Zvi Laron
John J. Kopchick
Editors

Laron Syndrome – From Man to Mouse

Lessons from Clinical and Experimental Experience

Springer

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(Editors)

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"I dedicate this book to the patients with Laron syndrome who heroically accepted many investigations to clarify their enigmatic disorder and subsequently to determine the therapeutic response to a new drug and to my devoted associates in our clinical and laboratory research"

Zvi Laron

"I would like to thank my 'extended laboratory family, including undergraduate and graduate students, technicians, postdoctoral fellows, visiting scientists, and collaborators worldwide'"

John J. Kopchick

Preface

It has been 50 years since the first family with Laron syndrome (LS, primary growth hormone (GH) insensitivity) was referred to the newly established Pediatric Endocrine Clinic at the Beilinson Hospital, Petah Tikva, Israel, a referral center now located at the Schneider Children's Medical Center on the same campus. Since then, 64 patients have been diagnosed by the same team, investigated, and the majority followed by them, most at regular intervals, throughout childhood into adult age. This was the reason to base this book on the data accumulated from this cohort of patients forthwith called the Israeli cohort.

It was also our privilege to be the first to determine its etiopathology and the first to study the effects of insulin-like growth factor-I (IGF-I) replacement therapy in these patients. The great amount of data accumulated during half a century in the large Israeli cohort of untreated and IGF-I treated LS patients is a unique source of knowledge which we thought should be shared and presented in a united manner.

The clinical portion of this book represents a compendium of the Israeli team's experience with LS from discovery, the struggle to define its pathogenesis, to determine the consequences if not treated by IGF-I, and the results of long-term IGF-I treatment. Reference to studies by other investigators are included when found appropriate.

During this 50 year-long journey, LS has proven to be a unique model to learn the effects of congenital IGF-I deficiency, the pharmacology of IGF-I, and the GH-IGF-I interrelationships. The fact that LS is a condition in which the action of pituitary GH is excluded permits the comparison between isolated GH deficiency and IGF-I deficiency as well as the comparison between the responses to IGF-I and hGH replacement therapies. To enable the comparison of findings in the same patient, we have identified certain patients by their initials.

In an attempt to generate an animal model of LS (GH resistance/insensitivity and IGF-I deficiency), the GH receptor gene disrupted the "knock-out" mouse (or Laron Mouse) was produced in the Kopchick laboratory in 1991. Experiments that could not be carried out in humans could now be advanced in this GH insensitive mouse. We are happy to review the results of studies using these mice on aging, adipose tissue, reproduction, metabolism, and cancer. Also, tissue-specific effects on the brain, heart, and bone are reviewed.

Thus, this book is a combination of data obtained in man on the Israeli cohort and the GHR−/−mouse. In each scenario, the action of GH is attenuated resulting in low levels of IGF-I. Similarities and differences between the mouse and human data are pointed out in Chapter 59. The data are both of academic as well as of practical clinical importance.

Professor Laron acknowledges all the early collaborators in the clinical studies, especially Prof. Athalia Pertzelan, Prof. Rivka Kauli, Dalia Peled, RN, Dr. Beatrice Klinger and Avinoam Galatzer, MA, (deceased), and Prof. Liora Kornreich. The contributions of the collaborators in our laboratory were crucial in elucidating the pathophysiology of the disease: immunology (Sara Assa, PhD), GH (Ruth Keret, MSc), IGF-I, and GHBP (Aviva Silbergeld, MSc), GH receptor (Rina Eshet, PhD) as well as our more recent collaborators in genetics and cancer (Orit Shevah, MSc) and (Pearl Lilos, MA, statistician). William H. Daughaday provided invaluable help in the early IGF-I (somatomedin-A) measurements and in the diagnosis of LS. John S. Parks collaborated in the early genetic evaluation. Thanks also to our coauthors of the present text and Mrs. Gila Waichman and Mrs. Rachel Ronen (Endocrinology and Diabetes Research Unit) for their technical assistance in the preparation of the clinical manuscripts and Ms. Irit Lis, Ms. Shlomit Offman, and Mr. Howard Martel from the Medical Photography and Graphic Department, Rabin Medical Center, for their tremendous help during many years. We also wish to acknowledge the generous supply of IGF-I from Fujisawa Pharmaceuticals, Osaka, Japan.

Professor Kopchick would like to cite his many colleagues over the years who helped with the work in the GH area. In particular, he is extremely proud of a young graduate student, Yihua Zhou, who first generated the mouse. Yihua went on to receive his PhD in Professor Kopchick's laboratory and MD degree from Washington University in St. Louis. Additionally, Professor Kopchick would like to recognize his many students (both graduate and undergraduate), technicians, postdoctoral fellows, faculty colleagues, and visiting scientists who helped with the work. Also, through scientific collaborations involving this mouse, the Ohio University group has been able to make many international friends and colleagues, most of whom are cited in the following chapters. If for some reason we "missed" a publication, we are very sorry. Finally, Professor Kopchick would like to acknowledge the many funding sources that helped advance our studies including the Ohio Eminent Scholar Program (that includes a gift from Milton and Lawrence Goll), which provided funding for his endowed Professorship; and The Edison Biotechnology Institute, Molecular and Cellular Biology Program, Biomedical Sciences Department in the College of Medicine, Diabetes Research Initiative, and BioMolecular Innovation and Technology Partnership at Ohio University; NIH; USDA; and several corporate sponsors including Pfizer, Merck, Sensus, and DiAthegen.

Last but not least, we wish to acknowledge the many people from Springer-Verlag GmbH, Berlin/Heidelberg, who assisted in the production of the book.

Petah Tikva, Israel — Zvi Laron
Athens, Ohio, USA — John J. Kopchick
December 2009

Contents

Part I

Clinical Aspects

History of the Israeli Cohort of Laron Syndrome Patients (1958–2009)

1

Zvi Laron

Core Message

› A concise history of the discovery of the Laron syndrome and the technological milestones enabling the elucidation of its etiopathology.

1.1 Introduction

In 1958, at a time when no radioimmunoassay for human growth hormone (hGH) was as yet available, three siblings (2 males, 1 female) from a consanguineous Yemenite Jewish family (the grandparents were first cousins) were referred to our newly established pediatric endocrine clinic. Their ages were 3.5, 1.5 years (Patients S.R. and SSi), and a newborn baby (SSh) (Fig. 1.1, Pedigree, see Chap. 5). They had five older siblings of normal stature (Laron et al. 1996).

The clinical history, appearance, and laboratory findings resembled those found in children with isolated growth hormone deficiency (IGHD) (Laron 1983) (Fig. 1.2). When radioimmunoassays for GH became available (Glick et al. 1963; Laron and Mannheimer 1966), we were astonished to find that their serum GH levels were very high. A short while thereafter, we were able to assemble 22 patients, all products of consanguineous Jewish families originating from mid-Eastern countries or North Africa (Laron et al. 1968) (Fig. 1.3). The striking finding was their typical appearance and the great resemblance between patients (Fig. 1.4).

Progressively more and more patients were referred to us comprising also of Moslem and Christian origin. It lasted 20 years from the first referrals until we were able to show that the pathogenicity of this disease is due to a lack of IGF-I (somatomedin A) generation (Daughaday et al. 1969; Laron et al. 1971) shown subsequently to be caused by a failure in the GH receptors in the liver (Eshet et al. 1984). Further 5 years of advances in laboratory technology were needed to permit the identification of the molecular defects in the

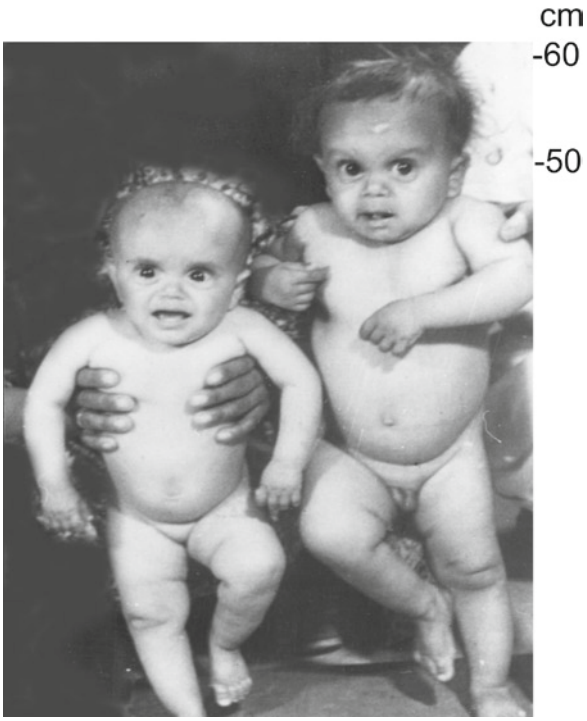

Fig. 1.1 Two of the 3 first patients with Laron syndrome (LS) referred to us in 1958. The girl (SR), 1.5 years old; and the boy (SSi), 3.5 years old. Note obesity, sparse hair, and saddle nose. Reproduced with permission from Laron (2004)

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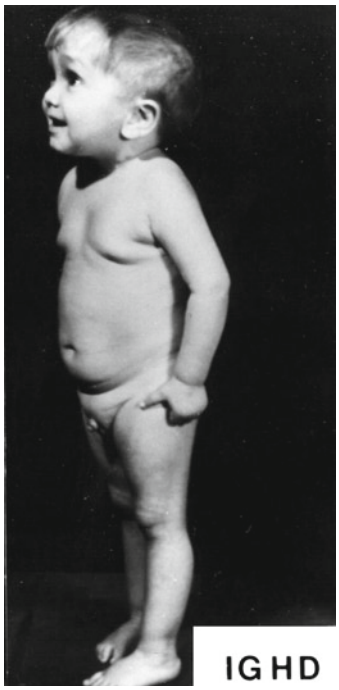

Fig. 1.2 A 4-year-old boy with isolated congenital GH deficiency (IGHD) due to GH gene deletion. Note resemblance with boy in Fig. 1.1: dwarfism, protruding forehead, obesity, and small penis

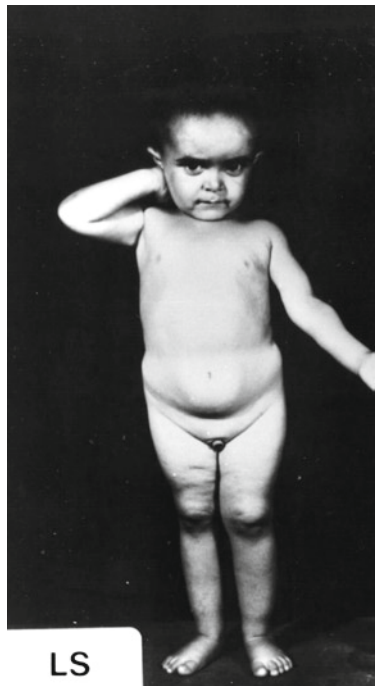

Fig. 1.4 Typical appearance of a 4-year-old boy with LS. Note obesity, protruding forehead, and saddle nose (YG)

Fig. 1.3 A group of LS patients (*front row*) with two fathers (extreme *left* and extreme *right – second row*) with four members of the medical team. The father on the *right* is himself a patient (MeS)

hGH receptor gene (Amselem et al. 1989; Godowski et al. 1989).

About the same time, IGF-I (somatomedin A) was synthesized (Niwa et al. 1986), and we were the first to administer IGF-I to children and initiate clinical trials in children with Laron syndrome (LS) with the new hormone (Laron et al. 1988; Laron et al. 1990; Laron et al. 1991; Laron et al. 1992). Over the years, further patients with this syndrome were referred to us. In 2009, our cohort consisted of 64 LS patients (33 males and 31 females) (Table 1.1 and Fig. 1.5), of these 60 living.

This forthwith called the "Israeli cohort" consists of patients residing in Israel at time of referral, of patients from other countries examined and followed in our clinic, and of patients seen or consulted by Z.L. and followed in collaboration with local physicians. Ten (possibly 11) patients are married, and 9 married couples have 20 children. The countries of origin of the patients are shown in Table 1.2.

Table 1.1 The Israeli cohort of 64 patients with Laron syndrome (2009)

	Males	Females
Total number[a]	33	31
Age over 30 years	12	17
Married	5	5
Have children	9	11

[a]Including four deceased

After our publications, patients with LS were diagnosed in many parts of the world, the majority of Mediterranean, Mid-Eastern or South Asian origin, or descendants from these geographical regions (Rosenfeld et al. 1994; Savage et al. 2006). Few patients have been diagnosed also in Japan (Iida et al. 1998) and China (Chen et al. 2003). The estimated number of LS patients in the world ranges between 300 and 500.

Table 1.2 Countries of origin at referral of patients with Laron syndrome

	Female	Male	Total
Israel	17	13	30
Palestine	1	4	5
Jordan	–	4	4
Lebanon	2	1	3
Iran	2	3	5
Malaya	–	2	2
Malta	1	–	1
Greece	1	–	1
Italy	4	–	4
Argentina	–	1	1
Ecuador	1	4	5
Peru	1	2	3
	30	34	64

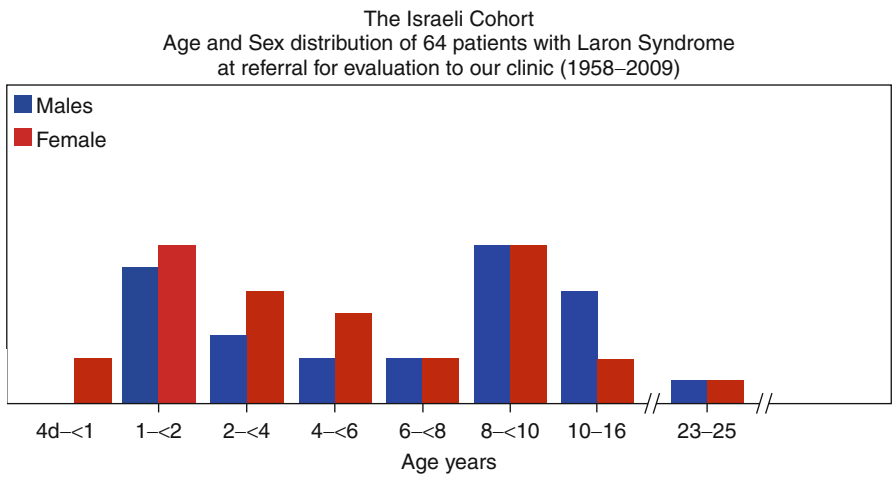

Fig. 1.5 Age and sex at referral of 64 patients with LS to the Beilinson-Schneider Pediatric Endocrinology Clinic

Table 1.3 Milestones in the description and elucidation of the pathology of the Laron syndrome

Description	References
Description of dwarfism with high serum GH levels	Laron et al. (1966)
Absence of IGF-I response to hGH	Laron et al. (1971)
Absence of GH binding to liver membranes	Eshet et al. (1984)
Description of serum GHBP	Herington et al. (1986)
Description of serum GHBP	Baumann et al. (1986)
Biosynthesis of IGF-I	Niwa et al. (1986)
Cloning of the GH receptor (GH-R)	Leung et al. (1987)
Initiation of IGF-I treatment in children	Laron et al. (1988)
Deletion of exons in GHR	Godowski et al. (1989)
Missense mutation in the GHR	Amselem et al. (1989)
Post-GHR defect	Laron et al. (1993a)
Asp 162His defect in dimerization	Duquesnoy et al. (1994)
GH-R knock-out mouse	Zhou et al. (1997)
Intronic mutation with high GHBP	Silbergeld et al. (1997)

It could be that there exist isolates with so far undiagnosed patients, and "de novo" mutations can be expected anywhere.

The main milestones in the history of this syndrome are shown in chronological order in Table 1.3.

1.2 Nomenclature

The name of the syndrome underwent several changes along its study: "genetic pituitary dwarfism" with high serum GH (Laron et al. 1966) was changed to Laron dwarfism (Elders et al. 1973); Laron-type dwarfism (Laron 1984) until it reached its present name "LS" (Laron syndrome – OMIM#262500) (Laron and Parks 1993). Other terms are growth hormone insensitivity (GHI) (Rosenfeld et al. 1994) or growth hormone receptor deficiency (GHRD) (Rosenbloom et al. 1999), the latter being an incorrect term as the receptor gene exists in a pathological form. Because GH insensitivity can be primary or secondary, a consensus classification and nomenclature of GH insensitivity states have been published in 1993 (Laron et al. 1993b). An updated version is shown in Table 1.4. In this book describing classical LS, congenital (primary) GH insensitivity or its shortened form "LS" is being used.

Table 1.4 Classification of hGH insensitivity (resistance)

Primary hGH Insensitivity (resistance) syndromes (hereditary and/or congenital conditionsx)
Laron syndrome
hGH receptor defects (classical Laron syndrome; OMIM#262500) hGH signal transduction defects (postreceptor defects, OMIM#245590)
Primary defects involving IGF-I
Abnormalities of the IGF-I gene IGF-I receptor defects (IGF-I insensitivity)
Secondary GH insensitivity(resistance) diseases (acquired conditions, sometimes transitory)
Circulating antibodies to hGH that inhibit GH action (hGH gene deletion patients treated with hGH) Antibodies to the hGH receptor Malnutrition Liver disease Uncontrolled diabetes mellitus Other conditions

hGH human growth hormone

Modified from Laron et al. (1993b)

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Early Investigations: Characterizations of the Circulating Growth Hormone

2

Zvi Laron

Core Messages

- The introduction of radioimmunoassays in the 1960ies led to the recognition that the patients who clinically resembled GH deficiency but had high serum hGH levels, had a new disease entity.
- This chapter describes the psychological pattern of circadian hGH secretion in Laron syndrome patients, and the response to stimulatory and suppressing agents. Immunological tests and radioreceptor assays showed that the hGH molecule is normal. Liver biopsies from 2 LS patients proved that the hGH receptor is defect.

2.1 Regulation of Growth Hormone Secretion

2.1.1 Introduction

Identification of patients who resembled GH deficiency, both clinically (dwarfism, obesity) and biochemically (tendency for hypoglycemia) (Fig. 2.1), and high FFA (Fig. 2.2), but who had abnormally high overnight fasting serum concentrations of circulating hGH (Laron and Mannheimer 1966; Laron et al. 1968; Laron 1984) (Fig. 2.3), led us to conclude that we had discovered a new disease entity (Laron et al. 1966). To explain the high serum human growth hormone (hGH), we considered two possibilities: (a) an abnormal hGH molecule or (b) a defect in the hGH receptors (Laron et al. 1966), leading to end organ resistance (Laron et al. 1971, 1980).
As the serum hGH levels in the same patient varied on different days, as seen in Fig. 2.3, possibly due to not complete fasting, previous exercise, stress, or physiological fluctuations, we investigated the diurnal secretion and regulation of GH secretion in these patients.

2.1.2 In Vivo 24 h Diurnal Pattern of hGH Secretion

Using a nonthrombogenic continuous blood withdrawal pump (Cormed, USA), the hGH secretory pattern was studied every 30 min in 3 patients with Laron syndrome and 2 age-matched controls (Keret et al. 1988). The radioimmunoassay (RIA) used was that of Laron and Mannheimer (1966). Figure 2.4 illustrates the 24-h hGH secretory profile in two young females and Fig. 2.5 that in an adult male with Laron syndrome. It is seen that in Laron syndrome patients similar to healthy individuals, hGH is secreted in pulses and that the number of daily peaks in the young adult patients is similar to healthy controls; however, both the daily peaks, and especially those during sleep, are significantly higher than those in the control subjects resembling those registered in acromegalic patients. The quantity of hGH secretion during a pulse as expressed by area under the curves and their average integrated concentrations are shown in Table 2.1.

It is further seen that both in the 27-year-old Laron syndrome patient as well as in its control subject the hGH values are lower than in the late pubertal or young adult patients, a decline known to occur with age in healthy individuals of both sexes (Zadik et al. 1985;

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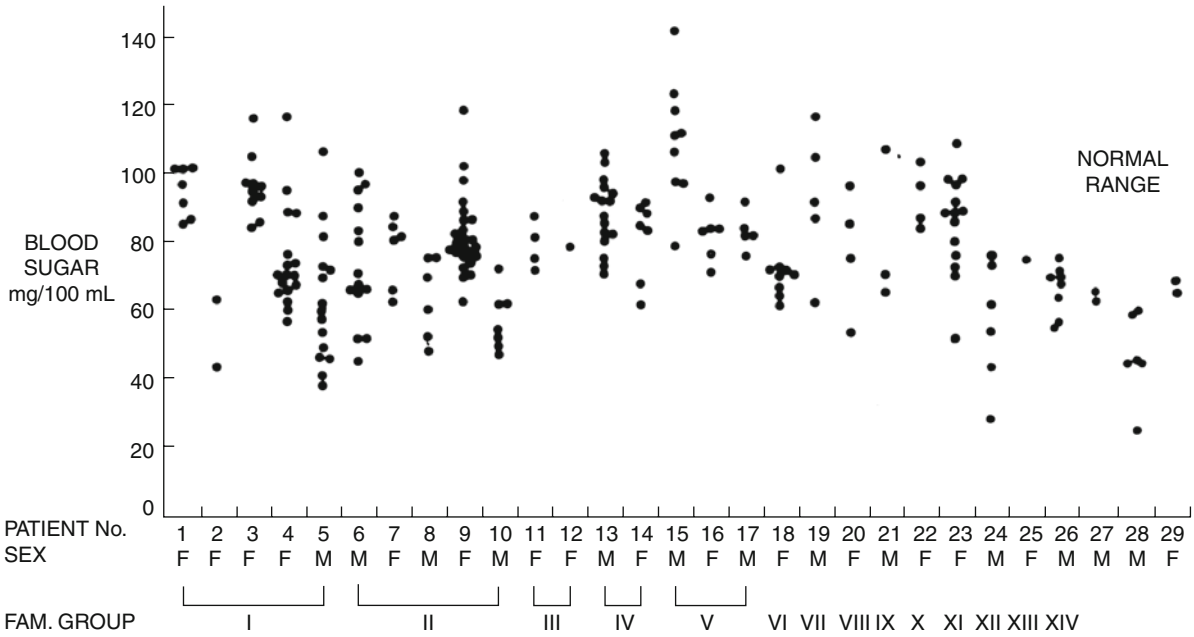

Fig. 2.1 Fasting blood glucose as total reducing substances in the first 29 patients. Reproduced with permission from Laron (1984)

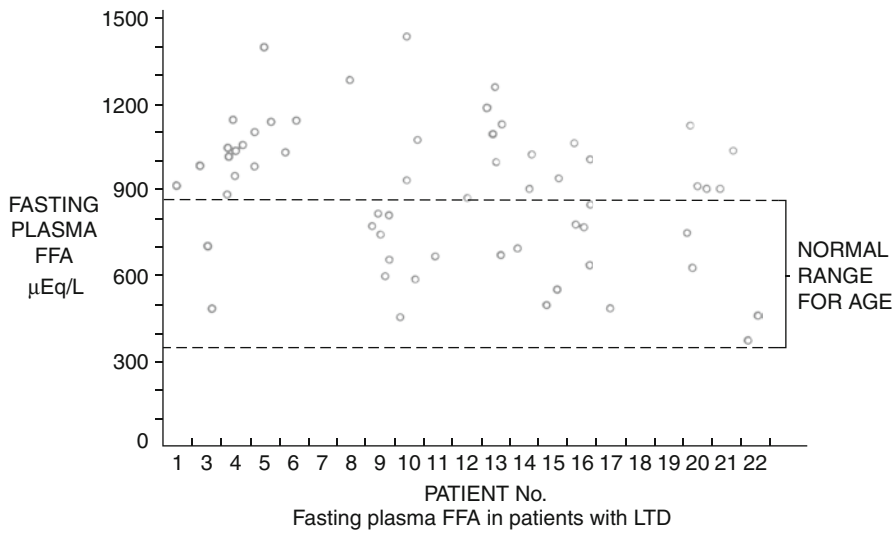

Fig. 2.2 Fasting plasma free fatty acids (FFA) in 22 patients with Laron syndrome. Reproduced with permission from Laron (1984)

Hartman 2000). Nevertheless, the secretion in the Laron syndrome patient is higher than that in the control subject (see also Chap. 26).

Calculating the production rate of hGH in Laron syndrome, we found it to be 2,480 ng/min, similar to that of patients with active acromegaly (MacGillivray et al. 1970); on the other hand, the metabolic clearance rate was half that of control subjects (Keret et al. 1988). This can be explained by the reduced GFR in IGF-I deficiency (see Chap. 34) and the lack of functioning GH receptors in the kidney. It is of note that the values fall within the lower limit of the normal range (Owens

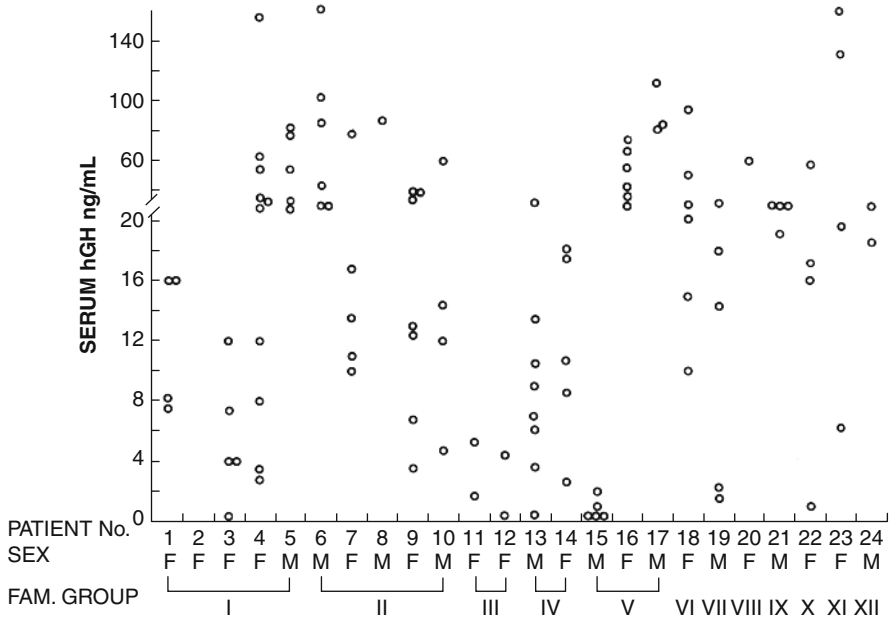

Fig. 2.3 Repeated serum growth hormone (hGH) levels in 24 patients with Laron syndrome. Reproduced with permission from Laron (1984)

et al. 1973). There is no doubt that both the increased production rate and reduced clearance rate contribute to the elevated serum hGH concentrations in Laron syndrome patients, which is primarily caused by the negative feedback induced by the IGF-I deficiency (Berelowitz et al. 1981).

2.1.3 Growth Hormone Stimulation Tests: The Insulin Tolerance Test (ITT)

In response to a bolus insulin (0.1 U/kg i.v.) to 17 children with Laron syndrome, there was a further rise in the elevated basal hGH levels up to 140–232 ng/mL. In 4 patients with high basal levels, there was a paradoxic response. Two patients had seizures so that future test doses were reduced to 0.05 U/kg i.v. (Laron et al. 1968). All children presented with hypoglycemia nonresponsiveness. Nowadays, we do not perform ITTs on Laron syndrome patients.

2.1.4 Arginine Test

Intravenous infusion of arginine (0.5 g/kg body weight infused over 30 min in a 10% solution) to 20 children with Laron syndrome showed a further increase of serum hGH in all Laron syndrome children (Fig. 2.6). No adverse effects were observed in this test.

2.1.5 Growth Hormone Suppression Tests

2.1.5.1 Somatostatin Administration

We studied the effect of intravenous injection of dihydrosomatostatin (SMS) (150 μg/m^2 over 5 min followed by 300 μg/m^2 over 40 min) on the hGH serum levels in 4 Laron syndrome patients (aged 7–18 years), one tested twice (Laron et al. 1977). The effect was compared to that obtained in one active acromegalic patient. The mean (±SD) results of the five tests on serum hGH, insulin, and glucagons in the Laron syndrome patients compared to an acromegalic patient are illustrated in Fig. 2.7.

It is seen that somatostatin acutely suppresses hGH, insulin, and glucagon. Due to discontinuation of the SMS infusion, the hGH levels made a significant rebound rise in both conditions, but more so in the Laron syndrome patients. The rebound of insulin after SMS was less in the Laron syndrome patients than in

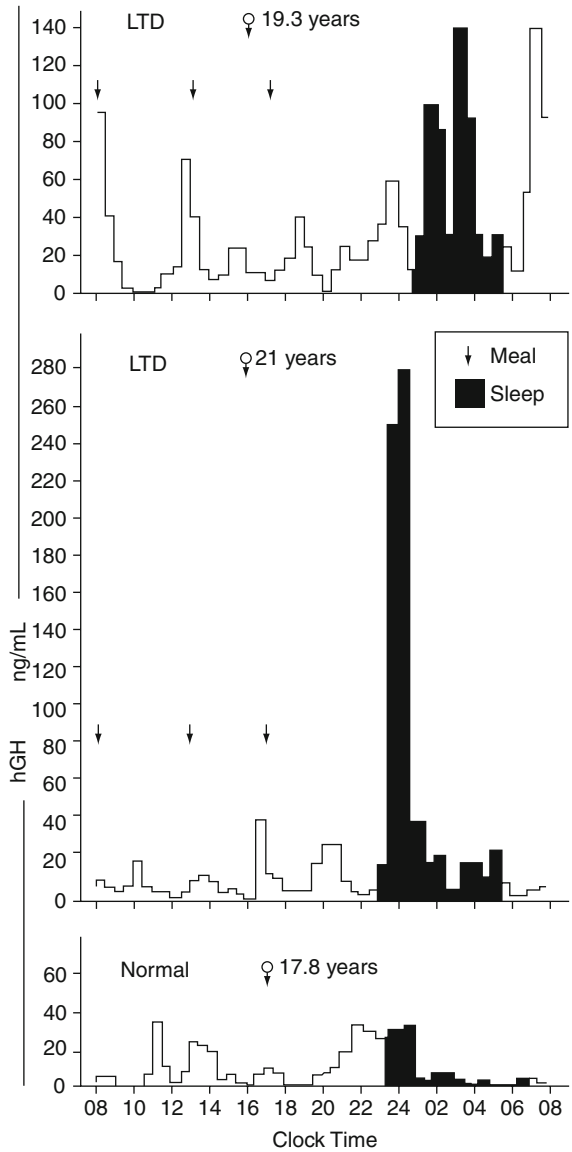

Fig. 2.4 24-h serum hGH profile in two young female adults with Laron syndrome compared to a healthy female control. Note the difference in hGH peak levels. Reproduced with permission from Keret et al. (1988)

the acromegalic patients. Somatostatin reduced also the serum TSH levels (not shown).

2.1.6 Oral Glucose Tolerance Test

Oral glucose administration (100 g/1.73 m^2 body surface) over 10–15 min in the fasting state to 10 Laron syndrome patients induced a reduction in serum hGH in variable degrees (Fig. 2.8).

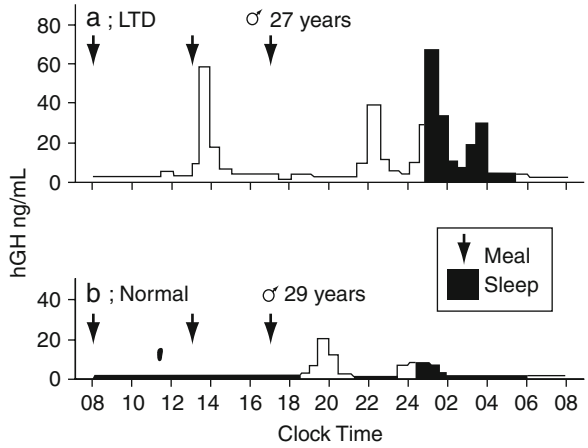

Fig. 2.5 24-h serum hGH profile in an adult male with Laron syndrome compared to a healthy control. Reproduced with permission from Keret et al. (1988)

2.1.7 Growth Hormone Response to Oral Corticosteroids

Administration of 6 alpha-methylprednisolone in divided doses of 10–15 mg daily for 3 days to 5 Laron syndrome children (4 females, 1 male) resulted in suppression of hGH in 2 patients and a paradoxic response in another 3 (Laron et al. 1968).

