodomain transcription factor
NIS	8	19p13	NIS; membrane protein with 13 putative transmembrane domains

formation of the thyroid diverticulum and the beginning of its migration is accompanied by the exclusive expression of these factors in thyroid primordium. In mice, the expression of Ttf-1, Ttf-2, and Pax-8 begins at the onset of thyroid migration on day 9.5 of gestation and these factors continue to be expressed throughout embryonic development [22–24]. The onset of thyroid differentiation is heralded by the expression of *Tshr*, *TPO*, and *TG*. For the rest of its life, a thyroid cell will be hallmarked by the simultaneous presence of Titf-1/Nkx2-1, Foxe1, Pax-8, and Hhex. The chromosomal localization of the genes of these transcription factors are presented in table 2 and their gene expression patterns in mice and humans in table 3. A summary of the different phases of thyroid development and the expression of relevant genes is presented in table 4. The specific role of these transcription factors in thyroid development have been confirmed by the generation of mouse knock-outs.

Genes Involved at Early Stages of the Morphogenesis

(a) Titf-1/Nkx2-1. The transcription factor Titf/Nkx2-1, responsible for thyroid specific expression of TG and TPO, is a homeobox transcription factor of the NK-2 gene family. The factor was originally called Ttf-1 (for thyroid transcription factor-1) and after reisolation as a protein binding to the enhancer of *TPO*, Ttf-1 has also been renamed T/EBP [25]. The official name for the mouse genetic locus is *Titf-1* (*Titf-1* for humans). The Titf/Nkx2-1 protein is encoded by a single gene (table 2). Human Ttf-1 is a single polypeptide 371 amino acids long and has two independent transcriptional activation domains located at the amino-terminal (N domain) and the carboxy-terminal (C domain) regions with respect to the DNA-binding homeodomain.

Table 3. Human and murine *Pax-8*, *Titf-1*, and *Foxe1* gene expression patterns

Features shared between human and mouse	Features observed in human or mouse only
Pax-8	
Thyroid	4th pharyngeal pouch in human
Brain and spinal cord	ureteric bud and derivatives in human
Otic vesicle	
Metanephric blastema and derivatives	
Titf-1	
Thyroid	4th pharyngeal pouch in mouse
Lung	
Ventral part of forebrain	
Foxe1	
Thyroid	later onset in the median thyroid primordium in human
Foregut	thymus in human

Table 4. Summary of the different phases of thyroid development, indicating the morphological features, the expression of relevant genes, and the capacity to produce thyroid hormones

Embryonic day	Morphology	Functional (terminal) differentiation		Thyroid hormones	Controller genes	
		TG, TPO, Tshr	*NIS*		*Titf-1/Nkx2-1, Fgfr-2, Foxe1, Pax-8, Hhex*	
E-8	undifferentiated endoderm	–	–	–	–	–
E-8.5	thyroid anlagen	–	–	–	+	–
E-9.5	thyroid bud	–	–	–	+	–
E-11.5–13.5	expansion of thyroid primordium	–	–	–	+	+
E-14.5–15	definitive bilobated shape	+	–	–	+	+
E-16	folliculogenesis	+	+	–	+	+
E-16.5	completion of organogenesis	+	+	+	+	+

+ = Present; – = absent.

Titf-1/Nkx2-1 Knock-Out. Heterozygous animals were initially described as having a normal euthyroid phenotype but were later found to have reduced motor coordination skills when compared with wild type mice. Kimura [26] announced in 1996 a mutant mouse lacking Ttf-1. Homozygous animals were stillborn, apparently owing to lack of a normal lung. Mutant mice may contain a rudimentary bronchial tree with severely abnormal epithelium. There is a reduction of the number of cartilage rings of the trachea probably due to the control of the expression of Bone morphogenetic protein (Bmp)-4 by Titf-1/Nkx2-1. In knock-out animals, thyroid follicular cells and C cells are completely absent. The latter feature, which is not shared by humans with thyroid dysgenesis, is consistent with the expression pattern of this factor in neuroectodermal tissue, as are the severe defects of the forebrain and hypothalamus in these animals. As concerns the pituitary gland, Titf-1/Nkx2-1 is exclusively detected in the posterior bud. The developing posterior pituitary expresses two growth factors, Bmp-4 and fibroblast growth factor (Fgf)-8, and is adjacent to Rathke's pouch, which expresses *Fgfr-2*, an Fgf receptor. In mice deprived of Ttf-1/Nkx2-1, Bmp-4 is still expressed and thus its expression is Titf-1/Nkx2-1 independent in the posterior pituitary. The *Fgf-8* expression is abolished in the posterior pituitary and the apoptosis of the anterior bud has been attributed to this fact. Later in development, no pituitary, either anterior or posterior, is present, thus showing that Titf-1/Nkx2-1 is required both for the development of the posterior bud and for controlling the expression of a signaling molecule, perhaps Fgf-8, that is essential for the survival of the anterior portion.

The appearance of Titf-1/Nkx2-1 in the thyroid anlagen coincides with the proliferation of the cells that give rise to the primitive thyroid bud. Titf-1/Nkx2-1 remains expressed in the TFC during all stages of development and in adulthood. This factor has been shown to function as a potent transcriptional activator of thyroid- and lung-specific genes [22]. In humans and rats, *Titf-1* transcripts are detected during lung development. It is known to regulate the transcription of *TG* and *TPO* genes, the *Tshr* gene in thyroid follicular cells, and the surfactant protein B (*SPB*) gene in epithelial lung cells (table 3). *Titf-1/Nkx2-1* mRNA was also identified in parafollicular C cells and in the epithelial cells of the ultimobranchial body. This transcription factor is first expressed in epithelial cells and becomes progressively restricted to distal branches. The absence of expression in main bronchial epithelial cells or in the proximal respiratory compartments of the fetal lung and its restriction to the distal part of the lung is also consistent with its role in surfactant production and regulation. Apart from lung and thyroid, this factor is expressed in the ventral forebrain. After birth and in adult organisms, Titf-1/Nkx2-1 is still present in the thyroid and lung epithelium and in the posterior pituitary, whereas its expression is reduced in the brain and is restricted to the periventricular regions and some

hypothalamic nuclei. However there is a transient increase before the first endocrine manifestations of puberty.

In conclusion, Titf-1/Nkx2-1 controls survival of thyroid cells at the beginning of organogenesis and the expression of TFC-specific genes in adult life, a role that cannot be investigated in knock-out mice because thyroid cells disappear before the onset of functional differentiation [22].

(b) Pax-8. Pax-8 (paired box gene 8) is a transcription factor, member of a family of 9 transcription factors and is also involved in the early stages of organogenesis [27]. In the endoderm Pax genes are essential for the differentiation of endocrine cells in the pancreas and follicular cells in the mature thyroid gland [27]. Pax-8 has a DNA binding domain at the amino terminal end, a carboxy terminal transcriptional activation domain, and a central homeodomain [27]. The gene encoding Pax-8 (called *Pax8* in mice and *Pax-8* in humans) is located on chromosome 2 in both species (table 2) and the *Pax-8* gene in humans consists of 11 exons. Pax-8 is expressed, as Titf/Nkx2-1, in the thyroid diverticulum and in the developing neural tube and excretory system. Like Titf-1/Nkx2-1, Pax-8 is detected in the developing thyroid from E8.5, i.e. at the time of specification. Expression of *Pax-8* is maintained in TFCs during all stages of development and in adulthood. In the mature TFC, Pax-8 regulates the expression of thyroglobulin and TPO genes [28, 29].

Pax-8 Knock-Out. Heterozygous *Pax-8*$^{+/-}$ mice show no specific phenotype. *Pax8*$^{+/-}$ mice have a higher prevalence of elevated plasma TSH than wild-type littermates, but their thyroid gland appears histologically normal. Homozygous *Pax8*$^{-/-}$ mice present with growth retardation and die within 2–3 weeks. Only the formation of the endoderm-derived follicular cells is affected (31 days) and thyroid glands are hypoplastic with absent median anlagen derivatives (i.e., follicular cells), whereas lateral anlagen derivatives (parafollicular calcitonin-producing C cells) are present [27]. The thyroid is composed completely of calcitonin producing cells. The early neonatal death of *Pax-8*$^{-/-}$ mice is due to their severe hypothyroidism (the administration of T_4 to *Pax-8*$^{-/-}$ mice allows the animals to survive), and to the retarded development of other organ systems (such as bone, spleen and intestine). The absence of Pax-8 is still compatible with very early stages of thyroid development (appearance of the thyroid diverticulum from endodermal cells of the primitive pharynx) but precludes further differentiation events to the mature TFC. The brain and kidneys, in which this transcription factor is expressed during development are normal. Furthermore, in the thyroid anlagen of *Pax-8*$^{-/-}$ mice, the expression of *Foxe1* and *Hhex* is strongly down-regulated.

The function of Pax-8 appears similar to that of Titf-1, i.e. it is not required for the initial specification of the thyroid anlagen, but is critical at later steps of development. It has been shown that Titf-1 and Pax-8 interact physically and

they might similarly cooperate to control thyroid differentiation [27] but the data are conflicting. The *Pax-8* gene has been implicated in the development and maintenance of the follicular cell phenotype by activating thyroperoxidase, sodium/iodide symporter, and thyroglobulin genes without apparent effect on C cell development. The expression of *Pax-8* gene expression observed in the thyroglossal duct cells suggests that this structure represents a cellular track left by the migrating thyroid anlagen rather than a pre-established pathway for thyroid migration, and its expression may explain the capacity of these cells to differentiate into follicular cells. During normal development, the thyroglossal duct disappears, but remnants may persist and form cysts anywhere along the course of thyroid migration. On the other hand, the expression pattern of the *Pax-8* gene in the central nervous system in human is similar to that observed in the mouse, i.e. restricted to the midbrain-hindbrain boundary, then to the myelencephalon and the spinal cord. In addition to being expressed in the condensed mesenchyme of the developing kidney, human *Pax-8* is expressed in the mesonephric duct, the ureteric bud, and the collecting ducts (but not at their tips). This is different from what has been described in the mouse.

(c) Foxe1. Foxe1 (formerly called Ttf-2 for thyroid transcription factor-2) was originally identified as a thyroid-specific nuclear protein that recognizes a DNA sequence present on both *TG* and *TPO* promoters under hormone stimulation [30]. It is a phosphoprotein that consists of an N-terminal region, a highly conserved forkhead domain, a helical polyalanine tract, and unique C terminal residues. The official name for the mouse genetic locus is *Foxe1* (*Foxe1* for the human locus). *Foxe1* is located on mouse chromosome 4 and the human gene is on chromosome 9q22 and consists of a single exon (table 2). *Foxe1* mRNA is detected at E-8.5 in all the endodermal cells of the floor of the foregut, including the thyroid anlagen. The expression of *Foxe-1* is limited posteriorly. Expression of *Foxe1* in the thyroid cell precursors is maintained during development and persists in adult TFCs.

Foxe1 (Ttf-2) Knock-Out. Heterozygous *Foxe1* knock-out mice are euthyroid, with no visible phenotype. In 50% of *Foxe1* null mice the thyroid disappears indicating that this gene, too, is implicated in the control of the survival of thyroid cells at a step different from those controlled by Titf-1/Nkx2-1 and Pax-8. Homozygous null mice have cleft palate and thyroid dysgenesis, consisting of either thyroid agenesis or an ectopic sublingual gland, which is often lethal in the neonatal period [22]. However their thyroid phenotype is complex [22]. Although they display no thyroid in its normal location and an absence of thyroid hormones, the elevated TSH levels suggests normal pituitary function. Death occurs within 48 h. In mouse embryos, Foxe1 is known to be expressed not only in the thyroid gland but also in the craniopharyngeal ectoderm involved in palate formation and in Rathke's pouch. In contrast to what is

observed in *Titf/Nkx2-1*$^{-/-}$ mice, C cells develop normally in *Foxe1*$^{-/-}$ mice. The budding of the thyroid primordium does not require Foxe1. However, at E-9.5 in *Foxe1* null embryos, thyroid precursor cells are still on the floor of the pharynx, whereas in wild-type embryos they are detached from the pharynx cavity and begin to descend. At later stages of development, in the absence of Foxe1, mutant mice exhibit either a small thyroid remnant still attached to the pharyngeal floor or no thyroid gland at all.

The role of Foxe1 in the adult gland is still a matter of study. In the adult, Foxe1 is still present in the thyroid, whereas the expression in the esophagus is faint. In ectoderm-derived structures, at an early stage of development, Foxe1 is present in the posterior stomatodeum, in the buccopharyngeal membrane, and in the cells of the roof of the oral cavity indenting to constitute Rathke's pouch, which will form the various components of the anterior pituitary. At later stages, *Foxe1* mRNA expression in the pituitary is downregulated [30], whereas it appears in the secondary palate, in the definitive choanae, and in the whiskers and hair follicles [31]. In humans, *Foxe1* mRNA is also detected in adult testis and several other tissues.

In conclusion, Foxe1 plays an essential role in promoting migration of TFC precursors, a role quite different from the previously mentioned transcription factors Tift-1/Nkx2-1 and Pax-8, which seem to be involved in the survival and/or differentiation of these cells [22].

(d) Hhex. Hhex (hematopoietically expressed homeobox) is a homeodomain-containing transcription factor that was first identified in hematopoietic cells. The genomic locus encoding Hhex is called *Hhex* in mice (located on chromosome 19) and *Hhex* in humans (located on chromosome 10q23.32) (table 2). The gene is split into four exons and codes for a protein 271 amino acids long in mice and 270 amino acids long in humans. *Hhex* mRNA is expressed in early mouse development in the primitive endoderm and at later stages in the ventral gut. From E8.5 onward it marks the primordium of several organs derived from the foregut, among which, both developing and adult thyroid express *Hhex* at the highest level. Hhex function is essential in definitive endoderm for normal development of the forebrain, liver and thyroid gland [32].

The role of Hhex in the adult thyroid gland cannot be studied in *Hhex* null mice because thyroid cells disappear at an early stage. In *Hhex*$^{-/-}$ embryos, at E-9.5 the thyroid primordium is absent or hypoplastic, still connected to the floor of the pharynx; notably, no expression of *Titf-1/Nkx2-1* and *Foxe1* mRNA is observed in the thyroid bud. A small thyroid primordium can nevertheless be identified in some *Hhex*$^{-/-}$ embryos before E-9 [33]. In the absence of Hhex, the thyroid anlagen is properly formed and expresses *Titf-1/Nkx2-1*, *Foxe1*, and *Pax-8*; at later stages, the expression of all these transcription factors is downregulated.

Hhex is an early marker of thyroid cells. The role of Hhex could me to maintain the expression of Titf/Nkx2-1, Foxe1 and Pax-8 mRNA in the thyroid anlage. On the other hand, Titf/Nkx2-1 and Pax-8 are both required to maintain the expression of Hhex. This regulatory network between transcription factors seems to be in place in differentiated TCFs also showing that Titf-1/Nkx2-1 regulates the activity of *Hhex* promoter in thyroid cell lines.

Genes Involved in the Late Stages of Thyroid Organogenesis

(a) Tshr. Tshr is localized on chromosome 14q31 in humans and chromosome 12 in mice. Tshr is a protein of 765 amino acids in humans and in mice and belongs to the superfamily of G-protein coupled receptors. The initiation of expression of the *Tshr* on day 14 of mouse embryogenesis, at the onset of thyroid differentiation after completion of gland migration [24] suggests that alterations in TSH signaling pathways could result in defective thyroid development. Alterations in the *Tshr* gene [34] may cause hypothyroidism. Both the $Tshr^{hyt/hyt}$ mice, characterized by a loss-of-function mutation in the fourth transmembrane domain of the *Tshr* and the *Tshr* null mice display severe hypothyroidism, associated with thyroid hypoplasia in adult life. However, at birth, in both these mutants, the size of the thyroid does not appear to be affected, and the gland displays only some alterations in its structure. The amount of TG does not change, whereas the expression of both *TPO* mRNA and NIS is strongly downregulated. During embryonic life the TSH/Tshr signaling is probably required to complete the differentiative program of the TFC, but, unlike what happens during adult life, this signaling is not relevant in controlling the growth of the gland. In contrast to mouse models, where an intact TSH-Tshr signaling pathway does not appear to be a prerequisite for the development of a normal sized thyroid gland in utero, this pathway is clearly important for the development of a normally sized fully differentiated gland in utero in humans. While the action of TSH through its receptor (Tshr) is essential for the proliferation and maintenance of differentiated function of thyroid follicular cells, it plays no role in the migration of the thyroid anlagen and the growth of thyroid gland. *Tshr* is therefore a candidate gene for thyroid hypoplasia, but not for thyroid ectopy.

(b) Hoxa3 and Eya1. The *Hox* genes belong to a large gene family in both mice and humans distributed in four different chromosomal complexes. *Hoxa3* is detected in the floor of the pharynx, in the developing thyroid, and in the mesenchymal, endodermal, and neural crest-derived cells of the fourth pharyngeal pouch [17].

Hoxa3 Null Mice. Hoxa3 is present both in thyroid diverticulum and the utimobranchial body and thus their products are defective in mutant mice [17]. *Hoxa3* mutant mice are athymic and show thyroid hyoplasia [17]. A more

detailed analysis of *Hoxa3*$^{-/-}$ mice revealed a variable expressivity and penetrance of the thyroid phenotype. The embryos show severe alterations in the development and migration of the ultimobranchial bodies, which do not fuse with the thyroid primordium (persistent ultimobranchial bodies), and a reduced or absent C cell population in the thyroid. Thus the ability of the neural crest population that is about to differentiate into C cells and migrate to its final position is affected. Both *Hoxb3*$^{-/-}$ and *Hoxd3*$^{-/-}$ single mutant mice have a thyroid gland that appears normal. However, both the double mutants *Hoxa3*$^{-/-}$ *Hoxb3*$^{-/-}$ and *Hoxa3*$^{-/-}$ *Hoxd3*$^{-/-}$ mice show a 100% penetrance of the thyroid and ultimobranchial body phenotype. Although *Hox3* paralogs do not play a direct role in the morphogenesis of the thyroid, they could have an important role in the normal development and migration of the ultimobranchial bodies [22].

In *Hox3*$^{-/-}$ mice, hemiagenesis occurs (absence of one of the thyroid lobes and, sometimes, of the isthmus) and defective fusion of the C cells with the thyroid lobes [17]; the latter feature is also seen in *Eya*$^{-/-}$ mice [33].This hypothesis is supported by the study of the phenotype of mouse embryos deprived of a functional Eya1 gene [33]. Eya1 control is critical in early inductive events involved in the morphogenesis of thymus, parathyroid and thyroid [33]. At an early stage of embryonic life, *Eya1* is expressed in the pharyngeal arches' mesenchyme, in the pouches' endoderm, and in the surface ectoderm of the clefts. Later, it is clearly evident in the thymus, parathyroid, and ultimobranchial bodies but is not detected in the developing thyroid. In *Eya1* null mice, the thyroid phenotype is almost identical to the phenotype displayed from *Hoxa3* mutants. Indeed, the embryos show persistent ultimobranchial bodies, hypoplasia of the lobes, absence of the isthmus, and a reduced number of follicular cells.

Other Genes

(*a*) *Fgfr2*. The Fgf family includes at least 22 peptide growth factors that bind and activate specific tyrosine kinase receptors (Fgfr). One of the receptors, the *Fgfr2-IIIb* isoform, is expressed in many types of epithelial cells and is activated by Fgfs (like Fgf-10) that are present in the surrounding mesenchyme. In many cases it has been shown that the activation of Fgfr-2-IIIb mediates the epithelium-mesenchyme cross-talk required for the development of different organs. In the thyroid Fgfr activation appears to be essential only after budding and initiation of migration. Both mutated mice expressing a soluble dominant negative form of Fgfr-2-IIIb receptor and mice deficient for the same isoform show absence of the thyroid. Furthermore, in *Fgf10* null mice the thyroid is missing. These data strongly suggest that the interaction of Fgf-10 with its receptor Fgfr-2-IIIb is relevant for thyroid organogenesis. It is possible that

Fgf-10/Fgfr signaling is required for the progression of already established differentiative programs [22].

(b) Nkx2-6, Nkx2-3 and Nx2-5. Other genes of the *Nkx2* family, such as *Nkx2-6*, *Nkx2-3*, and *Nkx2-5*, are expressed in the endodermal layer of the developing pharynx, including the thyroid anlagen, as well as in other tissues. *Nkx2-3* null mice the gland appears histologically normal despite the expression of *Nkx2-3* in the thyroid. Because of the early mortality of *Nkx2-5*$^{-/-}$ embryos, it is not easy to identify the role of this factor in thyroid morphogenesis. In the developing thyroid, the specific role of Nkx2-3 or Nkx2-5 has not yet been identified.

(c) Hepatic Nuclear Factor 3β (Hnf-3β). Hnf-3β has a wide and early expression in embryonic tissue including the developing thyroid. It is hard to identify the relevance of Hnf-3β during thyroid morphogenesis because the disruption causes an embryo-lethal phenotype at a stage preceding that of thyroid bud formation [22].

Thyroid-Specific Genes and Mature Thyroid Cell

The term 'thyroid-specific genes' applies to genes that encode proteins exclusively found in the thyroid (e.g. thyroglobulin and thyroperoxidase) or primarily involved in thyroid function (e.g. TSH receptor and sodium/iodide symporter). The transcription of these genes in the thyroid appears to rely on the coordinated action of transcription factors that includes at least Ttf-1, Pax-8, and perhaps also Ttf-2 [24]. TSH and the increase in intracellular cAMP, upregulates the expression of transcription factor Pax-8 as well as other transcription factors but it cannot account for the observed control on thyroglobulin gene transcription. TSH may also influence some post-transcriptional steps, as in the case of thyroglobulin. A positive in vivo effect of TSH on general protein synthesis, with stimulation of transcription and translation, has been well documented, an effect mimicked by cAMP agonists.

Ttf-1 and Pax-8 proteins exert a major control on thyroglobulin gene transcription [29] individually and in synergism, but other factors may contribute. The thyroglobulin gene is expressed in cells devoid of Ttf-2 protein. After the TSH receptor gene, the thyroglobulin gene is the most affected in its expression by a reduced Ttf-1 availability. There is probably a synergistic action of Ttf-1 and Pax-8 on gene transcription of TPO, but thyroperoxidase gene transcription is more rapidly and tightly controlled by TSH and cAMP. TSH signaling is indispensable for sodium/iodide symporter gene transcriptional activation in vivo [34], and iodide downregulates the expression of the gene. The control of Tshr gene seems to be quite complex. Additionally, growth factors, such as TGF-β and FGF, seem to be involved in the regulation of thyroid-specific genes [35].

In conclusion, it has been shown that, in mice, the thyroid anlagen, although distinguished by early expression of *Titf-1/Nkx2-1*, *Foxe1*, *Pax-8*, and *Hhex*, does not require these factors for the initial steps of morphogenesis [22]. Titf-1, Foxe1, Pax-8, and Hhex are transcription factors regulating the expression of downstream genes that ultimately activate the organogenesis of the gland. These thyroid specific genes are mentioned here because of their connection with congenital hypothyroidism. Nevertheless mutations of Ttf-1, Ttf-2 and Pax- 8 are found in $<10\%$ of patients with congenital hypothyroidism and these predominately have orthotopic hypoplasia, often associated with other malformations. The possibility of underestimation, considering that mutations have been searched mostly in the coding region, cannot be excluded. Additionally the discordance of more than 90% of monozygotic twin pairs suggests that isolated thyroid ectopy or athyreosis most often results from early somatic mutations, epigenetic modifications or stochastic developmental events [19].

Two more genes are worth mentioning, in respect to their relation with congenital hypothyroidism: (1) The stimulatory G-protein α subunit gene *(GNAS1)* is located on chromosome 20q13 and contains over 13 exons that encode Gsα, the α subunit of the heterotrimeric stimulatory G-protein. This protein has intrinsic GTPase activity. Apart from TSH receptors, TRH and LH and PTH receptors use these G-proteins for their signal. (2) The *PDS* gene is on chromosome 7q, contains 21 exons and is found to be expressed in the cochlea as well as in the thyroid. It encodes pendrin, 4,780 amino acid protein (molecular weight 86 kDa) with 11 transmembrane domains which functions as a chloride-iodide transporter.

The Maturation of Hypothalamic-Pituitary Axis – The Role of Placenta

At 10–12th week of gestation tiny follicle precursors can be seen, iodine binding can be identified and thyroglobulin detected in follicular spaces. Thyroid hormones (T_4 and T_3) are detectable in fetal serum by gestational age of 12 weeks, probably of maternal origin. Thyroid hormones and thyroxine-binding globulin (TBG) continue to increase gradually over the entire period of gestation. The serum TBG concentrations are higher in the infant than in adult humans ($\sim$300 nmol/l) as a consequence of placental estrogen effects on the fetal liver. In addition to the increase in total T_4, however, there is also a progressive increase of free T_4 concentrations between 18 and 36 weeks of gestation, indicating a maturation of the hypothalamic-pituitary-thyroid axis. While thyroglobulin can be identified in the fetal thyroid as early as the 5th week, and is certainly present in follicular spaces by 10–11 weeks, maturation of thyroglobulin secretion takes much longer and it is not known when circulating thyroglobulin first appears in the fetal serum. By the time of gestational age

27–28 weeks, however, thyroglobulin levels average approximately 100 ng/ml and remain approximately stable until the time of birth. Iodide concentrating capacity can be detected in the thyroid of the 10- to 11-week fetus, but 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 of gestation. TSH is detectable at levels of 3–4 mU/l at the 12th week of gestation. It increases moderately over the last two trimesters to levels of 6–8 mU/l at the time of delivery. The fetal thyrotroph responds to TRH as early as the 25th week of gestation. The maturation of the negative feedback control of thyroid hormone synthesis, occurs by approximately mid-gestation. The increase in serum TSH concentrations have been noted in infants as early as the 28th week of gestation. Serum levels of TRH are higher in the fetal circulation than in maternal blood, due to extrahypothalamic TRH production (placenta and pancreas) and the decreased TRH degrading-activity in fetal serum. All three iodothyronine deiodinases involved in the activation and inactivation of thyroid hormone are coordinately regulated during gestation for the proper supply of T_3 to developing tissues. Type 1 iodothyronine deiodinase (D1) is low throughout gestation. Consequently circulating T_3 concentrations in the fetus are quite low. The type 2 deiodinase (D2) is detectable by the 7th week of gestation. The type 3 or inner ring deiodinase (D3) is also expressed in fetal brain by the 7th week of gestation. D2 and D3 are the major isoforms present in the fetus and are especially important in defining the level of T_3 in the brain and pituitary [37]. The maturation of D2 activity in brain is tightly linked to thyroid hormone receptor ontogeny [37]. D2 expression with a precise timing is fundamental during critical periods of mammalian development. D3 is present in many fetal tissues and has a key role in protecting fetal tissues against high maternal T_4 concentrations present either in the placenta or in amniotic fluid.

The fetal hypothalamic-pituitary-thyroid axis develops relatively independent of maternal influence. The placenta is freely permeable to iodide which is essential for fetal thyroid hormone synthesis. On the other hand, maternal TSH does not cross the placenta, nor does thyroglobulin. Maternal thyroid function can play a critical role in the fetus and normal maternal T_4 concentrations seem to be important. T_4 is present in cord serum at concentrations between 25 and 50% of normal. Maternal-fetal T_4 transfer may occur in the first half of pregnancy, when fetal thyroid hormone levels are low prior to the onset of fetal thyroid function. An appropriate thyroid hormone level is critically important for the coordination of developmental processes in all vertebrate species. During embryogenesis, thyroid hormone acts primarily to promote differentiation and thus attenuate proliferation. As a result, either insufficient levels of T_3 or the premature exposure of the embryo to adult T_3 concentrations can be detrimental and can result in abnormal development [38]. In conclusion, the provision of

sufficient iodine from the placenta, probably appropriate maternal thyroid hormone levels and the normal maturation of the hypothalamic-pituitary-thyroid axis during gestation are important elements for the development of human embryos.

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Thyroid Disease during Pregnancy

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Thyroid disorders are common. The prevalence of hyperthyroidism is around 5 per 1,000 and hypothyroidism about 3 per 1,000 in women. As the conditions are generally much more common in the female it is to be expected that they will appear during pregnancy. Developments in our understanding of thyroid physiology [1] and immunology [2] in pregnancy as well as improvements in thyroid function testing [3] have highlighted the importance of recognising and providing appropriate therapy to women with gestational thyroid disorders.

Before considering the clinical entities occurring during and after pregnancy it is useful to briefly review thyroid physiology and immunology in relation to pregnancy.

Fetal-Maternal Relationships

Thyroid Function during Pregnancy

Iodine Metabolism

Pregnancy affects thyroid homeostasis. An increased excretion of iodine in the urine accounts for the increase in thyroid volume even in areas of moderate dietary iodine intake [4]. Some studies, however, do not show an increase in urinary iodine during pregnancy. In either case the increase in thyroid volume is the result of imbalance between the intake and increased requirements of iodine during pregnancy [5]. Iodine deficiency during pregnancy is associated with maternal goitre and reduced maternal thyroxine (T4) level. While thyroid size increases in areas of iodine deficiency it does not do so in those regions that are iodine sufficient; even in moderate iodine-deficient regions urinary iodine excretion is higher in all trimesters than in non-pregnant women and may be

causative in maternal goitre formation as assessed by ultrasound. The increase in thyroid volume already referred to is substantially greater in iodine-deficient areas. This gestational goitrogenesis is preventable by iodine supplementation not only in areas of severe iodine deficiency (24-hour urinary iodine less than 50 μg) but in areas such as Belgium and Denmark [6] where trials have shown clear beneficial effects on maternal thyroid size. The aim of these studies should be to increase the iodine supply to pregnant and lactating women to at least 250 μg/day, a level agreed by a recent concensus WHO meeting on iodine requirements in pregnancy and lactation [7]. Clinical studies of children born to mothers with known iodine deficiency clearly showed impaired neurointellectual development, sometimes to the extreme of cretinism in severe deficient states. These defects can be corrected by iodine administration before and even during gestation [8]. Urinary iodine excretion in pregnancy is characterised by maximum excretion in the first trimester followed by a decline in the second and third trimesters. Often there is an increase in urinary iodine in the first trimester compared to control non pregnant women but where the population has a high median iodine concentration this difference may not occur.

Thyroid Hormones

Thyroid hormone transport proteins particularly TBG (thyroxine-binding globulin) increase due to enhanced hepatic synthesis and a reduced degradation rate due to oligosaccharide modification. Serum concentration of free thyroid hormones has been reported to be decreased, increased or unchanged during gestation by different groups depending on the assays used [9]. However, there is general consensus that there is a transient rise in free thyroxine (FT4) in the first trimester due to the relatively high circulating hCG concentration and a decrease of FT4 in the second and third trimester albeit within the normal reference range. Recently, it has become apparent that there is a need for normative trimester-specific reference ranges for thyroid hormones [10]. Ideally these should be derived from iodine sufficient women who do not have any evidence of thyroid autoimmunity [11]. Changes in free triiodothyronine (FT3) concentration are also seen in which they broadly parallel the FT4, again within the normal range. The precise reason for the decline in free thyroid hormones is not clear. In iodine-deficient areas (including marginal iodine deficiency seen in many European countries) the pregnant woman may become significantly hypothyroxinaemic with preferential T3 secretion. The thyroidal 'stress' is also evidenced by a rise in the median TSH and serum thyroglobulin.

Thus, pregnancy is associated with significant, but reversible changes in thyroid function (table 1). The findings associated with the hypermetabolic state of normal pregnancy can overlap with the clinical signs and symptoms of thyroid disease.

Table 1. Physiologic changes in pregnancy that influence thyroid function tests

Physiologic change	Thyroid function test change
↑Thyroid-binding globulin (TBG)	↑Serum total T4 and T3 concentration
First-trimester hCG elevation	↑Free T4 and ↓TSH
↑Plasma volume	↑T4 and T3 pool size
↑Type III 5-deiodinase (inner ring deiodination) due to increased placental mass	↑T4 and T3 degradation resulting in requirement for increased hormone production
Thyroid enlargement (in some women)	↑Serum thyroglobulin
↑Iodine clearance	↓Hormone production in iodine-deficient areas

Immunological and Hormonal Aspects of Normal Pregnancy

Pregnancy has a significant effect on the immune system, in order to maintain the fetal-maternal allograft, which is not rejected despite displaying paternal histocompatability antigens [12]. While there is no overall immunosuppression during pregnancy, control or tolerization of anti-fetal T cells is critical [13]. Clinical improvement usually occurs in patients with immunological disorders such as rheumatoid arthritis [RA] when they become pregnant [14]. Clinical improvement occurs as well in psoriatic arthritis and Graves' disease. On the other hand, systemic lupus erythematosus (SLE) may flare during pregnancy.

The trophoblast does not express the classical major histocompatibility complex (MHC) class Ia or II which are needed to present antigenic peptides to cytotoxic cells and T helper cells, respectively. Instead HLA-G, a non-classical MHC Ib molecule is expressed which may be a ligand for the natural killer (NK) cell receptor so protecting the fetus from NK cell damage; it may also activate CD8+ T cells that may have a suppressor function. Human trophoblasts also express the Fas ligand abundantly, thereby contributing to the immune privilege in this unique environment possibly by mediating apoptosis of activated Fas expressing lymphocytes of maternal origin [15].

T cell subset studies in pregnancy are discrepant, as peripheral blood CD4+ and CD8+ cell levels have been variously reported to decline, remain unchanged and increase during pregnancy. Although, the distinction between Th1 (T cell helper 1) and Th2 (T cell helper 2) immune responses in humans remains less clear than in the mouse the general agreement is that in pregnancy there is a bias towards a Th2 response [16]. This seems to be achieved by the fetal/placental unit producing Th2 cytokines, which inhibit Th1. Th1 cytokines are potentially harmful to the fetus as, for example, interferon alpha is a known abortifacient.

Table 2. Immunological and hormonal features of pregnancy

Clinical: Improvement in Graves' hyperthyroidism
Rheumatoid arthritis
Psoriatic arthritis and other autoimmune diseases
Trophoblast: HLA G expression
Fas ligand expression
Lymphocytes: Th2 response
Th2 cytokines produced by the fetal/placental unit
Hormones: Progesterone increase – reduction in B cell activity
Oestrogen increase – fall in autoantibody levels
Cortisol, 1, 25-vitamin D and norepinephrine all affect the immune response

Sex steroids are powerful negative regulators of B cell activity. Oestrogen alone is effective in reducing B cell lymphopoiesis in pregnancy. Although progesterone is not effective on its own, it reduced the amount of oestrogen required for suppression by up to 90% in a mouse pregnancy model. The high concentrations of oestrogen produced in normal pregnancy almost certainly contribute to the fall in autoantibody levels observed in pregnant patients with autoimmune thyroid disease (AITD). Despite the fall in autoantibodies, there are no significant changes reported in the number of B cells in the circulation in normal human pregnancy. While progesterone may favour Th2 cells, evidence has indicated that oestrogen delivers a negative signal to B cell function during pregnancy and this showed a slow reversal in the postpartum period. In keeping with these observations, autoantibody titres and inflammation fall throughout pregnancy as observed in all autoimmune diseases investigated [17]. However, after most pregnancies, there is a marked increase in many different types of autoantibody secretion and an exacerbation of autoimmune diseases in the months after delivery. Recent data suggests that cortisol, norepinephrine and 1,25-dihydroxyvitamin-induced inhibition and subsequent rebound of interleukin-12 (IL-12) and tumour necrosis factor-α (TNFα) production may represent a major mechanism by which pregnancy and postpartum alters the course of or susceptibility to various autoimmune disorders [18]. Table 2 summarises relevant immunological changes in gestation.

Fetal Thyroid Development and Function

The fetal thyroid begins concentrating iodine at 10–12 weeks of gestation and is under control of fetal pituitary thyroid-stimulating hormone (TSH) by about 20 weeks of gestation [19]. Despite the fetus not possessing a functioning thyroid in early pregnancy there is good evidence that thyroid hormone is

important in the development of many organs including the brain. It is now well accepted that maternal circulating T4 crosses the placenta into the fetus at all stages of pregnancy, first shown by Vulsma et al. [20]. The precise mechanism of placental T4 transport is not clear but the important role of both the type 2 and type 3 deiodinase enzymes, both expressed in the placenta, has been recognised. Type 2 deiodinase is also located in the uterus and other parts of the genital tract and may have a role in fetal implantation [21]. In the fetus it is expressed in the brain and its action supplies that developing organ with T3. Type 3 deiodinase (D3), which degrades thyroid hormones, is also expressed in pregnant uterus, placenta, fetal and neonatal tissues. Analysis of a D3 knock out mouse has revealed a critical role for this enzyme in the maturation and function of the thyroid axis [22]. As thyroid hormone receptors have been localised in different brain areas well before fetal thyroid function occurs the supposition is that brain T3 derived from maternal T4 is active in promoting growth and differentiation in neural and other tissues [23]. Further understanding in relation to delivery of T3 to neurones following the deiodination of T4 in other nervous system cells has come from the discovery that, while there is absence of the type 2 deiodinase in neurones, a thyroid hormone transporter (MCT8) has been found to affect the entry of T3 into these neurones [24].

Thyroid Antibodies and Pregnancy Failure

Fertility is impaired in hypothyroid women with autoimmune thyroid disease and if such patients do achieve pregnancy the hypothyroid state is associated with a higher incidence of miscarriage early in pregnancy [reviewed in 14]. Thyroid autoimmunity, as evidenced by the presence of anti-thyroid antibodies, present during early pregnancy even in the euthyroid situation, is associated with an increased risk of subsequent miscarriage [25]. Thyroid autoantibody positive women miscarry at a rate of between 13 and 22% compared to 3.3–8.4% in control euthyroid antibody negative women [14]. While the association between thyroid antibodies and miscarriage is strong that between these antibodies and recurrent abortion is less so. In the euthyroid woman with thyroid antibodies no specific treatment can be offered to reduce the antibody titres; one uncontrolled study in euthyroid thyroid antibody positive women with recurrent abortion reported a significant success rate with thyroxine administration [26].

Hyperthyroidism and Pregnancy

Etiology

While the commonest cause of hyperthyroidism in pregnancy (which affects up to 0.2% of pregnant women) is Graves' disease (85–90%), other

Table 3. Causes of hyperthyroidism in pregnancy

Graves' disease
Transient gestational hyperthyroidism (associated with hyperemesis gravidarum)
Toxic multinodular goitre
Toxic adenoma
Subacute thyroiditis
Trophoblastic tumour
Iodide-induced hyperthyroidism
Struma ovarii
TSH receptor activation

causes such as hyperemesis gravidarum, toxic multinodular goitre, toxic adenoma and subacute thyroiditis may occur. It should be noted that most women with nausea and vomiting in pregnancy do not have hyperthyroidism. Rarer causes include struma ovarii, hydatidiform mole and one reported case of a TSH receptor mutation activated only during pregnancy [27] (table 3).

Diagnosis

The clinical suspicion of hyperthyroidism may not be obvious as symptoms of tachycardia, sweating, dyspnoea and nervousness are seen in normal pregnancy as are cardiac systolic flow murmurs. The diagnosis should always be confirmed by estimation of circulating thyroid hormone concentrations. It should be noted that serum thyroxine (both total and free) varies during normal gestation. Recent national and internationally agreed guidelines suggest that laboratories should be encouraged to develop normal ranges for total but more particularly free T4 and T3, as well as TSH after the 1st trimester during pregnancy, all of which may change during the course of gestation. Normally the TSH is suppressed in hyperthyroidism but in early pregnancy (approx. 9–12 weeks) TSH is usually suppressed by human chorionic gonadotrophin and may also be lowered due to non-specific illness such as vomiting as well as multiple pregnancy. This may lead to uncertainty in differentiating Graves' hyperthyroidism from gestational thyrotoxicosis due to hyperemesis gravidarum. The diagnosis of Graves' disease may be confirmed however by demonstrating the presence of TSH receptor stimulating antibodies which are also useful markers in the management of the condition.

Effects of Hyperthyroidism on Mother and Child

Several reviews of this subject are available [27–29]. Maternal complications of hyperthyroidism include miscarriage, placenta abruptio and preterm

delivery. Congestive heart failure and thyroid storm may also occur and the risk of pre-eclampsia is significantly higher in women with poorly controlled hyperthyroidism and low birth weight may be up to nine times as common. Neonatal hyperthyroidism, prematurity and intra-uterine growth retardation may be observed. A retrospective review documented a 5.6% incidence of fetal death or stillbirth in 249 pregnancies from hyperthyroid mothers and a further 5% fetal and neonatal abnormalities. Women with thyroid hormone resistance who, despite being euthyroid, had high levels of circulating T4 had a significantly increased miscarriage rate compared to euthyroid unaffected couples [30]. However, a recent study of women with subclinical hyperthyroidism, comprising 1.7% of women, showed no significant adverse pregnancy outcomes suggesting that treatment of this condition in pregnancy is not warranted [31]. Nevertheless, there is no doubt that overt clinical and biochemical hyperthyroidism should be treated to lessen the rate of complications described above. Gestational amelioration of Graves' disease is often associated with a reduction in titre of TSHR Ab and a change from stimulatory to blocking antibody activity [32]. A variety of TSHR Abs directed against the TSH receptor may occur in pregnant patients with Graves' disease. Zakarija et al. [33], e.g., reported the presence of high titres of two species of stimulating antibody in a patient who gave birth to 3 children with transient neonatal hyperthyroidism due to transplacental passage of the antibodies. A small number of newborns from mothers with Graves' disease develop central hypothyroidism. This is characterised by low FT4 concentrations in combination with suppressed TSH levels and a blunted TSH response after TRH administration. This situation may arise because of passively transferred thyroxine from the mother who is hyperthyroid in the short term or as a result of longer term (1 month) of neonatal hyperthyroidism due to passively transferred TsAb. There is a suggestion from the clinical description that maternal thyrotoxicosis before 32 weeks of gestation may be an important time point for the development of central hypothyroidism in the baby. The syndrome provides some indication of the effect of excess maternal thyroid hormones on the development of the hypothalamic pituitary thyroid axis as well as the effect of excess neonatal thyroid hormones on the same system [34].

Management of Graves' Hyperthyroidism

Preconception

There is a good case for a preconception clinic for patients with Graves' hyperthyroidism who wish to become pregnant. Firstly, education about the effects of the disease on maternal health and fetal well-being can be given to

Table 4. Guidelines for measurements of thyroid-stimulating hormone-receptor antibodies in a pregnant woman with Graves' disease (reproduced from Laurberg et al. [37], with permission from the Society of the European Journal of Endocrinology)

Patient status	Measurement
Euthyroid – previous ATD	not necessary
Euthyroid ± T4 therapy	check in early pregnancy: if low or absent no further testing
Previous radioiodine therapy/surgery	if high – check fetus and check antibodies in last trimester
Receiving ATD during pregnancy	measure in last trimester

ATD = Antithyroid drugs; T4 = thyroxine.

allay fears which are commonly present in these women. The patient's thyroid status should be checked frequently to minimise risk of miscarriage should she be hyperthyroid at the time of conception. If treatment had been commenced with methimazole or carbimazole a change to propylthiouracil (PTU) is recommended to reduce the admittedly rare occurrence of aplasia cutis [35] and the equally rare methimazole embryopathy [36] reported following the administration of the former drugs. The patient may have been rendered euthyroid by partial thyroidectomy or radioiodine therapy. However, if these procedures are performed the patient may require thyroxine therapy (with a requirement for an increase in dose and monitoring during gestation); in addition, there is still a small risk of neonatal hyperthyroidism even if the mother is euthyroid.

Previously Treated Patients with Graves' Disease

These patients may have received antithyroid drugs, surgery or radioiodine therapy and be euthyroid on or off thyroxine therapy. The important concern here is that neonatal hyperthyroidism may still occur. Guidelines [37] state that if previous antithyroid drugs alone have been used there is no need to measure TSH receptor antibodies as the maternal thyroid function gives a reliable estimate of fetal thyroid status and the risk of neonatal hyperthyroidism is very low (table 4). TSH receptor antibodies should be measured early in pregnancy in a euthyroid pregnant women previously treated by either of the other modalities. If the level is high (as defined by the local laboratory) at this time the fetus should be evaluated carefully during gestation normally by checking the fetal heart rate and the antibodies measured again in the last trimester (table 4). If there is a detectable titre of stimulating antibodies at 36 weeks the neonate should be checked for hyperthyroidism at birth.

Table 5. Management of Graves' hyperthyroidism in pregnancy

Confirm diagnosis
Start propylthiouracil
Render patient euthyroid – continue with low dose ATD up to and during labour
Monitor thyroid function regularly throughout gestation (4–6 times weekly) – adjust ATD if necessary
Check TSAb at 36 weeks of gestation
Discuss treatment with patient
Effect on patient
Effect on fetus
Breastfeeding
Inform obstetrician and paediatrician
Review postpartum – check for exacerbation

ATD = Antithyroid drugs; TSAb = Thyroid-stimulating antibodies.

Graves' Hyperthyroidism Inadvertently Treated with Radioiodine in Early Gestation

The practical procedures surrounding the administration of radioiodine therapy for Graves' disease vary widely. In many clinics routine pregnancy testing is not performed before ^{131}I administration. Despite denial of pregnancy several reports of inappropriate radioiodine administration have highlighted the concern about the fetal radiation risk [27]. The maternal thyroid uptake, the gestational age and the ability of the fetal thyroid to concentrate iodine are all vital in determining the radioiodine exposure in utero. The fetal thyroid concentrates iodide after 13–15 weeks of gestation with peak concentrations occurring at 20–24 weeks and is relatively more avid for iodine than the maternal thyroid [38]. The fetal tissues are also more radio-sensitive. Administration of up to 15 mCi (555 MBq) ^{131}I for hyperthyroidism up to 10 weeks of gestation does not compromise fetal thyroid function and the low fetal whole body irradiation is not considered sufficient to justify termination of pregnancy. Limited clinical data suggests that ^{131}I given after 10–12 weeks results in biochemical hypothyroidism in the neonate. In these cases management should maintain high normal maternal circulating thyroxine levels and ensure prompt treatment of the neonate with thyroxine. The availability of neonatal screening programmes for congential hypothyroidism ensures that mental retardation can be avoided by appropriate thyroxine treatment.

Patients Found to Have Hyperthyroidism during Pregnancy

Medical therapy is preferred by most clinicians as radioiodine is contraindicated and surgery requires pre-treatment with antithyroid drugs to render the patient euthyroid (table 5).

Propylthiouracil (PTU) should be given in a dose of 100–150 mg three times daily until the patient becomes euthyroid at which time the dose should be reduced to the lowest amount to maintain the euthyroid state with serum T4 at the upper end of normal and continued up to and through labour. PTU is preferred to MMI or carbimazole because there is (in contrast to MMI) no evidence of associated aplasia cutis [39]. There has been a suggestion of a specific methimazole embryopathy in children exposed to the drug during the first trimester of pregnancy which, although rare, has not been reported with PTU [36]. As these risks are very small the patient who receives MMI can be normally reassured. In terms of rapidity of action and fetal hypothyroidism inducing potential there is probably little reason to choose PTU over MMI. The so-called 'block and replace' regime in which thyroxine is given with antithyroid drug should not be used because the dose of antithyroid drug would inevitably be too high and cause fetal goitre and hypothyroidism. Hashizume et al. [40] reported that T4 administration to pregnant women with Graves' hyperthyroidism during pregnancy and after delivery, together with methimazole, was effective in reducing the incidence of postpartum recurrence of hyperthyroidism (vide infra) but these results have not been confirmed. Rarely an episode of infection or the development of pre-eclampsia may precipitate thyroid storm requiring the use of thionamides, iodides, beta-blockers, fluid replacement and possibly steroid therapy and plasmapheresis. PTU has a shorter half-life than methimazole and is not present in as high a concentration in breast milk. Hence women receiving PTU can breastfeed without significant risk to the neonate. Common complications of thionamide therapy include skin rash, arthralgia and nausea in about 2% of patients. A vasculitic syndrome may be more common with PTU. Methimazole (or carbimazole) may be used as an alternative in this situation with only a 33% chance of cross-reaction. Agranulocytosis is rare and is an indication for immediate withdrawal of the drug and possible treatment with granulocyte colony stimulating factor although the results are not always satisfactory. There is no benefit in routine monitoring of the white blood count as the fall in white blood count may be very rapid, but patients should of course be instructed to report immediately if they develop a sore throat with or without a fever.

There is no significant effect of antithyroid drugs in utero or during breastfeeding on the long-term health of the neonate or child assuming the dose during gestation has not caused iatrogenic fetal hypothyroidism [41]. Beta-adrenergic blocking agents such as propranolol may be used for a few weeks to ameliorate the peripheral sympathomimetic actions of excess thyroid hormone which is usually sufficient for the management of hyperthyroidism; prolonged use may result in retarded fetal growth, impaired response to anoxic stress together with postnatal bradycardia and hypoglycaemia. These drugs will need to be used in the uncommon instance of intolerance to both of the available thionamide

drugs. Lithium therapy for hyperthyroidism is contraindicated in pregnancy because of its known teratogenicity.

Monitoring of the Fetus in a Mother with Graves' Disease

As neonatal thyrotoxicosis is known to be associated with neurological impairment in some cases there is a requirement to monitor the fetus rather than wait till birth to diagnose thyroid dysfunction. The use of serial in utero ultrasonographic measurements has been shown to accurately measure fetal thyroid size [42]. If the fetal thyroid does not reduce in size in response to antithyroid drug administration then transplacental passage of TsAb causing fetal hyperthyroidism should be suspected. A recent comprehensive study by Luton et al. [43] showed that the sensitivity and specificity of fetal thyroid ultrasound at 32 weeks for the diagnosis of clinically relevant fetal thyroid dysfunction was 92 and 100%, respectively.

Graves Orbitopathy

Eye symptoms and signs of Graves' hyperthyroidism including excessive watering, pain and irritation as well as chemosis, periorbital oedema, proptosis and ophthalmoplegia may occur before, during or after the onset of hyperthyroidism and are more common in cigarette smokers. Treatment during pregnancy initially should be symptomatic with topical eye drops and elevation of the head of the bed. Careful monitoring is necessary to check for any signs of optic neuropathy. Oral or intravenous prednisone therapy is indicated in severe congestive ophthalmopathy but should be used sparingly in pregnancy. In line with the Graves' hyperthyroidism, the ophthalmopathy would be expected to improve during gestation.

Surgery

Subtotal thyroidectomy is indicated if control of the hyperthyroidism is poor on account of poor compliance or inability to take drugs. Patients with a very large goitre may also require surgery because of pressure symptoms. Surgery is preferred in the second trimester as there is a higher risk of associated abortion at an earlier stage of gestation. In general surgery should be avoided if it is considered that medical therapy has a reasonable chance of success.

Management of other causes of hyperthyroidism:

(a) Hyperemesis gravidarum is common and around 5% of cases require hospital admission because of dehydration and ketosis. Thyroid function should be checked in these patients; a correlation has been established between the severity of the hyperemesis and thyroid function with an elevated FT4 and FT3

with suppressed TSH. In those patients who are hyperthyroid antithyroid drugs may be given. The diagnosis of gestational thyrotoxicosis will be confirmed by noting the absence of TSH receptor stimulating antibodies.

(b) Toxic multinodular goitre and toxic adenoma: Radioiodine which may be a treatment of choice is absolutely contraindicated in pregnancy. The conditions may be managed with antithyroid drugs during gestation; if necessary surgery may be performed during the 2nd trimester but if possible it is better to postpone this till the postpartum period.

(c) Subacute thyroiditis: The diagnosis is suggested by the presentation of a painful thyroid in the presence of hyperthyroidism. As radionuclide evaluation (which would demonstrate a low iodine uptake) is contraindicated diagnosis may be made with a fine needle aspiration biopsy of the thyroid associated with an elevation in systemic markers of inflammation. Treatment is firstly with analgesics for pain and oral prednisolone therapy if inflammation is severe. Frequent monitoring of thyroid function is required as a small number of patients will develop hypothyroidism.

The other causes of hyperthyroidism listed are rare and referral to a specialist centre is advised.

Postpartum Graves' Disease

Patients with Graves' disease may develop Graves' hyperthyroidism as a post partum phenomenon due to the immune rebound of TSH receptor antibodies. In Graves' disease patients, TSHR Abs have been shown to decrease during late gestation with a significant rebound in the late postpartum [44]. In this situation the hyperthyroidism of Graves' disease may be followed immediately by transient hypothyroidism due to co-existing destructive postpartum thyroiditis during the early postpartum period despite increasing TSAb activity. This may be important when considering postpartum relapse of the disease. Screening for TSAb during pregnancy may detect patients with Graves' disease at risk of postpartum relapse and is also helpful when measured postpartum in diagnosing Graves' disease as the cause of hyperthyroidism as opposed to the hyperthyroid phase of postpartum thyroiditis [45]. The cost benefit of this proposed screening strategy is not available and it is probably not practical in most countries.

Hypothyroidism

The incidence of hypothyroidism during pregnancy is around 2.5% [46]. The aetiology is usually autoimmune thyroiditis characterised by the presence of anti-TPO antibodies. Significant titres of these antibodies are found in about 10% of women at about 14 weeks of gestation. Other causes of hypothyroidism in pregnancy include postoperative thyroid failure and non compliance with

existing thyroxine therapy. In areas of iodine deficiency the circulating maternal thyroxine concentrations are low although TSH is usually in the normal range. In this situation the incidence of thyroid abnormalities is higher and in particular thyroid autoimmunity may be associated with diminished thyroid reserve and an increase in spontaneous abortion.

The diagnosis of hypothyroidism is made by noting an elevated TSH accompanied by a low serum FT4. Subclinical hypothyroidism is recognised to be equally as important in its adverse effects, affecting mother and neonate as the full expression of the disease [47]. Maternal hypothyroxinaemia (without increased TSH) is also being increasingly accepted as deleterious to the neuropsychological development of the child [48]. Care should be taken in the interpretation of TSH concentrations in early gestation due to the thyrotrophic effects of hCG.

Previous studies have documented the effects of hypothyroidism on maternal and fetal well-being, drawing attention to increased incidence of abortion, obstetric complications and fetal abnormalities in untreated women. Women already receiving thyroxine for hypothyroidism require an increased dose during gestation. This is critical to ensure adequate maternal thyroxine levels for delivery to the fetus especially during the first trimester. The dose should normally be increased by 50–100 μg/day as soon as pregnancy is diagnosed; subsequent monitoring of TSH and FT4 is then necessary to ensure correct replacement dosage [49].

Maternal Thyroid Disease in Pregnancy: Effect on Child Development

Thyroid hormones are major factors for the normal development of the brain. The mechanisms of actions of thyroid hormones in the developing brain are mainly mediated through two ligand-activated thyroid hormone receptor isoforms [50]. It is known that thyroid hormone deficiency may cause severe neurological disorders resulting from the deficit of neuronal cell differentiation and migration, axonal and dendritic outgrowth, myelin formation and synaptogenesis [23]. This is the situation well documented in iodine-deficient areas where the maternal circulating thyroxine concentrations are too low to provide adequate fetal levels particularly in the first trimester. Recent work has raised concern that in an iodine-sufficient area maternal thyroid dysfunction (hypothyroidism, subclinical hypothyroidism or hypothyroxinaemia) during pregnancy results in neuro-intellectual impairment of the child. Two studies, have shown that a low thyroid hormone concentration in early gestation can be associated with significant decrements of IQ of the children when tested at

7 years and 10 months, respectively [51, 52]. Pop et al. [53] have also shown a significant decrement in IQ in children aged 5 years whose mothers were known to have circulating anti-TPO antibodies at 32 weeks gestation and were biochemically euthyroid. Haddow et al. [51] found that the full IQ scores of children whose mothers had a high TSH during gestation were 7 points lower than controls ($p < 0.005$) and that 19% of them had scores of less than 85 compared to 5% of controls ($p < 0.007$). More recently, the Dutch group [54] have again confirmed that maternal hypothyroxinaemia during early gestation is an independent determinant of neurodevelopmental delay. Further, they have suggested that when FT4 concentrations increase during gestation in women who have had low FT4 in early pregnancy infant development is not adversely affected [54]. The neurodevelopmental impairment is similar to that seen in iodine-deficient areas and implies that iodine status should be normalised in regions of deficiency. However, much of the USA and parts of Europe are not iodine-deficient which raises the question of routine screening of thyroid function during early pregnancy or even at preconception. Another reason for screening could be to focus on the risk for postpartum thyroiditis [55]. The following numerical issues should be considered in relation to such a strategy: the incidence of an elevated TSH in pregnancy is around 2.5%; the prevalence of anti-TPO antibodies is 10% as ascertained at a routine antenatal booking clinic; the incidence of thyroid dysfunction observed in anti-TPO-positive pregnancies is up to 15%. While these numbers are impressive the question as to whether there is any effective intervention must be addressed. Although one study has reported an improved psychological outcome in children from thyroxine-treated mothers (compared to those children from inadequately treated mothers) there are no results of any formal prospective randomised trials examining, for example the effect of T4 intervention therapy given to susceptible women on subsequent child development. These considerations emphasize that it is important to ensure an adequate thyroid hormone supply to the developing fetus in all areas of the world whether iodine-deficient or not [56]. Further studies in this area are required to answer questions relating to thyroid function screening before and during pregnancy.

Nodular Thyroid Disease

Thyroid nodules are claimed to be detected in up to 10% of pregnant women. Fine needle aspiration biopsy is the first investigation of choice which may yield a malignancy/suspicious result in 35% [57]. When malignancy is diagnosed it is usually a differentiated tumour which may be surgically resected in the second trimester or in some cases safely left until the postpartum period before therapy is started. The impact of pregnancy on thyroid cancer seems to be minimal in that there is no difference in rates of metastases or recurrence

compared to non-pregnant women with the same disease [58]. Whether women already treated for thyroid malignancy should become pregnant is of concern but current evidence suggests that differentiated thyroid cancer should not inhibit an intended pregnancy. Previous ^{131}I therapy does not result in demonstrable adverse events in subsequent pregnancies although miscarriage appears to be more frequent during the year preceding conception [59].

Neonatal Thyrotoxicosis

About 1–5% of children born to mothers with Graves' disease will develop neonatal thyrotoxicosis due to transplacental passage of maternal thyrotrophin receptor stimulating IgG antibodies [60]. It has been established that the presence of TsAb at 36 weeks gestation in women with Graves' disease has a significant positive predictive value for the probability of neonatal hyperthyroidism. Fetal hyperthyroidism is associated with intrauterine growth retardation, craniosynostosis and fetal death. In neonates cardiac failure, arrhythmias, hepatosplenomegaly and jaundice may be seen. In addition they may have vomiting, poor weight gain and be hyperkinetic. Treatment includes the administration of iodine, PTU, dexamethasone and adequate sedation. Reassurance may be given to the parents that the disease will remit permanently in 8–20 weeks due to the known half-life of IgG and remission by 10 months is nearly always achieved [61]. A subset of infants with neonatal hyperthyroidism appear to produce their own thyroid-stimulating immunoglobulins and therefore will not respond as readily to antithyroid drug therapy and require ablative treatment.

In the absence of maternal thyroid immune disorder, non autoimmune hyperthyroidism due to an activating thyrotropin receptor germ line gene mutation must be considered as a cause for neonatal hyperthyroidism. The condition may be sporadic or be inherited in an autosomal, dominant pattern and is characterised clinically by a variable severity of hyperthyroidism and goitre, absence of thyroid associated ophthalmopathy and dermopathy and negative thyroid antibodies [61]. Other clinical features including craniosynostosis, advanced bone age, low head circumference and psychomotor retardation have been described. A recent analysis of all reported cases of non autoimmune hyperthyroidism [62] drew attention to the observation that the mean gestation duration was significantly less than that seen in children with congenital hypothyroidism due to inactivating mutations of the TSH receptor (35.8 vs. 39.4 weeks, $p = 0.003$). The role of excess thyroid hormone in premature delivery is not yet established but is clearly relevant and requires further investigation. It is critical to determine if there is an activating TSH receptor mutation as the treatment in this case must be thyroid ablation to achieve long-term remission.

Neonatal hyperthyroidism has occurred due to the McCune-Albright syndrome [60], a condition characterised by a somatic activating mutation in the gene GNAS1 that encodes the α-subunit of GTP-binding protein that stimulates adenylate cyclase. In the murine D3 knock out mouse referred to previously [23], observation has shown that the lack of D3 function resulted in a probable overexposure of T3 during a critical period of thyroid axis development followed later by central hypothyroidism.

In conclusion a considerable increase in our appreciation of the physiology, immunology and clinical aspects of thyroid function in relation to gestation has occurred during the past decade. Important research into thyroidal influence on fetal development as well as delivery of thyroid hormones to the fetus will drive future clinical studies to improve recognition and management of thyroid disease before, during and after pregnancy.

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Thyroid Function in the Newborn and Infant

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The thyroid gland is an endocrine organ of vital importance for the neonate since normal thyroxine concentrations are essential for the normal neurodevelopment of the newborn and subsequently the infant and child. The recent advances in our understanding of fetal thyroid hormone physiology have also shown the importance of the placental transfer of maternal thyroid hormones and the normal function of the fetal thyroid gland for normal brain development of the fetus with subtle differences in the outcome of the term newborn as opposed to the preterm newborn.

Fetal Thyroid Function and Maternal Thyroid Hormones

The onset of active fetal thyroid function (FTF) coincides with full maturation of the pituitary portal vessels at 16–20 weeks of gestation [1]. Before the period of FTF, the neocortex of the fetal brain undergoes important phases of development which are largely dependent on the presence of thyroxine (T4) and triiodothyronine (T3) [1, 2]. Low concentrations of T4 and T3 are present in early embryonic and fetal tissue before the onset of FTF in concentrations that are directly influenced (especially the T4 levels) by those in the maternal circulation [3–14]. When maternal T4 and T3 concentrations are abnormally low in the first trimester, fetal brain development is adversely affected and there is a defect in the histogenesis and cerebral cortex cytoarchitecture, defective neuronal migration at the beginning of fetal neocorticogenesis, and a defective cortical expression of several genes in the fetal brain such as neuroendocrine-specific protein A [15, 16]. At the time of neural tube closure thyroid hormone receptor (TR) isoforms are already present in the fetal brain and these most likely mediate the biological effects of the T3 that has been locally generated from T4

transferred from the mother. Consequently, if the mother has T4 deficiency then the fetus will be T3-deficient even if maternal T3 is normal. This is because during early development serum-derived T3 essentially does not contribute to cerebral T3. Studies have shown that normal concentrations of T3 alone in the maternal or fetal circulation without normal T4 concentrations have no protective effect on the fetal brain because during fetal and postnatal development cerebral structures depend entirely on the local generation of T3 from T4 by type II 5′-iodothyronine deiodinase (D2), the activity of which is inversely related to the availability of T4 [17]. This might explain why in most cases of congenital hypothyroidism in a newborn with a mother who has normal thyroid function there is no permanent severe central nervous system damage when T4 is administered in the first 3 months of neonatal life. In this case, the fetal brain has not been severely damaged before birth and normal development can still be achieved by prompt administration of T4 [18–22]. The most severe brain damage occurs when both the mother and fetus have low T4 levels during the entire gestational period as occurs in iodine-deficient environments. Iodine deficiency during pregnancy can result in a global loss of 10–15 intellectual quotient points at a population level in the offspring and it constitutes the world's greatest single cause of preventable brain damage and mental retardation [23–27].