2.1.8 Conclusions

Both the secretory pattern and the regulatory behavior of hGH (stimulation and suppression) in patients with Laron syndrome were normal, but exaggerated. The next step we undertook was to find out whether the hGH of Laron syndrome patients is of normal structure. We decided to start with the immunological behavior of hGH in these patients.

2.2 Immunological Studies of the Serum GH from Patients with Laron Syndrome

2.2.1 Dilution Curves Using Radioimmunoassay

The shape of dilution curves obtained with serum hGH of patients with Laron syndrome (serum hGH

Table 2.1 Secretory dynamics of 24-h hGH secretion in three Laron Syndrome patients compared with normal subjects as controls

Subjects			Serum hGH				
Diagnosis	Sex	Age (year:months)	Baseline range (ng/mL)	Maximal amplitude (ng/mL)	AUC	AIC (ng/mL)	Pulses (number/24 h)
Laron syndrome	F	19[3]	10	164	560	33.9	9
Laron syndrome	F	21	10	280	780	23.4	7
Control	F	17[8]	1–3	135	268	11.3	6
Laron syndrome	M	27	1–3	67	231	9.9	4
Control	M	29	1–3	19	51	2.2	2

Modified from Keret et al (1988)
AUC area under the curve; *AIC* average integrated concentration

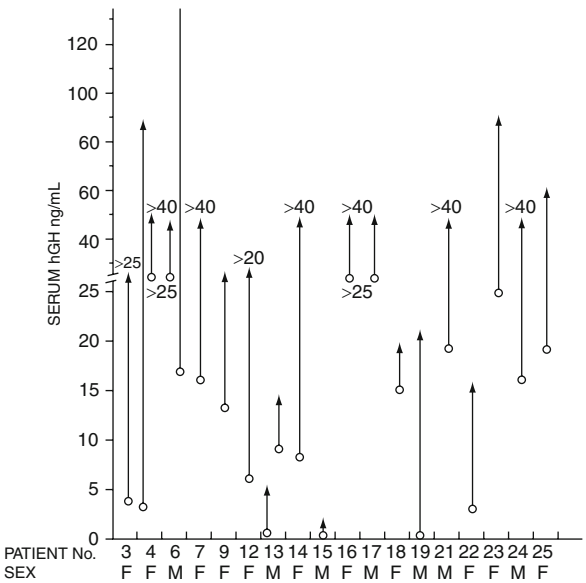

Fig. 2.6 Serum hGH response to arginine infusion in children with Laron syndrome. Reproduced with permission from Laron (1984)

concentrations of 10–68 ng/mL) was compared to that of serum hGH obtained from healthy children (stimulated serum hGH concentrations of 16–70 ng/mL), acromegalic patients (serum hGH 120–170 ng/mL), and healthy newborns (serum hGH concentrations of 16–70 ng/mL). The sera were tested with three different antisera (Eshet et al. 1973). The anti-hGH sera were one prepared by immunization of guinea pigs in our laboratory denoted AS-1 and another received from Yalow (NIH anti-hGH-2-5-19) denoted AS-2 and the serum of a patient who developed high titer of

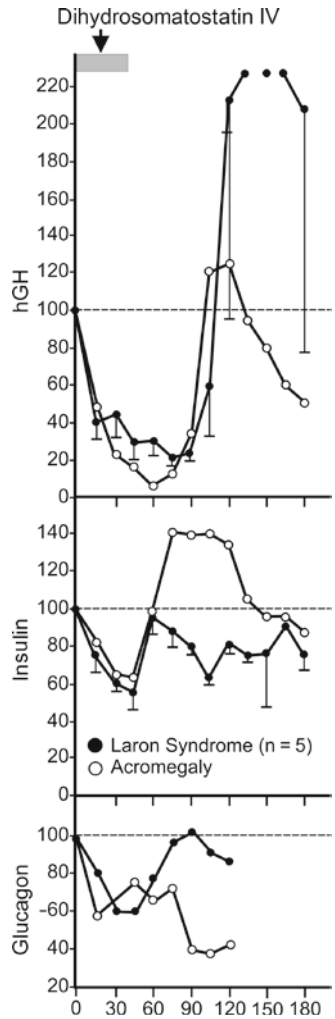

Fig. 2.7 Serum hGH, insulin, and glucagon response to intravenous somatostatin administration. Reproduced with permission from Laron (1984)

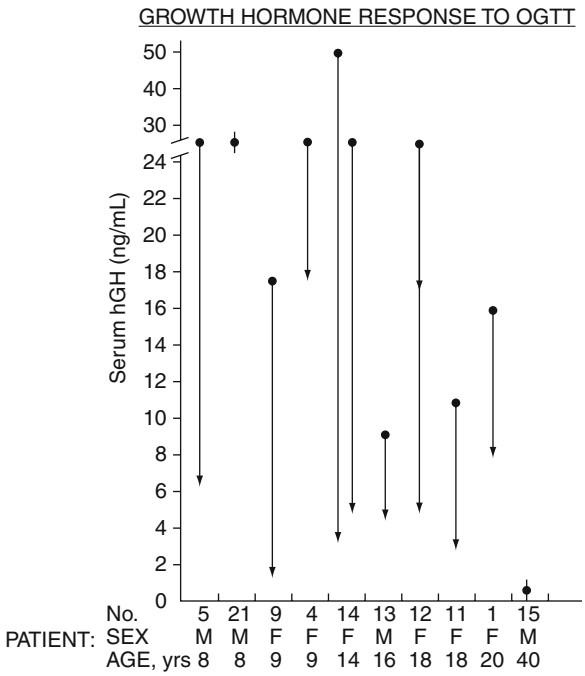

Fig. 2.8 Serum hGH response to an oral glucose load in 10 patients with Laron syndrome. Reproduced with permission from Laron (1984)

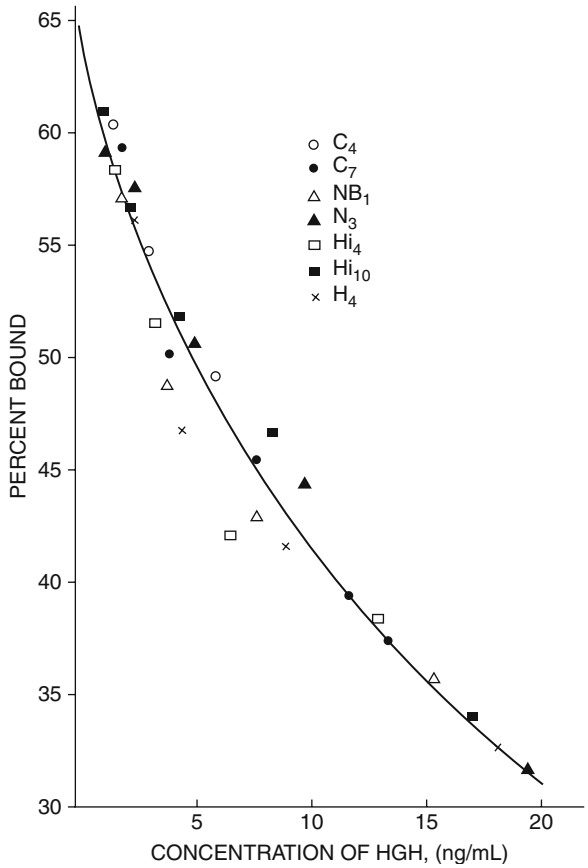

Fig. 2.9 Superimposition of dilution curves of serum hGH from Laron syndrome patients (*Hi*), compared with healthy children (*C*), newborns (*NB*), and acromegaly patients (*A*) on the standard curve using anti-hGH serum diluted $1{:}2\times10^{-6}$. The hGH of the various samples tested was brought to the same concentration (Eshet et al. 1973). Reproduced with permission from Laron (1984)

antibodies while on hGH therapy (kindly provided by Prader and Illig, Zurich) (AS-3). As all sera rendered similar results, we illustrate only the dilution curve with AS-1 (Fig. 2.9). Additional experiments used dilution curves of serum hGH of Laron syndrome patients in a hybrid system of RIA using ^{125}hGH and antiserum against HCS (human chorionic somatotropin) compared to anti-hGH serum. All the experiments revealed no differences in the immunological behavior of the hGH from patients with Laron syndrome compared to that in the control sera (Eshet et al. 1974).

2.2.2 Conclusions

The above findings were interpreted as suggestive that patients with Laron syndrome secrete hGH with a normal molecular structure. The next step to verify this assumption could be undertaken only when specific radioreceptor assays (RRA) for hGH became available.

2.3 Comparison Between Radioimmunoassays and Radioreceptor Assays to Measure Circulating High in Patients with Laron Syndrome

Using a RRA for hGH (Tsushima and Friesen 1973) with a 100,000-g pellet of human liver tissue (obtained from a male donor of a kidney transplantation), serum hGH concentrations of 6 Laron syndrome patients, of healthy children, and of acromegalic patients were compared both by RIA and RRA (Eshet et al. 1985).

2.3.1 Methods

The RRA assay employed was as follows:

2.3.1.1 Specific Binding of ^{125}I-hGH to Human Livers in the Microsomal Fraction

Specific binding of hGH was demonstrated in six human liver samples tested in 31 different binding assays. The specific binding of these six liver specimens ranged from 3.9 to 12% with a mean value of 7.2% per 6 mg/mL of microsomal fraction protein. The binding experiments were performed using the liver of a 13-year-old donor.

2.3.1.2 Radioreceptor Assay

Precision. The specific binding of various amounts of ^{125}I-hGH to different concentrations of human liver microsomal receptors was tested. The most precise standard curve was obtained with 585 μg of microsomal fraction protein and 12.5×10^3 cpm of ^{125}I-hGH (0.7 ng).

Sensitivity. In seven separate RRA with human liver receptors using 585 μg/tube of microsomal protein and 0.7 ng of ^{125}I-hGH, the total binding in the absence of unlabelled hormone was 17.79 ± 1.04%. Nonspecific binding, defined as the ^{125}I-hGH radioactivity bound in the presence of 5 μg/mL of unlabelled hormone, was 9.92 ± 0.57%. Specific binding, therefore, was 7.86 ± 0.82%.

Specificity. The ^{125}I-hGH bound by human liver receptors was displaced only by hGH. Other species of GH, e.g., bovine and ovine GH as well as hPRL, ovine and bovine PRL, hPL, did not inhibit the binding of ^{125}I-hGH to the human liver receptors even at a concentration of 10 μg/mL.

The results of the investigations are shown in Table 2.2. The hRRA/RIA ratio was better in the Laron syndrome patients than in the controls in whom the RIA values exceeded those found by the RRA. The dilution curves of the serum hGH from the Laron syndrome patients were parallel to the standard curve, denoting identical structure of the hGH at its binding sites.

2.3.1.3 Discussion

Previous reports of quantitative comparison of the hGH values obtained with the RIA and RRA in acromegalic and normal subjects using rabbit liver receptors showed a systematic overestimation by RIA (Herington et al. 1974; Sneid et al. 1975). In our study with hRRA, we made the same observation (Table 2.2). Testing of the serum of Laron syndrome patients, however, showed a higher value of hGH in the RRA in four of the six patients. It is of note that Jacobs et al. (1976), who prior to this study had used a rabbit liver assay to test the serum of another seven of our Laron syndrome patients, found also an overestimation of the hGH values by RRA in two of these patients. These findings may be due to the fact that Jacobs et al. (1976) used a pregnant rabbit liver RRA which also binds lactogenic

Table 2.2 Comparison of hGH concentrations in serum as measured by RIA and human liver hRRA in patients with Laron syndrome, acromegaly, and a healthy control

Subjects	Sample number	RIA (ng/mL)	hRRA (ng/mL)	hRRA/RIA ratio, %	Slope of regression curve	*F* statistics
Laron syndrome patients	1	105.0	18.0	20	−2.645	0.47
	2	12.0	20.0	170	−4.631	
	3	90.0	140.0	150	−1.255	
	4	80.0	130.0	150	−1.453	
	5	17.5	40.0	200	−1.226	
	6	20.0	23.0	100	−3.517	
Acromegaly	12	352.0	50.0	14.6		
		168.0	45.0	25		
Normal control[a]	1	58.0[a]	22.4	50		
Standard curve					−1.765	

The hGH values of each sample are expressed as the mean of three dilutionsModified from Eshet et al (1985)
[a]The values given for the normal subject is the peak value in a clonidine stimulation test

hormones in contradistinction to the human liver RRA we used and which was found specific for hGH (Eshet et al. 1985). The physiological meaning of discrepant findings is not clear and needs further investigation.

2.3.1.4 Conclusions

The immunological studies of hGH from Laron syndrome patients were interpreted as showing that the structure of the circulating pituitary GH of these patients is normal and that the RRA using human liver proved that their GH is biologically active.

2.4 Evidence that the Etiology of Laron Syndrome is a GH-Receptor Defect

2.4.1 Introduction

Having shown that the pituitary GH secreted by Laron syndrome patients has a normal immunological behavior and binds to human GH receptors, we disproved one of the early expressed possibilities that Laron syndrome patients secrete an abnormal GH. Thus, the second possibility of a GH resistant (insensitive) state had to exist (Laron et al. 1980). To prove this assumption, we convinced the mother of one 4-year-old Laron syndrome patient and a young adult Laron syndrome patient to agree to undergo an open liver biopsy to enable the preparation of GH receptors from Laron syndrome livers (Eshet et al. 1984).

Control liver tissue was obtained from kidney donors immediately after pronouncement of clinical death by traumatic causes (there were six donors, aged 13–47 years with a mean age of 24 years). The investigation was approved by the Ethical Hospital Committee.

2.4.2 Radioreceptor Assays Using Liver of Laron Syndrome Patients

2.4.2.1 Methods

The preparation of microsomes bearing hGH receptors was done as described byTsushima and Friesen (1973). The liver tissue was homogenized in a 0.25 mM sucrose solution and then centrifuged at 10,000 g for 10 min; the supernatant was then centrifuged at 100,000 g for 90 min yielding the microsomal pellet.

One gram of liver yielded 18 mg/mL of microsomal fraction protein. For the assay, 1.2 mg/100 μL of this liver microsomal pellet fraction was incubated in duplicate with 0.7 ng/100 μL of labeled hGH or insulin (specific activity 80 μCi/ μg and 90 μCi/ μg, respectively) in a 25 mM tris-HCl + 10 mM MgC2 buffer, pH 7.4 and 0.1% bovine serum albumin in a final volume of 0.5 mL. This mixture was incubated at 4°C with constant shaking for 48 h. Incubation was terminated by adding 2 mL ice-cold 0.1% bovine serum albumin tris/magnesium buffer. The fractions containing receptor-bound and free radioactivity were separated by centrifugation at 2,000 g for 30 min at 4°C. Radioactivity was measured in a 5260 Autogamma Scintillation Spectrometer (Packard, USA). Parallel incubations were made in the presence of excess (5 μg/mL) unlabeled hormone. Specific binding is the difference between radioactivity bound in the absence (total binding) and in the presence (nonspecific binding) of excess unlabeled hormone, (in this case hGH) and is expressed as a percentage of the total radioactivity in the incubation mixture. The receptor binding studies made with the liver tissue from the 2 Laron syndrome patients were carried out concomitantly with those of the control liver tissue of a 13-year-old boy. The microsome preparations from the liver tissue of the six healthy control subjects were studied repeatedly.

2.4.2.2 Results

The liver microsomes from the Laron syndrome patients showed almost no specific binding of ^{125}I-hGH, (Table 2.3 and Fig. 2.10) whereas those from the healthy liver tissue showed specific binding ranging from 7.9 to 24% (average 14.5 ± 3.2%) (Tables 2.3 and 2.4). Liver tissue from the 13-year-old control boy assayed concomitantly with that of the 2 Laron syndrome patients showed a specific binding of 28% for hGH. The microsomes of the Laron syndrome also showed binding of ^{125}I-insulin, which was comparable to those observed in healthy individuals. The serum levels of hGH prior to biopsy were 45 ng/mL in Patient 1 and 20 ng/mL in Patient 2; serum insulin was undetectable in Patient 1 and 48 mU/mL in Patient 2.

Table 2.3 Specific binding of ^{125}I-hGH and ^{125}I-insulin to liver microsomes from two patients with Laron syndrome and from one healthy control subject

Liver microsomes	Sex	Age (year)	Binding of ^{125}I-hGH (%)			Binding of ^{125}I-insulin (%)		
			Total	Nonspecific[a]	Specific	Total	Nonspecific[b]	Specific
Laron syndrome								
Patient 1	M	4	7.0	6.5	0.5	47.3	13.9	33.4
Patient 2	M	26	7.0	6.9	0.1	11.2	4.1	7.1
Healthy control subject	M	13	35.1	6.8	28.3	17.3	9.6	7.7

Liver tissue was tested per 12 mg/mL of microsomal fraction protein
Reprinted with permission from Eshet et al. (1984)
hGH human growth hormone; *M* male
[a]With 5 μg/mL hGH
[b]With 10 μg/mL insulin

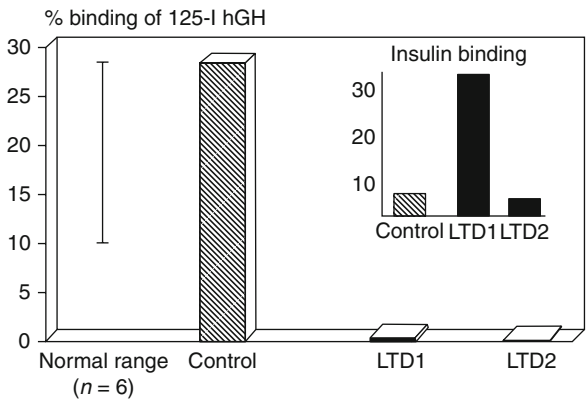

Fig. 2.10 Lack of ^{125}hGH binding to liver microsomes prepared from 2 Laron syndrome patients compared to liver microsomes from healthy controls. In contradistinction ^{125}labeled insulin bound to the liver microsomes from Laron syndrome patients

Table 2.4 Specific binding of ^{125}I-hGH to liver microsomes taken from six normal-kidney donors

Sex	Age (year)	Number of assays performed	Specific binding (%)
Male	13	10	24.1 ± 4.3[a]
Male	16	7	13.4 ± 3.0[a]
Male	19	2	15.6 & 10.8
Female	20	5	16.2 ± 4.5[a]
Male	30	2	7.3 & 8.6
Male	47	5	12.2 ± 3.2[a]

Liver tissue was expressed per 12 mg/mL of microsomal fraction protein
hGH human growth hormone
Reproduced with permission from Eshet et al (1984)
[a]Values are means ± SD

2.4.2.3 Discussion

Having shown that the liver of Laron syndrome patients is insensitive to hGH, the stimulus to generate IGF-I and the fact that the liver is the major source of IGF-I explain the lack of IGF-I secretion in this syndrome (Daughaday et al. 1969; Laron et al. 1971). This concept concurs with the findings of Golde et al. (1980) that erythroid colonies grown in vitro from 2 Laron syndrome patients were unresponsive to exogenous hGH in contrast to colonies from control subjects and patients with primary hGH deficiency. Further evidence found by us was that the addition of normal serum to cultures of fibroblasts from Laron syndrome patients enhanced the incorporation of ^{3}H-thymidine (Nevo et al. 1977; Laron et al. 1980) (Table 2.5), whereas the addition of serum from Laron

Table 2.5 Effect of sera (10%) from patients with Laron syndrome or, isolated GH deficiency (IGHD) and normal serum on the growth, in culture, of fibroblasts derived from 2 patients with Laron syndrome aged 10 and 12 years

Diagnosis (source of serum)	Number of sera	Protein per plate (μg ± SD)	^{3}H-thymidine (cpm/plate ± SD)
Laron syndrome	2	117 ± 13	927 ± 74
IGHD	7	105 ± 17	652 ± 168
Normal	Two pools	169 ± 6	1,251 ± 36
Without serum		8 ± 4	38 ± 21

All the trials were performed in triplicate.
Cells were allowed to grow for 72 h before harvest.
Statistical evaluation
Protein per plate: LS vs. control, $p < 0.05$; IGHD vs. control, $p < 0.05$ ^{3}H-thymidine incorporation: LS vs. control, $p < 0.002$; IGHD vs. control, $p < 0.002$

Table 2.6 Effect of IGF-I deficient sera on the growth of normal human fibroblasts in culture[a]

Source of serum	Number of sera[b]	Protein per plate (μg ± SD)	^{3}H-thymidine (cpm/plate ± SD)
Normal	One pool	200 ± 17	360 ± 23
Laron-type dwarfism	2	72 ± 16	200 ± 7
Isolated GH deficiency	5	118 ± 44	252 ± 82
Without serum		64 ± 3	45 ± 4

Statistical evaluation

Protein per plate: Laron syndrome vs. control, $p < 0.01$; IGHD vs. control, $p < 0.01$; ^{3}H-thymidine incorporation: Laron syndrome vs. control, $p < 0.005$; IGHD vs. control, $p < 0.05$

Modified from Nevo et al. (1977)

[a]The fibroblasts were cultured from a superficial skin biopsy from a 15-year-old boy. All the trials were performed in triplicate. Cells were allowed to grow for 48 h before harvest. Sera were used in 10% concentration

[b]Each patient's serum was investigated separately except those of the normal controls, which were pooled

syndrome and patients with IGHD caused a reduction in incorporation of ^{3}H-thymidine into normal fibroblasts as well as a decrease in protein content (Table 2.6). Serum from Laron syndrome patients also did not stimulate the uptake of ^{35}S and ^{3}H-D-glucosamine in calf-rib cartilage slices (Kleine et al. 1980). These findings indicate that the fibroblasts and the cartilage did not respond to IGF-I-deficient serum from patients with Laron syndrome in contradistinction to serum from control subjects containing active GH and IGF-I.

It can be assumed that the defect in hGH receptors in the Laron syndrome is not limited to the liver tissue and includes all tissues.

2.4.2.4 Conclusions

Our findings described were the first evidence that patients with Laron syndrome have a defect in the hGH receptors in their livers, which causes the resistance (insensitivity) to this hormone.

It thus took us 20 years from the description of this syndrome to obtain evidence of the etiopathology of the Laron syndrome, indicating that its defect is in the hGH receptors and explaining the lack of generation of IGF-I in the liver and may be other tissues.

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3 Clinical Evidence of Growth Hormone Resistance in Patients with Laron Syndrome

Zvi Laron

Core Message

› Lack of response to the administration of exogenous hGH was evidence that Laron syndrome patients suffer from GH resistance.

3.1 Metabolic Effects of Human Growth Hormone (hGH) Administration (Laron et al. 1971)

3.1.1 Subjects

Fifteen patients with Laron syndrome aged from 1 to 12[7] years (8 boys and 7 girls) and 6 patients with congenital isolated GH deficiency (IGHD) aged from 4[5] to 17[4] years (5 boys and 1 girl) as well as 5 patients with acquired IGHD (2 males and 3 females), 4 prepubertal, and one 20-year-old male were included in the study (Laron et al. 1971).

3.1.2 Methods

Human growth hormone (hGH) preparations produced in two different laboratories were used in this study. One was produced in our laboratory (batches scored A) and hGH obtained from the National Pituitary Agency, USA (scored NPA). Both products were prepared from acetone-preserved pituitaries and extracted by the Raben method (1957).

The effect of one-shot intravenous (i.v.) injection of hGH on the mobilization of plasma free fatty acids (FFA) was tested by measuring the level of FFA before hGH administration, after a 12–14-h overnight fast, and at hourly intervals for up to 4 h after the injection.

For the *metabolic balance studies,* the patients were kept on a constant diet, and after an equilibration period of 3–5 days, three 4-day periods were studied: a control period, a treatment period during which hGH was administered twice daily intramuscularly (i.m.), and a posttreatment period. Blood was drawn at the beginning and end of each period and analyzed for sugar as total reducing substances (TRS), urea, phosphorus, and creatinine. Plasma hGH was determined only at the beginning of each study. For sulfation factor (SF) activity, i.e., IGF-I, blood samples were drawn twice daily during the first day of treatment and daily thereafter for 4 or more days. Plasma samples of known volume were lyophilized and flown to St. Louis, MO, for determination in the laboratory of Daughaday (Washington University, St. Louis, MO). Urine was collected in 24-h periods and refrigerated throughout. After each 24-h period, a measured sample was removed for creatinine determination; sodium azide was added as a preservative. The remaining urine was acidified with concentrated HCl and kept frozen until analyzed for nitrogen, calcium, and phosphorus. Plasma FFA were determined according to Dole and Meinertz (1960). Blood sugar as TRS was measured by the method of Rappaport and Eichorn (1950) (normal range 80–120 mg/mL). Urea was estimated by a Technicon AutoAnalyzer according to Skeggs (1957) and March et al. (1957). Creatinine was estimated by the method of Popper et al. (1937), adapted for photocolorimetry. Urinary nitrogen was measured by the

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Koch micro-Kjeldahl method followed by direct nesslerization (Koch and Hanke 1953). In urine, the calcium was estimated by flame spectrophotometry (Liberman et al. 1967); the phosphorus according to the calorimetric method of Gomori (1953). Sulfation factor was determined by the method of Daughaday and Parker (1963). Serum growth hormone (hGH) was determined according to Laron and Mannheimer (1966).

3.1.3 Results

3.1.3.1 Effect of One i.v. Dose of hGH on Plasma FFA

The fat-mobilizing effect of hGH within 4 h after i.v. injection is shown in Table 3.1. The i.v. injection of hGH had a significantly greater ($p<0.01$) FFA-mobilizing effect in children with IGHD than in those with Laron syndrome who showed no increase.

3.1.3.2 Metabolic Studies

Table 3.2 summarizes the results of the chemical determinations in the urine during 15 metabolic balance studies performed in 3 patients with IGHD and 9 with Laron syndrome (in 2 patients of this group, balance studies were performed twice).

In the patients with Laron syndrome, the urinary nitrogen retention varied from none in 5 patients of 20% of control values in 3 to a slight response in 4 patients. The IGHD patients had nitrogen retention from 24 to 47%.

3.1.3.3 Blood Urea

In the patients with IGHD, there was a reduction in blood urea concentration during the administration of hGH. This response was less apparent and of shorter duration in the patients with Laron syndrome.

3.1.3.4 Urinary Phosphorus

The patients with IGHD showed some retention (3.7–13% over control values) in urinary phosphorus during the period of hGH administration. Of nine analyses made in the patients with Laron syndrome, only two showed phosphorus retention. One had a very slight retention (3.2%), and 6 excreted more phosphorus than in the control period.

3.1.3.5 Serum Inorganic Phosphorus

In patients with Laron syndrome, there was a very slight rise in serum phosphorus during hGH administration. These changes bore no relation to the urinary phosphorus or nitrogen.

3.1.3.6 Urinary Calcium Excretion

In the patients with IGHD, administration of hGH induced a marked calciuria (Table 3.2), which diminished after stopping hGH administration. A lesser degree of calciuria was observed in the patients with Laron syndrome.

3.1.3.7 Serum Sulfation Factor (SF, IGF-I)

All the patients with Laron syndrome had a low SF (IGF-I) activity, which remained low during the hGH administration. In the patients with IGHD, a rise of SF (IGF-I) was observed already after 48 h of hGH administration (Table 3.3) (see also Chap. 4).

Table 3.1 Effect of one intravenous dose of hGH on plasma free fatty acids (FFA) in patients with Laron syndrome and isolated GH deficiency (IGHD)

Group	Subjects		Fasting Levels			i.v. hGH Injection		
	Patients	*n*	Glucose (mg/100 mL)	hGH (ng/mL)	FFA (μEq/L)	FFA peak level (μEq/L)	ΔFFA` (μEq/L)	ΔFFA (%)
1	Laron syndrome	15	78.9±11.0	5–213	935.14±248.1	1,319.9±459.5	384.1±355.1	42.6±40.8
2	IGHD	11	76.3±15.4	0	853±266.0	1,791.4±331.2	1,006.5±324.9	143.4±96.7

Values are expressed as mean ± SD

Table 3.2 Effect of hGH on urinary nitrogen, phosphorus, calcium and hydroxyproline in patients with Laron syndrome and IGHD before and during hGH administration (2.5 mg bid for 4 days)

Patients		hGH injection, i.m.				Urinary nitrogen			Urinary phosphorus		Urinary calcium	
Name	Sex	Age at study h:months	Batch	Dose (mg q h)	Number of days	Fasting serum hGH at start (ng/mL)	Control period (mg/24 h)	hGH Rx % retention**	Control period (mg/24 h)	hGH Rx % retention**	Control period (mg/24 h)	hGH Rx % excretion**
Laron syndrome												
SnI	F	10:6	NPA	2.5 q 12	4	8	5.0	−6.0	530	22.0	11	72.7
BS	F	6:6	A_{12}	2.5 q 12	4	165	4.1	12.7	218	−5.0	–	–
BR	M	4:10	A_{12}	2.5 q 12	4	81	3.6	9.0	–	–	–	–
SSh	M	13:6	NPA	2.5 q 12	4	5	6.0	10.0	311	−5.7	10	9.9
SSi	M	2:5	A_3	3.0 q 24	4	–	2.1	−14.2	60	−11.6	13	23.0
YR	F	5:5	A_3	3.0 q 24	4	–	2.5	4.0	143	48.9	19	−63.1
YR		13:0	NPA	2.5 q 12	4	38	4.2	−4.7	213	−55.8	24	54.1
YG	M	3:3	A_4	2.0 q 24	4	–	2.4	3.7	65	−27.7	12	91.6
LY	M	9:0	A_5	5.0 q 24	4	–	1.0	−33.3	62	3.2	517	40.0
LY		13:0	A_{10}	3.0 q 24	4	9	2.1	−33.3	103	−65.0		41.1
MP	F	5:110	NPA	2.5 q 12	4	19	4.8	−6.2	–	–	–	–
IGHD												
ND	F	15:0	A_7	10.0 q 24	4	0	6.0	23.3	247	3.7	66	100.0
AE	M	12:9	A_7	2.5 q 12	4	0	2.8	42.8	109	11.9	58	132.7

** $p<0.01$

Table 3.3 Changes in plasma sulfation factor (SF, IGF-I) activity in patients with Laron syndrome and IGHD before and during hGH administration (2.5 mg bid for 4 days)

Subject			Serum hGH (ng/mL)	hGH injection		Plasma SF activity							
Name	Sex	Age (year:months)		Dose (mg q h)	Batch	Before hGH injection			During hGH injections (h)				
									4	24	48	72	96	192
Laron syndrome														
IS	F	10:6	7.4	2.5 q 12	NPA			0.32	0.21		0.12	0.17		0.25
SB	F	6:6	165	2.5 q 12	A_{12}			0.25	0.25	0.23	0.22	0.26		0.18
RB	M	4:10	81	2.5 q 12	A_{12}			0.13	0.17	0.17	0.11	0.18	0.18	0.22
ShS	M	13:6	5	2.5 q 12	NPA		0.07	0.11	0.07	0.07	0.02	0.05	0.07	0.05
RJ	F	13:0	38	2.5 q 12	NPA	0.13	0.13	0.15	0.11	0.11	0.13	0.10	0.16	0.31
AM	F	3:0	42	2.0 q 12	NPA			0.2					0.2	
RM	M	1:2	>50	2.0 q 12	NPA		0.05	0.16					0.11	
SE	M	6:5	27	2.0 q 12	NPA			0.27		0.19	0.18	0.23	0.27	
PM	F	6:11	19	2.5 q 12	NPA			0.26	0.45	0.28		0.32		0.26
IGHD														
RS	F	10:10	0	2.5 q 12	A_{12}		0.02	0.11			0.56	0.78		
JV	M	4:0	0	2.5 q 12	A_{12}			0.25	0.33	0.40	0.94	0.86	1.08	

3.1.3.8 Effect of Prolonged hGH Administration on Linear Growth

The effect of intramuscular hGH administration three times a week for several months on linear growth revealed that while 4 patients with IGHD treated for 6 months responded with an increased growth response, the 11 children with Laron syndrome treated for 3–10 months showed no response.