Maternal-Fetal Unit and the Function of the Fetal Thyroid Gland

During gestation the normal function of the maternal-fetal unit is crucial since it is the cornerstone for the physiological development of the fetus. Ultrasound-guided amniocentesis and cordocentesis have given researchers a greater insight into the mechanisms of maternal-fetal transfer of T4 and T3 which is crucial for normal brain physiology of the fetus. The human fetus is surrounded by two distinct fluid cavities for most of the first trimester: the inner (amniotic) cavity contains the fetus, and the outer (exocoelomic) cavity separates the amniotic cavity from the placenta and contains the secondary yolk sac (fig. 1a). The exocoelomic cavity is the site of important molecular exchanges between the mother and the fetus [28–30]. The coelomic fluid results from an ultrafiltrate of maternal serum with specific placental and secondary yolk sac bioproducts [30]. It has been shown that T4 (and possibly T3) is present in colelomic fluid as early as 5.6 weeks' gestation [29, 31]. Maternal T4 is transferred into the exocoelomic cavity and subsequently into the fetal gut and circulation via the secondary yolk sac. The second mode of transfer of maternal nutrients starts at the end of the first trimester. The secondary yolk sac and 2/3 of the placental mass degenerate, and the amniotic cavity containing the fetus

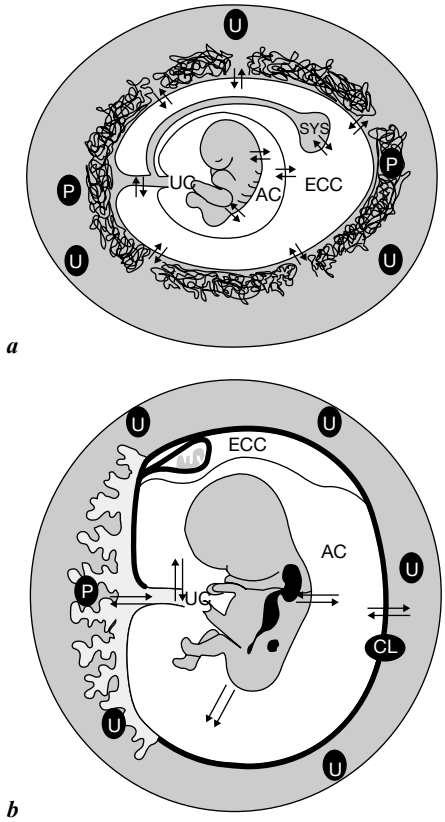

Fig. 1. Maternal-fetal unit during the first (***a***) and second (***b***) trimesters of pregnancy. AC = Amniotic cavity; CL = chorion laeve; ECC = exocoleomic cavity; P = placenta; SYS = secondary yolk sac; U = uterus; UC = umbilical chord. Adapted from [29] with permission.

grows and obliterates the exocoelomic cavity (fig. 1b) considerably changing the maternal-fetal exchange pathways. From the 11 to 12th weeks of gestation and onward, maternal nutrients, including thyroid hormone, are transferred by the placenta directly into the fetal circulation.

The placenta plays an important role in the development and function of the thyroid gland in the fetus. The placenta produces various hormones that can influence the fetal thyroid gland (e.g. chorionic gonadotropin, TRH). The most important role of the placenta though is in regulating the passage of hormones and drugs, from the mother to the embryo, which influence the fetal thyroid gland. For many years its was unknown how the very small amounts of maternal T4 which are allowed to pass the placental barrier (sometimes as low as 1% of the maternal concentrations in the first trimester) can play such a major role in the normal fetal physiology of the developing brain and fetal tissues. The answer came from studies which showed that fetal concentrations of total T4 were misleading because the proportion of T4 that is not bound to proteins

(FT4) is much higher than in adult sera and the concentrations of T4 that are available to developing tissues reach values that are comparable to those know to be biologically active in their mothers [28, 29] (fig. 2). The T4-binding proteins and the concentrations of maternal T4 or FT4 that are allowed to pass the placental barrier determine the concentrations of FT4 in the fetal fluids and this is determined ontogenically. Therefore, it has become clear why an efficient barrier to complete maternal thyroid hormone transfer is necessary as the same concentrations that are available in the maternal sera might possibly be toxic to the developing fetal tissues [22, 30]. However, if the fetus is hypothyroid the placenta allows T4 from the mother to pass to the fetus in larger quantities [31].

In contrast to what happens with thyroid hormones the placenta allows the free passage of TRH and iodine from the mother to the fetus. As mentioned previously, if there is iodine insufficiency in the mother the neonate may develop severe psychomotor retardation [23–27]. Furthermore, the placenta allows the passage of certain drugs (propylthiouracil and methimazole) and immunoglobulins (like TSH-receptor-stimulating antibodies) from the mother to the fetus which can influence the function of the thyroid gland of the fetus and the neonate.

Hypothalamic-Pituitary-Thyroid Axis during Gestation

Thyrotropin-releasing hormone (TRH) in the fetal hypothalamus regulates the thyroid-stimulating hormone (TSH) in the pituitary gland early on in the fetus. Pituitary TSH can be detected for the first time around the 10–12th week of gestation. Its concentrations in the serum of the fetus are approximately 3–8 mIU/l from week 12 and increase gradually during the final weeks of gestation to 10–12 mIU/l. This is accompanied by a parallel increase in fetal thyroid radioiodine uptake and by a progressive increase in the serum concentrations of both total T4 and FT4. It is intriguing that TSH bioactivity is greatly increased with respect to that circulating in the mother in spite of the increasing FT4 concentrations in the fetus (fig. 2).

This confirms the hypotheses that fetal serum TSH is not of maternal origin, that it is not under hypothalamic neuroendocrine control by the fetal hypothalamus and that it is not under negative feedback control by the thyroid hormones [26]. This raises the question though of the origin of fetal TSH. There have been reports of synthesis of TSH by the rat and monkey brain [32]. Also primary cultures of human astrocytes and early human fetal brain have shown the presence of a TSH receptor in these areas of the brain [33]. This receptor mediates extrathyroidal cAMP-independent biological effects of TSH, among which is the stimulation of type II deiodinase in astroglial cells. It has been

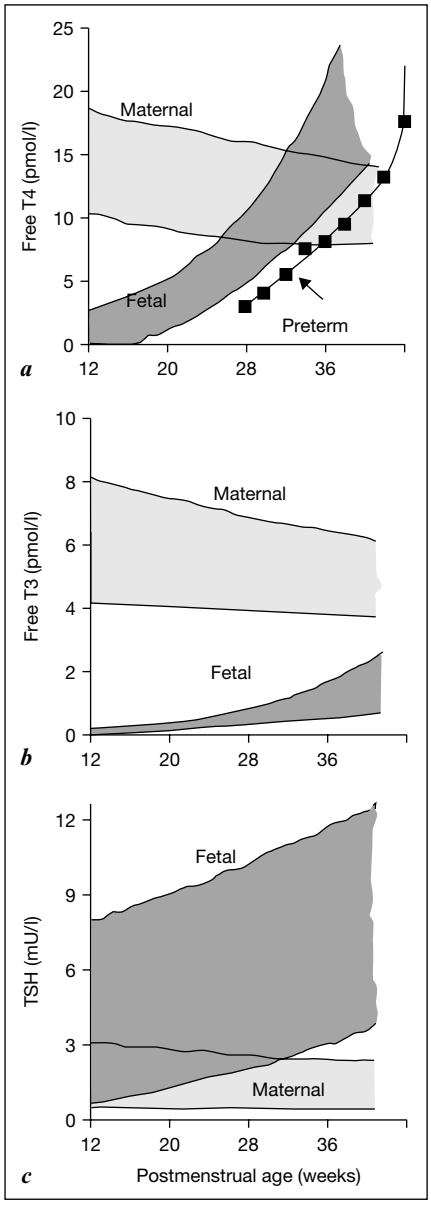

Fig. 2. Parameters of thyroid hormone status from 12 weeks Postmenstrual age until birth obtained in vivo by cordocentesis. ***a***, ***b*** Fetal FT4 serum concentrations reach maternal concentrations shortly after midgestation whereas those of FT3 are low throughout pregnancy. Also shows the FT4 levels found in sera from premature babies (■) as compared with those in utero. ***c*** Most fetal TSH levels are higher than those of the mother. Adapted from [17] with permission.

speculated that the possible extrathyroidal actions of TSH might be acting in brain development as a growth factor [34].

Thyroxine-binding globulin (TBG) also increases during this period as a result of the action of placental estrogens on the embryonic liver [35].

During the second trimester of gestation there is a gradual increase in the ratio of FT4/TSH in the embryonic serum [36]. The concentration of T3 in the serum of the fetus is low during the entire period of pregnancy because type I deiodinase has not matured yet [37].

The concentrations of TRH in the embryonic serum are higher than those in the mother because there is additional production of TRH by the placenta and because TRH is metabolized more slowly in the embryo [37]. It has been speculated that the reason for the significant decrease of the high TSH levels at birth may be the neonate's sudden severance from the placenta which produces high amounts of TRH-like peptides that might be stimulating extrapituitary synthesis of TSH or TSH-like proteins [26].

As mentioned earlier, the activity of the type I deiodinase is low during the entire period of pregnancy and consequently the concentrations of T3 in the fetus are low, i.e. 50–60 ng/dl when the neonate is born. The reason T3 is low during fetal life is not known but it is thought that it is low in order to avoid thermogenesis in the fetal tissues and in order to facilitate the anabolic functions of the rapidly developing fetus [36].

In contrast, types II and III deiodinase, which are expressed in the brain and the pituitary of the fetus, are activated mid-way through gestation. Consequently, the levels of T3 in the fetal brain are 60–80% of those in adults already from the 20–26th week of gestation despite T3 concentrations being low in the serum of the fetus [30]. If the fetus is hypothyroid then the action of type II deiodinase increases in the brain of the fetus while the action of type III deiodinase decreases. The reason for this is so that larger quantities of T3 can be produced in order to protect the brain as long as there are physiological levels of T4 in the mother [38].

Action of the Thyroid Hormones

The action of the thyroid hormones in the adipose tissue, the liver, the heart, the muscles and the bones are expressed during neonatal but not fetal life. It is not known whether this delay in the action of the thyroid hormones in these tissues is related to the maturation of the thyroid hormone signaling pathway at a molecular level or related to the maturation of thyroid hormone metabolism. The actions of the thyroid hormones, which are specific to each individual tissue, depend on the prevalent isoform of the thyroid hormone receptor (TR) which is expressed in each tissue and on cofactors at the site of action of the thyroid hormones. The highest concentrations of TRs are found in developing neurons and in various regions in the brain of the fetus and the neonate such as cortex, cerebellum, and visual and auditory cortices. There are many indications

that the TRβ1 isoform of the receptor is the one which promotes, via T3, the vital development of the central nervous system in the fetus, the neonate and the child in combination with the α1 receptor [39, 40]. It is of interest that the deafness that exists in the TRβ1 knockout rat also exists in endemic cretinism in some patients with resistance to thyroid hormones due to lack of the TRβ1 gene [41].

Thyroid Synthesis in the Full-Term Newborn

During the birth process many changes occur in the function of the thyroid gland in the full-term neonate. The most dramatic change is the abrupt increase in TSH which takes place in the first 30 min after parturition which can reach levels of 60–70 mIU/l. This increase causes a major stimulation of the thyroid gland with an increase in T4 in the serum by about 50% and a 3- to 4-fold increase in T3 within 24 h [36, 37]. Studies in experimental animals have shown that the increase in TSH is a consequence of the relative hypothermia that exists in the environment outside of the uterus. The increase in T3 occurs not only because TSH levels increase but also because of an increase in the action of type I deiodinase during birth. The high levels of reverse T3 (rT3) decrease relatively quickly during the neonatal period. The increase in the action of type II deiodinase causes an increase in T3 in the adipose tissue of the neonate which is necessary for thermogenesis and the synthesis of proteins in the neonate [31, 37].

Within the thyroid gland during the neonatal period in the full-term infant it has been shown that both the colloid content in the neonatal thyroid tissue and the amount of iodine in extracted proteins display transient variations.

Thyroid Synthesis in the Pre-Term Newborn

The function of the thyroid gland in the pre-term neonate reflects the immaturity of the hypothalamic-pituitary-thyroid axis which corresponds to the week of gestation of the pre-term neonate. There is a gradual increase in the concentration of TSH, TBG, T3 and T4 during gestation [42, 43]. After parturition there is an increase in T4 and TSH just as in full-term neonates, but the increase is much smaller in pre-term neonates than what it is in the full-term neonates and there is a dramatic decrease in the concentration of T4 during the following 1–2 weeks [44]. This decrease in T4 is more important in low birthweight and significantly premature neonates (<1.5 kg and <30 weeks of gestation) where the level of T4 may not be detectable [44, 45]. In most cases though total T4 is influenced and not FT4 as much since TBG is low in pre-term neonates due to

immaturity of the liver. Another reason for the fall in T4 in pre-term neonates is the reduced storage of iodine which exists due to the prematurity [31]. Preterm neonates have greater difficulty in maintaining a positive iodine balance than full-term neonates because pre-term neonates lose large quantities of iodine in the urine and because their iodine uptake system is immature [45–47]. Also because the requirement for thyroid hormones is considerably enhanced within the first few months of life it is normal that the turnover rate of thyroidal iodide increases. Even in the presence of TG with normal hormone content, the renewal rate of the intrathyroidal pool of T4 has to be very rapid to provide the premature infant with a normal hormone supply. This could be an important factor for increased risk of neonatal hypothyroxinemia in very premature infants [47].

Due to the immaturity of the thyroid gland preterm neonates have a reduced ability of adjusting to excessive amounts of iodine which are found in skin antiseptics which contain iodine and are frequently used in preterm neonatal units. That is why it is recommended that these should not be used.

Additionally, rT3 remains at higher levels and T3 remains at low levels for a longer time in pre-term than in full-term neonates because type I deiodinase is immature [31].

Function of the Thyroid Gland in the Neonate and Infant

After the large increase in the serum concentrations of the thyroid hormones and TSH which occur during the first days of neonatal life there is a gradual decrease in the levels of T4, T3 and TSH during the life of the neonate and infant. The most important difference between this period and adult life is that there is a larger production and utilization of T4 in the neonates and infants. The neonates produce 5–6 μg/kg/day of T4 with a gradual decrease during the first years of life to reach levels of 2–3 μg/kg/day of T4 at 3–9 years of age. This is in contrast to adults who produce 1.5 μg/kg/day of T4 [42].

The weight of the neonatal thyroid gland is a good indicator of maternal iodine intake during pregnancy. On an adequate maternal iodine intake, the weight of the neonatal thyroid is less than 1.5 g [48]. Due to the increased turnover of iodine and consequently of thyroglobulin in the early neonatal period, decreased iodine intake will bring about an increased consumption of reserve colloid, as a result of the increased activity of the follicular cells which would at the beginning cause a decrease in thyroid weight. Then due to the iodine deficiency, after prolonged TSH stimulation there might be hypertrophy of the follicular epithelium and hyperplasia may occur leading to an increase in thyroid weight. In areas of severe iodine deficiency, average thyroid weights in term newborn infants are approximately 3 g [49].

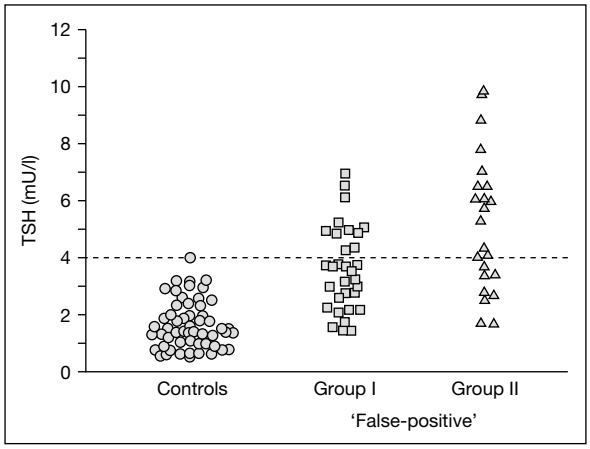

Fig. 3. A serum TSH concentration higher than normal (>3.9 mIU/l or the 99.7th percentile of the concentrations obtained in control infants) was found at 16–44 months of age in 28 of 56 infants who had high TSH at birth but normal FT4 concentrations. Adapted from [51] with permission.

The size of the normal thyroid gland increases gradually by approximately 1 g per year until the age of 15 when it has reached adult size, i.e. 15–20 g [31].

Recent studies have shown that neonates with pathological levels of TSH in the first days of life which have physiological levels of TSH at follow-up within the first or second month of life (transient hypothyroidism) have a 70% chance of having mild thyroid gland dysfunction (subclinical hypothyroidism) at 16 months of age and older (fig. 3). It is worthy to note that there is a high prevalence of antithyroid antibodies in the children who are false-positive at screening [50, 51].

It has also recently been shown that when there is intrauterine growth retardation, when neonates have a low birthweight and are short for gestational age (SGA), there is a considerable decrease in free T4 and free T3, and a moderate increase in TSH in childhood especially in the children that show blunted 'catch-up' growth [51–53]. Additionally, a significant reduction in the expression of thyroid receptor isoforms in the central nervous system of the SGA neonates was found which jeopardizes psychomotor development [54]. The reason for these changes seems to be that in SGA neonates, due to poor nutrition of the embryo, there is an intrauterine reprogramming of certain organs (such as the pancreas, liver and muscles) in order for the embryo to survive, and this reprogramming appears to include the thyroid gland [52]. The reprogramming of these organs in the SGA neonates appears to be permanent.

In conclusion, thyroid function and subsequent normal neurodevelopment in the neonate is greatly influenced by the conditions present in the fetal-maternal unit during gestation which are dependent upon normal maternal iodine and thyroxine status as well as a good nutritional capacity of the fetus.

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Pediatric Aspects of Thyroid Function and Iodine

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Iodine is a nonmetallic micronutrient present in the human body in minute amounts (15–20 mg), almost exclusively in the thyroid gland. It is an essential component of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3), comprising 65 and 59% of their respective weights. Thyroid hormones, and therefore iodine, regulate many key biochemical reactions, especially protein synthesis and enzymatic activity. They also play a determining role in the process of early growth and development of most organs, especially that of the brain, which occurs in humans during the fetal and first 2–3 years of postnatal life. Consequently, iodine deficiency, if severe enough to affect thyroid hormone synthesis during this critical period, will result in hypothyroidism and brain damage. The clinical consequence will be irreversible mental retardation [1].

Iodine is found in relative abundance in marine plants and animals, in the thyroid gland of vertebrates, in deposits of organic origin, in certain natural mineral water, in sedimentary phosphate rock, and in association with certain mineral deposits. Most of the Earth's iodine is found in its oceans and most of the iodine ingested by humans comes from food of animal and plant origin. This iodine, in turn, is derived from the soil. In general, the older an exposed soil surface, the more likely the iodine has been leached away by erosion. Only a relatively small fraction is derived from drinking water. A most important factor in the depletion of iodine has been glaciation, which removes old soil and scrapes bare the virgin rocks, which have iodine concentrations far lower than those of the covering soil. This situation is found in regions that remained longest under Quaternary glaciers and lost their iodine when the ice thawed. Mountainous regions, such as those found in the Himalayas, the Andes, the Alps, Vietnam, China, Indonesia and Africa and also in flooded river valleys,

Table 1. Recommended iodine allowance (RDA) and urinary iodine concentration for different age groups (adapted from [6–8])

Life stage	Age	Estimated iodine intake μg/day	Urinary iodine concentration μg/l
Premature infants	0–6 months	90	<100
Term infants	0–6 months	90	<100
Children	6–12 months	90	<100
Children	1–3 years	90	100–150
Children	4–8 years	90	100–150
Children	9–13 years	120	100–150
Adolescents	14–18 years	150–200	100–200
Adults	19 years and older	150–299	100–299
Pregnant women	all ages	230–300	200–300
Lactating women	all ages	260–300	200–300
Recent ICCIDD RDA for pregnant and lactating women	all ages	250–350	200–300

such as the Ganges, are among the most severely iodine-deficient areas in the world [2, 3].

Optimal Iodine Intake

The recommended daily iodine intake is variable depending the age of the subject (table 1). The recommended intake of iodine in neonates reflects the observed mean iodine intake of young infants exclusively fed human milk in iodine-replete areas [4, 5]. However, it is well established that the iodine content of breast milk is critically influenced by the dietary intake of the pregnant and lactating mother [4, 5]. The iodine intake required in order to achieve a positive iodine balance and to insure a progressively increasing intrathyroidal iodine pool in the growing infant is at least 15 μg/kg/day in full-term and 30 μg/kg/day in preterm infants; this corresponds approximately to 90 μg/day [6].

These recommendations derive from consensus statements by several groups, including the International Council for Control of Iodine Deficiency Disorders, the World Health Organization, UNICEF, and the Food and Nutrition Board of the US National Academy of Sciences. The amounts are based on the following: the calculated daily thyroid hormone turnover in euthyroidism, the iodine intake producing the lowest values for serum thyrotropin (TSH) and for serum thyroglobulin (TG), the amount of thyroid hormone replacement

necessary to restore euthyroidism to athyreotic subjects, the iodine intake associated with the smallest thyroid volumes in populations, and the lowest incidence of transient hypothyroidism in neonatal screening with blood spot TSH. About 90% of iodine is eventually excreted in the urine. The median urinary iodine concentration in casual ('spot') samples, expressed as micrograms per liter (μg/l), is currently the most practical biochemical laboratory marker of community iodine nutrition. It is more useful and much simpler than measuring 24-hour samples or calculating urinary iodine/creatinine ratios. Recommendations by the International Council for the Control of Iodine Deficiency Disorders, WHO, and UNICEF [9] set 100 μg/l as the minimal urinary iodine concentration for iodine sufficiency. This figure corresponds roughly to a daily intake of 150 μg iodine. The upper limit for safe iodine intake is uncertain and varies widely among individuals and populations. Occasional intake up to 1 mg iodine per day may be safe for most people, and much higher amounts are usually tolerated for a brief period of time, without major problems.

Iodine Deficiency

When the aforementioned physiological requirements are not met in a given population, a series of functional and developmental abnormalities occur, including thyroid function abnormalities.

Iodine deficiency is now accepted as the most common cause of preventable brain damage in the world. According to the World Health Organization (WHO), iodine deficiency disorders (IDD) affect 740 million people throughout the world, and nearly 50 million people suffer from some degree of IDD-related brain damage. The spectrum of IDD includes endemic goiter and cretinism, endemic mental retardation, decreased fertility rate, increased perinatal death and infant mortality, and varying degrees of other growth and developmental abnormalities (table 2). Nearly 2.2 billion people throughout the world live in areas of iodine deficiency and risk its consequences. Major international efforts have produced dramatic improvements in the correction of iodine deficiency in the 1990 decade mainly through the use of iodized salt and iodized vegetable oil in iodine deficient countries [7].

The mechanism by which the thyroid gland adapts to an insufficient iodine supply is to increase the trapping of iodide as well as the subsequent steps of the intrathyroidal metabolism of iodine leading to preferential synthesis and secretion of T3. They are triggered and maintained by increased secretion of TSH, which is ultimately responsible for the development of goiter. The acceleration of the main steps of iodine kinetics and the degree of hyperstimulation by TSH are much more marked in the pediatric age groups, including neonates, and the

Table 2. The spectrum of IDD across the life-span (adapted from [10])

Fetus	abortions deaf mutism stillbirths congenital anomalies increased perinatal mortality endemic cretinism deaf mutism
Neonate	neonatal goiter neonatal hypothyroidism endemic mental retardation increased susceptibility of the thyroid gland to nuclear radiation
Child and adolescent	goiter (subclinical) hypothyroidism impaired mental function retarded physical development increased susceptibility of the thyroid gland to nuclear radiation
Adult	goiter, with its complications hypothyroidism impaired mental function hyperthyroidism in the elderly (after iodized salt)

development of goiter appears as an unfavorable side effect in the process of adaptation to iodine deficiency during growth, because it leads to a vicious cycle of iodine loss and defective thyroid hormones synthesis [11] (table 3).

Endemic goiter is one of the earliest and most visible sign of iodine deficiency [12]. According to iodine deficiency level this response may be adequate to preserve euthyroidism, but at the cost of an enlarged thyroid and the attendant risks of neck compression and eventual hyperfunctioning autonomous nodules with hyperthyroidism. An insufficient adaptation in adults produces hypothyroidism with its usual clinical stigmata. The damage is greater when iodine deficiency provokes hypothyroidism during fetal or early postnatal life, because thyroid hormone is necessary for proper development of the central nervous system, particularly its myelination. Individuals who were hypothyroid at this critical period frequently have permanent mental retardation, which cannot be corrected by later administration of thyroid hormone or iodine.

Most of the populations which live in areas of iodine deficiency are in developing countries, but many in the large industrialized countries of Europe

Table 3. Summary of mechanisms involved in the adaptation to iodine deficiency (adapted from [32])

Increased thyroid clearance of plasma inorganic iodine
Hyperplasia of the thyroid and morphologic abnormalities
Changes in iodine stores and thyroglobulin synthesis
Modifications of the iodoamino acid content of the gland
Enrichment of thyroid secretion in T3
Enhanced peripheral conversion of T4 to T3 in some tissues
Increased thyroid-stimulating hormone production

are also affected. Correcting this public health problem is the goal of a massive global campaign that is showing remarkable progress so far. But despite its importance to most other countries, iodine deficiency receives little attention in the United States because its elimination years ago has been widely assumed [3].

Health Consequences of Iodine Deficiency by Developmental Stage

Prenatal Development

Fetal iodine deficiency is caused by iodine deficiency in the mother. The result of iodine deficiency during pregnancy is impaired synthesis of thyroid hormones by the mother and the fetus. An insufficient supply of thyroid hormones to the developing brain may ensue in mental retardation [13–15].

An important issue on thyroid function and regulation in the fetus is the concept that during the first half of gestation the thyroid hormone available to the fetus is predominantly of maternal origin. T4 from the mother is the most important source of T3 for the fetal brain and protects it from a possible hormone deficiency until birth [16, 17]. Once the fetal thyroid secretion starts, fetal supplies are of mixed fetal and maternal origin. Although fetal thyroidal secretion is believed to constitute an increasing proportion of the hormone available to the developing fetus, maternal transfer of T4 may still contribute significantly to fetal needs (20–50% of normal values) up to term, mitigating the consequences of inadequate fetal thyroid function [17, 18]. The iodine content of the fetal thyroid increases progressively from less than 2 μg at 17 weeks of gestation up to 300 μg at term [6].

In conditions of mild iodine deficiency (iodine intake: 50–99 μg/day) [12], the serum levels of free T4 steadily decrease during gestation while, in iodine sufficiency, there is only a slight (15%) decrease by the end of gestation. As a consequence, serum TSH levels increase progressively. This situation of chronic thyroid hyper stimulation results in an increase in serum TG and in an increase in thyroid volume by 20–30% during gestation, a figure twice higher than in conditions of normal iodine supply [19].

In moderate iodine deficiency (iodine intake: 20–49 μg/day), the anomalies are of the same nature but more marked. The few studies conducted in populations with severe iodine deficiency [13] showed that the prevalence of goiter reaches peak values of up to 90% in females of child bearing age and that during pregnancy, serum T4 is extremely low and serum TSH is extremely high. Comparative studies carried out in New Guinea and the Democratic Republic of Congo showed that, in spite of the fact that the two areas are submitted to a similar degree of severe iodine deficiency (iodine intake <20 μg iodine/day), serum T4 in pregnant women is much higher in the Congo (8.0 μg/dl) than in New Guinea (3.0–5.0 μg/dl). This discrepancy was understood only when it was demonstrated that in the Congo, iodine deficiency is aggravated by selenium deficiency and thiocyanate overload.

Accordingly, iodine deficiency results in relative hypothyroxinemia during pregnancy, thus leading to enhanced thyroidal stimulation (through the TSH feedback mechanisms) and goitrogenesis in both the mother and fetus. Goiter formation is the most directly 'visible' consequence of iodine deprivation, and pregnancy should therefore be viewed as an environmental factor to trigger the glandular machinery and induce functional and anatomical abnormalities of the thyroid in areas with a reduced iodine intake.

Newborns and Infants

Infant mortality is increased in areas of iodine deficiency, and several studies have demonstrated an increase in childhood survival when iodine deficiency is corrected [20]. Infancy is a period of rapid brain growth and development. Even in the absence of congenital hypothyroidism, iodine deficiency during infancy may result in abnormal brain development and, consequently, impaired intellectual development [21].

In mild iodine deficiency, the serum concentrations of TSH and TG are still higher in neonates than in their mothers. The frequency distribution of neonatal TSH on day 5, at the time of systematic screening for congenital hypothyroidism, is shifted towards elevated values. The frequency of values above 5 μU/ml (blood) is 4.5%, while the normal value is below 3% [22].

In moderate iodine deficiency, the anomalies are of the same nature, but more drastic. The frequency of neonatal TSH above 20–25 μU/ml (blood), that is above the cut-off point used for recalling the neonates because of suspicion of congenital hypothyroidism in programs of systematic screening for congenital hypothyroidism, is increased. This frequency is inversely related to the median urinary iodine of populations of neonates used as an index of their iodine intake. In addition, transient neonatal hypothyroidism can occur with a frequency approximately 6 times higher in Europe than in the United States, where the iodine intake is much elevated.

In severe iodine deficiency, the biochemical picture of neonatal hypothyroidism is exaggerated. In Congo, as many as 11% of the neonates have both a cord serum TSH above 100 μU/ml and a cord T4 below 3.0 μg/dl, i.e. a situation similar to the one found in thyroid agenesis.

The changes in neonatal TSH and thyroid function in the neonates in all conditions of iodine deficiency are much more frequent and severe than in their mothers. The hypersensitivity of neonates to iodine deficiency is explained by their very small intrathyroidal iodine pool, which requires increased TSH stimulation and a fast turnover rate in order to maintain a normal secretion of thyroid hormones.

The most important and frequent alterations of thyroid function due to iodine deficiency in Europe occur in neonates and young infants. The frequency of transient primary hypothyroidism is almost 8 times higher in Europe than in North America [23]. This syndrome is characterized by postnatally acquired severe primary hypothyroidism lasting for a few weeks and requiring substitutive therapy [24]. The risk of transient hypothyroidism in neonates increases with the degree of prematurity [25]. The specific role played by iodine deficiency in the etiology of this type of hypothyroidism is demonstrated by the disappearance of neonatal transient thyroid failure in Belgian pre-terms following systematic supplementation with 30 μg potassium iodide/day. In Toronto, where the iodine intake is elevated, the iodine content of the thyroid in full-term infants is 292 μg. In Brussels, with a borderline iodine intake, the iodine content of the thyroid is 81 μg and in Leipzig, which used to be severely iodine-deficient, the content is only 43 μg. As the turnover rate of intrathyroidal iodine is markedly accelerated in iodine-deficient neonates, thyroid failure is more likely to occur. These neonatal data contrast with adult data which have shown that the iodine stores of the thyroid are not affected by iodine deficiency unless the degree of deficiency is severe [8].

Contrasting with the plentiful data on the consequences of iodine deficiency on thyroid function during pregnancy, in the neonate and in adults, there are few data on the impact of the deficiency on thyroid function in the young infant.

In conditions of mild iodine deficiency, as indicated earlier, the frequency distribution of neonatal TSH is shifted towards elevated values and the frequency of transient hyperthyrotropinemia and transient primary hypothyroidism is much higher than in iodine-replete areas [24]. In particular, thyroid function of preterm infants is characterized by a biochemical picture including low total and free T4, elevated TSH and exaggerated TSH response to TRH. This picture of primary subclinical hypothyroidism is in contrast with the picture of tertiary hypothyroidism evidenced in preterm infants in iodine-replete areas, characterized by the fact that TSH remains normal in spite of low free T4.

In conditions of severe iodine deficiency, the data in infants are still scantier: in Congo, it was found that the frequency of biochemical signs of congenital hypothyroidism (9.0%) was as frequent in infants aged 5 days as in neonates [26]. Follow-up studies showed that in some of these infants, the signs spontaneously corrected within a few weeks. The transient character of hypothyroidism in some of these infants may explain why the incidence of congenital hypothyroidism (close to 10%) is almost ten times higher than the prevalence of myxedematous endemic cretinism in the general population of the Ubangi area of northern Congo (1%). Another factor could be the high mortality rate of hypothyroid newborns and young infants [26]. It was proposed that transient neonatal and infantile hypothyroidism in Congo resulted in endemic mental retardation while permanent hypothyroidism occurring during this critical period resulted in the long-term development of endemic cretinism [26].

Mechanisms of Brain Damage due to Iodine Deficiency during the Perinatal Period

As mentioned, thyroid hormones are crucial for brain development both during fetal and early postnatal life [14]. Type II 5′-iodothyronine deiodinase (DIO_2) activity, which generates T3 from T4, is found during this period in the human fetal cerebral cortex [27]. The effects of T3 on the central nervous system are mediated by the regulation of the expression of genes that synthesize proteins implicated in cerebral neurogenesis, neuronal migration and differentiation, axonal outgrowth, dendritic ontogeny and synaptogenesis. They are also necessary for cerebellar neurogenesis (predominantly during early postnatal life), gliogenesis (predominantly during late fetal life to 6 months postnatally) and myelogenesis (during the second trimester of gestation to 2 years of postnatal life). From clinical studies on the effect of iodine deficiency of both mother and fetus it becomes clear that T4 is required for brain development during gestation [18, 28, 29]. Low T4 levels during neonatal life, especially if persistent, could be a negative factor contributing to the neurodevelopmental problems of very preterm infants. Indeed, retrospective studies have shown a relationship between hypothyroxinemia and developmental delay and a increased risk of disabling cerebral palsy [30, 31]. In agreement, the most dramatic consequence of iodine deficiency on brain and physical development is endemic cretinism [32]. This is a polymorphous clinical entity, which happen in remote, underdeveloped areas of the Third World and may affect up to 15% of populations living in conditions of severe iodine deficiency [33, 34] (fig. 1).

The disorder is found in India, Indonesia, China, Oceania (Papua New Guinea), Africa (Congo), and South America (Ecuador, Peru, Bolivia). In all these locations, with the exception of Congo, neurological features are predominant [33, 35]. Endemic cretinism may be defined essentially by severe and irreversible

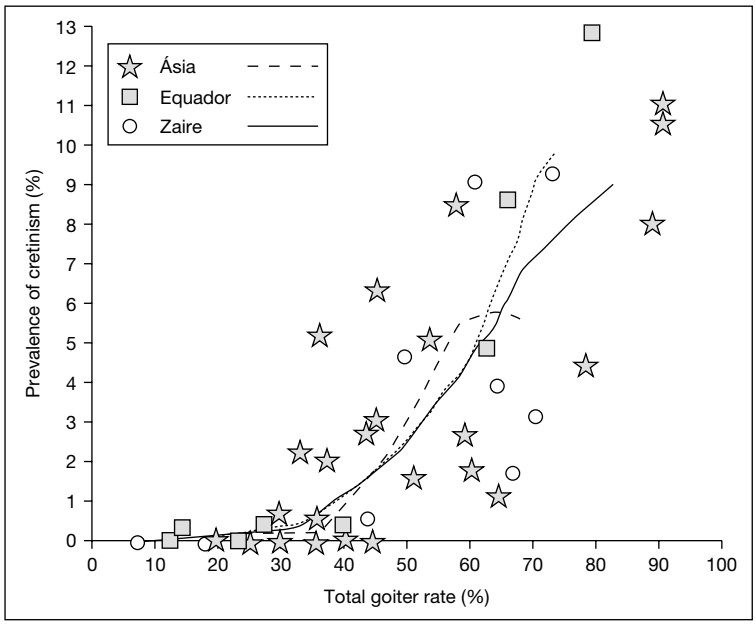

Fig. 1. As the total goiter rate (TGR) increases in a given population due to chronic iodine deficiency there is sharp increase in the prevalence of endemic cretinism (% of all newborns). As depicted in this figure there is no significant difference between geographical areas (Asia, South America and Africa). Adapted from [92].

changes in mental development in individuals born in an area of endemic goiter; such individuals exhibit a combination of some of the following characteristics not explained by other causes: (1) a predominantly neurological syndrome consisting of defects of hearing and speech associated or not with characteristic disorders of stance and gait of varying degree; (2) stunted growth; (3) mental deficiency; (4) hypothyroidism, and (5) sexual immaturity. In its fully developed form, mental deficiency, deaf-mutism, and motor spastic diplegia are associated with or without goiter. This condition is referred to as the *neurological form* of endemic cretinism, in contrast to the myxedematous form [34, 36] (fig. 2). The typical myxedematous cretin has mental retardation, severe hypothyroidism and non palpable thyroid. This division of the syndrome into two broad categories has been the subject of some confusion and disagreement which undoubtedly originates from the repeated observation of the occurrence of neurological signs in myxedematous cretins, indicating that the two physiognomic forms of the syndrome varied from one geographical area to another with mixed clinical characteristics. Although the myxedematous type is more

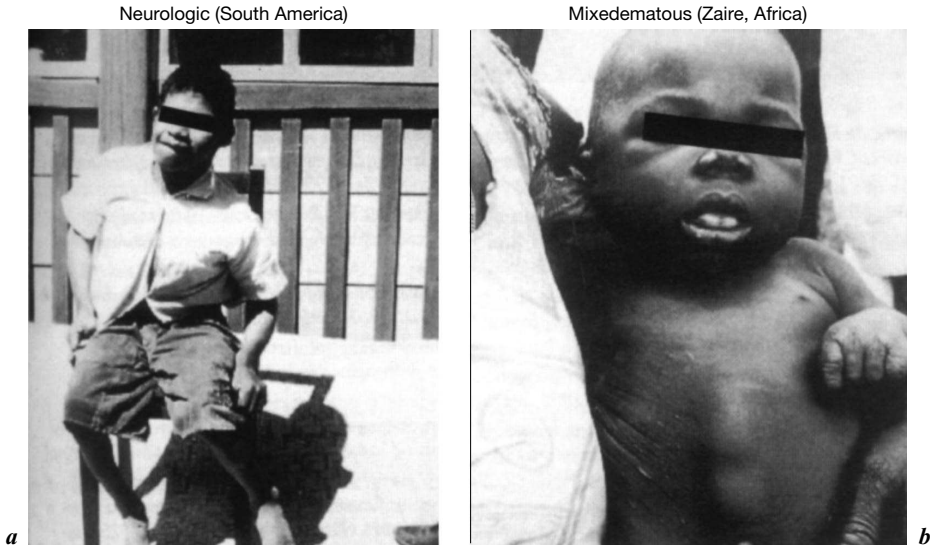

Fig. 2. ***a*** The predominantly neurological syndrome of Endemic Cretinism consists in mental deficiency, hearing defect (resulting in deaf-mutism), motor spastic diplegia, with characteristic disorders of stance and gait as it was seen in this young boy from Equador. ***b*** The typical myxedematous cretin has mental and physical retardation, severe hypothyroidism and non palpable thyroid (the child from Zaire, Africa). Adapted from [32].

common in Congo, the condition may be found in the Himalayas, the Hetian and Luopu districts of Xing-Jiang (China), Sicily (Italy), and South America (Bolivia and Peru) (fig. 2).

Based on epidemiological studies and on experimental aspects of thyroid homeostasis during the perinatal period in conditions of iodine deficiency it was possible to reconcile the physiopathologic events to explain the clinical picture features of endemic cretinism [13–15].

In severe iodine deficiency, maternal hypothyroidism does occur during pregnancy and the contribution of maternal thyroxine to the saturation of the T3 receptors of the brain of the growing fetus is decreased, resulting in the development of the neurological features of endemic cretinism. The particular pattern commonly found in Africa (i.e. myxedematous cretinism) might be explained by the fact that in this area iodine deficiency is complicated by selenium deficiency. Selenium deficiency results in the accumulation of peroxide in the hyperstimulated thyroid glands, and excess peroxide induces thyroid cell destruction, thus leading to parenchymal fibrosis and hypothyroidism [37]. On

the other hand, deficiency in type I 5-desalogenase (DIO1) in pregnant mothers induced by selenium deficiency causes decreased catabolism of T4 to T3 and thus increased availability of maternal T4 for the fetus and its brain [26]. These aspects explains why in situations characterized by isolated severe iodine deficiency such as New Guinea, China, Indonesia and Thailand, the clinical picture of endemic cretinism is characterized by a dominant neurological picture and why, when selenium deficiency and SCN overload are added, as in Congo, the neurological signs are mitigated and the picture is dominated by severe hypothyroidism. The role of SCN in the etiology of endemic cretinism in Africa has been proposed because of the observation that people in areas with severe uniform iodine deficiency exhibit cretinism only when a certain critical level threshold in the dietary supply of SCN (through cassava consumption, a staple food in these areas) is reached. The action of SCN is entirely due to an aggravation of iodine deficiency resulting in fetal hypothyroidism [26].

Thus, the neurological form is the result of maternal iodine deficiency that affects the fetus before its own thyroid is functional. In the myxedematous or hypothyroid form in addition to iodine deficiency, selenium deficiency and the presence of goitrogens (SCN) in the diet interfere with thyroid hormone production [38]. Endemic cretinism, therefore, constitutes the extreme expression of a spectrum of abnormalities in the physical and intellectual development in children, as well as diminished functional capacity of the thyroid gland, observed in inhabitants of areas with severe iodine deficiency and endemic goiter (fig. 3).

Children and Adolescents

Iodine deficiency in children and adolescents is often associated with goiter. The incidence of goiter peaks in adolescence and is more common in girls. School children in iodine deficient areas show poorer school performance, lower IQs, and a higher incidence of learning disabilities than matched groups from iodine-sufficient areas. A recent meta-analysis of 18 studies concluded that iodine deficiency alone lowered mean IQ scores in children by 13.5 points [39, 40].

The view that endemic goiter constitutes the most efficient mechanism of adaptation to iodine deficiency is based, with a few exceptions [41] on information available only in adults. But a study of the time course as a function of age from 3 to 22 years of the main variables exploring thyroid function in two populations submitted to a similar degree of iodine deficiency, but with markedly different prevalences of goiter, showed that goiter constitutes, rather, an unfavorable side effect to the mechanism of adaptation to iodine deficiency which is increased trapping of iodide by the thyroid, as indicated by an elevated thyroidal uptake of radioiodine [41]. It was also shown that the highest values of serum TSH were observed in the youngest infants and children in spite of the fact that they had also

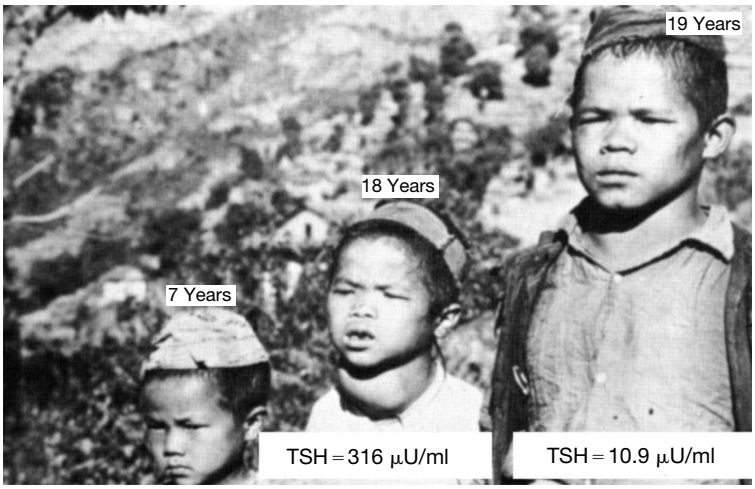

Fig. 3. The spectrum of clinical presentation of iodine deficiency in children and adolescents. The three siblings lived all their existence in iodine deficiency area of Nepal. The eldest brother has mental retardation and hearing was impaired. A small goiter was present and hypothyroidism considered relatively mild. The second sibling has severe hypothyroidism stunted growth, a very large goiter, severe mental retardation, spastic diplegia and was deaf-mute. The youngest brother had no goiter or hypothyroidism although stunted growth was present with mild degree of mental retardation. Adapted from [93].

the highest serum T4 values. These variations of the TSH/T4 ratio as a function of age could reflect the increase with age of the iodine content of the thyroid and/or changes in the sensitivity of the thyroid to TSH [41].

Euthyroid pubertal goiter is especially frequent in adolescents and occasionally requires substitutive therapy by T4 or iodide. Its main cause is iodine deficiency although thyroiditis has to be carefully considered [42]. Iodine metabolism is accelerated during this period of life [8]. A very important issue is the demonstration that even in Europe today, clinically euthyroid schoolchildren born and living in an iodine-deficient environment exhibit subtle or even overt neuropsychointellectual deficits as compared with controls (not subjected to iodine deficiency) living in the same ethnic, demographic, nutritional and socioeconomic system. These deficits are of the same nature as those found in schoolchildren in areas with severe iodine deficiency and endemic mental retardation, although they are less marked [13]. As demonstrated in severe endemic goiter, such deficits could result from transient thyroid failure occurring during fetal or early postnatal life, i.e. during the critical period of brain development [43].

Nutrient Interactions

Besides selenium shortage [44], deficiencies of vitamin A, zinc or iron may also exacerbate the effects of iodine deficiency [7].

Deficiencies of selenium [45] and iron [46] can act in concert with iodine deficiency to impair thyroid metabolism and modify the response to prophylactic iodine [47]. Iron deficiency impairs thyroid hormone synthesis by reducing activity of heme-dependent thyroid peroxidase. Iron deficiency anemia blunts and iron supplementation improves the efficacy of iodine supplementation [48]. The clinical consequences of selenium deficiency include cardiomyopathy (Keshan disease) [49], which is caused by a Coxsackie B virus infection under conditions of selenium deficiency without concomitant iodine deficiency [50], hypothyroid cretinism (in some parts of central Africa) and Kashin-Beck disease, an osteoarthropathy of the hands, fingers, elbows, knees, and ankles in children and adolescents [51]. Recent studies in Tibet have suggested that this disorder results from a combination of selenium and iodine deficiency [52]. One possibility is that necrosis of the growth plate and epiphyseal chondrocytes is dependent on locally produced T3 and sensitive to oxidative damage. Thus, deficiency of iodothyronine deiodinase and GPx might result in local thyroid hormone deficiency and cellular injury, a combination that causes chondronecrosis.

Vitamin A supply affects thyroid function. The most vulnerable groups are women of reproductive age and young children [53]. In rural Côte d'Ivoire, 32–50% of school-age children suffer from both vitamin A deficiency and goiter [45]. In northern Morocco, 41% of children have vitamin A deficiency and 50% are goitrous [54]. In animals, vitamin A deficiency has multiple effects on thyroid metabolism. Vitamin A deficiency decreases thyroidal iodine uptake, impairs thyroglobulin synthesis, and increases thyroid size. In the periphery, vitamin A deficiency increases free and total circulating thyroid hormone, and binding of transthyretin to retinol-binding protein decreases vitamin A turnover and enhances vitamin A delivery. Centrally, because retinoic acid suppresses transcription of the pituitary TSH-β gene through activation of the retinoid X receptor, vitamin A status may modulate T4 feedback of TSH secretion. Vitamin A deficiency in rats increases pituitary TSH beta mRNA and TSH secretion; both return to normal after treatment with retinoic acid [55].

Although the literature has limited information, zinc status seems to affect the metabolism of thyroid hormones [56] and zinc supplement also appears to induce a cellular iron deficiency and, possibly, further reduce iron status [57]. In zinc-deficient rats, decreased DIO1 activity, lower T3 and free T4 serum concentrations, and marked alterations of follicular cellular architecture, including signs of apoptosis, were found [58].

Goitrogens

Some foodstuffs (cassava, millet, babassu coconut, piñon, vegetables from the genus *Brassica* and soybean) contain substances that interfere with iodine utilization or thyroid hormone production, known as goitrogens [31, 59]. The goitrogenic factor in cassava is related to the hydrocyanic acid liberated from the cyanogenetic glucoside (linamarin) and endogenously changed to thiocyanate, which competitively inhibits trapping and promotes the efflux of intrathyroidal iodine. Pearl millet is one of the most important food crops in the semiarid tropics (large portions of Africa and Asia) [60]. Millet porridge is rich in C-glucosylflavones and also contains thiocyanate. Both are additive in their antithyroid effects. Babassu coconut is largely consumed in northern Brazil, and studies have demonstrated the possible presence of flavonoids in the edible part of the nut [32]. Thus, in areas where millet and babassu coconut are a major component of the diet, their ingestion may contribute to the genesis of goiter. Furthermore, flavonoids, besides being potent inhibitors of thyroid peroxidase, also interact with thyroid hormone at the peripheral level. From turnips the compound 1–5-vinyl-2-thiooxazolidone (VTO, goitrin) was isolated; it is similar in action and potency to synthetic antithyroid drugs. The soybean isoflavones, genistein and daidzein, have also been found to inhibit thyroid hormone synthesis [61, 62]. Most of these goitrogens are not of clinical importance unless they are consumed in large amounts or there is coexisting iodine deficiency. Recent findings also indicate that tobacco smoking may be associated with an increased risk of goiter in iodine-deficient areas [63].

Individuals at Risk of Iodine Deficiency

While the risk of iodine deficiency for populations living in iodine-deficient areas without adequate iodine fortification programs is well recognized, concerns have been raised that certain subpopulations may not consume adequate iodine in countries considered iodine-sufficient. Vegetarian and nonvegetarian diets that exclude iodized salt, fish, and seaweed have been found to contain very little iodine [64, 65]. Urinary iodine excretion studies suggest that iodine intakes are declining in Switzerland, New Zealand, and the US, possibly due to increased adherence to dietary recommendations to reduce salt intake. Although iodine intake in the US remains sufficient, further monitoring of iodine intake has been recommended [66, 67].

Iodine Excess

The thyroid gland has intrinsic regulatory mechanisms that maintain normal thyroid function even in the presence of iodine excess. When large amounts

of iodine are given to subjects with normal thyroid function a transient decrease in the synthesis of the thyroid hormones occurs for ~48 h. This acute inhibitory effect of iodine on thyroid hormone synthesis is called the acute Wolff-Chaikoff effect and is due to increased intrathyroid iodine concentrations. The escape from or adaptation to the acute Wolff-Chaikoff effect is a decrease in the thyroid iodide trap, thereby decreasing the intrathyroid iodide concentration [68], due to a decrease in the sodium iodide symporter (NIS) mRNA and protein expression [69]. For this reason, most people can tolerate high doses of iodine without developing thyroid abnormalities. Excess iodine ingestion (up to 1.5–3.0 mg/day) also decreases the release of T4 and T3 from the thyroid resulting in small decreases in serum T4 and T3 concentrations with compensatory increases in basal and TRH-stimulated TSH concentrations, all values remaining well within the normal range. These iodine-treated subjects remained euthyroid although they continued to ingest the excess iodide and serum thyroid hormone and TSH values returned to basal levels when the iodide was discontinued. These subtle changes in thyroid function were accompanied by increased thyroid volume assessed by echography and a decrease in thyroid blood flow determined by color Doppler flow imaging [70].

The smallest quantity of iodine, exceeding that consumed with the diet in the United States, for instance, that does not affect thyroid function is 500 μg/day. The administration of 1 mg of iodine per week for 6 weeks followed by the administration of 2 mg of iodine weekly for another 6 weeks did not affect thyroid function. Other studies have suggested that the administration of 500 μg iodine daily induced a small but significant increment of basal and TRH-stimulated serum TSH concentrations. Ingestion of 1,500 μg of iodine per day for 15 days by euthyroid subjects invariably resulted in a significant decrease in serum free T4 concentrations and FT4 Index with a significant compensatory rise in basal and TRH-stimulated serum TSH concentrations [70]. There are adequate data to demonstrate that thyroid ^{131}I uptake or thyroid clearance of iodide decreases with increases in serum iodine levels. Single doses >10 mg suppress the uptake of radioactive iodine to ≤1.5% within 24 h, and daily doses of ≥15 mg will maintain uptake below 2% [71].

Evaluation of iodine nutrition using the ThyroMobil model in 35,223 schoolchildren at 378 sites of 28 countries has shown that many previously iodine-deficient parts of the world now have median urinary iodine concentrations well above 300 μg/l, indicating iodine excess which carries the risk of adverse health consequences [72]. Table 4 shows the epidemiological criteria for assessing iodine nutrition, based on median urinary iodine (UI) concentrations in school-age children. These introduce a clear distinction between iodine intake and its impact, i.e. the status of iodine nutrition. It was agreed that the

Table 4. Adequate daily iodine intake and the consequences of excessive nutritional iodine intake [World Health Organization, 5th Report on Word Nutrition, Geneva, Switzerland, March 2004]

Urinary iodine	Nutritional iodine intake	Clinical effects
<20 μg/l	totally deficient	severe iodine deficiency
20–49 μg/l	insufficient	moderate iodine deficiency
50–99 μg/l	insufficient	mild iodine deficiency
100–299 μg/l	ideal situation	None
≥300 μg/l	excessive	clinical increasing risks for autoimmune thyroiditis and hyperthyroidism (mostly in the elderly)

optimal status of iodine nutrition corresponds to a UI concentration in school-children situated between 100 and 200 μg/l [12].

Health Consequences of Excessive Iodine Intake

Excessive dietary iodine may increase the risk of thyroiditis, hyperthyroidism, hypothyroidism, and goiter [73]. In healthy adults, short-term iodine intakes of 500–1,500 μg/day have mild inhibitory effects on thyroid function. The consequences of prolonged exposure to high intakes of iodine, particularly in children, are less clear. Endemic goiter in children has been described in coastal Japan, where iodine intake from seaweed was >10,000 μg/day. Lower intakes, in the range of 400–1,300 μg/day, from iodine-rich drinking water, were associated with increased serum thyrotropin and thyroid volume in a small sample of Chinese children.

In children, excess dietary iodine has been associated with goiter and thyroid dysfunction. In a report of what the authors called ‘endemic coastal goiter’ in Hokkaido, Japan [74], the traditional local diet was high in iodine-rich seaweed. UI excretion in children consuming the local diet was ~23,000 μg/day. The overall prevalence of visible goiter in children was 3–9%, but, in several villages, about 25% of the children had visible goiter. Most of the goiters responded to the administration of thyroid hormone, restriction of dietary iodine intake, or both. TSH assays were not available, but it was suggested that an increase in serum TSH was involved in the generation of goiter. No cases of clinical hypothyroidism or hyperthyroidism were reported.

Goiter in children may also be precipitated by iodine intake well below the high amount described in the studies from Hokkaido. Li et al. [75] examined thyroid status in 171 Chinese children from 2 villages where the iodine

concentrations in drinking water were 462 and 54 μg/l, and the children's mean UI concentrations were 1,235 and 428 μg/g creatinine, respectively. The mean serum TSH concentration (7.8 μU/ml) was high in the first village and normal (3.9 μU/ml) in the second village. In the first village, the goiter rate was >60% and mean thyroid volume (tvol) was 13.3 ml, whereas the goiter rate was 15–20% and mean tvol was 5.9 ml in the second village. However, only those who developed goiter had positive antimicrosomal and TSH-binding antibodies. There were no signs of neurological deficits in the children. In other report from China, drinking water with iodine concentrations of >300 μg/l resulted in UI concentrations >900 μg/l and a goiter rate of >10% [76]. Although the mechanism remains unclear, increased thyroid size associated with high iodine intake may be due to autoimmune-mediated lymphoid infiltration of the thyroid [77, 78], inhibition of thyroid hormone release that increases serum TSH and thyroid stimulation [79], or both. Taken together, the Chinese studies suggest that goiter and thyroid dysfunction may occur in children at iodine intakes in the range of 400–1,300 μg/day. The mechanisms possibly involved in the role of iodine in thyroid autoimmunity include the damage to the thyroid by the generation of free radicals, a direct injury to the thyrocytes through the strong necrotic effect of iodide and an enhancement of autoimmunogenic properties of TG [79].

It is worth to mention that perinatal exposure to excess iodine can lead to transient hypothyroidism in the newborn. In Japan, large quantities of iodine-rich seaweed such as kombu (*Laminaria japonica*) are consumed. The concentration of iodine in serum, urine, and breast milk in addition to TSH, free T4, and TG was measured in 34 infants who were positive at congenital hypothyroidism screening. Based on the concentration of iodine in the urine, 15 infants were diagnosed with hyperthyrotropinemia caused by the excess ingestion of iodine by their mothers during their pregnancy. According to serum iodine concentrations, these infants were classified into group A (over 170 μg/l) and group B (under 170 μg/l) of serum iodine. During their pregnancies these mothers consumed kombu, other seaweeds, and instant kombu soups containing a high level of iodine. It was calculated that the mothers of group A infants ingested approximately 2,300–3,200 μg of iodine, and the mothers of group B infants approximately 820–1,400 μg of iodine per day during their pregnancies. Twelve of 15 infants have required levo-thyroxine because hypothyroxinemia or persistent hyperthyrotropinemia was present. In addition, consumption of iodine by the postnatal child and susceptibility to the inhibitory effect of iodine may contribute in part to the persistent hyperthyrotropinemia. It was proposed that hyperthyrotropinemia related to excessive iodine ingestion by the mother during pregnancy in some cases may not be transient [80] (table 5).

Pharmacological quantities of iodine are almost always due to the administration of inorganic and organic medicinal compounds. Iodine-induced

Table 5. Iodine-induced alterations of thyroid function in newborn infants after prenatal and perinatal exposure to povidone-iodine. Adapted from [94]

Thyroid function	After birth				
	Day 3		Day 5		
	Controls	**Neonates exposed**	Controls	**Neonates exposed**	p
TSH, μU/ml	4.3	**13.8***	2.0	**8.0***	<0.001
Total T4, μg/dl	18.1	**13.7***	2.0	**13.8***	<0.001
Free T4, ng/dl	3.1	**2.3***	2.7	**2.4***	<0.001
T3, ng/dl	144.0	**126.0**	166.0	**140.0**	NS
Reverse T3, ng/dl	273.0	**177.0***	214.0	**138.0***	<0.001

Median values (*$p < 0.001$). Note that exposure to povidone-iodine (neonates exposed) induces an elevated serum TSH and lower free T4 and total T4 concentrations. Furthermore, 20% of the infants had serum TSH values above 20 μU/ml (day 3) returning to normal (day 14) after two weeks.

hypothyroidism (IIH) has been observed in 20% of children chronically treated with amiodarone [81]; a drug extensively used as an antiarrhythmic agent which contains 75 mg of iodine per 200 mg tablet, is known to affect thyroid homeostasis by competitive inhibition of DIO1, which converts T4 to T3 and (reverse T3) rT3. In contrast, the administration of a single dose of 50–70 mg of potassium iodide KI to children to prevent radioactive contamination of the thyroid from the Chernobyl reactor accident did not induce significant change in serum TSH concentrations [82]. IIH may develop in children with cystic fibrosis, especially when iodine was given along with sulfisoxazole [83]. Also, it has been observed in children and adults with beta-thalassemia major requiring blood transfusions. It is likely that hemosiderosis of the thyroid was the predisposing factor [84].

More Than Adequate Iodine Intake

Although not excessive, studies in more than adequate iodine intake (see table 4) following iodine prophylaxis, also pointed out the possible development of thyroid autoantibodies. Zois et al. [85] investigated the iodine status and the impact of iodine prophylaxis on the prevalence of autoimmune thyroiditis among schoolchildren in a formerly iodine-deficient community in northwestern Greece. The findings were compared to those obtained from a similar survey

carried out 7 years previously in the same area. A total of 302 schoolchildren (12–18 years of age) from a mountainous area of northwestern Greece were examined for the presence of goiter, and blood and urine samples were collected for assessment of thyroid function, antithyroid antibodies and urinary iodine excretion. Median urinary iodine concentration in the children was ~200 μg/l. Thyroid function was normal in all but 7 children, who had subclinical hypothyroidism (2.5%). Antithyroid antibodies (antithyroid peroxidase and/or antithyroglobulin) were positive in 32 children, including those with subclinical hypothyroidism (10.6%). Twenty-nine of these children (9.6%) also had the characteristic hypoechoic pattern of thyroiditis on ultrasound studies and were diagnosed to have autoimmune thyroiditis (AIT). It was concluded that iodine prophylaxis has resulted in the elimination of iodine deficiency in this region of Greece but this has been accompanied by an increase in the prevalence of AIT. These authors followed up 29 children (12–18 years old) with AIT for 5 years to track its course in the postiodination era [86]. At diagnosis, thyroid peroxidase autoantibodies (TPOAb) were positive in 25 children (86%) and became positive in all children during follow-up. Thyroglobulin autoantibodies (TGAb) were positive in 17 children at diagnosis (59%) and became positive in 3 more children (69%). Both antibody types increased by the end of the observation period. Regarding thyroid function, 7 children (24%) at diagnosis had subclinical hypothyroidism that persisted and 4 more children developed subclinical hypothyroidism during the study period (38%). Only 5 of these children (45%) had positive TGAb. There was an increase in thyrotropin (TSH) so that at the end of the study all children had TSH greater than 2.5 μU/ml but none developed overt hypothyroidism. Thyroid hypoechogenicity that increased over time was seen in all children, especially in those with subclinical hypothyroidism. They concluded that both antibody types increased in frequency and level, but TPOAb were the predominant autoimmunity marker predictive of impending thyroid failure in children with AIT, as was thyroid hypoechogenicity on ultrasound.

Although the short-term effects of iodine in inducing thyroid autoimmunity by enhancing the immunogenicity of thyroglobulin are properly understood, the long-term effects of dietary iodine in modulating the autoimmune process are debated [87]. In this regard, a recent study suggested that thyroid autoimmunity markers may evolve during the course of iodine prophylaxis [88]. In particular, the authors reported a high prevalence of thyroid autoantibodies among schoolgirls 5 years after the introduction of an iodine prophylaxis program in Sri Lanka. The predominant antibodies were against thyroglobulin (TGAb), whereas thyroid peroxidase autoantibodies (TPOAb) were less frequent [88]. Interestingly, 3 years later, a shift in the pattern of autoantibodies was observed with a significant reduction in the frequency of TGAb and the predominance of TPOAb [89]. In a study from Epirus, an area under salt iodization

for three decades, overall prevalence of juvenile AIT, as diagnosed by assessment of thyroid antibodies was 3.3% and the goiter specific prevalence was 16.5% [90]. This scenario resembles what is currently occurring in India [91]. In a countrywide study to assess the thyroid status on Indian schoolchildren in the post-salt iodization phase, the authors demonstrated that there was a residual goiter prevalence ranging from ~12 to ~31% (mostly grade 1, WHO definition) [9] in different age groups of boys and girls, striking relationship between residual goiter prevalence and urinary thyocyanate excretion and significantly higher thyroid autoimmunity markers and functional abnormalities among goitrous children when compared to nongoitrous controls. Thus, after the elimination of iodine deficiency, at least in the above mentioned areas, the occurrence of clinically significant iodine-induced AIT appears to be a persistent and progressive phenomenon.

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Thyroid Hormone Transport and Actions

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Thyroid hormones (TH) are essential for normal development, differentiation growth and metabolism of every cell in the body. The pro-hormone thyroxine (T4) is synthesized by the thyroid follicles together with a small amount of the biologically active hormone triiodothyronine (T3), which derives mainly from tissue T4 deiodination. Approximately 0.03% of total T4 and 0.3% of total T3 in serum are circulating in a free or unbound form while the major part of TH is bound to circulating plasma proteins. These plasma proteins are responsible for the maintenance of the large extrathyroidal pool of TH, but their function is otherwise not quite clear, since wide differences in their concentrations do not influence the thyroid functional status of the individual to any large degree [1, 2].

Thyroid Hormone Transport

Transport in the Blood

More than 99% of the circulating thyroid hormone is bound to plasma proteins but can be liberated with great rapidity for entry into cells. The thyroid hormone-binding proteins are comprised of thyroxine-binding globulin (TBG), transthyretin (TTR or thyroxine-binding prealbumin), human serum albumin (HSA) and lipoproteins. Their functions are most probably to ensure a constant supply of TH to the cells and tissues by preventing urinary loss [3], protect the organism against abrupt changes in thyroid hormone production and degradation, protect against iodine deficiency [2] and target the amount of TH delivery by ensuring a site-specific, enzymatic alteration of TBG [4]. TBG has by far the highest affinity for T4, the result of which being that TBG binds 75% of serum T4, whereas TTR binds 20% and HSA 5% [2]. Some of the properties of the binding proteins are displayed in table 1.

Table 1. Some properties and metabolic parameters of the prinicpal thyroid hormone-binding proteins in serum

	TBG	TTR	HSA
Molecular weight, kDa	54*	55	66.5
Structure	monomer	tetramer	monomer
Carbohydrate content, %	20	–	–
Number of binding sites for T4 and T3	1	2	several
Association constant, K_a (M-1)			
For T4	1×10^{10}	2×10^{8}**	1.5×10^{6}**
For T3	1×10^{9}	1×10^{6}	2×10^{5}
Concentration in serum (mean normal, mg/l)	16	250	40,000
Relative distribution of T4 and T3 in serum, %			
T4	75	20	5
T3	75	<5	20
In vivo survival			
Half-life, days	5***	2	15
Degradation rate, mg/day	15	650	17,000

HSA = human serum albumin; TBG = Thyroxine-binding globulin; TTR = transthyretin.
* Apparent molecular weight on acrylamide gel electrophoresis 60 kDa.
** Value given is for the high affinity binding site only.
*** Longer under the influence of estrogen.
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Thyroxine-Binding Globulin

TBG carries the major part of both circulating T4 and T3 (as well as reverse T3), and therefore quantitative or qualitative changes in TBG concentration have a high impact on total serum T4 and T3. The protein is encoded by a single gene on the X-chromosome and is produced and cleared by the liver. It has a single iodothyronine-binding site with a slightly higher affinity for T4 compared to T3 [5]. When it is fully saturated it carries approximately 200 μg T4/l. The TBG concentration in serum is between 11 and 21 mg/l (180–350 nmol/l), present from 12th week of fetal life and 1.5 times higher in newborns and children until 2–3 years of age [6]. Estrogen has a marked effect on TBG by prolonging the biological half-life from the normal 5 days, thus resulting in increased plasma concentrations of TBG and total TH [7] while testosterone has the opposite effect [8]. In children and adolescents this may have an implication in diseases with a severe sex hormone overproduction related to the age, as well as oral contraceptives and pregnancy in adolescent girls.

Inherited TBG excess was first described in 1959 [9], and several familial X-chromosome-linked TBG abnormalities have been described [10, 11]. A rare TBG abnormality is seen in carbohydrate-deficient glycoprotein syndrome, which is associated with severe mental and motor retardation [12]. Acquired TBG abnormalities are mostly resulting in altered synthesis and/or degradation and caused by, e.g., severe terminal illness, hypo- and hyperthyroidism, severe liver disease and a variety of critical non-thyroidal illnesses [2, 13]. The latter may be mediated by interleukin-6 or other cytokines suppressing acute-phase reactants [14].

Transthyretin

TTR, previously called thyroxine-binding prealbumin binds only about 15–20% of the circulating TH and has a lower affinity for the hormones thus dissociating from them more rapidly and thus responsible for much of the immediate delivery of T4 and T3. Transthyretin is the major thyroid hormone-binding protein in cerebrospinal fluid. It is synthesized in the liver and the choroids plexus and secreted into the blood and cerebrospinal fluid, respectively. Only 0.5% of the circulating TTR is occupied by T4 and it has a rapid turnover of 2 days in plasma. Hence, acute reduction of the rate of synthesis results in a rapid decrease of its serum concentration [2]. Acquired abnormalities in TTR include major illness, nephrotic syndrome, liver disease, cystic fibrosis, protein fasting and hyperthyroidism. However, changes in TTR concentrations have little effect on the serum concentrations of TH [15].

Albumin

HSA binds about 5% of the circulating T4 and T3. Its affinity for the hormones is even lower, and since HSA associates with a wide variety of substances, including a number of different hormones and drugs, the association between TH and HSA can hardly be regarded specific. Even marked fluctuations in serum HSA concentrations have no effect on TH levels [16].

Lipoproteins

Lipoproteins transport a minor fraction of circulating T4 and to some extent T3 [17]. The binding site for TH on apolipoprotein A1 is distinct from that which binds to cellular protein receptors.

Consequences of Abnormal Binding Protein Concentrations

Abnormalities of the TH-binding proteins do not cause alterations in the metabolic state of the individual nor do they result in thyroid disease. Thus, abnormal concentrations of these binding proteins, due to changed synthesis, degradation or stability, result in maintaining normal free TH concentrations.

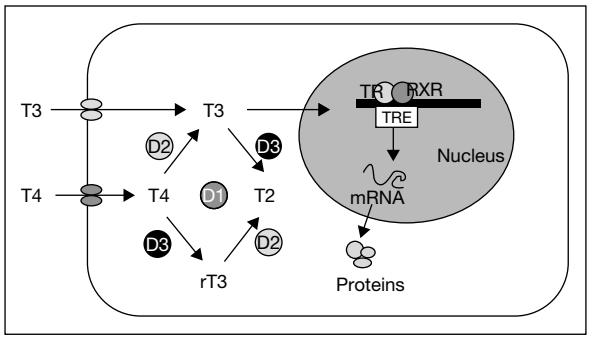

Fig. 1. Thyroid hormone transport and metabolism in a 3,3′,5-triiodothyronine (T3) target cell. Reproduced with kind permission from Jansen et al. [21].

However, they do give rise to misinterpretation of most of the measurements of serum levels of TH by available techniques. Depending on the severity of the abnormality only total TH concentrations are affected, but also the measured free TH levels by automated currently used methods give rise to incorrect results [18]. In such cases, it may be necessary to provide a free TH estimate by quantifying total hormone concentration with a subsequent estimate of the available binding places by use of a TH uptake test or direct measurement of TBG [2]. Even better is measurement of free TH concentrations by equilibrium dialysis or ultrafiltration, but not many laboratories in the world perform these measurements anymore.

Transport Across the Cell Membrane

The deiodinases involved in T4 to T3 conversion and T4 and T3 degradation as well as the T3 receptors are located intracellularly. Therefore, both action and metabolism of thyroid hormones are intracellular events requiring transport of iodothyronines across the cell membrane. For a long time it was believed that TH diffused passively over the cell membrane, but recent years of research has made it increasingly clear that cellular transmembrane transport of TH is mediated by transporters, that these transporters determine the availability of iodothyronines to the intracellular sites for metabolism and action [19], and that the TH transport is energy dependent [20] (fig. 1). Recently, specific transporters (organic anion transporters and amino acid transporters) known to facilitate cellular thyroid hormone uptake have been identified [20–22]. Hennemann and Visser [22] have defined requirements for (patho)physiological significance of thyroid

hormone plasma membrane transport in the terms that it should be specific, without significant diffusion, plasma membrane transport subject to regulation, transport rate limiting on subsequent metabolism, and changes in transport should be appropriate from the (patho)physiological point of view.

Organic Anion Transporters

These mediate uptake of iodothyronines and their sulphonated derivatives and they are members of the Na^+/taurocholate cotransporting polypeptide (NTCP) and the Na^+-independent organic anion transporting polypeptide (OATP) families [23, 24]. NTCP is only expressed on hepatocytes and is the major transporter of conjugated bile acids in the liver. The OATPs are a large family responsible for transmembrane transport of a number of compounds including TH. The most interesting OATP superfamily members in terms of TH transport are OATP1C1 and OATP14. The former has been demonstrated to be widely expressed both in human brain and the Leydig cells of testis [25]. In the brain they seem to participate in maintaining the T3 concentration along with parallel changes in D2 expression. It has been demonstrated that the thyroid state modulates OATP1C1, and by doing so counteracts the effects of alterations in circulating T4 levels on brain T4 uptake [26, 27]. In humans, OATP1C1 is also expressed in the testis where also D2 expression has been demonstrated [28]. This combination supports a role of TH in development, growth and differentiation of Leydig cells. In particular T3 is very important for testosterone biosynthesis and may therefore have an important role in male puberty. Other OATPs have been demonstrated in a number of other tissues and may exert a variety of effects, but this is not well clarified, and they are possibly less tissue-specific considering the widespread expression [21]. Some characteristics of the transporters are shown in table 2 [29–39].

Amino Acid Transporters

Iodothyronines are a particular class of amino acids built from two tyrosine residues implying transport by specific amino acid transporters, in particular the L and T type amino acid transporters, which therefore are involved in TH uptake into several tissues [40–44]. Among those are members of the heterodimeric amino acid transporter (HAT) family. Their exact role is not clear, but it has been demonstrated that overexpression of the heterodimer L-type transporter in cells resulted in increased intracellular T3 availability and a consequent augmentation of T3 action [45]. Evidence has also been presented to suggest a role of members from the HAT family in supplying the placenta and developing fetus with thyroid hormone [46].

The monocarboxylate transporter (MCT) family comprise to date 14 identified members in various tissues from different species [21]. MCTs are dispersed over autosomal chromosomes, except MCT8, which is X-linked [47]

Table 2. Characteristics of thyroid hormone transporters

Gene	Protein	Species	Accession code	Chromosome	Tissue distribution	Iodothyroine transport	Ref.
SLC10A1	NTCP	human	NP_003040	14q24.1	liver	T4, T3, rT3, T2	[28, 29]
SLC10A1	NTCP	rat	NP_058743	6q24	liver, kidney, CP	T4, rT3, T3, T2	[29]
SLCO1A1	OATP1A1	rat	NP_058807	4q44			
SLCO1A2	OATP1A2	human	NP_602307	12p12	brain, kidney, liver	T3, T2, T4, rT3	[29–31]
SLCO1A4	OATP1A4	rat	NP_571981	4	liver, brain, retina	T4, T2, T3, rT3	[29, 32]
SLCO1A5	OATP1A5	rat	NP_110465	4q44	kidney, retina, liver	T3, T4	[30, 32]
SLCO1B1	OATP1B1	human	NP_006437	12p	liver	T3, T4	[30, 33]
SLCO1B2	OATP1B2	rat	NP_113838	4q44	liver	T3, T4	[34]
SLCO1B3	OATP1B3	human	NP_062818	12p12	liver	T3, T4	[30]
SLCO1C1	OATP1C1	human	NP_059131	12p12.3	brain, cochlea	T4, rT3, T3	[25]
SLCO1C1	OATP1C1	rat	NP_445893	4q44	brain	T4, rT3, T3	[26]
SLCO4A1	OATP4A1	human	NP_057438	20q13.33	multiple	T3, T4, rT3	[31]
SLCO4A1	OATP4A1	rat	NP_598292	3q43	multiple	T3 (T4, rT3 NT)	[31]
SLCO4C1	OATP4C1	human	NP_851322	5q21.2	kidney, other	T3, T4	[35]
SLCO4C1	OATP4C1	rat	AAQ04697	9		T3, (T4 NT)	[35]
SLCO6B1	OATP6B1	rat	NP_596903	9q36	testis	T4, T3	[36]
SLCO6C1	OATP6C1	rat	NP_775460	9q36	testis	T4, T3	[36]
SLC7A5	LAT1	human	NP_003477	16q24.3	multiple (not liver), tumors	T2, rT3, T3, T4	[37]
SLC7A5	LAT1	rat	NP_059049	19q12			
SLC7A8	LAT2	human	NP_036376	14q11.2	multiple, tumors	T2, rT3, T3, T4	[37]
SLC7A8	LAT2	rat	NP_445894	15p13			
SLC16A2	MCT8	human	NP_006508	Xq13.2	brain, liver, kidney, heart, thyroid, eye, pituitary, other	T3, T2, T4, rT3	[38, 39]
SLC16A2	MCT8	rat	NP_671749	Xq31			

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and a specific TH transporter [38]. Compared to other TH transporters the rate of T3 and T4 transport is much higher and follows the criteria set down for requirements of a transporter. The MCT8 gene is located in the region of the X-chromosome associated with X-linked diseases [47], and it was therefore hypothesized that a mutation in this gene would result in an X-linked form of thyroid hormone resistance. Indeed, this hypothesis was verified first in a 6-year-old boy with highly elevated serum T3 and severe psychomotor retardation of unknown origin, where a deletion of the first exon of the MCT8 gene was demonstrated [39]. Since then the same group have described 5 unrelated

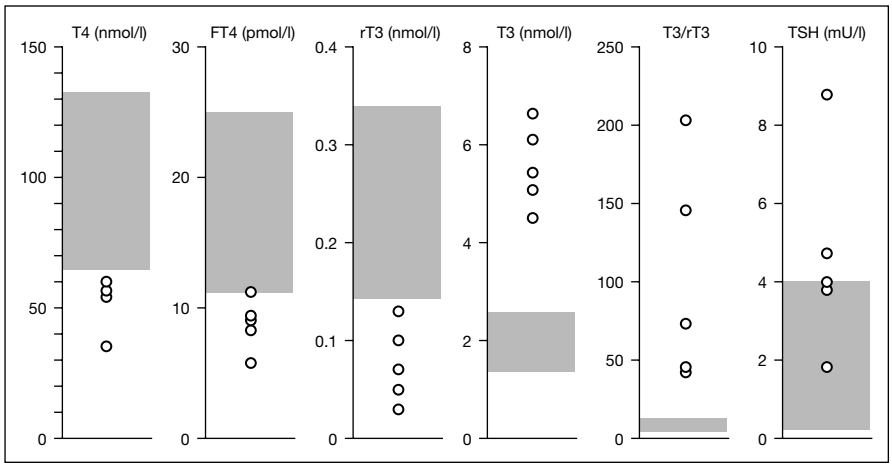

Fig. 2. Thyroid hormone serum levels in patients with mutations in MCT8. Hatched areas indicate normal reference ranges for each analyte. Reproduced with kind permission from Jansen et al. [21].

young boys aged 1.5–6 years with mutations or deletions in the MCT8 gene. They all had a uniform type of severe psychomotor retardation of hitherto unknown origin. The described phenotype comprised symptoms such as severe proximal hypotonia with poor head control and lack of verticalization, absence of targeted grasping, severe mental retardation with only rudimentary communicative skills and movement-induced increase in tone in the extremities [39]. Concerning thyroid function variables, T3 was invariably strongly elevated in all the patients, T4 and free T4 were mildly increased while thyroid-stimulating hormone (TSH) was in the normal range for age in 4 patients and increased in one (fig. 2). The various mutations have been described in more detail in a recent review [21]. All the mothers of the 5 patients were proven to be carriers, all of them with normal thyroid hormone levels and without psychomotor retardation. Another group has described two other cases with different mutations [48]. By studying the complex clinical picture of these patients it was assumed that MCT8 had an important role in TH-dependent processes of brain development. To provide a clue to the cellular function of MCT8 in brain, the expression of MCT8 mRNA in the murine central nervous system was studied by in situ hybridization histochemistry [49]. In addition to the choroid plexus structures, the highest transcript levels were found in neo- and allocortical regions (e.g. olfactory bulb, cerebral cortex, hippocampus, and amygdala), moderate

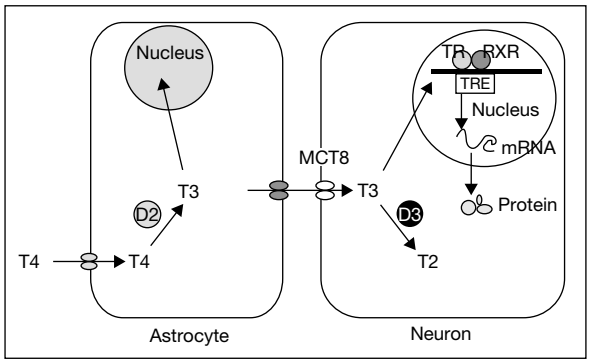

Fig. 3. Role of MCT8 in the neuronal uptake of T3. Reproduced with kind permission from Jansen et al. [21].

signal intensities in striatum and cerebellum, and low levels in a few neuroendocrine nuclei. Co-localization studies revealed that MCT8 was predominantly expressed in neurons. Together with the spatiotemporal expression pattern of MCT8 during the perinatal period, these results strongly indicated that MCT8 plays an important role for proper central nervous system development by transporting TH into neurons as its main target cells [49]. Another hypothesis raised by these clinical pictures was that MCT8 must play an essential role in the supply of T3 to neurons in the central nervous system (fig. 3). T3 binds to nuclear receptors in neurons, which are a primary action site for T3. The action of T3 is terminated by deiodination by D3, which is expressed in the neurons. However, for local production of T3 the neurons are dependent on neighboring astrocytes expressing D2, which is necessary for the local deiodination (fig. 3). Inactivation of MCT8 by mutation in the gene will result in an impaired supply of T3 to the neuron, as well as a decrease in T3 clearance due to block of T3 access to D3 with a possible subsequent increase in serum T3, consequently stimulating a further expression of D1 in the liver and kidney. The resulting increase in conversion of T4 to T3 and breakdown of reverse T3 explains the serum thyroid hormone concentrations in these patients.

The mutations in the MCT8 gene thus resulted in a severe hypothyroidism in the brain with the consequent phenotype, but other tissues and organs did not demonstrate signs of hypothyroidism e.g. bones and metabolism. It therefore seems that other tissues than the brain, are not dependent on MCT8 for uptake of TH. The elevated T3 did not exert any symptoms of hyperthyroidism in the patients, indicating that other yet unknown regulating mechanisms must be in place.

Deiodination of Iodothyronines

Deiodination is the foremost pathway of thyroid hormone metabolism both in quantitative terms but also through activation of T4 by outer ring deiodination to T3, as well as inactivation of both T4 and T3 by inner ring deiodination [reviewed in 50]. Three iodothyronine deiodinases (D1-D3) are identified as seleno cysteino-containing membrane proteins with their active enzymatic sites located in the cytoplasma. D1 and D2 convert T4 to T3, while D3 has only inner ring deiodination activity and inactivates T4 and T3 to rT3 and T2, respectively (fig. 1). D1 is expressed in liver, kidney and the thyroid, while D2 is expressed in the brain, pituitary, thyroid gland and skeletal muscle. In contrast to the rat, humans do not express D1 in the central nervous system. D3 is expressed in brain and fetal tissues, placenta and pregnant uterus. Other characteristics of the deiodinases are presented in table 3.

D1 has both outer and inner ring deiodination activities, but appears particularly important for the generation of plasma T3 and clearance of reverse T3 by outer ring deiodination. D1 is positively regulated at the pretranscriptional level by T3, and is very potently inhibited by the antithyroid drug propylthiouracil. In humans, therefore, it might be expected that hyperthyroidism would induce D1 with subsequent relative increase in T3 production. Hyperthyroidism is indeed commonly associated with a higher increase in plasma T3 compared to T4, and D1 activity has also been demonstrated to be approximately 3-fold elevated in thyroid glands from Graves' disease compared to euthyroid control glands [51]. D2, on the other hand, has only outer ring deiodination activity, preferring T4 over reverse T3 as substrate, and it is increased in hypo- and decreased in hyperthyroidism. This regulation by the thyroid state can occur both by pre- and posttranslational mechanisms. D2 is particularly important for local T3 production in the brain as mentioned previously (fig. 3). D3 has only inner ring deiodination activity and is therefore crucial for inactivation of TH, with preference for T3 over T4 as the substrate. In fetal life, D3 probably serves to protect against undue overexposure to active TH, which may be damaging to the development in particular of the brain. D3 is higher in the brain in hyperthyroidism and lower in hypothyroidism, the reason for which is unclear.

A high degree of similarities has been demonstrated between the structures of the deiodinases and the reactions they catalyze [50]. Yet, there are also important differences in their catalytic properties (table 3; fig. 4). D1 catalyzes both outer and inner ring deiodination while D2 only outer ring and D3 inner ring deiodination, respectively [52]. In addition to deiodination, iodothyronines are metabolized by conjugation of the phenolic hydroxyl group with sulphate or glucoronic acid (so-called phase II detoxification reactions) [53, 54]. The purpose of this is to increase the water solubility of the substrates and thereby to

Table 3. Characteristics of the three iodothyronine deiodinases

	D1	D2	D3
Deiodination	ORD+IRD	ORD	IRD
Preferred substrate	rT3>T4, T3	T4>rT3	T3>T4
Sulfation of substrates	stimulation	inhibition	inhibition
Kinetic mechanism	ping-pong	sequential	sequential
Inhibitors			
Propylthiouracil	10	>1,000	>1,000
Iodoacetate	1	>1,000	>1,000
Gold thioglucose	0.02	1	1

IRD = inner ring deiodination; ORD = Outer ring deiodination; rT3 = reverse triiodothyronine; T4 = thyroxine; T3 = triiodothyronine.

Reproduced with kind permission from Kuiper et al. [50].

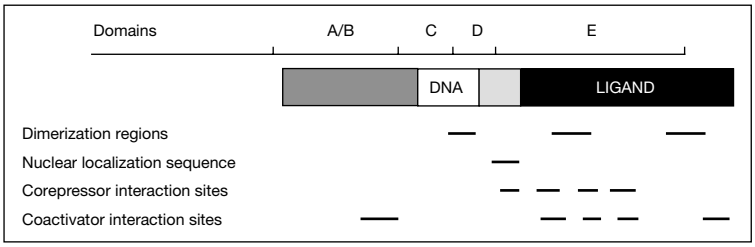

Fig. 4. Functional domains of the TH receptor. The TH receptor is depicted schematically. The zinc finger DNA-binding domain is denoted along with the carbo-terminal ligand-binding domain. Other functional domains and interaction sites are indicated. Reproduced with kind permission from Yen [96].

facilitate their biliary and/or urinary clearance. The iodothyronine sulphate levels are normally very low in plasma, bile and urine, because they are rapidly degraded by D1, indicating that sulphate conjugation is the first step leading to irreversible inactivation of TH [54]. Going back to the differences between the three deiodinases, the effects of sulfation of the substrate vary as does the effect of inhibitors (table 3). Most pronounced is the difference in the reaction to propylthiouracil, which inhibits D1 very potently but does not inhibit D2 and D3 [50]. Plasma T3 levels are decreased more by the D1 inhibitor propylthiouracil in hyperthyroid patients than in normal individuals, indicating that D1 makes a larger contribution to plasma T3 concentrations in hyperthyroidism

compared to the euthyroid state [52]. So, although propylthiouracil is used to treat hyperthyroidism mainly due to its inhibitory action on the enzyme, thyroid peroxidase, propylthiouracil at high doses also inhibits D1 activity [55].

The production of TH is regulated by the hypothalamo-pituitary-thyroid axis, while the biological activity of TH, i.e. the tissue availability of T3 is mainly regulated by the three deiodinases [56]. The serum concentrations of thyroid function variables are regulated very closely within the individual, while there is a substantial interindividual variation in serum levels of both T4, T3, TSH and thyroglobulin. This was first demonstrated by Feldt-Rasmussen et al. [57] in 1979, and has later been verified by others [58, 59]. This pattern indicates an important genetic component in the regulation of serum concentrations of thyroid function variables, with an individual set-point for thyroid function. A classical twin study demonstrated results to support this by finding approximately 67% heritability accounting for the variations in plasma concentrations of TSH, and free T3 and T4 [60], and in a population study of Mexican Americans 26–64% of the interindividual variation was suggested to be due to heredity [61]. Finally, Spencer has in a guideline publication with Baloch as first author [18] described an individual TSH-free T4 log-linear set point ratio as further support of this concept.

Along these lines polymorphisms have recently been identified in the D1 gene [62]. The T-allele of one of them (D1a) was dose dependently associated with increasing plasma reverse T3 levels and decreasing T3/reverse T3 ratio, while the G-allele of the other (D1b) showed the opposite. Since D1 physiologically plays a key role in production of serum T3 and in the clearance of reverse T3, it might be assumed that the D1a-T variant has a negative effect on tissue D1 activity, while the D1b-G variant could be responsible for a positive effect [63]. Another study performed in a different population showed a dose effect from D1a-T allele on serum T3 concentration and thus supported this hypothesis [64]. In performing such studies it is important to pay attention to the age distribution of the population since a decreased T3 production by D1 may be masked by the production of T3 by skeletal muscle D2 in young subjects [64, 65]. Skeletal muscle size and strength increase during childhood and in young adults, and again gradually declines throughout adult life. D1 activity increases during childhood and adolescence and again decreases during ageing, but the relative contribution of D2 to serum T3 production may be more important in young compared to elderly subjects, resulting in a relatively smaller contribution to T3 production from D1 in young persons.

A polymorphism in the D2 gene did not demonstrate any relationship with plasma concentrations of T3 or reverse T3 [62], which is possibly explained by the fact that D2 plays the major role in local T3 production in D2-containing tissues. It would therefore not be expected to find polymorphism relations to

plasma concentrations, and an effect of the polymorphism on intracellular T3 cannot be excluded. One study described a correlation between the same polymorphism and insulin resistance in obese women [66]. Since there was no concomitant association with their body composition, it was hypothesized that the results might be explained by a linkage to another polymorphism [62].

No significant association between concentrations of TH and a polymorphism in the D3 gene have been described and there have been no descriptions of deficiencies of deiodinases neither in humans nor in animals [67]. The present conclusion of studies over the recent years have clarified that genetic variation by polymorphisms plays an important role in the serum concentrations of thyroid function variables, and that deiodination of the iodothyronines are crucial players in this unique set-point. In adults it is becoming increasingly clear also, that only minor modifications from this set-point resulting in mild (or subclinical) hypo- or hyperthyroidism, may induce alterations in thyroid hormone bioactivity with consequences for clinical end-points such as bone mineral density, atherosclerosis and heart rate, with increased morbidity and even increased mortality [68, 69]. How frequent such alterations are in children has not been investigated, and therefore it is unknown if, e.g., polymorphisms in the deiodinase genes may have an impact on bone development in children and adolescents.

Because D1 is a selenoprotein, one might expect to find decreased D1 enzyme activity in selenium deficiency, and in rats this was indeed demonstrated for hepatic and renal D1 [70, 71]. There are, however, differences in the organ sensitivity to selenium deficiency, so studies may show difference in results depending on the organs studied. Furthermore, it is difficult to study in humans, because it is difficult to find pure, isolated selenium deficiency. Yet, mildly elevated serum T4 levels have been described in selenium-deficient humans [70–73]. Selenium supplementation in an area with both iodine and selenium deficiency has resulted in an unexpected reduction of serum T4, and in some an increase of serum TSH as indication of worsening of hypothyroidism [74, 75]. This reaction might be explained by selenium deficiency causing reduced D1-catalyzed inner ring deiodination of iodothyronines, thereby protecting against hypothyroidism. These results are in contrast to a study by Roti et al. [76], who examined the effect of selenium supplementation in an area with mild iodine deficiency. The eight female subjects had a positive perchlorate discharge test after a previous episode of subacute or postpartum thyroiditis and thus might have been at risk of developing thyroid dysfunction, but they all remained with normal TH concentrations after selenium supplementation.

Nonetheless, it seems that restoration of adequate iodine supply is essential before selenium intake is increased, thereby avoiding selenium-dependent deiodinative degradation of TH, subsequent urinary loss of iodine and TSH stimulation of an iodine depleted thyroid gland [77].

The issue of selenium intoxication is still controversial, and intakes of selenium up to 400 μg/day have not resulted in any adverse effects [78]. Signs of reversible intoxication have been reported by ingesting more than 1,000 μg/day over a long time [78]. Nevertheless, paramedication, over-the-counter administration, and uncontrolled use of selenium containing preparations with accompanying strong advertisements on the Internet should be monitored and restricted in order to avoid uncontrolled distribution of selenium and its accumulation into body proteins. These commercial preparations are marketed and sold under names such as Thyroid Helper, Daily Energy, Daily Protector, Thyroid Booster and many more.

During critical illness at any age, pronounced alterations in plasma thyroid hormone concentrations occur. It is a whole body response to virtually any serious illness and covers synonyms such as nonthyroidal illness (NTI), low T3 syndrome and euthyroid sick syndrome [79]. The validity of thyroid hormone measurements was initially described as questionable [80], and although it is generally accepted that a low free T3 perhaps together with low free T4 and TSH at later stages is a hallmark of the disease, the interpretation of serum values of thyroid function variables is still questionable [18]. In fact, an estimate of free TH concentration by total hormone measurement and correction for binding sites on the binding proteins by a TH uptake test is superior to the so-called 'direct' free TH measurements by automated analyses, since the direction of changes of each of the measurements will indicate whether the encountered abnormality is within (i.e. thyroid dysfunction) or outside the thyroid gland (i.e. NTI) [18].

The typical changes of NTI have initially been described as low T3 (later also T4) together with elevated reverse T3, and studies on the role of deiodinases during critical illness focused on D1 and D2, since the reduction of circulating T3 was thought to be due to decreased peripheral deiodination by D1, D2 or both [52, 81, 82]. It is, however, also possible that D3 is induced in the liver and abundant tissues such as skeletal muscle, thereby decreasing the ratio between T3 and reverse T3, a mechanism that might have been underestimated in the previous studies [50]. Cytokines, in particular interleukin-6, may be responsible for part of these changes in NTI, but cannot explain the full effect [83, 84]. Pulsatility of hypothalamic-pituitary hormones including TRH-TSH is almost abolished in this syndrome, and restoration of all the axes by injection of hypothalamic hormones can restore the abnormalities almost completely [85, 86]. Whether this also involves pituitary D1 and D2 is not fully clarified [87].

Genomic and Nongenomic Actions of Thyroid Hormones

As mentioned above, the biological activity of thyroid hormones is largely exerted by T3 and is determined by the intracellular T3 concentration, which is

dependent on a number of factors: the circulating concentration of T3 and its precursor T4, the activity of transporters mediating the cellular uptake of T4 and T3 and the relative activities of the iodinases catalyzing the outer-ring deiodination of T4 to T3 and the inner ring deiodination of T4 and T3 to inactive metabolites.

Most thyroid hormone actions are initiated by an interaction of T3 with specific nuclear receptors, which act largely as transcription factors exerting a modifying effect on the expression of a variety of genes, the genomic actions. However, extranuclear processes may also contribute to the overall biologic actions of thyroid hormones [88–90]. These effects occur rapidly and are shown to be unaffected by inhibitors of transcription and translation suggesting that thyroid hormones may also mediate non-genomic actions [reviewed in 91].

The heart is a major target organ for thyroid hormone action, and the T3 effects are shown to be mediated by both nuclear and extranuclear mechanisms leading to enhanced velocity of cardiac contraction and increased speed of diastolic relaxation [reviewed in 92].

Receptor-Specific Nuclear Actions (Genomic Actions)

Thyroid hormone receptors belong to a large superfamily of nuclear hormone receptors that include the steroid hormone, retinoic acid, vitamin D and peroxysomal proliferator receptors (PPARs). The receptors have a central DNA-binding domain and a carboxy-terminal ligand-binding domain (fig. 4). The two major isoforms, the thyroid hormone receptor α-1, α-2 (TRα) and β-1, β-2, β-3 (TRβ) have a high homology in these two domains, while the amino-terminal regions are more variable. Two thyroid hormone receptor genes located on chromosomes 17 and 3, respectively [89, 93–94] encoding for TRα and TRβ, respectively. TRα-1, TRα-2, TRβ-1 and TRβ-3 are expressed widely, whereas TRβ-2 is predominantly restricted to the hypothalamic/pituitary axis in the negative feedback regulation of TSH.

T3 binds to TR-α and TR-β resulting in nuclear gene expression. The receptors are ligand-regulatable transcription factors that recognize and interact with specific DNA sequences (thyroid hormone response elements) in the promoter region of target genes leading to consequent effects on transcription [95, 96] (fig. 5a). The transcriptional activity of target genes is either increased or decreased. Examples of target genes that are positively regulated by TH are: fatty acid synthetase, growth hormone, lysosome silencer, malic enzyme, type I 5′-deiodinase and negative regulated: epidermal growth factor receptor, prolactin, TSH, thyrotropin-releasing hormones, type II 5′-diodinase [96] The genomic effects have response times of hours to days. After TR binding to TH

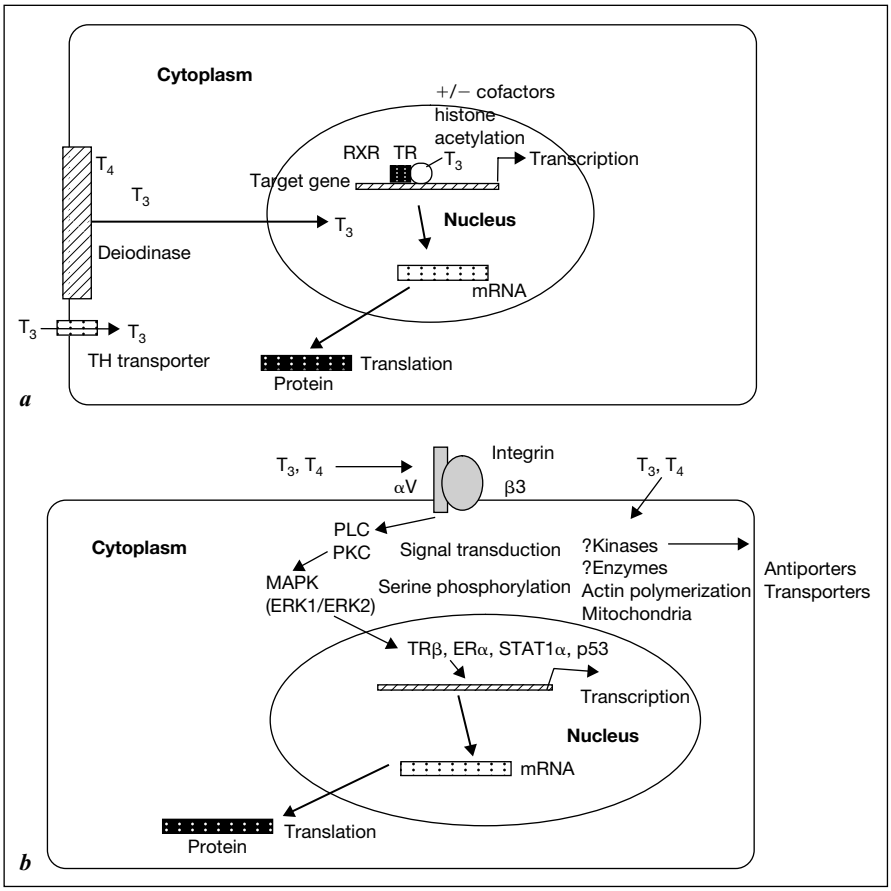

Fig. 5. ***a*** The genomic pathway of TH action. T3 is converted from T4 by deiodinase or transported directly into the cell whereupon it binds to nuclear TRs. In positively regulated target genes, corepressors are subsequently released and coactivators recruited, resulting in histone acetylation and RNA polymerase II-mediated transcription. ***b*** Schematic representation of the proposed model of the nongenomic pathway of thyroid hormone action. T4 and T3 binds to integrin αVβ3 and activates the MAPK pathway. It is possible that nuclear hormone receptors are serine phosphorylated and with down-stream transcriptional regulation result in angiogenesis. Other TH-regulated pathways have been depicted but little is known about their mechanisms. ERα = estrogen receptor α; PLC = Phospholipase C; PKC = protein kinase C; STAT1α = signal transducer and activator of transcription 1α. Reproduced with kind permission from Yen [109].

response elements the transcriptional activity is altered by an interaction directly or indirectly with a complex array of transcriptional cofactors including corepressors, coactivators, integrators. Even unliganded TRs interact with corepressors and repress expression rather than being an inactive passive receptor. This also explains that TR knockout mice are not suffering from as pronounced a hypothyroidism as might be expected [97].

Mutations have been demonstrated in the TR-β gene with resultant familial resistance to thyroid hormones. These patients are identified by their persistent elevation of circulating free T3 and T4 without a suppressed TSH concentration. The thyroid hormone resistance syndrome will be dealt with in more detail in a subsequent chapter.

Nongenomic Actions (Extranuclear Actions)

A number of T3 effects occur rapidly and are unaffected by inhibitors of transcription and protein synthesis. The site of these actions has been localized to the plasma membrane, cytoplasm and cellular organelles. The nongenomic actions often have a short latency. Cell culture studies suggest that thyroid hormones rapidly, and nongenomically regulate the Ca^{2+} ATPase enzyme, the Na^{+} channel via protein kinase C (PKC), the K^{+} channel via phosphatidyl-inositol 3 (PI3)-kinase, the Na^{+}/H^{+} antiporter via PKC and mitogen-activated protein kinase (MAPK) [98]. The nongenomic actions thus presumably include the regulation of ion channels, oxidative phosphorylation and mitochondrial gene transcription and involve the generation of intracellular secondary messengers signaling pathways including induction of calcium, cyclic AMP or protein kinase signaling cascades [91, 98–100]. Recently, integrin αVβ3, has been identified as a plasma membrane TH-binding site [101]. Furthermore, it has been shown that both T4 and T3 activate MAPK activity leading to phosphorylation of TRβ [90]. Additionally Davis and colleagues [102, 103] showed a proangiogenic action of the thyroid hormone analogues GC-1 and 3,5′-diiodothyropropionic acid (DITPA) initiated at the cell surface interacting with integrin. The proposed model (represented schematically in fig. 5b) thus includes that TH activates the MAPK cascade and promotes angiogenesis via TH binding to membrane-bound integrin αVβ3.

TH Analogs, Metabolites, and Antagonists

Several tissue- and TR isoform-specific compounds have been developed as potential treatments for hypercholesterolemia, obesity, and heart failure [reviewed in 96]. In the development of these compounds it is attempted to use

information on tissue-specific uptake of the compound. One of the initial compounds was investigated in mice, who subsequently had lower serum cholesterol levels without cardiotoxicity. Recently, several other TH analogs have been described that have compared to TRα. Since thyroid hormone receptors in the liver, isoform-selective affinity for TRβ is approximately 90% TRβ, and in the heart mostly TRα, these isoform-selective compounds may serve as novel agents to lower serum cholesterol with minimal cardiotoxicity. Recently, KB141 was shown to be a potential treatment for obesity by decreasing body weight via stimulation of metabolic rate and oxygen consumption.

Some TH analogs and derivatives can also bind specifically to proteins other than thyroid hormone receptors, and are involved in nongenomic cell signaling pathways. Recently, Scanlan et al. [104] identified 3-iodothyronamine, which is a naturally occurring byproduct of TH, with interesting physiological actions as it produced a rapid drop in body temperature and heart rate when injected intraperitoneally in mice. These physiological actions are thus opposite of those observed for T3, and may provide a counter-regulation to the transcriptional effects of TH by nuclear thyroid hormone receptors.

The TH-related compound demonstrated with low metabolic activity and low affinity for nuclear thyroid hormone receptors, DITPA was able to increase cardiac contractility and peripheral circulation without significant effects on heart rate as well as improve hemodynamic performance in animal models of congestive heart failure after myocardial infarction [105]. Preliminary studies have been performed in patients with heart failure demonstrating a significant improvement in systolic cardiac index and systemic vascular resistance [106]. Future studies are needed with this and similar compounds to clarify if such drugs may represent a novel class of drugs for the treatment of heart failure.

Mitochondrial Actions of Thyroid Hormone

Both genomic and nongenomic actions of thyroid hormones may mediate mitochondrial effects regulating metabolism, cellular proliferation, differentiation and apoptosis [107]. It has long been known that TH has profound effects on mitochondrial activity and cellular energy state [108].

Summary and Conclusions

The functions of binding to plasma proteins are most likely a protection from fluctuation in TH production and degradation, a projection against environmental

deficient supply of e.g. iodine, and possibly also a protection of urinary loss of the smaller molecules of unbound TH compared to the bound forms [2]. The normal human organism has a high capacity for compensating to a maintained normal thyroid function by almost any reduction in the plasma binding proteins.

Several transporters that mediate the cellular entry of TH have been identified, but most of them are not specific for thyroid hormones. Up to now only two truly TH-specific transporters have been found: OATP1C1 with high preference for T4 and MCT8 with preference for T3 as the ligand [21]. Since delivery of TH to the cells is a crucial mechanism for subsequent TH action, abnormalities in these transporters probably result in disease, e.g. a described mutation in the MCT8 caused tissue-specific hypothyroidism in the brain with milder affection of other organs [39].

In both qualitative and quantitative terms, deiodination is by far the most important pathway of thyroid hormone metabolism. Deiodination by the deiodinases D1-D3 are extremely important for TH delivery to its intracellular action mechanisms. The deiodinase activities are actively regulated in a variety of fashions, and active differentially in various tissues. Clinically, the importance of the deiodinases in the regulation of thyroid hormone bioactivity becomes apparent when their activity is affected by pathophysiological conditions, such as thyroidal and non-thyroidal illness and malnutrition. The selenium containing deiodinases are important players both in the physiological regulations of thyroid function, e.g. with relations to fetal development in general and brain development in particular, and in responses to antithyroid drug therapy such as propylthiouracil. In conditions of limited and inadequate supply of both iodine and selenium, complex rearrangements of TH metabolism enable adaptation to this unfavorable situation by increasing retention of selenium in the brain, the endocrine tissues, and especially in the thyroid gland.

During nonthyroidal critical illness at all ages a series of typical changes of serum concentrations of thyroid-related function tests are found, which are probably ascribed to both downregulation of D1 and possibly D2, but recently also induction of D3 has been suggested to play an important role, which has probably been underestimated in previous studies. Possibly, both cytokines and the hypothalamo-pituitary axes also play important roles in this complex condition.

Independent of the mechanisms and consequences of thyroid function test abnormalities in transport binding protein levels, thyroid hormone resistance or NTI, it is important for all clinicians to be aware of the pitfalls in the use of routine methods for measurement of circulating variables of thyroid function such as TSH, and total and free T3 and T4.

The increased knowledge of the molecular mechanisms of thyroid hormone receptor structure and isoforms together with TH actions mediated by nuclear and extranuclear pathways has a high potential for opening for possibilities to

design new therapeutic agents, e.g. for treatment of cardiac failure, hypercholesterolemia, or for treatment of obesity without the central effect that most other anti-obesity drugs display. This could be a very important pharmaceutical progress in the solution of the increasing obesity epidemic in the Western world.

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The Thyroid and Autoimmunity in Children and Adolescents

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Thyroid autoimmunity is the commonest disease process to affect thyroid function. The prevalence of thyroid autoimmunity increases throughout life, with a possible decline in frequency in the very old as a 'healthy survivor' effect. The mere presence of thyroid autoimmunity, as demonstrated by the presence of thyroid autoantibodies or focal thyroiditis, for example, does not equal thyroid disease, since the majority of people with focal thyroiditis do not become hypothyroid [1]. On the other hand, as far as we know the formation of thyroid-stimulating antibodies (TSAb) leads to Graves' disease in the great majority of subjects, even if in rare cases their levels may oscillate and be associated with a fluctuating clinical course. After a brief review of the basic immunological mechanisms which underlie autoimmune thyroid diseases, this chapter will focus on the comparatively few studies which have looked specifically at the pathogenic mechanisms in these disorders in children and adolescents, and then look at the autoimmune disease associations which have considerable clinical relevance to the management of such patients.

Mechanisms of Thyroid Autoimmunity

Predisposition

It is well established that a complex interplay of diverse environmental and genetic susceptibility factors interact in predisposing an individual to autoimmune thyroid disease (fig. 1). Moreover, the contribution that each factor makes varies from patient to patient, and as yet there are no clear genotype-phenotype correlations. We have shown that polymorphisms in the thyroid stimulating hormone receptor (TSH-R) are associated with Graves' disease but not autoimmune

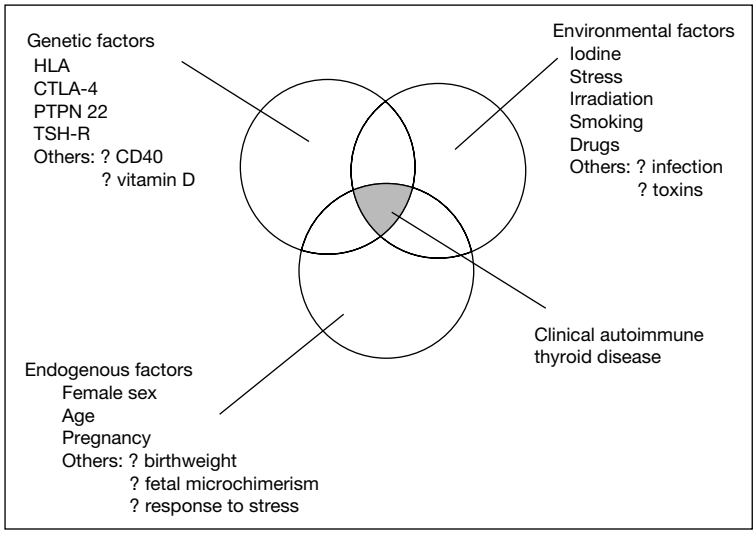

Fig. 1. Interaction of factors predisposing to autoimmune thyroid disease.

hypothyroidism [2]. The other known genetic loci associated with thyroid autoimmunity, namely HLA, *CTLA-4* and *PTPN22*, are shared between these 2 thyroid conditions, as well as many other autoimmune diseases [reviewed in 3]. Several environmental factors have been delineated but some of these remain controversial and of unknown action, such as smoking and stress [4, 5]. Evidence for the involvement of infections is lacking (thyroid autoimmunity rarely follows subacute thyroiditis, for instance), but there does appear to be an association between congenital rubella infection and subsequent thyroid autoimmunity [6].

Failure of Self-Tolerance

Genetic and environmental factors predispose to autoimmune disease through their effects on immunological tolerance (table 1). It is well established that most autoreactive T cells are deleted in the thymus, and this involves the intrathymic expression of self-antigens during development. This process is most clearly demonstrated in autoimmune polyglandular syndrome type 1, in which there is a defect in the autoimmune regulator (*AIRE*) gene, which prevents transcription of self antigens in medullary thymic epithelial cells and, as a result, there is a failure to negatively select organ-specific thymocytes [7]. However, the main autoimmune endocrinopathies in this syndrome do not include thyroid disease, although there is a slightly higher frequency of this disorder than expected in patients with the syndrome. Therefore, the expression of

Table 1. Mechanisms to ensure immunological self tolerance and prevent autoimmune disease

Deletion or anergy of autoreactive T and B cells during fetal life
Peripheral tolerance, including deletion or anergy of T cells by antigen presentation in the absence of a co stimulatory signal
Sequestration of autoantigen, including tissue expression of Fas ligand (immunological privilege) causing apoptosis in Fas-expressing autoreactive T cells
Clonal ignorance; absence of activated CD4+ cells required for CD8+ T or B cells
Active suppression of autoreactive T cells; particularly by CD4+, CD25+ T regulatory cells
Mutual inhibition of Th1 and Th2 cytokine pathways

thyroid autoantigens in the thymus may be regulated by other transcription factors, or other mechanisms may be important in regulating tolerance.

One likely additional mechanism involves T regulatory cells. Once again, a disorder caused by a single gene defect in man in revealing in illustrating the importance of this type of tolerance mechanism. In the IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, there is a defect in the *FOXP3* gene which encodes a transcription factor essential for the function of CD4+, CD25+ T cells with immunoregulatory properties, and such patients have a fatal disorder with severe autoimmune disease including that against the thyroid [8]. In fact, the existence of T regulatory cells was first defined by elegant experiments on experimental autoimmune thyroiditis induced in rats by neonatal thymectomy and sublethal irradiation [9]. Disease could be prevented by transfer of cells from healthy donors, which subsequently led to identification of this important CD4+, CD25+ subset.

Another clinical illustration of this pathway appears to be the common autosomal dominant condition, autoimmune polyglandular syndrome type 2, which of course includes thyroid autoimmunity as 1 of the 3 cardinal endocrinopathies, alongside Addison's disease and type 1 diabetes mellitus. Although there are no quantitative differences, CD4+, CD25+ T cells from patients with this syndrome have markedly reduced suppressive capacity compared to controls or patients with isolated endocrinopathies [10]. Disturbances in these or other populations of immunoregulatory T cells may be responsible to 'reconstitution' Graves' disease, in which thyroid disease appears as lymphocyte counts rise in patients with previously low counts, such as occurs after HAART treatment in HIV disease [11].

A final important pathway for T cell tolerance is likely to be induced by the expression of HLA class II molecules on thyroid epithelial cells in response to γ-interferon released by any local inflammation. In the absence of costimulation mediated through CD80 or CD82 (which thyroid cells do not express),

antigen presented by class II thyroid cells is able to induce anergy and tolerance in naïve T cells, rather than their activation [12]. Unfortunately, in an already initiated autoimmune response, in which autoreactive, memory T cells have been exposed to costimulation delivered by professional antigen-presenting cells, HLA class II+ thyroid cells are able to induce further T cell activation, leading to exacerbation of the autoimmune response. Overall the relative importance of these and other tolerogenic pathways in thyroid autoimmunity is unclear, but unlikely to be similar in all patients.

Mechanisms of Disease

Although thyroglobulin (TG) and thyroid peroxidase (TPO) autoantibodies are useful diagnostic markers, their role in causing tissue injury, at least primarily, is minimal. They may, however, be important in causing secondary damage, through antibody dependent cellular cytotoxicity (ADCC) or complement fixation (TPO antibodies) [13]. TSAb are obviously central to the pathogenesis of Graves' disease and there have been several recent studies which have shown the potency of monoclonal TSAb in causing thyroid cell activation [14]. Indeed, there is now a real issue over the exact relationship between the hyperthyroidism and the thyroid lymphocytic infiltrate which is so frequently accepted as an inevitable accompaniment of Graves' disease [15]. Perhaps Graves' disease is a 'pure' B cell-mediated disorder that is very frequently associated with T cell-dependent thyroiditis, and whether one leads to the other becomes a critical question. The main mechanism of thyroid destruction in autoimmune thyroid disease is probably T cell-mediated cytotoxicity, but a number of pathways of tissue injury are involved (fig. 2) [15].

Autoimmunity in Juveniles

Probably the most frequent clinical presentation of thyroid autoimmunity in children and adolescents is with a small asymptomatic goitre typically appearing around 11 to 12 years of age and comprising a mild lymphocytic thyroiditis. Patients are usually euthyroid. This entity was characterised by Hazard [17] as showing little if any Ashkenazy cell metaplasia, marked colloid phagocytosis in affected follicles and areas of epithelial hyperplasia. The levels of antibodies against TG and TPO are typically much lower than in the adult and there is a tendency to spontaneous remission [18]. There is still a female to male preponderance in children, but perhaps 3-fold less than in adults.

The overall clinical course is variable and may fluctuate, including periods of thyrotoxicosis [19]. Even patients with severe hypothyroidism may be come euthyroid. In 15 patients with overt hypothyroidism from Japan, reversibility

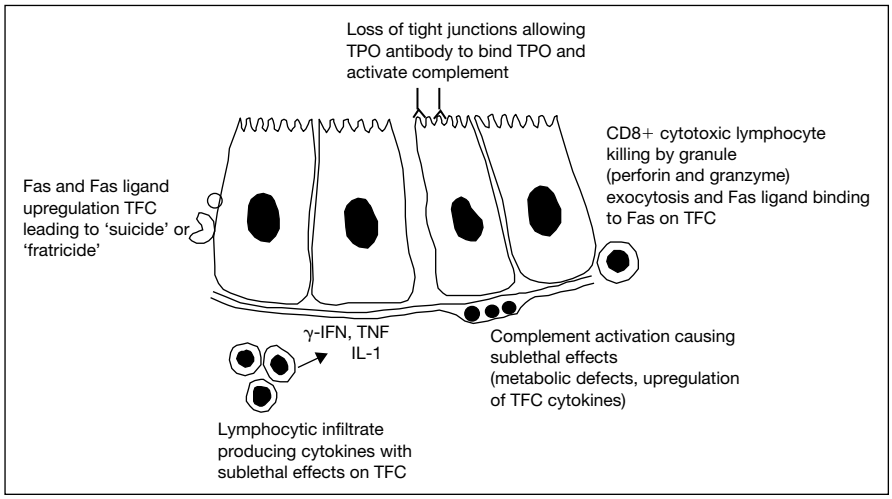

Fig. 2. Pathogenic mechanisms in autoimmune hypothyroidism. From Weetman [16], with permission.

was associated with iodine restriction in the diet and disappearance of antibodies capable of blocking the TSH-R [20]. In another study of 21 children with atrophic thyroiditis and 48 children with a lymphocytic goitre, all treated with thyroxine, five of the goitrous patients recovered normal thyroid function [21].

These clinical observations indicate that autoimmune thyroiditis in children and adolescents is typically less severe than in adults, with lower levels of autoantibodies and a more fluctuating course which includes spontaneous recovery. There do not appear to be good, very long-term follow-up studies which show what happens to these individuals over subsequent decades.

Predisposing Factors

Children not only encounter a somewhat different range of environmental factors to adults, but also have overall a lower chance of encountering aetiological agents simply because of their shorter period of exposure. In turn, this has led to the suggestion that genetic factors are likely to play a larger role in childhood thyroid autoimmunity than in adults, while environmental factors would have an increasing role in adults as they age. Despite possible ascertainment artefacts, initial studies have certainly shown that children and adolescents with autoimmune thyroiditis have strikingly strong family histories of thyroid and other autoimmune disease, including those in the non-organ-specific category. For instance, in 35 such juvenile patients, there was a family history of thyroid

disease in 27% (compared to 17% in adult Hashimoto patients) and there was a 28% frequency of positive antinuclear factor antibodies, compared to 9% in adults with thyroiditis [22]. In another study of 20 child probands with chronic lymphocytic thyroiditis and 18 with Graves' disease, there was a considerable risk of developing thyroid autoimmunity in their siblings, which was demonstrated by the 50% prevalence of thyroid antibodies in the siblings of probands with either type of thyroid disease [23]. When one or both parents also had antibodies, there was a significantly greater risk of thyroid autoimmunity in their offspring. An even stronger familial clustering of thyroid autoimmunity is apparent when detection of TPO antibodies is combined with fine needle aspiration biopsy [24].

There have been relatively few studies looking specifically at the genetic associations of autoimmune thyroid disease in children. Those studies which have been conducted suffer from the limitations of small sample size to an even greater degree than those in adults, in turn related to the relative infrequency of such conditions in children, and presumably ethical constraints as well. The entire thyroid and other autoimmune literature contains many examples of genetic associations which have not been replicated, as a result of inadequate power and population stratification [25]. As well as relatively simple association studies using a candidate gene approach, there have been attempts to identify novel genes which contribute using genome-wide screening, but again these have not been replicated, and indeed the problem of sample size is magnified in such approaches [26].

HLA alleles confer the best established and strongest susceptibility to autoimmune thyroid disease. In Caucasians, the HLA-DR3 specificity is most consistently associated with both Graves' disease and Hashimoto's thyroiditis and there are conflicting reports concerning a possible role of HLA-DR4 or DR5 in the latter [29]. In a study of 18 patients with juvenile autoimmune thyroiditis, 63% were HLA-DR4, but obviously this is too small a sample to draw firm conclusions, and a direct comparison with adult cases would have been desirable [28]. In another study of 91 juvenile patients, there was evidence of a higher risk conferred by the presence of HLA-DR3, DQ2, as well as positive TPO antibodies, in the fathers of the children [29].

Most recently, the association of the HLA-A1, B8, DR3 (DRB1*0301) haplotype with autoimmune thyroid disease was confirmed in 90 Italian children (mean age 11 years) [30]. Intriguingly there was a significant interaction between DRB1*0301 and infection with *Helicobacter pylori* in the children with autoimmune thyroid disease but not controls. It seems premature to speculate on a causal relationship between thyroid disease and *H. pylori* based on such data, rather than there simply being a shared predisposition (perhaps linked to HLA), but further studies are clearly warranted.

Many genetic studies in children have focussed on type 1 diabetes mellitus, for obvious reasons, and coincidental autoimmune thyroid disease has been co-analysed in this context. How representative such patients are is unclear, as they constitute an example of autoimmune polyglandular syndrome type 2. This is considered further below, but in the context of genetic associations, the presence of TPO antibodies in juveniles with type 1 diabetes is associated with the HLA-DQA1*0301, DQB1*0302 haplotype in Caucasians [31]. These results have been confirmed subsequently but the association does not extend to the presence of parietal cell antibodies [32].

It is well recognised that different racial groups have different susceptibility factors for the same autoimmune disease, and in Korean children with type 1 diabetes, DRB1*0401 was associated with presence of autoimmune thyroid disease [33]. In non-diabetic children DQA1*0301 was associated with thyroid disease, but again small numbers (n = 21) limit the conclusions that can be drawn.

As in adults, *CTLA-4* polymorphisms are also associated with type 1 diabetes and other autoimmune disorders, but within a group of Japanese diabetic children there was an association between polymorphism in exon 1 (G/G genotype) and the co-existence of thyroid autoimmunity, as well as with younger age of onset of diabetes [34]. It has been claimed that childhood and adult Graves' disease may be more genetically different. In Japanese children, there was a similar association with *CTLA-4* polymorphism to adults, but the HLA association was with DRB1*0405 and DQB1*0401 [35]. However only 43 children were analysed and there was no direct comparison with adult patients. A larger study of 65 Chinese children with Graves' disease found that HLA-DQB1*0303 was increased and DQB1*0201 was decreased, and these results also are somewhat different to those of local adult patients, but again there was no direct comparison [36]. Reports of an association between polymorphisms in the MICA (major histocompatibility complex class I chain related gene A) gene and Graves' disease in juveniles may reflect linkage disequilibrium with other, more important HLA genes [37]. Finally, in Caucasian children DRB1*0301 was associated with Graves' disease, as in adults, but the strength of association appeared to be even greater in children [38].

One very long established genetic association remains unexplained, namely the increased frequency of autoimmune thyroid disease in Turner's syndrome [39]. In a typical series of 84 girls with this condition, evaluated at mean age 10 and followed for a mean of 8 years, hypothyroidism was detected in 24% and hyperthyroidism in 2.5% [40]. In 42% there were thyroid autoantibodies and thyroid dysfunction first became apparent at 8 years of age. Although these clinical observations are secure, it is still unclear why the association exists, but together with the increased frequency of both autoimmune hypothyroidism and Graves' disease in prepubertal children, this seems to argue in favour of

a genetic susceptibility effect conferred by the X-chromosome. In the case of Turner's syndrome, this might involve loss of some important autoimmune regulatory function.

In relation to environmental factors, diffuse autoimmune thyroiditis and high levels of thyroid autoantibodies are rare in children in moderately iodine-deficient areas, although TG and TPO antibodies occur at low levels quite frequently [41]. Overall however the prevalence of thyroid antibodies in children in relation to iodine intake is not well established, although pilot data show equal prevalence of TPO antibodies in iodine replete and moderately iodine-deficient patients [42]. Overall, therefore, the effect of dietary iodine on thyroid autoimmunity appears, at best, modest.

Children do seem more susceptible than adults to develop thyroid autoimmunity after fallout radiation or as a side effect of irradiation given for treatment of head and neck lesions. Hashimoto's thyroiditis occurred in 30% of 90 patients who had received head and neck irradiation as children or adolescents; the mean length of follow-up was 26 years [43]. A careful follow-up of children exposed to fallout after the Chernobyl nuclear reactor accident found a significantly higher frequency of thyroid antibodies in children aged 7–14 years compared to unexposed controls (81 vs. 17%) and ultrasonographic abnormalities compatible with lymphocytic thyroiditis were also increased [44]. The dose of ^{131}I that the children had been exposed to correlated with thyroid antibody levels, up to a thyroid gland dose of 4 Gy.

Perhaps the most striking indication for a likely role of environmental factors has been demonstration of a five-fold higher frequency of juvenile Graves' disease in Hong Kong compared to Denmark [45]. Although it is conceivable that this could have a partial genetic basis, this explanation seems far less likely as there does not seem to be such a difference in adults and the pace of change is rapid. In this series, there was a female preponderance of Graves' disease but this increased at adolescence, suggesting the involvement of a sex chromosome-encoded factor and, later, sex steroids then operate as susceptibility factors.

Finally, a survey of physicians' experience of childhood Graves' ophthalmopathy has confirmed that this is uncommon but appears to be found more frequently in countries in which there is a high prevalence of teenagers who smoke [46]. This fits with the fact that smoking is a well known risk factor in adults, for reasons which are still unclear [4]. There is also indirect evidence from this survey of a possible adverse effect of passive smoking in children younger than 10 years of age.

Pathogenesis

Apart from the tendency to spontaneous remission, which is in part related to fluctuation in the level of TSH-R blocking antibodies [2], and the lower levels

of thyroid autoantibodies, there are no particularly distinct pathogenetic features of autoimmune thyroiditis which have been delineated. However, these clinical observations do suggest that the autoimmune response is not fully developed and is susceptible to modulation. Further work to define how this modulation occurs (and why it fails to prevent some children developing permanent hypothyroidism) would be very useful.