3.1.4 Conclusion

In contradistinction to patients with congenital or acquired isolated GH deficiency, patients with Laron syndrome proved resistant (insensitive) to exogenous hGH administration, both to metabolic balance effects and to linear growth stimulation. These findings are explained by their inability to generate IGF-I.

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Diagnosis of Laron Syndrome

4

Zvi Laron

Core Message

› Description of phenotypic characteristics leading to suspicion of Laron syndrome and means of its diagnosis

The diagnosis of Laron syndrome should be suspected in infants, older children, or adults who present with a height deficit of −4 to −10 height SDS and with the typical features (shown in Figs 1.1 and 1.2 in Chap. 1) (facial phenotype, dwarfism, obesity, hypogenitalism) identical with those of congenital isolated growth hormone (GH) deficiency (cIGHD), but by laboratory screening, having elevated serum hGH concentration in young age reaching acromegalic levels (see Chap. 2, Fig. 2.3) in the presence of very low or undetectable serum IGF-I. As in neonates and especially premature babies, blood hGH levels are high; determination of serum hGH at this age is useful only for determining the hGH deficiency (Laron et al. 2007).

Evidence for hGH insensitivity (resistance) is obtained by performing an IGF-I generation (stimulation test), i.e., administration of hGH 33 μg/kg s.c. for 4 or preferably 7 days, and determining serum IGF-I before, on day 5 and day 8 (i.e., the morning after the last injection). Lack of rise of the serum IGF-I is diagnostic for Laron syndrome (Fig 4.1) (Daughaday et al. 1969; Laron et al. 1971). The definite diagnosis is the documentation of a molecular defect in the hGH receptor (hGH-R) gene.

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The location of the molecular genetic defect in the hGH receptor (Shevah et al. 2004a; Shevah et al. 2004b; Shevah and Laron 2006) gene requires preparation of DNA, PCR and sequencing (see Chap. 5 page 33). Lack of serum GHBP (growth hormone-binding protein), a rarely performed determination, discloses that the defect is in the extracellular domain of the GH-R

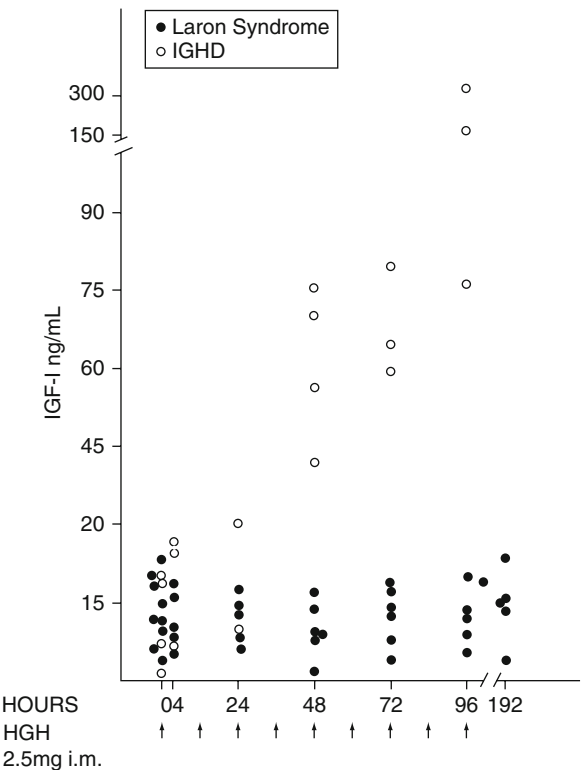

Fig 4.1 IGF-I stimulation (generation) test in patients with Laron syndrome compared to patients with IGHD (congenital hGH gene deletion). Note: Lack of rise of very low serum IGF-I during exogenous daily hGH administration in patients with Laron syndrome in contradistinction to the marked rise in the IGHD patients. Modified from Laron et al. (1971)

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Table 4.1 Comparison between clinical features and diagnostic laboratory characteristics in untreated Laron syndrome (primary GH insensitivity) and isolated GH deficiency (IGHD)

	Laron syndrome	IGHD
Clinical features	+	+
Dwarfism	+	+
Obesity	+	+
Sparse hair	+	+
Small head circumference	+	+
Frontal bossing, sunset sign	+	+
Crowded, defect teeth	+	+
Acromicria	+	+
Micropenis	+	+
High-pitched voice	+	+
Retarded skeletal maturation	+	+
Slow motor development	+	+
Laboratory		
Hypoglycaemia in infancy	+	+
Low serum IGF-I	+	+
High serum hGH	+	–
IGF-I rise upon hGH administration	–	+
Serum GHBP	– (or +)[a]	+
Final height – 4 to –10 SDS	+	+

[a]For further explanation see Chap. 5

(see pages 31–46). Exception to this seems to be patients with GH receptor mutations in intron 6 and the activation of a pseudo exon resulting in normal GHBP levels (David et al. 2007) in whom GHBP is positive. Mutations in the transmembrane or intracellular domains of the GH-R permitting GH binding to its receptor result in normal or high serum GHBP concentrations (Silbergeld et al. 1997). Low serum levels of IGFBP-3 (Laron et al. 1992a) and/or low IGF-I levels are not diagnostic (Laron et al. 1992b; Laron et al. 2007).

Table 4.1 summarizes the clinical and main laboratory features of untreated Laron Syndrome as compared to congenital isolated GH deficiency (IGHD). It is seen that the only diagnostic distinctions are the high serum hGH levels and lack of rise of IGF-I upon hGH administration. The final proof is obtained by genetic analysis of the hGH receptor gene.

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Genetic Aspects

5

Orit Shevah and Zvi Laron

Core Messages

- For the 64 patients with Laron syndrome (LS) in Israel, we were able to obtain 34 pedigrees. We performed molecular genetic analysis of the GH receptor in 35 patients and 32 family members. We found 16 different molecular defects, of them 3 novel mutations.
- This chapter also provides a hypothesis on the origin of LS and discusses whether the short stature of the African Pygmies is due to a defect in the growth hormone receptor similar or identical as this of the LS.

5.1 Genetic Investigations

Orit Shevah and Zvi Laron

Many studies have been performed on the hereditary forms of GH deficiency (Parks et al. 1999; Cogan and Phillips 2006; Mullis 2007) among others. The number of publications relating to those aspects in Laron syndrome (LS, OMIM #262500) is small (Adam et al. 1981; Woods and Savage 1996; Silbergeld et al. 1997; Rosenbloom and Guevara-Aguirre 1998; Shevah and Laron 2006).

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Forthwith the updated detailed genetic analysis of the Israeli cohort of 64 Laron syndrome patients and their family members.

5.1.1 Inheritance

The fact that from the start families with more than one child were referred to our clinic and that the majority of these families were consanguineous pointed to the genetic origin of this form of dwarfism. The genetic analyses of the patients and their families were made in several stages depending on the available knowledge and technologies.

The first genetic study and analysis performed in 1968 (Pertzelan et al. 1968) comprised 20 Laron syndrome patients (7 males, 13 females) belonging to ten families and two sibships, with strong consanguineous links, as well as 22 family members (Table 5.1). All of these families were Jews of Oriental (predominantly Mid-Eastern) origin.

The occurrence of affected siblings of both sexes, the high proportion of consanguineous parents, and the fact that the parents were not affected (with one exception who himself was late found to be homozygous for a stop mutation in the GH-R: Table 5.1, sibship #6 and Fig. 5.3b) pointed to an autosomal recessive inheritance. An additional criterion for a fully penetrate recessive gene is a ratio of 0.25 or more, i.e., one affected to three normal sibs within the affected families. As Table 5.1 shows, the ratio is 20/42 = 0.475 for LS families. Such a marked excess of affected members is a usual finding in many series of recessive diseases.

The expected number of affected children was calculated by the Bernstein-Lenz-Hogben method (Lenz 1963), which gives the average expected number of affected sibs

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Table 5.1 Pertinent genetic data of the first 20 Jewish patients with Laron syndrome

Sibship no.	Country of origin	Number of siblings									Parental consanguinity
		Affected			Normal			Total			
		M	F	Total	M	F	Total	M	F	Total	
1[a]	Iraq		2	2			0[b]		2	2	First cousins
2[a]	Iraq	1	1	2			0[b]	1	1	2	Distant relatives
3[a]	Iraq		1	1			0[b]		1	1	First cousins
4	Iraq	1	1	2			0[b]	1	1	2	First cousins
5	Iraq	1		1	2	3	5	3	3	6	First cousins
6[c]	Iraq		1	1			0		1	1	
7	Iran	1		1			0[b]	1		1	First cousins
8	Iran		1	1	1		1	1	1	2	
9	Iran		1	1	1	1	2	1	2	3	
10	Afghanistan		2	2	2	2	4	2	4	6	Double first cousins
11	Algeria		1	1		2	2		3	3	First cousins
12[d]	Yemen	2	1	3	2	2	4	4	3	7	Third cousins
13[d]	Yemen	1	1	2		4	4	1	5	6	Father is half-brother of maternal grandmother

(modified from Pertzelan et al. 1968)
M male; *F* female
[a]Related sibships
[b]Parents decided not to have more children following the birth of the affected child
[c]Father and daughter are affected
[d]Related sibships

in families with normal (heterozygous) parents, at least two offsprings and at least one affected, homozygous child. In our series of LS patients, there were six such families. The observed and expected numbers of affected children in each group were the same ($n=10$).

Even the odd occurrence of the LS-affected father whose daughter also had Laron syndrome was found compatible with a recessive mode of inheritance. The father was indeed a homozygote for W-15X mutation in the GH-R, and his second wife, the mother of the affected girl, was a heterozygote for the same defect (see pedigree of Family Me., in Fig. 5.3b).

5.1.2 Ethnic Details of the Israeli Cohort of Patients with Laron Syndrome

Our cohort of patients consisted of 64 patients (33 males and 31 females) up to March 2009. Their ages range from 2 to 75 years. The overall ethnic origin is shown in Table 5.2. The majority of the patients or their families originate from the Middle-East, Central Asia, and the Mediterranean region and were described in detail by Shevah and Laron 2006.

Forty-three patients are Israelis and Palestinians and 21 are from other countries. The patients belong to 42 families from various ethnic origins. The Jewish families originate from Iran (11 families), Iraq (seven), Yemen (two), Afghanistan (one), and Morocco (one). None of the Jewish families is Ashkenazi. The non-Jewish families comprise nine Arabs and one Druze; the additional ten families are from South-America, Italy, Greece, Malta, and Malaysia.

5.1.3 The Elucidation of the Molecular Pathology of Laron Syndrome

5.1.3.1 The IGF-I, hGH, and hCS Genes in Patients with Laron Syndrome

Using southern blotting of restriction endonuclease digests of genomic DNA from five patients originally

Table 5.2 Distribution of Ethnic origins in the Israeli cohort of Laron syndrome patients

	Total number of patients (M: F)	No. of families
	64[a] (33 : 31)	
Jews of oriental origin	31[a] (13 : 18)	Iran – 11 Iraq – 7 Yemen – 2 Morocco – 1 Afghanistan – 1
Arabs (including Israelis, Palestinians, and others)	16 (11 : 5)	Moslems – 6 Christians – 1 Maronite – 1
Druze	1F	Druze – 1
Malaysia	2M	Moslems – 2
Other ethnic origins (from: South America, Italy, Greece, Malta)	14 (7 : 7)	

[a]Including four deceased

described in Laron et al. (1968) and two of their family members, Russell et al. (1989) found that the Laron syndrome patients do not have deletions in the IGF-I gene and that their pattern and that of the hGH and hCS genes are normal.

5.1.3.2 Investigation of the Human Growth Hormone Receptor in Laron Syndrome Patients

Having shown that the etiology of Laron syndrome resides at the site of the GH receptor (Eshet et al. 1984), investigations at the molecular level of the hGH receptor were planned.

Cloning and sequencing of the human growth hormone receptor (hGH-R) cDNA by Leung et al. (1987) provided the means to study the possible molecular abnormalities of the GH-R in LS patients. Using restriction enzyme hybridization techniques on cDNA, Godowski et al. (1989) described the genomic organization of the human GH-R gene of nine of our Laron syndrome patients. The gene consists of 10 exons distributed over a distance of more than 87 Kb (Fig. 5.1). Transcription processing generates an mRNA that is 4.3 kb in length and encodes the 620 amino acid GH-receptor protein. Exon 2 comprises an 18 amino acid signal peptide and the first six amino acids of the extracellular domain. Exons 3–7 encode the remainder of the extracellular domain. The growth hormone binding protein (GHBP) is derived from this domain (Herington et al. 1986). The single, hydrophobic, transmembrane domain is encoded by exon 8, and the cytoplasmic domain is encoded by exons 9 and 10. Mature mRNA also contains more than 2 kb of 3' nontranslated sequence downstream from exon 10. The highly polymorphic 333 bp intervening sequences used by Amselem et al. (1989) for linkage analysis is equivalent to intron 9. Barton et al. (1989) have mapped the human GH-R gene to chromosome 5 between bands p12 and p13.1.

In two of the nine patients, Godowski et al. (1989) found a noncontinuous deletion of exons 3, 5, and 6 (Fig. 5.2). This was the first specific proof of a molecular defect in the GH-R gene in patients with Laron syndrome. The other patients did not carry the "deletion" alleles, demonstrating that LS is caused by a heterogeneity of gene defects. These findings were repeated by Brown et al. (1993).

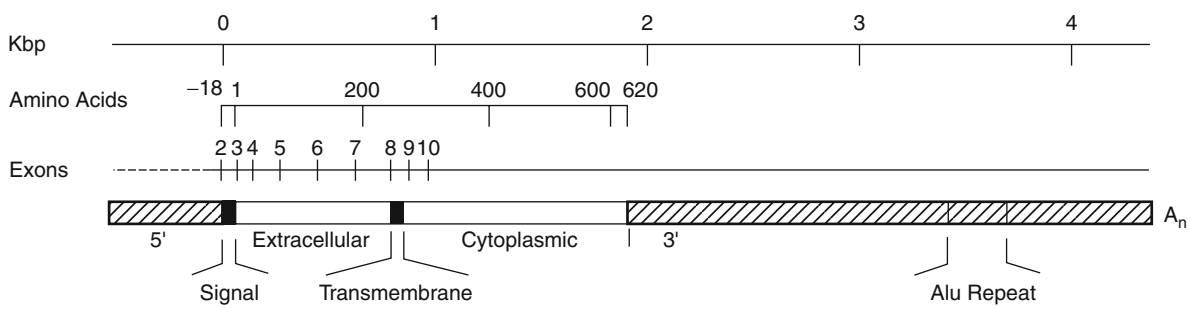

Fig. 5.1 Schematic representation of the GH-receptor mRNA and protein. Shown are scales of nucleotides and amino acids, the location of the exon boundaries, and the major feature of the protein and mRNA (from Godowski et al. 1989)

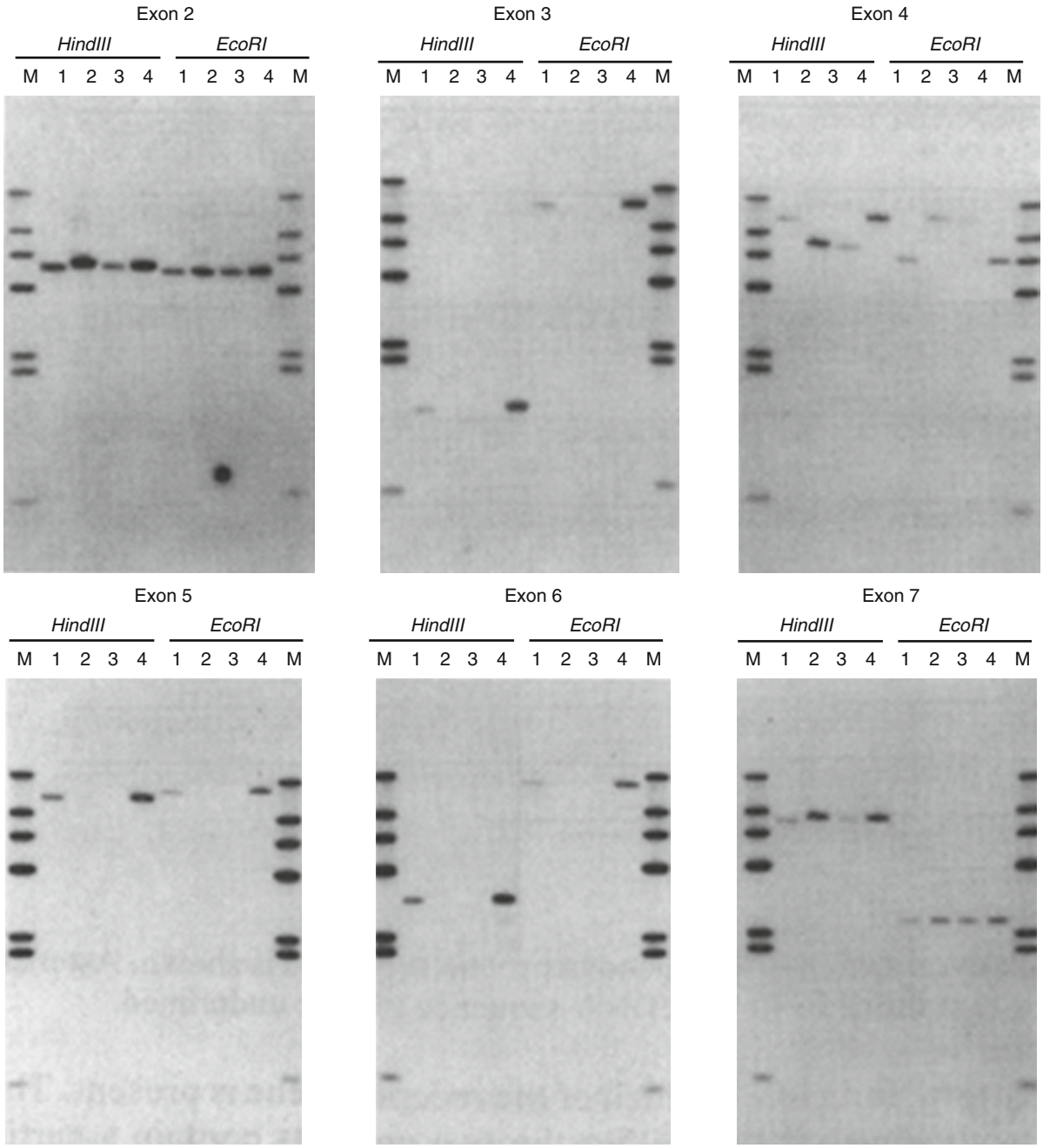

Fig. 5.2 Genomic blots hybridized with exon-specific probes. Blots containing DNA from two normal individuals (lanes 1 and 4) or LS patients (lanes 2 and 3) were hybridized with exon-specific probes. DNAs from LS patients failed to hybridize with the exon 3, 5, and 6 probes. (modified from Godowski et al. 1989)

Using at that time the newly method of polymerase chain reaction (PCR) and reverse transcriptase PCR (RT-PCR), Amselem et al. (1989) found point mutations in the GH-R in Laron syndrome patients originating from North-Africa. Both PCR and the technology of DNA sequencing enabled us to investigate a large number of Laron syndrome patients from our cohort (Godowski et al. 1989; Meacham et al. 1993; Berg et al.

Table 5.3 Primers sequences for PCR amplification of the GH-R gene

Primer sequence	Primer name[a]	Exon amplified
5′-TTTCATGATAATGGTCTGCTTTTA	2A	
5′-GAATACAGTTCAGTGTTGTTTCA	2B	2
5′-AGGATCACATATGACTCACCTG	4A	
5′-AGGAAAATCAGAAAGGCATGATG	4B	4
5′-ACTTAAGCTACAACATGATTTTTG	5A	
5′-GCTTCCCCATTTATTTAGTCTA	5B	5
5′-ATTAAATTGTGTCTGTCTGTGTACT	6A	
5′-GAAAGAAAAGTCAAAGTGTAAGGTG	6B	6
5′-TAGTGTTCATTGGCATTGAGT	7A	
5′-ACAAAAGCCAGGTTAGCTAC	7B	7
5′-ACTAGTCGTAATTCTGAAAGC	8A	
5′-AGGTCTAACACAACTGGTAC	8B	8
5′-GCTATAATTGAGAATATGTAGC	9A	
5′-TGACAGGAGTCTTCAGGTG	9B	9
5′-TATTATGAGTTTCTTTTCATAGATC	10A	
5′-ATTTTGATTCTTCAGGTCAAGGC	26P(reverse)	10

[a]A = forward; B = reverse

1994; Silbergeld et al. 1997; Gastier et al. 2000; Shevah et al. 2002; Shevah et al. 2004).

5.1.4 Genetic Analysis of the GH-R gene of Laron Syndrome Patients

Progress in technology and the availability of commercial kits for DNA analysis permitted further molecular studies of the GH-R.

5.1.4.1 Methods

DNA Extraction

Genomic DNA was extracted from peripheral blood leukocytes, using "nucleus buffer" (0.32M Sucrose, 10 mM Tris pH = 7.5, 5 mM $MgCl_2$, 1% Triton) for separation of the white blood cells. Then, the cells were incubated with 20% sodium dodocyl sulfate (SDS), lysis buffer (10 mM Tris pH = 7.5, 24 mM EDTA pH = 8.0, 75 mM NaCl), and Proteinase K (10 mg/mL). Finally, a visible thread of DNA was obtained by mixing the solution with 100% Ethanol.

Polymerase Chain Reaction (PCR) Amplification

PCR is a primer-directed enzymatic amplification of a specific DNA sequence. The DNA is combined with specific primers (oligonucleotides), deoxyribonucleotides (A, T, G, and C) for synthesis of new DNA strands, a buffer, and a DNA polymerase. PCR amplification is done by rapidly increasing or decreasing the reaction temperature.

For the amplification of exons 2–10 (including exon-intron boundaries) of the GH-R gene, we used intronic primers deduced from the published sequence by Godowski et al. 1989 (Table 5.3). The PCR conditions for specific amplification of exons 2, 4–10 are shown in Table 5.4. Amplified products were analyzed by electrophoresis on 2% agarose gel for determination of fragment size.

Table 5.4 PCR conditions for specific amplification of the GH-R exons

Exon	Annealing Temperature (°C)	$MgCl_2$ (mM)	PCR product (bp)
2	54	3	201
4	56	3	216
5	56	3	289
6	62	3	269
7	60	2.5	278
8	48	2.5	193
9	58	2.5	194
10	58	2	398

Table 5.5 Electrophoresis conditions in the SSCP analyses of the GH-R exons

Exon	Running Temperature (°C)	[TBE] mM	Running voltage	Running time (h)
2	Room	0.6	90	16
4	4	0.5	120	17
5	4	0.6	120	19.5
6	4	0.6	90	18
7	4	0.6	120	18.5
8	4	0.6	120	19.5
9	4	0.5	120	17
10	4	0.6	120	18

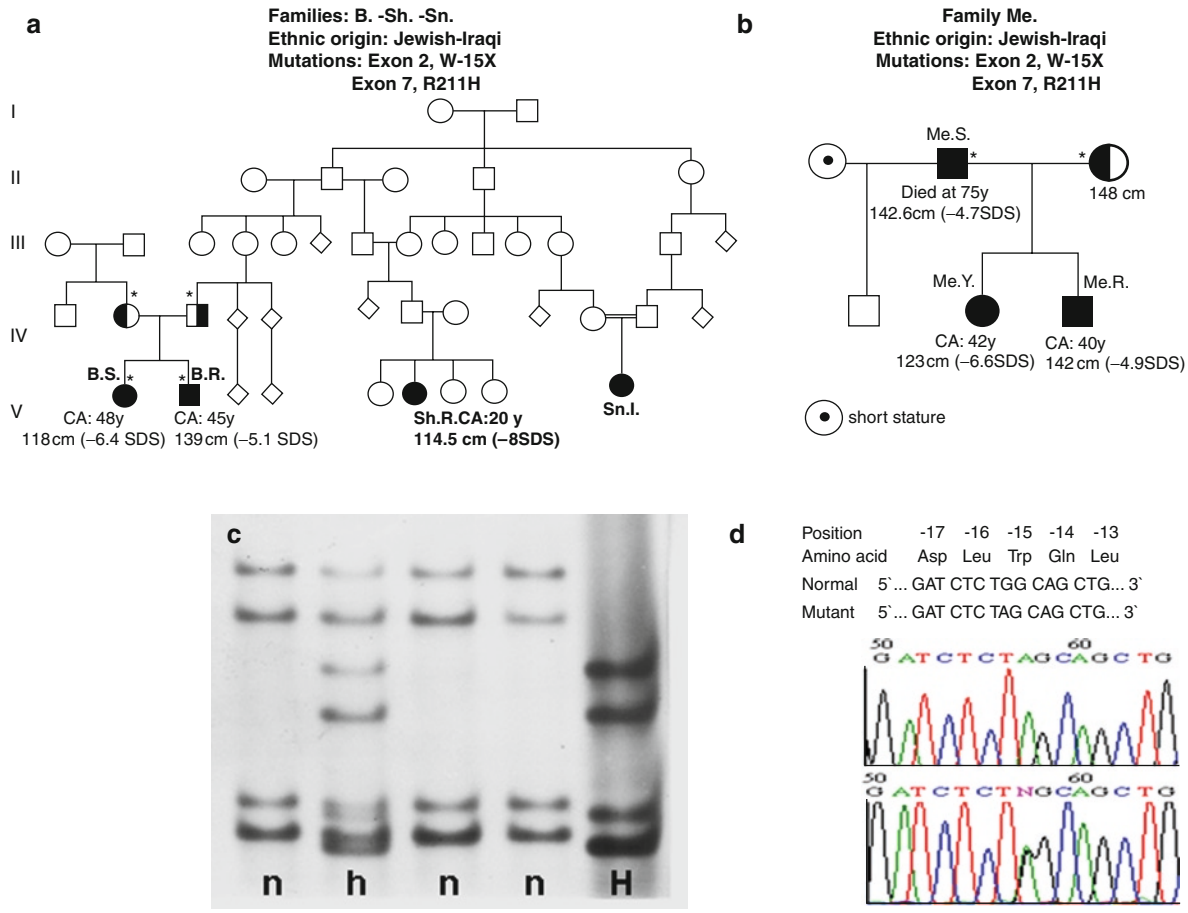

Fig. 5.3 (**a**, **b**) Pedigrees of two Jewish-Iraqi families carrying the same molecular defect in exon 2 of the GH-R and additional mutation in exon 7. The defect in exon 2 is responsible for the phenotype because it abolishes almost the entire exon. The first pedigree (Fig. 5.3a) is a clan comprising three related families. *Black* symbols indicate LS patients, *half-black* symbols indicate heterozygous carriers. Subjects marked with *asterisks* were genetically examined. (**c**) SSCP analysis of the PCR product of exon 2 showing normal (n), homozygous (H), and heterozygous (h) patterns. (**d**) Sequence of a homozygous mutated fragment of Exon 2 (upper chromatograph) compared with heterozygous sequence (lower chromatograph), showing a G to A substitution. This leads to replacement of Tryptophan with a termination codon at the fourth codon of the signal peptide (*W-15X*)

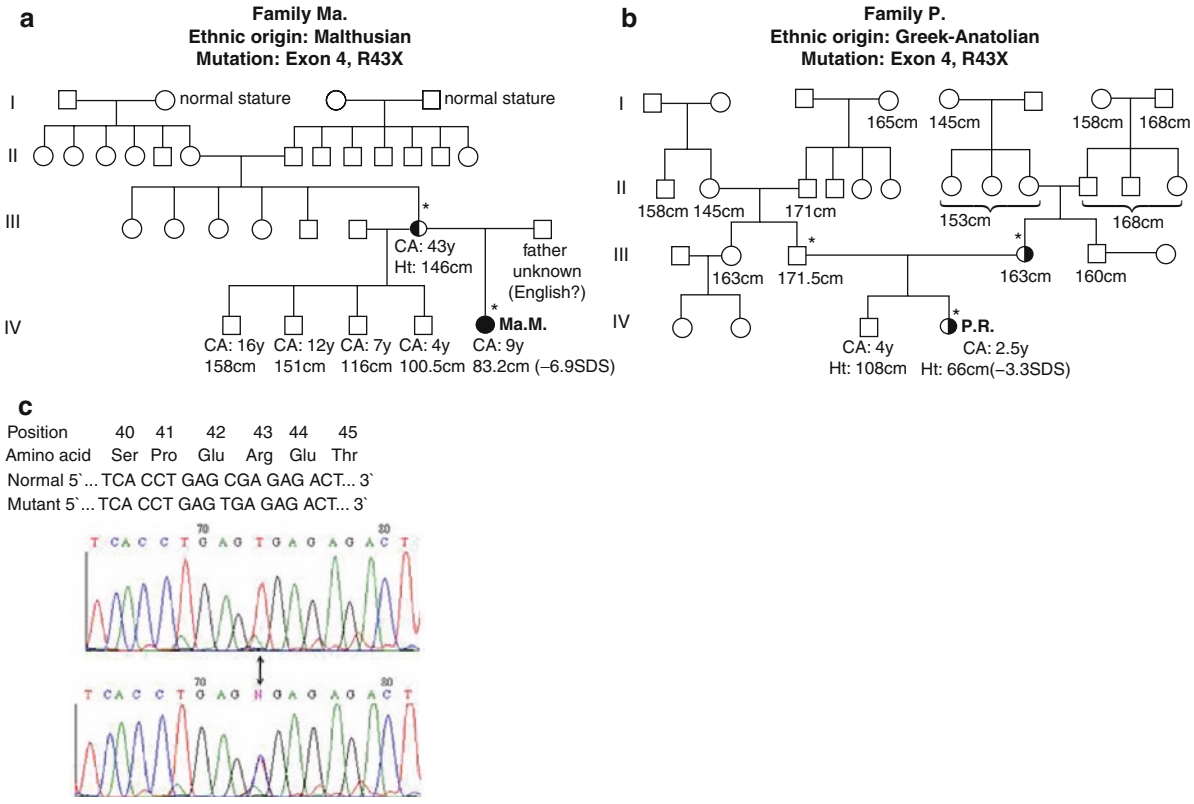

Fig. 5.4 (**a**, **b**) Pedigrees of two families from Malta and Greece with a mutation in exon 4 of the GH-R. *Black* symbols indicate LS patients, *half-black* symbols indicate heterozygous carriers. Subjects marked with *asterisks* were genetically examined. (**c**) Sequence of the homozygous mutated fragment of Exon 4 (upper chromatograph) compared with heterozygous sequence (lower chromatograph), showing a C to T substitution. This leads to replacement of Arginine with a termination codon in position 43 (*R43X*)

Analysis of Single Strand Conformation Polymorphism (SSCP)

SSCP is a common analysis for the detection of mutations based on conformation changes of the double-stranded DNA (Orita et al. 1989). SSCP was performed to all PCR products to detect aberrant bands, using 3 μL of PCR product + 20μL of loading buffer loaded onto 0.5xMDE gel (AT Biochem) (after denaturation at 100°C for 3 min). Electrophoresis conditions are shown in Table 5.5. The gels were silver stained in order to see the typical pattern of normal and abnormal fragments.

DNA Sequencing

Samples that presented an abnormal pattern were subjected to DNA sequence analysis on a capillary electrophoresis-based analyzer. Sequencing was performed on both the sense and the antisense strands, in collaboration with Prof. M. Rubinstein, of The Weizmann Institute of Science, Rehovot, Israel.

5.1.4.2 Results

Molecular Analysis Results

Thirty-five patients (out of 64) and 32 of their relatives, from 27 families, underwent molecular analysis of the GH-R by our group and by others. Their pedigrees and molecular analysis are illustrated in Figs. 5.3–5.15 (arranged according to the exon number). The remaining patients and families either did not collaborate or had left the country.

Sixteen different molecular defects of the GH-R were found in the Israeli LS cohort (Fig. 5.16), three of the mutations were novel (Shevah et al. 2003; Shevah et al. 2004). Twenty-eight patients were homozygous for a single mutation or deletion, one patient was a compound

heterozygote (Fig. 5.11b), and in another patient, three different mutations were detected (Fig. 5.14). Two patients were heterozygotes; one of them carried a nonsense mutation in exon 4 (R43X) and a polymorphism in exon 6 (Gly168Gly), both in the maternal allele (Fig. 5.4b). Three Palestinian-Arab siblings (Fig. 5.13) had normal serum GHBP and IGFBP-3 levels; thus, a postreceptor defect was considered (Laron et al. 1993). However, we did not detect any mutation in the GH-R. Subsequently, one of these siblings was examined in London and was found to have an intronic mutation in the GH-R gene, resulting in the activation of a pseudoexon (6ψ) and inclusion of 36 amino acids (David et al. 2007).

In all the families, the 28 out of 32 relatives studied were heterozygous for the GH-R molecular defect of the proband (four had a normal GH-R gene). In the Druze family, ten members, including the proband's sister and her parents (but not her brother) who were heterozygotes, had high GHBP levels. It is of note that all of them were of normal stature (Fig. 5.12a, Silbergeld et al. 1997).

Patients with No Molecular Analysis

In seven patients followed since birth or early childhood, contact was lost due to marriage, emigration, etc. With four families, renewal of contact was successful but the patients did not agree to a genetic analysis. Their pedigrees are shown in Fig. 5.17. Additionally, we have medical information on 15 patients who were referred for treatment and counseling. The pedigrees of four families are shown in Fig. 5.18.

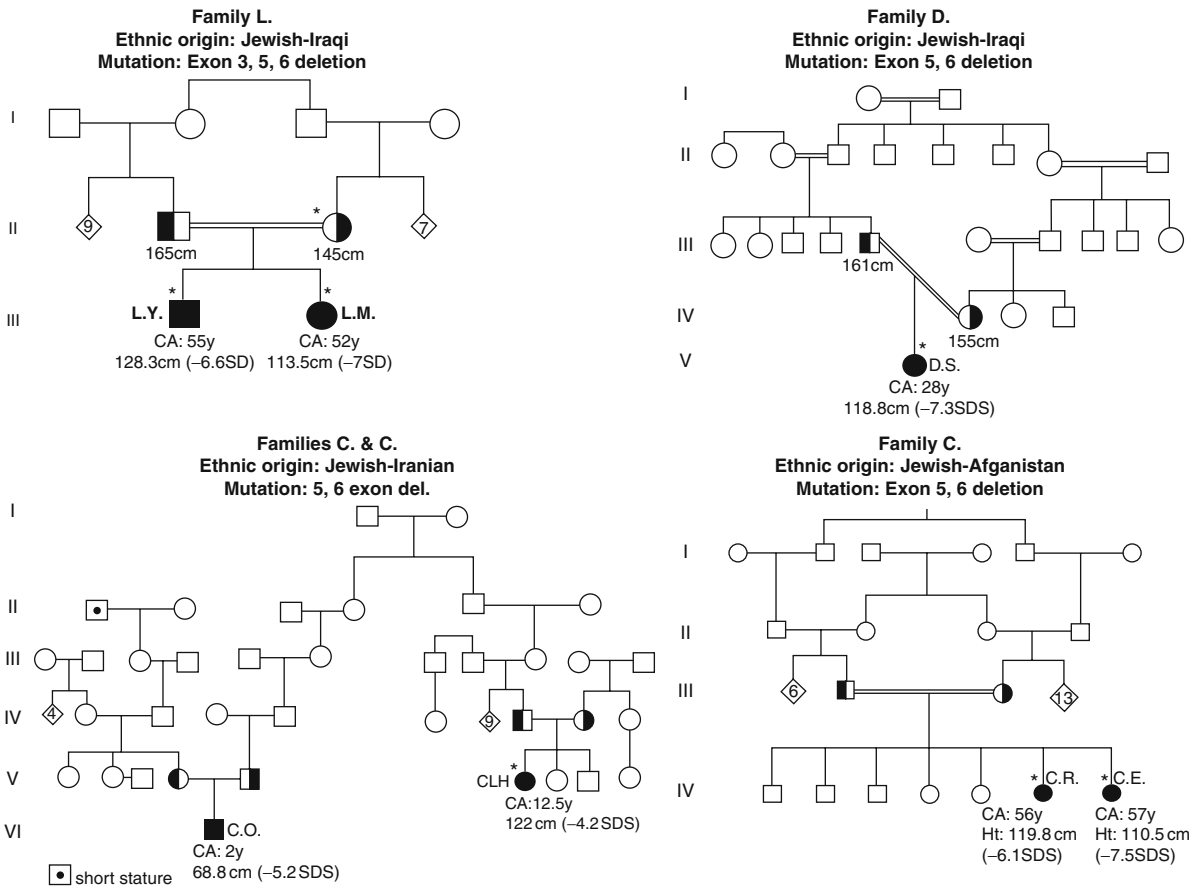

Fig. 5.5 Pedigrees of six Jewish families originating from Iraq, Iran, and Afghanistan. The LS probands are homozygous for the *3, 5, and 6 exon deletion*. *Black* symbols indicate LS patients, *half-black* symbols indicate heterozygous carriers. Numbers in diamonds indicate additional siblings. Subjects marked with *asterisks* were genetically examined. Family L was the first to be diagnosed with this large deletion by a genomic blot hybridization technique (see Fig. 5.2)

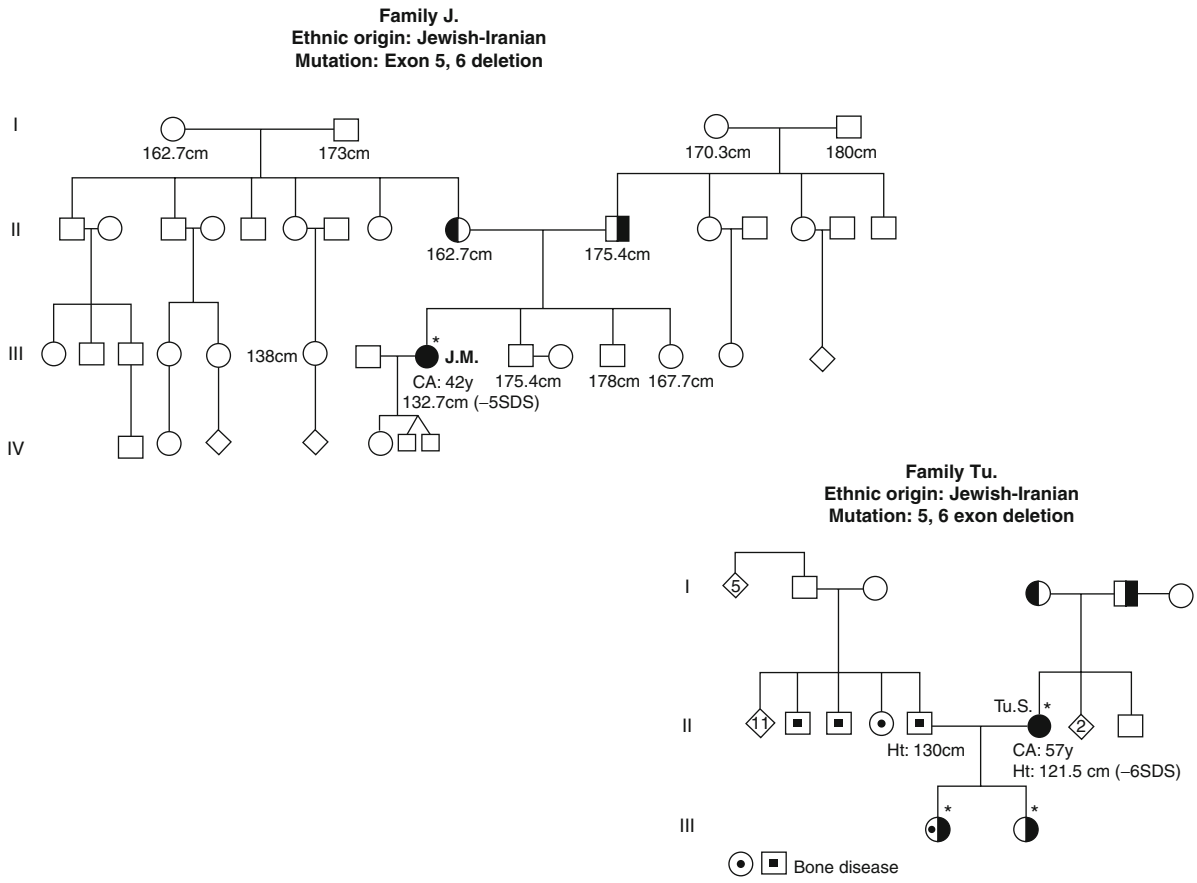

Fig. 5.5 (continued)

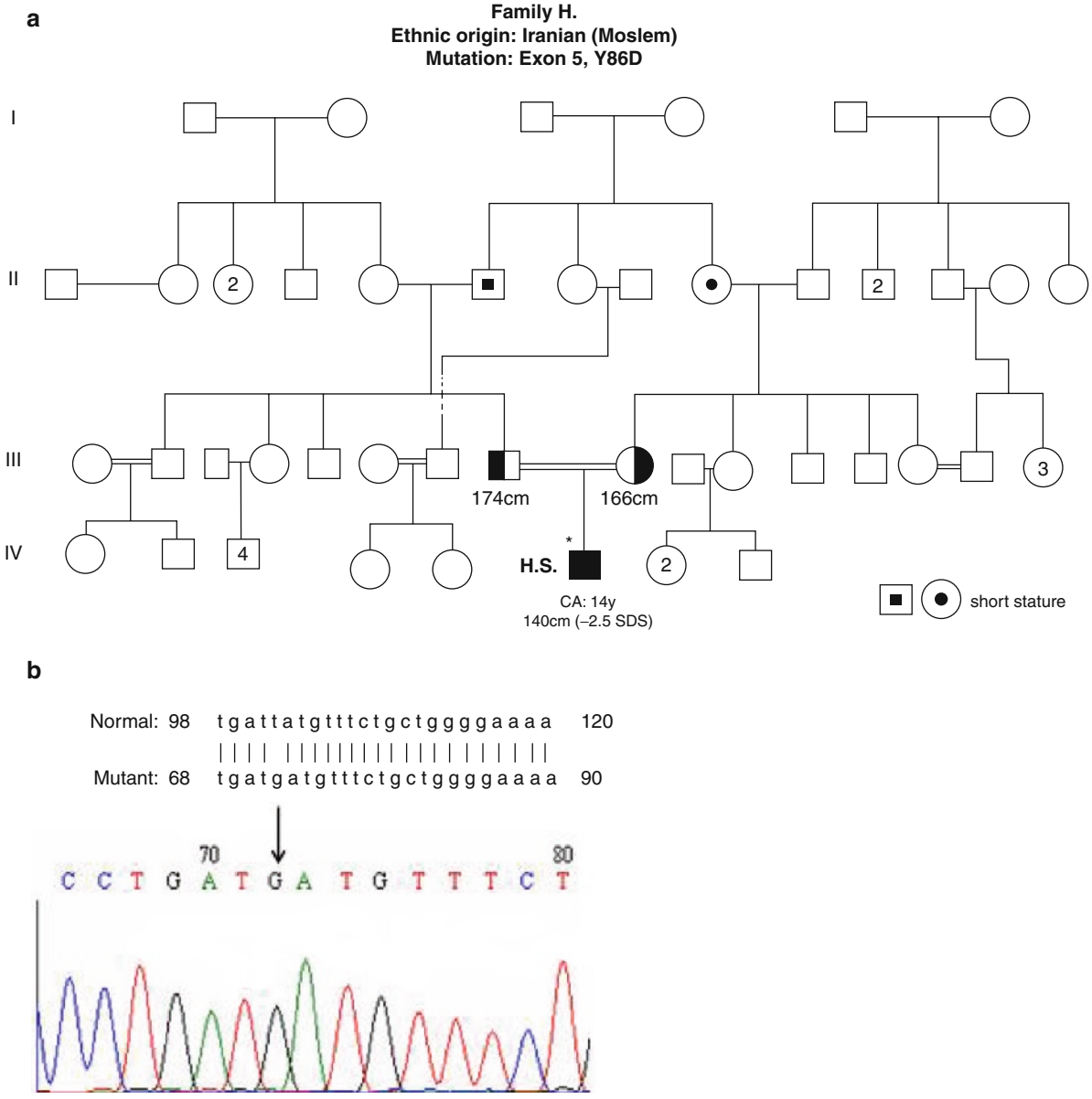

Fig. 5.6 (**a**) Pedigree of an Iranian family with high degree of consanguinity (parents connected by a *doubled line*). The proband is homozygous for a missense mutation in exon 5. *Black* symbols indicate LS patients, *half-black* symbols indicate heterozygous carriers. Subjects marked with *asterisks* were genetically examined. (**b**) Sequence of the normal fragment of exon 5 compared with homozygous mutated sequence (lower line and chromatograph), showing a T to G substitution. This leads to replacement of Tyrosine with Aspartate in codon 86 (*Y86D*)

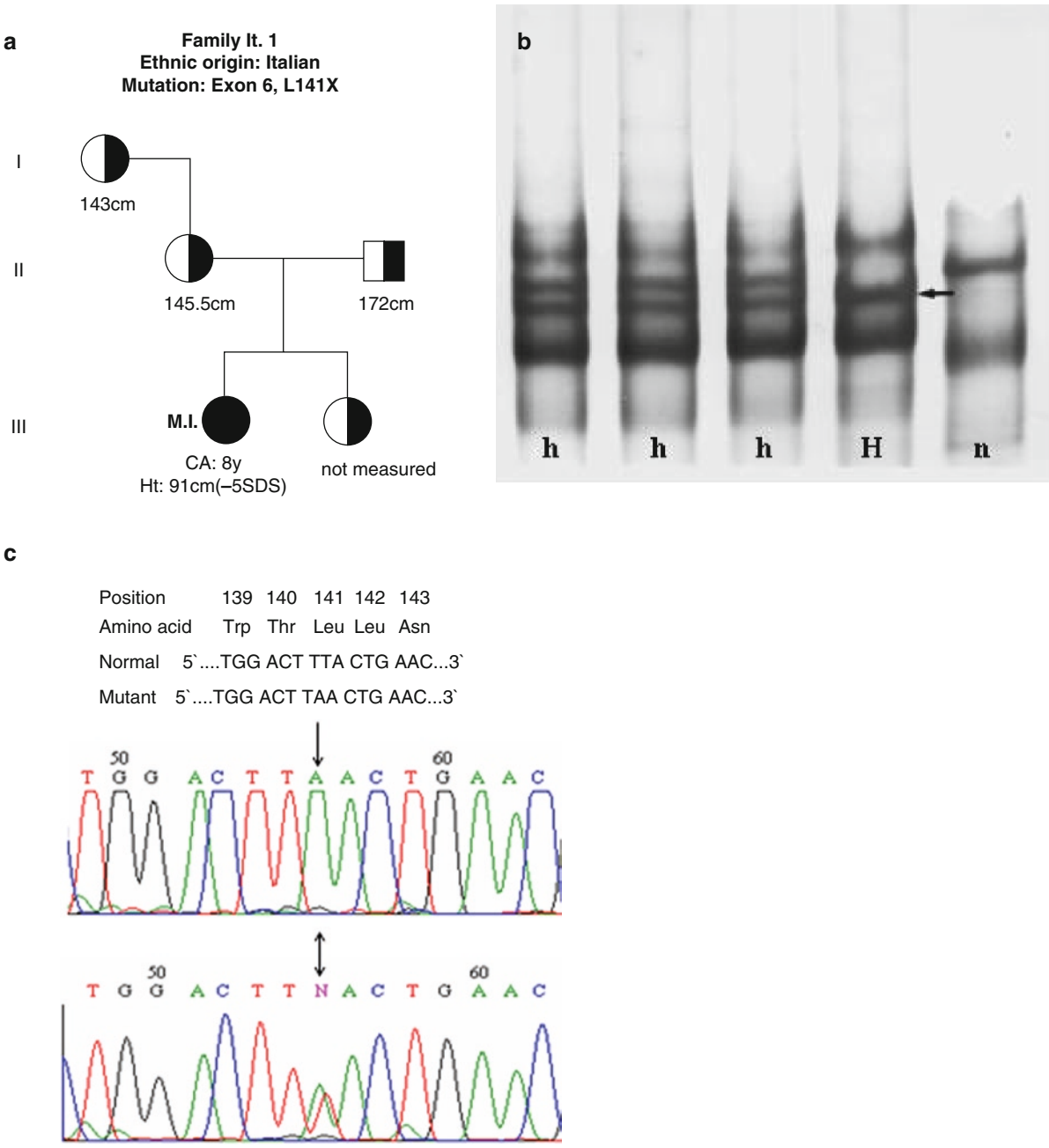

Fig. 5.7 (**a**) Pedigree of an Italian family. All subjects were genetically analyzed. *Black* symbol indicates the homozygous proband, *half-black* symbols indicate the heterozygous relatives. (**b**) SSCP analysis of the PCR product of exon 6 showing normal (n), homozygous (H), and heterozygous (h) patterns. *Arrow* points at the single-bold band which characterizes the homozygous patient. The heterozygous family members are characterized by two bands. (**c**) Sequence of the homozygous mutated fragment of exon 6 (upper chromatograph) compared with heterozygous sequence (lower chromatograph), showing a T to A substitution. This leads to replacement of Leucine with a termination codon in position 141 (*L141X*)

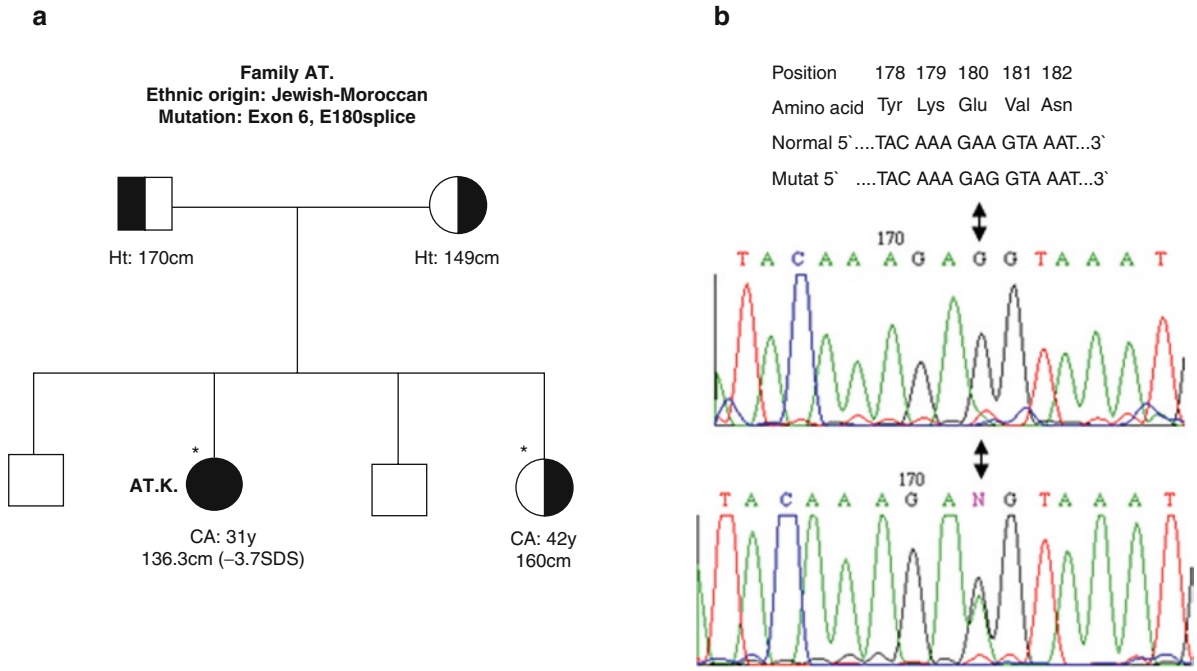

Fig. 5.8 (**a**) Pedigree of a Jewish-Moroccan family carrying a mutation in exon 6 of the GH-R. *Black* symbol indicates LS patient, *half-black* symbols indicate heterozygous carriers. Subjects marked with *asterisks* were genetically examined. (**b**) Sequence of the homozygous mutated fragment of exon 6 (upper chromatograph) compared with heterozygous sequence (lower chromatograph). The substitution of A to G led to a cryptic splice site between exon 6 and intron 6, thus causing a 24 nucleotides (eight amino acids) deletion and the destruction of the GH-R (*E180 splice*)

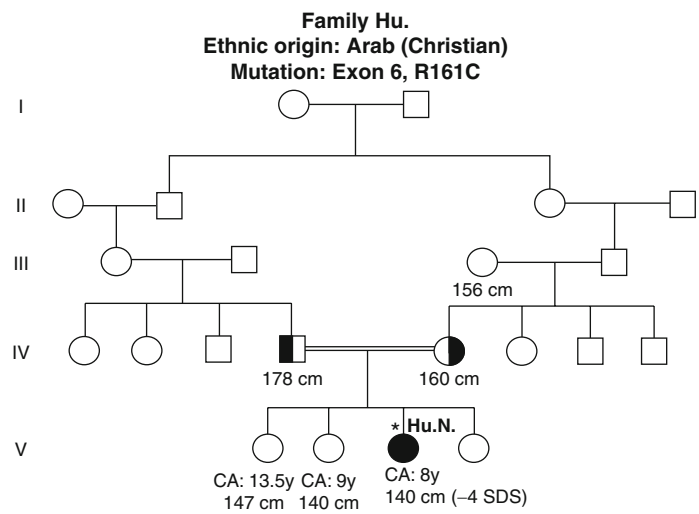

Fig. 5.9 Pedigree of an Israeli-Arab family. *Black* symbols indicate LS patients, *half-black* symbols indicate heterozygous carriers. The proband was genetically analyzed* in another clinic and was found homozygous for the *R161C* mutation in exon 6

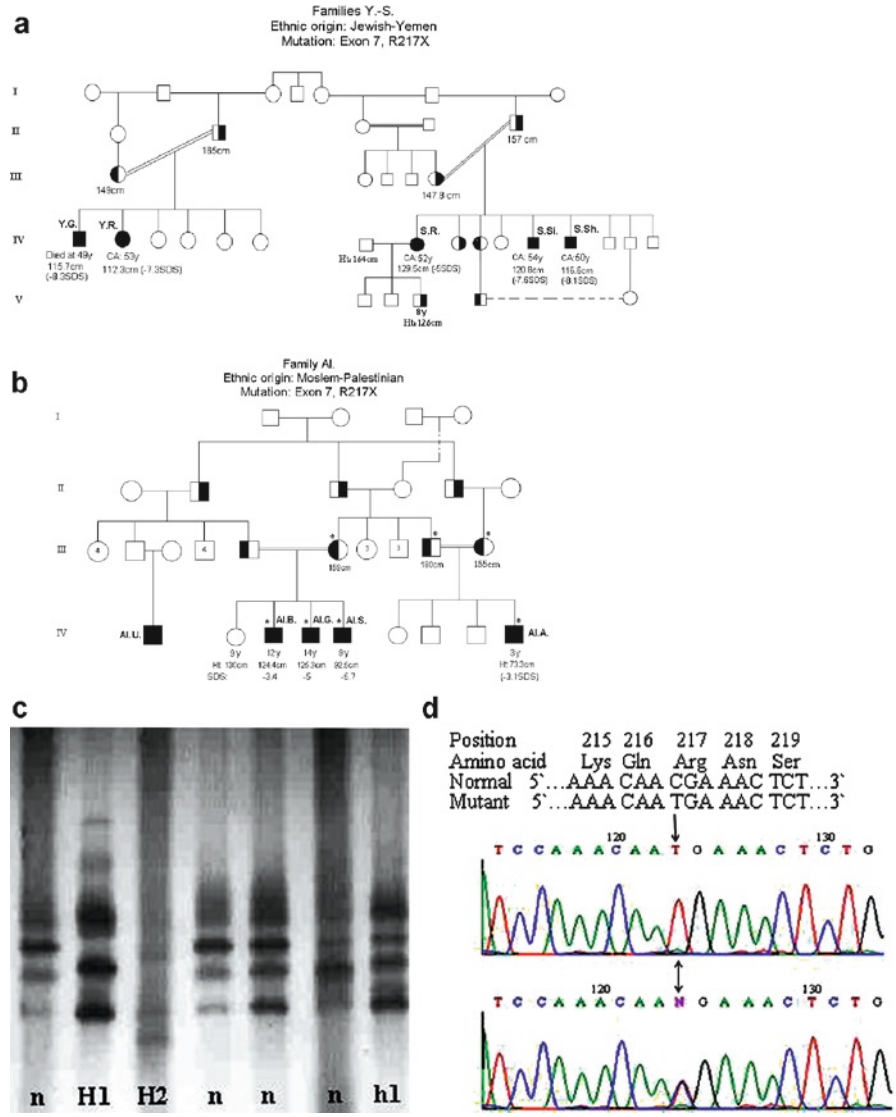

Fig. 5.10 (**a**, **b**) Two pedigrees of highly consanguineous families, a Jewish-Yemenite clan (**a**) comprising two related families and a Moslem-Palestinian family (**b**) carrying the same molecular defect in exon 7 of the GH-R. *Black* symbols indicate LS patients, *half-black* symbols indicate heterozygous carriers. Subjects marked with *asterisks* were genetically examined. (**c**) SSCP analysis of the PCR product of exon 7 showing normal (n), homozygous (H1 for R217X, H2 for R211H), and a heterozygous (h1) patterns. (**d**) Sequence of the homozygous mutated fragment of exon 7 (upper chromatograph) compared with heterozygous sequence (lower chromatograph), showing a C to T substitution. This leads to replacement of Arginine with a termination codon in position 217 (*R217X*)

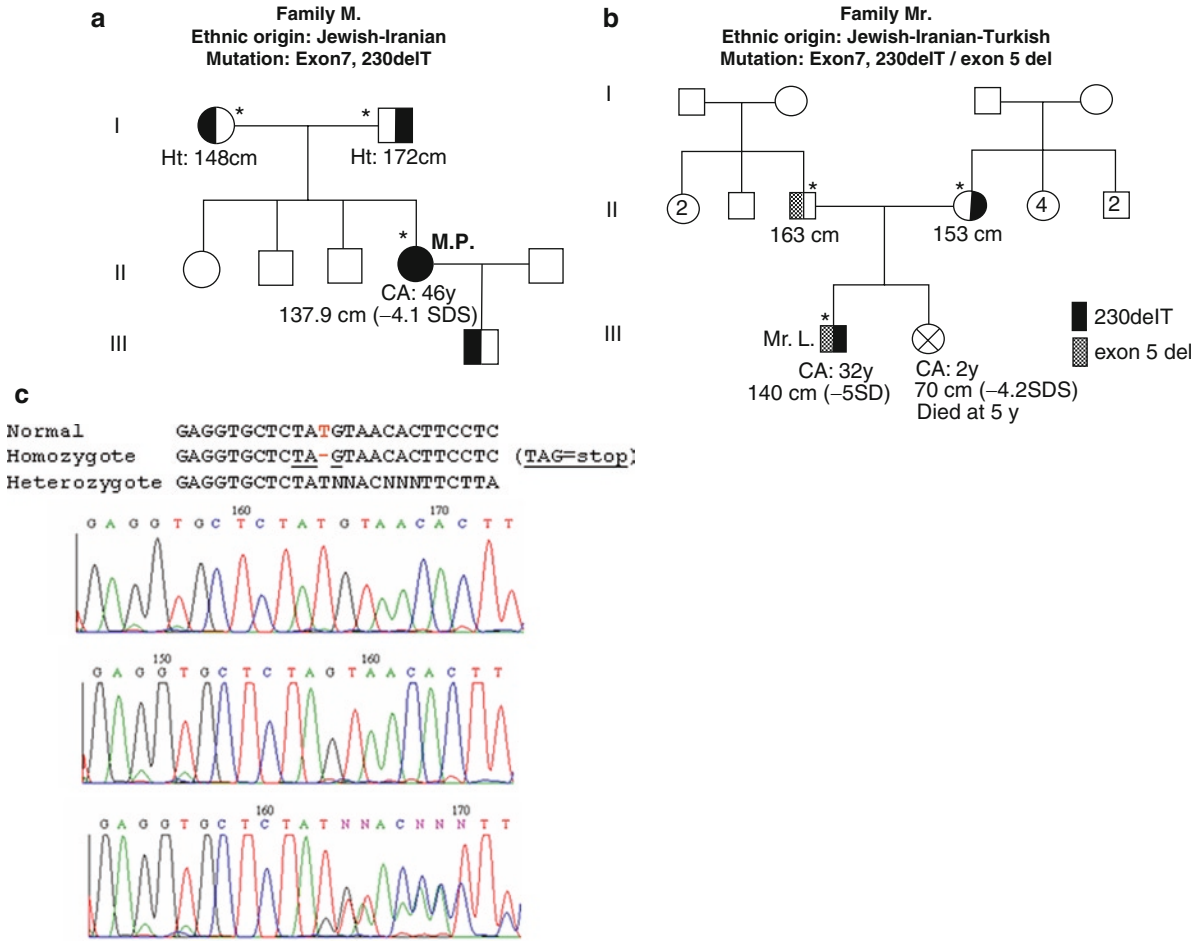

Fig. 5.11 (**a**) Pedigree of a Jewish-Iranian family in whom the proband was found homozygous for a frameshift mutation in exon 7 and her parents, heterozygotes. (**b**) Pedigree of a Jewish-Iranian/Turkish family in whom the proband is a compound heterozygote: he carries a frameshift mutation in exon 7 on the maternal allele and an exon 5 deletion on the paternal allele. His mother, from Iran, and father, from Turkey, are heterozygous for the respective mutations. *Black* symbols indicate LS patients, *half-black* symbols indicate heterozygous carriers. Subjects marked with *asterisks* were genetically examined. (**c**) Comparison between normal (upper chromatograph), homozygous mutated (*middle*), and heterozygous (*lower*) sequences of the PCR product of exon 7. Deletion of one nucleotide, T in amino acid 230, shifts the reading-frame that terminates in a stop codon (*230delT*)