Such studies as there are have not compared children and adults with thyroid disease directly making conclusions about any differences tenuous. Clear phenotypic differences exist between circulating lymphocyte subsets in children with Graves' disease and in healthy age-matched controls, including an increase in CD19+ (B cells), CD4+, CD45R0+ (T memory cells) and a decrease in CD8+ T cells, but how such changes relate to intrathyroidal autoimmune events has not been established [47]. The same group has more recently shown a positive correlation between the level of TSAb and circulating T cell expression of CTLA-4 (CD152) in children with Graves' disease [48]. It is difficult to envisage how these two parameters may relate, and more work is required on the intrathyroidal T cell populations which are more clearly involved in the autoimmune response.

Other Autoimmune Disorders

Probably the bulk of immunologically related studies in children and adolescents with autoimmune thyroid disease have addressed the frequency of association with other autoimmune disorders, especially type 1 diabetes mellitus. Although there are few lessons to be gleaned from such reports in a narrow immunological sense, given the fact that all such disorders share similar genetic susceptibility factors, there are clear implications for screening, which in turn frequently leads to questions over the utility of TG and TPO antibody testing. The effectiveness of screening strategies for measuring non-thyroid autoantibodies in autoimmune thyroid disease has been reviewed recently [49]. Major difficulties in such association studies concern adequate population size and inclusion of suitably matched contemporary controls. In attempting to establish baseline frequencies for thyroid antibodies in the healthy population it is clear that age is crucial, since in females, but not males, the prevalence of thyroid antibodies increases at puberty and there is unexplained geographical heterogeneity which is not related to goitre prevalence or iodine intake [50].

Type 1 Diabetes Mellitus and Thyroid Autoimmunity

It is clear that thyroid autoimmunity is more frequent than expected in type 1 diabetes. However, the frequency of autoantibodies in diabetic patients which

are directed against glutamic acid decarboxylase (65-kDa isoform) and IA-2 does not differ between those with or without other autoimmune disorders, including thyroid disease [51]. In a series of 216 diabetic children (mean age 13 years), 10% had TPO antibodies, 8.7% had TG antibodies and 5.9% had both autoantibodies [52]. Around half of those with thyroid antibodies had an elevated TSH and/or echographic features of thyroiditis on ultrasound, or developed these within a mean of 3.5 years of follow-up, and there was an increased risk in those with the highest antibody levels. A similar set of findings have come from a 3-year follow-up of 105 diabetic children with a mean age of 12.7 years at the beginning of the study; the prevalence of thyroid dysfunction rose from 5 to 8%, while the prevalence of TPO antibodies remained constant at 13% and TG antibody positively declined from 14 to 7% [53]. An even higher figure for thyroid autoantibody positivity (25%) was reported in 109 children with a mean age of 13, and the frequency in their first-degree relatives was 27% compared to half the prevalence in controls [54]. Another series found that 18.4% of 197 diabetic children had thyroid antibodies, compared to 7.8% of first-degree relatives and 3.2% of controls [55].

Both series also make clear that these patients are at a significantly increased risk of coeliac disease as well, and support the case for consideration of screening for both coeliac disease and thyroid disease in children with type 1 diabetes mellitus. Parietal cell antibodies are also found in around 20% of diabetic patients, but occur in a somewhat older patient population; those with concurrent thyroid autoantibodies are at 50% greater risk of developing parietal cell antibodies [56].

Other Diseases

Many other diseases are associated with autoimmune thyroid disease in adults but rather few studies have examined these specifically in children [57]. Pernicious anaemia is rare in the young, but in 129 children, mean age 9.7 years, with autoimmune thyroid disease, parietal cell antibodies were present in 30%, and almost half of these had elevated gastric levels [58]. In 80 Kuwaiti children aged less than 12 years with alopecia areata, 17.5% had some evidence biochemical evidence or positive thyroid autoantibodies [59]. Thyroid autoimmunity is also more common than expected in juvenile rheumatoid arthritis, and 25% of the relatives of such patients have autoimmune thyroid disease [60].

Conclusion

Autoimmune thyroid disease is uncommon in children and adolescents, but there is a significant prevalence of self-limiting autoimmune thyroiditis

with positive thyroid autoantibodies and biochemical thyroid dysfunction. There is some evidence that the genetic predisposition to thyroid autoimmunity differs between adults and children, and there are specific, clinically important associations with other autoimmune disease in children. The area still requires further association studies, in particular examining larger cohorts and directly comparing results to series of adult patients from the same area, to elucidate fully the differences, which might enhance our understanding of how the autoimmune response can be modulated for therapeutic benefit.

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Congenital Hypothyroidism

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Epidemiology of Congenital Hypothyroidism

The incidence of congenital hypothyroidism (CH) as detected by common neonatal screening programs, is approximately 1:3,000–1:4,000 in live births [1]. Very recently, in the Netherlands a higher incidence of 1:1,800 was observed using a screening based on thyroxine (T4), thyrotropin (TSH) and thyroxine-binding globulin (TBG) measurement [2]. With this strategy, the incidence of various types of CH was estimated 1:2,200 for permanent CH with 1:2,500 of thyroidal origin, 1:21,000 of central origin, and 1:12,000 for transient hypothyroidism. For unknown reasons, the female/male ratio in CH is consistently 2:1. Newborn infants with Down syndrome have an increased risk for CH of approximately 1:140.

Genetic and Other Causes of Congenital Hypothyroidism

CH represents a heterogeneous group of thyroidal and non-thyroidal disorders (fig. 1), leading to decreased or absent thyroid hormone action and clinical sequelae. In 70–80% of the cases thyroid dysgenesis is found due to agenesis (30%), ectopic gland (48%) or hypoplastic, eutopic gland (5%) [3]. A normal (11%) or enlarged thyroid gland (6%) is observed in children with disorders of thyroid hormone synthesis. Up to 15% of cases with CH occur on a hereditary basis (table 1), while the remaining majority of cases are considered sporadic forms.

Severe central hypothyroidism due to isolated TSH deficiency frequently results from TSH-β subunit (*TSHB*) mutations [4, 5]. Similarly, TSH deficiency may be found as a component of combined pituitary hormone deficiencies

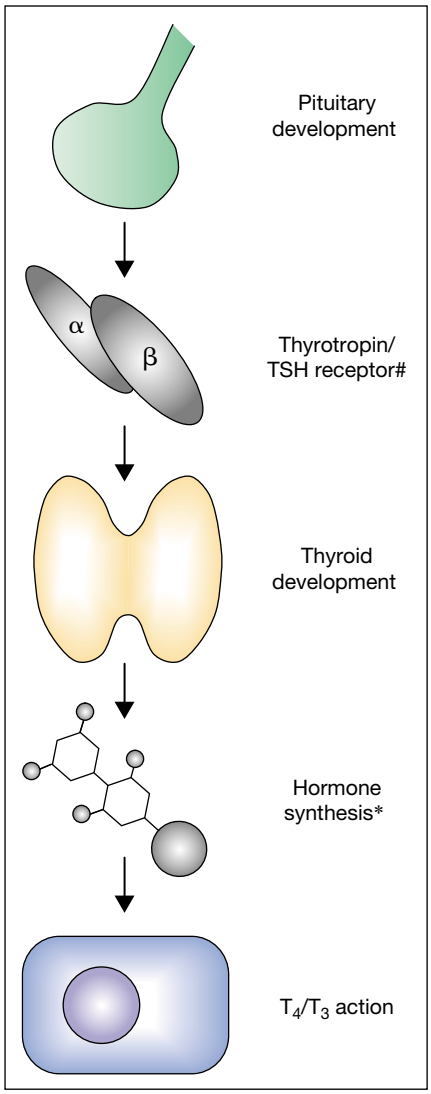

Fig. 1. Anatomic and functional levels at which CH may occur, including exogenous and maternal causes of transient CH. *Antithyroid medication, iodine deficiency; #blocking TSHR antibodies.

(CPHD). In some of these patients, mutations in homeobox genes including *POU1F1*, *PROP1*, *LHX3*, *LHX4*, *HESX1* and *PHF6* have been identified [6–8], with *PROP1* mutations accounting for the majority of cases with familial CPHD.

Isolated thyroid dysgenesis may be caused by inactivating ('loss-of-function') mutations of the TSH receptor (*TSHR*) (fig. 2). CH in association with various complex syndromes is found in patients carrying mutations of thyroid transcription factors *PAX8*, *TITF1*, *TITF2* or the stimulatory G protein α-subunit

Table 1. Genetic causes of congenital hypothyroidism

Gene	Protein function	Heredity	Thyroid volume	Associated malformation
Central (pituitary) hypothyroidism				
TSHB	TSH subunit	AR	↓ – n	–
TRHR	TRH receptor	AR	↓ – n	–
POU1F1	pituitary transcription factors	AR/AD	↓ – n	GH, PRL deficiency
PROP1		AR	↓ – n	CPHD, pituitary mass
LHX3		AR	↓ – n	CPHD, pituitary mass, rigid cervical spine
LHX4		AD	↓ – n	CPHD, hindbrain-, sella turcica defect
HESX1		AR/AD	↓ – n	CPHD, septooptic dysplasia
PHF6		X-linked	↓ – n	CPHD, epilepsy, septo-optic dysplasia
Thyroid aplasia or hypoplasia				
TSHR	thyrotropin receptor	AR	↓, ↑, or n	–
PAX8	thyroid transcription factors	AD	↓	renal agenesis
TITF1		AD	↓ – n	choreoathetosis, pulmonary disease
TITF2		AR	↓	cleft palate, choanal atresia
GNAS1	signalling protein	AD	n	osteodystrophy
Abnormal thyroid hormone synthesis				
TPO	peroxidase	AR	↑	–
THOX2	oxidase	AR	↑ – n	–
TG	storage protein	AR	↑ – n	–
Pendrin	anion transporter	AR	↑ – n	sensineural hearing loss
NIS	Na^+/I^- symporter	AR	↑ – n	–
DEHAL1	iodine recycling	AR	↑ – n	–
Defects of thyroid hormone action				
MCT8	transmembrane T3 transporter	X-linked	↑ – n	severe neurological abnormalities
THRB	nuclear thyroid hormone receptor	AD/AR	↑ – n	hyperactivity, learning disorder

AD = Autosomal-dominant; AR = autosomal-recessive; GH = growth hormone; n = normal; PRL = prolactin.

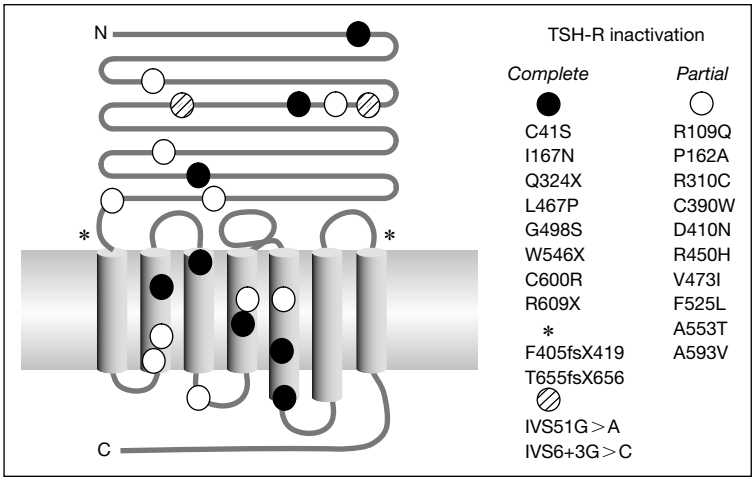

Fig. 2. Schematic illustration of the thyrotropin receptor (TSH-R), a heptahelical G protein-coupled receptor. The localization of inactivating TSH-R mutations is shown by symbols. Mutations have been categorized as partial or complete loss-of-function variants according to clinical and/or functional in vitro data.

gene (*GNAS1*) [9]. Although thyroid dysgenesis is the most common cause of CH, mutations in thyroid transcription factors or *TSHR* gene are found in less than 10%.

Inborn errors of T4 synthesis are frequently caused by inactivating mutations of the thyroid peroxidase (*TPO*). A positive perchlorate discharge indicating total iodine organification defect is suggestive of a defect of TPO or, more rarely, thyroid oxidase 2 (*THOX2*) function [10]. Low serum levels of thyroglobulin associated with enlarged thyroid gland and elevated TSH are typical for thyroglobulin (*TG*) defects, while sensorineural deafness is a frequent finding in Pendred's syndrome. CH caused by mutations of the sodium iodine symporter (*NIS*) is characterized by low radionuclide uptake in the thyroid [9].

Mutations in the iodotyrosine dehalogenase gene, *DEHAL1*, leading to a iodine recycling defect have recently been identified in patients with goitrous CH and presence of mono- and di-iodotyrosines in urine [11]. Iodothyronine transporter defects associated with severe neurological abnormalities have been described due to mutations of the monocarboxylate transporter gene (*MCT8*) [12]. Hypothyroidism is usually mild with normal or elevated TSH. Familial thyroid hormone resistance, caused by various thyroid hormone receptor (*THRB*) defects, is paradoxically associated with elevated serum thyroid hormone levels and mild-to-moderate hypothyroidism.

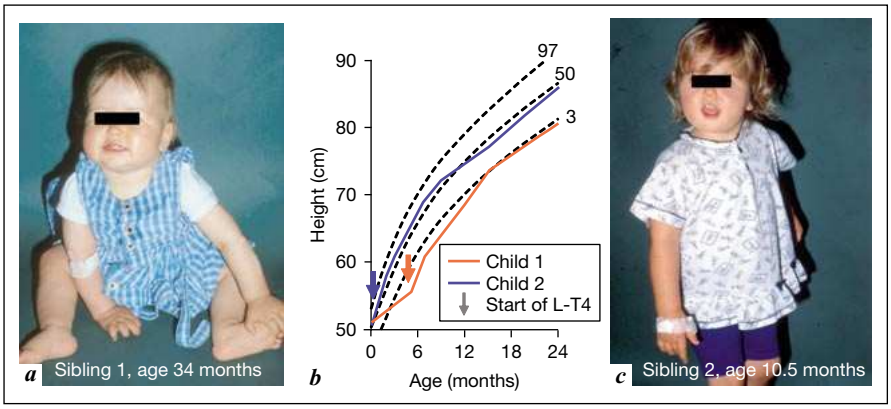

Fig. 3. Clinical consequences of delayed versus early thyroxine treatment in two sisters with congenital hypothyroidism caused by inactivating TSH-β mutation (C105Vfs114X). In child 1, thyroxine was initiated at 5 months of age. At 34 months of age, she is characterized by severe psychomotor retardation, difficulties to stand and walk (***a***), and growth retardation (***b***). In contrast, thyroxine treatment was started immediately after birth in child 2, followed by normal development (***b***, ***c***).

Less commonly, hypothyroidism is transient and may be attributable to transplacental passage of maternal antithyroid medication, blocking TSHR antibodies, iodine deficiency or excess, or heterozygous *THOX2* gene mutations.

Debate on Newborn Screening Programs

To detect CH, primary TSH screening is used in most European countries, Japan and Australia. However, using this approach, some forms of CH including delayed TSH elevation in infants with TBG deficiency or low birth weight, central hypothyroidism and hypothyroxinemia are missed. In North America, a T4-based program with additional measurement of TSH in samples with lowest T4 concentration is commonly used [1]. Primary T4 screening with backup TSH measurements has the potential to detect primary hypothyroidism, TBG deficiency and central hypothyroidism. The recall rate for primary hypothyroidism in both approaches is 0.05%, and the rate of false positive results is higher using the primary T4 strategy.

Although both screening strategies detect CH of thyroidal origin, they may miss patients with central CH because T4 may be only moderately decreased and TSH is not elevated. In such patients, however, early diagnosis is crucial not only for early and appropriate thyroxine replacement (fig. 3), but

also to detect or rule out CPHD for which adequate and timely treatment is fundamental.

In the Netherlands, a T4-TSH-TBG-based screening strategy has been implemented which has been shown to detect CH of variable origin and severity [2] with a sensitivity of 95.8% and specificity of 99.9% [13], associated with the highest incidence rates worldwide. A high rate of false-positive results mainly due to severe illness or TBG deficiency, and occasional false negative cases in very mild forms of CH with normal T4 levels or in premature neonates are pitfalls of this strategy that have to be addressed in the future.

Preterm infants with CH may have a delayed TSH increase owing to the immaturity of the hypothalamic-pituitary-thyroid axis, and may thus be missed by laboratory screening procedures. Therefore, a routine second screening between 2 and 6 weeks of age has been suggested in preterm neonates [14] leading to a reported additional 10% of cases.

Clinical Outcomes of Congenital Hypothyroidism

Longitudinal growth, final height and pubertal development are typically normal in male and female individuals with CH in whom L-T4 therapy is maintained as recommended [15, 16]. Pubertal timing and final height are independent of etiology, severity of CH and the start of L-T4 treatment, but girls with a higher initial dose L-T4 (>8 μg/kg/d) had an earlier onset of puberty [15].

In contrast to physical signs, the neurodevelopmental outcome of patients with CH largely depends on the early initiation and maintenance of adequate postnatal L-T4 therapy, especially in cases of severe hypothyroidism (T4 < 5 μg/dl). Despite neonatal screening, 10% of early treated infants with severe hypothyroidism are likely to require special education [17]. Subtle differences in intelligence, school performance and neuropsychological tests in comparison to control individuals, classmates and siblings have been detected in adults with CH despite early L-T4 treatment [18, 19]. While in some studies the severity of CH was correlated with poor developmental outcome, recent observations indicate that delayed and inadequate hormone substitution is a main predictor of clinical outcome [18, 20].

Children with CH may have selective deficits on visual, language, motor, attention and memory abilities [21]. Auditory brainstem evoked potentials were abnormal in 25% of early-treated patients with CH [22]. Recent studies have comprehensively analyzed the temporal patterns of thyroid hormone action in the developing brain [21]. Hypothyroidism in early pregnancy is related to impaired visual attention and processing as well as gross motor abilities.

Exposure to maternal hypothyroxinemia in later pregnancy is linked to an additional risk of subnormal visual skills, including impaired contrast sensitivity, slower response speeds and fine motor deficits [23]. In case that hypothyroidism occurs after birth, language and memory are brain functions predominantly affected.

It has to be considered that syndromatic forms of CH due to functional defects of thyroidal transcription factors or the iodothyronine transporter (table 1) may adversely affect CNS development independent of circulating thyroid hormone levels. The long-term perspective for normal mental and neurologic development is poor for infants with CH not detected by newborn screening. Physical symptoms and growth may normalise when L-T4 treatment is started later but within the first months of life but infants with severe perinatal hypothyroidism frequently have low-to-normal IQ [1].

Less favorable neurodevelopmental outcome is related to late treatment start, inadequate L-T4 dosage, poor social-economic environment, compliance problems and severity of CH. A better neurodevelopmental outcome was obtained with higher initial L-T4 dose of 11.6 μg/kg/day [24, 25] and faster time to normalize thyroid function (<2 weeks) [26]. Since thyroid hormone replacement is now more vigorous in achieving early correction than in previous decades, neonates with CH today may have eventually better intellectual and neurological long-term outcomes.

Diagnostic Work-Up of Congenital Hypothyroidism

A positive newborn screening result calls for immediate diagnostic work-up. Information on maternal medication or morbidity should be obtained to assess the infant's prenatal thyroid status. Clinical examination should be performed to document signs and symptoms of CH and possible associated malformations. There is an increased risk for other congenital anomalies (8.4%), including cardiovascular, musculoskeletal and CNS malformations [3].

Confirmatory serum measurements of TSH and T4 are required, along with thyroid hormone binding proteins and serum free T4. In cases of maternal autoimmune thyroid disorder, assessment of TSHR blocking antibodies may indicate a transient form of CH. Thyroglobulin levels tend to be high in dyshormonogenesis and low in thyroid agenesis. Thyroid ultrasonography and/or thyroid scan are considered optional for management of CH [1] but are necessary to clarify the underlying source of CH, to distinguish between thyroid aplasia, ectopy or inborn errors of T4 synthesis. Testing thyroid function in first degree relatives may be informative because of the variable penetrance of inherited CH. Measurement of iodine or iodotyrosines in

urine are helpful if iodine exposure, iodine deficiency or recycling defects are considered.

It is clinically important to distinguish permanent or transient forms of CH. If imaging studies reveal ectopic or absent thyroid tissue, hypothyroidism is probably permanent. If initial TSH is below 50 mU/l and there is no increase after the neonatal period, at 3 years of age discontinuation of L-T4 may be considered [1]. If TSH increases after 1 month discontinuation, permanent hypothyroidism is probable, and L-T4 treatment must be resumed. Regular follow-up visits are essential to ensure optimal growth and development including auditory and visual abilities and neuropsychological skills.

In recent years genetic studies have revealed a variety of molecular defects underlying CH. In the clinical management of patients with CH, however, genetic testing is currently not yet recommended on a routine basis.

Treatment Recommendations

As the result of newborn TSH screening is available within 10–14 days, treatment of CH is commonly initiated within the first 2 weeks of life. An initial dosage of 10–15 μg/kg/d L-T4 per os is recommended [1]. T4 and TSH should be normalized within 2 and 4 weeks of L-T4 therapy, respectively. Serum total T4 or free T4 should be maintained in the upper half of the reference range (10–16 μg/dl [130–204 nmol/l] or 1.2–2.3 ng/dl [18–30 pmol/l]) during the first 3 years of life with a low normal serum TSH concentration [1].

To ensure optimal dosage and compliance, frequent evaluations of thyroid hormone serum levels are necessary. These tests should be obtained 2 and 4 weeks after L-T4 start, every 1–2 months during the first year of life, every 3–4 months between 1–3 years of age and 2–4 weeks after any change in dosage [1]. During L-T4 therapy, 4 or more episodes of elevated TSH (>5 mU/l) after the age of 6 months were associated with inferior school performance [27]. These episodes may be caused by poor parental empowerment or impaired T4 bioavailability. The latter may be caused by inhibited intestinal uptake of T4 through soy or fiber and medications with iron or calcium, malabsorption or increased degradation by anticonvulsants.

Because poor compliance has major consequences, initial and ongoing counseling of parents is of utmost importance. Education of parents by trained professionals should address the etiology of hypothyroidism, the benefit of early diagnosis in preventing mental retardation, the appropriate L-T4 application, and the importance to follow treatment regimens and regular visits. Thus, the pediatrician plays a central role to provide a medical home for every child with CH to coordinate care and lifelong disease management.

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Newborn Screening, Hypothyroidism in Infants, Children and Adolescents

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Newborn Screening

Newborn screening (NS) for congenital hypothyroidism (CH) is one of the major achievements of preventive medicine [1, 2]. Although since 1972 the problem of CH has been resolved in developed countries by the implementation of NS for CH, the problem exists for developing countries that still have no NS programs for CH [2, 3]. Since diagnosis based on clinical findings is delayed in most instances because of few symptoms and signs, hypothyroidism in the newborn period is almost always overlooked and delayed diagnosis leads to the most severe outcome of CH, mental retardation. In a study from Denmark, it was emphasized that only 10% of the affected infants were diagnosed within the first month of life, 35% within 3 months. 70% were diagnosed within the first year, while in the remainder the diagnosis was delayed to the 3rd and 4th years of life [4]. In a retrospective analysis of 1,000 cases of CH from Turkey, the mean age at diagnosis was 49 months and only 3.1% of cases were diagnosed within the first month, whereas 55.4% were diagnosed after 2 years of age [5].

The first CH screening was performed by Dussault and Laberge [6, 7], in Quebec, Canada in 1972. They detected 7 hypothyroid infants among 47,000 newborns during 3 years. The high frequency of false-positives delayed the diagnosis and increased the cost and they arranged the cutoffs used for recall. The babies recalled underwent thyroid hormones and TSH blood studies. In the meantime, radioactively labeled antibodies for determining T4 in dried blood spots began regionally in the USA and Europe. They went parallel with screening programs of PKU. In the initial report by Dussault et al. [8], the method was recommended as a confirmatory test knowing that it would miss cases with hypothlamic-pituitary hypothyroidism which they reported to be 10% of the

cases. In 1976, it was reported in the *Lancet* that cord blood TSH measurements were shown to have greater sensitivity and specificity to cord blood T4 and blood spot T4 (collected on 3- to 4-day-old newborns) and false-positives were high in T4 method with high costs [9]. Walfish [9], suggested routine T4 screening supplemented by TSH estimation be used in mass screening.

Blood spot T4 or TSH or both could be used in NS for CH. The latter, which is more sensitive, is not cost effective so the first two are used in different programs around the world. North America usually prefers primary T4 testing supplemented with TSH and Europe prefers primary TSH in the detection of CH [10–14]. TSH screening was shown to be more specific in the diagnosis of CH, T4 screening was more sensitive in detecting newborns especially with rare hypothalamic-pituitary hypothyroidism but less specific with a high frequency of false-positives mainly in low-birth-weight and premature babies. Thyroxine-binding globulin (TBG)-deficient babies who are euthyroid could be detected by T4 screening who are not targets for NS.

1982, a Neonatal Thyroid Screening Conference held in Tokyo recommended NS programs oriented to detect infants with elevated serum concentrations of TSH [15]. They suggested that this could be accomplished by measuring TSH in filter paper blood spot or by measuring T4 supplemented by TSH on the same blood spot of infants who have T4 values in the lower 3rd to 10th percentile.

Methods

The aim is to detect all cases with the disease, as early as possible, with an acceptable cost-benefit ratio and to avoid false-positive results. Today more sensitive and automated methods (chemiluminescence, fluoroimmunoassay, etc.) for determining both T4 and TSH in dried blood spots have been introduced [16–21]. They have increased sensitivity and specificity. Besides the development of more accurate test programs, some children may still be missed in any screening program. The reasons could be failure of sample collection, unsatisfactory samples, misinterpretation of samples and unsatisfactory recalls.

The ideal time to obtain the blood spot is 3–5 days after birth to minimize the false-positive high TSH due to the physiological neonatal TSH surge that elevates TSH levels and causes dynamic T4 and T3 changes in the first 1 or 2 days after birth. Early discharge of mothers postpartum has increased the ratio of false-positive TSH elevations. The difficulty in screening for CH using cord blood samples is in the handling and transport of the samples, making it an impractical method for mass screening [22].

Whichever method is used, babies whose initial TSH is $>50\ \mu U/l$ are most likely to have permanent CH, whereas a TSH between 20 and 49 $\mu U/l$ is frequently a false-positive or represents transient hypothyroidism. Transient CH is particularly common in premature infants in borderline iodine deficient areas.

In the primary TSH method, when 15 μU/l (immunofluorometric method) or 20 μU/l (radioimmunological method) is used as cutoff, the recall rate is quite low to be 0.05%. Iodine deficiency could increase false-positives and increase recall rate. The sensitivity of TSH method for CH is suggested to be 97.5% and specificity 99% [23, 24].

Neonatal screening with the primary TSH method detects:

(a) overt and compensated primary hypothyroidism.

Neonatal screening with the primary TSH method misses:

(a) secondary-tertiary hypothyroidism;
(b) TBG deficiency;
(c) premature babies with very LBW with a delayed TSH surge.

In primary T4 screening, performed in some states of the USA, cutoff to the 10th percentile resulted in 1.5% missed cases, whereas cutoff to the 5th percentile T4 values resulted in 3.5% cases. Only 0.2% of cases were missing using the 20th percentile as a cutoff, but off course with increased cost in terms of repeat testing [25]. Optimal screening requires initial T4 determination to be followed by TSH determinations on low T4 samples.

Neonatal screening with the primary T4 method detects:

(a) overt primary hypothyroidism;
(b) secondary-tertiary hypothyroidism (1 in 50,000–100,000 live births);
(c) hypothyroxinemia in a sick and preterm newborn;
(d) TBG deficiency;
(e) hyperthyroxinemia.

Neonatal screening with the primary T4 method misses:

(a) compensatory hypothyroidism with subnormal T4 and elevated TSH levels;
(b) transient hyperthyrotropinemia where iodine deficiency is present.

Reliability of the laboratories is as crucial as the reliability of detection methods (with emphasis on sensitivity, specificity and positive predictive value). According to the recommendations of the working group of NS of ESPE (European Society for Pediatric Endocrinology), screening should be conducted in centralized laboratories covering 100,000 newborns per year [26]. These laboratories should participate in international control programs. In North America it is estimated that 6–12% of the neonates with CH are missed due to biological factors and screening errors [27, 28].

Neonatal Screening Results

Hypothyroxinemia (Low T4 and Normal TSH)

It occurs most commonly in premature infants, in whom it is found in 50% of babies of less than 30 weeks' gestation [26]. Screening programs that employ

primary TSH analysis will miss these infants because of normal TSH levels. Often the free T4 is less affected than the total T4. The reasons for the hypothyroxinemia of prematurity are complex. In addition to hypothalamo-pituitary immaturity, low TBG levels and decreased conversion of T4 to T3 exists in prematures. Numerous studies have shown that there is a correlation between the degree of lowering of T4 and negative outcomes; both mortality and developmental problems. Systematic supplementation of all low-birth-weight babies is not recommended at this time [23, 29, 30].

Other causes of low T4 in the face of normal TSH are euthyroid sick syndrome, TBG deficiency, laboratory errors and central hypothyroidism [3]. Immature liver function, undernutrition and illness are the reasons for low T4 and normal TSH levels in euthyroid sick syndrome. Euthyroid sick syndrome may be seen in the sick term newborns as well [23]. TBG deficiency is an X-linked condition discovered only by screening programs using the primary T4 approach. It does not require treatment since the plasma levels of free thyroid hormone levels are normal and subjects are euthyroid. Its incidence is estimated to be 1 in 2,800 [31]. TBG deficiency should be estimated especially in male infants with low T4 and normal TSH and could be confirmed by measuring TBG levels in the serum. Loss of protein from nephrotic syndrome may also lead to low total T4. Errors in measurement may be caused by errors in sample gathering, impregnation with water due to improper sample handling or less amounts of blood spots or extremes hematocrit values which adversely affect the measurements.

In a term neonate with a low free T4 but normal TSH level, true central hypothyroidism, which is quite rare, should be ruled out. Mutations in the gene coding for the beta subunit of TSH or the TRH receptor could be the causes [32, 33]. Central hypothyroidism could coincide with other anterior pituitary hormone deficiencies: hypoglycemia, microphallus, prolonged jaundice and/or cryptorchidism [34–36].

Isolated Hyperthyrotropinemia (Normal T4 and Elevated TSH)

Elevated TSH, despite a normal or low normal T4 indicates inadequate hormone production. It is most common in premature babies. Although some babies have compensated hypothyroidism, the etiology is not clear in the others. In early discharged babies (in the first day or two), because of the cold-induced TSH surge, TSH values are found to be elevated. It could be a transient finding due to goitrogens, iodine deficiency or medications. Genetic defects of hormone biosynthesis and also dysgenesis especially ectopia could be the causes. TSH rises with normal T4 levels could persist for years [37].

Table 1. Causes of transient hypothyroidism

Maternal antithyroid medication
Exposure of topical iodine
Maternal iodine deficiency or excess
Maternal TSH receptor blocking antibodies
Medications (dopamine, steroid)
Prematurity (<30 weeks)

Table 2. Causes of childhood hypothyroidism

(a) Congenital hypothyroidism
(b) Acquired hypothyroidism
– Autoimmunity (Hashimoto thyroiditis)
– Drug-induced hypothyroidism
Antithyroid
Anti-TBC
Iodine compounds
Lithium, cobalt, sulfonamides
– Thyroidectomy
– Endemic goiter
Iodine deficiency
Environmental goitrogens
– Irradiation of thyroid
Therapeutic radioiodine
External irradiation of nonthyroid tumors
– Infiltrative disorders
Amyloidosis
Histiocytosis
Cystinosis

Low T4 and Elevated TSH

The most common cause is primary CH. There might be transient cases as shown in table 1.

Although transient hypothyroidism may occur frequently, all the suspected infants should be treated as CH for the first 3 years of life by taking into account the risks of mental retardation. A re-evaluation after 3 years is needed in such patients [1, 38–40].

Hypothyroidism in Infancy, Childhood and Adolescence

Hypothyroidism during childhood and adolescence can result from a variety of congenital or acquired defects (table 2).

Table 3. Symptoms of childhood hypothyroidism

Growth and developmental delay
Short stature
Infantilism in anthropometric ratios
Bone age delay
Motor developmental delay
Skin and hair
Pale, coarse, dry and cold skin
Hypertricosis in forehead and neck
Rare, dry, thick hair
Myopathy and muscular pseudohypertrophy
Delayed puberty
Rarely precocious puberty
Sluggish motor performance, sleepiness, cold intolerance

Some children present with an asymptomatic goiter, whereas others may present with mild tenderness or a sensation of fullness in the anterior neck [41]. The course of hypothyroidism is often so insidious that neither the child nor the parents are aware of the physical changes that have occurred. These children often have marked growth retardation before the disease is recognized, and the expected effect on linear growth emphasizes the importance of serial growth measurements in all children. Children who develop hypothyroidism before age 2 years may suffer some irreversible central nervous system damage and developmental delay, the onset of hypothyroidism developed after infancy does not cause mental retardation [42] (table 3).

Deceleration of linear growth is an important sign that is helpful in the early recognition of this disease. Affected children are relatively overweight for their height, although they are rarely obese. If hypothyroidism is severe and long-standing, immature facies and immature body proportion (increased upper/lower body ratio) may be noted with delay in dental and skeletal maturation. The children have cold intolerance, dry skin and dry hair texture. In patients with severe long-standing hypothyroidism, muscular pseudohypertrophy gives a Herculean appearance called Kocher-Debre-Semelaign syndrome [23].

The child with severe primary hypothyroidism may develop enlargement of the cella tursica. After radiologic examination, the detected mass represents hypertrophy and hyperplasia of thyrotrophs in response to lack of negative feedback by thyroid hormones [43]. In laboratory evaluation they have high levels of TSH with low levels of T4.

Puberty tends to be delayed in hypothyroid children, although sexual precocity has been described too [44]. The cause of precocious puberty in primary

Table 4. Na L-T4 dosages with respect to age in hypothyroidism

1–3 years	4–6 μg/kg
3–10 years	3–5 μg/kg
10–16 years	2–4 μg/kg

hypothyroidism is presumed to be from chronic TRH stimulation of the pituitary which could cause galactorrhea in girls with elevated prolactin levels [45, 46]. More recent studies have shown that TSH can bind and activate both LH and FSH receptors and elevated TSH levels in stimulation of both LH and FSH receptors could contribute to the development of precocious puberty [47, 48].

Diagnostic Evaluation

Measurement of TSH and thyroid hormones, antithyroid antibodies namely thyroperoxidase (TPOAb) and thyroglobulin (TGAb) should be obtained. The presence of the antibodies permits the diagnosis of autoimmune thyroiditis. A hypothalamic cause vs. pituitary origin of the hypothyroidism with low serum free T4 and TSH levels can be distinguished by TRH testing. In children with hypothalamic hypothyroidism the peak serum TSH response to TRH is often delayed beyond 30 min, and the TSH response may be prolonged with serum TSH values that remain elevated for 2–3 h. In hypopituitarism, there is little or no TSH response to TRH. Thyroid hormone resistance is characterized by elevated levels of T4 and T3 and an inappropriately normal or elevated TSH concentration.

Treatment

The aim of treatment of hypothyroidism in childhood is to attain normal growth, neurological and pubertal development. The drug of choice is Na L-thyroxine (T4). It should be given once daily, half an hour before breakfast. Iron, calcium and colestiramin interfere with the drug absorption.

If hypothyroidism exists for long periods, Na L-T4 treatment should be given with gradual increments, beginning with small doses to prevent hyperactivity, unsleepiness and school performance deterioration (table 4).

The mean dosage of Na L-T4 could be calculated as 100 $\mu g/m^2$. The dosage should be arranged by the regular follow-ups with T4 and TSH measurements.

Hashimoto Thyroiditis

Chronic lymphocytic thyroiditis (Hashimoto) is an autoimmune disease closely related to Graves disease [49]. It was first described by Hashimoto [50]

in 1912. Although lymphocyte and cytokine-mediated thyroid destruction predominates in Hashimoto thyroiditis (HT), antibody-mediated thyroid stimulation occurs in Graves disease and overlap may occur in some patients. HT arises from a combination of genetic traits that heighten susceptibility in conjunction with some environmental trigger.

HT occurs in 1% of children and adolescents and is the most common cause of acquired hypothyroidism in the pediatric population [51]. The disease has a predilection for females 4 to 7 times and a family history is present in 30–40% of patients. The prevalence increases with age with the common age of adolescence [52]. HT accounts for many of the enlarged thyroids formerly designated as adolescent or simple goiter [53]. Goiter is present in two thirds of children, resulting from lymphocytic infiltration and from the stimulatory effect of TSH. The remaining one third of children have no goiter [54]. The patients could be euthyroid, hypothyroid or hyperthyroid. Opthalmopathy may occur in HT in the absence of Graves disease [55]. The course is variable. The goiter may become smaller or may disappear spontaneously or it may persist unchanged for years while the patients remain euthyroid. Some euthyroid children acquire hypothyroidism gradually within months or years, and some adolescent patients achieve spontaneous remission. Thyroid function tests are often normal in HT, although the level of TSH may be slightly or moderately elevated in some individuals. Thyroid scintigraphy can be entirely normal, but in most instances the radioiodine uptake is decreased. Early in the course of the disease, increase uptake could be noted. Thyroid ultrasonography shows scattered hypoechogenicity in most patients [56].

Genetic susceptibility is present in HT. Associations have been observed between HT and HLA-DR3, DR4 or DR5 [57, 58]. Familial clusters of HT are common. The incidence in siblings or parents of affected children may be as high as 25% [57]. TPOAbs are demonstrable in the sera of 90% of children with HT. TGAbs occur in a smaller percentage of affected children but much more common in adults. Thyrotropin receptor-blocking antibodies are frequently present especially in hypothyroid HT patients and believed to be the cause of hypothyroidism [51].

HT, a typical organ-specific autoimmune disease, is characterized histologically by lymphocytic infiltration of the thyroid. There is infiltration of lymphocytes and plasma cells between follicles and atrophy and fibrosis of the follicles are present. HT is seen more frequently with type 1 DM, celiac disease, Addison, autoimmune atrophic gastritis, chronic candidiasis and hypoparathyroidism, and juvenile chronic arthritis [59–61]. HT is also associated with certain chromosomal aberrations, in particular Turner, Down and Klinefelter syndromes [51, 53]. Progressive dementia and Hashimoto-related encephalopathy has been reported in some HT patients [62, 63].

Because the disease may be self-limited in some instances, there should be periodic checks in treatment. Untreated patients should also be checked periodically. A TSH level greater than 10 μU/ml warrants treatment with Na L-T4. The initial dose should be arranged according to the age of the patient (25 μg/day to 100–150 μg/day). The goiter may decrease in size as may persist for years. Antibody titers fluctuate in both treated and untreated patients and persist for years.

Iodine Deficiency

Iodine is essential for thyroid hormone synthesis and is present in soil, water and air. Iodine deficiency disorders (IDD), which was referred to as endemic goiter up to thirty years ago refers to iodine deficiency that can be prevented by ensuring an adequate intake of iodine in population [64].

Goiter is the most frequent and visible manifestation of IDD and is an important health problem. It effects intellectual growth in neonates and children and almost 20 million people living in developing countries have some degree of brain damage due to the effects of iodine deficiency (ID). ID in the mother results in deficiency of the neonate. The most striking feature of ID is endemic cretinism. In severe iodine deficiency, endemic goiter and cretinism; increased perinatal death, decreased fertility rate and increased infant mortality occur. Combined iodine and selenium deficiency causes a severe form of cretinism in some areas. Two types of endemic cretinism have been defined [65–67]. In neurological cretinism, the number of neuronal cells are decreased, brain weight is reduced. Myxedematous cretinism has a less severe degree of mental retardation than neurological cretinism. Iodine deficiency in children is characteristically associated with goiter. Goiter rate increases with age and reaches a maximum at adolescence [68–70].

Iodine is present in the human body in minute amounts (10–20 μg). The recommended dietary allowance is 60–100 μg/daily for 1–10 years of age and 100 μg/daily for adolescents and adults. Urinary iodine (UI) excretion provides a measure of the nutritional status of iodine in a population. Dietary iodine intake is positively correlated with its urinary excretion in iodine-repleted areas. 24-hour iodine excreted in the urine shows the iodine nutritional status, but it is impractical and can be unreliable. If nutrition is adequate UI/creatinine is considered a more reliable measure of iodine excretion than random spot UI concentration measurement since there are variations in iodine intake [71, 72]. There are several methods used to detect iodine in urine with different sensitivities; spectrophotometric method, HPLC, mass spectrometry and laser spectrometry [73–75]. UI excretion 50–99 μg/l is defined as mild iodine deficiency,

Table 5. Prevalence of IDD in school-aged children (WHO)

Region	UI <100 μg, %	UI <100 μg, millions
Africa	47.6	48,342
The Americas	14.1	9,995
East Mediterranean	55.4	40,224
Europe	59.9	42,206
SE Asia	39.9	95,628
West Pacific	19.7	36,082
Total	36.9	272,438

20–49 μg/l is defined as moderate iodine deficiency and UI excretion <20 μg/l is severe iodine deficiency (table 5) [76, 77].

Prevention of Iodine Deficiency

(a) *Iodized Salt*. The daily recommended level of salt is 3–5 g. The level of iodization of salt has to be sufficient to cover the requirement together with losses from the point of production to the point of consumption. The packing of salt is important, it loses some of its activity with boiling. Iodized salt was used for the first time in 1920s in Switzerland and USA and in 1950s in Europe. But despite the elimination programs ID still exists in different parts of the world [78–81].

Problems with the iodization of salt are [80]:

- Not reaching all target communities
- Plethora of small scale salt producers makes salt iodization programmes difficult to implement in some countries
- Some salt producers are unwilling to pay for potassium iodate, which is recommended agent for iodization or use less amounts
- Frequently unacceptable variation in the quality of iodized salt
- Some iodization programmes are not being adequately monitored
- Lack of laboratory facilities in many countries for monitoring salt and urinary iodine levels
- Transient increase in the incidence of hyperthyroidism in some countries after salt iodization

(b) *Iodized Oil.* Iodized oil (lipiodol) was first used in Papua New Guinea. The effectiveness of the single dose iodized oil injection (4 ml) corrects iodine deficiency for a period of 4.5 years [82]. Refrigeration is not required and the cost is low with respect to iodized salt. It could be taken orally too [78]. Iodized oil should be used in severe IDD areas until an effected program is introduced.

(c) *Iodized Bread, Iodized Milk, Iodized Water, Iodine Tablets*. Used in different countries as iodine sources [83, 84].

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Resistance to Thyroid Hormone in Childhood

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Occasionally, a patient is seen in the clinic with apparent hypo- and/or hyperthyroid symptoms but with conflicting results of thyroid function tests: they have a nonsuppressed or even slightly increased TSH inappropriate for the invariably increased free T4 in serum. This combination of hormone levels can have several causes but there are two major ones namely a TSH-producing pituitary adenoma and resistance to thyroid hormone (RTH). The latter will be the subject of this chapter. The basic problem in RTH is a decreased sensitivity of tissues to thyroid hormone. The decreased sensitivity is also present in the pituitary, where it leads to a blunting of the feedback of thyroid hormone on the pituitary. This in turn results in the above mentioned increased secretion of TSH and thereby of T4. As will be explained below, the insensitivity is caused by mutations in the thyroid hormone receptor beta isoform that reduce thyroid hormone binding affinity. This does not just lead to a presentation resembling hypothyroidism as would be expected – many patients present with symptoms reminiscent of hyperthyroidism, especially with tachycardia.

Normally the net effects of thyroid hormone are brought about by the positive or negative changes it causes in the expression of T3-responsive genes in target tissues. For instance the rise in LDL-cholesterol found in hypothyroid patients can be attributed to a decrease in LDL-receptor protein expression. The gene for this protein is sensitive to thyroid hormone. The presence of thyroid hormone is signaled by nuclear thyroid hormone receptors (TR) of which at least five isoforms exist (fig. 1). These are members of the so-called nuclear receptor family of which the steroid, vitamin D and retinoic acid receptors are also a member. These receptors influence gene expression by binding to specific DNA elements as dimers. TR can bind as a homodimer (two identical monomers) or as a heterodimer (two different

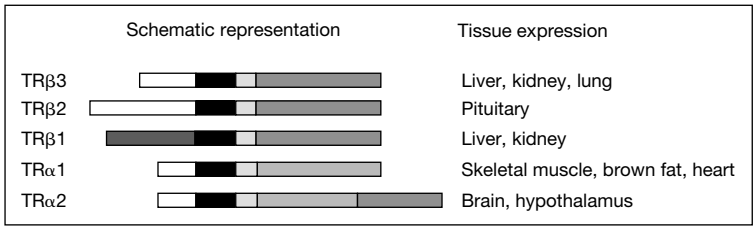

Fig. 1. Schematic representation of the five thyroid hormone receptor (TR) isoforms. The receptor isoforms are shown schematically with the tissues where their expression is most prominent. The black box represents the DNA-binding domain. TRβ1, TRβ2 and TRβ3 differ in their N-terminal domain whereas TRα1 and TRα2 differ in their C-terminal domain. In both cases, an mRNA is transcribed from one gene which due to alternative splicing or alternative promoter usage yields the α or β isoforms.

monomers) to these specific DNA elements which are called thyroid-response elements (TRE), located in the promoter region of T3-responsive genes. There are at least four active (=T3 inducible) TR namely TRα1, TRβ1, TRβ2 and TRβ3 which bind T3 and one inactive one, TRα2 which does not bind T3. They are derived from two different genes located on chromosomes 17 and 3, respectively. The TR genes are expressed at different levels in different tissues (fig. 1). Furthermore, a number of different TRE can bind the receptors, sometimes in specific combinations, so a plethora of regulatory possibilities is present. This explains how TR (after binding T3) can both activate or repress a gene. The latter happens for instance in the case of the feedback to the pituitary where the ligand-bound TR binds to a special TRE of the TSHα and β-subunit genes and thereby shuts the gene down.

Thyroid hormone receptors are rather unique among their family in that they can influence gene expression with or without ligand. This is because the TR can bind to a TRE without hormone. When it does, it recruits a so-called co-repressor protein which silences the gene. Upon binding of the hormone the receptor homodimer releases the co-repressor and then falls apart. The ligand-bound TR monomer then heterodimerizes with the retinoic X receptor (RXR) and binds again to the same TRE but due to a conformational change is now able to recruit a coactivator and so can increase gene transcription (fig. 2).

After the three dimensional structure of the thyroid hormone receptor had been solved [1] it became apparent that thyroid hormone is tightly packed inside the receptor molecule and that the last few amino acids of the receptor protein (helix 12) act like a lid which closes the box into which the hormone fits. From these studies, it has also become clear that the closure of the lid is necessary for the formation of the binding site of the coactivator.

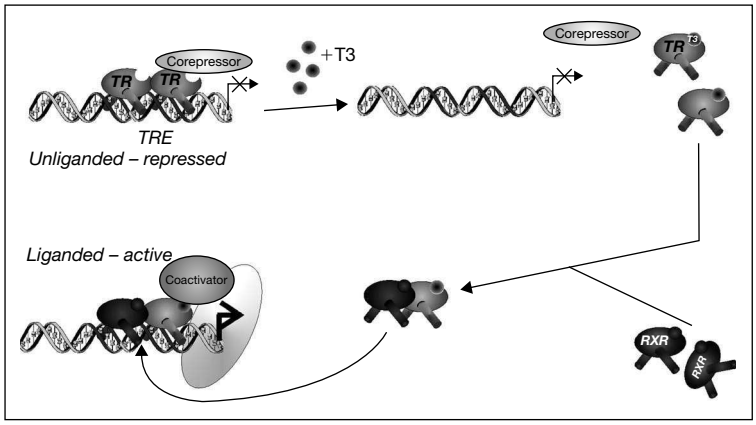

Fig. 2. Model for gene activation by the TR. When the TR is unliganded it binds to the TRE of the gene as a homodimer (two identical TR monomers) and it represses gene expression by binding to a corepressor. When hormone binds to TR, the corepressor is released and the homodimer falls apart. The TR receptor monomer then acquires another heterodimerization partner RXR (the retinoic X receptor) and then binds again to the TRE as a heterodimer (two different monomers) and attracts a coactivator which will signal to the transcription machinery. As a result the gene is actively transcribed.

Clinical

The first patients described with RTH had a very specific phenotype consisting of short stature, delayed bone maturation, deaf-mutism and very obvious winged scapulae [2]. Further research has shown, however, that a wide variety of symptoms exists in this patient group (table 1) [3]. Up until now about 700 cases have been described [4] and the prevalence of the syndrome is estimated at about 1 in 40,000 [5].

The clinical presentation is heterogeneous. Some patients have no or minor symptoms, others have more marked symptoms which can be of a hypo- or hyperthyroid nature. It is even possible that the two co-exist within 1 patient. Depending on the clinical presentation RTH has in the past been divided into two classes. Patients who are able to maintain peripheral euthyroidism by increasing the T4 production thus compensating for the decreased tissue sensitivity or who present with hypothyroid symptoms, were classified as generalized resistance (GRTH). Those patients who presented with hyperthyroid symptoms were classified as having pituitary resistance (PRTH). Unfortunately, the distinction is not as definite as it may appear and has no firm pathophysiological basis. Hyperthyroid symptoms have also been found in patients defined as having GRTH; furthermore

Table 1. Features of RTH

Biochemical	Normal	RTH	Non-RTH
Raised free T4, pmol/l	12.8–24.4	41±2.1	17.9±0.5
Raised free T3, pmol/l	3.8–8.4	11.4±1.5	6.5±0.4
Normal or slightly elevated TSH, mU/l	0.5–4.5	3.15±0.3	2.5±0.2
Clinical	**Frequency**		
Goitre	65–95%		
Tachycardia	<50–80%		
Emotional disturbances	73%		
Recurrent ear, nose and throat infections	47%		
ADHD	45%		
Hyperactivity/learning disorder	19–42%		
Low IQ (<85)	35–50%		
Delayed bone age	29–47		
Hearing loss	21%		
Short stature	18–26%		

Normal indicates the normal range observed in the general population. RTH = Values as found in RTH patients; non-RTH = values as found in nonaffected relatives of RTH patients. Adapted from Brucker-Davis et al. [8] and Weiss and Refetoff [47].

no significant differences between GRTH and PRTH exist when parameters like age, sex, goiter frequency, and FT3, FT4 and TSH levels are compared.

About two-thirds of the cases patients present with a goiter (table 1). When the goiter is combined with resting tachycardia, palpitations and high T4 serum concentrations, the wrong diagnosis of Graves' hyperthyroidism has often been made in adults [6]. This is now less of a risk since the advent of sensitive TSH assays. One thing to keep in mind is that it has been shown that the bioactivity of serum TSH in RTH patients is higher than normal even though the immunoreactive TSH is normal, stimulating thyroid growth and T4 and T3 secretion [7].

In children, attention-deficit hyperactivity disorder (ADHD) has been found more often (75%) in RTH patients than in their unaffected relatives (15%). Furthermore, in RTH children problems occur in the areas of reading skills and articulation [8]. One third of RTH patients have an IQ <85 which could manifest as a learning disability, and it has been shown in one family that RTH cosegregates with a lower IQ. The relation to ADHD should not be overinterpreted since two studies have shown that in two different cohorts of children with ADHD no biochemical evidence was found for any RTH patient among them [9, 10]. Other features that have been reported include reduced intrauterine

growth, low body mass index (30% of cases), childhood short stature and delayed bone age [8]. Final adult height is often not affected. No effects of RTH have been found on pubertal development, fertility and life expectancy. Furthermore, recurrent pulmonary and upper respiratory tract infections have been reported, as well as hearing defects which may be the result of recurrent ear infections during childhood [8]. Atrial fibrillation is often found in older patients.

Diagnosis

An increased level of free T3 and T4 in combination with nonsuppressed TSH in serum is indicative of RTH, but is also observed in TSH-secreting pituitary adenomas. There are, however, a number of other conditions which can give rise to spuriously elevated T3 and T4 levels with normal TSH. It is therefore important to first rule out any of these other possibilities before embarking on the path to RTH. Of course a careful check of the history will exclude causes like drugs (amiodarone, iodine-containing X-ray contrast agents) and nonthyroidal illness which often are associated with a high serum FT4 but low FT3. Familial dysalbuminic hyperthyroxinemia gives rise to markedly elevated total T4 but normal FT4 levels in serum. Endogenous anti-T3 and anti-T4 antibodies or heterophilic anti-TSH antibodies in the serum cause spurious results; a simple test to rule out the presence of such antibodies is diluting the serum and checking that the level of the analyte measured decreases linearly with the dilution steps.

Having ascertained the validity of the obtained hormone test results, a distinction must be made between a TSH-secreting pituitary adenoma and RTH. This can be difficult when imaging of the pituitary does not show a tumor. In both cases TSH is refractory to thyroid hormone feedback. A TRH test can be helpful. In the case of RTH there will be a response of TSH to the TRH which will be less so when an autonomous pituitary tumor is present. Furthermore, the α subunit to TSH ratio is normal in RTH whereas it will be elevated in TSH-secreting tumors [11, 12]. When the differential diagnosis clearly points to RTH, the patients genomic DNA can be sequenced, in particular exons 7–10, to confirm the diagnosis.

In cases where the diagnosis is not clear or when no mutation is found in the receptor gene, tests aimed at measuring the effect of T3 in peripheral tissues can be used. These tests were developed by Refetoff et al. [13], although not many publications exist using the protocol in children. The scheme [13, for details] consists of administering increasing doses of T3 in an in-patient setting (0.5× dose, 1× dose, 2× dose, each given for 3-day periods). The daily doses

of L-T3 in children are: 25 μg for ages 1–3 years (body weight 8–15 kg); 50 μg for ages 4–9 years (body weight 16–25 kg), and 75 μg for ages 10–14 years (body weight 26–45 kg). The initial dose is halved and the last dose is doubled. At the end of each 3-day period various T3-dependent peripheral tissue function tests are done [13, for details]. Using this protocol significant changes in these parameters can be found, especially when comparing RTH patients with nonaffected subjects (when possible family members) and a diagnosis of RTH can be made.

Management

Most patients have corrected themselves by increasing their serum thyroid hormones in the presence of normal TSH [12, 14]. No treatment is necessary in these cases. When the patient presents with hyperthyroid symptoms, especially tachycardia, beta-blockers can be used. The thyroid hormone analogue 3,5,3′-triiodothyroacetic acid (TRIAC) has also been used successfully to treat some symptoms of RTH in children such as increased TSH and goiter [15–18]. This is due to the fact that TRIAC has a higher affinity for the TRβ then for the TRα, and is metabolized more rapidly then T3. Because of this it has a limited effect on organs like the heart with a predominance of TRα. Similar treatment successes with D-T4 [19, 20] have no clear explanation.

In cases where previous erroneous diagnosis has occurred resulting in postsurgical or postradiation hypothyroidism, treatment with thyroid hormone can be started. As an outcome for successful therapy serum TSH can be used; required T4 doses can be as high as 1000 μg/day [12]. In the case of ADHD in RTH children it was found that T3 treatment improved symptoms [21]. Recently, ADHD symptoms in a child-bearing mutation F455I were successfully treated with TRIAC [22]. In another report, TRIAC was used to treat a fetus harboring a TRβ mutation in utero to reduce fetal goiter [23]. Although treatment was successful up to a point, some controversy has arisen due to the fact that repeated chordocentesis was necessary (with all risks attached to it) and that we do not know enough about placental TRIAC transport and metabolism [12].

All in all it is clear that much more clinical groundwork is needed.

Molecular Issues

The first patients described with RTH in 1967 had, as mentioned above, a very particular phenotype. The inheritance in these cases was autosomal-recessive.

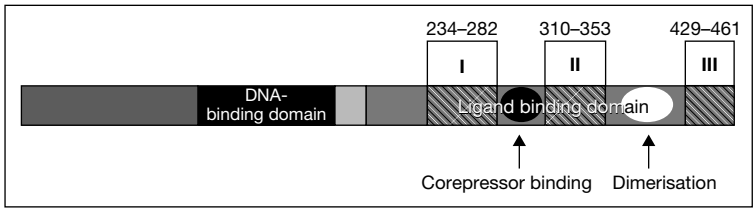

Fig. 3. Position of the mutations in the ligand-binding domain of the TRβ. The mutations that have been found in the TRβ cluster in three areas of the ligand binding domain straddling the sites for corepressor binding and dimerization. The amino acid positions between which the mutations are found are indicated above the boxes.

After a tight linkage was reported between the TRβ locus [24] and RTH about 700 other cases have been described. One of the first patients had a homozygous deletion of the TRβ1 allele, which as it turned out was the exception to the rule since all other cases had point mutations or small deletions in the TRβ1 gene. As a result of these changes in the TRβ1 gene amino acids change or the synthesis of the receptor protein is stopped prematurely. Interestingly, all mutations found to date cluster in three areas of the receptor with some amino acid positions very prone to mutation (fig. 3) [25 and references therein]. The inheritance of the point mutations is autosomal-dominant and patients are heterozygous for the mutation. In 15% of cases RTH is sporadic and a mutation has arisen de novo.

How do the mutant receptors give rise to the resistance phenomenon? From the first case described, it is clear that losing the complete gene is only a problem when both alleles are lost. The dominant nature of the inheritance of all other mutants described indicates that the mutated receptors do interfere with the action of their normal counterparts. From in vitro experiments it has become clear that this is indeed the case and that the mutated receptors act in a dominant negative manner, i.e. they decrease the effect of the hormone even though the normal receptor is present [26, 27]. For the mutant receptor to act in a dominant-negative manner, DNA binding and heterodimer formation are essential [28]. When mutations which abolish either of the two are tested they will not work as dominant-negatives.

On the basis of this, the interference with the normal way in which the TR works can be envisaged to take place at several different levels. As shown in figure 3 all mutations cluster in three particular areas. When these areas are plotted onto the 3D structure of the TRβ, it becomes apparent that they are surrounding the binding site for thyroid hormone (fig. 4). Therefore the first possibility is loss of hormone binding. In this case the hormone cannot bind to

Fig. 4. Three-dimensional structure of the TRβ ligand-binding domain. The 3D structure of the TRβ ligand-binding domain is depicted as a ribbon following the peptide backbone (derived from NCBI-MMDB database, structure number 1BSX). The top of the structure is the side of the DNA-binding domain. The dark ribbons indicate the three areas where mutations preferentially occur. It can be clearly seen that these areas concentrate themselves around the T3-binding site.

the receptor which will then not be able to release the corepressor and the result of this is that the gene is not activated. Other receptor mutants have been found that do bind T3 but which release the corepressor slower than normal [29, 30]. This will also lead to a decrease in gene activation. Another possibility is that the mutant receptor molecules form heterodimers with RXR and go back to the TRE but then fail to attract the coactivator in which case the gene remains silent.

Disruption of the TRβ gene in mouse models shows a phenotype reminiscent of the first RTH patients identified to harbor a homozygous deletion [31]. These animals also have serious hearing defects. When the deletion is heterozygous, normal thyroid test results are found. The expression of mutant TRβ in mice results in an animal model of RTH with lower body weight, hyperactivity and learning problems, similar to the problems found in humans [32–35].

Another interesting point is that of the variation in peripheral symptoms encountered which brings us back to the GRTH/PRTH distinction. The same

mutation has led to the diagnosis PRTH in one family whereas it led to GRTH in another. There are even reports that PRTH and GRTH can exist within one family harboring the same TRβ mutation. It has therefore been argued that the distinction between the two is an artifact based on the poor definition of the symptoms. PRTH and GRTH can be viewed as two sides of a spectrum of a single gene disease. However, it recently emerged that a novel mutation found an newborn with severe RTH due to a frame-shift mutation gave rise to symptoms which point to predominantly pituitary RTH [36]. Wu et al. [36] also showed that the mutation leads to an impaired interaction with the co-repressor SMRT.

A possible reason for the variability in the symptoms with which patients present could be that not all individuals express the same levels of TR (both mutant and normal) in their tissues [37]. When the ratio between mutant and normal TR changes so will the final effect of the mutant receptor. Furthermore, not all mutations have the same effect on T3 binding [38]. Recently, it was found that there is a relation between the T3-binding impairment and the outcome of thyroid function tests [39]. Another factor could be the different tissue distribution of the TR (fig. 1). In liver, the TRβ is the predominant isoform which, however, is expressed in a zonal fashion, indicating that not every liver cell will be sensitive to a mutated TRβ [40]. The heart on the other hand is a predominantly TRα tissue. Since the TRα is normal in RTH patients but their FT3 levels are high, it can be expected that they will react to the extra amount of T3 in a hyperthyroid manner as far as the heart is concerned. Recently, it appeared that the TRβ1 is expressed in the ventricles in only a subset of cardiac cells which form the peripheral ventricular conduction system [41]. A mutated TRβ will therefore probably only affect this subset of cells. Furthermore, not every individual will express the same amount of TR or corepressors/coactivators in a particular tissue leading to differences between individuals. An interesting observation in this context is that some mutations are more deleterious when present in the TRβ2 then in the TRβ1. Since the TRβ2 expression is restricted to the pituitary it may be expected to give rise to a 'PRTH' phenotype.

A number of cases have been reported where no mutation has been found in the TRβ gene even though the biochemical evidence was there [42]. It has been argued that the origin of the resistance in these cases is a faulty cofactor [42–44]. This is supported by the recent finding that RXRγ knock-out mice display PRTH-like symptoms [45], and that SRC-1 knock-out mice manifest RTH symptoms [46].

With the rapid increase of our knowledge of receptor structure and the way agonists and antagonists interact with the receptors it can be envisaged that it would be possible to design receptor agonists which will correct the receptor defect. It has been shown for instance that a shift of 0.3 Å of helix 6 of the TR due

to the mutation of alanine 317 to threonine is the cause of the decrease in T3 binding. If an agonist were found which can 'live' with this small shift and thus activate the receptor, patients harboring this particular mutation could be treated.

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Pendred Syndrome

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History

The syndrome of familial profound congenital hearing loss associated with large, multinodular goiter was first described by Vauhan Pendred [1] in 1896 (OMIM 274600). From its initial description until 1996, a large number of articles were written describing novel cases and suggesting possible pathophysiologic mechanisms for the syndrome. However, all of these were based on speculation, since the precise cause of the syndrome remained unknown for 100 years. Then, in 1996, using linkage analysis, 2 groups independently identified the genetic locus responsible for the syndrome [2, 3]. The discovery of the precise gene mutated in Pendred syndrome, only 1 year later, opened a new phase in the history of the syndrome [4].

Pendred syndrome is caused by loss-of-function mutations in the novel protein called pendrin, which is encoded by the gene SLC26A4 (PDS). Pendrin functions as an anion transporter and is expressed in the thyroid, the inner ear, the kidney and the placenta. The precise mechanism by which mutations in this single protein cause both defective thyroid function and profound hearing loss has been the topic of extensive research ever since the discovery of the gene.

Clinical Syndrome

The hallmark of Pendred syndrome is the combination of sensorineural deafness and goiter in the presence of a positive perchlorate discharge test. The clinical manifestations of this syndrome can be highly variable between affected families and even within the same family [5].

The true incidence of the syndrome is not known, and depends in part on whether it is defined on a purely clinical basis, or on a genetic basis. The incidence of congenital deafness has been reported to be between 1:1,000 and 1:2,000, and 1–8% of patients with congenital hearing loss are thought to have Pendred syndrome [6, 7]. This suggests that the incidence of Pendred syndrome may range from 1:12,500 to 1:200,000. However, this estimate is based on data collected long before the genetic etiology of Pendred syndrome was known. New studies are needed to determine what percentage of patients with congenital hearing loss have mutations in the PDS gene, and what percentage of these have complete syndrome (see below).

Most patients with Pendred syndrome are born with severe to profound bilateral sensorineural hearing loss, although some appear to have normal hearing initially and lose their hearing suddenly or gradually later in childhood or adolescence.

In contrast, the thyroid disease associated with Pendred syndrome rarely presents in infancy, usually appearing in late childhood or early adolescence as diffuse or multinodular goiter. Typically, the patients are clinically euthyroid, although mild, compensated hypothyroidism, characterized by elevated TSH with normal thyroxin and tri-iodothyronine levels, is often present. However, clinical presentation is variable and Massa et al. recently described a case of documented Pendred syndrome in whom the presenting thyroid pathology was a painless, benign solitary thyroid nodule that resolved after hormone replacement [8]. The size of the goiter is also variable, and may be small, detectable only on close physical examination, or may reach massive proportions, causing significant cosmetic problems or even tracheal compression (fig. 1) [9]. Although the goiter tends to recur after surgery, partial thyroidectomy may be required. The histological appearance of the thyroid tissue is characterized by hyperplastic, diffuse goiter that develops into a multinodular pattern later in life [10] (fig. 2). Frank hypothyroidism can occur after thyroidectomy, presumably due to acute loss of thyroid mass, and full hormone replacement is advised regardless of the extent of the surgical resection. The incidence and severity of the goiter may be related in part to iodine intake, and high levels of dietary iodine intake, such as typically seen in Japan, may protect against the goiter [11, 12].

Patients with Pendred syndrome typically have elevated or high-normal TSH levels in the setting of normal or low-normal levels of T4, with high T3/T4 ratios (fig. 3) [3]. Thyroglobulin levels are frequently elevated and may be extremely high. However, the laboratory findings are of minor clinical significance since there is considerable overlap between patients and unaffected individuals. Furthermore, elevated TG levels are not specific and can be found in MNG from any cause [9]. Although association has been made between the

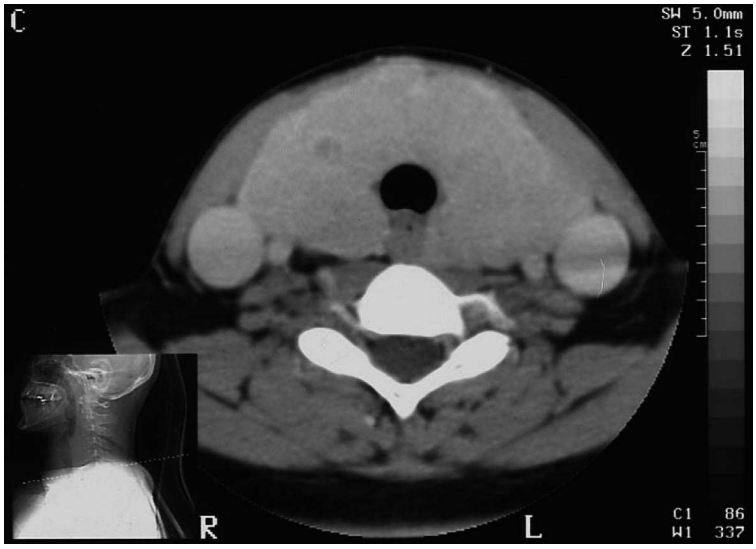

Fig. 1. CT of neck of Pendred syndrome patient with large multinodular goiter. In this case, the goiter is symmetrical and does not cause any significant displacement or constriction of the trachea. In some cases, critical tracheal compression may occur and thyroidectomy may be required to prevent upper airway obstruction.

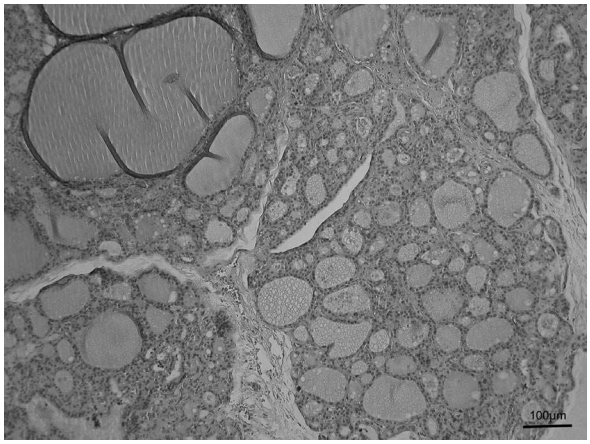

Fig. 2. The histological appearance of thyroid tissue from a patient with Pendred syndrome and multinodular goiter. HE staining showing thyroid nodules surrounded by fibrous capsules of different shapes and sizes as well as marked hyperplasia of the follicular epithelial cells. The wide variability in size and shape of the colloid follicles is also demonstrated.

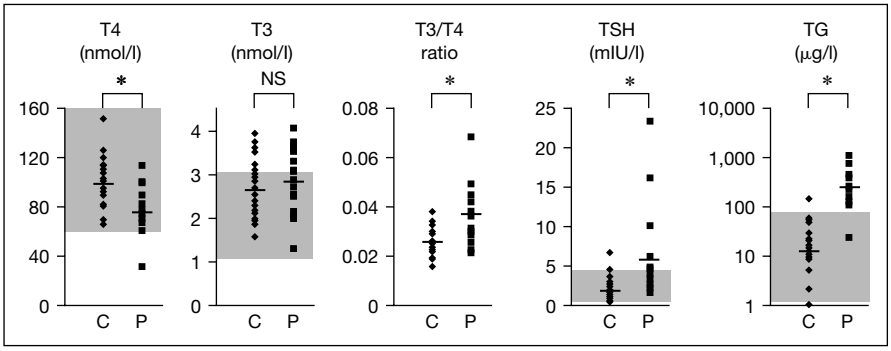

Fig. 3. Thyroid function tests in patients with Pendred syndrome but with no prior surgery (P, black squares) and unaffected family members (C, black diamonds). The reference rage is shown by light gray shading. The slightly elevated TSH and TG seen in some of the controls may be related to the fact that some are heterozygous for the PDS mutation and that these families live in an area of relatively low iodine intake and high incidence of multinodular goiter. Significant differences were determined using the Mann-Whitney nonparametric test (*$p < 0.001$).

alterations in the expression of the gene responsible for Pendred syndrome and thyroid cancers [13], it is not known if the incidence of cancer is increased in this disease. Among 35 patients from a genetic isolate in Northern Israel, 1 was diagnosed with papillary thyroid carcinoma [unpubl. observations].

Until genetic analysis for mutations in the gene responsible for Pendred syndrome became available, the diagnosis relied on the constellation of clinical and laboratory findings, in addition to a positive perchlorate discharge test. Perchlorate is a competitive inhibitor of sodium-iodide symporter (NIS), the thyroid cell surface protein responsible for transporting iodide from the plasma into the thyrocyte. It has no effect on the iodination process itself; rather, it displaces iodide by competitive uptake at the NIS. When the NIS is blocked by perchlorate, free iodide in the cytosol diffuses out of the cell. Under normal circumstances virtually all iodide transported into the cell is immediately organified, leaving very little free in the cytosol. However, in the presence of any abnormality in the organification process, free iodide accumulates, and will diffuse out if the NIS is blocked by perchlorate.

The test is performed by administering 1 g potassium perchlorate 2 h after a tracer dose of ^{131}I. Thyroidal radioactive iodine uptake is measured immediately before perchlorate administration and at 15 min intervals thereafter. In normal individuals, after radioactive iodide uptake into the thyroid gland is blocked by the administration of potassium perchlorate, there is little loss of the accumulated thyroidal radioactivity since virtually all of it is fully organified.

However, in individuals with Pendred syndrome, significant stores of unorganified iodide are present in the gland, and after potassium perchlorate administration, 10–80% of accumulated radioactivity may be discharged.

This test is of limited specificity and sensitivity for the diagnosis of Pendred syndrome. Specificity is particularly poor, since a positive result can be obtained in patients with any thyroid disease associated with an iodide organification defect, including rare genetic diseases such as that caused by thyroid peroxidase mutations (OMIM 274500), as well as very common diseases such as Hashimoto's thyroiditis and Graves' disease [14]. Treatment with lithium or antithyroid drugs will also cause abnormal organification and a positive test [15]. Sensitivity is also limited, since a negative perchlorate discharge test has been reported in a patient with genetically proven Pendred syndrome [16].

In as many as 50% of patients with congenital deafness and goiter, clinically suspected to have Pendred syndrome, no PDS gene mutations can be found. In some, this may be due to technical limitations of the methods used to detect mutations, whereas in others, mutations in different genes may result in similar clinical picture (genetic heterogeneity). Alternatively, the association of sensorineural deafness and goiter may be a random phenomenon, since goiter is a common finding, particularly in some regions of the world (phenocopies). Recently, congenital goiterous hypothyroidism and deafness was described in a patient who was heterozygous for a recessive pendrin mutation, and compound heterozygous for 2 different mutations in the thyroid peroxidase gene (TPO, OMIM 274500). For this patient, the presence of congenital overt hypothyroidism and goiter suggested clinically that the syndrome was not caused by PDS mutations alone, since as described above, typical Pendred syndrome patients are clinically euthyroid and the goiter is not present in the neonatal period. In another case, the coexistence of sensorineural deafness and goiter were thought to be related to an autoimmune phenomenon [17]. Thus, genetic heterogeneity and phenocopies of Pendred syndrome may be common, and genetic analysis is required for definitive diagnosis. This may have important implications in terms of genetic counseling and family planning when the precise genetic diagnosis is not known.

Molecular Genetics

The gene associated with Pendred syndrome (SLC26A4, PDS) was mapped to chromosome 7q13 in 1997 and cloned 1 year later using newly available data and technology provided by the human genome project [2–4]. The gene spans 57 kb of genomic DNA and contains 21 exons. The 4,930 basepair-long mRNA codes for a 780 amino acid protein, pendrin, which is predicted to contain 11 or 12 transmembrane domains [4, 18]. Immunohistochemical studies demonstrated

that the mature protein is expressed on the apical membrane of the follicular thyroid epithelial cells, in cells lining the endolymphatic duct, endolymphatic sac and organ of Corti in the inner ear, in the intercalated cells of the kidney and in trophoblast cells [4, 19, 20]. In the thyroid, pendrin expression is regulated by TTF-1 and thyroglobulin, but not by TSH, sodium iodide or insulin [18, 21, 22].

The pendrin gene, SLC26A4, belongs to a larger family of ion transporters that is currently thought to include 10 members, SLC26A1–11 (SLC26A10 is a pseudogene). Early homology studies suggested the pendrin may function as a sulfate transporter [4], but this was soon proven unlikely, since sulfate transport was shown to be entirely normal in thyroid cells obtained from Pendred syndrome patients [23]. In vitro expression studies subsequently documented that the protein forms a channel that can function either as a chloride-iodide transporter in the thyroid [24] or a $Cl^-/OH^-/HCO_3^-$ exchanger in the kidney [25]. The functional importance of pendrin in the placenta is not known, although no abnormality in reproductive function has been reported in women with Pendred syndrome.

More than 100 different PDS (SLC26A4) mutations have been reported in patients with Pendred syndrome and nonsyndromic deafness (see below) (fig. 4). Most of these are seen in only a single family, although 4 specific mutations (E384G, L236P, T416P, and 1001 + 1G) are commonly seen, and are estimated to be responsible for 50–60% of the Pendred syndrome cases in the Caucasian population [26, 27]. Haplotype analysis suggests that these are founder mutations in the Northern European Caucasian population and not mutation hot spots. Another founder mutation, a single base deletion causing a frame shift and truncated protein (1220delt), was identified in a large Bedouin tribe from Northern Israel with more than 35 patients diagnosed with Pendred syndrome.

All forms of mutations have been found, including deletions, insertions, missense and nonsense mutations. Elegant work by Rotman-Pikielny et al. [28] demonstrated that at least some of the missense mutations result in defective peptide processing, causing the protein to be trapped in the Golgi apparatus or in the endoplasmic reticulum. Although of little clinical relevance at the present time, this could become important in the future, since it may be possible to develop chaperone proteins that can correct the secondary structure of these mutant proteins, thus allowing them to be transported to the membrane, thereby recovering at least some function.

Pendrin's Function in the Thyroid

The exact mechanism by which pendrin functions in the thyroid is still debated. Based on homology with sulfate transporters, it was initially hypothesized that

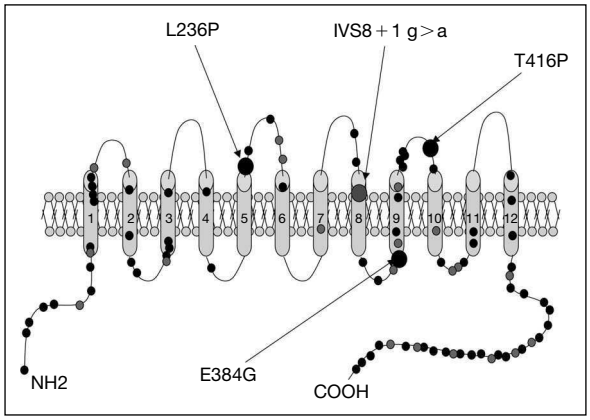

Fig. 4. Structure of the pendrin protein showing 12 transmembrane domains as proposed by Royaux et al. [18]. Circles show some of the more than 100 different mutations identified in patients with Pendred syndrome and DNFB4. The black circles indicate missense and non-sense mutations whereas the gray circles indicate other mutations types including splice-site mutations and micro deletions or insertions. The 4 large circles indicate 4 most common mutations. Together, these 4 mutations are responsible for up to 60% of the Caucasian Pendred syndrome patients diagnosed to date. The diagram is modified from that on the University of Iowa Otolaryngology Research Laboratories Web-site (http://www.medicine.uiowa.edu/pendredandbor/slc26a4_mutations.htm).

pendrin belongs to this family of channels [4]. Scott et al. [24] induced the expression of pendrin in *Xenopus laevis* oocytes and Sf9 cells and reported 3 major findings: firstly, there was no increase in sulfate transport; second, the rates of transport for iodide and chloride were significantly increased and third, pendrin transports iodide and chloride in a competitive manner. Yoshida et al. [29] reported that pendrin is responsible for iodide efflux from the follicular cells into the colloid. They also report that in the thyroid, iodine is transported in exchange for chloride, whereas in other tissues, it is hypothesized that pendrin's main function is to transport chloride through exchange with other anions [30].

In normal thyroid, pendrin is expressed at low levels on the apical membrane of follicular cells. Thyroids from patients with Graves' disease display a similar, albeit more extensive, expression of pendrin when compared to normal thyroid tissue, especially in areas with increased proliferation of the follicular cells. In contrast, immunohistochemical staining was absent and mRNA levels were significantly lower in papillary carcinoma when compared to normal and other neoplastic diseases of the thyroid. These findings suggest a correlation between pendrin expression and hormonogenesis [31].

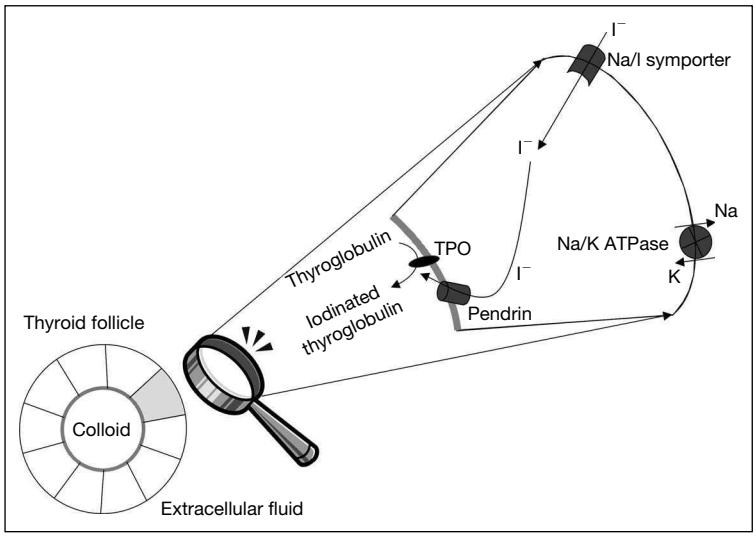

Fig. 5. Schematic diagram showing iodide transport within the thyrocyte. The Sodium-Iodide Symporter (NIS) pumps iodide into the cell against a concentration gradient. The cytosolic iodide must exit the cell to interact with thyroid peroxidase on the extracellular side of the apical membrane. This task is accomplished, at least in part, by pendrin.

Why, then, does the thyroid follicular cell require a second iodide transporter, and why in the apical membrane? The sodium iodine symporter (NIS), cloned in 1996 [32], and located on the basolateral membrane of the thyrocyte, actively transports iodide against a concentration gradient into the cytoplasm of thyroid cells. However, the iodination of thyroglobulin is carried out by the enzyme thyroid peroxidase (TPO) located on the colloidal side of the follicular cell apical membrane. Therefore, before the iodide can be organified, it must be transported out of the cytosol into the colloid space (fig. 5). Studying thyroid plasma membrane vesicles, Golstein et al. [33] proposed the existence of a channel in the apical plasma membrane that accomplishes this function. Subsequent studies in polarized monolayers showed that iodide exited the cell via the apical membrane and that this process was rapidly accelerated by thyrotropin [34, 35]. The identification of pendrin and the protein product of PDS gene provided a mechanism for iodine transport from the thyroid cells into the colloid space [36]. It is proposed that pendrin promotes the transfer of iodide across the apical membrane (fig. 4), and that its absence or dysfunction leads to insufficient delivery of iodide to the iodination site and thus to an organification defect [30]. Both TPO and NIS are absolutely required for successful

iodination of thyroglobulin and formation of thyroid hormone. Thus, patients with severe mutations in either will have severe congenital hypothyroidism. In contrast, in the total absence of pendrin, organification is only partially inhibited and most patients remain clinically euthyroid. Thus, an alternative, as yet unidentified, pathway, or pathways, must exist by which iodide can exit the apical border of the cell. It is possible that mutations in the components of these other pathways may explain the disease in patients with clinical Pendred syndrome, but without PDS mutations.

Pendrin and the Ear

Since its initial description in 1896 [1], the mechanistic connection between the defective thyroid function and sensorineural deafness was not clear. Thyroid dysfunction per se clearly could not be blamed, since most patients with congenital hypothyroidism do not have significant hearing loss [37] and, as described above, most patients with Pendred syndrome are born with normal or near-normal thyroid function. The high incidence of deaf-mutism in patients with neurologic cretinism is thought to be related to maternal hypothryoidism early in the pregnancy and not directly to fetal thyroid dysfunction [38]. With the identification of the Pendrin gene in 1997 [4], it became possible to begin to study this connection.

Soon after the discovery of pendrin, it became apparent that not all patients with PDS mutations have thyroid abnormalities. Nonsyndromic congenital deafness previously linked to the same region of chromosome 7 (DFNB4), was shown to be caused by PDS mutations [39]. In some cases, this apparent dissociation of clinical findings may be temporary, since the deafness typically is present at birth, or shortly thereafter, while the goiter may appear later in life, often during adolescence or even later [40]. The converse may also be true, since in some patients with proven PDS mutations, the hearing loss may fluctuate, or may occur abruptly later in childhood, associated with an acute illness or surgery [3]. Cremers et al. [41] described 14 patients with Pendred syndrome in whom hearing loss was first suspected as early as 6 months or as late as 6 years, and was progressive in all. Goiter was diagnosed before the hearing loss in 4 patients.

The incidence of PDS mutations in patients with non-syndromic deafness is not known. Scott et al. [39] screened 20 such patients and identified 3 with novel PDS mutations. Functional analysis of these mutations and comparison with mutations found in Pendred syndrome patients, showed that mutations that cause Pendred syndrome have no in vitro function at all, whereas those associated with non-syndromic deafness retain some, albeit much reduced, function. This suggests that the ear is more sensitive to changes in pendrin expression

and action than is the thyroid. Hearing loss may also be partial and progressive. Sugiura et al. [11] tested 17 patients with bilateral enlarged ventricular aqueducts, a hallmark of Pendred syndrome (see below) and identified *PDS* mutations in 14. Many of these patients had fluctuations in hearing loss that were associated with vertigo. Hearing loss was first diagnosed after the age of 3 years in 3 of the patients, the oldest being 17 years old at the time of diagnosis. Six of the 14 patients had moderate hearing loss or better at the beginning of the study (aged 3–18 years). In 5 of these, hearing deteriorated significantly over 5–25 years of follow-up. Only 1 patient had a goiter, but in 8 of the 11 patients studied there was an indication of abnormal iodine organification demonstrated by an abnormal perchlorate discharge test. Thirteen of the patients had a single mutation H723R that has been previously been associated with a higher rate of goiter in other populations. The patients reported by Sugiura were Japanese, and the relatively high iodine intake in the typical Japanese diet may explain the lack of goiter. Of the 14 patients with *PDS* mutations in this study, in 8 only a single mutant allele was identified. This finding is difficult to explain, since the mutations identified were previously associated with recessive disease. It is possible that a mutation or mutations in the regulatory regions of the gene, which were not analyzed in these patients, may have been missed by the genetic analysis. Alternatively, mutations in other genes may interact with recessive PDS mutations to cause hearing loss.

What, then, is the cause of the hearing loss in patients with *PDS* gene mutations? The structural abnormality of the inner ear associated with Pendred syndrome is variable. A particularly malformation of the cochlea, known as the Mondini malformation, has been reported in some, but not all ears of patients with Pendred syndrome. Enlargement of the vestibular aqueduct (EVA) appears to be a more constant finding [41–43]. Significant differences can be seen between the 2 ears of a single patient with profound bilateral hearing loss. Using high-resolution CT to evaluate a cohort of Pendred syndrome patients from a genetic isolate in the Middle East, we recently showed that the most common structural abnormalities were an enlarged vestibulum and abnormal modiolus, both of which were found in 100% of affected ears (fig. 6). In contrast, an enlarged aqueduct and an absent interscalar septum were found in only 80 and 75% of affected ears, respectively [44].

Pendrin is expressed in cells lining the endolymphatic duct and sac, the organ of Corti and in distinct areas of the utricle, saccule and cochlea suggesting that the hearing defect is cause by a primary defect within the ear [19, 45]. In order to better understand the precise mechanism causing the profound hearing loss, Everett et al. [36] generated a mouse with targeted disruption of the mouse PDS gene. These animals are completely deaf and have vestibular dysfunction. Interestingly, the middle ear develops normally until embryonic day 15,

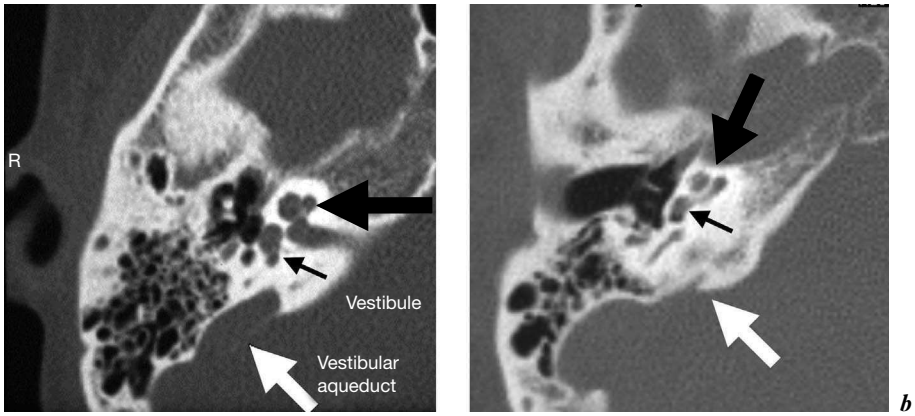

Fig. 6. High-resolution thin section CT image of inner ear. Note the markedly enlarged vestibular aqueduct (white arrow), the enlarged vestibule (thin black arrow) and the abnormal modiolus (thick black arrow) in the Pendred patient (***a***). Corresponding structures are shown in a normal individual for comparison (***b***). Images provided by Dr. Moshe Goldfeld, Western Galali Hospital, Nahariya, Israel [9].

after which endolymphatic dilatation occurs. Sensory cell degeneration and malformation of the inner ear develop during the 2nd postnatal week. Mice deficient in pendrin show evidence of vestibular dysfunction. In contrast, this is not clinically evident in most patients with Pendred syndrome, although only a minority of patients have undergone rigorous testing of vestibular function.

Mice lacking the transcription factor Foxi1 have a similar phenotype and lack pendrin expression in the ear during development, suggesting that this is an upstream regulator of PDS expression. Mutations in this gene could conceivably cause a Pendred-like phenotype in man.

The clinical findings described above, along with the data from the mouse model provide evidence that the structural defect of the inner ear may not be directly genetically defined, but could be a secondary phenomenon. Taken together with the presumed function of pendrin as an ion transporter or channel, these findings suggest that the structural anomalies and hearing defect caused by mutations in this gene may be caused by abnormal endolymphatic pressure leading to dilatation of the vestibulum and vestibular aqueduct and degeneration of the sensory cells. This may explain the variability in structural malformations seen in man, and may explain the occasional occurrence of postlingual deafness in patients with PDS mutations. More importantly, this finding may have therapeutic implications, since there may be a window of opportunity during which therapy could be given to correct endolymphatic pressure and rescue the sensory cells from destruction.

Pendrin's Function in the Kidney

Intercalated cells are located in the distal nephron of man and rodents, representing a minority cell type that appears to be important for acid-base balance. Three types of intercalated cells have been identified, type A, type B and non-A/non-B. The major differences between these cell types relates to the expression and sub-cellular localization of several specific ion channels [see 46 for a recent comprehensive review]. Type A intercalated cell excrete protons through the apical H^+-ATPase. Disruption of this channel results in a net decrease in H^+ secretion. Type B intercalated cells express pendrin, which acts as a HCO^-/Cl^- exchanger, on their apical membrane [25, 47]. Disruption of this channel results in decreased bicarbonate secretion and a tendency toward metabolic alkylosis [47]. In the mouse kidney, pendrin expression is regulated and can be modified by changes in acid-base status [48].

Mice deficient in pendrin (*slc26a4*$^{-/-}$) have normal pH, renal function and fluid balance under non-stimulated conditions. However, during NaCl restriction, slc26a4$^{-/-}$ mice have elevated urinary volume and Cl^- excretion and develop metabolic alkylosis, volume depletion and relative hypotension [49]. Stimulation with the aldosterone analogue diozycorticosterone pivalate (DOCP) results in weight gain and hypertension in normal mice, but not in scl26a4$^{-/-}$ mice [50]. These findings suggest that pendrin may play a role in the pathogenesis of mineralocorticoid-mediated hypertension.

To date, no fluid or electrolyte abnormality has been reported in patients with Pendred syndrome, although rigorous studies have yet to be reported, and it seems likely that subtle abnormalities will be found under certain stress conditions. Common polymorphisms have been found in *PDS*, including at least 2 non-synonymous coding variants. It is possible that these or other genetic variants in this gene affect the genetic risk of developing fluid and electrolyte imbalances or hypertension. Large-scale association studies are needed to establish or to refute this potential association between pendrin and the commonly seen essential hypertension.

Directions for the Future

The discovery of the genetic cause of Pendred syndrome opened up new opportunities in the study of thyroid, ear and kidney physiology. The next challenge is to translate these findings into clinically relevant interventions. Genetic testing can identify carriers in high-risk populations, and this information can then be used for genetic counseling and family planning. The thyroid disease per se does not cause overwhelming disability; however, early, complete thyroid

hormone replacement may prevent or delay the development of goiter, thus obviating the need for surgery and the morbidity associated with it. Careful prospective studies are needed to test this hypothesis. Most importantly, however, it may be possible to develop pharmacologic interventions that can prevent or minimize the damage to the inner ear, the most debilitating defect associated with the syndrome. Further basic and clinical studies are urgently needed in this direction.

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Treatment of Hyperthyroidism Due to Graves' Disease in Children

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Current treatment approaches involving antithyroid medications, surgery, and radioactive iodine have been used for more than five decades for the treatment of hyperthyroidism due to Graves' disease in children, adolescents, and adults [1–4]. Although additional studies are needed, the collective observations of thousands of children with Graves' disease have spawned a generous body of literature detailing the natural history of Graves' disease, along with treatment outcome and complications [5, 6]. Based on this reported experience, the following generalizations can be made.

Long-term, spontaneous remission of Graves' disease occurs in less than 30% of children. Thus, the vast majority of children with Graves' disease will need definitive, curative therapy, either in the form of surgery or radioactive iodine.

- There is little evidence that use of antithyroid medications beyond 1 or 2 years increases the likelihood of spontaneous, long-term remission.
- Antithyroid medication use in children and adolescents is associated with minor and major side effects. Although the use of antithyroid medications is standard practice, the use of antithyroid medications involves definite risks.
- Total thyroidectomy is an effective treatment of Graves' disease, with a low rate of disease recurrence. Long-term complications include recurrent laryngeal nerve paresis in 2% or more of individuals, permanent hypoparathyroidism in 1% or more, and hypertrophic and non-hypertrophic scars. Surgery is the preferred definitive treatment for the very large thyroid gland and when the individual is considered too young for ^{131}I.
- When used at doses that deliver 150 Gy, or more (>150 μCi ^{131}I/g thyroid tissue), radioactive iodine is an effective cure for Graves disease and is associated with few acute side effects. Potential long-term adverse side

effects, including thyroid cancer and genetic damage, have yet to be observed in individuals treated as children or adolescents with ^{131}I.

Childhood Hyperthyroidism

Hyperthyroidism occurs much less commonly in children than hypothyroidism, yet is a far more virulent condition [7, 8]. In children the most common cause of childhood thyrotoxicosis is Graves' disease, which is characterized by diffuse goiter, hyperthyroidism and occasionally ophthalmopathy [6, 9–11]. Other causes of childhood hyperthyroidism include toxic nodules, toxic multinodular goiters, acute and subacute thyroiditis, and the ingestion of thyroid hormone [6, 9–11].

Untreated, hyperthyroidism is associated with excessive activity, tremor, tachycardia, flushing, palpitations, accelerated linear growth, weight loss, impaired skeletal mineralization, and poor school performance [6, 9–11]. Because Graves' disease, toxic nodules and toxic multinodular goiters only rarely spontaneously resolve within a short period, treatment of hyperthyroidism is essential. Current treatment options include the use of radioactive iodine, surgery, and antithyroid medications.

Central to considering the use of radioactive iodine and other treatment options in Graves disease in the pediatric population, is recognition of the natural history of the autoimmune disorder. One must also consider how long antithyroid drug therapy should be continued before moving on to definitive therapy.

Spontaneous remission of Graves' disease in the pediatric population occurs in the minority of individuals. Published remission rates are usually less than 25% after several years of antithyroid therapy [5, 6, 12]. The most extensive long-term study of this issue involving nearly 200 children showed that less than 20% of children treated medically achieved remission lasting greater than 2 years [13]. In another large series of 186 children, less than 30% of children went into remission [14]. When responses to medical therapy between prepubertal and pubertal children are compared, remission rates are even less in prepubertal than pubertal children, with remission occurring in fewer than 15% of prepubescent children [15, 16].

When spontaneous remission of Graves' disease does not occur, prolonged drug therapy will control the hyperthyroid state and is used by some clinicians; however, years of treatment with antithyroid drugs do not appear to increase the likelihood of lasting remission. More than two decades ago, Greer et al. [17] showed that the likelihood of spontaneous remission of hyperthyroidism was similar when antithyroid medications were used for 6 or 36 months. Most recently, Weetman [18] reviewed prospective trials comparing different durations of

treatment in adult subjects. In one French study involving 94 patients, following treatment for 6 or 18 months, remission rates were 42 and 62%, respectively, 2 years after discontinuing treatment [19]. In 52 Spanish patients, following treatment for 12 or 24 months, remission rates were 46 and 54% at 2 years after cessation of therapy [20]. This difference was not significant, and by 5 years, the relapse rate was 85%. Another study of 134 French patients found no benefit of 18 vs. 43 months of treatment [21]. It is notable that remission rates in these cohorts of adults are considerably greater than those reported in children, suggesting that the younger one is when Graves' disease occurs, the more lasting it will be.

Radioactive Iodine

Origins of ^{131}I Therapy

The use of radioactive iodine grew out of collaborative efforts in the 1930s and 1940s of physicists at the Massachusetts Institute of Technology (MIT) and clinicians at the Massachusetts General Hospital (MGH; Drs. J. Howard Means, Earle M. Chapman, and others) [3]. The first patient treated with radioactive iodine alone with the intent of curing Graves' disease, was a 55-year-old man who received two doses in 1943 of the very short half-life isotope ^{130}I ($t_{1/2}$ = 12 h). Between 1943 and 1945, an additional 22 patients were treated with a short half-life radioactive iodine at the MGH with good outcome [3].

When the US Atomic Energy Commission was allowed to supply uranium fission products for medical use, ^{131}I, with a half-life of 8 days became available for the treatment of Graves' disease. In 1946, a patient at Barnard Hospital in St. Louis with thyroid cancer became the first to be treated with the long half-life nuclide [3]. Because of the inherent advantages the longer half-life isotope, ^{131}I rapidly became the preferred iodine isotope for treating hyperthyroidism and thyroid cancer.

About 10 years after the first adult was treated with radioactive iodine for Graves' disease, Drs. John D. Crawford and Chapman at the MGH treated the first child with Graves' disease with radioactive iodine. The child faced unremitting hyperthyroidism in the face of toxic reactions to antithyroid medications. In the 1960s and 1970s, several groups reported their experience using radioactive iodine to treat childhood Graves' disease [22–24]. These reports showed both safety and efficacy in children. When radioiodine was not associated with an increased risk of thyroid cancer or genetic damage to the offspring of treated children and adolescents [25], radioiodine therapy use became more widespread and extended to progressively younger children.

Iodine-131

Because the uptake of radioactive iodine by the thyroid is indistinguishable from ordinary iodine, radioactive iodine is trapped in thyroid cells [26]. When taken up by thyroid cells, beta emissions from radioactive iodine result in the destruction of the trapping cell and cells in close proximity. Because the thyroid gland has extremely high affinity for iodine in comparison with other tissues, the use of radioactive iodine results in selective ablation of thyroid tissue [26].

About ten different isotopes of iodine have been used medically. ^{123}I is the most frequently used isotope for diagnostic studies of thyroid function and structure [26]. This isotope has a short half-life (13.3 h) and emits X-rays, gamma-photons, yet no beta particles. In comparison, ^{131}I has a half-life of about 6–8 days and emits beta particles and gamma rays. The beta particles result in local thyroid damage; gamma emissions facilitate external diagnostic imaging.

It has been suggested that doses (administered activities) delivering 30,000–40,000 cGy (rad) to the thyroid are required to ablate the thyroid gland [27, 28]. However, doses delivering 10,000–20,000 cGy to the thyroid are more commonly used and may result in complete or partial destruction of the thyroid [6, 12, 29].

Administered thyroid doses of 150 μCi/g (5.5 MBq/g) typically yield radiation doses of 12,000 cGy to the thyroid [30]. Following ^{131}I treatment, radiation exposures to the stomach, marrow, liver, and gonads are about 14, 6.8, 4.8 and 2.5 cGy per organ, respectively. The total body exposure is about 4.0 cGy [30]. Because of the risk to the fetus, ^{131}I should not be given to pregnant women.

^{131}I Therapy

Thyroid destruction is strongly influenced by rates of iodine uptake and the amount of thyroid tissue. Doses of radioiodine administered to the patient are therefore based on gland size and iodine uptake using the Quimby-Marinelli equation: dose ∃ + (radiation; in Gy) = 90 × {oral iodine-131 dose (μCi) × oral 24-hour uptake (%)/gland mass (g) × 100%}; assuming an effective $T_{1/2}$ of 6.0 days for iodine-131 [31]. Thyroid size is determined by palpation or ultrasound (ultrasound volume = 0.48 × length × width × depth) [32]. For example, if a dose of 300 μCi/g of thyroid tissue is desired for a patient with a 20-gram thyroid gland and a 50% radioiodine uptake at 24 h, the dose will be 12 μCi.

When calculating ^{131}I doses, thyroid size can be assessed clinically relative to the size of a normal thyroid gland size (0.5–1 g per year of age; 15–20 g for adults) or by ultrasound, which is preferred to provide a more accurate size determination [30, 33, 34]. However, even when gland size, uptake, and effective ^{131}I half-times are measured with a high degree of accuracy, the outcome is still imprecise due to individual variation in the sensitivity of the thyroid to radioiodine [32].

If a patient is taking antithyroid medication, as is often the case, treatment should be stopped 3–5 days before the administration of radioactive iodine. If antithyroid medication is stopped too soon before radioactive iodine administration, there can be accumulation of thyroid hormones within the gland leading to thyroid storm following radioactive iodine treatment [35]. After ^{131}I administration, circulating levels of thyroid hormones may then rise within 4–10 days as thyroid hormone is released from degenerating follicular cells [36]. Progressive decline in thyroid hormone levels will then occur.

Until the patient becomes biochemically euthyroid or hypothyroid, which usually takes 6–12 weeks after treatment, symptoms of hyperthyroidism can be controlled using beta-blockers [36–38]. The use of SSKI or Lugol's solution started one week after the administration of radioactive iodine will also attenuate biochemical hyperthyroidism and not adversely affect the outcome of radioiodine therapy [38]. In some patients, transient biochemical hypothyroidism can develop by 8 weeks, and hyperthyroidism will recur [39]. In up to 5–20% of patients (varying with dose), hyperthyroidism will persist; a second dose of radioiodine is recommended for these patients [12]. Additional doses of radioactive iodine are not usually given until 6 months after initial therapy.

Long-Term Cure Rates

Long term cure rates are generally higher in patients treated with larger than smaller amounts of radioactive iodine [6]. In adults treated with low doses of ^{131}I (50–75 μCi/g), hyperthyroidism persists in 30–50% 1 year after therapy [40–43] and hypothyroidism will develop in 7–20% of patients [40, 41]. In comparison, after treatment with higher ^{131}I doses (150–250 μCi/g), only 5–10% of patients are hyperthyroid at one year, and 40–80% become hypothyroid [30, 44, 45].

The success of radioiodine therapy is influenced by the size of the thyroid gland and possibly by circulating levels of TRAb. Responses to ^{131}I therapy are lower in patients with very large glands (>80 g) and high TRAb levels than in patients with smaller glands [29, 46–49]. (At present the basis for lower efficacy in the presence of high TRAb levels is not known.) Thus, surgical thyroidectomy should be considered with for persistently large glands. Responses to radioactive iodine may also be less favorable after treatment with PTU [48, 50, 51] than after MMI treatment [52].

Radioactive Iodine Use in Children

The details of ^{131}I therapy for childhood Graves' disease have been reported in several studies [13, 22, 24, 53–57]. Patients as young as 1 year of age have been treated with ^{131}I with excellent outcomes [23, 57]. ^{131}I doses in children and adolescents have ranged from 100 to 400 μCi/g thyroid tissue [6]. As in adults,

Table 1. Outcome of ^{131}I treatment as related to dose

^{131}I dose, μCi/g	Radiation dose, Gy	Outcome, %		
mean + SEM (range)	mean + SEM (range)	hyperthyroid	euthyroid	hypothyroid
92.1 ± 8.1 (80–120)	82.9 ± 7.3 (72–108)	28.6	28.6	42.8
222.7 ± 12.3 (200–250)	200 ± 11.1 (180–225)	37.5	0.0	62.5
365.0 ± 11.5 (300–405)	325 ± 10.8 (270–364)	0.0	6.25	93.75

From Rivkees and Cornelius [59].

responses to ^{131}I therapy are related to dose and gland size. In children treated with 50–100 μCi/g thyroid tissue, 25–40% are hyperthyroid several years after therapy [58]. In children treated with a single dose of 150–200 μCi/g thyroid, hyperthyroidism persists in 5–20%, and 60–90% become hypothyroid [6, 12, 22, 23].

We have analyzed the outcomes of 31 children (ages 7–15) treated with radioactive iodine therapy at Yale New Haven Hospital over the past 7 years to assess effectiveness of therapy as related to dose and gland size [59] (table 1). When children were treated with 80–120 μCi ^{131}I/g thyroid tissue at 6–12 months after treatment, 28% are hyperthyroid, 28% are euthyroid, and 42% are hypothyroid. When children are treated with 200–250 μCi/g thyroid tissue, 37% are hyperthyroid, 0% euthyroid, and 62%. When children were treated with 300–400 μCi/g thyroid tissue, 0% are hyperthyroid, 7% euthyroid, and 93% are hypothyroid. When we compare these data with those of Peter and co-workers [29, 32, 59], it appears that thyroid tissue of children and adolescents is more sensitive to ^{131}I than in adults, as hypothyroidism occurs at lower ^{131}I doses (fig. 1).

We also find that gland size influences therapy outcomes, especially at lower doses (fig. 2). For children treated with the low or moderate doses, 53% developed hypothyroidism when the thyroid gland is moderately enlarged (~30 g) and when the thyroid gland is quite large (50–80 g), about 60% remain eu- or hyperthyroid. Yet, when high doses are used, hypothyroidism occurs in 93% of patients, irrespective of gland size up to ~80 g of thyroid tissue.

Complication Rates

Acute complications of ^{131}I therapy have been reported, but the incidence of these is low and not well defined [6]. In children, very few acute adverse responses to ^{131}I therapy of Graves' disease have been described [6].

In adults, transient nausea has been reported after radioiodine administration, and mild pain over the thyroid gland, reflecting radiation thyroiditis, may develop one to three days after a therapeutic dose [36]. These side effects are

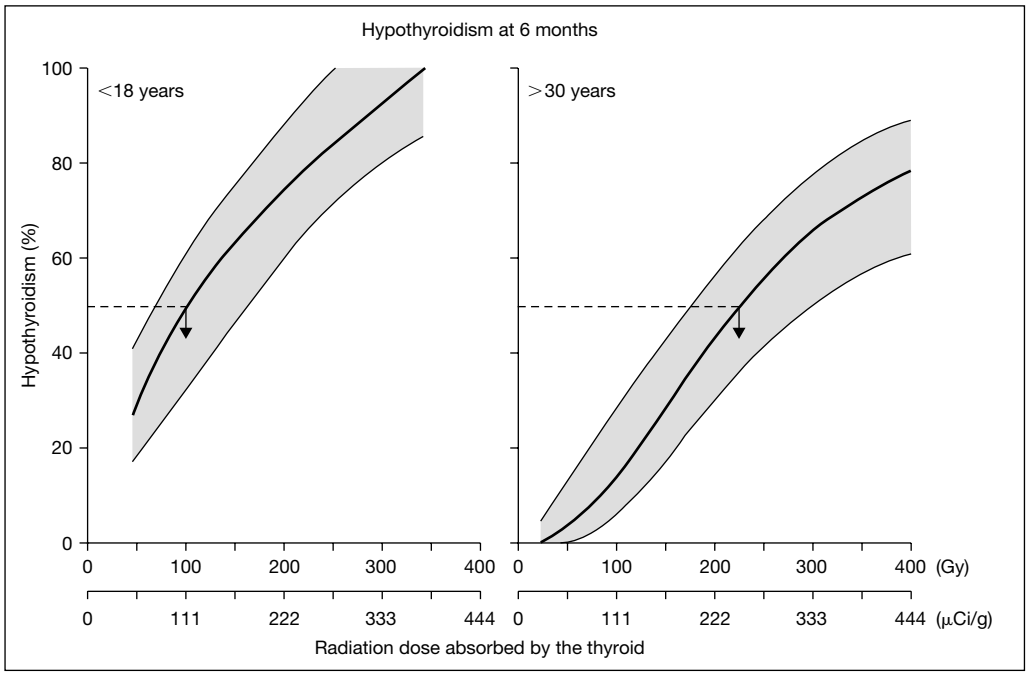

Fig. 1. Relationship between thyroid radiation dose and hypothyroidism rate in individuals less than 18 years of age (left panel) as compared to outcomes observed in adults. Based on published data of Peters et al. [32]. The shaded area shows the 95% CI; $r = 0.98$, $p < 0.01$.

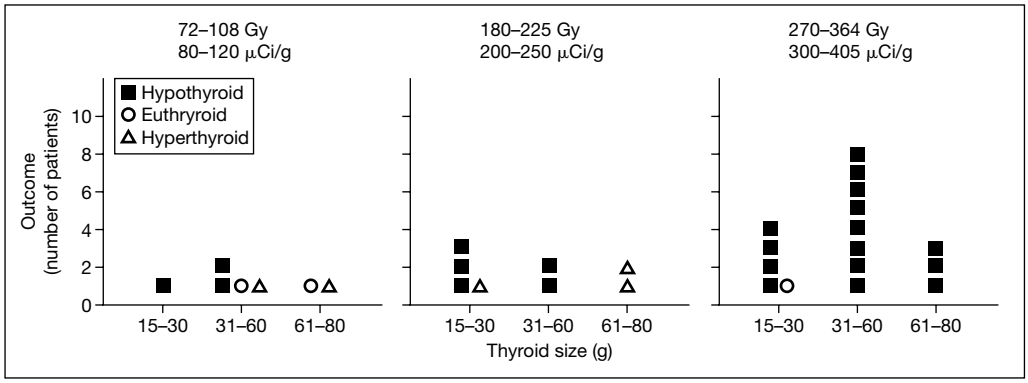

Fig. 2. Therapy outcome as related to dose and thyroid gland size. Each character represents an individual patient. From Rivkees and Cornelius [59].

self-limited and respond to treatment with nonsteroidal anti-inflammatory agents [36]. Severe neck swelling and tracheal compression have been reported rarely in patients with very large goiters after ^{131}I administration and can be controlled with large doses of corticosteroids [36]. However, neck swelling after radioactive iodine treatment typically occurs with doses greater than 50,000 cGy; such doses are much greater that those needed for Graves' therapy [60]. Vocal cord paresis occurs very rarely [61].

Thyroid storm has been reported to develop between one and fourteen days after ^{131}I treatment in a small number of patients [62]. This complication is rare and no cases were reported among 7,000 patients treated with ^{131}I at one center [12]. Patients with severe thyrotoxicosis and very large goiters may be at higher risk for thyroid storm. In this setting, antithyroid drugs can be administered for several weeks before radioactive iodine therapy to deplete stores of hormones before radioactive iodine therapy [62]. However, if medication is stopped too soon, thyroid hormone stores will be replenished and can lead to thyroid storm [35].

Recent discussions have focused on the association of ^{131}I therapy of Graves' disease with the development or progression of ophthalmopathy in adult patients [63, 64]. In contrast to adults, children rarely develop severe ophthalmopathy and proptosis is generally mild [65, 66]. Of 87 children treated with ^{131}I for Graves' disease at one center, eye signs improved in 90% of children, did not change in 7.5%, and worsened in 3% after treatment [23, 45]. In 45 children with ophthalmopathy at the onset of treatment, eye disease improved in 73% and worsened in 2% after 1 year or more of drug therapy [67]. Following subtotal thyroidectomy in 80 children, eye disease worsened in 9% [68]. In contrast, eye disease was stable in 60 (75%) children after total surgical thyroidectomy [68]. Thus, eye disease worsens in only a small percentage of children following medical, radioactive iodine, or surgical therapy of Graves' disease.

It has been suggested that the development and progression of ophthalmopathy prevented by treatment with prednisone for 3 months after radioiodine therapy [69]. However, adjunctive prednisone therapy is not routinely recommended for most children since long-term progression of ophthalmopathy occurs infrequently and unpredictably after radioiodine [69]. Prolonged prednisone administration is also associated with weight gain, immune suppression, and growth failure in children. However, prednisone may be useful after radioiodine therapy for the pediatric patient with severe eye disease.

Post ^{131}I Cancer Risks

The increased risk of thyroid cancer after thyroid irradiation in childhood has been recognized for nearly 50 years [70]. Thus, a major concern of ^{131}I therapy

relates to the risk of thyroid cancer. Detractors of ^{131}I therapy point to the increased rates of thyroid cancer and thyroid nodules observed in young children exposed to radiation from nuclear fallout at Hiroshima or after the Chernobyl nuclear reactor explosion.

The thyroid gland is unique in its developmental sensitivity to malignancy following radiation exposure. Individuals older than 20 years of age do not have an increased risk of thyroid cancer when exposed to low-level thyroid irradiation [71–73]. Yet, when individuals are less than 20 years of age at the time of low-level thyroid irradiation, the thyroid cancer risks increases the younger one is [71–73].

In addition to age, the radiation dose plays a major role in cancer risk [70–73]. The risk of thyroid cancer and thyroid nodules is highest with exposure to low or moderate levels of external radiation (0.1–25 Gy), and not with the considerably higher doses used internally to treat Graves' disease (>150 Gy) [70–74].

It is important to note that iodine deficiency and exposure to nuclides other than ^{131}I may have contributed to the increased risk of thyroid cancer in the young following the Chernobyl reactor explosion [70–72]. In comparison, rates of thyroid cancer were not increased in the more than 3,000 children exposed to ^{131}I from the Hanford reactor site in an iodine replete region [75]. An increase in thyroid cancer has not been observed in about 6,000 children who received ^{131}I for diagnostic procedures [72, 76].

The Cooperative Thyrotoxicosis Therapy Follow-up Study showed that long-term thyroid problems occur in children treated with lower, rather than higher doses of ^{131}I. Thyroid adenomas developed in 30% of 30 children treated in one center with low doses of ^{131}I estimated to result in thyroid exposure of 25 Gy [33, 50]. Yet, when children are treated with higher doses of ^{131}I (100–200 Gy), the incidence of thyroid neoplasms was not increased [77].

Outcomes after ^{131}I treatment of more than 1,200 children and adolescents treated with higher doses of radioiodine for Graves' disease have been reported [6]. The duration of follow-up in these studies ranged from <5 to 15 years, with some subjects followed for more than 20 years. These studies have not revealed an increased risk of thyroid malignancy. The longest follow-up studies of children recently treated with ^{131}I come from Read et al. [78]. When more than 100 patients were surveyed nearly four decades after receiving radioactive iodine at ages ranging from 3 to 19 years, no adverse events or deaths could be attributed to ^{131}I therapy [78]. None of the patients developed thyroid cancer or leukemia. One individual developed breast cancer, and one individual developed colon cancer, numbers in keeping with the incidence of these malignancies in the population at large.

We are aware of four reported cases of thyroid malignancy in children previously treated with ^{131}I (5 years of age at treatment with 50 μCi/g; 9 years

of age at treatment with 5.4 μCi; 11 years of age at treatment with 1.25 μCi; 16 years of age at treatment with 3.2 μCi) [6]. These individuals were treated with low doses of ^{131}I. We are not aware of reports of thyroid cancer in patients treated with >100 Gy of radioactive iodine for childhood Graves' disease that can be attributed to radioactive iodine therapy. Thus, low doses of ^{131}I in children should be avoided. Ablation of the thyroid gland will decrease the risks of tumors and recurrence of hyperthyroidism. The child will need long-term thyroid hormone replacement, but such will be the situation if total thyroidectomy is performed.

Although radioactive iodine is being used in progressively younger ages, we do not know if there is an age below which high-dose ^{131}I therapy should be avoided. Risks of thyroid cancer after external irradiation are highest in children less than 5 years of age and progressively decline with advancing age [70, 72, 78, 79]. If there is residual thyroid tissue in young children after radioactive iodine treatment, there is a theoretical risk of thyroid cancer. It may therefore be prudent to avoid radioactive iodine therapy in children less than 5 years. However, children as young as 1 year have been treated with radioactive iodine with excellent outcomes [6, 23].

Radiation exposure of the gonads during ^{131}I therapy approximates 2.5 cGy, which is comparable to the gonadal exposure from a barium enema or an intravenous pyelogram [80]. The literature contains data on 500 offspring born to approximately 370 subjects treated with ^{131}I for hyperthyroidism during childhood and adolescence [6]. The incidence of congenital anomalies reported among the offspring of patients treated with radioiodine does not differ from the incidence in the general population. In addition, there was no increased prevalence of congenital anomalies in the offspring of 77 patients treated for thyroid cancer in childhood with 80–700 μCi of ^{131}I [81]. There is also no evidence of an increased rate of birth defects in survivors of the Hiroshima and Nagasaki atomic bomb blasts who were exposed to higher levels of external irradiation of the gonads than are associated with radioactive iodine therapy [25, 82].

In addition to thyroid cancer, potential influences of ^{131}I therapy on other cancers need to be considered. Follow-up from the large cohort of the Cooperative Thyrotoxicosis Therapy Follow-up Study did not find increased risks of leukemia in the ^{131}I-treated group, as compared with the drug and surgery treated groups [83]. No increase in overall cancer mortality was seen in the ^{131}I-treated patients either [84]. In other studies, excess thyroid cancer mortality following ^{131}I therapy for Graves' disease was observed during early, but not later, years of follow-up [85]. This observation is believed to reflect mythological issues related to increased cancer surveillance and detection, rather than ^{131}I effects [85].

Total-body radiation doses after ^{131}I vary with age, and the same absolute dose of ^{131}I will result in more radiation exposure to a young child than to an adolescent or adult [10, 59, 80, 86]. At 0, 1, 5, 10, 15 years of age, and in adulthood, respective total body radiation doses are 11.1, 4.6, 2.4, 1.45, 0.90, and 0.85 rem per μCi of ^{131}I [80]. Based on the Biological Effects of Ionizing Radiation Committee V (BEIR V) analysis of external radiation exposure, the theoretical risk of cancer death following acute radiation exposure is 0.16% per rem for children and 0.08% per rem for adults [87–89], although there is uncertainty associated with these projections [87–89]. Thus, if the same 10-μCi dose is given to a 10-year-old child and an adult, total-body doses will be 14.5 and 8.5 rem, respectively, and the theoretical risks of cancer mortality will be 2.2 and 0.68%. These values can be compared with the natural life-time risk for cancer death of 20% [87, 89].

We do not have good dosimetry information regarding ^{131}I use in children with Graves' disease to assess actual total body exposure and the long-term theoretical risks associated with this exposure, especially in young children. At present, data are not available to assess actual lifetime cancer risks in children treated with ^{131}I or medication for Graves' disease.

Health of Offspring

Radiation exposure of the gonads during ^{131}I therapy approximates 2.5 cGy, which is comparable to the gonadal exposure from a barium enema or an intravenous pyelogram [25]. The literature contains data on 500 offspring born to approximately 370 subjects treated with ^{131}I for hyperthyroidism during childhood and adolescence [6]. The incidence of congenital anomalies reported among the offspring of patients treated with radioiodine does not differ from the incidence in the general population. In addition, there was no increased prevalence of congenital anomalies in the offspring of 77 patients treated in childhood with 80–700 μCi of ^{131}I [81]. Furthermore, there was no evidence of an increased rate of birth defects in survivors of the Hiroshima and Nagasaki atomic bomb blasts who were exposed to higher levels of external irradiation of the gonads than are associated with radioactive iodine therapy [82].

Thyroidectomy

Surgery is the oldest form of definitive therapy of Graves' disease with the Nobel Prize in Physiology and Medicine awarded to Koker in 1909 for developments in this field [90]. Whether total or subtotal thyroidectomy should be performed has been the focus of past and recent debate. The higher relapse rates seen with subtotal thyroidectomy have resulted in the recommendation that

total thyroidectomy is the procedure of choice for Graves' disease [68, 91, 92]. New surgical techniques, such as minimally invasive thyroidectomy and minimally invasive video-assisted thyroidectomy have recently been described [93]. Whereas it can take several months for the hyperthyroid state to remit after ^{131}I treatment, the hypothyroid state occurs much sooner after surgery, being dependent on the clearance of circulating thyroid hormone.

In preparation for surgery, the child should be rendered euthyroid. This is typically done with either PTU or MMI. One week before surgery, adding iodine to the treatment 5–10 drops, t.i.d. may be desirable. This treatment causes the gland to become firmer and less vascular, facilitating surgery.

Following subtotal thyroidectomy, relief of hyperthyroidism is achieved in about 80% of children and adults, and hypothyroidism develops in about 60% of individuals [94, 95]. Hyperthyroidism recurs in about 10–15% of patients after subtotal thyroidectomy [68, 94, 95]. In comparison, hyperthyroidism recurs in less than 3% of children and adults who undergo total thyroidectomy, and hypothyroidism is nearly universal [68, 94–96].

Even in centers with considerable experience in thyroid surgery, acute and long-term complications are reported. Acute complications include hypocalcemia (40%), hematomas (2%), and recurrent laryngeal nerve paresis (2%) [68, 91, 93]. Long-term reported complications include permanent hypoparathyroidism in 1% of patients, which is treatable with vitamin D or vitamin D analogues, and recurrent laryngeal nerve injury in 2% [97]. Surgery is associated with a neck scar ranging from about 2.5–7.0 cm, that we find socially conscious teenagers and young adults try to hide with necklaces, scarves and high collars. Hypertrophic scars can also occur following thyroidectomy. Associated with surgery are the acute postoperative pain or discomfort, and time lost from school, work or activity. Surgery is expensive with collective costs of thyroidectomy often topping USD 7,000.

Of considerable importance in evaluating surgical outcome of Graves' disease, is the experience and expertise of the surgical center and surgeon. The above complication rates pertain to expert surgical centers. We know little about current complication rates following pediatric thyroidectomy performed by non-endocrine surgeons.

Antithyroid Drug Therapy

Medical treatment in the first half of the century consisted of bed rest, quinine, and iodine in the form of Lugol's solution [98]. Partial thyroidectomy was used to provide permanent cures [98]. With the advent of thiouracil and propylthiouracil (PTU) in the mid-1940s, medical therapy of Graves' improved

markedly [99]. Because of the relatively high incidence of toxic reactions that developed following the administration of thiouracil including agranulocytosis, leukopenia, and drug fever, PTU became the mainstay of medical therapy [99] and was later joined by methimazole (MMI) as an effective treatment option.

PTU and MMI reduce thyroid hormone synthesis by inhibiting the oxidation and organic binding of thyroid iodide [100, 101]. These medications are not curative. Rather, they palliate the hyperthyroid state until it spontaneously resolves or definitive treatment is rendered.

MMI is tenfold more potent than PTU and has a longer half-life [100, 101]. Recommended doses for initial therapy are 5–10 mg/kg per day for PTU and 0.5 to 1.0 mg/kg per day for MMI [102]. Yet, even lower doses of PTU or MMI may be effective for induction or maintenance therapy.

To control the hyperthyroid state, PTU and MMI are typically given every eight hours. However, once-a-day dosing may bring remission as rapidly as divided doses [102–104] and is well suited for maintenance therapy [105, 106]. Because MMI pills (5 or 10 mg) are smaller than PTU tablets (50 mg), and fewer MMI pills are generally need, MMI may be more convenient.

In contrast to oral iodine therapy (see below), thiouracil drugs do not prevent thyroid gland hyperplasia. Thus, thyroid enlargement may occur during therapy. The thyroid gland may become softer and the outlines of the gland more difficult to distinguish [99]. Because radioactive iodine is less effective in large than in small glands [59, 99, 107], thyroid size should be continuously monitored for progressive thyroid enlargement that may make the patient an unsuitable candidate for radioactive iodine treatment. If the gland enlarges, this may also be due to hypothyroidism. Thus, patients should be monitored for TSH elevations.

Although MMI and PTU promptly inhibit hormone formation, they do not inhibit hormone release. Thus, levels of circulating thyroid hormones may remain elevated for several weeks as stored hormone is released. Until circulating levels of thyroid hormones normalize, the signs and symptoms of hyperthyroidism may be controlled with beta-blockers such as atenolol (25 or 50 mg, QD or BID) or propranolol (2.5–10 mg b.i.d. or t.i.d.). If the child has reactive airway disease, beta-blocker therapy may trigger acute exacerbations of asthma. In this setting we have had success using metoprolol, which is a cardiac-selective beta-blocker.

Thyrotoxicosis can be controlled more quickly than with thionamides using solutions of saturated potassium iodine (SSKI or Lugol's solution; 1–3 drops t.i.d.) which blocks the release of stored hormones. Side effects of iodine are uncommon and include acneiform eruptions, fever, coryza, and salivation [99]. Severe and fatal allergic reactions to iodine have also been observed [99].

When combined thionamide and iodine therapy is used, PTU or MMI should be given a few hours before iodine to prevent iodine-induced increases in thyroid hormone synthesis [99].

After initiation of treatment with PTU or MMI, maximal clinical responses are seen after 4–6 weeks, at which time biochemical hypothyroidism develops. The thionamide dose can then be reduced 30–50%. To achieve a euthyroid state, the dose of MMI or PTU can either be reduced further, or supplementation with L-thyroxine started.

Complications of PTU and MMI

An apparent difference between the adult and pediatric populations is the higher incidence of adverse side effects of antithyroid medications in the young. Published studies including 500 children [6, 13, 59, 108, 109], show that complications of drug therapy include increases in liver enzymes (28%) and leukopenia (25%). Up to 0.5% of propylthiouracil (PTU) or methimazole (MMI)-treated children will develop serious complications [6, 10]. By 1998, 36 serious adverse events and two deaths from liver failure (from PTU) due to antithyroid drug therapy of childhood Graves' disease had been reported to the FDA MedWatch Program, which is very prone to under reporting [6]. In addition, at least five other deaths related to antithyroid medication therapy in children have been reported to me by professionals. Other rare and serious adverse effects of thionamide drugs include periarteritis nodosa, other forms of vasculitis, nephrotic syndrome, hypothrombinemia, and aplastic anemia [6].

Most side effects of antithyroid drugs develop within eight weeks of starting therapy. However, adverse effects may develop later. Parents should be instructed to contact their physician promptly if fever, sore throat, oral ulceration, rash, joint pain, nausea, abdominal pain, or any other unusual symptoms develop, and stop medical therapy.

When an adverse event related to either PTU or MMI occurs, some physicians will switch to another thionamide. Published data about the risks of changing to another medication following the occurrence of toxic reactions in children are limited [16]. Thus, faced with major or minor side effects in up to 20% of patients in the midst of a course of drug therapy, physicians will be faced with either electing for definitive therapy or an alternative medication.