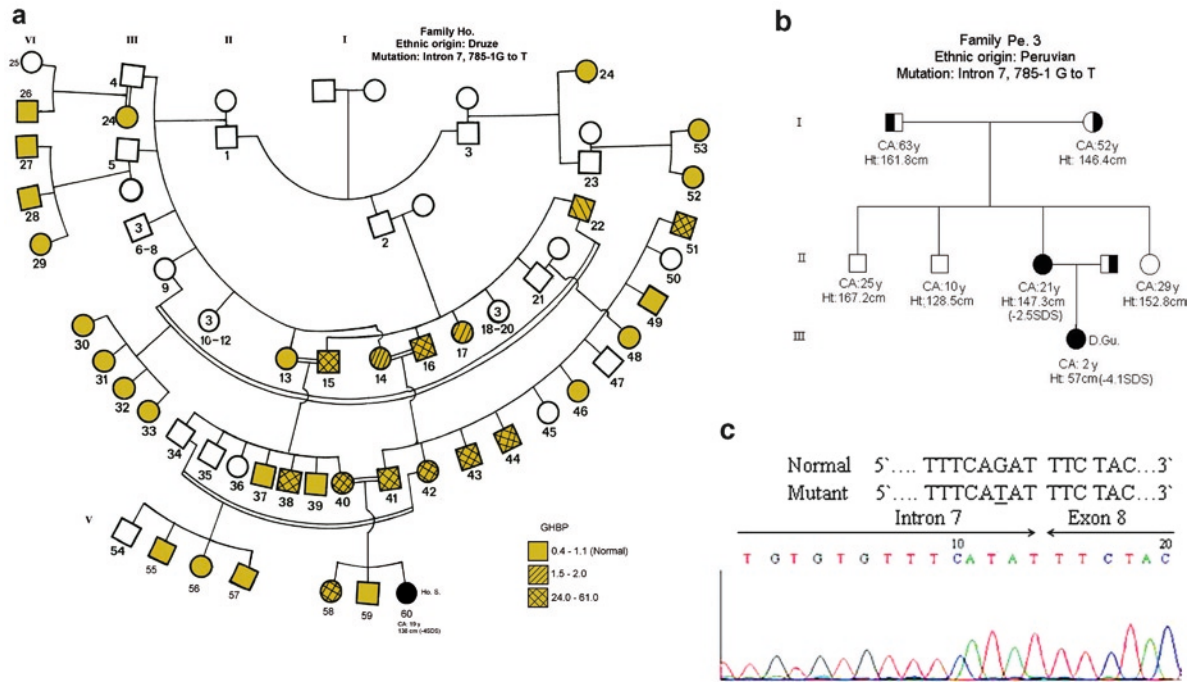

Fig. 5.12 (**a**) Pedigree of a highly consanguineous Druze family. The proband (*black symbol*) is homozygous for a splice site mutation in intron 7. Her parents and sister are heterozygotes. The mutation results in very high serum GHBP levels in heterozygous subjects (Silbergeld et al. 1997). (**b**) Pedigree of a family from Peru with the same intronic splice mutation, detected in the proband and her mother. (**c**) Sequence of the PCR product of exon 8 showing a homozygous G to T substitution in the splice acceptor site, at nucleotide 785-1, preceding exon 8. This substitution destroys the invariant AG dinucleotide of the splice acceptor site and is responsible for skipping exon 8, a sequence that mostly encodes the transmembrane domain

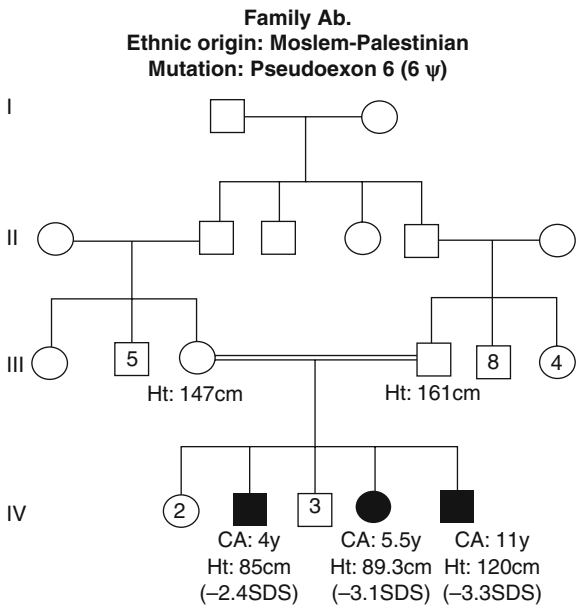

Fig. 5.13 Pedigree of a consanguineous Palestinian family with three LS patients. *Black* symbols indicate LS patients. One of the siblings underwent genetic analysis of the GH-R abroad and was found to have a mutation in intron 6, which leads to an inclusion of 36 amino acids and activation of a pseudoexon (6ψ)

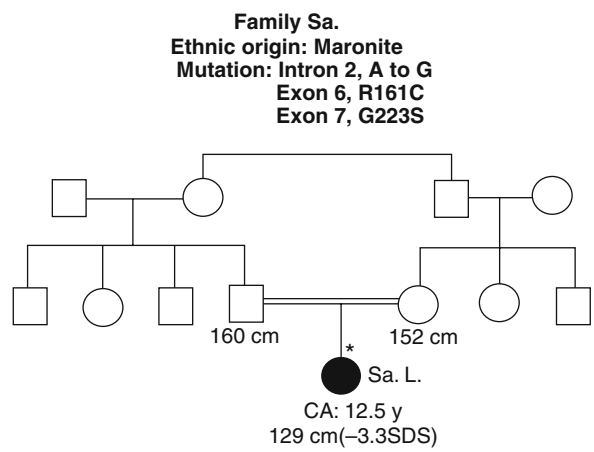

Fig. 5.14 Pedigree of a consanguineous Maronite family. *Black* symbol indicates LS patient Genetic analysis (*) of the proband's GH-R revealed three different mutations. A to G substitution in intron 2, a missense mutation in exon 6 – *R161C*, and a missense mutation in exon 7 – *G223S* (patient of Wakim, personal communication)

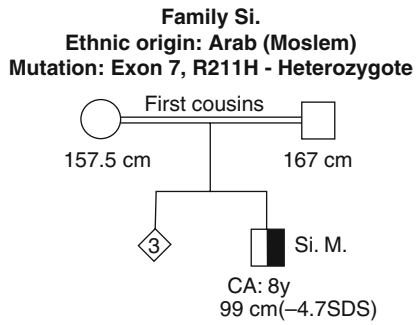

Fig. 5.15 Partial pedigree of an Arab family in whom the proband is heterozygous for a missense mutation in exon 7 – *R211H* (patient of Tenenbaum-Rakover, personal communication)

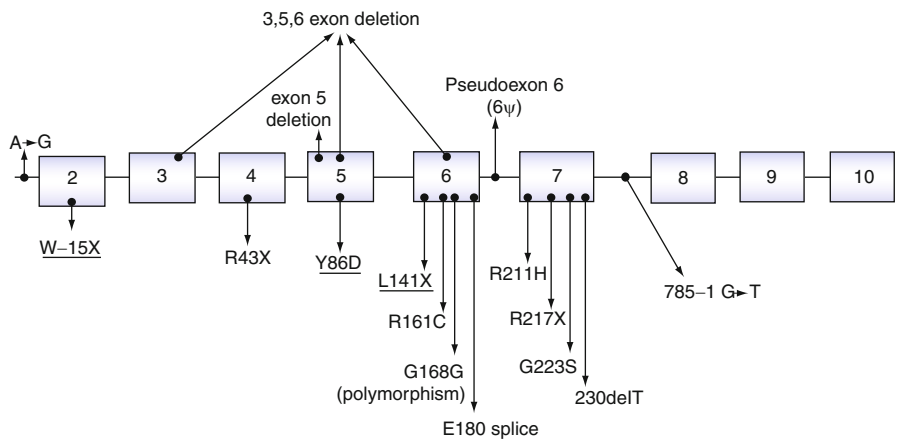

Fig. 5.16 Schematic representation of sixteen molecular defects detected in 27 families with Laron syndrome in the Israeli cohort. Underlined are new mutations described by our group

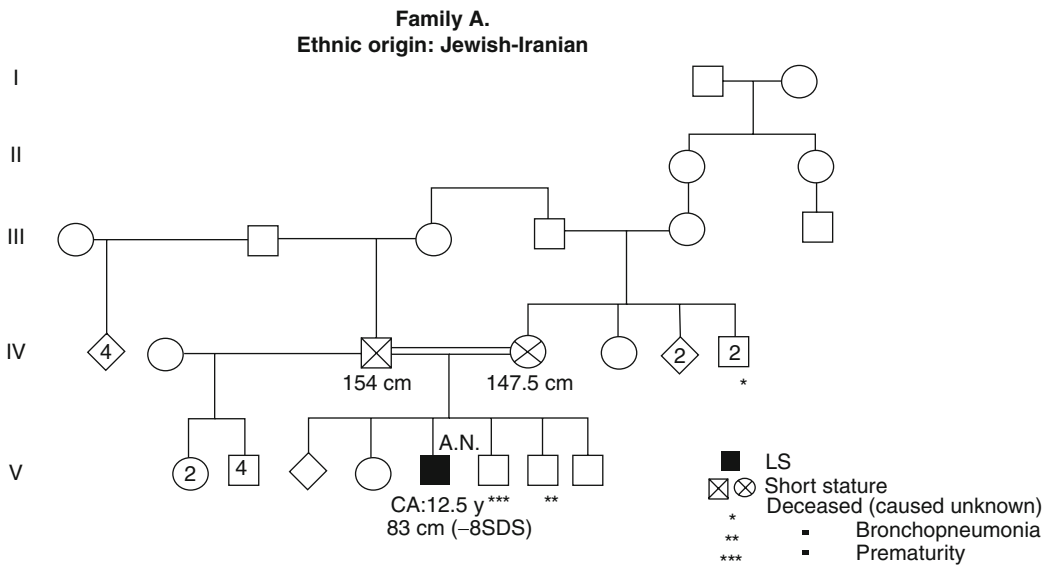

Fig. 5.17 Pedigrees of seven families in whom molecular analysis was not performed. All families are characterized with high degree of consanguinity. Five families are of a Jewish-Iranian origin, one is Jewish-Iraqi, and one is Arab-Moslem

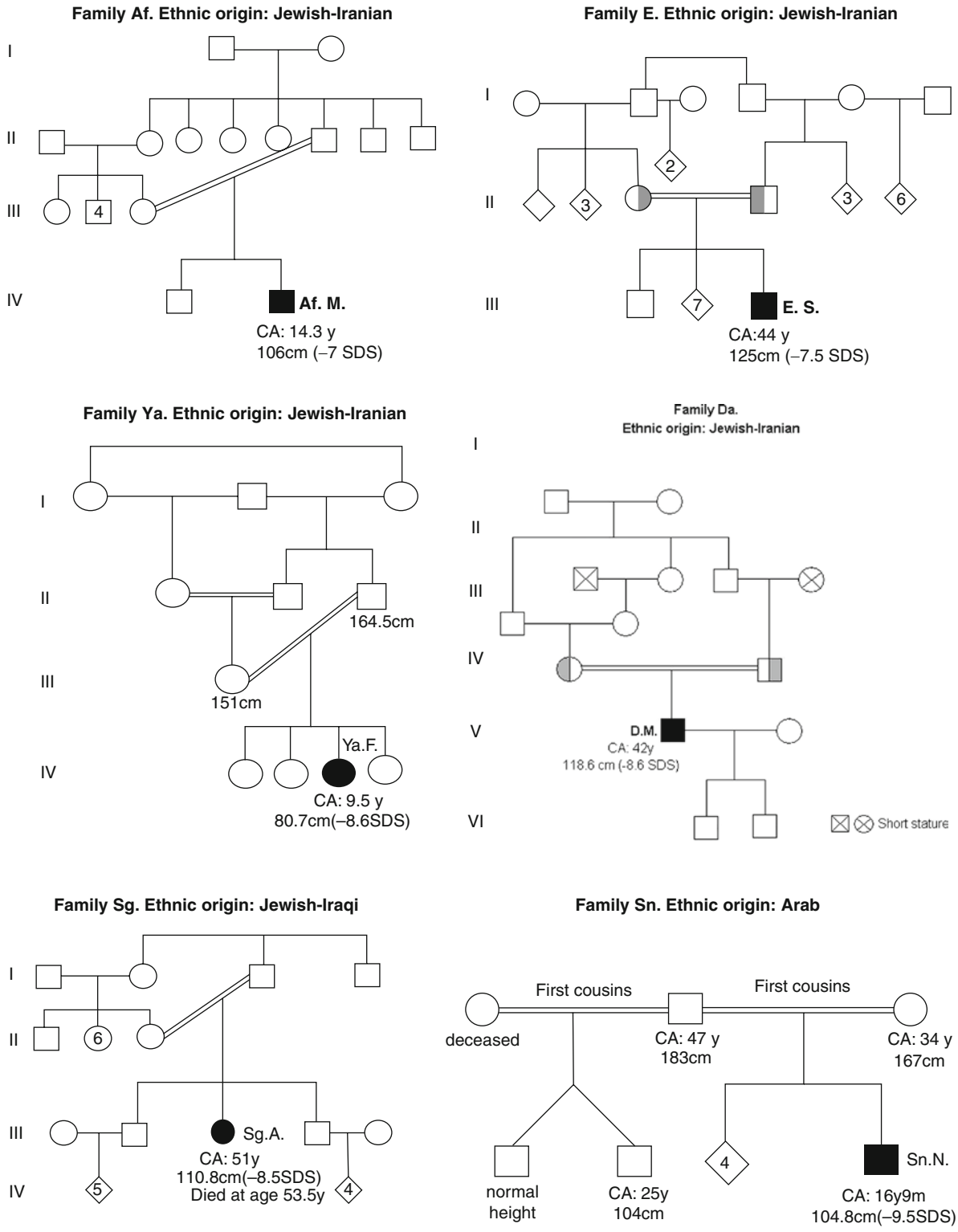

Fig. 5.17 (continued)

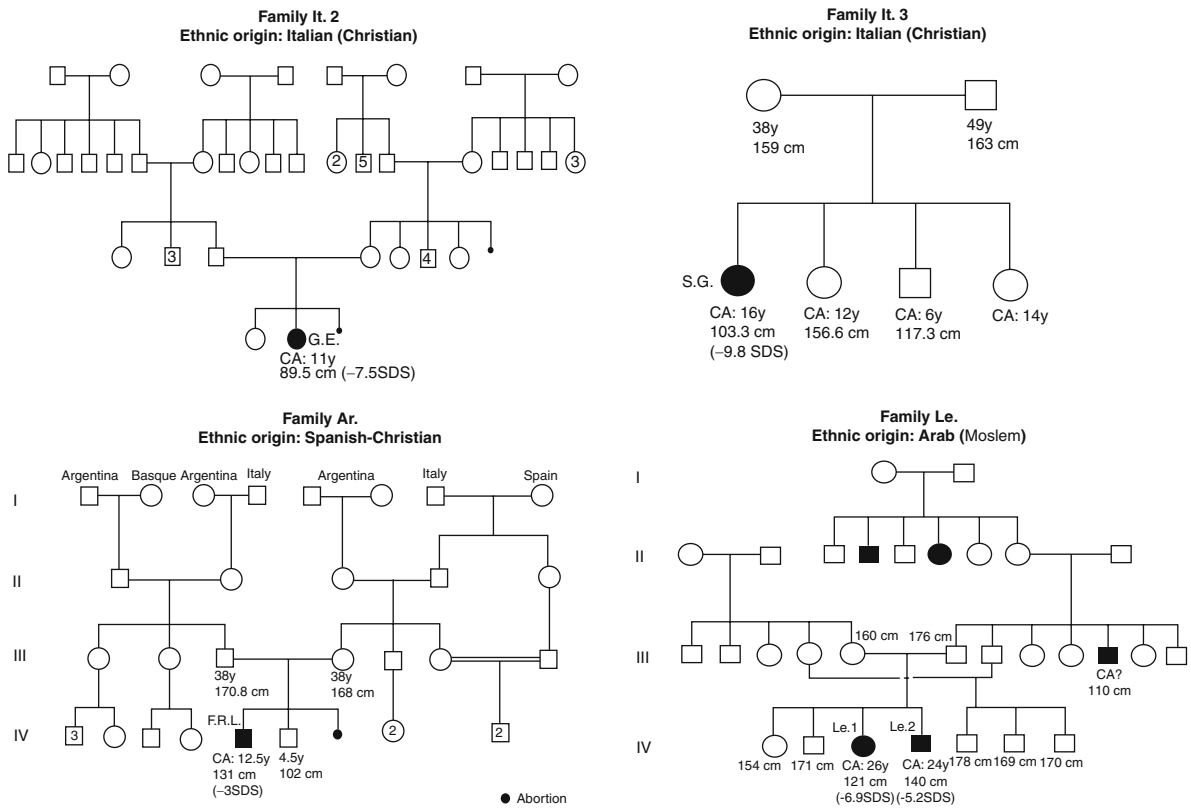

Fig. 5.18 Four pedigrees of families from abroad referred to our clinic for consultation. *Black* symbols indicate LS patients, *half-black* symbols indicate heterozygous carriers

5.1.4.3 Consanguinity

We found consanguinity in 21 of the families. The consanguineous families are marked in Table 5.6. The high degree of consanguinity is partly explained by the origin from small isolates in the Middle-East, as well as by tradition and customs in those populations. This phenomenon does not seem to be extinguished: we encountered two cousins from a Jewish-Yemenite family who intended to marry and consulted us. Molecular analysis of the GH-R revealed that the male was heterozygous for the R217X mutation in exon 7 and the female was normal (see pedigree in Fig. 5.10a, family S). We explained to the future parents that 25% of their offsprings might be heterozygotes for Laron syndrome.

5.1.4.4 Family Planning

Genetic counseling reduced the number of children in consanguineous families so that heavily affected families did not have further children. Relatives of affected families have inquired about genetic counseling, which in the future may include prenatal diagnosis.

5.1.5 Discussion

In contradistinction to the members of the Ecuadorian cohort (Rosenbloom et al. 1990) who lived in isolates, the population of the Israeli cohort originates from different areas and ethnic groups. This may explain the diversity of the molecular defects in the GH receptor in our cohort. The role of epigenetic phenomena in the diversification of the GH-R gene defects needs to be considered.

5.1.6 Acknowledgment

We are indebted to Prof. Menachem Rubinstein of the Dept. of Molecular Genetics, Weizmann Institute of

Table 5.6 Genetic information and consanguinity status of the LS patients of the Israeli cohort

Family name (patient initials)	Number of LS patients	Ethnic origin	Mutation (location)	Consanguinity	Heterozygous relatives identified
B	2	Jewish-Iraqi	W-15X (signal peptide)	Yes	2 (parents)
Sh	1	Jewish-Iraqi	ND	No	ND
Sn	1	Jewish-Iraqi	ND	Yes	ND
Me	3	Jewish-Iraqi	W-15X (signal peptide)	No	1 (wife)
Ma	1	Malthusian	R43X (Exon 4)	No	1 (mother)
P	1	Greek-Anatolian	R43X (Exon 4)/normal	No	1 (mothers)
L	2	Jewish-Iraqi	3, 5, 6 exon deletion	Yes	1 (mother)
Tu	1	Jewish-Iranian	5, 6 exon deletion	No	2 (daughters)
J	1	Jewish-Iranian	5, 6 exon deletion	No	ND
D	1	Jewish-Iraqi	5, 6 exon deletion	Yes	ND
C (LH)	1	Jewish- Iranian	5, 6 exon deletion	No	ND
C (O)	1	Jewish- Iranian	5, 6 exon deletion	No	ND
C (E&R)	2	Jewish-Afghani	5, 6 exon deletion	Yes	ND
H	1	Iranian	Y86D (Exon 5)	Yes	ND
It.1	1	Italian	L141X (Exon 6)	No	4 (parents, GM, sister)
AT	1	Jewish-Moroccan	E180 splice (Intron 6)	No	1 (sister)
Hu	1	Christian-Arab	R161C	Yes	ND
Y	2	Jewish-Yemenite	R217X (Exon 7)	Yes	ND
S	3	Jewish-Yemenite	R217X (Exon 7)	Yes	4 (mother, sister, nephew, proband`s son)
Al	4	Palestinian Arab	R217X (Exon 7)	Yes	3 (parents and mother)
M	1	Jewish-Iranian	230delT	No	2 (parents)
Mr	1	Jewish-Iranian/ Turkish	230delT/Exon 5 del.	No	2 (parents)
Ho	1	Druze	785-1G toT (Intron 7)	Yes	3 (parents, sister)
P3	1	Peruvian	785-1G toT (Intron 7)	No	1 (mother)
Ab	3	Palestinian Arab	Pseudoexon 6	Yes	ND
Sa	1	Maronite	Intron 2 A to G, R161C, G223S	Yes	ND
Si	1	Arab	R211H heterozygous	Yes	ND
A	1	Jewish-Iranian	ND	Yes	ND
E	1	Jewish-Iranian	ND	Yes	ND
Da	1	Jewish-Iranian	ND	Yes	ND
Af	1	Jewish-Iranian	ND	Yes	ND
Ya	1	Jewish-Iranian	ND	Yes	ND
Sg	1	Jewish-Iraqi	ND	Yes	ND
Sn	1	Arab	ND	Yes	ND

ND not done

Science, Rehovot, Israel, who collaborated with us in the sequencing of the GH-receptor gene.

5.2 Hypothesis of the Origin of Laron Syndrome

Zvi Laron and Orit Shevah

Analyzing the ethnic origin of our cohort of patients with Laron syndrome (LS) (Shevah and Laron. 2006), we realized that Jewish patients from Afghanistan, Iraq, and Morocco have the same genetic defect, similarly to Yemenite Jews and Palestinian Arabs (partly originating from the Arab peninsula). Also the patients from Malta and Greece, both from the Mediterranean area, have the same growth hormone receptor (GH-R) defect as does a Druze patient and one patient from Peru (Table 5.6). The common GH-receptor defects in patients from adjacent geographical regions, or along known commercial routes (spice and silk) or emigration routes (Jews from Spain and Portugal took refuge in South-America during the Inquisition; or Arabs and Jews from the Middle-East emigrated to South-America from nineteenth to twentieth century (Rosenbloom et al. 1990)), make one realize that the GH-R defects may have traveled during centuries between continents and between countries. Indeed, travel and migration are not new phenomena but go back to Biblical times and even earlier. So it seems that the origin of many diseases including Laron syndrome is ancient.

The existence of dwarfs with the appearance of Laron syndrome in the middle ages is plastically exemplified in the painting by Mantegna from 1454 in the Palace of Mantova (Battin 1996) (Fig 5.19), but the history of this syndrome seems to go further back.

Comparison between the roentgenological findings of the skeleton of our large cohort of patients with Laron syndrome (Chap. 20) and the skeletal remains of "Homo floresiensis" in Indonesia (Brown et al. 2004), thought to be 18–20,000 years old and characterized by dwarfism (body height 106 cm) and a small head (Morwood et al. 2005), revealed many resemblances between that skeleton and Laron syndrome (Hershkovitz et al. 2007). As Laron syndrome occurs more often in consanguineous families (Shevah et al. 2006) and isolates such as

Fig. 5.19 The enlarged picture of a dwarfed lady in a painting by Mantegna 1454. Note the typical facial features

in the Bahamas (Baumbach et al. 1997) or Ecuador (Rosenbloom et al. 1990), and having been described in patients from several countries in South East Asia, including Pakistan (Rosenfeld et al. 1994), India (Desai et al. 1993), Cambodia (Vesterhus 1997), Vietnam (Walker et al. 1998), and Malaysia (Harun, personal communication), we put forward the hypothesis that "homo floresiensis" does not belong to a "new species" but belongs to a locally inbred population of "homo sapiens" in whom a GH-receptor mutation has occurred in old times (Hershkovitz et al. 2007) and has been genetically transmitted in that isolate for many generations. This so far unidentified mutation(s) harboring possibly the "founder gene" for Laron syndrome has wandered with time along the "spice and silk roads" from Southern Asia to the Middle East and the Mediterranean region, and from there to South and Central America (Fig. 5.20). On its way, it has encountered epigenetic changes leading to different "de novo" mutations.

Support for our hypothesis are the following recent findings: Investigating our Laron syndrome patient,

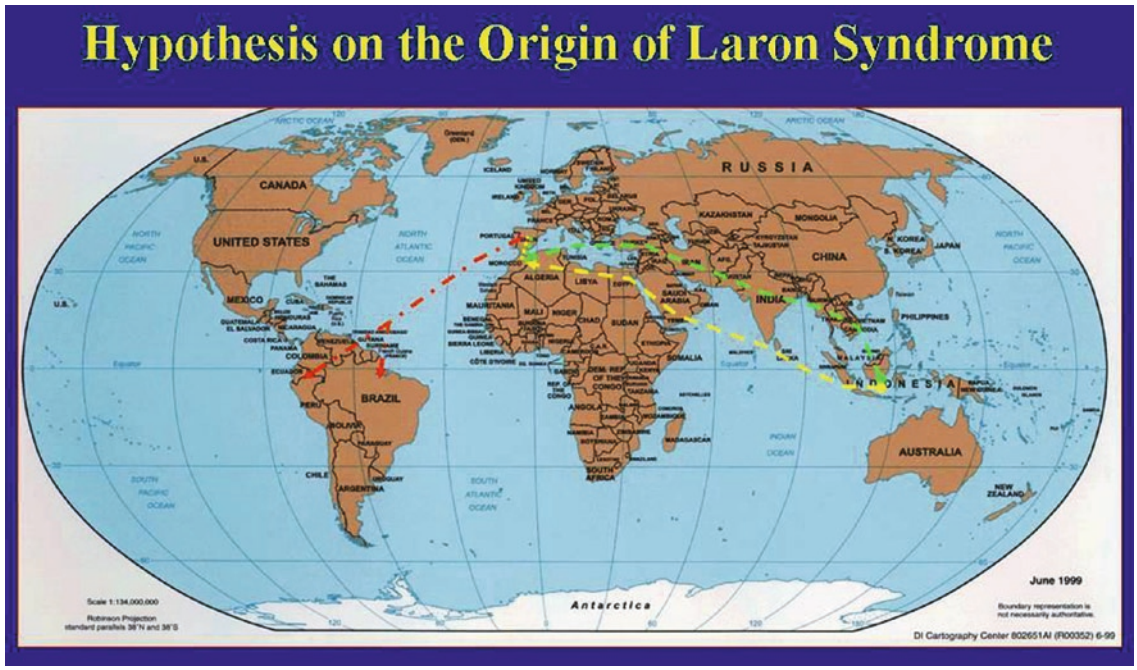

Fig. 5.20 The hypothetical journey of the Laron syndrome gene defect from the Far-East to the Middle-East and Europe, and from there to South-America

homozygous for the E180 splice mutation, and 9 Laron syndrome patients with the same mutation from Brazil, compared to five patients (three from Israel and two from Brazil) with other GH-R mutations, Pinto et al. (2008) found that unrelated patients carrying the E180 mutation carried the following polymorphic markers in the short arm of chromosome 5: D5S2082, D5S665, and D5S2087. This can be considered an evidence that the Laron syndrome population carrying the E180 splice mutation on Exon 6 and occurring in our patient from Morocco and patients in Brazil (Jorge et al. 2005), Ecuador (Berg et al. 1992), and Chile (Espinosa et al. 2008) originates from Spanish Jews dispersed during the Inquisition in Spain and Portugal in the sixteenth century (Rosenbloom and Guevara-Aguirre 2008). Furthermore, the same polymorphic markers were described by David et al. (2007) in patients with an intronic GH-R mutation originating in the Middle East and Pakistan. One example of gene survival is that of sickle cell anemia. It survived because it provided protection against another disease (malaria). Maybe the genes of the GH-receptor defects survived because they provided protection against malignancy (Shevah and Laron 2007).

5.3 Are the Pygmies Laron Syndrome Patients?

Zvi Laron

The Pygmies, inbred short people in Central Africa, have been described in ancient times (Turnbull 1961). Merimee and Laron (1996) reviewed the state of research on this population (Fig. 5.21). The evidence for a GH-receptor defect leading to IGF-I deficiency is based so far on indirect findings: birth length is reduced (44.7 ± 3.2 cm) compared to neighboring tribes, and the mean linear height for females was found to be 137.4 ± 4.3 cm and that for males, 144.2 ± 6.0 cm. The evidences are reduced expression of the GH-R, low IGF-I levels (Bozzola et al. 2009), and the highly reduced serum GH binding protein levels. Major evidence for a GH-R defect are highly reduced serum GH binding protein levels (Figs 5.22 and 5.23) (Baumann et al. 1989; Merimee et al. 1990). DNA studies are needed to find out whether the Pygmies are either true or a variant of Laron syndrome and if indeed, their gene has been brought from the Persian Gulf or the

Fig. 5.21 The late Dr. Thomas Merimee with an adult female Pygmy in Africa

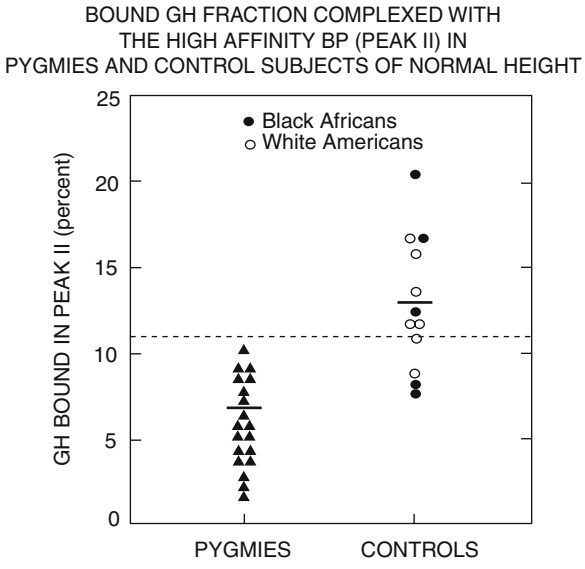

Fig. 5.22 The percent of GH bound to a high affinity binding protein in the serum of Pygmies and controls ($p<0.001$) (modified from Baumann et al. 1989)

Arabian Peninsula, like the Lemba tribe in South Africa who claim to be descendants from ancient Jews (Partiff 2003). As the Pygmies live in tropical forests, presently involved in wars, further investigations have been postponed. There are very short inbred

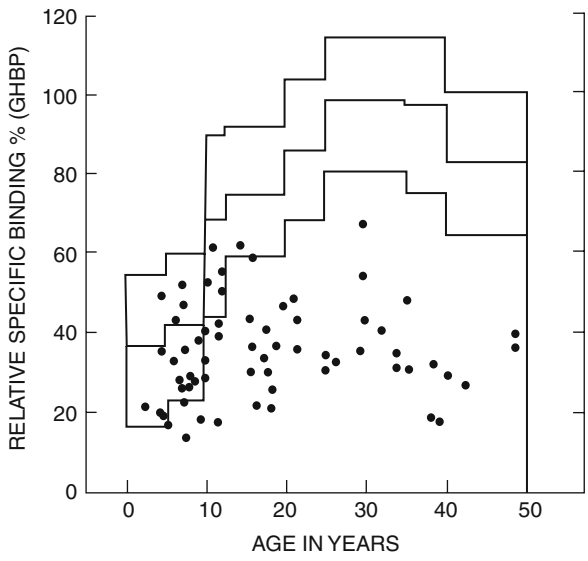

Fig. 5.23 Binding of ^{125}I-hGH by a high affinity GHBP of serum from Pygmies compared to controls (framed area) (modified from Merimee et al. 1990 and Merimee and Laron 1996)

populations also in other regions of the world such as the Pacific Ocean Islands, but none has been studied in detail.

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Clinical Evaluation

6

Zvi Laron

Core Message

› This chapter describes the anthropological measurements performed at each visit during the long-term follow-up of the patients and the construction of specific growth charts for girls and boys with Laron syndrome.

6.1 Methods of Examination and Follow-Up of Patients

6.1.1 Family History

An extensive family history was taken at the first visit and completed at subsequent visits. The parents and older patients were asked to draw a family tree registering height and chronic diseases as well as degree of relationships (consanguinity, etc.) (see Chap. 5, page 29).

6.1.2 Physical Examination

A complete physical examination was performed at each visit, and positive and negative findings were registered on our follow-up form (Fig. 6.1) (Laron et al. 1998). Additional information registered in the blank spaces included hearing, eyesight, skin or skeletal abnormalities, age at menarche, age at first ejaculation, sexual relations, drugs, etc. Throughout follow-up, the patients were examined by a limited number of physicians (Zvi Laron, Athalia Pertzelan, Beatrice Klinger).

6.1.3 Anthropometric Measurements

Height and body length were measured in stretched positions using Harpenden anthropometers (Holtain Ltd., Crymych, UK). Sitting height was also measured, and the upper/lower body segment was calculated by subtracting the sitting height from the body length (Arad and Laron 1979). The measurements were made by a restricted number of nurses (Dalia Peled, Clara Weininger, Sara Anin) or the physicians.

6.1.4 Body Weight

Body weight was measured in underwear mostly in the morning after voiding in the fasting state. Young children were weighed naked.

6.1.5 Degree of Body Adiposity

Skinfolds in the anterior iliac, midtriceps, and subscapular regions were measured by the physician using a Harpenden caliper (Holtain Ltd., Crymych, UK).

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a

Endocrinology & Diabetes Research Unit
Schneider Children's Medical Center
Petah-Tikva, Israel

Family name ——————
Given name ——————
Sex ——————
ID ——————

Date of birth ——————
Place ——————

Date	CA Yr	BA Yr				Weight kg	Height cm	Length cm	Sitting Height	Shoe / FL	Head circum-ference	Blood pressure	Pulse		Teeth	Neck	Heart & Lungs	Abdomen

CA=Chronological Age BA=Bone Age FL=Foot Length

b

Date	Distribution of body fat	S.C. mm			Voice	HAIR				Striae	Pre-acne	Acne	BREAST or TESTIS			Scrotum	Penis / Clitoris	VULVA				REMARKS & TREATMENT
		Iliac	Triceps	Subscapular		Moust	Beard	Ax	Pub					R	L			Redness	Secretion	Lab. Min.	Lab. Maj.	

Fig. 6.1 Examination and follow-up prestructured form (**a** and **b**) modified from Laron et al. (1998)

6.1.6 Sexual Development

Appearance of preacne (microcomedones), acne, facial, axial, and pubic hair were graded from 0 to +++, so were the development of rugae of the scrotum and that of female nipple and areolae and genitalia. Breast half circumference (North-South) was measured with a tape and expressed in cm. The stretched penis length and width was measured by a caliper. Penis circumference was calculated: width multiplied by π. The testicular volume in ml was evaluated by bi-manual palpation using a Prader orchidometer (Prader 1966).

6.1.7 Head Circumference

Head circumference was measured with a tape measure from the most prominent occipital to the frontal protrusion.

6.1.8 Hand Size

Hand size was estimated using routine hand X-rays (Konen et al. 2009) (Chap. 25, page 231).

6.1.9 Foot Length

Foot size was measured by a tape from the tip of the big toe to the end of the heel. In a minority of instances, the shoe size was converted to foot length using the European shoe size on the Brannock scale (The Brannock Device Co. Inc., Liverpool, NY, USA) (Silbergeld et al. 2007) (see Chap. 25, page 231).

6.1.10 Blood Examinations

All routine blood analysis and tests were performed after an overnight fast of 12–14 h. In small children a

light meal before midnight was permitted. Water was allowed ad libidum unless contraindicated.

6.1.11 Imaging

X-rays and MRIs were performed at the Pediatric Imaging Department, Beilinson Hospital, and since 1991 at the Schneider Children's Medical Center. Bone (skeletal) age was determined by Zvi Laron, Athalia Pertzelan, and/or Liora Kornreich from hand and wrist X-rays using the Atlas of Greulich and Pyle (1959).

6.1.12 Photography

Medical photography was performed at the Photography and Graphic Institute, Beilinson Hospital.

6.1.13 Ethical Committee

All examinations and investigations were approved by the Hospital Ethical (Helsinki) Committee and Ministry of Health. The parents of minor patients and adult patients signed an informed consent form for all investigations including photography.

6.2 Design of the Laron Syndrome-Specific Growth Charts

The long-term follow-up from infancy into adult age of 24 patients with Laron syndrome (10 boys and 14 girls) and their frequent measurements by the same nurses enabled us to construct growth charts for this syndrome (Laron et al. 1993). Further experience showed that they fit all syndromes of true congenital GH/IGF-I

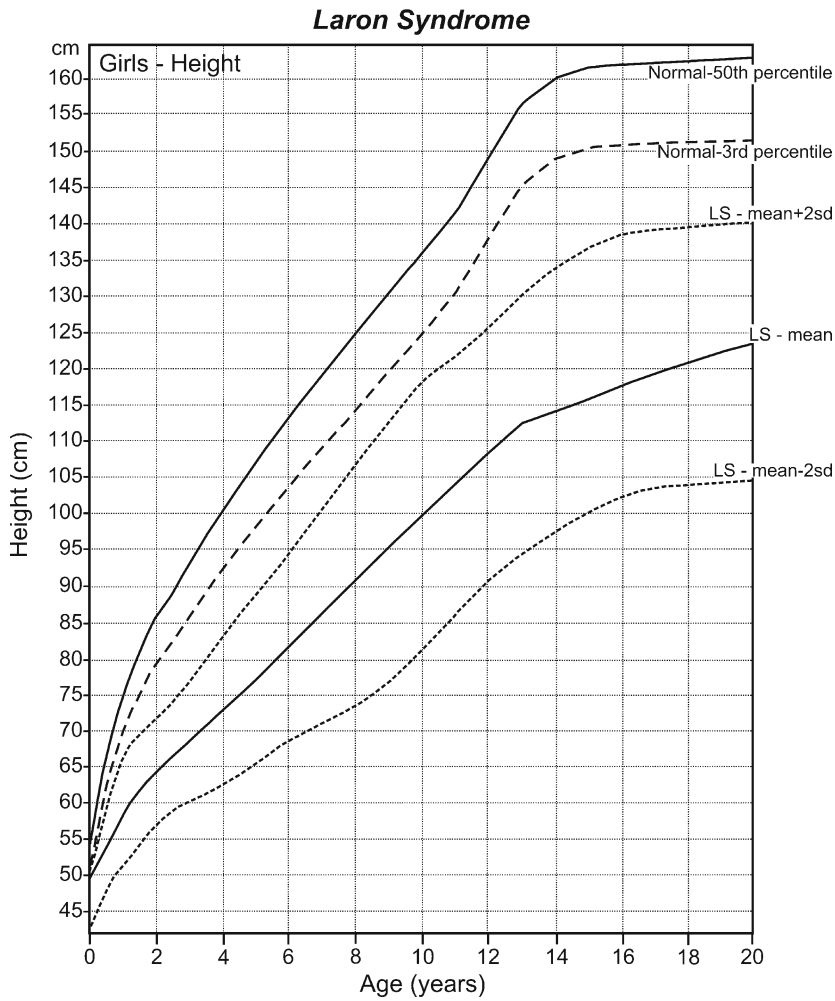

Fig. 6.2 Laron syndrome-specific growth chart for height in girls from birth to 20 years (Laron et al. 1993)

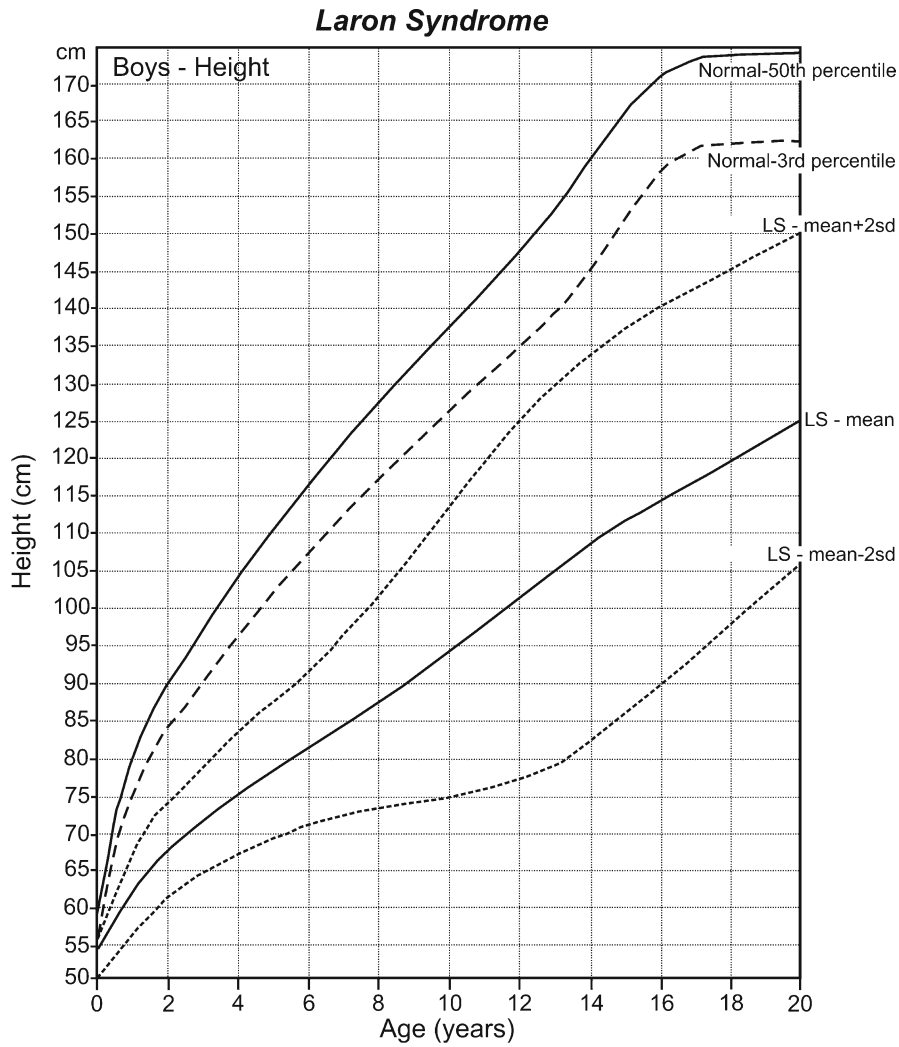

Fig. 6.3 Laron syndrome - specific growth chart for height in boys from birth to 20 years (Laron et al. 1993)

deficiency except ALS (acid labile subunit of IGFBP-3) mutations (Laron and Silbergeld 2006).

6.2.1 Methods

The growth increments over periods of 1–3 years were calculated for each sex separately. These increments enabled us to plot the growth velocity curve.

The height curves were constructed using the Tanner-Gupta method (Tanner 1951) as follows: the mean height was calculated for boys at the age of 10 years and for girls at the age of 8 years. From these points, the mean calculated yearly growth increments were added or subtracted going backwards to birth (age 0) or forward up to the age of 20 years. Both the growth velocity and height curves were smoothed using the "Lowess" method (Cleveland 1979). The final height was established when the subjects grew less than 1 cm during 1 year.

Figures 6.2 and 6.3 represent the smoothed height growth charts as mean (±2 SD) for girls and boys with Laron syndrome (Laron et al. 1993). From the two charts it is seen that the spread of the mean (2 SD) is wider in boys than in girls. Postnatally, the growth velocity slows and after the age of 3 maintains a

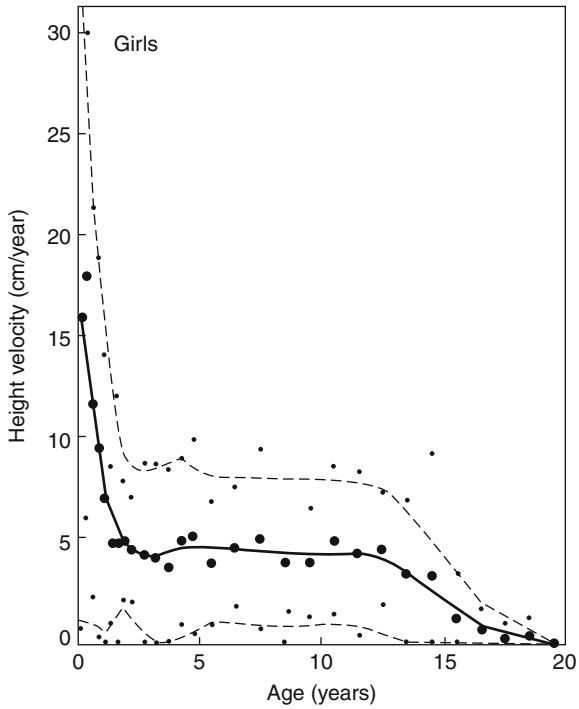

Fig. 6.4 Growth velocity curve in girls with Laron syndrome from birth to 20 years

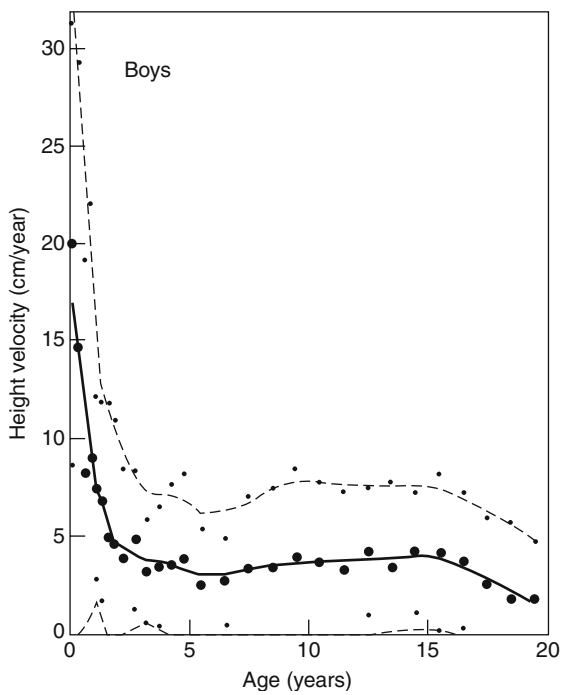

Fig. 6.5 Growth velocity curve in boys with Laron syndrome from birth to 20 years

velocity of 4–5 cm/year in girls and 3–4 cm in boys. This difference in growth velocity makes the mean height of girls greater than that of boys between ages 5 and 15 years, but the girls lose again by their earlier sexual development and earlier finalization of adult height.

The girls reached their final height between ages 16 and 19 years, whereas the boys continued to grow beyond the age of 20 years. The mean (SD) final height of the girls was 119.5 (±8.5) cm and that of the boys (reached around the age of 24 years and not shown on the graphs) was 124.1 (±8.5) cm. Figures 6.4 and 6.5 illustrate the growth velocity curves of the girls and boys with Laron syndrome (also shown in Chap. 10). It is evident that both sexes lack the typical pubertal growth spurt (Tanner et al. 1976). The mean age at onset of puberty in girls was 10.7 (+0.7) and in boys 15.6 (±2.6) years (see also Chap. 10, pages 102–104).

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Perinatal Development in Laron Syndrome

7

Zvi Laron and Rivka Kauli

Core Message

› The description of birth length and weight, congenital malformations and postnatal neurodevelopmental milestones of Laron syndrome patients.

7.1 Introduction

Pituitary growth hormone (GH) and its anabolic effector hormone, insulin-like growth factor-I (IGF-I), are considered the major postnatal growth factors (Daughaday 1994; Kaplan and Cohen 2007), with added sex hormones acting during puberty (Buckler 2007) and insulin playing a modulator role (Laron 2008). We (Laron 2000) and others (Gluckman 1994) have demonstrated that congenital GH and/or IGF-I deficiency also affect intrauterine growth.

Laron syndrome patients with congenital IGF-I deficiencies, and GH insensitivity, are a unique model to learn how growth proceeds without these hormones on one hand and how IGF-I replacement therapy without the interference of endogenous GH stimulates growth parameters on the other.

Patients with Laron syndrome suffer from growth retardation which starts in utero followed by progressive slow postnatal growth resulting in severe dwarfism if untreated. Figure 7.1 illustrates the final height achieved in a girl without GH/IGF-I activity as compared to a normal same-aged girl, demonstrating the difference in postnatal growth due to hGH and IGF-I. Following are the detailed data obtained from our cohort of patients.

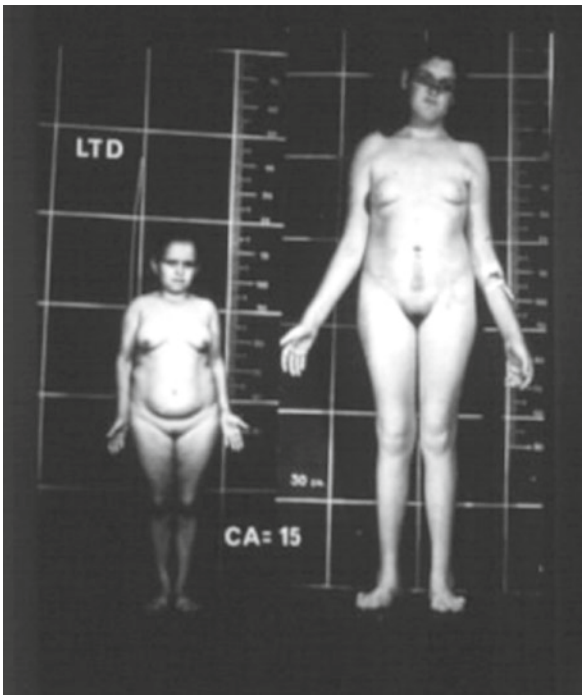

Fig. 7.1 The difference in final height between an untreated 16-year-old girl with Laron syndrome and a normal same-aged control

7.2 Perinatal Period

7.2.1 Gestation

The total number of pregnancies in which information was available was 48 in 36 mothers. All were reported

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to have been uneventful and full term with one exception – Pt TAK was born premature in the 34th week. There were 42 vaginal deliveries, two of them by forceps and six by cesarean section.
Some mothers mentioned weaker movements of the fetus compared with normal siblings (see also Sect. 10.4, Chap. 10, page 116).

7.2.2 Birth Length

We have accurate measurements in 26 newborns with Laron syndrome (14 males and 12 females); 20 were below 48 cm in length, 2 measured 48 cm and 4 more (Fig. 7.2).

7.2.3 Birth Weight

Data on birth weight was available for 44 newborns (20 males and 23 females). All but five weighed between 2,500 and 4,000 g; three weighed 1,800 g and 2,100 g and one 4,700 g (Fig. 7.2).

7.3 Comment

Our data shows that congenital IGF-I deficiency affects fetal length even when compared with the norms for Jews of Oriental origin (Zaizov and Laron 1966) but had no significant effect on fetal weight.

Considering that the Laron syndrome infants have delicate bones and an underdeveloped muscular system (see Chap. 17, page 161), the increased birth weight/birth length ratio denotes already an "in utero obesity" as illustrated by the double chin in a 13-day-old Laron syndrome baby (Fig. 7.3; Pt. SSh).

Considering the importance of birth length as a diagnostic indication of "in utero" growth (Laron 2000), we recommend nurseries to measure birth length in addition to birth weight and head circumference.

7.4 Congenital Malformations

Among our 64 patients with Laron syndrome we registered the following congenital malformations:

Two males with congenital dislocation of the hip, both not operated.

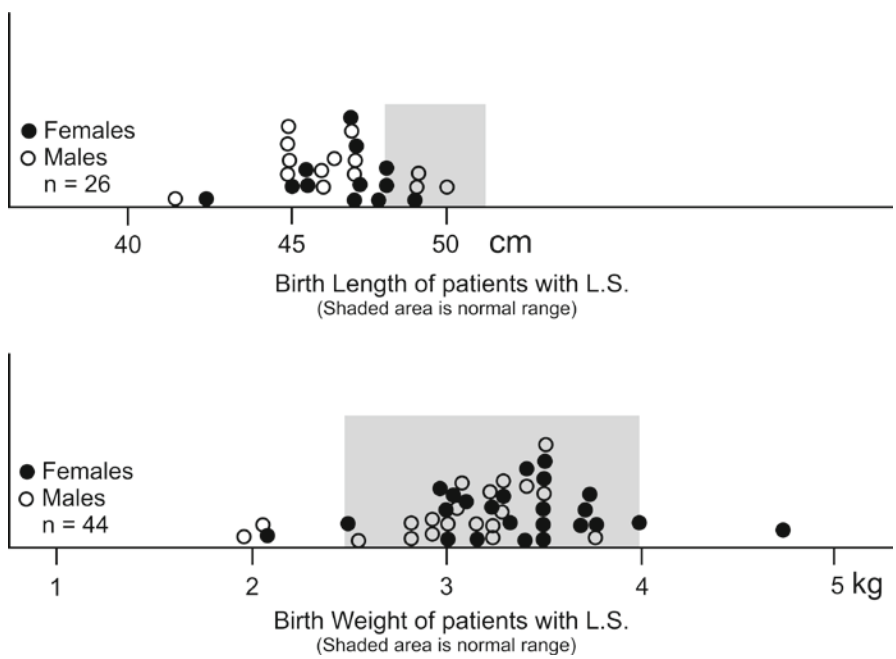

Fig. 7.2 Birth length and weight of neonates with Laron syndrome

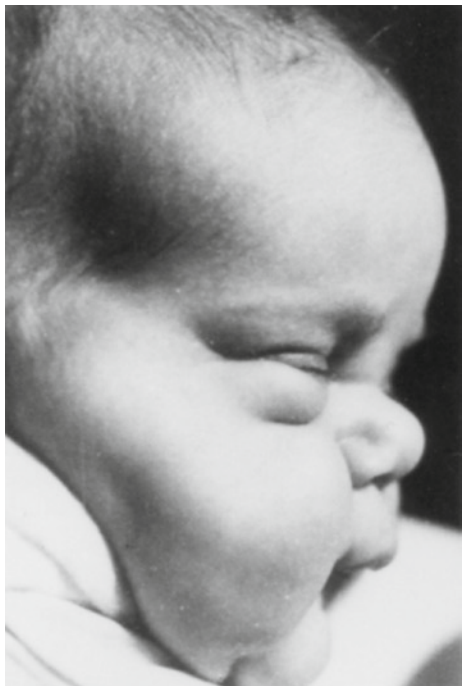

Fig. 7.3 A 13-day-old male baby with Laron syndrome. Note sparse hair, protruding forehead, saddle nose, puffy cheeks, and double chin (Pt. SSh)

One male with unilateral Perthes' disease (operated).
Two males with patent ductus arteriosus (one corrected by surgery); one of them had Erb's palsy.
One female with congenital coloboma of the eye.
One female with strabismus.
One female with harelip (operated).
Two males with one hypoplastic kidney.
Two males with undescended testicles (operated).
One male with coronal hypospadias.
One male with an ectopic posterior pituitary (but normal pituitary hormone function).

In addition, the following skeletal anomalies were found in several patients of both sexes (see also, Chap. 20) Kornreich et al. (2008).

(a) Underdevelopment of the facial bones.
(b) Lack of development of frontal sinuses.
(c) Malformation of the dens of the first vertebra.
(d) Spinal stenosis.
(e) Abnormal rotation of the humerus leading to an inability of full extension of the upper limbs (Hershkowitz et al. 2007).

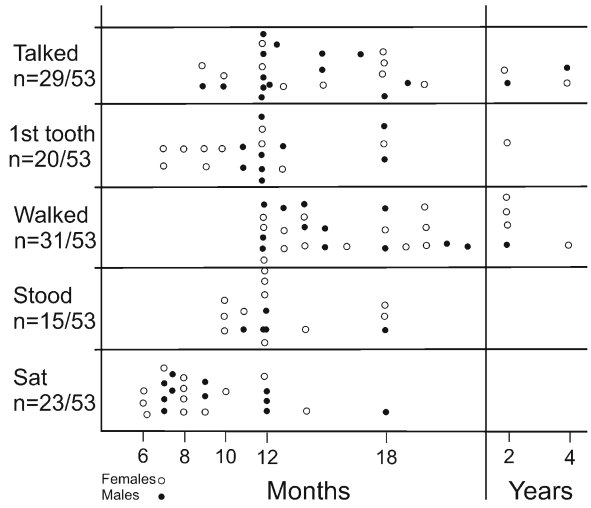

Fig. 7.4 Age at neuro-motor developmental milestones in infants with Laron syndrome

7.4.1 Comment

Rosenbloom et al. (1993) also reported a number of congenital malformations in the Ecuadorian cohort of Laron syndrome patients. Congenital dislocation of the hip has also been reported in congenital GH deficiency (Nishi et al. 1998).

7.4.2 Conclusions

It is obvious that congenital IGF-I deficiency, such as in Laron syndrome affects intrauterine growth and adiposity and causes a higher number of malformations than in the general populations (2–4.5%).

7.5 Neuro-Motor Developmental Milestones

Data on early development was retrieved in 53 out of 64 Laron syndrome patients. The progress of developmental neuro-motor milestones of infants with Laron syndrome from whom accurate data was obtained during follow-up are shown in Fig. 7.4.

It is seen that each milestone presents a wide range and that few Laron syndrome infants had late development.

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Linear Growth Pattern of Untreated Laron Syndrome Patients

8

Zvi Laron and Rivka Kauli

Core Message

› Description with examples of the disproportional linear growth retardation of untreated boys and girls with Laron syndrome.

A slower than normal growth velocity becomes apparent already during infancy, and progresses through prepuberty and puberty, resulting in a height deficit ranging from −4 to −10 SDS below the median of normal height for age. Due to the much delayed puberty in boys, they do not show a pubertal growth spurt (see Chap. 10). The girls also present a reduced pubertal growth spurt (see Chap. 10) but to a lesser degree.

If untreated, as, unfortunately, most patients in many countries still are, the final height ranges from 113 to 142 cm in male Laron syndrome patients (Figs. 8.1–8.10) and from 108 to 139 in females (Figs. 8.11–8.20) (Laron 2002, 2004). The adult stature in the Ecuadorian cohort was reported as 106–141 in males and 95–124 in females (Rosenbloom et al. 1998).

It is noteworthy that Laron syndrome children positive for GHBP (i.e., the molecular defect is in the transmembrane or intracellular domain of the GH receptor) are slightly less growth retarded than those with absent GHBP (i.e., a molecular defect in the extracellular domain of the GH receptor) (Laron et al. 1997) (Table 8.1). Similar observations were reported by Woods and Savage (1996). It is of interest that siblings of the same sex (SSh and SSi) had a similar height deficit, whereas siblings of differing gender (BR – male and BS, female) varied greatly in their height, with the boy being taller.

8.1 Parental Height of Patients with Laron Syndrome

Accurate measurements made by us are available for 33 parents (not always pairs). The height of mothers is illustrated in Fig. 8.21 and that of fathers in Fig. 8.22. Both parents are heterozygote for the molecular defect of the GH-R, and this may be the cause that the height of all but 4/33 mothers are below the 50th centile and 13 below the third centile. The height of all but 3/33 fathers were below the 50th centile and of these 7 were below the third centile.

It thus seems that heterozygocity has an influence on height as shown by the different distribution of the parents' height compared to the norm. No significant statistical differences were found when correlating the individual parental heights with the type of GH-R defect.

8.2 Upper to Lower Body Segment Ratios (U/L)

These measurements were available for 34 Laron syndrome patients.

The U/L body ratio (Arad and Laron 1979) at referral of 8 boys and 6 male adult patients with Laron syndrome is illustrated in Fig. 8.23 and that of 11 girls and 9 adult females in Fig. 8.24. It is seen that all male

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Laron syndrome patients except one boy have a ratio at or above 2 SD of the normal mean. Fifteen of the 20 female patients (6 girls and 9 adult females) showed the same, whereas 4 girls had a ratio slightly below the +2 SD. One 10-month-old girl had a normal U/L ratio. Figure 8.25 illustrates the upper to lower body segment ratio from childhood to puberty in a boy with Laron syndrome plotted on the Arad and Laron chart (1979). Figure 8.26 illustrates the upper to lower body segment ratio from childhood to adult age in a female with Laron syndrome plotted on the Arad and Laron chart (1979). Guevarra-Aguirre et al. (1993) have reported that arm span measurements in adult Ecuadorian Laron syndrome patients were reduced.