Serious side effects of antithyroid drugs often develop within the first few months of therapy onset; however, adverse effects may develop after several years of antithyroid therapy. Increasing reports describe the development of anti-neutrophil-cytoplasmic antibodies (ANCAs) with prolonged medical therapy of Graves' disease [110–112], which are associated with vasculitis. In adults, up to 15% of individuals treated with PTU, develop ANCAs after 2 years

of therapy [110, 111]. MMI use is associated with the occurrence of ANCAs, albeit with a lower incidence than PTU [110, 111]. In the pediatric population, ANCA-mediated disease has been observed in patients treated with PTU or MMI [113, 114]. Because these antibodies can trigger serious vasculitis events, elimination of the trigger of ANCA induction, i.e. antithyroid medications, must be considered [115].

Long-Term Efficacy of Antithyroid Drugs

In children, published remission rates after several years of drug therapy are usually less than 25% [13, 59, 67, 95, 116–118]. It has been suggested that after 2 years of treatment remission rates are 25%, that 4 years of drug therapy are needed to achieve 50% remission rates [109], and that 10 years of drug therapy can achieve remission in 75% of children [109]. However, although widely cited, these theoretical projections have not been substantiated. The most extensive long-term study of this issue involving near 200 children with Graves' disease shows that less than 20% of children treated medically achieve remission lasting greater than 2 years [13]. When responses to medical therapy between prepubertal and pubertal children are compared, 1 year remission rates are also less in prepubertal than in pubertal children [15, 16].

The efficacies of antithyroid drugs appear to be inversely related to serum levels of thyroid-stimulating antibodies (TSAb) or thyrotropin receptor antibodies (TRAbs) [119–123]. After several years of antithyroid therapy, remission rates in adults range from 15% in individuals with high levels of TRAb at the time of diagnosis, to 50% in individuals with low pretreatment TRAb levels [119]. In our experience, if remission occurs with medical therapy (about 15% of our patients; n = 30 patients), it is in the setting of patient with a small thyroid gland (<20 g) and low levels of TRAbs (<110% of control).

It has been suggested that long-term remission rates can be predicted by observing responses to short-term (ca. 6 months) antithyroid drug therapy [17, 124, 125]. Short-term therapy appears to work as well as long-term therapy in patients with mild hyperthyroidism and small goiters, but neither short- nor long-term antithyroid drug therapy is likely to lead to a lasting remission in patients with severe thyrotoxicosis and a large goiter [17, 124, 125]. Although most of the evidence supports the efficacy of short term therapy, some investigators have noted higher relapse rates after short-term than long-term treatment [126, 127].

Risks of Cancer after Drug Therapy

Antithyroid drugs are preferred to radioactive iodine therapy by many clinicians based on the assumption that cancer risk is less after drug therapy than after radioactive iodine. However, data do not support that this assumption.

The largest long-term follow-up study of thyroid cancer risks after treatment of Graves' disease by the (CTSG), revealed that the incidence of thyroid carcinomas over 10–20 years of follow-up (not lifetime incidence) is fivefold higher in adults with Graves' disease treated with thionamide drugs (follow-up period normalized incidence rate = 1 case per 332 individuals) than in patients treated with ^{131}I (1/1,783), and eightfold higher than in patients treated surgically (1/2,820) [77]. The incidence of thyroid adenomas are also 10 and 20 times higher among the adults treated with antithyroid drugs (1/76) than in patients treated with ^{131}I (1/802) or surgery (1/1,692), respectively [77]. Rather than reflecting a causative role for medical therapy in the pathogenesis of thyroid neoplasia, these observations may reflect the persistence of more thyroid tissue in patients treated with drugs than in individuals treated with radioactive iodine or surgery.

Although CTSG data show an increased rate of thyroid cancer in the drug-treated patients [77], it is important to note that thyroid cancer mortality rates were not increased in the CTSG patients treated with drugs [84]. We are also unaware of thyroid cancer cases in the large numbers of children treated with antithyroid drugs alone.

Treatment Approaches for Children

Based on what is now known about the risks and benefits of different treatments and the pathogenesis of Graves' disease, we can now be more selective in our approach to therapy. To reduce treatment risks and expedite cures, the treatment of the child or adolescent with Graves' disease can be guided by the patient's age and the nature of the intrinsic autoimmune disease.

For children less than 5 years of age, we consider antithyroid medications as a first line therapy. Although radioactive iodine has also been successfully used in this age group without an apparent increase in cancer rates, it may be best to defer radioactive iodine therapy because of the possible increased risks of thyroid cancer after radiation exposure in very young children in the event that any thyroid tissue remains after radioactive iodine therapy.

Because young children are less likely to have remission than older children on drug treatment [15, 16], prolonged drug therapy may be needed. If there are no toxic effects, continuing antithyroid drugs is reasonable until the child is considered old enough for radioactive iodine therapy. Alternatively, thyroidectomy or ablative radioactive iodine therapy can be considered if reactions to medications develop or there is the desire to avoid prolonged drug use.

Fortunately, less than 5% of children with Graves' disease present at 5 years of age or younger [8].

Fifteen percent of children with Graves' disease will present between 6 and 10 years of age [8]. Considering drug therapy as a first-line measure for this age group is reasonable. Yet, as 10 years of age are approached, either radioactive iodine or drug therapy can be considered as initial therapy, as the risks of thyroid cancer in remaining irradiated thyroid tissue is expected to be less at 10 than at 5 years and there will be lower whole-body radionuclide exposure at 10 than at 5 years.

Children 10 years of age and older account for 80% of the pediatric cases of Graves' disease. For this age group, radioactive iodine or antithyroid drugs can be considered as first-line treatment options. In determining if drug therapy is likely to be successful, TRAb levels and thyroid size may be predictive of remission rates. The presence of low TRAb levels and a small thyroid suggests the possibility of remission on medical therapy. Yet, if TRAb levels are high and the thyroid is large, the odds of spontaneous remission are low [119, 121, 123]. However, TRAb levels and thyroid size may not always be indicative of remission likelihood.

The critical issue about drug therapy is whether a lasting cure can be achieved after using medications to palliate the hyperthyroid state. Thus, for patients with normal TRAb levels and a small thyroid size, it seems reasonable for to treat for 6–12 months and stop the drug when a clinical remission has been achieved. If a relapse occurs, medical treatment can be resumed or an alternative form of therapy chosen. For patients with elevated TRAb levels and a large thyroid, it is much less likely that remission will occurs after short-term or long-term medical therapy, and consideration should be given to definitive treatment after euthyroidism is achieved.

When radioactive iodine is used, it is important that higher doses of ^{131}I be used in children. The goal of radioactive iodine therapy in children should be to ablate thyroid gland and achieve hypothyroidism. If no thyroid tissue remains, the risk of thyroid cancer will be very small if present at all. To achieve this goal we now use doses of ^{131}I of 250–300 μCi/g thyroid tissue.

Finally, irrespective of the treatment option selected, careful follow-up is needed for all patients treated for Graves' disease. Long-term follow-up should include regular examination of the thyroid gland and measurement of circulating levels of thyroid hormones once or twice a year. All newly appearing thyroid nodules should be biopsied or excised.

Choosing a treatment approach for childhood Graves' disease is often a difficult and highly personal decision. Discussion of the advantages and risks of each therapeutic option by the physician is essential to help the patient and family select a treatment option (table 2).

Table 2. Graves' disease treatments

	Medical	Surgery	Radioactive iodine
Long-term remission rates	15–25%	90–100%	90–100%
Minor side effects	20–30% rash/urticaria arthralgia leukopenia	100% pain 5% transient hypocalcemia	5% pain
Major side effects	0.8% severe hepatitis agranolocytosis	1–5% vocal cord paresis 1–5% hypoparathyroidism	0.01% thyroid storm
Reported mortality	13 children	1/1,000 children	none
Long-term thyroid cancer risks	0.3%	0.03%	0.05%

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Thyroid-Associated Ophthalmopathy in Juvenile Graves' Disease: Clinical, Endocrine and Therapeutic Aspects

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Thyroid-associated ophthalmopathy or Graves' ophthalmopathy (GO), or thyroid eye disease (TED) refers to the eye changes observed in Graves' disease (GD). The orbital involvement is characterized by lymphocytic infiltration and edema of the retrobulbar tissues, resulting in marked swelling of extraocular muscles and orbital fat. Due to the increased volume or orbital contents the retrobulbar pressure rises, interfering with venous drainage (causing lid swelling) and pushing the globe forwards (causing proptosis or exophthalmos) [1, 2]. In severe cases, direct pressure on the optic nerve may result in loss of visual functions. The swelling of eye muscles hampers muscle motility, associated with double vision. The clinical manifestations of GO can thus be understood from a mechanistic point of view. However, the immunopathogenesis of GO remains largely unknown despite considerable progress made in this field in the last decade [3]. In this communication we review the pediatric aspects of GO and provide the latest information regarding the therapeutic approach of this disease.

Immunopathogenesis of Thyroid Eye Disease

The orbital fibroblasts are widely viewed as the target cells of the autoimmune attack in GO. During the early stages of the disease, macrophages, highly specialized T cells, mast cells, and occasional plasma cells infiltrate the orbital connective, adipose, and muscle tissues [4, 5]. Activation of T cells directed against a thyroid follicular cell antigen(s) that then recognizes and binds to a similar antigen(s) in orbital tissue is a probable but yet unproven theory [2].

Alternatively, macrophages and dendritic cells may nonspecifically initiate the orbital immune response, which is then propagated by recruitment of sensitized T cells. Several cytokines have been associated with the evolution of the orbital tissue changes in TED [6, 7]. These include interferon-γ [8], tumor necrosis factor-α, interleukin-1 (IL-1), and transforming growth factor-β [9] as well as other growth factors such as insulin-like growth factor-I (IGF-I) [10, 11] and platelet-derived growth factor [12, 13]. These compounds are now known to be produced both by infiltrating immunocompetent cells and by residential fibroblasts, adipocytes, myocytes, and microvascular endothelial cells. These cytokines and growth factors stimulate cell proliferation, glycosaminoglycan (GAG) synthesis, and expression of immunomodulatory molecules in orbital fibroblasts and microvascular endothelial cells [13–15]. An increase in connective tissue and extraocular muscle volume within the bony orbits caused by accumulating hydrophilic compounds (predominantly GAG, the hydrophilic nature of which can attract water by osmosis) leads to the clinical manifestations of TED and causes proptosis, extraocular muscle dysfunction, and periorbital edema [1, 2].

The orbital fibroblasts do express functional TSH receptors (TSH-R). This recent finding has led to the currently favored view that the TSH-R is the long sought after shared antigen between the thyroid and the orbit and that the TSH-R is the autoantigen involved in GO. Indeed, cytokine-induced differentiation of a particular subset of orbital fibroblasts into adipocytes is associated with increased TSH-R expression and adipogenesis [16].

Furthermore, TSH-R immunization of experimental animals results in histological changes in orbital tissues resembling GO [17].

A causative role of stimulating TSH-R antibodies (TSI) in the development of GO is very attractive as it allows a unifying hypothesis for the various clinical manifestations of GD: Graves' hyperthyroidism (GH), GO and thyroid dermopathy. Arguments against such a hypothesis cannot, however, be dismissed. TSI, in contrast to T cells, cross the placenta and may cause fetal and neonatal hyperthyroidism. GO, however, has never been observed in neonatal thyrotoxicosis TSI are almost always present in GH, but clinically apparent GO develops only in a subset of the patients. Lastly, serum TSI are only slightly related to the severity of GO, although more so to the activity of the eye disease [18]. Whereas TSI might contribute to further progression of GO, it remains doubtful if TSI act as the primary mediator in the immunopathogenesis of GO.

Consequently, the search for other antigens and antibodies involved in GO continues. Graves' IgG added to a culture of human skin fibroblasts increased the synthesis of collagen. The effect was not mimicked by TSH and rather specific for GO as IgG of Graves' hyperthyroid patients without GO were not

active in this respect [19]. Another study demonstrated that Graves' IgG was able to induce the release of T-cell chemoattractants from cultured orbital fibrobasts, notably IL-16 (a CD+ ligand that activates T cells) and RANTES (a C-C type chemokine) [20]. The authors postulated IgG binding to a surface receptor of the fibroblasts distinct from the TSH-R, because TSH had no effect and there was no relation with TSH-R antibodies. The induction of IL-16 and RANTES could be blocked by rapamycin and the authors speculated the surface receptor could be the IGF-I receptor as IGF-I post-receptor signaling is also blocked by rapamycin.

Several antibody markers of immune-mediated damage to eye muscle have also been identified and the great majority of patients with active ophthalmopathy have antibodies against one or more eye muscle antigens. However, none of the target antigens are localized exclusively in the eye muscle and all are intracellular, indicating that their exposure to the immune system would be a consequence of eye muscle fiber damage rather than its cause [21].

Activity and Severity of TED

The majority of Graves' patients have a mild and nonprogressive ocular involvement that does not require any specific or aggressive treatment, also because non-severe GO often tends to improve spontaneously. When evaluating a patient with TED, two basic questions have to be addressed. First, does the patient needs treatment for TED and, in a positive answer, which kind of treatment is indicated.

The decision of whether ophthalmopathy must be treated should rely on the assessment of two different parameters, the activity and severity of the disease. The activity of the disease is neither synonymous nor coincident with the severity of the disease. In other words, an individual patient may have severe ocular manifestations but the disease may be inactive (fig. 1). To assess the activity of ophthalmopathy, Mourits et al. [22] proposed a clinical activity score (CAS), which in its original formulation included 10 different items (table 1) mainly, but not solely, reflecting inflammatory changes: giving one point to each manifestation, a score is obtained, with a range from 0 (no activity) to 10 (highest activity). A slightly modified CAS which does not include some of the items originally proposed by Mourits et al. [22] was proposed by an ad hoc committee of the four thyroid societies as a tool to record ocular changes over time after treatment of ophthalmopathy [23] (table 1). Definition of severity of GO is somehow arbitrary (table 2). Undoubtedly, optic neuropathy which can be subclinical and heralded only by changes in the visual evoked potentials, depicts per se a situation that can be sight threatening, especially if it is associated

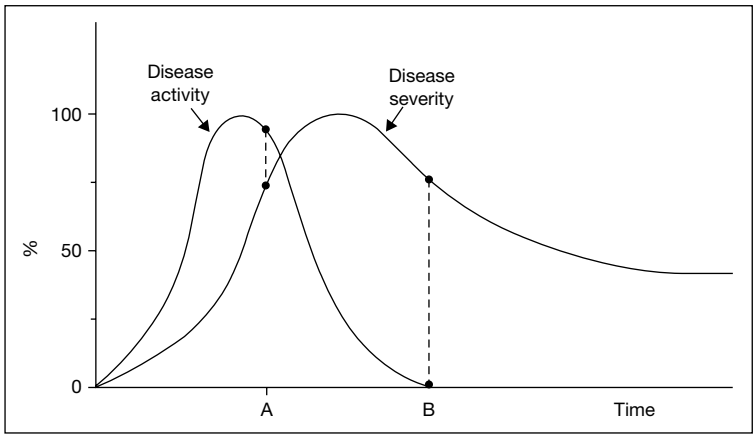

Fig. 1. Hypothetical relationship between disease activity and severity in the natural history of TED [60].

Table 1. Clinical activity score

Original formulation [22]	Revised formulation [23]
Painful, oppressive feeling on or behind the globe	spontaneous retrobulbar pain
Pain on attempted up, side, or down gaze	pain on eye movements
Redness of the eyelids	eyelid erythema
Diffuse redness of the conjuctiva	conjuctival injection
Chemosis	chemosis
Swollen caruncle	swelling of the caruncle
Edema of the eyelids	eyelid edema or fullness
Increase of 2 mm or more in proptosis in the last 1–3 months	
Decrease in visual acuity in the last 1–3 months	
Decrease in eye movements of 5 degrees or more in the last 1–3 months	

with an evident reduction of visual acuity. It has to be remembered that immunosuppression treatment is effective only in patients with active disease.

Juvenile Graves' Ophthalmopathy. Incidence and Symptomatology

The most accurate data on the incidence of GO is derived from a population-based cohort study in Olmsted County, Minn., USA [24]. The overall age-adjusted incidence rate was 16.0 cases for women and 2.9 cases for men per 100,000 population per year. Peak incidence rates were observed in the age groups

Table 2. Assessment of severity of Graves' ophthalmopathy

Degree of involvement	Parameter		
	proptosis[a]	diplopia[b]	optic neuropathy
Mild	19–20	intermittent	subclinical[c]
Moderate	21–23	inconstant	visual acuity 8/10–5/10
Marked	>23	constant	visual acuity <5/10
Severe ophthalmopathy: at least one marked, or two moderate, or one moderate and two mild manifestations[d]			

[a]Proptosis by exophthalmometer readings or CT/MRI measurements. Median normal value in our Italian population is 15 mm. Normal values show racial variation; accordingly, abnormal values should be considered those 4 mm or more above the respective median value.

[b]Diplopia: intermittent, present only when fatigued; inconstant, present in secondary positions of gaze; constant, present in primary and reading positions.

[c]Abnormal visual-evoked potentials or other tests, with normal or slightly reduced (9/10) visual acuity.

[d]Patients with severe GO will need either medical or surgical treatment depending on the activity of eye disease.

Reproduced from Bartalena et al. [61].

40–49 and 60–69 years. The incidence rates start to increase as of the age of 20 years. Below the age of 20 years the occurrence of GO is a rare event. Incidence rates (cases per 100,000 population per year) are in the age groups 5–9, 10–14, and 15–19 years for females 3.5, 1.8 and 3.3, respectively, and for males 0, 1.7 and 0, respectively. Only 6 of the 120 incident cases of GO observed in this cohort study were below the age of 20 years. A more detailed study published recently from the same department found that of 1,662 cases ages <18 years, with thyroid-related abnormalities, evaluated at the Mayo Clinic in Rochester, Minn., USA, during the 15-year interval (1985 to 1999), 35 children with GO were identified. Of these, 6 had received radioactive iodine (RAI), 1 patient had RAI plus antithyroid drugs, 9 had partial or total thyroidectomy, and the rest antithyroid medications for their thyroid problem. Four patients did not require treatment. Of the 35 children with GO, 31 required no therapy with only supportive measures, 1 had eyelid surgery, and 3 had orbital decompression. None of the patients received steroids or external radiotherapy. They concluded that although the pediatric population has similar clinical manifestations of GO to adults, the disorder is less severe in children [25]. The low incidence of childhood GO might be related to the low incidence of Graves' disease during childhood. To analyze this further, we compared the prevalence

Table 3. Relative frequencies (%) of eye changes in patients with Graves' ophthalmopathy with onset in childhood or adulthood

	Childhood onset [26–29] (n = 42)	Adulthood onset [34] (n = 152)
Soft tissue involvement	48	75
Proptosis	36	63
Extraocular muscle involvement	2	49
Corneal involvement	26	16
Optic nerve involvement	0	21

of clinically apparent GO in young or adult consecutive patients with GH. Lid retraction by itself did not qualify for the diagnosis of GO, as this sign can be attributed to the hyperthyroid state, disappearing spontaneously once the euthyroid state has been reached. GO was present in 42 of 182 (23%) patients with childhood GH [26–29] and in 118 of 1,050 (18%) adult patients with GH [30–33]. It follows that children have about the same risk (or slightly increased) as adults to develop GO once they have contracted Graves' hyperthyroidism.

The severity of childhood GO appears to be less than that of adulthood GO. This is evident from a comparison of the relative frequency of the various eye changes between children and adults with GO. Taking together the 42 childhood GO cases from the four studies published so far [26–29] and contrasting then with 152 new consecutively referred adult GO patients [34], it is clear that soft tissue involvement and proptosis are the predominant changes in childhood GO whereas the more severe manifestations of restricted eye muscle motility and optic nerve dysfunction almost never occur in children (table 3). Remarkable is the high frequency of corneal involvement in children. This was, however, limited to punctate epithelial erosions and all cases originated from one study on Chinese children [29], whereas corneal involvement was absent in the three other studies on childhood GO [26–28].

Very recently, we embarked on a questionnaire study among members of the European Society for Paediatric Endocrinology (ESPE) and the European Thyroid Association (ETA) with the following specific aims. First, we wanted to know the proportion of GO cases among patients with Graves' hyperthyroidism in the age group of 18 years and younger. Second, we were curious whether childhood GO could be related to smoking prevalence. Third, we wanted to record the diagnostic and therapeutic approaches to a standard case (and some variants) of a 13-year-old girl with Graves' hyperthyroidism and

Table 4. The childhood Graves' ophthalmopathy questionnaire (reproduced from Krassas et al. [35])

General questions

(1) How many cases of childhood Graves' ophthalmopathy (patients up to 10 years old in prepubertal stage) have been seen in your institution in the last 10 years and how many among adolescents (11–18 years old)?
(2) How many cases of Graves' hyperthyroidism (up to 18 years old) have been seen in your institution in the last 10 years?
(3) Is there an official figure on the percentage of smokers among teenagers in your country?

Standard case

A 13-year-old Caucasian girl developed over the last 6 months lack of ability to concentrate in school, failure in school, weight loss and nervousness. Pulse rate of 120/min, diffuse goiter around 30 g, and signs of moderately severe and active thyroid ophthalmopathy. Specifically, she had moderately severe eyelid swelling, some chemosis and redness of the eyes, but not caruncle swelling, no pain behind the eyes and no redness of the eyelids. Exophthalmometer reading was 21 mm for both eyes. She also had impairment of elevation for both eyes with inconstant diplopia. She is not a smoker.
(1) What is your diagnostic approach?
(2) What is your therapeutic approach?

Case variants

Is your therapeutic approach to the standard case changed in case of:
(1) Slight worsening of GO after 4 months
(2) Age of 7 years
(3) Age of 15 years, recurrent hyperthyroidism after a course of antithyroid drugs, still active GO
(4) Age of 15 years, euthyroid, active GO
(5) Driving problems, because of mild diplopia

moderately severe active GO [35]. The study design allowed evaluating any differences in approaches between pediatricians and endocrinologists.

For this purpose, questionnaires were sent between November 2004 and January 2005 to approximately 300 members of ESPE and ETA who had an electronic address. The questionnaire contained three general questions and a standard case of a 13-year-old girl with Graves' hyperthyroidism and moderately severe active GO (table 4) [35].

Physicians were asked to outline their diagnostic and therapeutic approaches to the standard case according to a list of given biochemical thyroid-function tests, imaging techniques, specific eye investigations and various therapeutic options. Five variants of the standard case were presented, and physicians were asked whether case variants would change their therapeutic approach chosen for the standard case.

119 questionnaires were returned but 52 respondents indicated they had no experience with the treatment of Graves' disease in childhood. The analysis was thus restricted to 67 returned and completed questionnaires, originating from 23 ESPE members (called paediatricians) and 44 ETA members (called endocrinologists). It should be noted that the ETA membership list does not discriminate between basic scientists and clinicians, so in reality the response rate was much higher. Respondents came from 25 countries, predominantly from Europe but also included one from Brazil, three from the USA and two from Japan. Leaving out the results from these six respondents from outside Europe did not make any real difference in the overall results. A total of 1,963 patients with juvenile Graves' hyperthyroidism had been encountered by respondents over the last 10 years; on average 4.6 cases per year by each pediatrician and 2.3 cases per year by each internist. One-third of the patients with Graves' hyperthyroidism had GO. Among the patients with GO, one-third were ≤10 years old (77% of them being seen by pediatricians) and two-thirds were in the age group of 11–18 years (56% of them being seen by pediatricians). The answers of respondents with regard to smoking prevalence among teenagers in their country were incomplete and mostly based educated guesswork. Therefore, we grouped countries of respondents according to smoking prevalence among teenagers as given by official data from the World Health Organization [WHO; regional office for Europe, tobacco control database, 2003]. A higher prevalence of smoking was associated with a higher frequency of GO among juvenile patients with Graves' hyperthyroidism ($p < 0.0001$ by χ^2 test). Whereas in countries with a smoking prevalence among teenagers of ≥25% the distribution of GO cases was 36.6% (236 cases), in countries with a smoking prevalence of <20% the distribution was 25.9% (117 cases; table 5) [35].

Regarding the diagnostic approaches to the standard case, on average, five biochemical thyroid function tests were requested by respondents, paediatricians asking one test more than internists (5.6 compared with 4.6, $p < 0.005$). Thyroid-stimulating hormone (TSH), free thyroxine (FT4) and TSHR-Ab were almost universally ordered, and thyroperoxidase antibodies (TPO-Ab) and FT3 by about 60%. Thyroid imaging was requested by 56 of 67 respondents (84%), with ultrasound by 46 and with scan by 10. Orbital imaging was asked by 59 of 67 respondents (88%), with magnetic resonance imaging or computed tomography by 42, with ultrasound by 14 and with octreoscan by 3. The preferred treatment of Graves' hyperthyroidism of the standard case was clearly antithyroid drugs, chosen by 94% of respondents. A wait-and-see policy was recommended for the co-existing GO of the standard case by 70%, and corticosteroids by 28%. The therapeutic approach did not differ between paediatricians and internists. With regard to the therapeutic approach of case variants, a younger age of 7 years did not affect management very much. Antithyroid drugs were still the treatment of choice (66%) for recurrent hyperthyroidism, whereas

Table 5. Occurrence of childhood Graves' ophthalmopathy in Graves' hyperthyroidism as a function of smoking prevalence among teenagers in their country of origin (reproduced from Krassas et al. [37])

Smoking prevalence among teenagers (%)	Graves' hyperthyroidism (%)	Graves' ophthalmopathy (%)	Graves' ophthalmopathy	
			≤10 years (%)	11–18 years (%)
≥25[a]	644 (100)	236 (36.6)	52	48
20–25[b]	818 (100)	223 (27.3)	15	85
<20[c]	452 (100)	117 (25.9)	24	76

Data per country based on WHO regional office for Europe, tobacco control database, 2003. Internet: http://data.euro.WHO.int/tobacco

[a]Turkey, Bulgaria, Germany, Czech Republic, Spain, Hungary, France.

[b]Switzerland, UK, The Netherlands, Romania, Belgium, Canada, Russia, Portugal, Poland.

[c]Denmark, Italy, Serbia, Sweden, USA, Brazil, Greece.

^{131}I therapy was now chosen by 25% and thyroidectomy by 9%. Worsening of GO after 4 months or still-active GO when euthyroid was viewed by 68 and 63% of respondents, respectively, as an indication to start specific eye treatment, mainly with steroids. In case of driving problems, 36.5% recommended eye muscle surgery and 21% prisms. One respondent remarked that diplopia in his experience is never seen in childhood GO [35].

From all the above data it is clear that the incidence of ophthalmopathy in childhood GD is more or less the same as in adults. However, it is less severe and more likely to remit completely [26–29, 34]. The question then arises why childhood GO is less severe. The female preponderance is similar between children and adults with GH (87 and 83%, respectively), but the prevalence of smoking is much lower in children than in adults (4 and 47%, respectively) [29, 35].

Smoking is a risk factor for GO, and the odds increase significantly with increasing severity of GO [36]. One study observes that the manifestations of GO begin to resemble more closely the adult findings when adolescence approaches [26]; conceivably this could be explained by increasing smoking prevalence with age.

Our recent study [35] supports the above data and provides a very interesting clue: the difference might be caused by exposure to tobacco smoke. Specifically, of 1,914 patients with childhood GH seen by respondents 576 (30%) had GO. When grouped according to smoking prevalence among teenagers in the country of origin, it became evident that the proportion of GO patients among children

with GH is highest in countries in which teenagers smoke most (table 5). What is striking is that 52% of the children with GO in these countries (smoking prevalence ≥25%) are 10 years old or younger, whereas the figure (19%) is much lower in countries in which smoking prevalence among teenagers is less than 25%. It is unlikely that children ≤10 years of age smoke themselves; the high proportion of GO in this group is thus best explained by passive smoking as a result of living in an environment in which 25% or more of their peers smoke. It is of interest that, based on the WHO regional office for Europe, tobacco control database, 2003, all the countries (100%) that are included in the first group have a smoking prevalence higher than 25% among adults, while only 50 and 40% of the countries in the second and third groups exhibit such a prevalence (table 5) [37].

The conclusion is that passive smoking may also have a deleterious effect on childhood GO [37].

Treatment of Thyroid Eye Disease in Childhood

Corticosteroids

As the expectation remains that the expression of GO in children is, in most instances, both mild and transient most of the physicians who are dealing with such cases prefer the 'wait-and-see' policy. Indeed, in our recent study [35] 70% of the respondents recommended such a policy for the eye changes. Active intervention (predominantly with steroids) is considered appropriate in case of worsening of eye changes or no improvement of eye changes when the patient has become euthyroid [35]. Doses between 5 and 20 mg prednisone daily are used depending on the severity of the case. Our policy in moderately severe cases is to start with 20 mg daily for 4–6 weeks when usually a beneficial effect is expected and then we tapering the dose accordingly. We are reluctant to use higher doses of glucocorticoids (GC) as well as intravenous glucocorticosteroids. It has to be kept in mind that prolonged prednisone administration, which should be used in some severe cases of TED, is associated with weight gain, immune suppression and growth failure in children [38]. Retrobulbar irradiation, which has been proved beneficial in adult cases with TED [2], has no place in the treatment of juvenile GO in view of the theoretical risk of tumor induction [3].

One important issue is the use of steroids in patients with TED who received radioiodine treatment (RAI) for hyperthyroidism. Two randomized, prospective, controlled clinical trials by Tallstedt et al. [33] and Bartalena et al. [39] clearly demonstrated in adults that radioiodine administration may be associated with a progression of ophthalmopathy in a small proportion of patients (≈15%). GC can prevent, at relatively low doses and for short periods of time, exacerbation of eye disease and can effectively cure pre-existing ocular manifestations.

Table 6. Relative abundance of somatostatin receptor mRNA expression in retrobulbar fibroblasts and lymphocytes obtained from Graves' ophthalmopathy and control patients (reproduced from Pasquali et al. [42, 43])

	Fibroblasts [42]		Lymphocytes [43]	
	GO (n = 10)	controls (n = 6)	GO (n = 10)	controls (n = 2)
sst1	++	–	+++	–
sst2	+++	+++	++	−/+
sst3	++	++	+	+
sst4	−/+	–	++	−/+
sst5	++	–	+	–

Recently, Perros et al. [40] showed that RAI is not associated with deterioration of TED in patients with minimally active eye disease when post radioiodine hypothyroidism is prevented. The message from all relevant studies published so far is that RAI in adults can cause TED progression in a certain proportion of Graves' patients [41]. Patients who smoke or have active (although mild to moderate) TED or severe hyperthyroidism are good candidates for receiving GC coverage. Unfortunately, similar data are not available for adolescents for two main reasons. First, RAI as treatment of hyperthyroidism in the pediatric age is unpopular in Europe and some other continents and second the incidence of GO during childhood is low which might be related to the low incidence of GD during childhood.

Somatostatin Receptors in Retrobulbar Tissues

Somatostatin and somatostatin receptor gene transcripts can be detected in primary cultures of fibroblasts obtained from retrobulbar connective tissue samples of Graves' ophthalmopathy and controls patients (table 6). Somatostatin receptor subtypes 2 and 3 were present in GO and control fibroblasts, but sst1 and SST5 only in GO fibroblasts. Somatostatin-14 and octreotide inhibited the binding of radiolabeled somatostatin-14 with half-maximal inhibition of binding (IC50) of 0.80 ± 0.37 and 33.7 ± 33.1 nmol/l respectively in GO fibroblast cultures [42]. Octreotide (10^{-7} M) significantly decreased forskolin-induced but not basal cAMP accumulation. It inhibited cell growth and induced apoptosis of the fibroblasts [42].

Lymphocytes recovered from retrobulbar tissues of GO or control patients also express sst transcripts (table 6). All five sst subtypes were present in GO lymphocytes, in contrast to control lymphocytes which expressed preferentially sst3 [43].

The presence of somatostatin receptors in retrobulbar tissues of GO patients and the inhibitory effects of octreotide on immune functions and

fibroblasts growth and activity provide a sound biologic rationale for the application of somatostatin analogues in the diagnosis and treatment of GO.

Orbital Octreoscan for Assessment of Disease Activity

By radiolabeling octreotide, tissues that express somatostatin receptors can be visualized. By applying [^{111}In-DPTA-D-Phe] octreotide scintigraphy, specific uptake of the radiolabel was observed in the orbits of some but not all patients with GO [44]. The orbital uptake can be explained from binding of somatostatin receptors on activated T lymphocytes and fibroblasts in the orbit and from local blood pooling due to venous stasis. Systemic hypercirculation seems only partly responsible, as evident from the rather low orbital uptake in Graves' hyperthyroid patients without GO.

Some but not all studies report a direct relation between orbital octreotide accumulation and the severity of GO [44–46]. In contrast, the activity of the eye disease is always found to be related to orbital octreotide uptake. This is evident from a direct relation between orbital uptake and various parameters of disease activity in GO like the clinical activity score [44, 46] and the T2 relaxation time of the inferior rectus muscle on MRI [47]. The lower uptake in patients with inactive GO is close to that in controls subjects in whom no specific uptake is observed [45]. A positive orbital octreoscan might thus indicate active GO which – unlike the inactive end stage of the disease with fibrosis – is susceptible to immunosuppressive treatment [48]. Indeed, successful immunosuppression is associated with a fall in orbital octreotide uptake. Orbital octreoscan could consequently be used to select those GO patients, who are likely to benefit from immunosuppression [48].

Potential Role of IGF-I

IGF-I immunoreactivity is increased in eye muscle cells, fat cells and retrobulbar inflammatory cells of GO patients [10]. IGF-I stimulates the synthesis of collagen and glycosaminoglycans by orbital fibroblasts in vitro [49]. Graves IgG inhibit the binding of radiolabeled IGF-I to orbital fibroblasts in culture, although without discrimination between IgGs obtained from Graves' patients with or without GO [50]. This finding is reminiscent of the inhibition of radiolabeled TSH to the TSH-R by Graves IgG, suggesting the possibility that there might be IGF-I receptor stimulating autoantibodies in GD. These findings on IGF-I in GO have so far not been confirmed by others. In patients with active GO – all euthyroid while receiving methimazole treatment – serum concentrations of free and total IGF-I and IGF-II and of the three IGF binding proteins 1, 2 and 3 were all similar to those of matched controls [51], thus excluding serum as the origin of any upregulated IGF-I in orbital tissues of GO patients. The increased IGF levels in retrobulbar tissues may represent autocrine and/or paracrine activity, in theory susceptible to reduction by somatostatin analogues [52].

Therapeutic Approach of TED by using Somatostatin Analogs

Somatostatin (SM), a peptide inhibiting the release of GH, is present and plays an inhibiting role in the regulation of several organ systems in men and other species. Various SM analogs (SM-as) have been developed and used in clinical practice because the short half-life of SM makes it unsuitable for routine treatment [53]. Recently, it has been shown that SM-as might be of therapeutic value in the treatment of active TED in adults. However, most of the initial studies were uncontrolled, not randomized, and included only small number of patients. We had the opportunity to treat 3 adolescents (2F, 1M) with moderate severe TED with SM-a aged 14, 15 and 16 years old [54]. All had an increased clinical activity score (CAS) – 4, 5 and 6, respectively. All were on antithyroid therapy and euthyroid at the time of the initiation of treatment. They received 20 mg octreotide (sandostatin-LAR) i.m. one injection every 30 days for 4 months. Their ophthalmopathy improved substantially and CAS decreased in all patients [54].

Recently, 4 double-blind, randomized, placebo-controlled clinical studies were published. In the first [55], 50 euthyroid patients (11 males, 39 females, age 22–74 years, median 50 years) with active TED (clinical activity score [CAS] ≥3, NOSPECS 2a–5a, of median duration 0.9 years) received either 30 g LAR or placebo every 4 weeks for 16 weeks. Both groups then received 30 g LAR for weeks 16–32 and were followed-up without treatment for a further 24 weeks. Objective assessments included all individual parameters of TED, CAS, and derived scores for soft tissue inflammation (STI) and ophthalmopathy index (OI). During weeks 0–16 there was significant reduction in STI, subjective diplopia, and CAS in LAR treated patients; STI and CAS were also reduced with placebo. The OI reduced by –1.12 in LAR ($p = 0.0017$) vs. -0.23 in placebo ($p = 0.33$), giving a barely significant treatment effect by Wilcoxon's rank sum test ($p = 0.043$), but analysis of covariance failed to confirm this ($p = 0.16$). During weeks 16–32 there was no significant change in OI in either group. The overall results (weeks 0–32) showed reduction in STI and CAS in both groups. They concluded that no significant therapeutic effect of octreotide LAR was seen in patients with moderately severe TED. The improvement in both treated and placebo groups emphasize that the results of open studies must be viewed with caution.

In the second study of a long-acting SM-a (16 weeks of long-acting release formulation of octreotide [octreotide-LAR]), which was conducted in 51 patients with mild active TED and aimed in preventing deterioration and precluding the need for more aggressive therapeutic modalities, such as glucocorticoids or radiotherapy, no treatment effect was observed for the primary end point [56]. The clinical activity score was reduced for patients treated with octreotide-LAR, but without any significant difference with respect to patients receiving placebo. However, octreotide-LAR significantly reduced proptosis (as measured by exophthalmometry). This was associated with non-significant

differences in favor of octreotide-LAR in a series of proptosis-related parameters. These included class III grade, opening of the upper eyelid, the difference in ocular pressure before primary position and upgaze, and extraocular muscle involvement. Evaluating the extraocular muscle volume by magnetic resonance imaging showed a nonsignificant reduction. No significant correlation between the initial uptake of octreoscan and the response to treatment was observed.

The inference was that in this study, octreotide-LAR did not seem suitable to mitigate activity in mild TED. However, proptosis, one of the most debilitating symptoms of TED, was significantly reduced. The sustained effect on proptosis of just 16 weeks of octreotide-LAR treatment is an encouraging preliminary result in light of the serious lack of therapeutic options for this condition.

Very recently a third similar study was published, in which lanreotide 20 mg every 2 weeks was used in a randomized fashion. A total of 60 patients were investigated. The inference was that lanreotide had no effect on CAS in patients with TED [57]. Finally, in a randomized controlled study from the Endocrinology Department of the Mayo Clinic, Minn., USA, which has just been published, 29 patients with moderately severe TED were investigated and a significant improvement in clinical activity score and lid fissure width in patients who received sandostatin LAR 20 mg was found [58].

Future Perspectives of Somatostatin Analogs

One may raise the question why the efficacy of long-acting SM-a is not so strong, given the well established biologic rationale for this therapeutic modality in GO. One of the answers might be that octreotide and lanreotide have a high affinity only for sst2, a low affinity for sst3 and sst5 and an almost absent affinity for sst1 and sst4 (table 6) [59]. This is unfortunate in view of the expression of all five subtypes of the somatostatin receptors in retrobulbar fibroblasts and lymphocytes of GO patients. The newly developed SM-a SOM 230 has, in contrast, a rather high affinity for all sst subtypes except sst4 (table 6) [59]. It is thus plausible to assume that SOM230 might be much more effective in the treatment of GO. If so, this might be especially relevant for the treatment of GO in children, in whom one might be reluctant to administer high doses of glucocorticoids (in view of the adverse effects of longitudinal bone growth) or retrobulbar irradiation (in view of the theoretical risk of tumor induction).

Conclusions

Children have about the same risk (or slightly increased) as adults to develop GO once they have contracted Graves' hyperthyroidism. The severity of

childhood GO appears to be less than that of adulthood GO. The female preponderate is similar between children and adults with GH (87 and 83%, respectively), but the prevalence of smoking is much lower in children than in adults (4 and 47%, respectively). Smoking is a risk factor for GO, and the odds increase significantly with increasing severity of GO. It has also been shown that the manifestation of GO begin to resemble more closely the adult findings when adolescence approaches. This could be explained by increasing smoking prevalence with age. Our recent study supports the above data and provides a very interesting clue: the difference might be caused by exposure to tobacco smoke.

Regarding treatment of TED in childhood, most physicians who are dealing with such cases prefer the 'wait-and-see' policy. Indeed, in our recent study 70% of the respondents recommended such a policy for the eye changes. Pharmacological intervention, predominantly with steroids is considered appropriate in case of worsening of eye changes or no improvement of eye changes when the patient has become euthyroid. Doses between 5 and 20 g prednisone daily are used depending on the severity of the case. It has to be kept in mind that prolonged prednisone administration, which should be used in some severe cases of TED, is associated with weight gain, immune suppression and growth failure in children. Retrobulbar irradiation has no place in the treatment of juvenile GO in view of the theoretical risk of tumor induction.

SM, a peptide inhibiting the release of GH, is present and plays an inhibiting role in the regulation of several organ systems in men and other species. Various SM-as have been developed and used in clinical practice because the short half-life of SM makes it unsuitable for routine treatment. Recently, it has been shown that SM-as might be of therapeutic value in the treatment of active TED in adults. However, most of the initial studies were uncontrolled, not randomized, and included only small number of patients. Very recently four double-blind, placebo-controlled clinical studies were published, which have demonstrated only a modest improvement in proptosis and lid fissure width. However, it is encouraging that some benefit may be derived from SM-as. The current range of SM-as drugs target two of four somatostatin receptors present in orbital fibroblast and two of five receptors found in the lymphocytes of TED patients. Therefore, there is a reason to believe that newer generations of SM-as that target a wider range of somatostatin receptors may show markedly superior results in the treatment of TED. SOM230 is a SM-a, that is still being tested, which targets a greater range of somatostatin receptor seen in TED patients. Currently, the available assortment of SM-as should be considered in those patients with persistent proptosis that is unresponsive to other therapies. The generally mild variety of adverse effects that SM-as elicit indicates that concurrent use with other therapies may be palatable from the patients' perspective, even though current benefits are small.

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Differentiated Thyroid Carcinoma in Pediatric Age

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Epidemiology

Differentiated thyroid carcinoma (papillary and follicular thyroid carcinoma) is rare during childhood and adolescence. It comprises 90–95% of all pediatric thyroid cancers; medullary thyroid carcinoma is present in 5–8%, and undifferentiated anaplastic carcinoma is extremely rare. The annual incidence of differentiated thyroid carcinoma in children below 16 years of age is between 0.02 and 0.3 cases per 100,000, whereas the annual incidence per 100,000 in the general population ranges from 1.2 to 2.6 in men and from 2.0 to 3.8 in women [1]. In large retrospective surveys of differentiated thyroid carcinoma, 72 of 1,500 cases (4.8%) occurred in children of ≤16 years at the Institut Gustave-Roussy in Villejuif [2], and 140 of 1,599 cases (8.8%) in children of ≤19 years at the M.D. Anderson Cancer Center in Houston [3]. Most affected children are older than 10 years, and the occurrence of differentiated thyroid cancer below the age of 10 years is exceptional [1, 4]. Although juvenile thyroid cancer is rare, it accounts for about 35% of all carcinomas in children [5].

In the USA about 350 subjects younger than 20 years are diagnosed with thyroid carcinoma every year [5].

Differentiated thyroid carcinoma is in general 2–4 times more frequent in females than in males [1], but the sex difference in frequency is less marked in children below the age of 10 years [3, 4]. Age-specific incidence rates diverge for males and females starting at the age of 10 years, and increase substantially for females from age 13–14 years [5–7].

Over the past 60 years pediatric thyroid cancer incidence has had two distinct peaks [4]. The first occurred around the mid-20th century and was due to irradiation of benign conditions like tinea capitis, acne, chronic tonsillitis and

thymus enlargement. Thyroid cancer incidence rates decreased when external neck irradiation for benign conditions was abandoned in view of its recognized causal relationship [8]. The second peak occurred in the early 1990s caused by environmental contamination with radioactive iodine from the 1986 Chernobyl nuclear power plant catastrophe, reaching its maximum in the mid 1990s [9]. Thyroid cancer developed mainly in children <5 years at exposure, with onset before the age of 14 years. Girls were at greater risk than boys, with a 30-fold increase of thyroid cancer. Others also observed that children under 5 years of age at the time of exposure are the most vulnerable to the effects of ionizing radiation, girls more so than boys [10]. This may be due to age- and sex-related differences in metabolic activity of the thyroid gland: follicles less than 100 μm in size are presumably active and more prevalent in children <12 years old, whereas follicles >200 μm considered to be hypofunctional are more frequent in adults up to the age of 40 years [11]. A comparative study on differentiated thyroid carcinoma among children and adolescents living in either Belarus or France/Italy demonstrated that the post-Chernobyl thyroid carcinomas in Belarussian children were less influenced by sex, occurred in younger children, had greater aggressiveness at presentation, were more frequently papillary, and were more frequently associated with thyroid autoimmunity than the naturally occurring thyroid carcinomas in French and Italian children [12].

Thyroid cancer can occur after other childhood malignancies that involve radiation to the neck region, including tumors of the central nervous system, acute lymphoblastic leukemia, non-Hodgkin lymphoma, Ewing's sarcoma and Wilms' tumor [13]. The median latent interval between therapeutic irradiation for childhood malignancy and diagnosis of thyroid cancer is 13 years (range 6–30 years) [14]. Total body irradiation before allogeneic bone-marrow transplantation carries also a risk for thyroid cancer [15]. The risk of thyroid cancer after childhood exposure to thyroid irradiation increases with doses up to 20–29 Gy (odds ratio 9.8, 95% CI 3.2–34.8) [16]. At dosis >30 Gy a fall in the dose-response relation is seen, consistent with a cell-killing effect. Both increased and decreased risks are more pronounced in children diagnosed with a first primary malignant disease before age 10 years than in those older than 10 years.

In approximately 5% of children there is a family history of papillary thyroid carcinoma. In some families this is related to adenomatous polyposis or Cowden's disease, but in other families there are no associated lesions.

Pathology

Combining three large surveys of differentiated thyroid carcinoma in children and adolescents, 107 of the 137 cases had papillary carcinoma (78%) and

30 had follicular carcinoma (22%) [1, 17, 18]; these figures are remarkably similar to 81% papillary and 19% follicular carcinomas among differentiated thyroid cancers in the general population [1, 3]. The data do not support a higher prevalence of papillary thyroid carcinoma in children than in adults, as stated by some authors [19].

Papillary thyroid carcinomas from children and adolescents contain more numerous lymphocytes than those from adults: nearly half contain CD4+ T helper cells, CD8+ killer cells or CD19+ B cells [20, 21]. This may be related to the more favorable prognosis of differentiated thyroid cancer in children and adolescents than in adults, in line with the notion that the immune response to thyroid cancer appears to be important in preventing metastasis and recurrence. Pediatric papillary thyroid carcinomas with the most numerous proliferating lymphocytes have indeed the longest disease-free survival [20]. Consistent with this effect is the greatest risk of recurrence in those pediatric papillary thyroid carcinomas which intensely express the B7–2 coactivator: B7–2 suppresses T cell growth by binding to the CTLA-4 receptor on T cells [22].

Differentiated thyroid carcinomas in general have a lower expression of the sodium iodide symporter (NIS) than normal thyrocytes, but this appears less so in childhood: NIS expression is absent or subnormal in about 90% of adult patients, in contrast to undetectable NIS expression in about 60% in patients $<$20 years of age [11, 23]. The greater NIS expression in juvenile than in adult cancer implies greater differentiation and radioiodine responsiveness at a younger age; indeed recurrence risk in young patients is lower in NIS-positive than in NIS-negative tumors [23].

Tumorigenesis of thyroid carcinomas is explained mainly by two mechanisms: activation of proto-oncogenes (e.g. the *RET* gene in papillary thyroid carcinoma) and inactivation of tumour suppressor genes (e.g. *p53* and *PTEN* in follicular thyroid carcinoma). Pediatric differentiated thyroid carcinoma differs in many aspects from carcinomas in adults: in children, the cancer has a larger size and is already more widespread at presentation than in adults (vide infra). The difference calls for a biologic explanation. *RET* mutations can initiate papillary thyroid carcinoma, and they occur nearly always already in childhood; these mutations are less likely to be transmitted to later generations of cells after puberty in view of the early expiration of the potency of thyrocytes to divide [4]. Thus the papillary carcinomas with the fastest onset become detectable in children.

Many studies have looked after molecular-biologic differences between pediatric and adult thyroid cancers. In papillary thyroid carcinoma, mutations in *RET, NTRK, BRAF* (and rarely *RAS*) activate the *MAP* kinase cascade, resulting in increased transcription of growth and proliferation genes and thereby initiating tumorigenesis. *RET* rearrangements result from the fusion of the *RET* tyrosine

kinase domain with the N-terminus part of different proteins, creating chimeric oncogenes with constitutive activity, named *RET/PTC*. At least 15 different *RET/PTC* variants have been described so far involving rearrangements with 10 different genes. A higher frequency of rearrangement of the *RET/PTC* oncogenes [24–26] and lower frequency of *BRAF* mutations [27] have been observed in childhood than in adult papillary thyroid cancer, but these data have not been confirmed by others [28, 29]. The higher frequency of *RET* rearrangements in radiation-induced cancer may be linked to the particular effectiveness of radiation in causing double-strand breaks (and thereby in gene rearrangements) rather than point mutations [30]. *RET-PTC* and *BRAF* mutations are mutually exclusive in papillary carcinomas, both activating constitutively the *RET/PTC-RAS-BRAF-MAP* pathway. Gene expression in post-Chernobyl cancer is similar to that in sporadic papillary carcinoma as analysed by cDNA and Affymetric microarrays [30]. Radiation-induced thyroid cancers and sporadic papillary carcinomas thus most likely represent the same disease. A relationship between *RET* and *NTRK* positive cases and more advanced disease or worse prognosis is found in some [24] but not all studies [26, 29]. Likewise, increased expression of the tyrosine kinase receptor cMET and its ligand hepatocyte growth factor/scatter factor in papillary thyroid carcinoma in children and young adults is associated with a high risk for metastasis and recurrence [31], but later studies observed overexpression of *MET* in the majority of papillary thyroid carcinomas [4, 32]. *RAS* and *PPARG* are involved in follicular thyroid carcinogenesis, and it has been claimed that *PPARG* rearrangement is more frequent in cancers at a younger age [33]. Taken together, it is clear that much still has to be learned on the biology of these tumors in order to fully understand differences in the clinical course of these tumors between pediatric and adult age.

Clinical Presentation

The most common clinical presentation of childhood thyroid cancer is a palpable thyroid nodule; it is the first sign of the disease in 73–87% of the cases [8, 18]. Most thyroid cancers in children are asymptomatic, but palpable thyroid nodules are more frequently malignant in children than in adults [19, 34]. As with adults, hoarseness, dysphagia or a hard fixed nodule may be indicative of an underlying thyroid malignancy. Fine-needle aspiration cytology of the nodule should confirm the diagnosis. The size of newly diagnosed papillary thyroid tumors in childhood is larger than in adulthood: a size of >4 cm is found in 36% of children vs. 15% of adults, and a size of <1 cm occurs in 9% of children vs. 22% of adults [35]. Invasion of contiguous structures in papillary thyroid carcinoma is also more frequent in children than in adults (24 vs. 16%) [35].

Neck node involvement is quite common in childhood papillary thyroid carcinoma, in the order of 60–90% [2–4, 8, 35]; palpable cervical lypmphadenopathy occurs usually in the presence of a palpable thyroid nodule, whereas palpable lymph nodes in the absence of a palpable thyroid nodule is uncommon [18]. Neck node involvement is much more frequent in children than in adults. Among 1,039 consecutive patients with papillary thyroid carcinoma treated in the Mayo Clinics, nodal metastases were present in 90% of children vs. 35% in adults, and the same was true for distant metastases (7% in children vs. 2% in adults) [34, 35]. Similar findings have been reported in other large series [2, 3]. The distant metastases occur almost always in the lungs; they are rare outside the lungs. In contrast to adult lesions, pediatric pulmonary metastases are overwhelmingly miliary and seldom nodular; they may not be detected on standard chest radiographs or even on spiral computed tomography scans, becoming visible only at postablation ^{131}I whole-body scans [36–39]; they are almost always functional [4].

It might well be that children with differentiated thyroid carcinoma nowadays present with less advanced disease than in the past, possibly reflecting increased awareness on the part of pediatricians and family physicians [34]. Nevertheless, one must conclude that differentiated thyroid carcinoma in children and adolescents is associated with a much higher frequency of cervical lymph node and distant (pulmonary) metastases at clinical presentation than in adults. The paradox of this more widespread disease in children is its association with a better prognosis than in adults (vide infra).

Management

The goals of primary treatment of differentiated thyroid carcinoma are to eradicate disease and extend recurrence-free survival [4]. Childhood differentiated thyroid carcinoma is, however, a rare disease, and it may take decades even at large referral centers to accumulate large series from which meaningful conclusions on the most appropriate treatment regimen can be derived. No randomized controlled trials are available. Guidelines consequently rely on adult and more specifically pediatric outcomes literature, which has been summarized in two recent publications [4, 34].

Thyroidectomy

The general consensus is that total or near-total thyroidectomy is the best operation in experienced hands. Reasons to perform a complete thyroidectomy are first the high prevalence of multifocality and bilaterality in papillary thyroid carcinoma, due to intrathyroidal lymphatic spread or de novo tumors

Table 1. Predictors of recurrence-free survival in 274 patients with differentiated thyroid carcinoma (103 children ≤18 years old and 171 adults 19–28 years old) [4, 40]

Predictor	RR (95% CI)	p value*
Age at diagnosis (19–28 vs. ≤18 years)	0.99 (0.92–1.0)	NS
Gender (male vs. female)	0.97 (0.38–2.4)	NS
Histology (follicular vs. papillary)	0.51 (0.23–1.1)	NS
Lymph node metastases (present vs. absent)	3.1 (1.3–7.2)	0.027
Thyroidectomy (less than total vs. total)	6.2 (2.8–13.7)	<0.001
Radioiodine ablation (no vs. yes)	5.8 (2.4–14.1)	<0.001

*Cox multiple regression analysis.

arising in a synchronous or metachronous (possible due to *RET/PTC* rearrangements) fashion [34]. A second compelling argument is the longer recurrence-free survival after total vs. less than total thyroidectomy (table 1) [4, 40]. Completion thyroidectomy has been associated with lower mortality rates in adults with papillary thyroid carcinoma and children and adolescents with radiation-induced papillary thyroid carcinoma as well [41]. Less extensive surgery has been supported by the outcome of an American multi-institutional cohort of 329 patients diagnosed when <21 years old: progression-free survival did not differ in relation to the extent of surgery [42]; however, total thyroidectomy was more often applied to later-stage patients, jeopardizing the claim of no benefit from more intense treatment [4]. Lobectomy for microcarcinomas (<1 cm) remains a controversial issue, and is better avoided in radiation-induced cancer.

Thyroidectomy should be accompanied routinely by en bloc dissection of the central neck compartment with clearing of lymphatic and soft tissue. Modified lateral neck dissection is advocated in case of metastases to lateral lymph node compartments (as diagnosed clinically, by ultrasound or intraoperative biopsy). Mere 'berry picking' does not alter long-term survival, and may actually increase the risk of nodal recurrence [34]. There seems never a need for radical neck dissection in a child with papillary thyroid carcinoma [43]. Care should be taken to protect the laryngeal nerves and the parathyroid glands; devitalized parathyroids must be autotransplanted.

Thyroid Remnant Ablation

Meaningful ^{131}I uptake (>0.3% at 24 h) by thyroid remnants can usually be demonstrated even after the most meticulous 'total thyroidectomy'. Reasons to

apply routine ^{131}I remnant ablation are: (1) a longer recurrence-free survival in comparison with no ablation (table 1); (2) increased sensitivity of subsequent diagnostic ^{131}I whole-body scans to detect (pulmonary) metastases; (3) render serum thyroglobulin (Tg) a highly sensitive marker for residual recurrent disease during long-term follow-up [4, 40, 44]. Consequently, radioiodine remnant ablation in children is the rule rather than the exception at most centers worldwide. However, some authors advocate a more conservative approach, restricting the procedure to selected high-risk patients [34]. Most children should be included in the high-risk group in view of the frequent extrathyroidal invasion, lymph node metastases and distant metastases, but most staging systems because of the good overall survival of children will classify them as stage I and only as high-risk stage II in case of distant metastases [4]. A recent paper on 60 children and adolescents with differentiated thyroid carcinoma reinforces the benefits of radioiodine remnant ablation: local relapse was reduced from 42% to 6.3% when ^{131}I was administered postoperatively, 10-year locoregional failure-free survival (in children without distant metastases at diagnosis) was 86.5 vs. 71.9% without ablation ($p = 0.04$), and distant failure-free rate was 100 vs. 94% without ablation (not significant) [45]. According to a recent meta-analysis, remnant ablation improves outcomes in patients with differentiated thyroid carcinoma of all ages by reducing locoregional and distant recurrence risk [46].

Current recommendations are to perform ablation 6 weeks after surgery. Children are placed on T3 1 μg/kg/day in two or three divided doses for the first 4 weeks, followed by a 2-week period of withdrawal [44]. By doing so, serum TSH will rise to levels of >25 mU/l allowing maximal radioiodine uptake by the thyroid remnant. Recent studies suggest that adequate hyperthyrotropinemia can be reached in 14 days after total thyroidectomy. In adult patients serum TSH concentrations of >30 mU/l were reached in 74% after 9–11 days, in 93% after 15–17 days, and in 98% after 22 days after total thyroidectomy; these figures were 16, 65 and 97%, respectively, after withdrawal of suppressive T4 therapy [47]. Compared with adults, T4 clearance rates and serum TSH to free T4 ratios are higher in children, implying the possibility of shorter T4 withdrawal periods. Indeed in children on suppressive T4 therapy (mean TSH 0.26 mU/l, range 0.01–1.37 mU/l) the mean interval to reach a serum TSH >30 mU/l after thyroxine withdrawal was 12.4 ± 0.8 days; serum TSH >25 mU/l was documented in all patients by day 14 of withdrawal [48]. In this study, T4 was stopped on day −14, low-iodine diet was instituted as of day −7, TSH was measured on days −14, −7 and −1, a diagnostic whole-body scan with ^{123}I was done on day 0, dose determination on day 1, and the therapeutic ^{131}I dose was given on day 2. A low iodine diet – at least in adults – improves the efficacy of thyroid remnant ablation [49]. The diagnostic whole-body scan just prior to remnant ablation should employ 300–400 mCi ^{123}I or 0.5–2.0 mCi ^{131}I; higher doses of ^{131}I might

be associated with thyroid stunning, i.e. a lower uptake of a subsequent (therapeutic) dose of ^{131}I [44]. The ablation dose in adults varies between 25 and 100 mCi ^{131}I. A large randomized clinical trial in 509 patients (mostly adults but also including children) concludes that doses between 25 and 50 mCi are equally effective for remnant ablation, which was successful in 82%. In pediatric patients thyroid remnant ablation is successful in the majority after a single dose of 30 mCi ^{131}I [34, 44], but others use higher doses of ~60 mCi in view of the high frequency of locally advanced disease and distant metastases in children [4]. Still others use body weight-based formulas, like 1 mCi/kg with a range of 0.5–2 mCi/kg [2, 44]. Most institutions treat pediatric patients with fixed empiric doses of ^{131}I, and do not apply dosimetry to determine minimally effective doses [34]. But all centers agree to perform a postablation or posttherapy whole-body scan 5–7 days later, especially to detect pulmonary metastases. In a study of 28 children and adolescents with pulmonary metastases, whole-body scan revealed the pulmonary metastases in all patients but chest X-rays only in 7 cases (25%); 18 of the 21 children with normal chest X-rays underwent chest CT scan, which detected micronodular pulmonary shadows only in 5 children (28%) [51].

Follow-Up

Following radioiodine remnant ablation, patients are placed on TSH-suppressive doses of levothyroxine aiming at serum TSH levels of ≤0.1 mU/l. In patients with low risk papillary thyroid carcinoma and no evidence of remaining disease the target could be TSH values between 0.1 and 0.4 mU/l for several years, followed by replacement doses of levothyroxine [34, 44]. These recommendations have been extrapolated from adults to children and adolescents because scientific data at the pediatric age are lacking. High risk patients should be maintained at TSH levels of ≤0.1 mU/l, but children may suffer from headaches, insomnia and attention deficit disorders which should be taken into account in delineating the levothyroxine dose. Children require higher L-T4 doses per kg body weight to reach TSH levels of ≤0.1 mU/l: 3–4 μg/kg/day in children below the age of 10 years, but at the age of 16–18 years 2.4–2.8 μg/kg/day may be sufficient [19]. Growth rate and puberty are usually normal, with the expected height reached at adult age.

The success of radioiodine remnant ablation is judged about 6 months later by a diagnostic whole-body scan (uptake should be ~<0.1%) or increasingly by TSH-stimulated serum Tg (Tg should be undetectable). The protocol for a diagnostic whole-body scan after T4 withdrawal has been given above [48]. Prolonged T4 withdrawal is often poorly tolerated by children, and for this reason the use of recombinant human TSH (rhTSH) may be particularly beneficial. rhTSH is licensed in Europe and the USA as an adjunct to diagnostic whole-body scan or serum Tg testing and (in Europe only) as an adjunct to radioiodine

ablation, but in both settings the licensing covers only adults; thus, rhTSH administration in children is 'off-label' [4]. rhTSH has been successfully used in a limited number of children so far [4, 52]. Serum peak TSH levels after rhTSH are negatively related to body surface area ($r = -0.72$, $p < 0.0001$), implying the need for a personalized rhTSH dose [53]. Mean TSH levels achieved in children after rhTSH, however, appear to be remarkably similar to values previously reported in adults [54]. The data suggest that no alterations in dose (0.9 mg intramuscularly on two consecutive days) may be necessary when rhTSH is used in children and adolescents.

Neck ultrasonography should be included in the follow-up, as it can detect lymph node metastases that are not suspected by palpation, diagnostic whole-body scan, or serum Tg determination [55]. When no evidence of still existing disease is found at 6 months using palpation, neck ultrasonography, whole-body scan and serum Tg, the patient can be followed under a lower levothyroxine dose. Serum Tg under levothyroxine treatment and neck ultrasonography should be repeated every year, and with longer time intervals after 'no evidence of disease' status for 2 years. Follow-up should probably be life-long.

Neck lymph node metastases are approached surgically, in which the extent of excision depends on the extent of the disease; complete resection of neoplastic foci is obtained in the majority of patients [19]. Microscopic neck metastases can be treated with ^{131}I. ^{131}I treatment should always be administered for inoperable functional distant metastases. Pulmonary metastasis are typically treated with 175–200 mCi ^{131}I. Others apply 1 mCi/kg body weight to be repeated every 6 months until the posttreatment scan no longer shows any uptake. This schedule diminishes the risk on pulmonary fibrosis, and after four to six courses of ^{131}I 80% of children seem to be cured [19]. Therapy is carried out following thyroid hormone withdrawal on a low-iodine diet [4, 34, 44]. Total cumulative doses of ^{131}I should be kept below 500 mCi in children and 800 mCi in adolescents. All care is best delivered by a multidisciplinary specialized team.