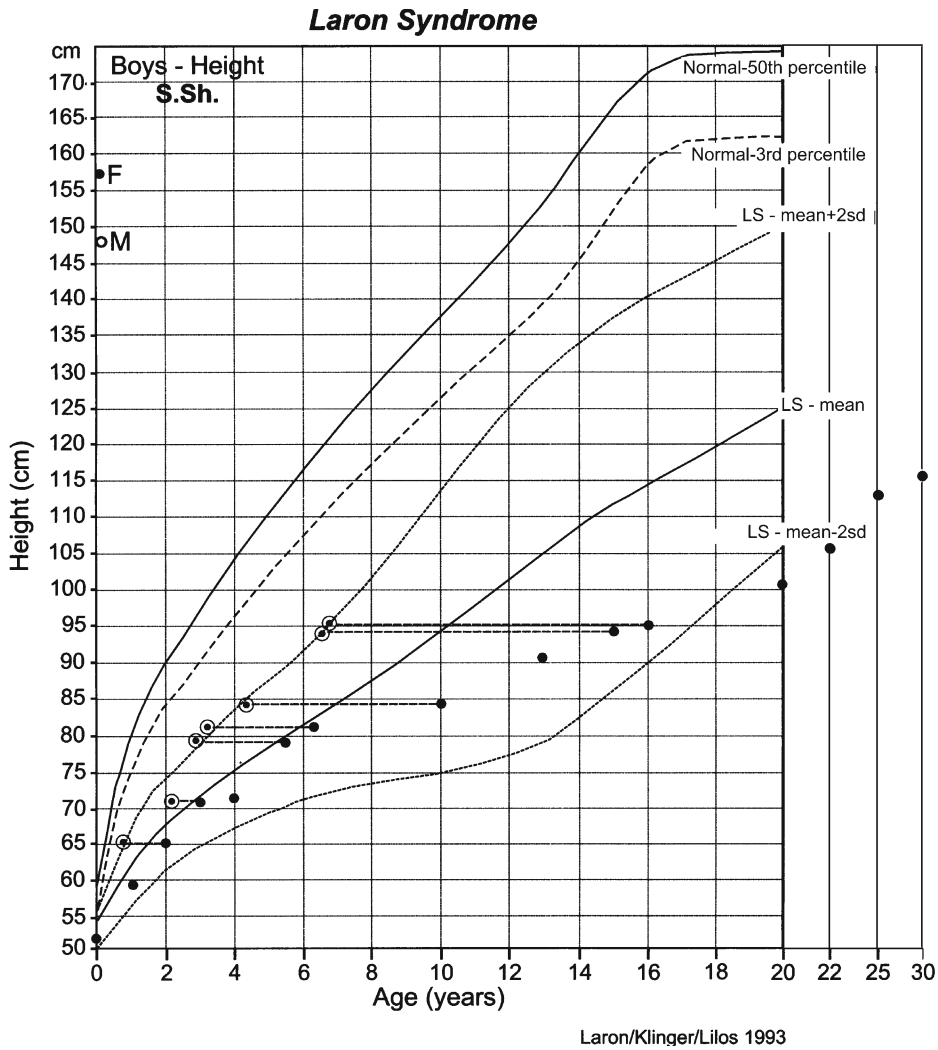

Fig. 8.1 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

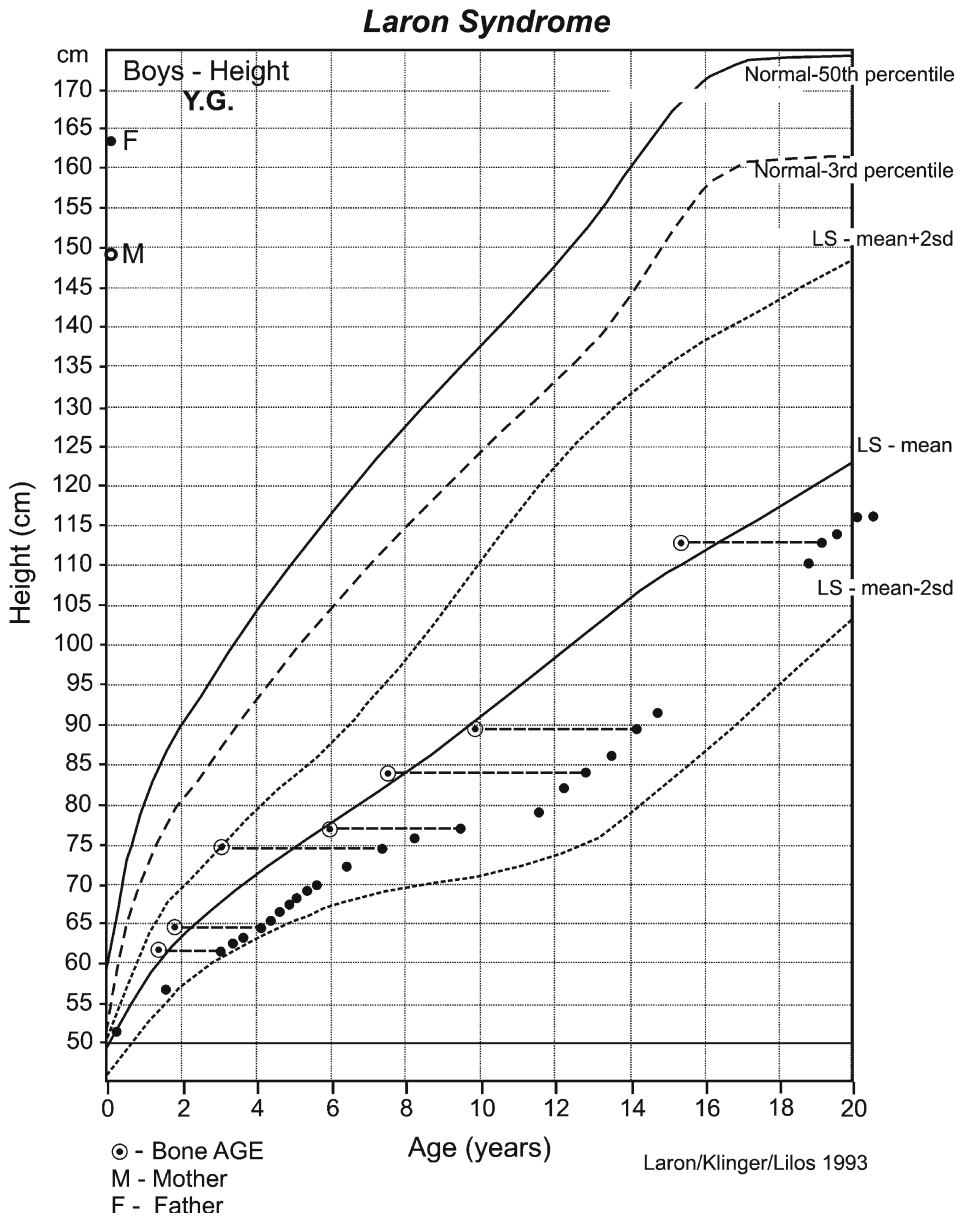

Fig. 8.2 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

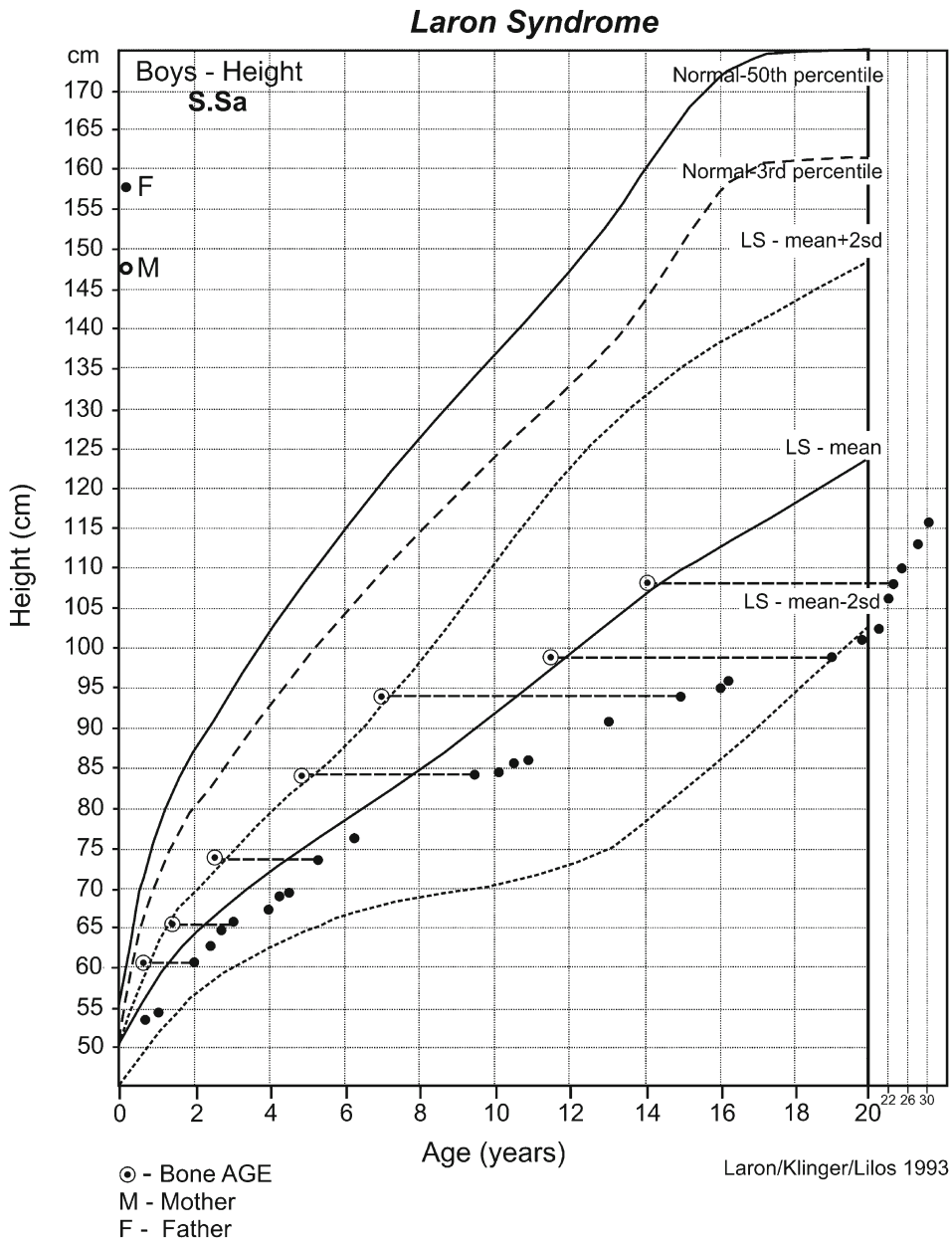

Fig. 8.3 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

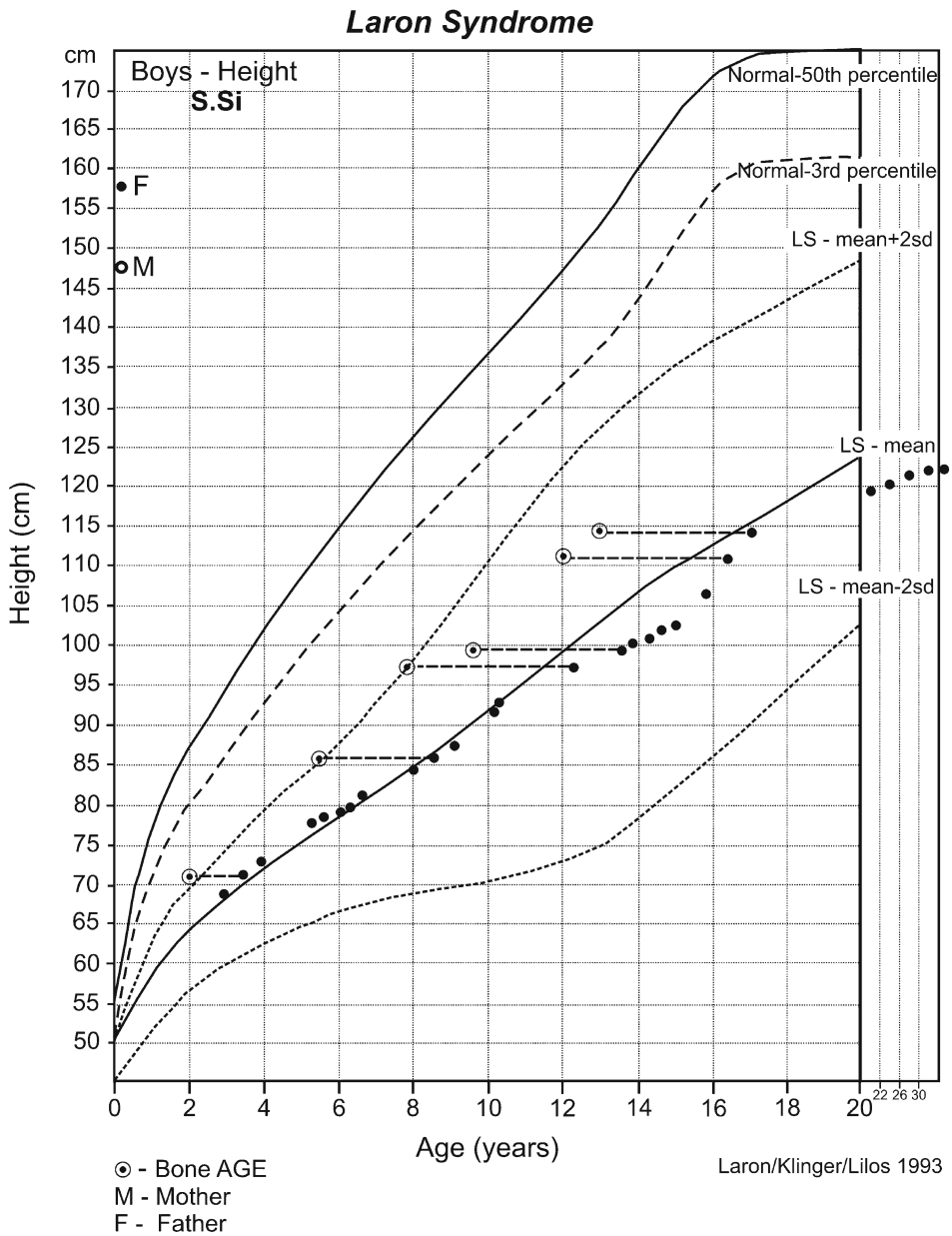

Fig. 8.4 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

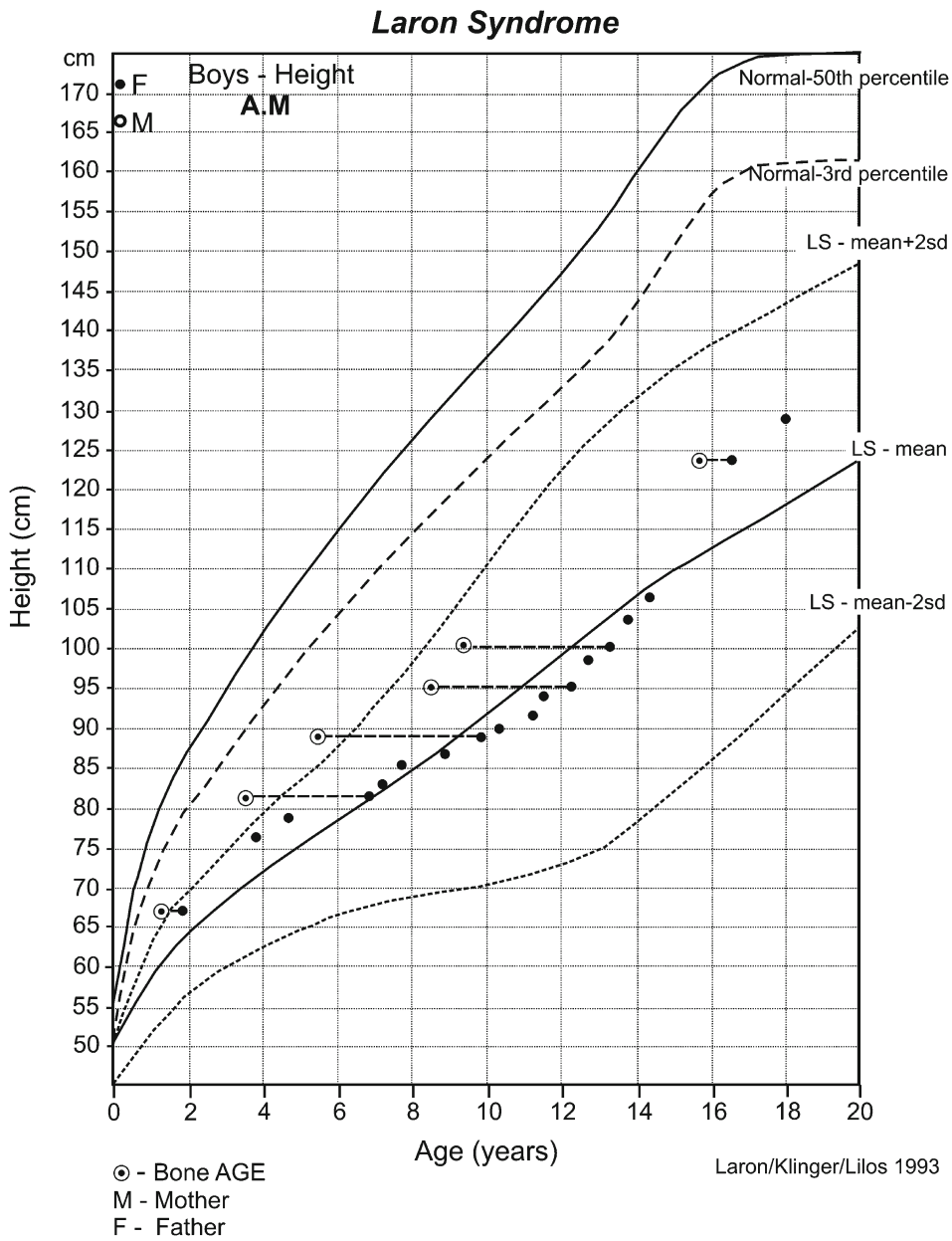

Fig. 8.5 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

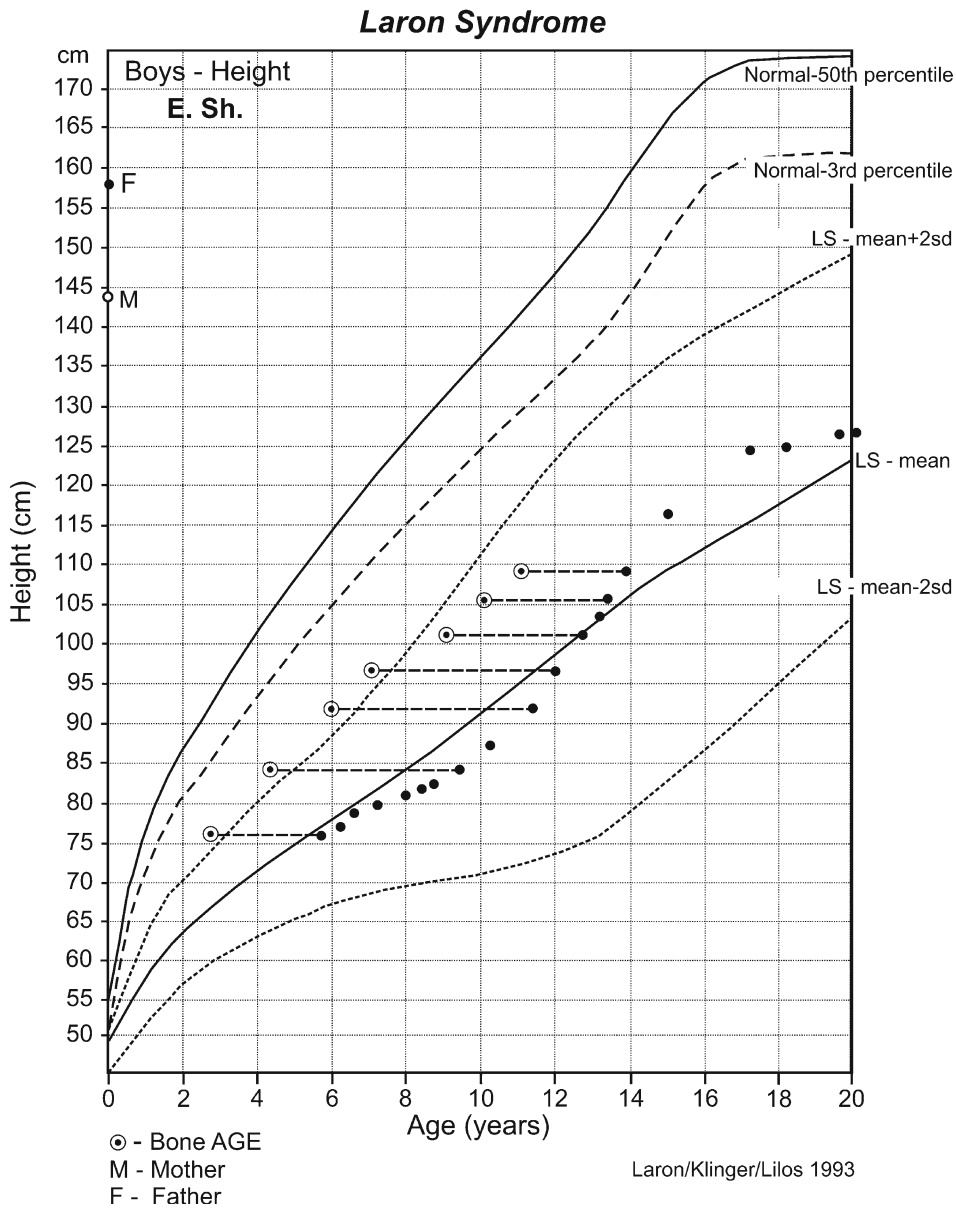

Fig. 8.6 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

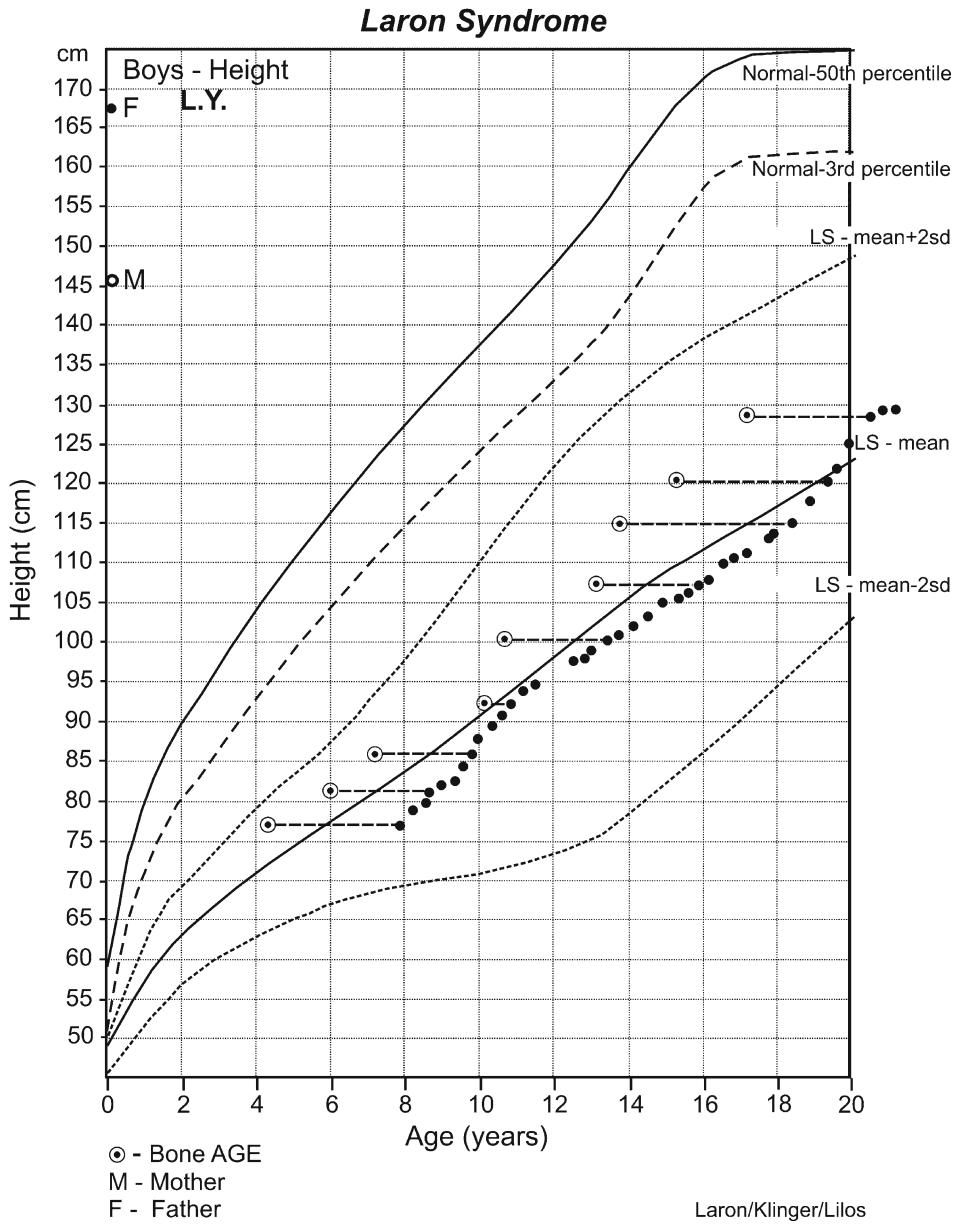

Fig. 8.7 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

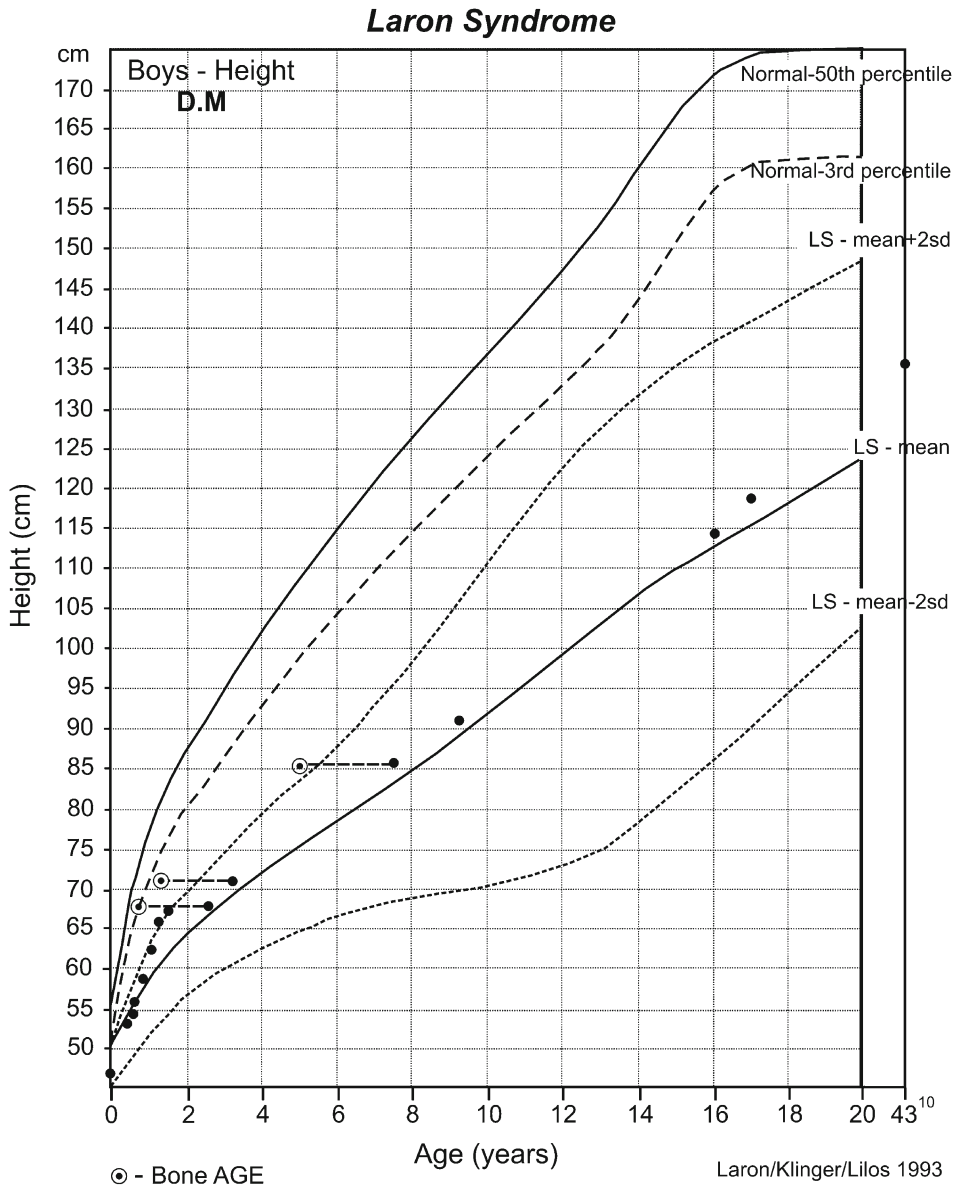

Fig. 8.8 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

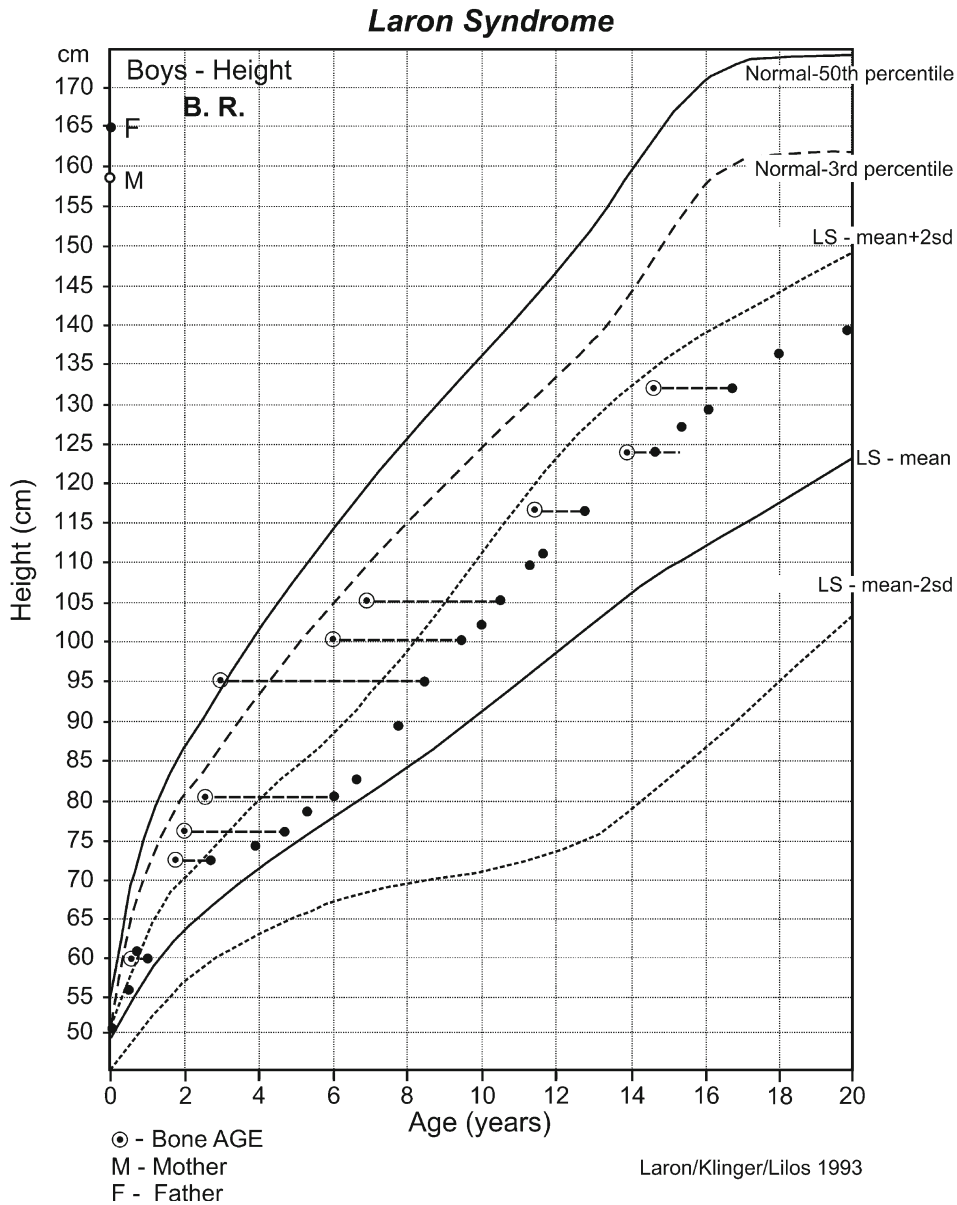

Fig. 8.9 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

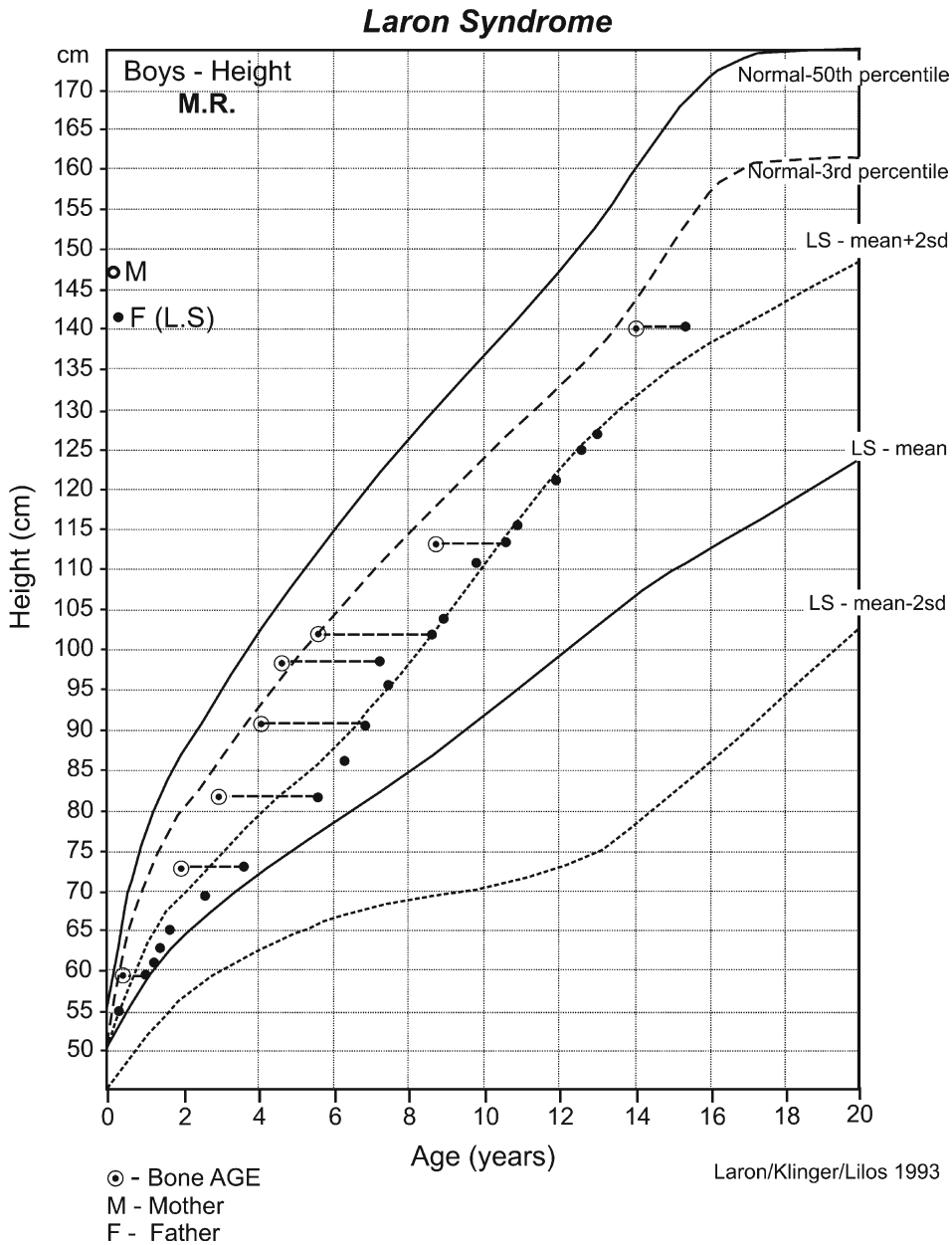

Fig. 8.10 Growth pattern of a boy with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