Prognosis

In 1994, Mazzaferri and Jhiang [56] already noticed a very high recurrence rate but low mortality rate in children and adolescents with differentiated thyroid carcinoma. This was confirmed by Samaan et al. [3] in 1992 in a comparative study on 140 patients below 20 years of age and 1,459 patients of ≥20 years with differentiated thyroid carcinoma. In both groups the frequency of papillary carcinoma (86 vs. 80%), thyroidectomy (73 vs. 65%) and ^{131}I therapy (48 vs. 45%) was similar, but extrathyroidal spread was more prevalent in the younger age group (74 vs. 57%). Recurrences were more frequent at age

<20 years than in the older group (37 vs. 22%), but mortality was lower (3.6 vs. 11.3%). The number of actual recurrences in the children was higher than expected (48 vs. 30, $p < 0.001$) but not so in the adults (301 vs. 319), and the number of actual deaths in the children was lower than expected (5 vs. 18, $p < 0.001$) but not so in the adults (166 vs. 153). The median follow-up in this study was 11 years, with a range of 1–43 years.

Although the above findings are reconfirmed by many other smaller series, the low mortality rate of pediatric differentiated thyroid carcinoma might to some extent reflect relatively short follow-ups compared with patients' lifespans [4]. Most reports have a median follow-up of ≤15 years, but cause-specific deaths may occur after longer time intervals [57]: e.g. mortality was 10% in 34 patients followed up for ≥20 years [6], and 15% of 40 patients diagnosed at age <12 years died after 12–33 years [2]. The relatively short follow-up also may lead to underestimation of the recurrence rate. The median time to recurrence is 7 years, but events occur up to 44 years after presentation [58, 59]. In the historical series of the Royal Marsden Hospital the median overall survival was 53 years; presentation with distant metastases predicted poorer survival, and recurrences had also a higher risk of death with a median survival of 30 years [58]. A 100% survival at 10 years' follow-up seems to be the rule rather than the exception. Disease-free survival at 5 and 10 years follow-up is 80 and 61%, respectively [40]. The majority of children with lung metastases achieve complete remission, and even partial responders rarely progress [2, 36, 40]. Over a 20-year follow-up, few if any cause-specific deaths were noted in pediatric patients with lung metastases, in contrast to the 10-year mortality rate of 30–60% in adults with lung metastases [4, 59].

The risk of developing recurrent disease is increased by lymph node metastases at presentation, less than total thyroidectomy, and no radioiodine ablation as observed in many studies (table 1). In the recent multivariate regression analysis by Jarzab et al. [4] presented in table 1, age is not an independent risk factor in this respect. In contrast, age is a major determinant of recurrence risk in many other reports [19, 40, 43, 45, 58]: e.g. 20-year recurrence-free interval was 10% in patients aged <10 years and 48% in patients aged 10–18 years at diagnosis [60]. Among 137 cases of papillary thyroid carcinomas ≤21 years of age with a median follow-up of 6.6 years, univariate analysis demonstrated recurrence to be more common in patients with multifocal disease (OR 7.5) or large tumors >2 cm (OR 4.1), and in those with palpable cervical lymphadenopathy (OR 3.0) or distant metastases at diagnosis (OR 2.8); by multivariate analysis the only significant predictor of recurrence was multifocality, which was also true for the 38 patients with follicular carcinoma (OR 22) [61].

Most of the outcome studies described so far, refer to patient series collected in the distant past. The outcome of patients who were diagnosed more

Table 2. Differentiated thyroid carcinoma in children and adolescents: clinical presentation, initial treatment and long-term outcome in studies reported after 2000 [18, 62–67]

Clinical presentation
Median age 16 years
73% females, 27% males
89% papillary, 11% follicular carcinomas
26% extrathyroidal invasion
53% lymph node metastases
16% lung metastases
Initial treatment (n = 308)
80% (near) total thyroidectomy
64% ^{131}I ablation
Long-term outcome (n = 281)
Median follow-up 65 months
22% recurrences
0.3% mortality
16.4% residual disease
83.3% disease-free

recently might be different in view of the recommendations to perform (near) total thyroidectomy and radioiodine ablation postoperatively. This was evaluated by summarizing seven studies on children and adolescents with differentiated thyroid carcinoma published after 2000 (table 2) [18, 62–67]. Sex distribution and histology type are in agreement with previous series. At clinical presentation, the cancer was already widespread as evident from a high frequency of extrathyroidal invasion, lymph node and distant metastases, again confirming more advanced disease in children with differentiated thyroid carcinoma than in adults. Remarkably in comparison with past figures is the higher frequency of near-total, total or completion thyroidectomy (80%) and of direct postoperative ^{131}I therapy for thyroid ablation and metastases (64%). The outcome after a median follow-up is reassuring: only one child died and 83% had become disease-free. It can be concluded that the prognosis of children and adolescents with differentiated thyroid carcinoma is in general rather good.

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Imaging of the Normal and Affected Thyroid in Childhood

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Imaging has undergone major advances over the past three decades and has revolutionized the evaluation of patients with thyroid disease. However, the use of thyroid imaging is in general not evidence-based, and there have been few cost-benefit evaluations of medical imaging [1].

The thyroid gland can be evaluated by several imaging techniques: (1) radionuclide imaging, and (2) nonisotopic imaging comprising (a) ultrasonography (US); (b) computed tomography (CT), and (c) magnetic resonance imaging (MRI). A recent development has been the combination of PET (positron emission tomography) and CT for oncologic imaging. Each has advantages and limitations, and there is no absolute clinical indication for performing any of them in the majority of patients [1, 2]. In this chapter emphasis will be on the clinical use of US in childhood.

Radionuclide Imaging

In regions with adequate dietary iodine intake, the 24-hour uptake of oral radioiodine is 10–35%. The trapping mechanism is the sodium-iodide symporter (NIS), which is regulated by thyrotropin (TSH) [3]. There are more than 20 radionuclides of iodine, but only ^{123}I and ^{131}I are in widespread clinical use. ^{123}I has a relatively short half-life and emits only γ photons and is used for thyroid uptake measurements and scintigraphy. In contrast, ^{131}I has a half-life of 8 days and emits β particles as well as high-energy γ photons. ^{131}I is therefore suited for therapy, but the γ photons can be imaged, which explains why ^{131}I is used for diagnostic and post-treatment whole-body scanning in patients with thyroid

cancer. The use of ^{131}I for routine thyroid scintigraphy is discouraged because the radiation dose is about 100 times greater than that of ^{123}I. Based on low cost, availability and an even lower radiation dose, technetium (^{99m}Tc) pertecnetate is an attractive alternative to ^{123}I and consequently recommended for routine thyroid imaging by authorities in most European countries. ^{99m}Tc is administered intravenously, and uptake and scan are obtained after 15–20 min. In children the radiation exposure to the gland is three- to fivefold higher than in adults.

For routine imaging a gamma camera with a pin-hole collimator is most often used. The patient lies in the supine position with the neck extended. Markers can be used to identify anatomic sites, such as the manubrium, or can be placed at the edge of a palpable nodule. Additional SPECT (single photon emission computed tomography), where the camera head rotates 180–360° around the patient, improves resolution and can provide volumetric estimates, but is not performed routinely [4, 5].

Thyroid uptake is influenced by the serum inorganic iodine level, which is dependent on the intake of iodine. A number of factors can influence the uptake. Thus, it is generally increased in hyperthyroid patients with Graves' disease or toxic nodular goiter, and decreased in patients with subacute or silent thyroiditis as well as in those with hypothyroidism (table 1) [6].

Indications for Thyroid Uptake and Imaging

When patients are referred for uptake and/or scan, it is important to ensure that they are not taking thyroid hormone. However, in congenital hypothyroidism, L-thyroxine therapy need not be delayed while awaiting scintigraphy, since scintigram validity depends on a normal or elevated TSH level, which is the case for many days after onset of treatment, during which time scintigraphy can be performed. It is also important to avoid the ingestion of excess iodine, and to secure that female adolescents are not pregnant.

Measurements of thyroid uptake and imaging give valuable information in several clinical situations (table 2). A known activity of tracer is administered orally, and the percentage accumulated at designated times is measured using either a probe or a gamma camera. It is almost standard procedure to obtain a 24-hour measurement, but the early 4- to 6-hour measurements allows the clinician to identify a thyroid with rapid turnover. Some obtain only an early measurement and by extrapolation calculate the 24-hour value [7]. The uptake is often used to determine therapy doses of ^{131}I to treat patients with Graves' disease or toxic nodular goiter [8].

Only few studies, all retrospective, that describe the role of scintigraphy in the evaluation of the spectrum of pediatric thyroid disorders, have been published. In one study, comprising 280 children, indication for scintigraphy included hypothyroidism, neck masses, and hyperthyroidism [9] and was

Table 1. Factors that influence thyroid radioiodine uptake

Causes of increased uptake
Hyperthyroidism
Iodine deficiency
Rebound after withdrawal of antithyroid medication
Rebound after suppression of thyrotropin
Recovery phase of subacute, silent, or postpartum thyroiditis
Inborn errors of thyroid hormogenesis (apart from trapping defects)
Pregnancy (use of radiopharmaceuticals contraindicated during pregnancy)
Lithium carbonate therapy
Some patients with Hashimoto's thyroiditis
Causes of decreased uptake
Primary hypothyroidism
Destructive thyroiditis (subacute thyroiditis, silent thyroiditis, postpartum thyroiditis)
Thyroidectomy, ^{131}I treatment or external neck irradiation
Thyroid hormone
Antithyroid drugs
Excess iodine, including dietary supplements with iodine
Radiological contrast media
Amiodarone
Topical iodine
Perchlorate, thiocyanate
Sulphonamides, sulphonylurea
High-dose glucocorticosteroids

Table 2. Indications for thyroid uptake and imaging

Indications for measuring thyroid uptake
Confirm the diagnosis of hyperthyroidism
Differentiate different types of thyrotoxicosis
Provide data for calculation of a therapeutic dose of ^{131}I
Detect intrathyroidal defects in organification
Follow-up of patients treated for thyroid cancer
Indications for thyroid scintigraphy
Depict structure and function of the thyroid
Differentiate different types of thyrotoxicosis
Determine whether a nodule is functioning
Determine whether a cervical or mediastinal mass contains functioning thyroid
Identify ectopic thyroid
Aid in the diagnosis of congenital hypothyroidism
Identify thyroid metastases
Determine whether ablation therapy of thyroid cancer has been successful

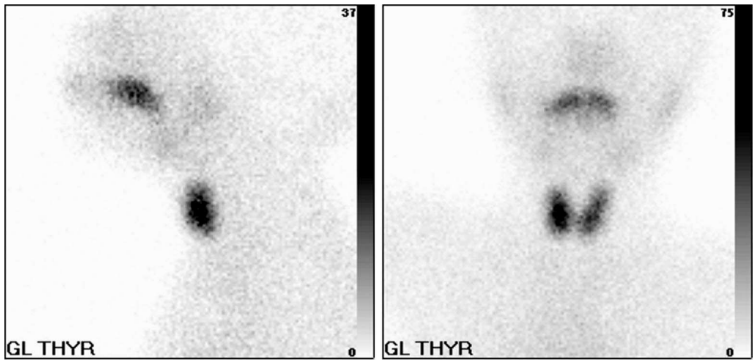


Fig. 1. Normal thyroid gland in a 4-year-old girl 15 min after intravenous administration of 50 MBq ^{99m}Tc pertechnetate. Left panel: lateral view; right panel: anterior view.

considered helpful in the work-up. Scintigraphy added little to the management of children with post-irradiation hypothyroidism, Hashimoto's thyroiditis, or Graves' disease, when the clinical diagnosis was straightforward.

The Normal Thyroid Scintigraphy

The thyroid gland is located in the antero-inferior part of the neck (infrahyoid compartment) (fig. 1). There are two lobes and an isthmus. 10–40% have a small pyramidal lobe, arising from the superior part of the isthmus, which is occasionally identified on scintigraphy.

Congenital Defects

Congenital defects include anatomic and inherited disorders [10]. Anatomic defects comprise agenesis (fig. 2), hemiagenesis and maldescent of the gland, which is positioned along the tract of the thyroglossal duct. Rarely, congenital cysts of the thyroid are seen. The clinical consequences are highly variable, from severe hypothyroidism due to thyroid agenesis to moderate hypothyroidism due to ectopic (usually lingual) (fig. 3) thyroid rudiments or thyroid hypoplasia (fig. 4) and, finally, to subclinical hypothyroidism (high serum TSH with normal serum free T4 and free T3 concentrations) in patients with thyroid hemiagenesis. Imaging is valuable in defining agenesis of the thyroid. On ^{99m}Tc scintigraphy, the thyroid is not identified, but there is uptake by salivary glands [11]. Anatomic defects such as hemiagenesis are infrequently identified because they are rare and seldom result in subclinical or clinical hypothyroidism. Rare cases of coexisting hyperthyroidism [12], including TSH

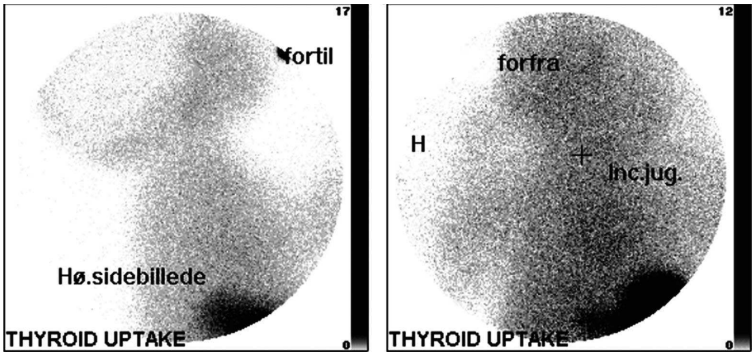


Fig. 2. ^{99m}Tc pertechnetate thyroid scintigraphy demonstrating absence of thyroid uptake in a 10-day-old girl with congenital hypothyroidism due to thyroid agenesis (lateral and anterior view).

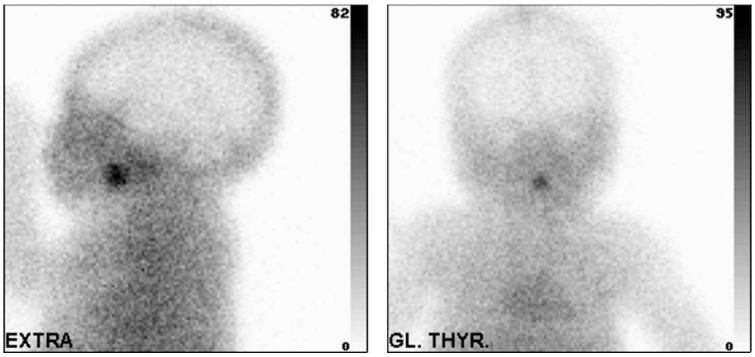


Fig. 3. ^{99m}Tc pertechnetate thyroid scintigraphy showing uptake in the lingual region in a neonate (lateral and anterior view) with congenital hypothyroidism.

receptor antibodies resulting in Graves' disease have been described [13]. Most often congenital defects are found incidentally in patients having imaging of the neck for other reasons.

The introduction of neonatal screening programs has enabled early diagnosis and treatment of infants with congenital hypothyroidism (CH) and the prevention of mental retardation [14]. Patients with CH are classified as having developmental abnormalities of the thyroid gland in 85% of the cases. These include ectopic thyroid tissue, aplasia or hypoplasia of the thyroid or a normally located gland with hypothyroidism caused by dyshormogenesis [15] (fig. 5).

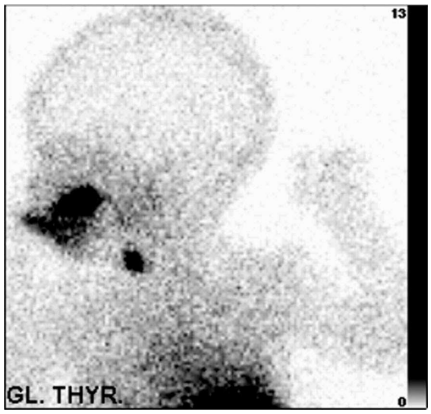


Fig. 4. ^{99m}Tc pertechnetate thyroid scintigraphy (lateral view) showing reduced uptake in a cervical thyroid in a 13-day-old boy with mild congenital hypothyroidism. Additional US demonstrated a 5×5 mm large normoechoic thyroid in the midline, not divided into two lobes – findings compatible with thyroid hypoplasia.

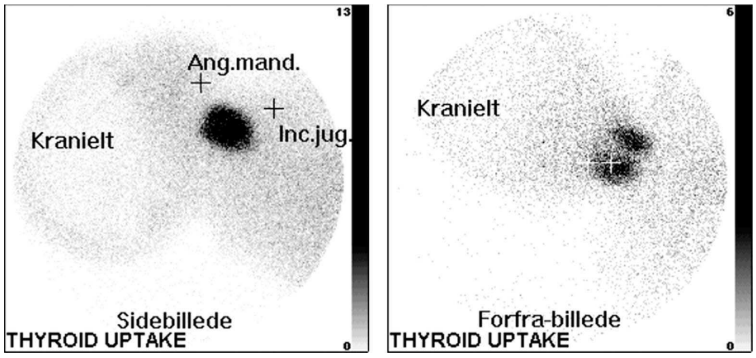


Fig. 5. ^{99m}Tc pertechnetate thyroid scan in an 11-day-old girl with diffuse goiter on palpation and congenital hypothyroidism due to dyshormogenesis. Increased and diffuse uptake in an enlarged cervical thyroid gland can be seen (lateral and anterior view).

Although thyroid imaging with ^{123}I or ^{99m}Tc has been available for decades, these techniques are not routinely used in newborn infants diagnosed by screening as having CH. Guidelines on CH have described thyroid imaging in newborns as optional [16, 17] and some argue that presence, absence, or abnormal location of a thyroid does not alter management of CH. Others believe that optimal counseling of parents, and management, implies obtaining a scintigraphy [18]. The latter authors recommend ^{123}I rather than ^{99m}Tc in cases of CH, arguing that ^{99m}Tc is valid only in cases of absent or normal-appearing thyroid glands and more often misdiagnoses ectopic thyroid tissue [18]. However, these results have not been confirmed by others [19, 20]. Thyroglobulin (Tg) has been

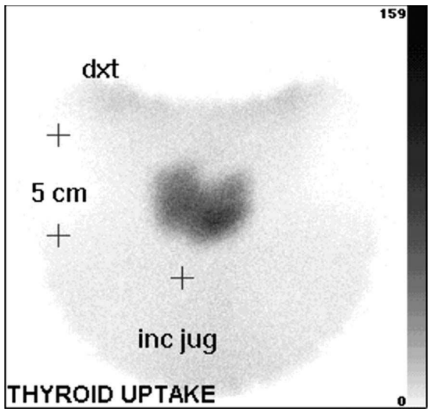


Fig. 6. ^{99m}Tc pertechnetate thyroid scintigraphy in a 7-year-old boy with congenital hypothyroidism and a palpable diffuse goiter (scan performed without preceding thyroxine withdrawal). Diffuse uptake in an enlarged gland can be seen. Two weeks later an additional perchlorate discharge test was performed: injection of 10 MBq ^{123}I showed diffuse uptake after 60 min at which time sodium perchlorate (20 mg/kg) was administered. Uptake measurements every 15 min the following hour showed increased discharge reaching a level of 43% of the maximum uptake after 30–45 min. Results are compatible with a partial thyroid peroxidase defect explaining the goiter and concomitant hypothyroidism.

found in varying concentrations in infants and children with congenital hypothyroidism. While a comparative study found that Tg was a more reliable marker for the presence or absence of a thyroid gland it cannot substitute scintigraphy as a first line diagnostic tool in the management of CH [20].

Inborn errors of synthesis of thyroid hormones can be diagnosed by clinical findings, biochemical results and uptake and scintigraphy. Future improvements in obtaining a definite diagnosis will be based on genetic testing. Absent trapping, due to mutations in the NIS gene, results in absent thyroid uptake, and additionally lack of trapping in salivary glands. A defect in thyroid peroxidase can be identified by active trapping by the thyroid and a positive perchlorate discharge test [21] (fig. 6).

An ectopic thyroid, located at the base of the tongue, is called a lingual thyroid and occurs in 1 in 100,000 persons [22]. It can be seen with or without other functioning thyroid tissue located at the usual site of the thyroid gland or anywhere else between the foramen cecum and the normal position. One third of the patients with an ectopic thyroid have hypothyroidism at the time of diagnosis [23]. In the majority it is often asymptomatic until physiologic stress, such as severe systemic disease or pregnancy [24], causes enlargement of the ectopic tissue. It may be associated with hypothyroidism, with or without thyroid

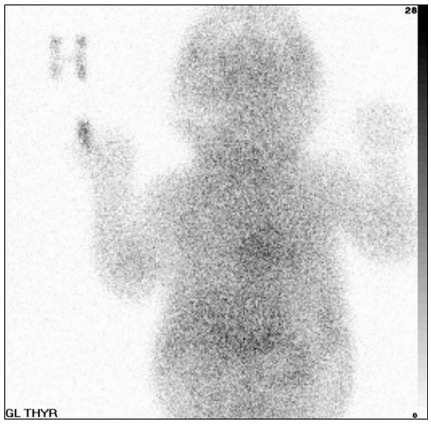


Fig. 7. ^{99m}Tc pertechnetate thyroid scintigraphy in an 11-day-old girl with a small palpable goiter and mild hypothyrodism due to maternal prenatal ingestion of excess iodine. Normal uptake in the gut and the bladder. Image cannot distinguish between thyroid aplasia and iodine contamination, but additional US demonstrated a normal thyroid gland. Thyroxine could be withdrawn 3 months postpartum.

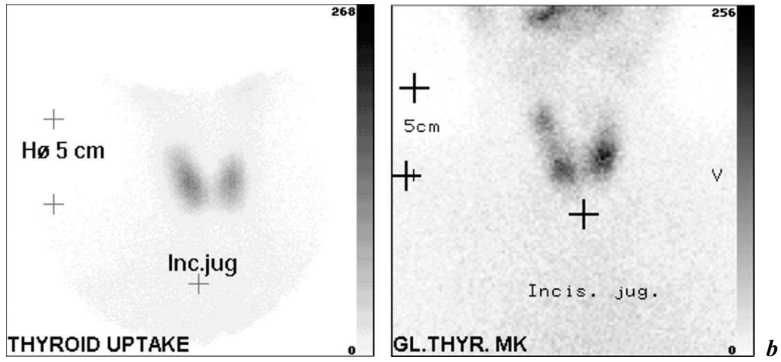
enlargement, or in case of swelling, with dysphagia, dysphonia, or dyspnea [25]. Ectopic thyroid tissue, including lingual thyroid disease, can be diagnosed efficiently by ^{99m}Tc scintigraphy [26, 27].

Congenital Goiter

Congenital goiters may be secondary to a number of diseases. Goiters secondary to enzyme deficiencies [28] (fig. 6) may be present at birth. However, most of these develop in the early months and years of extrauterine life [28]. In the absence of maternal thyroid autoantibodies, further evaluation of goiter in the newborn is based on thyroid function tests in addition to ^{123}I or ^{99m}Tc scintigraphy. Infants born to mothers with hyperthyroidism secondary to TSH receptor antibodies can have goiter [29] and do not warrant imaging. Other causes include maternal prenatal ingestion of excess iodine (fig. 7), antithyroid medication, lithium and other goitrogens [29, 30]. Scintigraphy is of limited value in these cases.

Hypothyroidism

Hashimoto's thyroiditis is the most common cause of thyroid disease in children and adolescents and also the most common cause of acquired hypothyroidism with or without goiter [31]. Often a continuum from normal to slightly reduced homogeneous distribution of tracer on thyroid scintigraphy is found (fig. 8a), unlike the heterogeneous distribution (fig. 8b) more often reported in



a *b*

Fig. 8. ^{99m}Tc pertechnetate thyroid scintigraphy in a 15-year-old girl with Hashimoto's thyroiditis (hypothyroidism and high concentrations of thyroid peroxidase antibodies) (***a***) – slightly reduced homogeneous distribution of tracer in a small thyroid gland, and a 10-year-old girl with Hashimoto's thyroiditis (elevated thyroid peroxidase antibodies and hypothyroidism) (***b***) – heterogeneous distribution of tracer in a normal-sized thyroid gland.

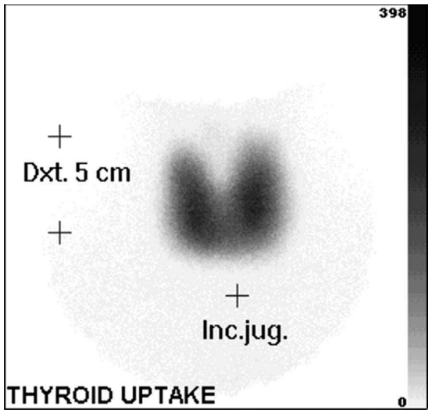


Fig. 9. ^{99m}Tc pertechnetate thyroid scintigraphy showing diffuse and increased uptake in a thyroid gland with enlarged and symmetric lobes.

adults [32]. However, in children and adolescents, thyroid scintigraphy is not helpful in the diagnosis of typical Hashimoto's thyroiditis [33].

Hyperthyroidism

In children, hyperthyroidism is a result of Graves' disease or an autonomous hyperfunctioning thyroid nodule [34]. The latter is extremely rare in childhood. Figure 9 shows the typical scintigraphic appearance of Graves'

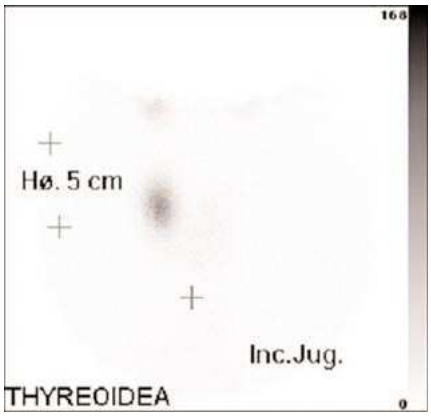


Fig. 10. ^{99m}Tc pertechnetate thyroid scan of a hyperfunctioning nodule in the right lobe with complete suppression of uptake in the remainder of the thyroid.

disease. Compared with a normal thyroid, the thyroid lobes are slightly larger in all dimensions, and the early and late uptakes are higher. Figure 10 shows an autonomous hyperfunctioning nodule with suppression of extranodular thyroid tissue. If thyrotoxicosis is confirmed biochemically, in addition to elevated levels of TSH receptor antibodies and a nonpalpable thyroid gland, there is no absolute indication to measure uptake or obtain a scan.

Subacute thyroiditis is rare in childhood [35] and most often presents with thyrotoxicosis and elevated erythrocyte sedimentation rate and is associated with neck pain and tenderness. Reduced or no thyroid uptake on a scintiscan supports the diagnosis (fig. 11).

Single and Multiple Thyroid Nodules

In general, diffuse enlargement of the thyroid is of benign origin, whereas a solitary nodule must be evaluated carefully. Discrete thyroid nodules are uncommon in children, especially in the prepubescent child [36]. Figure 12 shows a multinodular goiter in a euthyroid prepubescent girl with familial occurrence of nontoxic and toxic multinodular goiter in several female probands. The prevalence of thyroid nodularity in children is considerably lower than in an adult population and has been estimated to be approximately 1.8% [37]. In both nonpalpable (incidentally found by neck imaging for other reasons) and palpable nodules larger than 1 cm, a ^{99m}Tc scintigraphy is recommended by the authors [37]. A solitary nodule with low-uptake (cold) and a nodule with normal uptake are shown in figure 13. In the adult population the likelihood of a cold nodule being malignant is low (5% in a recent review [38]) and in the view of many clinicians it adds little valuable information to a US-guided fine-needle aspiration

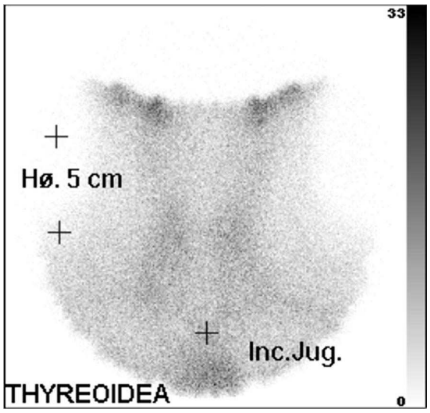


Fig. 11. ^{99m}Tc pertechnetate thyroid scintigraphy of a 14-year-old girl with transient thyrotoxicosis and painful swelling of the thyroid gland following an episode of flu-like symptoms. Low tracer uptake, compatible with subacute thyroiditis, is seen.

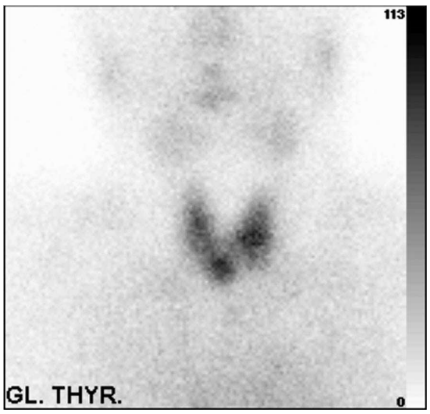


Fig. 12. ^{99m}Tc pertechnetate thyroid scintigraphy in an 11-year-old girl with nontoxic multinodular goiter. Heterogeneous uptake, bilaterally.

biopsy [39]. However, the a priori risk of a solitary nodule being malignant is higher in childhood, with an estimated risk of 18–46% [37, 40]. This risk increases if there is a history of previous radiation therapy to the cervical region [41], or if the patient is a male [42]. Therefore, the functional status of a solitary nodule should be evaluated, and non-functioning nodules are biopsied. A rare cause of a solitary cold thyroid nodule in childhood is the thyroglossal duct cysts, which often appears as a palpable neck mass [43].

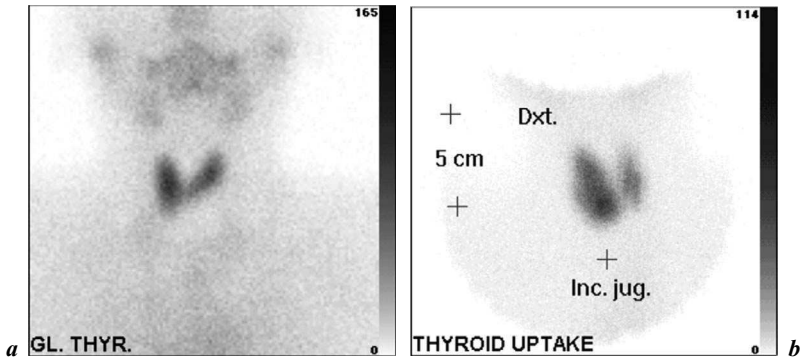


Fig. 13. ***a*** A nonfunctioning nodule in the left lower pole is shown. The patient, an 8-year-old boy, had a benign solitary 3 × 3 cm large cyst as evidenced by US and US-guided aspiration. ***b*** An enlarged right lobe and normal uptake in a 2 × 2 cm large nodule can be seen in the right lower pole. An 11-year-old boy with a palpable solitary solid homogeneous well-defined nodule. Biopsy was performed due to previous neck irradiation and showed benign cytology.

PET and PET/CT

Cancer-seeking radiopharmaceuticals have been evaluated for more than a decade to improve differentiation between benign and malignant thyroid nodules. Positron emission tomography with fluorine 18-fluorodeoxyglucose (FDG) is well established as a functional imaging tool for diagnostic oncologic imaging. It yields metabolic information about lesions that is not provided with conventional morphologic imaging modalities such as US, CT and MRI [44]. Studies using FDG PET for tumor staging and restaging, monitoring treatment, and predicting the prognosis in patients with head and neck cancers, have been published [45, 46]. To interpret PET images accurately, it is essential to be fully familiar with the normal patterns, intensities, and frequencies of FDG distribution in the head and neck area. PET evaluations of physiologic tracer uptake in the head and neck region, with or without image fusion techniques involving the use of conventional cross-sectional modalities to assist in locating structures and lesions seen on PET images, have been described [47, 48].

Combined PET/CT scanners that enable highly precise localization of the metabolic abnormalities seen on PET and high-spatial-resolution CT images have been developed [49]. The PET/CT fusion imaging is a novel multimodality technology that allows the correlation of findings from two concurrent imaging modalities in a comprehensive examination (fig. 14). The CT demonstrates exquisite anatomic detail but does not provide functional information,

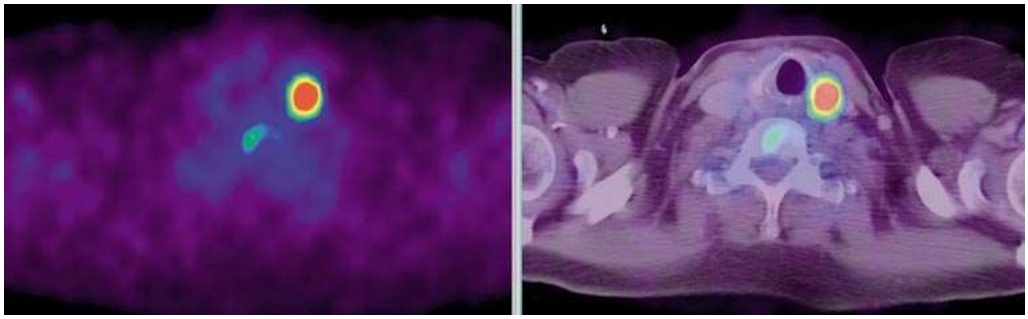


Fig. 14. Left panel: PET image 45 min after injection of 400 MBq 18 FDG shows increased focal uptake on the left side of the neck. Right panel: PET/CT fusion image shows that the increased uptake is localized in the left thyroid lobe. At surgery a papillary carcinoma was found.

whereas FDG PET reveals aspects of tumor function and allows metabolic measurements.

In a recent retrospective review of PET/CT images, obtained in 78 patients with non-head and neck cancers, the accumulation of FDG was described [46]. Intense tracer uptake is usually seen in the palatine tonsils, soft palate, and lingual tonsils. In the normal thyroid gland, the tongue, and inferior conchae the uptake is minimal. FDG accumulation is variable in the sublingual, submandibular and parotid glands [46]. Thus, the normal thyroid shows very low-grade FDG uptake, and is usually not visualized on the whole-body FDG-PET scan. Diffuse thyroid FDG uptake is usually an indicator of chronic autoimmune thyroiditis, as supported by the presence of thyroid autoantibodies and changes on sonography in one study [50]. Occasionally, focally or diffusely increased FDG uptake is seen as an incidental finding in the thyroid. The dilemma is to differentiate physiologic from pathologic FDG uptake [51]. Although a high FDG uptake in a thyroid tumor suggests malignancy even low levels of FDG uptake cannot completely rule out malignancy [52–54]. A cytologic diagnosis of focal thyroid FDG uptake in incidentalomas is mandatory, as cancer is confirmed in a significant number [55]. Despite limited data, PET and PET/CT have proved valuable in the evaluation of recurrent thyroid carcinoma [56]. So far, implementation of PET/CT in the routine evaluation of thyroid nodules in children awaits larger studies because of the considerable overlap in uptake between malignant and benign nodules.

In hereditary thyroid cancer the role of imaging in gene carriers is controversial. In one study CT and MRI failed to locate tumors <5 mm in diameter but whole-body FDG PET and adjunctive cervical US helped stage individuals

carrying mutant genes, thus verifying multiple endocrine neoplasia (MEN)2A or familial medullary thyroid carcinoma (FMTC) [57].

Ultrasonography

Because of the superficial location of the thyroid gland, high-resolution real-time gray-scale and color Doppler sonography can demonstrate the normal thyroid anatomy and pathologic conditions with remarkable clarity [58]. With increasing availability, this technique has come to play an ever more important role in the diagnostic evaluation of thyroid diseases. High-frequency transducers (7.5–15.0 MHz) provide both deep ultrasound penetration (up to 5 cm) and a high-definition image, with a resolution of 0.7–1.0 mm. It can distinguish solid nodules from cysts and allows accurate estimation of size, shows vascular flow (Doppler), and aids in the accurate placing of needles for diagnostic or therapeutic purposes [59]. It is also an excellent tool for use in the follow-up for estimation of changes in size of a lesion or the entire thyroid gland over time. Finally, it allows in utero investigation of the fetal thyroid [60] and can be helpful in fetal diagnosis of thyroid dysfunction [61]. The major limitations of sonography are the high degree of observer variability [62] and the inability to identify retrotracheal, retroclavicular, or intrathoracic extension of the thyroid [2, 59].

Examination is performed with the patient in the supine position and the neck hyperextended. A small pillow may be placed under the shoulders to provide better exposure of the neck. The thyroid gland must be examined in both transverse and longitudinal planes. The examination should be extended laterally to include the region of the carotid artery and jugular vein to identify enlarged jugular chain lymph nodes, superiorly to visualize submandibular adenopathy, and inferiorly to define any pathologic supraclavicular lymph nodes.

Indications for Thyroid Sonography

It is important to remember that thyroid scintigraphy (imaging providing information on functionality and to some degree anatomy) and sonography (providing information on morphology and anatomy) are complementary imaging modalities. Based on the lack of prospective comparative studies in childhood thyroid disease, indications for each will often be based on local traditions and nuclear medicine and radiology facilities and expertise. The evasion of ionizing radiation and sedation, in addition to a short examination time and wide availability, makes ultrasound an ideal initial examination in children [36]. Sonography will provide valuable diagnostic information in a number of

Table 3. Indications for thyroid US

Aid in the diagnosis of congenital hypothyroidism
Differentiate different types of thyrotoxicosis
Differentiate thyroid masses
Guide biopsy of nodules
Aspirate thyroid cysts
Guide interventional procedures (e.g. laser ablation)
Identify ectopic thyroid
Identify thyroid metastases
Identify recurrence in the follow-up of patients treated for thyroid cancer

clinical situations (table 3). In one series, one third of pediatric neck masses were located in the thyroid gland [36]. Sonography, and sonography-guided fine-needle aspiration biopsy, often has substantial impact on the final diagnosis of a thyroid mass (table 4). Sonographic tissue characteristics aid in classifying the lesion as inflammatory, neoplastic, congenital, traumatic, or vascular, and are diagnostic in the majority of cases [63]. In some genetic disorders attention must be drawn to the frequent involvement of the thyroid. For instance, Cowden syndrome, a rare autosomal-dominant disease, is characterized by multiple hamartomas of the skin and often (two-thirds of the patients) coexisting benign thyroid nodules, but also increased risk of nonmedullary thyroid carcinoma [64]. Genetic confirmation of Cowden syndrome warrants regular thyroid US because of the increased risk of thyroid malignancy.

Normal Thyroid Sonography

The thyroid gland is made up of two lobes located along either side of the trachea (seen in the midline of the lower neck as a markedly echogenic area with shadowing), and connected across the midline by the isthmus (fig. 15). The pyramidal lobe can often be visualized in younger patients, but it undergoes progressive atrophy in adulthood and eventually becomes invisible. Generally, the parathyroid glands are not identified.

The size and shape of the thyroid lobes vary widely. In the newborn, the gland is 18–20 mm long, with an anteroposterior diameter of 8–9 mm. By 1 year of age, the mean length is 25 mm and the anteroposterior diameter is 12–15 mm [58].

Sonography is an accurate method for calculating thyroid volume. The most common mathematical method is based on the ellipsoid formula (length $\times$ width $\times$ thickness $\times$ $\pi/6$ for each lobe) (fig. 16). This method has an estimated mean error of 15% [58] but the accuracy decreases with increasing size, irregularity of the thyroid, and with retroclavicular extension [1]. The most

Table 4. Potential causes of thyroid masses in childhood and adolescence

Acute suppurative thyroiditis
Subacute thyroiditis (DeQuervain)
Congenital goiter (often diffuse)
Diffuse goiter
Nodular goiter (uni- or multinodular):
Benign thyroid nodules
• Colloid/hyperplastic nodule
• Follicular adenoma
• Hürthle-cell adenoma
• Thyroid teratomas
• Lymphocytic thyroiditis
• Thyroglossal duct cyst
Malignant thyroid nodules
• Papillary carcinoma
• Follicular carcinoma
• Hürthle-cell carcinoma
• Anaplastic carcinoma (extremely rare in childhood)
• Medullary carcinoma
• Lymphoma
• Cancer metastatic to the thyroid
Nonthyroid lesions (clinically mistaken for being of thyroid origin)
• Branchial cleft cyst and other epithelial cysts
• Parathyroid adenoma or cyst (rarely palpable)
• Lymph node

precise mathematical method is the integration of partial volume estimates obtained at cross-sectional scans of the thyroid gland through evenly spaced sonographic scans [65]. This method has an estimated error of 5–10%. Modern three-dimensional ultrasound technology permits the simultaneous measurement of the three orthogonal planes of each thyroid lobe [66]. Planimetric three-dimensional sonography seems less observer-dependent and is more accurate than conventional sonography with an intraobserver variability of 5% [67].

Goiter prevalence in school-age children is an important indicator of iodine deficiency disorders in a population. The 1994 WHO criteria provides an acceptable estimate of goiter prevalence in areas of severe iodine deficiency, but in areas of mild iodine deficiency sonography-determined thyroid volume is the method of choice [68]. Thyroid volume is correlated with iodine status, age, weight, height, sex and body surface area in non-iodine-deficient areas [69]. Thyroid volumes increase with advancing age with a relative sudden increase between the age of 11 and 12 in girls and between 13 and 14 in boys

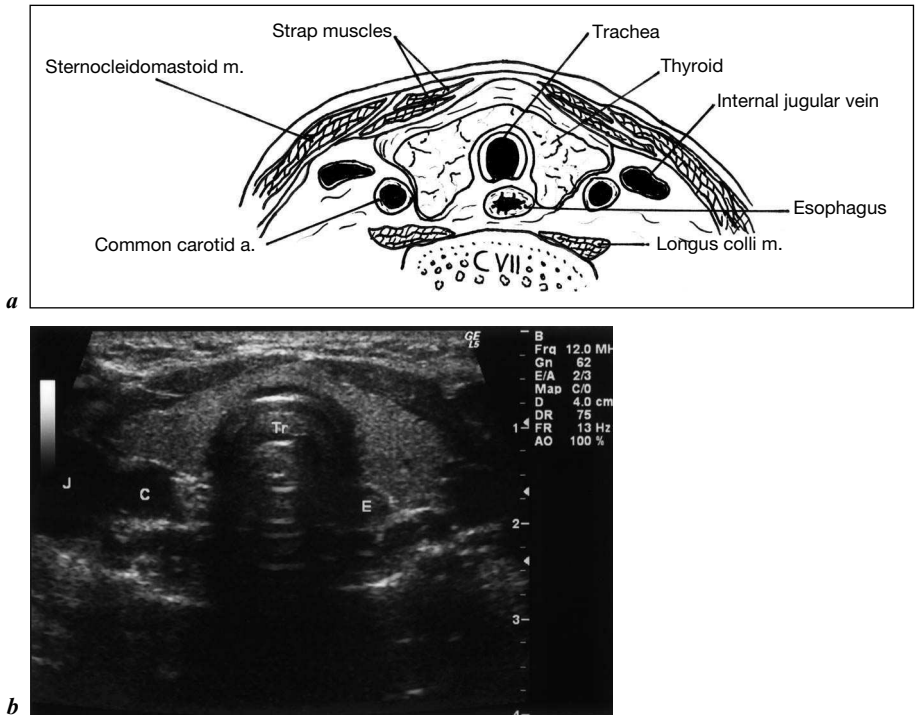


Fig. 15. Anatomy of the neck. Transverse section through the thyroid at the level of the 7th cervical vertebra CVII. Strap muscles: sternohyoid and sternothyroid muscles. ***a*** Anatomic drawing (modified from [58]). ***b*** Corresponding transsectional sonogram. C = Common carotid artery; E = esophagus (often deviating to the left at this level); J = jugular vein; Tr = trachea.

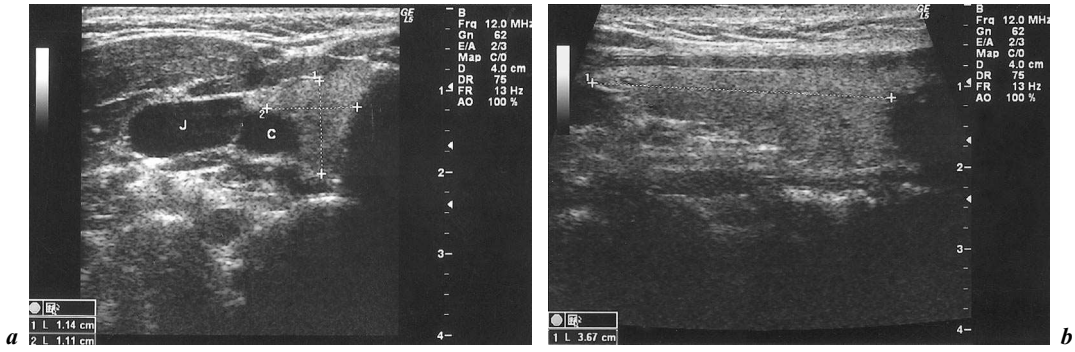


Fig. 16. Volume measurement of the thyroid gland. Transverse (***a***) and longitudinal (***b***) images show callipers at the boundaries of the right thyroid lobe. The calculated thyroid volume is based on the ellipsoid formula with a correction factor (length × width × thickness × π/6 for each lobe). C = Carotid artery; J = jugular vein.

Table 5. Median thyroid volume in a cohort of Dutch schoolchildren

Age	Thyroid volume, ml	
	boys	girls
6 years	3	3
8 years	4	4
10 years	5	5
12 years	6	8
14 years	10	9
16 years	10	9
18 years	12	9

Median thyroid size determined by ultrasound in Dutch schoolchildren (408 boys and 529 girls) according to age.

[70]. Thyroid volume is sex-independent up to the age of about 11, but at ages 12 and 13, girls have a slightly larger thyroid volume (associated with an increase in body surface area). The subsequent larger increase in body surface area in boys results in larger thyroid volumes from the age of 14. The sex difference in thyroid volume is less marked if expressed by body surface area than by age, but both indicate larger thyroid glands in 14 year old males than in females [70]. In a study of Dutch schoolchildren, median US-determined thyroid volume was approximately 3 ml at the age of six, 5 ml at the age of ten, 9 ml and 12 ml at the age of 18 in girls and boys, respectively (table 5).

Normal thyroid parenchyma has a characteristic homogenous medium-level echogenicity (fig. 15b), whereas that of the muscles anterior (m. sternothyroideus and m. sternohyoideus) and anterolateral (m. sternocleidomastoideus) to the thyroid appear hypoechoic (fig. 15b). The thin hyperechoic line that bounds the thyroid lobes is the capsule which is often identifiable by sonography. The rich vascularity of the gland is easily detected with currently available high-sensitivity Doppler instruments (fig. 17).

Congenital Defects

Congenital hypothyroidism is one of the more common congenital endocrine disorders, with an incidence of around 1 in 3,800 live births. Patients are classified as: (1) having developmental abnormalities of the thyroid gland (85% of cases) [15], which include ectopic thyroid tissue, aplasia, or hypoplasia

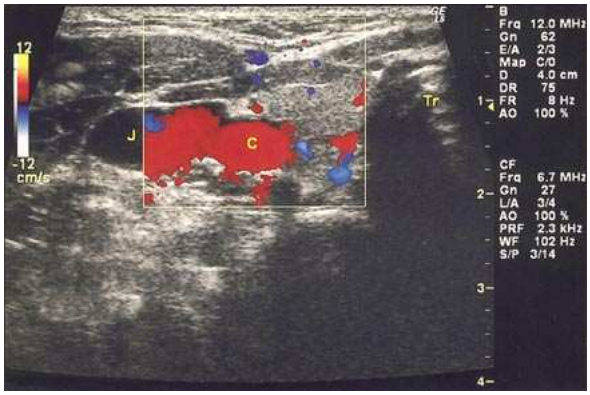


Fig. 17. Normal thyroid vascularity on color Doppler US. C = Carotid artery; J = jugular vein; Tr = tracheal air shadow.

of the thyroid gland, or (2) having a normally located gland mostly related to thyroid dyshormogenesis. It is generally accepted that scintigraphy is indispensable in the correct diagnostic work up of congenital hypothyroidism [21, 71]. However, scintigraphy has to be performed within the first week after starting thyroxine treatment, to prevent an inhibited uptake of the isotope, and is not always performed. Sonography has been evaluated and found valuable for obtaining an etiologic diagnosis [72, 73], but not reliable for detecting ectopia or for differentiating ectopia from aplasia [74]. This was confirmed in a recent study of 66 neonates with an established diagnosis of congenital hypothyroidism resulting in a diagnosis of ectopic thyroid tissue in 42 of them (64%). Confirmation was obtained by scintigraphy, but sonographically confirmed in only 9 of 42 cases [75].

More recent advances in US technology, including color Doppler and high-resolution gray-scale US, have led to a reevaluation of US in congenital hypothyroidism. In a recent study from Japan color Doppler US was found superior to gray-scale US and MRI (sensitivity 90, 70 and 70%, respectively) [76]. In a comparative study of US and scintigraphy in 88 patients with congenital hypothyroidism, it was confirmed that sonography failed to distinguish between thyroid aplasia and ectopia but did distinguish between presence and absence of thyroid tissue [77]. The authors conclude that sonography is an accurate method to establish the presence of dysgenesis of the thyroid gland and might be used as the first imaging tool in patients with CH, whereas scintigraphy should be used mainly to distinguish agenesis from ectopia, whenever there is no thyroid tissue present at US [77].

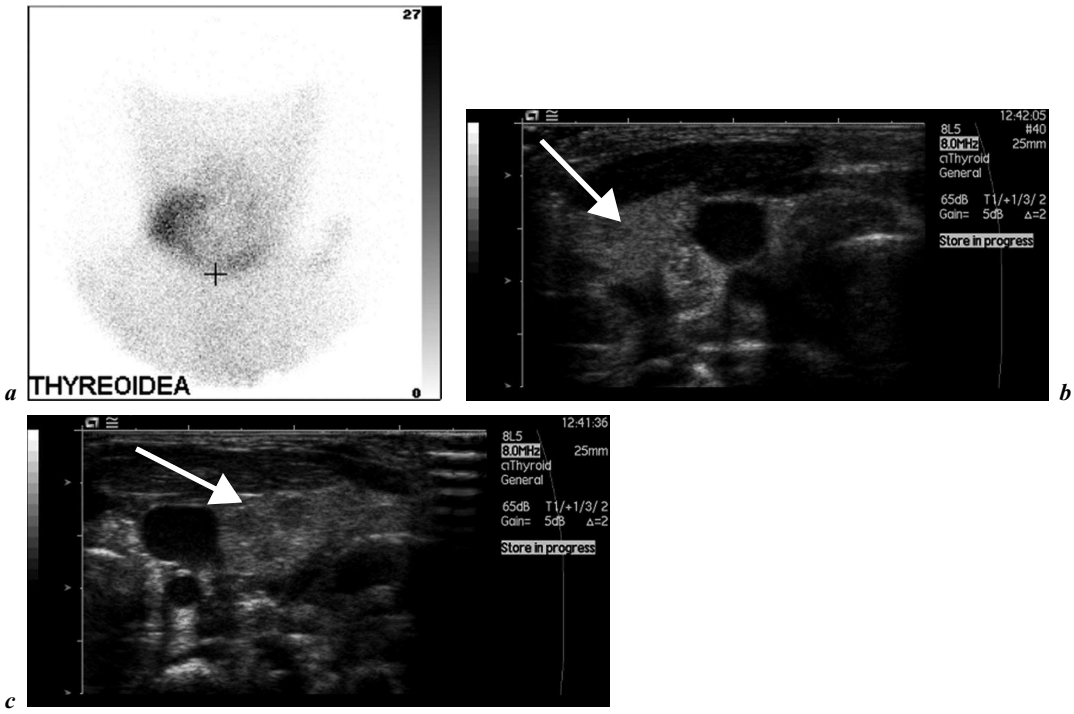


Fig. 18. ***a*** ^{99m}Tc pertechnetate scintigraphy demonstrating heterogeneous and reduced uptake in the lateral neck, primarily on the right side. Additional trans-sectional US demonstrated well-defined thyroid tissue (arrows) lateral to the jugular vein on both the right (***b***) and the left side (***c***).

Ectopic thyroid tissue is the most frequent cause of congenital hypothyroidism (two-thirds of cases) and although sonography results in a low detection rate compared to radionuclide scanning, it adds etiological information based on location, echogenicity and vascularity [78]. Figure 18 shows ectopic thyroid tissue in the lateral neck, confirmed by scintigraphy (fig. 18a) as well as by US (fig. 18b, c), in a neonate with congenital hypothyroidism. The presence of cysts, detected by sonography, within the empty thyroid area in two-thirds of patients with thyroid dysgenesis, is a novel observation [79] but does not alter management.

Syndromes like Williams' syndrome (incidence of 1:10,000 live births, characterized by facial dysmorphisms, heart defects, short stature and mental retardation) can show thyroid disorders including thyroid ectopia, hemiagenesis and thyroid hypoplasia in addition to subclinical or overt hypothyroidism. In

this syndrome, abnormalities of thyroid morphology are best detected by US [80]. Thyroid hemiagenesis is often an incidental finding on sonography with a higher incidence of agenesis of the left lobe [81].

Seventy percent of congenital anomalies in the neck are thyroglossal duct remnants or cysts [82, 83]. In the young child, thyroglossal duct cysts often appear as a firm midline mass with a variable sonographic appearance. The majority are pseudosolid rather than anechoic and closely related to the hyoid bone [84]. Cysts can be located anywhere from the base of the tongue to the thyroid isthmus [82], but also at the level of the hyoid or infrahyoid.

Congenital Goiter

Congenital goiters may be secondary to a number of diseases [28]. Goiters secondary to enzyme deficiencies, e.g. mutations in the thyroid peroxidase gene resulting in iodide organification defects, may be present at birth. In a cohort study of newborns with congenital hypothyroidism and normally located thyroid, 50% were classified as having goiter [85]. In the group with permanent congenital hypothyroidism and goiter, one-third had an iodine organification defect, one-fourth a defect of thyroglobulin synthesis and 5% had Pendred's syndrome.

Sonography can be used to differentiate goitrous hypothyroidism (gland enlargement) from agenesis (absent gland). Morphology in congenital goiters will often show homogeneous normal or slightly reduced echogenicity.

Most of the congenital goiters develop in the early months and years of extrauterine life. Infants born to mothers with Graves' disease (circulating TSH receptor antibodies that cross the placenta) can have fetal goiter [86]. Other causes include prenatal ingestion of iodine [87], including administration of amiodarone during pregnancy [88], lithium [30] and antithyroid drugs [89].

Diffuse Thyroid Disease

Inflammatory disorders of the thyroid include acute (suppurative), subacute (de Quervain), and chronic autoimmune (Hashimoto's) thyroiditis. Even with less evident clinical signs of local infection, a complex hypoechoic mass seen sonographically raises the suspicion of acute suppurative thyroiditis with abscess formation [90]. When the left lobe of the thyroid is involved, the possibility of a remnant of the left third pharyngeal pouch, which results in a fistula between this lobe and the ipsilateral piriform sinus, should be considered [91, 92]. When acute symptoms have subsided, a barium swallow should be performed to identify any hypopharyngeal fistula [90]. Thyroglossal duct remnants or cysts pose a risk of fistulas that may develop with infection and should be evaluated with fistulograms [93]. Still, the ability of the thyroid gland to withstand infection is well known and abscess formation is rare [94].

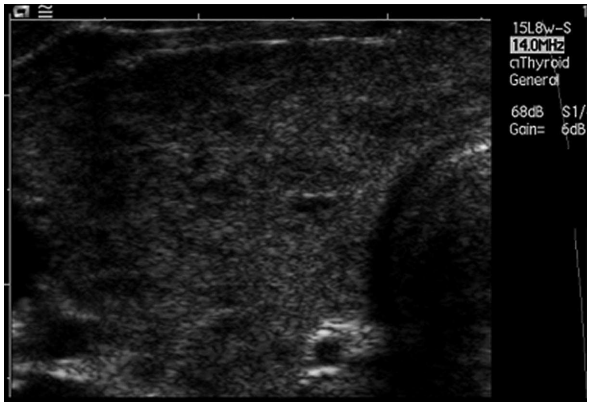


Fig. 19. Trans-sectional gray-scale US of the right lobe showing marked hypoechogenicity. A 14-year-old girl with subacute thyroiditis (for a corresponding scintigraphy of the patient, see fig. 11).

Subacute thyroiditis is extremely uncommon during childhood [35] and the incidence is lower than that of acute suppurative thyroiditis. However, it is an important differential diagnosis even with a unilateral painful enlargement of a lobe presenting as a solitary cold thyroid nodule [95]. A radionuclide scan showing 'no uptake' supports the diagnosis (fig. 11), as does sonography, showing marked hypoechogenicity – focal or diffuse – with reduced vascularity (fig. 19) [96]. With recovery, size decreases, but areas of hypoechogenicity may be detected for many months [96].

The majority of patients in the pediatric age group, mostly older children, have an autoimmune chronic lymphocytic thyroiditis (Hashimoto's thyroiditis) and it is also the most common cause of acquired hypothyroidism with or without goiter [31]. This condition is often associated with other autoimmune disorders, e.g. type 1 diabetes mellitus, celiac disease and also Turner's syndrome and Down's syndrome. Conversely, in a high proportion of young patients with type 1 diabetes without any clinical signs of thyroid disease, markers of thyroid autoimmunity have been found [97]. More than 40% showed degrees of thyroid hypoechogenicity on sonography and 16% had thyroid autoantibodies. The typical sonographic signs in Hashimoto's thyroiditis are marked diffuse or inhomogeneous hypoechogenicity or patchy echo pattern (fig. 20) [1, 98]. Sonography cannot differentiate between goitrous autoimmune thyroiditis and lymphoma. Therefore, growth of a goiter, especially in euthyroid subjects on L-thyroxine therapy, should raise suspicion of lymphoma and lead to large-needle biopsy [99].

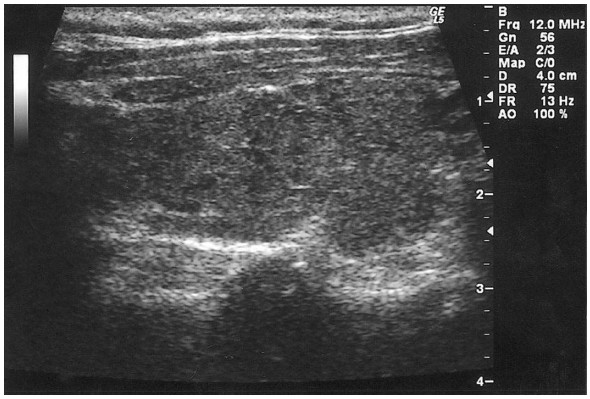
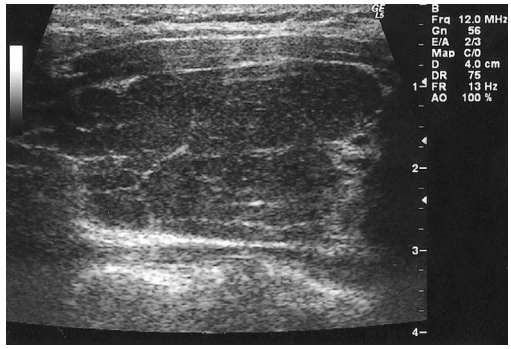


Fig. 20. Longitudinal US image of a 15-year-old girl with Hashimoto's thyroiditis, depicting reduced and diffuse echogenicity in an enlarged thyroid.



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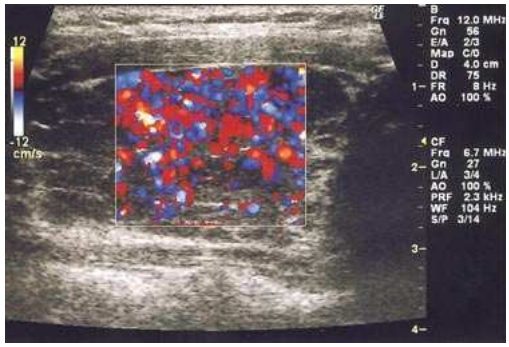

b

Fig. 21. Graves' disease. ***a*** Longitudinal US showing an enlarged and diffusely hypoechoic thyroid lobe. ***b*** Corresponding Doppler image with increased vascularity indicating an acute stage (debut) of the disease. There are multiple linear bright echoes throughout the hypoechoic parenchyma with coarse septations.

In conjunction with presence or absence of thyroid autoantibodies the clinical utility of US is imperative. In contrast to the abnormal echogenicity in all patients with Hashimoto's thyroiditis, patients with a diffuse colloid goiter have normal echogenicity [100, 101].

In patients with Graves' disease, the thyroid is usually enlarged and the echo pattern is homogeneous and diffusely hypoechoic (fig. 21a). Color Doppler sonography demonstrates a hypervascular pattern often referred to as 'thyroid inferno' (fig. 21b). A significant decrease in flow velocities after

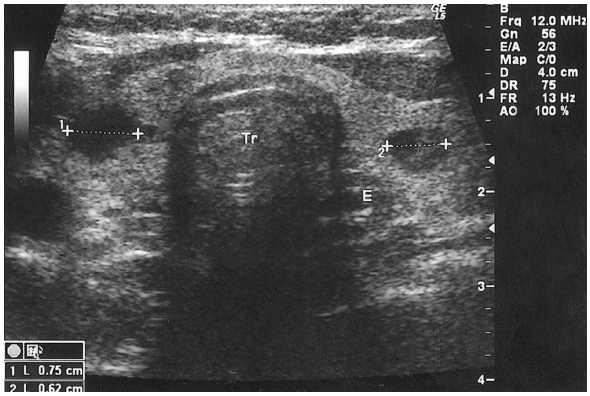

Fig. 22. Multinodular goiter. Transverse US shows two small (less than 1 cm in diameter) cystic nodules. E = Esophagus; Tr = tracheal air shadow.

medical treatment is often seen. However, there is no correlation between the degree of biochemical hyperfunction and the degree of hypervascularity or blood flow velocity. On the other hand, preliminary data suggest that quantification of blood flow can predict recurrence following withdrawal of medical treatment with a sensitivity of 71% [102].

Routine thyroid imaging (radionuclide scanning and US) is not indicated in all disorders accompanied by diffuse thyroid enlargement, when there is no clinically detectable focal thyroid abnormality, unless presence of features suggestive of acute or subacute thyroiditis or malignancy, e.g. a history of radiation exposure.

Nodular Thyroid Disease

In children, nodular thyroid disease may appear clinically as either a single thyroid nodule (more common) or as a multinodular thyroid gland (less common) [103, 104]. Nodular thyroid disease in childhood differs from that in adulthood in two aspects. First, it is far less common in younger individuals and increases in frequency with age. Second, thyroid carcinoma is much more likely to be present in children than in adults with thyroid nodules. US is very helpful in differentiating multinodular goiters (fig. 22) from single thyroid nodules and diffuse thyroid disease.

Multinodular thyroid disease in children is often associated with other disorders, e.g. renal or digital anomalies, McCune-Albright syndrome and Hashimoto's thyroiditis, but equally important with a non-negligible incidence of thyroid malignancy [103]. Furthermore, there are several families in whom multinodular goiter has been described and the genetic loci identified [105],

including the rare autosomal-dominant Cowden disease [106]. The echographic structure of multinodular or adenomatous goiter may be heterogeneous without well-defined nodules (can have the same appearance of inhomogeneity as with Hashimoto's thyroiditis), or there may be two or more nodules within an otherwise normal-appearing gland. Often patients evaluated for a solitary nodule have additional small thyroid nodules detected by US but this finding does not exclude carcinoma [107]. A solitary (by palpation) low-uptake thyroid nodule with or without coexisting nodules warrants US-guided fine-needle aspiration biopsy and a lower, compared with adults, threshold for diagnostic surgery [38].

Thyrotoxicosis in children is most often a result of Graves' disease or rarely an autonomous hyperfunctioning nodule but almost never due to toxic multinodular goiter [108]. Toxic multinodular goiter in childhood has also been described in association with nonautoimmune activating TSH receptor mutations [109]. In the absence of thyroid autoantibodies, scintigraphy and US are helpful in establishing a final diagnosis in order to guide treatment. The sonographic appearance most often is that of multiple (two or more) discrete nodules with increased blood flow on color Doppler sonography.

Single Thyroid Nodules

Although most single thyroid nodules in childhood are benign (e.g. colloid nodules, follicular adenoma), thyroid carcinoma has been reported in 7–30% of nodules in this population [104]. The clinical challenge is to distinguish the malignant nodules from the many benign ones, and thus, to identify those patients for whom surgical excision is indicated. The combination of sonography with fine needle aspiration biopsy provides a sensitive and specific approach to the child with a single thyroid nodule [110] and can at best avoid unnecessary thyroid surgery [111].

Thyroid nodules on US may have one of three echo patterns: solid (echogenic), cystic (echo-free), or a mixed solid-cystic appearance (fig. 23). Mixed solid-cystic nodules are more likely to be neoplasms (follicular adenoma or carcinoma) than are purely cystic lesions [112]. The majority of thyroid nodules are due to hyperplasia, and are often referred to as hyperplastic, adenomatous, or colloid nodules. Most of the cystic lesions are hyperplastic (colloid) nodules that have undergone liquefactive degeneration. Most solid colloid nodules appear iso- or hypoechoic on US (fig. 24) often with a thin peripheral hypoechoic halo.

The benign follicular adenoma is a true thyroid neoplasm that has a fibrous encapsulation, often appears as a solid iso- or hypoechoic mass (fig. 23a) but without specific sonographic features to distinguish it from carcinoma. Various subtypes include fetal adenoma, Hürthle cell adenoma, and embryonal adenoma. The cytologic features of follicular adenomas are indistinguishable from

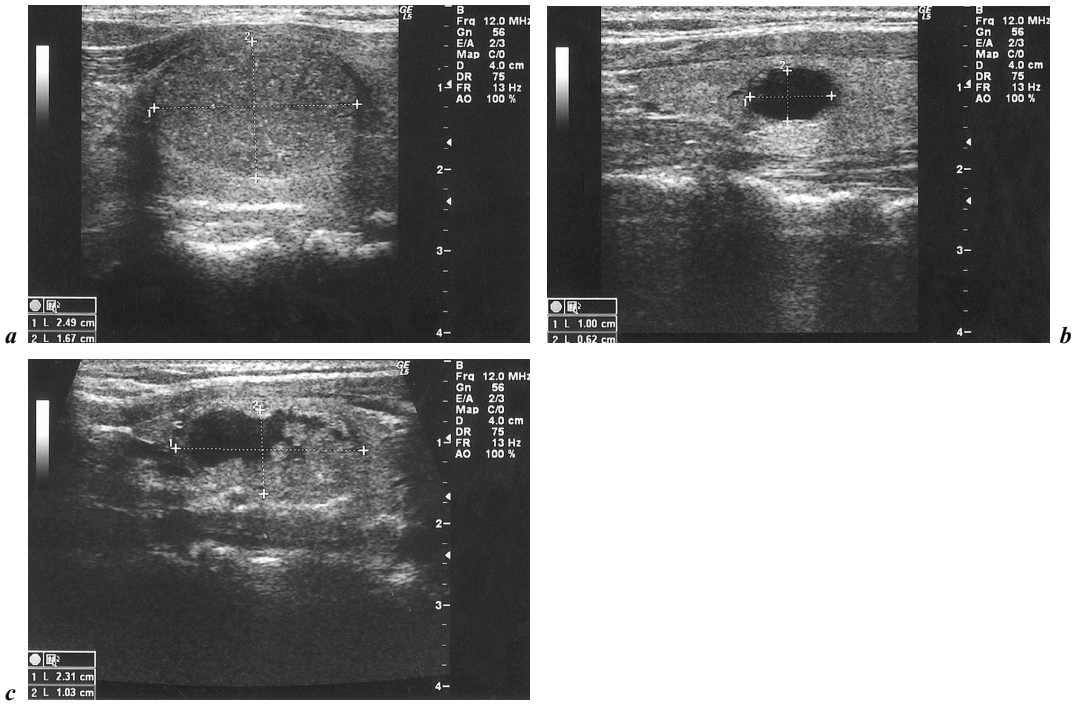

Fig. 23. Longitudinal US images of young patients with (***a***) a solid homogenous, oval and well-defined hypoechoic thyroid nodule (benign follicular adenoma); (***b***) a small (hypoechoic) well-defined cyst, and (***c***) a mixed solid-cystic nodule.

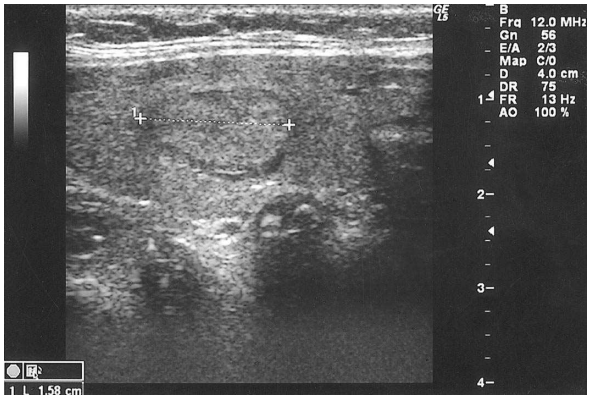

Fig. 24. A benign colloid thyroid nodule. Longitudinal image of a homogeneous isoechoic round to oval mass with a thin halo surrounding the nodule.

those of follicular carcinoma. Vascular and capsular invasion are the hallmarks of follicular carcinoma and implies surgical excision for complete histological investigation. Preoperative selection of these patients based on TPO immunostaining of biopsies has been proposed to improve sensitivity (more than 80% staining suggesting a benign nodule) [113], but others have found that TPO expression has limited value for the differential diagnosis of follicular thyroid carcinoma from the thyroid adenoma [114].

Rare cases of primary thyroid teratomas have been described and are important in the differential diagnosis because of the potential of malignant transformation [115].

Since the extent of primary surgical treatment is closely related to the overall prognosis, preoperative diagnosis becomes essential in the management of thyroid neoplasms in young patients. The preoperative workup of children and adolescents with thyroid nodules requires the collaboration of an experienced team of professionals, and US and US-guided FNAB are important in the initial evaluation [116].

Thyroid Carcinoma

In childhood the most common malignant tumors in the head and neck region are lymphomas and rhabdomyosarcomas whereas thyroid cancer in childhood is rare, representing 1.4% of all pediatric malignancies in the USA [117]. Its incidence rises after the age of 5 and its overall incidence in children in England and Wales is 0.5 per million per year [118]. Papillary carcinoma accounts for more than 90% of all pediatric thyroid cancers and 75% of these have metastasized at presentation [119]. The commonest clinical presentation of a thyroid malignancy is a palpable, asymptomatic, solitary nodule in the thyroid [120]. A solitary thyroid nodule in a child is alarming, since the incidence of malignancy in such a nodule is higher than in adults and, at least in older series, varies from 18 to 46% [37, 40].

A thyroid nodule that clinically appears solitary, solid or mixed solid-cystic on ultrasound and hypofunctioning on scintigraphy is highly suspicious for malignancy and warrants US-guided FNAB. Ultrasound guidance is recommended because it facilitates accurate sampling of the lesion and reduces the risk of false-negative results [121].

Sonographically, most carcinomas appear hypoechoic compared with the remaining gland but so do the majority of colloid nodules. No single sonographic criterion distinguishes benign thyroid nodules from malignant nodules with complete reliability [122]. However, certain sonographic features are more commonly found in benign or malignant nodules and thus can be suggestive of either (table 6). The fundamental morphological features recorded on high-resolution and color Doppler sonography should include:

Table 6. Sonographic features suggestive of a benign versus a malignant thyroid nodule

Feature	Pathologic diagnosis	
	benign	malignant
Purely cystic content	++++	+
Mixed solid-cystic	+++	++
Hypoechoic	+++	+++
Thin halo	++++	+
Well-defined margin	+++	++
Poorly defined margin	++	+++
Microcalcifications	++	+++
Increased peripheral flow	+++	++
Increased intranodular flow	++	+++
Subcapsular location	++	+++
Lymphadenopathies	+	++++
Invasion of adjacent structures	++++	

$^{+}$Rare (<1%); $^{++}$low probability (<15%); $^{+++}$intermediate probability (16–84%); $^{++++}$high probability (>85%).

Adapted from [58, 125, 126].

- internal consistency (solid, mixed solid-cystic, or purely cystic)
- echogenicity hyper (= increased), iso (= same), or hypoechogenicity (= decreased) relative to the adjacent thyroid parenchyma
- halo (present or absent, complete or incomplete)
- margin (well-defined vs. poorly defined)
- presence and pattern (coarse or fine) of calcifications
- presence and distribution of blood flow signals
- location (subcapsular or intervening thyroid parenchyma)
- lymphadenopathies
- invasion of adjacent structures

A nodule that has a significant cystic component is usually a benign colloid nodule that has undergone degeneration or hemorrhage. FNAB of both the cystic and the solid part is mandatory. Papillary carcinomas may exhibit partly cystic degeneration [123] and cervical lymph nodes with metastases may also show a cystic pattern.

A peripheral sonolucent halo that completely surrounds a thyroid nodule may be present in 60–80% of benign nodules and 15% of thyroid cancers [58]. The vast majority of benign thyroid nodules tend to have a sharp and well-defined margin, whereas malignant lesions tend to have irregular or poorly

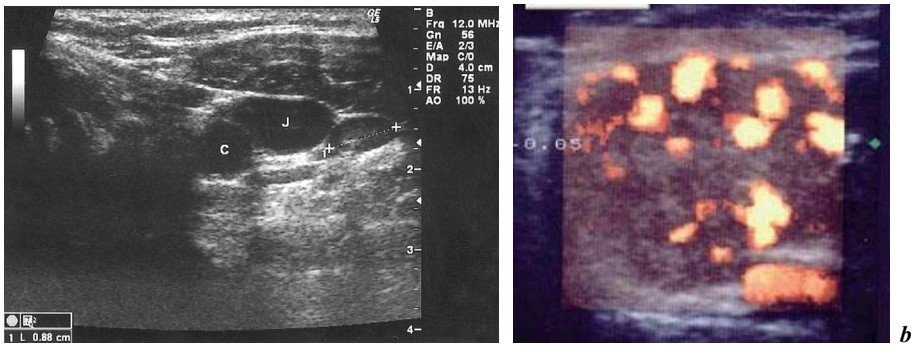

Fig. 25. a A normal oval lymph node with an echogenic linear hilus centrally. ***b*** Longitudinal image near the carotid artery and jugular vein, showing a large hypoechoic round metastatic lymph node with anarchic vascularization (power Doppler). C = Carotid artery; J = jugular vein.

defined margins, but nevertheless a finding with poor predictive value. Intranodular calcifications are detected in more than 10% of thyroid nodules in the adult population [58]. Although indicative of malignancy it is also frequently seen in benign nodules as reported in one study of 159 adult patients operated on. In this study a preoperative US detected calcifications in three fourths of the malignant and one third of the benign nodules [124]. In thyroid cancer in children it is a less frequent finding and in one study of 103 consecutive pediatric patients with solid thyroid nodules, microcalcification was found in only 5 and 3% of malignant and benign nodules, respectively [125]. In the same study, increased intranodular vascularity (sensitivity of 70% and specificity of 88%), a subcapsular location (sensitivity of 65% and specificity of 86%) together with an irregular outline were the most reliable diagnostic markers for cancer in smaller nodules (diameter less than 15 mm). The only sonographic features with a very high probability of malignancy are pathological appearing ipsilateral lymph node(s) and features of invasion of adjacent structures [126]. Certain features of enlarged cervical lymph nodes are indicative of malignancy and include round shape (rather than oval), absent hilus, intranodal necrosis, calcification, matting, soft-tissue edema, and peripheral vascularity (fig. 25) [127].

Papillary carcinoma has specific histologic (fibrous capsule, microcalcifications) and cytologic features (‘ground glass’ nuclei, cytoplasmic inclusions in the nucleus) that often allow a relatively easy pathologic diagnosis. Some of the US characteristics of papillary carcinoma include hypoechogenicity (90%), microcalcifications, hypervascularity (often disorganized), and cervical lymph node metastases that may contain microcalcifications or may be partly cystic. The minimally invasive follicular carcinoma is encapsulated, and only the

histological demonstration of focal invasion of the capsule itself, or of capsular blood vessels, permits differentiation from follicular adenoma. Sonographically, it will often appear solid and iso- or hypoechoic with a thick hypoechoic halo. Often vessels pass from the periphery to the center of the nodule. The widely invasive follicular carcinomas are not well encapsulated and invasion of the vessels and the adjacent thyroid can sometimes be apparent on US, showing an irregular tumor margin in addition to a chaotic arrangement of internal blood vessels [58]. The sonographic appearance of medullary carcinoma is usually similar to that of papillary carcinoma and is most often seen as a hypoechoic solid mass, occasionally with more coarse calcifications. It is important to remember that the disease is often multicentric and/or bilateral in about 90% of the familial cases and a high incidence of lymph node involvement is seen. Extranodal thyroid lymphomatous involvement (non-Hodgkin's lymphoma) is rare in childhood and sonographically appears as a markedly hypoechoic and lobulated mass which is mostly hypovascular. In the adult population it can arise from a preexisting Hashimoto's thyroiditis [128].

Routine thyroid US is recommended for surveillance of children and adolescents who have had neck irradiation for other childhood cancers [129]. A baseline study one year after irradiation is recommended with ongoing follow-up US depending on the radiation dose and the patient's age at the time of irradiation [104]. Thyroid US seems more sensitive than physical examination or scintigraphy in the follow-up of patients exposed to head-and-neck irradiation during childhood for benign conditions [130].

Computed Tomography

Computed tomography offers excellent anatomic resolution because of its ability to identify small differences in density between different tissues [131]. It is highly sensitive for detecting thyroid nodules, but as with US, benign nodules cannot be distinguished from carcinomas [132]. It can distinguish solid from cystic and mixed solid-cystic nodules and thyroid volume can be determined. It is superior to US in detecting thyroid tissue in the retrotracheal, retroclavicular and intrathoracic regions and for evaluation of metastatic disease in the neck and thorax [133]. The limitations of CT are cost, limited availability, length of the procedure, need for patient cooperation, artifacts caused by swallowing or breathing, and exposure to ionizing irradiation (1–4 rad) [132]. It has been suggested that the higher doses and increased lifetime radiation risks in children will actually produce a sharp increase, relative to adults, in estimated risk of lifetime cancer from CT [134]. This fact may stimulate a more active approach toward reduction of CT exposure in pediatric patients, which is definitely

supported by the availability of equal, or in some instances superior, imaging modalities. Furthermore, the need for sedation in newborn and smaller children makes CT less attractive compared to US, as an initial examination of neck masses in children. CT-guided biopsy is possible but more cumbersome than is US-guided biopsy. It can be valuable in poorly accessible or deep-seated lesions of the neck [135]. Neither CT nor MRI is routinely indicated in the pediatric population with thyroid disorders, and never in case of hyper- or hypothyroidism.

Indications for CT of the Thyroid

Localization of thyroid tissue is valuable in the workup of hypothyroidism (including congenital hypothyroidism) during childhood or in rare cases of stridor [136]. For this purpose, US is recommended as an initial screening examination in addition to scintigraphy. However, in a small series of 19 patients with congenital hypothyroidism, enhanced CT (Omnipaque intravenously) identified ectopic (sublingual) thyroid tissue in 7 patients, which was missed by US as well as by scintigraphy [137]. Enhanced CT seems to be of value in identifying thyroid tissue when US and scintigraphy fails.

CT can estimate the extent of tracheal compression by a goiter and can provide information on retroclavicular extension of the thyroid. Some recommend preoperative US as well as CT of the neck and chest to delineate the extent of the disease [104]. Furthermore, CT is of value in the follow-up of patients with thyroid carcinoma because of its sensitivity for detecting recurrent carcinoma in the neck, and metastases elsewhere. Recurrent carcinoma appears as discrete low-density lesions within or outside the thyroid bed, and lymph node metastases show no enhancement after contrast injection [138]. CT can complement whole-body scintigraphy in the follow-up of these patients, especially if recurrence is suspected.

Normal Thyroid

The normal thyroid gland is easily seen on CT, and its density is always higher than that of the surrounding tissues (fig. 26). CT density of the thyroid is closely related to its iodine content and reduced density is the hallmark of many thyroid disorders, but still not specific for any thyroid disorder.

Developmental Abnormalities

Ectopic thyroid tissue may be located anywhere from the foramen coecum, at the base of the tongue, to the anterior mediastinum. Scintigraphy is the imaging procedure of choice but CT can aid in localization if radionuclide uptake is poor [1]. CT enables the differentiation of thyroglossal duct cysts from other neck lesions based on location, CT values, and alterations in the adjacent soft

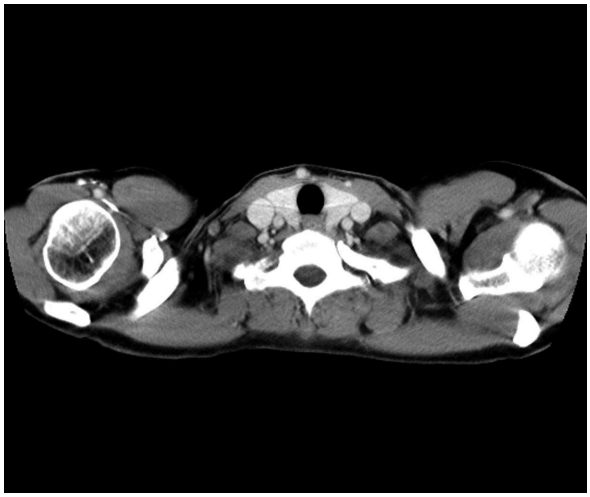

Fig. 26. Transverse sectional computed tomography of the neck showing a normal homogeneous thyroid gland.

tissues [139]. Carcinoma within the thyroglossal duct is a very rare pediatric tumor and so far only 22 cases have been reported in the literature [140]. Calcification within the cyst, or a dense enhancing nodule seen on enhanced CT, raises suspicion of a carcinoma.

Diffuse Thyroid Disease

In patients with Graves' disease, the density is decreased by 50–70% due to decreased iodine stores, and the tissue may be slightly inhomogeneous [141]. In patients with Hashimoto's thyroiditis the density is reduced and is lowest in patients with hypothyroidism [142]. Asymmetric low-density areas should raise the suspicion of lymphoma or carcinoma [143]. In the initial phases acute suppurative thyroiditis shows non-specific morphological alterations, but as infection progresses loculated hypodense areas (abscess) may appear [144].

Nodular Thyroid Disease

Multinodular goiters are usually seen as an enlarged asymmetric thyroid gland with multiple areas of varying degrees of density (fig. 27) [1]. However, medullary thyroid carcinoma can appear as single but also multiple low-density lesions of variable size, in one or both lobes, and be misclassified as 'benign-appearing' multinodular goiter [145]. Compression of the trachea (fig. 28), esophagus, and great vessels is easily detected, and CT is ideal for estimating the extent of tracheal compression by a goiter [146].

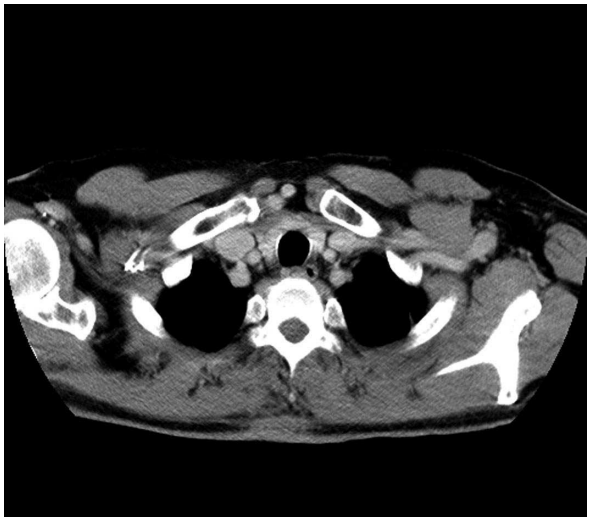

Fig. 27. CT of the neck showing areas of varying degrees of density in a slightly enlarged thyroid gland compatible with multinodularity.

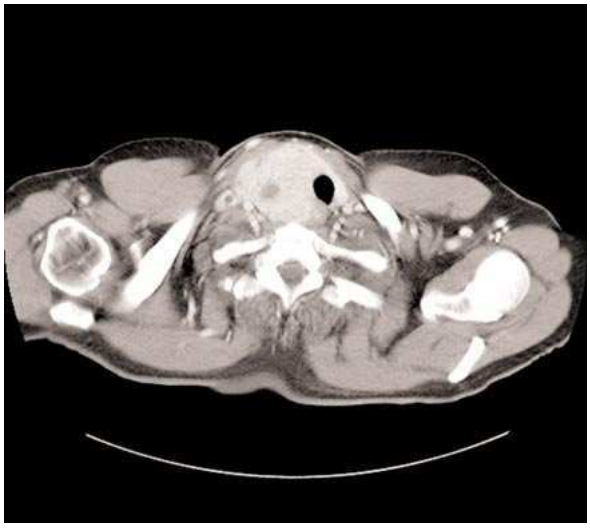

Fig. 28. CT of the neck showing the trachea is displaced to the left and with compressed lumen due to thyroid carcinoma.

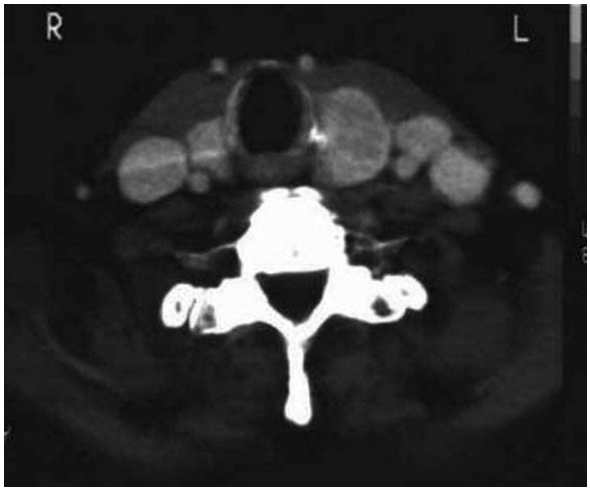

Fig. 29. Medullary carcinoma appearing as a heterogeneous and enlarged left thyroid lobe with ipsilateral lymph node metastasis lateral to the jugular vein. The trachea is displaced slightly to the right.

The complete extent of larger lesions is – in some cases – better evaluated with CT (or MRI).

Thyroid nodules often appear as low density lesions but CT cannot differentiate benign nodules from papillary and follicular carcinomas. As with US, calcifications are easily detected and invasive growth into surrounding structures, as well as lymph node metastases (neck and mediastinum), can be revealed by CT (fig. 29) [138].

Magnetic Resonance Imaging

MRI offers excellent anatomic resolution and generation of images in multiple planes. Conventional T_1- and T_2-weighted imaging is highly sensitive but just as nonspecific as US and CT in differentiating benign thyroid nodules from carcinomas. Sensitivity does not increase with additional gadolinium-enhancement but primary thyroid lymphoma enhances less than other solid thyroid tumors [147]. MRI can distinguish solid from cystic nodules (fig. 30) [132]. Like CT, it provides highly accurate estimates of thyroid volume with a low observer variability and is useful, especially in irregularly enlarged goiters [148]. As CT, and in contrast to US, it can identify thyroid tissue in the retrotracheal and intrathoracic regions (fig. 31). The obvious limitations of MRI are its cost, limited

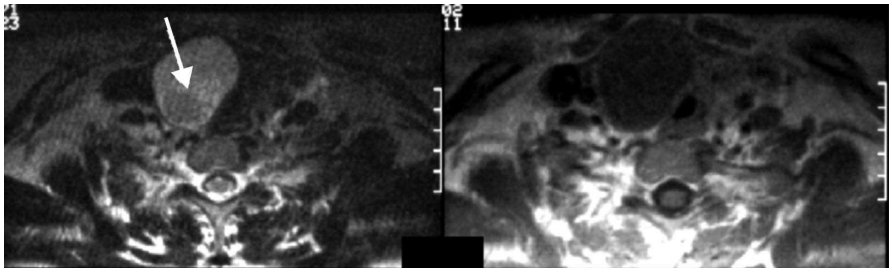

Fig. 30. Axial MR examination with T_2- (on the left side) and T_1-weighted (on the right side) scans of a cystic-solid thyroid nodule in the right thyroid lobe. In the picture on the left side a hypointense solid component (arrow) can be seen in comparison with the relatively hyperintense fluid. In the T_1-weighted picture on the right side, the lesion can not be recognized in the hypointense fluid.

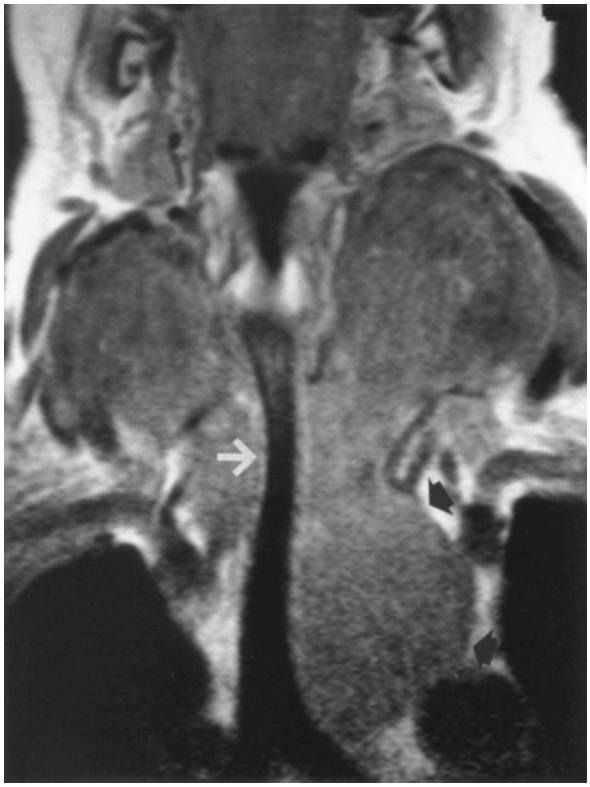

Fig. 31. Coronal T1-weighted MRI of a large multinodular goiter shows compression of trachea (white arrow) and left-sided substernal extension (black arrows).

availability, length of the procedure, need for preparation and patient cooperation – the examination cannot be carried through in 5–10% of adult patients due to claustrophobia – and anesthesia is required in early childhood [149]. Tissue movement decreases image quality, and calcifications are better seen with CT [150].

Indications for Thyroid MRI

MRI is rarely required to define anatomy and parenchyma of the thyroid gland itself, but is more useful in defining the exact extension of very large thyroid glands and large masses caused by lymphadenopathy, which may be difficult to achieve with US alone. Metastatic lymph nodes in the neck as well as invasion of the aerodigestive tract are also in the realm of MR imaging [142]. In this context, the extent of thyroid carcinoma can be determined preoperatively, which may be useful in planning surgery. Another potential implication of MRI is for the detection of the site of recurrent carcinoma in thyroglobulin-positive patients with normal clinical examinations. Features such as asymmetry, increased signal intensity in the thyroid bed, and invasion or displacement of adjacent tissue, as well as enlarged lymph nodes with increased signal intensity suggest recurrent carcinoma [151]. Additional gadolinium injection may be useful because enhancement is seen in recurrent carcinoma and also in metastatic nodes [152].

Acute suppurative thyroiditis and thyroid abscess are rare disorders and congenital pyriform fistula should be suspected, especially in case of recurrent infections on the left side. MRI or CT is valuable in addition to barium esophagography in the workup of such patients [153].

Normal Thyroid

On T_1-weighted images the normal thyroid gland has a nearly homogeneous signal with an intensity similar to that of the adjacent neck muscles (fig. 32) [154]. Air, blood, and vessels usually appear black. On T_2-weighted images, the normal thyroid gland has a greater signal intensity than the adjacent muscles. Blood vessels, lymph nodes, fat, and muscle are clearly identified and distinguished from the thyroid.

Developmental Defects

Ectopic thyroid tissue may be encountered in the tongue (foramen cecum), along the midline between the posterior tongue and the isthmus of the thyroid gland, but also in the oral cavity, lateral neck and mediastinum. Scintigraphy is the first-line imaging modality. MRI, however, is also useful as demonstrated in a small series of 21 patients with submucosal lesions in the base of the tongue [155]. MRI depicted lingual thyroid and additional ectopic thyroid tissue in the floor of

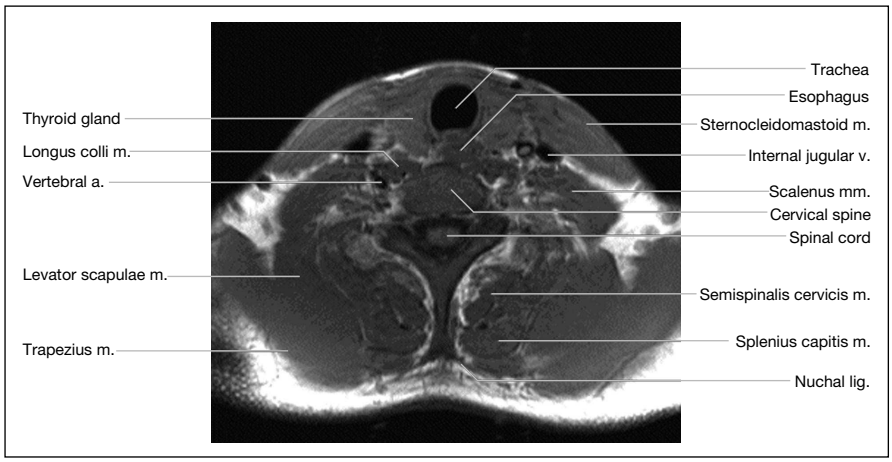

Fig. 32. Axial MRI of the neck showing a T_1-weighted image with a normal thyroid gland appearing homogeneous and with signal intensity similar to that of the adjacent neck muscles.

the mouth and lateral neck in concordance with the scintigraphic findings. Ectopic thyroid glands appear isointense or hyperintense relative to muscle tissue on T_1-weighted images and show slight to moderate contrast enhancement, and the T_2 signal appears low to intermediate. In the same study all ectopic thyroid tissue had well-defined margins on MRI and in case of ill-defined margins malignancy with invasion of adjacent structures was confirmed surgically [155]. Although rare, goiter and malignant tumors may develop in ectopic thyroid tissue [142].

Diffuse Thyroid Disease

In Graves' disease both T_1- and T_2-weighted images show a diffusely increased but slightly heterogeneous signal [156]. Dilated vessels within the thyroid can often be identified [157]. In autoimmune thyroiditis the thyroid appears heterogeneous on T_1-weighted images and often with diffusely increased signal on T_2-weighted images [157]. A morphological overlap on T_1- and T_2-weighted images is seen between patients with Graves' disease, subacute thyroiditis and Hashimoto's thyroiditis, but additional calculation of the diffusion coefficient can distinguish Graves' (highest values) from the other two [158]. In subacute thyroiditis T_1-weighted images demonstrate regions of abnormality with irregular margins and slightly high intensity while on T_2-weighted images, markedly increased intensity can be seen in the same sites [159].

Infiltration of adjacent neck structures and hypointensity on T_1- and T_2-weighted images are suggestive of Riedel's thyroiditis [160].

Nodular Thyroid Disease

Multinodular goiters have various degrees of heterogeneity and low to increased signal intensity on T_1-weighted images [154]. Focal hemorrhage and areas of cystic degeneration, often seen in multinodular goiters, are characterized by high signal intensity [157]. Nodules are better visualized on T_2-weighted images [157] and simple cysts show a homogeneous high-intensity signal (increases with increasing protein and lipid content) on both T_1- and T_2-weighted images. The MR characteristics of hyper- or hypofunctioning nodules do not differ. Hyperplastic-colloid nodules and benign adenomas appear round or oval with a heterogeneous signal equal to or greater than that of normal thyroid tissue [156].

No MRI characteristics accurately distinguish between benign nodules and carcinomas, although a nodule with a smoother, more uniform, and thicker capsule is more likely to be benign [161]. Thyroid carcinomas appear as focal or multifocal lesions of variable size, and iso- or slightly hyperintense on T_1-weighted images and hyperintense on T_2-weighted images. MRI is valuable to assess extracapsular spread, especially into the trachea, larynx, esophagus, vessels, and muscles [162].

The complete extent of larger lesions is – most often – better evaluated with MRI or CT than with US.

On MRI, metastatic lymph nodes can have low to intermediate T_1- and high T_2-weighted signal intensities or high T_1- and T_2-weighted signal intensities, the latter reflecting primarily a high thyroglobulin content. The metastatic nodes in papillary carcinoma may enhance markedly (hypervascular) [142].

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Thyroid and Other Autoimmune Diseases with Emphasis on Type 1 Diabetes Mellitus and Turner Syndrome

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Modern diabetes management facilitates normal growth and development in children and adolescents with diabetes. However, comorbidity including autoimmune disorders like autoimmune thyroiditis, adrenal insufficiency, or celiac disease may lead to disturbance of growth and pubertal development of children and adolescents with diabetes. Such comorbidity could also have a negative impact on metabolic control. Therefore awareness of these complications and monitoring are mandatory in clinical diabetes management [14]. If several of autoimmune diseases are present in a patient autoimmune polyglandular syndrome (APS) should be considered. APS type 1 also known as autoimmune polyendocrinopathy-candidiasis ectodemal dystrophy (APECED) is a rare syndrome that combines mucocutaneous candidiasis, hypoparathyroidism and adrenal insufficiency. Inheritance is autosomal-recessive with a mutation of the autoimmune regulatory gene (AIRE) on chromosome 21. In contrast, APS type 2 shows various combinations of adrenal insufficiency, autoimmune thyroid disease and type 1 diabetes. This syndrome with polygenetic inheritance has a prevalence of 1–2/100,000 [5, 13, 31]. For girls with Turner syndrome autoimmune hypothyroidism is an even more common comorbidity. Thyroid function should therefore performed routinely in the long term care of Turner patients. Additionally, there is evidence for a 4- to 8-fold higher incidence of celiac disease in females with Turner syndrome than in the non-Turner population. The prevalence of other autoantibodies to endocrine organs (pituitary, adrenocortical cells, gastric parietal cells) is not increased in Turner patients [10].

Thyroid Disease in Diabetes Mellitus

The most frequent autoimmune disease in type 1 diabetes affects the thyroid. The etiology of autoimmunity in pancreas and thyroid is a T cell-mediated disease and seems to be due to common genetic susceptibility. Two immune regulatory genes (HLA = human leukocyte antigen and CTLA-4 = cytotoxic T lymphocyte-associated protein 4) contribute to the susceptibility for both diseases [5, 23]. This locus, also known as the IDDM 12 gene, seems to play a major role in development of autoimmune polyglandular syndrome type 2 (APS-2).

Autoimmunthyroiditis describes a group of thyroid diseases with destruction of thyroid tissue due to an autoimmune reaction. Classification of these diseases is not consistent in the literature. Most frequently, Hashimoto thyroiditis with antibodies against thyroid antigens is found. These antibodies are directed towards thyroid peroxidase (TPO-Ab), thyreoglobulin (TG-Ab) and/or TSH-receptor antigen (TRAK).

Positivity for thyroid auto antibodies in children with type 1 diabetes shows considerable variability in different countries. Incidence and prevalence numbers vary between 3 and 50% [3, 18, 19, 33, 36] compared to a suggested rate of 3–10% in non diabetic children and adolescents [17, 26, 38]. The largest cohort analysis was published by Kordonouri et al. [19] reporting a rate of 21.6% of thyroid antibodies in a group of 7,097 children and adolescents with type 1 diabetes. In this study patients with antibody positivity were older, had longer diabetes duration and had developed diabetes later in life. 63% of patients with positive thyroid antibodies were female.

The majority of patients with positive thyroid antibodies have normal thyroid function. Elevated TSH levels as a marker for subclinical hypothyroidism are found in about 15% in the antibody positive patient group. Overt primary hypothyroidism due to autoimmune thyroiditis is seen in 3–5% of patients [3, 8, 19]. Clinical findings of hypothyroidism like goiter, weight gain, fatigue, cold intolerance and bradycardia are rare because of screening for TSH and autoantibodies in patients with type 1 diabetes (table 1).

In the study of Kaspers et al. [16], evidence for thyroid disease was significantly more often observed in patients when celiac disease was present (6.3 vs. 2.3%).

Since screening is both efficient and cost effective there is no controversy about thyroid antibody screening in patients with type 1 diabetes anymore. Screening is performed in our institution once a year. In case of significant antibody levels (especially thyroperoxidase antibodies) a longitudinal survey of diabetic children over 5 years showed a higher risk of later development of TSH elevation and subclinical or clinical hypothyroidism [18]. These data were confirmed by a recently published study from Australia over a follow-up period of 13 years [9]. Therefore, in patients with elevated TPO/TGA antibodies thyroid

Table 1. Prevalence of hypothyroidism or hyperthyroidism in patients with type 1 diabetes mellitus in different countries

Country	Number of patients (age, years)	Hypothyroidism male:female	Hyperthyroidism male:female	Follow-up years	Reference
UK	509 (16–45)	20 (3.9%) 1:5.6	8 (1.6%) 1:1	8	[20]
USA	58	18 (31%) 1:2.6	1 (1.7%)	18	[35]
USA	204, <20 years	28 (14%) 6 subclinical	18 (9%) 3 subclinical	–	[34]
Germany	216 (1–22)	8 (3.7%) 1:1.6 all subclinical	0	4–13	[18]
Italy	1,419 (1–18)	55 (3.9%)	0	–	[33]
Italy	212 (1.2–21)	3 (1.5%) 9 with thyroiditis in biopsy	1 (0.5%)	3–10	[25]

function (TSH and free T4) should be measured routinely. Ultrasound of the thyroid gland could provide further information on the development of Hashimoto's disease with typical patterns like increased volume of the gland and areas of lower echogenity within the thyroid. Hansen et al. [11] found sonographic abnormalities in 42% of children with type 1 diabetes in comparison to 11% in the control group. In long-term follow-up after 3 years prevalence of these sonographic findings increased up to 50% in diabetic patients. However, 9% of diabetic patients with abnormalities at baseline had a normal ultrasound of the thyroid at follow-up.

There is no consensus on the time point of introduction of treatment with thyroxin! In our opinion treatment with thyroxin is recommended in the case of subclinical or clinical hypothyroidism or significant antibody levels plus ultrasound findings.

The impact of subclinical hypothyroidism on metabolic control in children and adolescents with type 1 diabetes mellitus was studied by Mohn et al. [30]. In this retrospective case control study, 13 patients with subclinical hypothyroidism had significantly more symptomatic and severe hypoglycemic events during 12 months prior to the diagnosis of thyroid disease. There was no difference in HbA1c, insulin requirement or growth between the two groups as had also

been found in the cohort of Kordonouri et al. [19]. After introduction of thyroxin substitution the rate of hypoglycemia decreased rapidly and after 6 months there was no difference between the groups anymore.

Hyperthyroidism is less common than hypothyroidism in association with diabetes but still more common than in the general population. There is less published data available with a frequency of subclinical disease in about 2–3% and overt hyperthyroidism or thyrotoxicosis in only a few patients [25, 34, 35]. Hyperthyroidism may be due to Grave's disease or hyperthyroid phase of Hashimoto's thyroiditis. It should be considered if there is unexplained weight loss with normal appetite, agitation, sweating, tachycardia, tremor or unexplained problems with metabolic control.

There is no difference in treatment strategies between patients with diabetes and the nondiabetic population [20]. Therefore, antithyroid drugs still remain the initial treatment of choice. However, in non-European countries (especially the USA) radioactive iodine is used more frequently. There is no long-term safety data available until now and radioactive iodine has not been shown to be superior to antithyroid drug treatment at the moment.

Thyroid Disease in Turner Syndrome

An association between Turner syndrome (TS) and thyroid disease was first suggested by Atria et al. [1] in 1948 when they reported post mortem findings of a small thyroid gland with lymphocytic infiltration in a young woman with Turner syndrome.

Many authors reported on a higher prevalence of hypothyroidism and an association with positive thyroid antibodies in TS patients (table 2) [2, 6, 7, 24, 29, 32, 37]. Hypothyroidism is found in up to 35% of TS patients. Thyroid autoimmunity seems to be even more common in females with Turner syndrome with a prevalence of up to 52% [15]. A positive family history was reported by Wilson et al. [37]. This group found an increased incidence of thyroid antibodies in patients with TS and their first degree relatives. The incidence of thyroid antibodies was 30% in patients compared to 1.7% in an age matched control group and 22% in the mothers of the TS patients (vs. 6.6% in the controls). Larissa et al. [21] found a preferential parental segregation of autoimmunity in their study.

There is no clear explanation for the higher frequency of thyroid autoimmunity in Turner syndrome. The positive family history could give a link to genetic co-etiology. HLA association is discussed very controversially in the literature. In the Italian study of Larizza et al. [21], an association of HLA-DR7/DQ2 and DR7/DQ9 haplotypes with autoantibodies was detected. These haplotypes have been reported to be associated with autoimmune disorders. Other

Table 2. Prevalence of hypothyroidism or hyperthyroidism in patients with Turner syndrome in different countries

Country	Number of patients (age)	Hypothyroidism	Autoantibodies	Hyperthyroidism	Reference
Greece	84 (1–19)	20 (24%)	35 (42%)	2 (2.5%)	[24]
UK	60	–	18 (30%)	–	[37]
UK	145 (16–52)	22 (15%)	60 (41%)	1 (0.7%)	[7]
Sweden	91 (0–37)	23 (25%) follow up: 34 (37%)	25 (28%)	3 (3.3%)	[6]
Brazil	71 (0–20)	11 (15.5%)	17 (23.9%)	–	[29]
Italy	478 (3–25)	29 (6.1%) 27 subclinical	106 (22.2%)	3 (0.6%)	[32]
Germany	120 (16–23)	42 (35%)	43 (35.8%)	–	[2]
Total	1,049	147/989 (14.9%)	304 (29%)	9/798 (1.2%)	

chromosomal aberrations like Down syndrome also tend to be associated with thyroid autoimmunity.

In order to evaluate the functionality of the hypothamic-hypophyseal-thyroid axis, Mazzilli et al. [28] studied 27 children and adolescents with TRH test and compared these data with an age- and sex-matched control group. There were no differences between the two groups in TSH levels or areas under the curve after the injection of TRH.

The age of onset of thyroid abnormalities has been reported to a variable degree in the literature. Many authors reported (laboratory) onset before the age of 5 years [29, 32]. As seen in the normal population there is a rise in incidence of thyroid autoimmunity and hypothyroidism until puberty [29, 32]. The annual incidence is estimated to be 3.2% in females with Turner syndrome [6].

The clinical findings in Turner females in comparison to laboratory abnormalities were examined in a large cohort of 478 patients by Radetti et al. [32]. Of the 106 patients with positive thyroid antibodies 49 patients had a positive ultrasound indicating autoimmune thyroid disease. Of those 49 patients 17 were euthyroid, 27 had compensated subclinical hypothyroidism, 2 were hypothyroid and 3 were hyperthyroid. Goiter was found on clinical examination in 16 patients. There were no symptoms of hypothyroidism in any patient. However,

in the three hyperthyroid patients irritability, sweatiness, diarrhea, weight loss, tremor and sleep disorders were found [33].

Many studies tried to find an association between clinical symptoms and the karyotype of X-chromosome [2, 7, 29, 33]. The risk of developing autoimmune thyroid disease may be particularly high in patients with Turner syndrome with an X-isochromosome [7, 21]. Ivarson et al. [15] also found a higher incidence of thyroid autoantibodies in females with isochromosome X and in patients with 45 X0 compared to mosaicism or structural disturbances of the X chromosome. Other authors [6, 29, 33] could not find such associations. The risk of developing hypothyroidism therefore appears to be high for all TS women, independent of the karyotype.

Short stature in Turner syndrome can be successfully treated with growth hormone. Normalization of height can be achieved when growth hormone treatment is started at a young age and pharmacological doses are applied [4]. However, the growth hormone response in patients with TS seems to be rather variable. A transient alteration of thyroid status with a slight decrease of T4 levels after introduction of growth hormone administration has been described [27]. Bettendorf et al. [2] found an association of the gain over projected height (PAH) after growth hormone treatment with autoimmunity. The PAH was 6.56 cm in TS patients without autoantibody titers while patients with positive TPO/TG or tissue-transglutaminase antibodies had a PAH of only 4.16 cm ($p < 0.01$ cm). This could indicate an association of growth hormone effects with autoimmunity and especially subclinical hypothyroidism. On the other hand, a higher risk to develop thyroid autoimmunity due to growth-promoting treatment has not been found.

In conclusion, screening for thyroid function and thyroid autoimmunity in females with Turner syndrome is recommended from age 4–5 years upward. Measurement of TSH and free T4 should be conducted annually. There is no consensus whether or not autoantibodies should also be screened for. In our opinion, in patients at high risk for the development of hypothyroidism elevated autoantibody titers may precede the development of hypothyroidism.

Treatment is not different from the guidelines for the general population. However, one should keep in mind the association of thyroid function and autoimmunity to growth hormone response in some studies. Therefore, treatment of subclinical hypothyroidism should immediately be introduced.

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Thyroid and Trace Elements in Children and Adolescents

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Iodine Deficiency and Supplementation

Iodine deficiency produces a spectrum of disorders – endemic goiter, hypothyroidism, cretinism, and congenital anomalies – that are termed the iodine deficiency disorders. Despite substantial global progress against iodine deficiency, it is estimated that 750 million people worldwide, or approximately 15% of the population, remain iodine deficient and goitrous. In iodine-deficient areas, multiple nutritional and environmental influences contribute to the prevalence and severity of iodine deficiency disorders. Even nowadays, iodine nutrition in children and iodine supplementation of pregnant women remains a medical challenge and ought to be optimized. In Europe, nearly two-thirds of the populations live in countries that are iodine deficient. Damage to reproductive function and to the development of the fetus and newborn is the most important consequence of iodine deficiency. The fetal brain is particularly vulnerable to maternal hypothyroidism in iodine deficiency, and iodine deficiency is the leading cause worldwide of mental retardation. Even mild or sub clinical maternal hypothyroidism during pregnancy can impair mental development of the newborn. The recommended daily intake during pregnancy from the World Health Organization/United Nations Children's fund/International Council for Control of iodine deficiency disorders (WHO/UNICEF/ICCIDD) is 0.2 mg, while the United States Institute of Medicine (IOM) suggests a recommended dietary allowance (RDA) during pregnancy of 0.22 mg or 220 μg. The 'VERA' study in Germany reported a median (range) iodine intake of 100 (33–284) μg/day in 19- to 24-year-old women. Recent studies reporting low urinary iodine in pregnant women in Europe reinforce the dietary intake data. Urinary iodine excretion is an accurate indicator of dietary iodine intake as more than 90% of ingested

iodine is excreted in the urine and urinary iodine is highly sensitive to recent changes in iodine intake. In eight iodine-deficient countries, urinary iodine concentrations indicate that iodine intakes are clearly inadequate. Studies of thyroid size in pregnancy measured by ultrasonography also indicate iodine nutrition is suboptimal in much of Europe. In countries affected by mild or moderate iodine deficiency (e.g. Germany, Belgium, Italy, Denmark), thyroid volume increases 14–30% during pregnancy, while in iodine-sufficient countries (Finland, Holland), there is no increase in thyroid volume during pregnancy.

Six randomized, controlled trials of iodine supplementation in pregnancy have been published, involving 450 women with mild-to-moderate iodine deficiency. In all six trials, supplementation resulted in a significant increase in maternal urinary iodine. Iodine doses varied between 50 and 230 μg/day, and the data indicate no clear dose-response relationship for urinary iodine, TSH, thyroglobulin, thyroid hormone or thyroid volume. For the newborn, most data suggest supplementation is safe and efficacious. The studies also suggest an increase in newborn thyroid volume and thyroglobulin can be prevented or minimized by supplementation, which has little or no impact on newborn total or free thyroid hormone levels. There are no clinical data on the effect of supplementation on birth weight or prematurity, and no data on long-term outcomes, such as thyroid autoimmunity, or child development.

The labeled iodine content of multivitamin/minerals marketed as prenatal supplements in Europe varies widely. Many commonly used products contain no iodine, while others contain 200 or even 300 μg. The actual iodine content in supplements is determined not only by the original amount added, but also by the stability of the compound, the time elapsed since manufacture, and the conditions under which the product is stored. The iodine content of kelp supplements, a popular supplemental form, is highly variable. The median iodine content (range) of the manufacturer's recommended daily supplement regimen was 1,005 μg (210–3,840 μg) or 1 mg. The mean measured content (as a percent of declared content) was 137% (45–914%). For half of the kelp supplements, the manufacturer's recommended daily dose was greater than 1,100 μg/day, the recommended safe upper limit for pregnancy. In addition, bioavailability of iodine from supplements has not been tested. Bioavailability can be influenced by the physical form of the product, e.g. tablets vs. gelatin capsule, the substance used in coating and thickness of coat, the amount of pressure used to form the tablet, the disintegration and dissolution of the tablet, and other nutrients or substances present which may interfere with bioavailability. Until recently, there was no specific European Union regulation of multivitamin/mineral supplements. They were classified as foods, and had to comply with relevant EU food legislation and individual member state's internal legislation. In 2002, the European Parliament approved a common position which states that the label of the supplement must contain clear

instructions for daily dosage, and a warning about possible health risk from excess use. Provisions specific for iodine state: supplements may contain iodine; the amount present should be labeled in μg; the only iodine compounds permitted are potassium iodide, potassium iodate, and sodium iodate.

Finally, 26 prospective controlled trials regarding iodine deficiency in childhood and related disorders, assessing 29,613 children, were recently reviewed. The results suggest that iodine supplementation, especially iodized oil, is an effective means of decreasing goiter rates and improving iodine status in children. Indications of positive effects on physical and mental development and mortality were seen. Adverse effects, noted in 1.8% only of the children investigated, were generally minor and transient. Results for differences in cognitive and psychomotor measures were mixed, with studies showing a positive intervention effect. Most studies showed a significant increase in urinary iodine excretion and levels recommended by the WHO were reached in most cases after iodine supplementation [1–20].

Selenium Deficiency and Supplementation

The essential trace element selenium is involved in thyroid hormone synthesis, metabolism and action. In several regions of the world people are exposed to inadequate selenium supply because selenium contents of surface soils have been depleted by erosion and glacial washout similar to iodine. Therefore, plant and animal food chains contain inadequate amounts of both of these elements. Deficiencies of selenium and iron can act in concert with iodine deficiency to impair thyroid metabolism and modify the response to prophylactic iodine. The effects of selenium and iron status on iodine and thyroid metabolism share certain parallels. Selenium deficiency reduces the activity of the selenium-dependent deiodinase and peroxidase enzymes and thereby impairs thyroid metabolism in iodine-deficient populations [21–24]. Similarly, iron deficiency reduces heme-dependent thyroperoxidase activity, impairs thyroid metabolism, and influences the response to iodine in iodine deficiency disorders.

Combined selenium and iodine deficiency are etiologic factors involved in the pathogenesis of myxedematous cretinism in central Africa. Additional factors such as dietary consumption of goitrogens, e.g. thiocyanate contained in or released from staple foods of these regions, may contribute to the selenium and iodide interaction. In a longitudinal intervention trial in goitrous, nonanemic children living in an iodine- and selenium-deficient area in Cote d'Ivoire, oral iodized oil was administered and thyroid size and thyroid hormone metabolism was analyzed. Positive thyroid response to iodine supplementation, decreased thyroid volume and serum TSH, at 50 weeks was significantly impaired

depending on the extent of selenium deficiency, but no adverse effect of administration of iodized oil were observed. In rodent models, studies revealed necrosis and infiltration by mononuclear inflammatory cells in the affected selenium deficient thyroid glands after administration of high doses of iodide. No such destructive processes were observed when high iodide doses were given to selenium adequate rats.

Relations between selenium and iodine status and thyroid hormone levels were also examined in goitrous children in comparison to a control group. Blood selenium and plasma glutathione activities were lower in the goitrous group than in the controls but differences of free T4 and TSH levels were only identified in girls belonging to the low and high selenium quartiles without evidence for altered iodine status. Provided iodine supply reaches minimal critical levels or low intake as in many parts of Europe additional selenium supplementation is not harmful as described in Zaire where selenium-enhanced degradation of thyroid hormones by deiodination occurred in treated children. Thus, low-dose selenium administration does not cause thyroid insufficiency in subjects with mild iodine deficiency.

It is known that selenium intake and plasma selenium levels decline in infants fed selenium-poor milk formula before meat-derived food additives are fed as 'beikost' compared to breastfed babies. Nevertheless, selenium supplementation appears not indicated in premature babies provided adequate nutrition is achieved. However, during pregnancy and the postpartum period the maternal plasma selenium status is decreasing because of considerable transfer of the trace element to the growing fetus via the placenta (1–4 μg of selenium per day) and via breast milk (3–6 μg of selenium per day) to the feeding baby in addition to enhanced maternal urinary losses. Therefore, adequate supplementation of both trace elements to the pregnant and lactating mother is indicated in areas of limited or inadequate supply of selenium and/or iodine. Selenium supplementation in children with congenital hypothyroidism on T4 treatment did not affect serum thyroid hormone concentrations or the impaired T3/T4 ratio but decreased thyroglobulin levels and normalized the TSH difference observed between matched euthyroid controls and children with congenital hypothyroidism, indicating improvement of the central thyroid hormone feedback and decreased thyroid stimulation.

Iron Deficiency and Supplementation

Worldwide, more than two billion people – mainly children and young women – are iron deficient. In developing countries, 40–50% of school-age children are anemic, approximately 50% because of iron deficiency. Iron and

iodine deficiencies often coexist; in regions of West and North Africa, 20–30% of school-age children suffer from both goiter and iron-deficiency anemia. Data from animal studies indicate that iron deficiency, with or without anemia, impairs thyroid metabolism. Iron deficiency also impairs thyroid metabolism in human trials. Overall, these studies suggest that iron deficiency blunts the thyrotropic response to exogenous TRH; lowers serum T3 and T4 levels, and lowers utilization of thyroid hormones [25–30].

Clinical trials were done in primary schools in an area of endemic goiter in the mountains of Cote d'Ivoire. At that time, the median urinary iodine concentration and the goiter rate by palpation in school-aged children in this region were 28 μg/l and 45%, respectively, indicating moderate to severe iodine deficiency. Goitrous, school-aged children were divided into two groups: nonanemic or with iron deficiency anemia. All children received an oral dose of 0.4 ml iodized poppy seed oil (Lipiodol®) containing 200 mg or 0.2 g of iodine. At 15 and 30 weeks, thyroid volume was significantly reduced in the nonanemic group compared to the group with iron deficiency. A sharp difference in goiter prevalence was apparent at 15 and 30 weeks, when goiter rates were 62 and 64% in the anemic group but only 31 and 12% in the nonanemic children. Median TSH values were lower ($p < 0.01$), and T4 values were greater ($p < 0.01$) in the nonanemic children. Thus, in this study, both anatomic (thyroid size) and biochemical (TSH, T4) measures indicated that treatment with iodized oil significantly improved thyroid function in the nonanemic children compared to the children with iron deficiency. Goiter prevalence in the anemic children group was reduced after iron supplementation from 64 to 20% at 65 weeks.

In a second study, goitrous, iron-deficient children randomly received either oral iron sulfate (60 mg elemental iron) 4 tablets per week for 16 weeks or placebo. Thyroid volume was significantly reduced in the iron-treated group (mean % delta thyroid volume –22.8 (SD 10.7%) compared to placebo (-12.7%, $p < 0.02$). The final study was done in an area of endemic goiter in northern Morocco. In a 9-month, randomized, double-blind trial, 6- to 15-year-old children were given iodized salt (25 μg iodine per gram of salt) or dual fortified salt with iodine (25 μg iodine per gram of salt) and iron (1 mg iron per gram of salt) as ferrous sulfate encapsulated with partially hydrogenated vegetable oil. In the children group with dual fortified salt, hemoglobin and iron status improved significantly compared to the iodized salt group. Addition of encapsulated iron to iodized salt improved the efficacy of iodine in goitrous children with a high prevalence of anemia. Taken together, these data demonstrate that iron deficiency anemia blunts the efficacy of iodine prophylaxis while iron supplementation improves the efficacy of oral iodized oil and iodized salt in goitrous children with iron deficiency anemia. This suggests that

a high prevalence of iodine deficiency anemia among children in areas of endemic goiter may reduce the effectiveness of iodized salt programs. Iron deficiency anemia may have a greater impact on iodine deficiency than previously described goitrogens because of its high prevalence in vulnerable groups. The data also argue strongly for the dual fortification of salt with iodine and iron, not only to reduce the prevalence of iron deficiency but also to increase the efficacy of the iodine in populations that are both iron deficient and goitrous.

Vitamin A Supply and Zinc Status

In developing countries, children are at high risk for vitamin A deficiency, a leading cause of preventable blindness in children and increased morbidity and mortality from serious infections. In rural Cote d'Ivoire, 32–50% of school-age children suffer from both vitamin A deficiency and goiter. In northern Morocco, 41% of children have vitamin A deficiency, and 50% are goitrous. In animals, vitamin A deficiency has multiple effects on thyroid metabolism: it decreases thyroidal iodine uptake, impairs thyroglobulin synthesis, and increases thyroid size. In the periphery, vitamin A deficiency increases free and total circulating thyroid hormone, and vitamin A status may modulate T4 feedback of TSH secretion. Finally, vitamin A deficiency in rats increases pituitary TSHβ mRNA and TSH secretion; both return to normal after treatment with retinoic acid [31].

In a double-blind, randomized clinical trial, children with vitamin deficiency were given iodized salt and either vitamin A or placebo at 0 and 5 months. At baseline, increasing severity of vitamin A deficiency was a predictor of greater thyroid volume and higher concentrations of TSH and thyroglobulin. In children with vitamin A deficiency, the odds ratio for goiter was 6.51 (95% CI 2.94–14.41). Severity of vitamin A deficiency was also a strong predictor of higher concentrations of total T4; the odds ratio for hypothyroidism in vitamin A deficiency was 0.06 (95% CI 0.03–0.14). During the intervention, mean thyroglobulin, median TSH, and the goiter rate significantly decreased in the vitamin A-treated group compared with those in the placebo group. The findings indicate that vitamin A deficiency in severely iodine deficient children increases TSH stimulation and thyroid size and reduces the risk for hypothyroidism. This effect could be due to decreased vitamin A-mediated suppression of the pituitary TSHβ gene. Therefore, in children with iodine and vitamin A deficiencies receiving iodized salt, concurrent vitamin A supplementation improves iodine efficacy.

Finally, zinc status also affects thyroid function [32, 33]. For example, in zinc deficient rats decreased 5′ deoidinase activity, lower T3 and free T4 serum

concentrations and marked alterations of follicle cellular architecture including signs of thyroid cell apoptosis were found.

Conclusions

Despite significant progress, deficiencies of iodine and other trace elements, e.g. selenium and iron, remain major public health problems affecting more than 30% of the global population. These deficiencies often coexist in children. Recent studies have demonstrated that a high prevalence of iron deficiency among children in areas of endemic goiter may reduce the effectiveness of iodized salt programs. These findings argue strongly for improving iron status in areas of overlapping deficiency, not only to combat anemia but also to increase the efficacy of iodine prophylaxis. The dual fortification of salt with iodine and iron may prove to be an effective and sustainable method to accomplish these important goals. Iron deficiency impairs thyroid hormone synthesis by reducing activity of heme-dependent thyroid peroxidase. Iron-deficiency anemia blunts and iron supplementation improves the efficacy of iodine supplementation. Combined selenium and iodine deficiency leads to myxedematous cretinism. The normal thyroid gland retains high selenium concentrations even under conditions of inadequate selenium supply and expresses many of the known seleno-cysteine-containing proteins. Among these selenoproteins are the glutathione peroxidase, deiodinase, and thioredoxine reductase families of enzymes. Adequate selenium nutrition supports efficient thyroid hormone synthesis and metabolism and protects the thyroid gland from damage by excessive iodide exposure. In regions of combined severe iodine and selenium deficiency, normalization of iodine supply is mandatory before initiation of selenium supplementation in order to prevent hypothyroidism. Selenium deficiency and disturbed thyroid hormone economy may develop under conditions of special dietary regimens such as long-term total nutrition, or may be the result of imbalanced nutrition in children.

Iodine deficiency during pregnancy adversely affects thyroid function of the newborn and mental development of the offspring and these adverse effects can be prevented or minimized by supplementation. Although most women in Europe are iodine deficient during pregnancy, less than 50% receive supplementation with iodine. There are no data on the effect of iodine supplementation on long-term child outcomes. The iodine content of prenatal supplements in Europe varies widely; many commonly used products contain no iodine. This is why the European Union is developing legislation to establish permissible levels for iodine in food supplements. Therefore, in most European countries, pregnant women and women planning a pregnancy should receive an iodine-containing supplement (approximately 150 μg daily). Kelp and seaweed-based

products, because of unacceptable variability in their iodine content, should be avoided. Prenatal supplement manufacturers should be encouraged to include adequate iodine (150 μg/day) in their products. Also, professional organizations should influence EU legislation to ensure optimal doses for iodine in prenatal vitamin-mineral supplements.

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