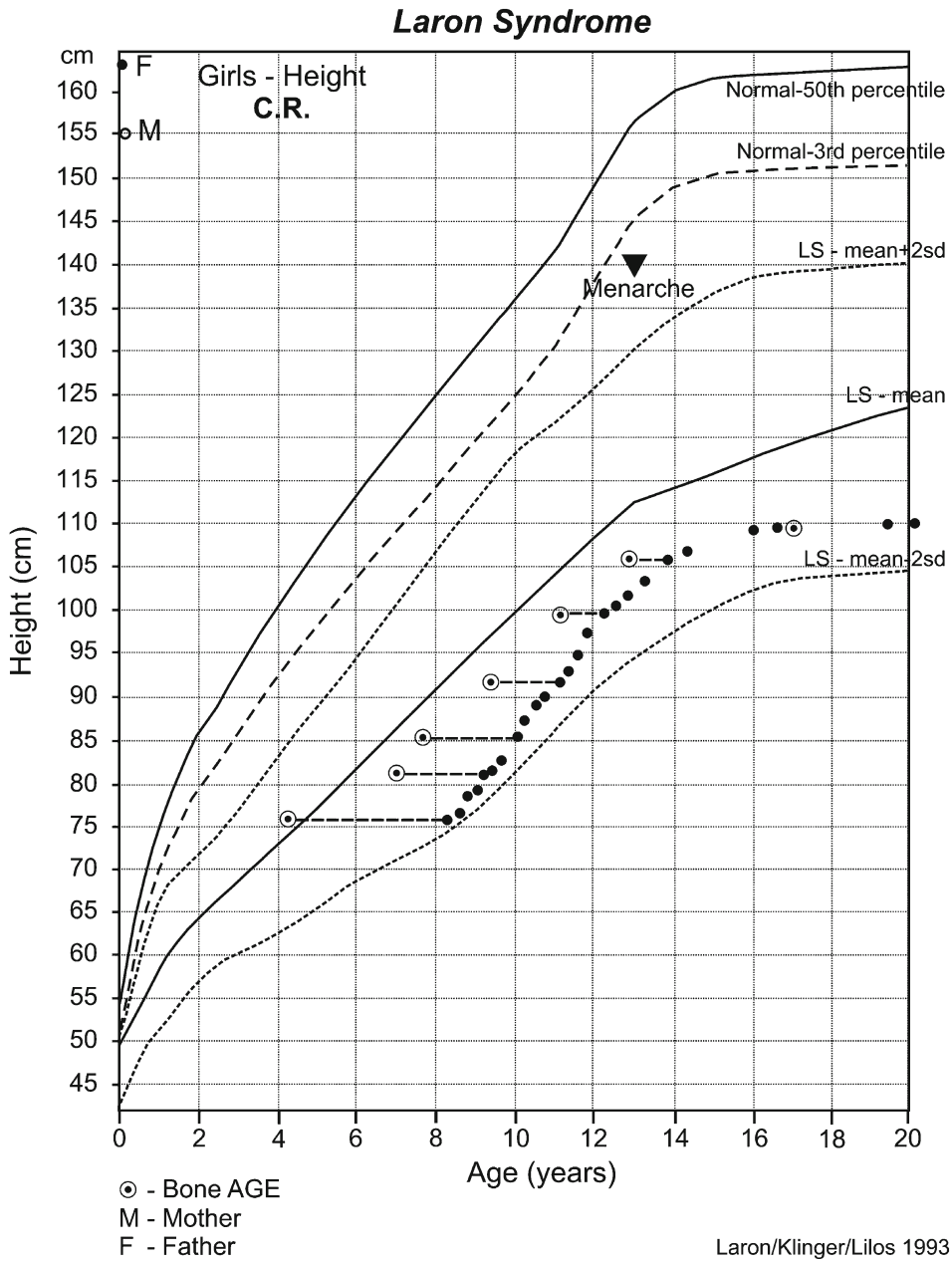

Fig. 8.11 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

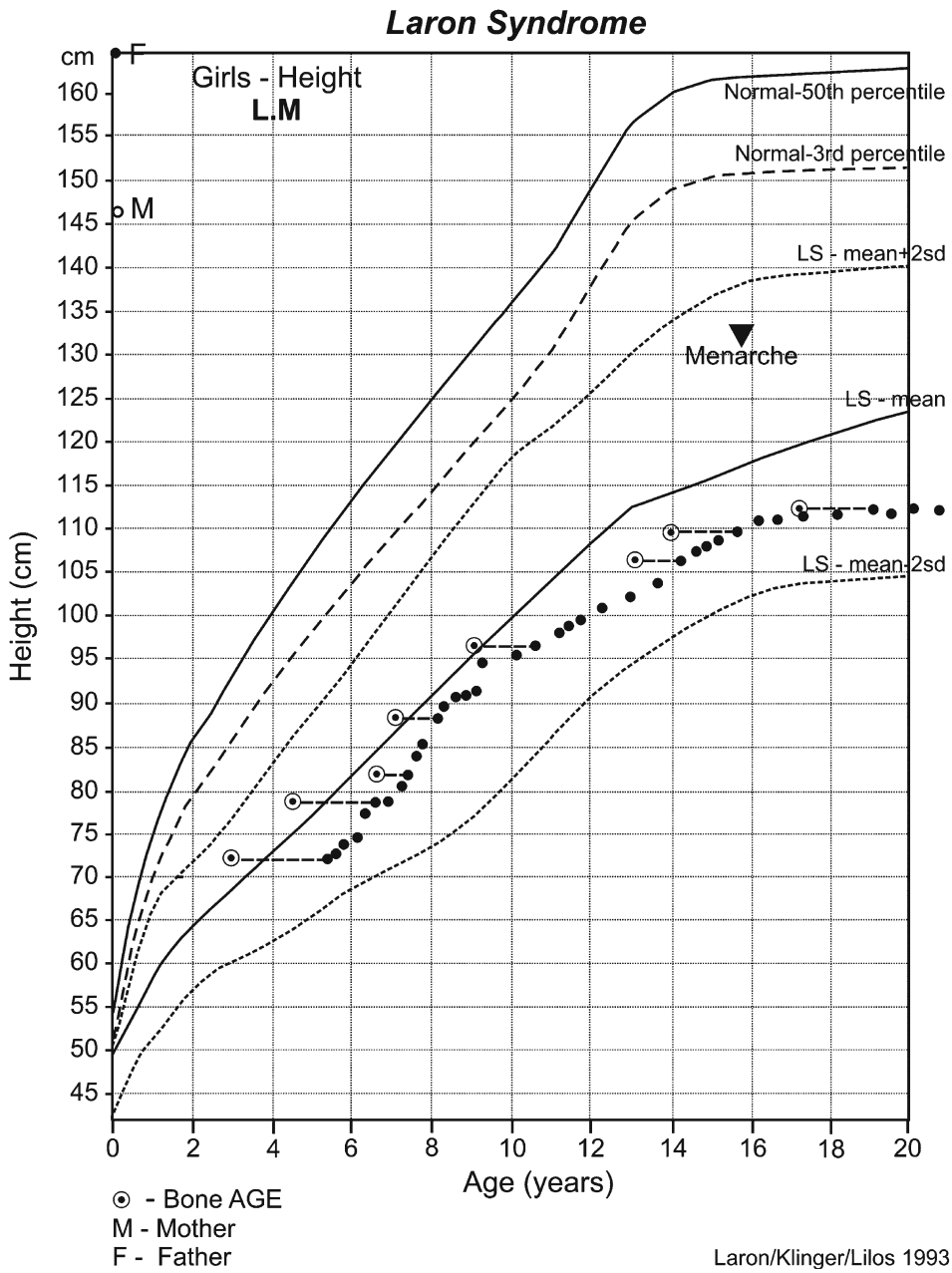

Fig. 8.12 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

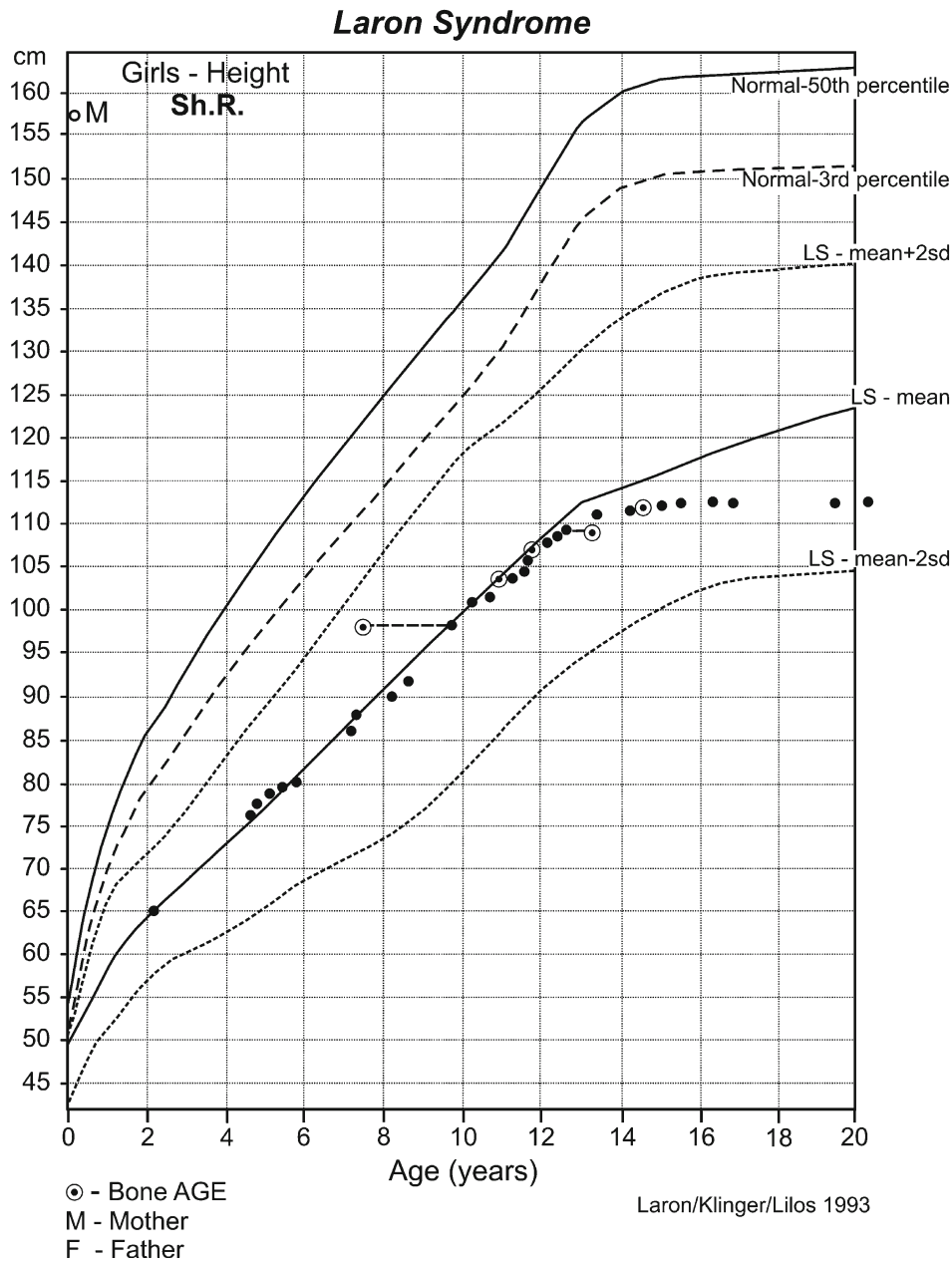

Fig. 8.13 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

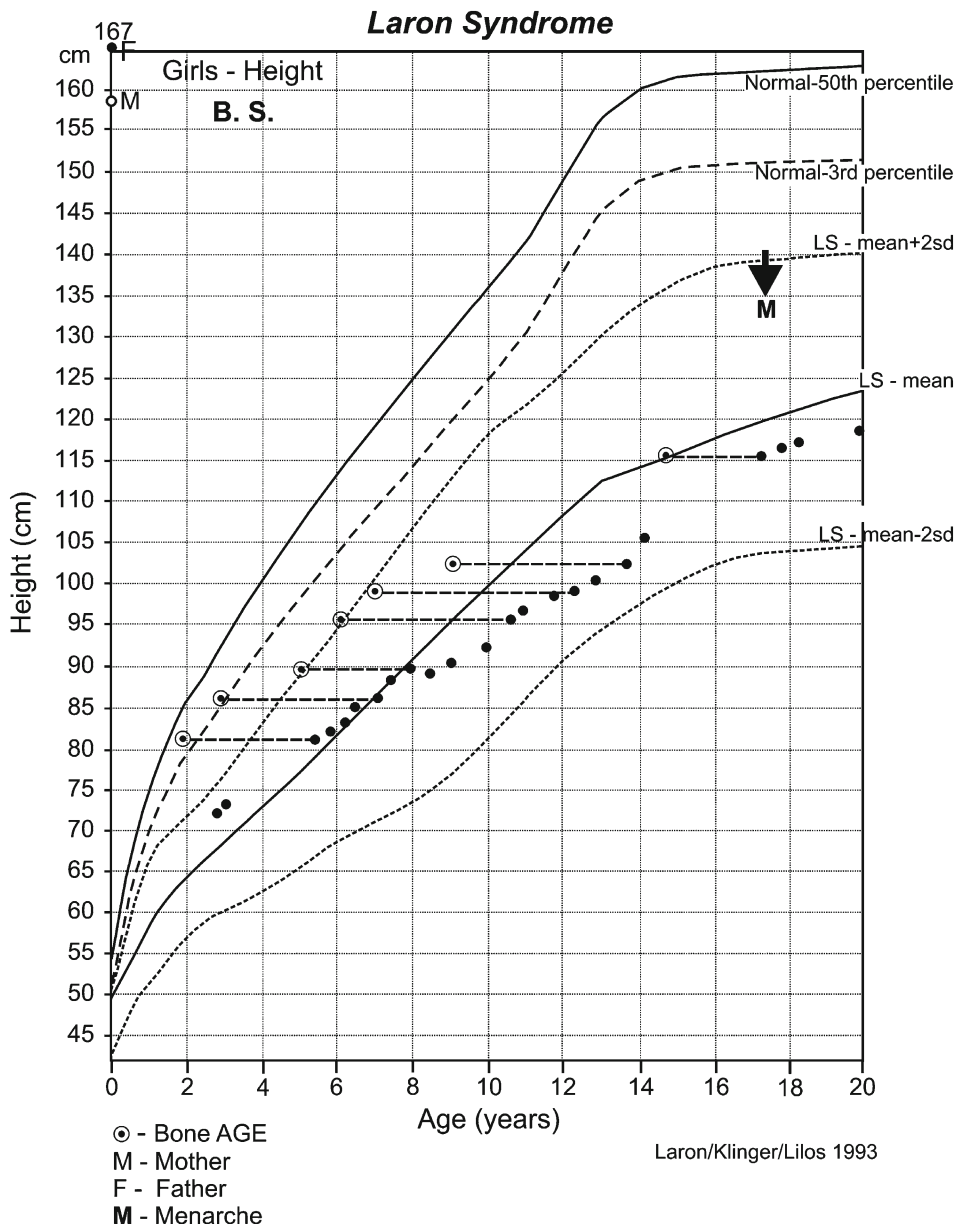

Fig. 8.14 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

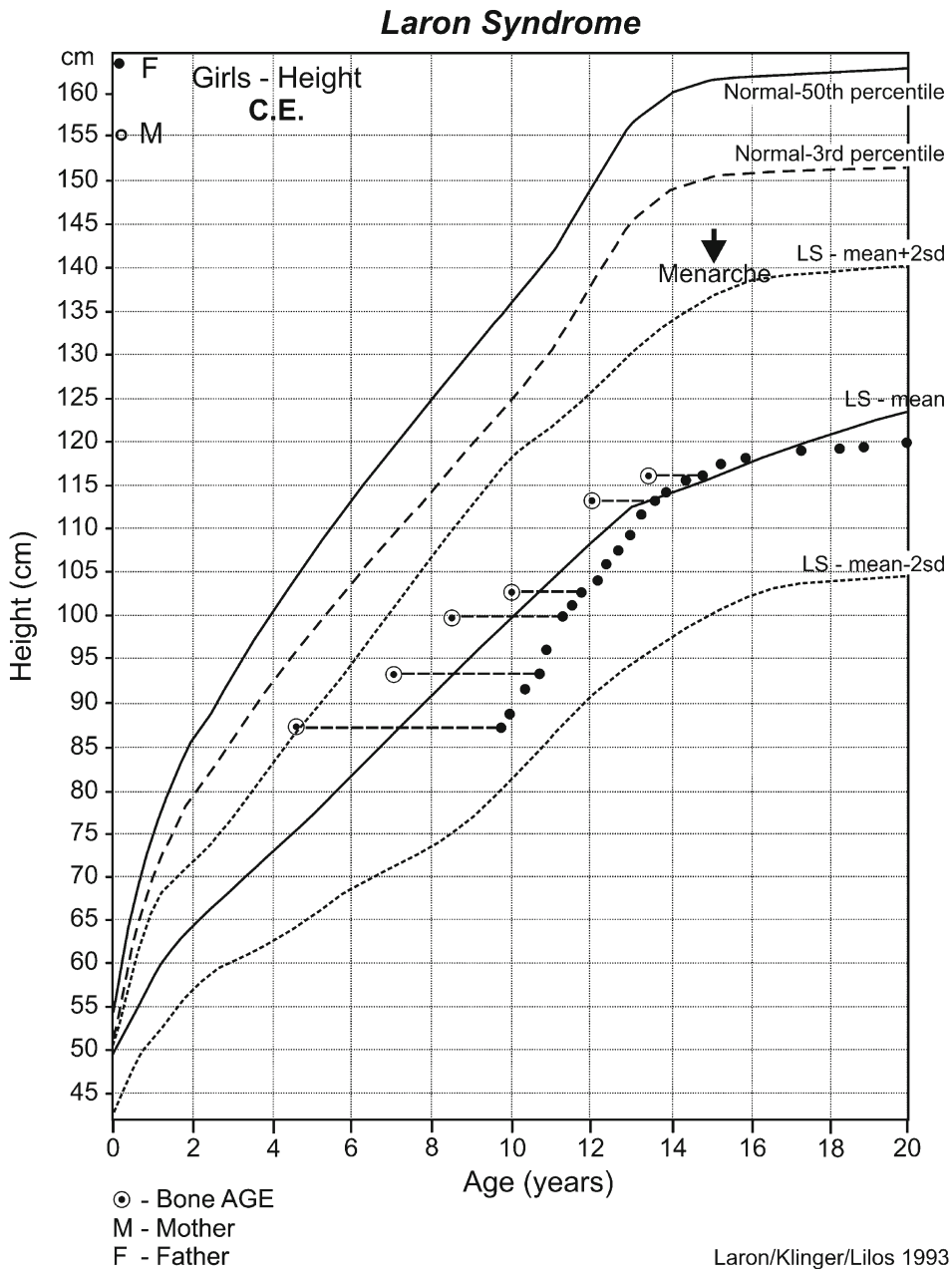

Fig. 8.15 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

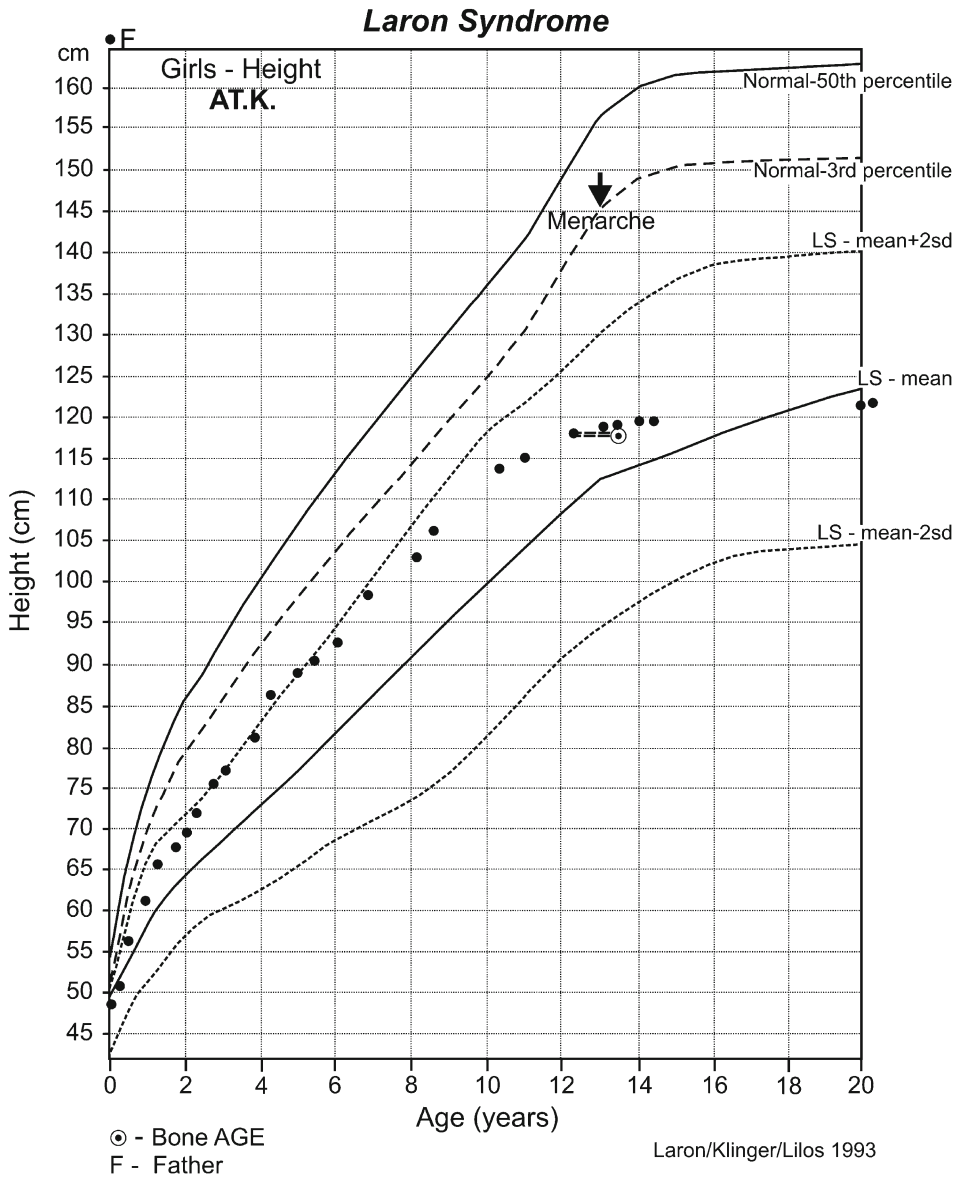

Fig. 8.16 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

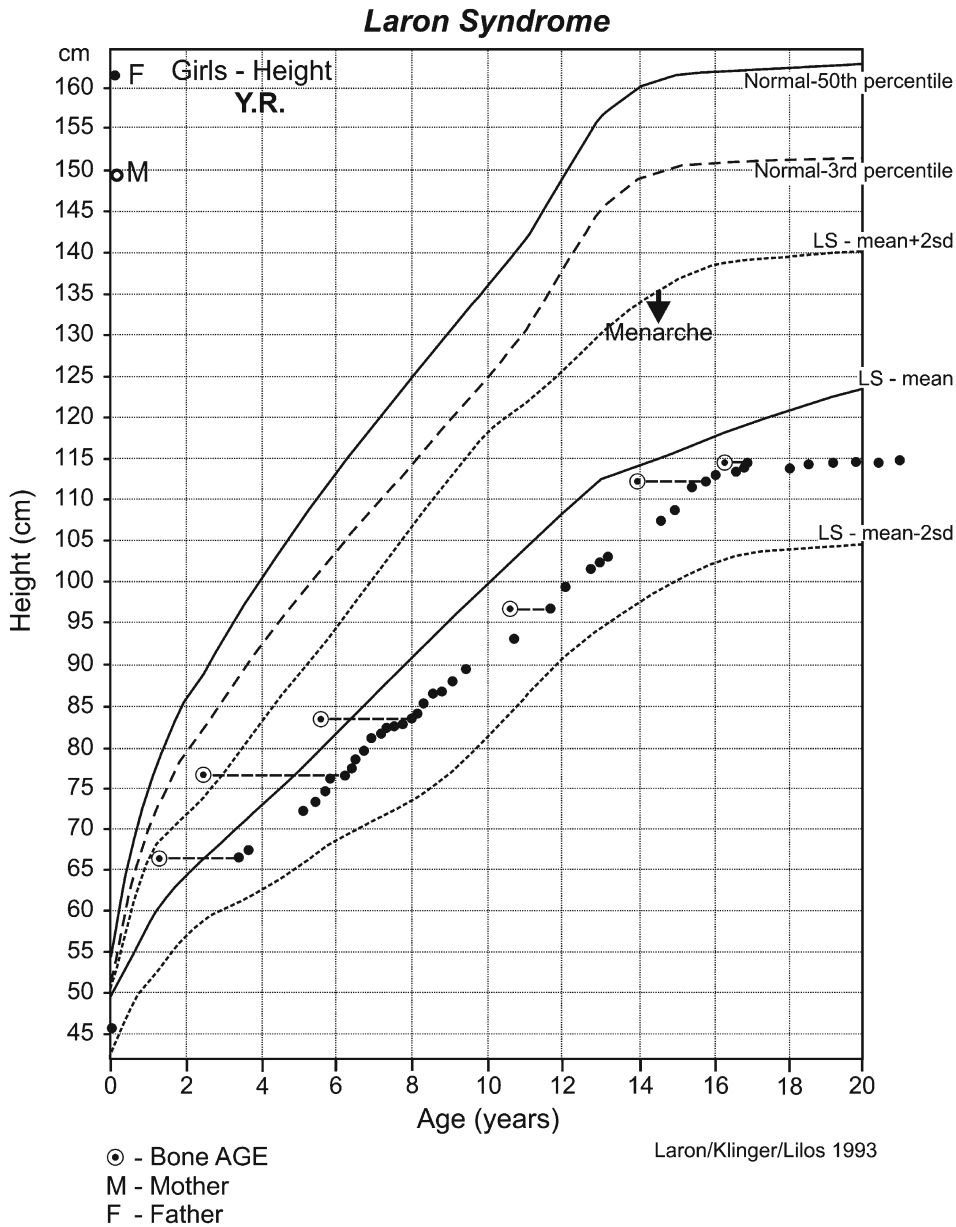

Fig. 8.17 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

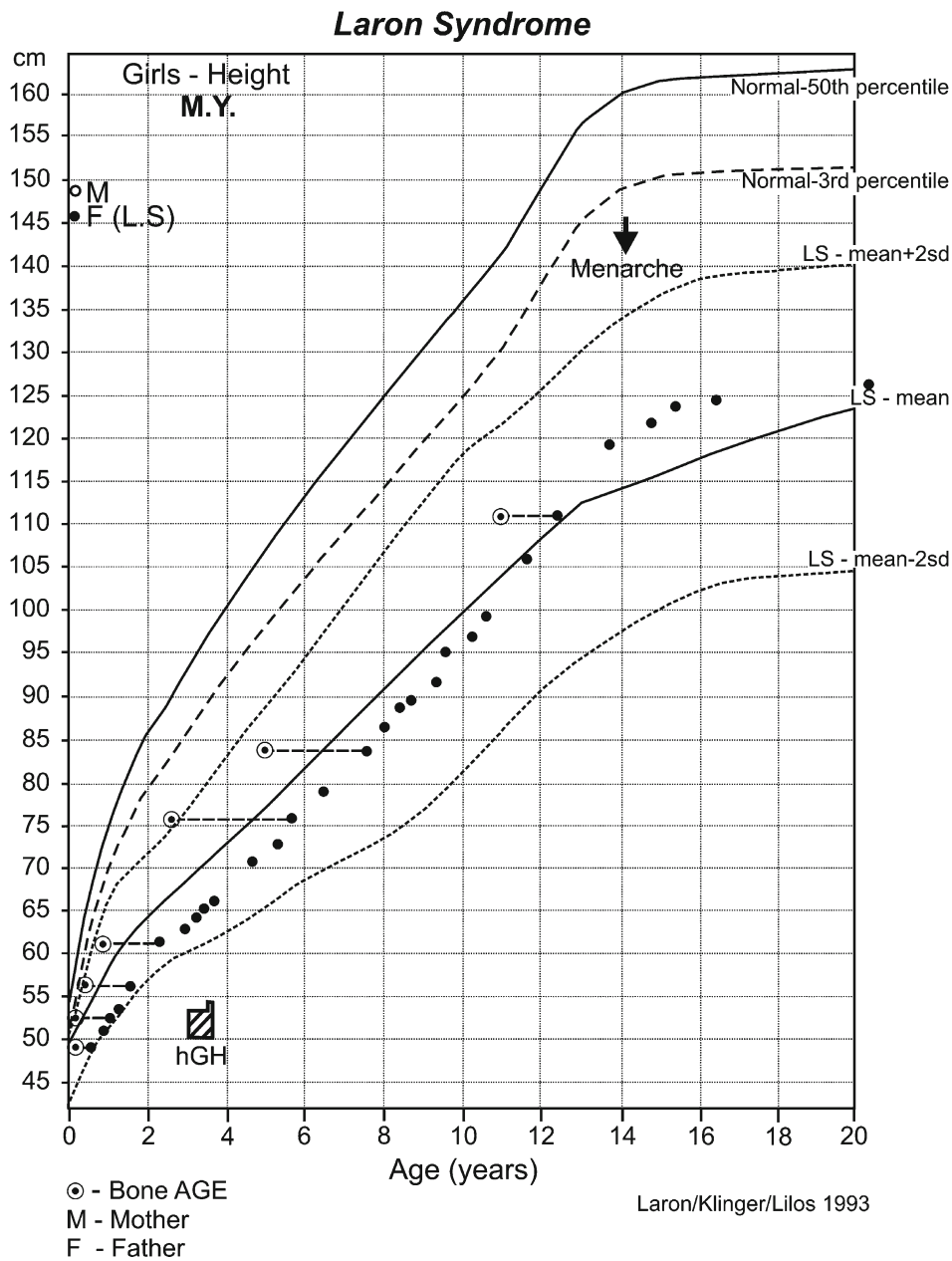

Fig. 8.18 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

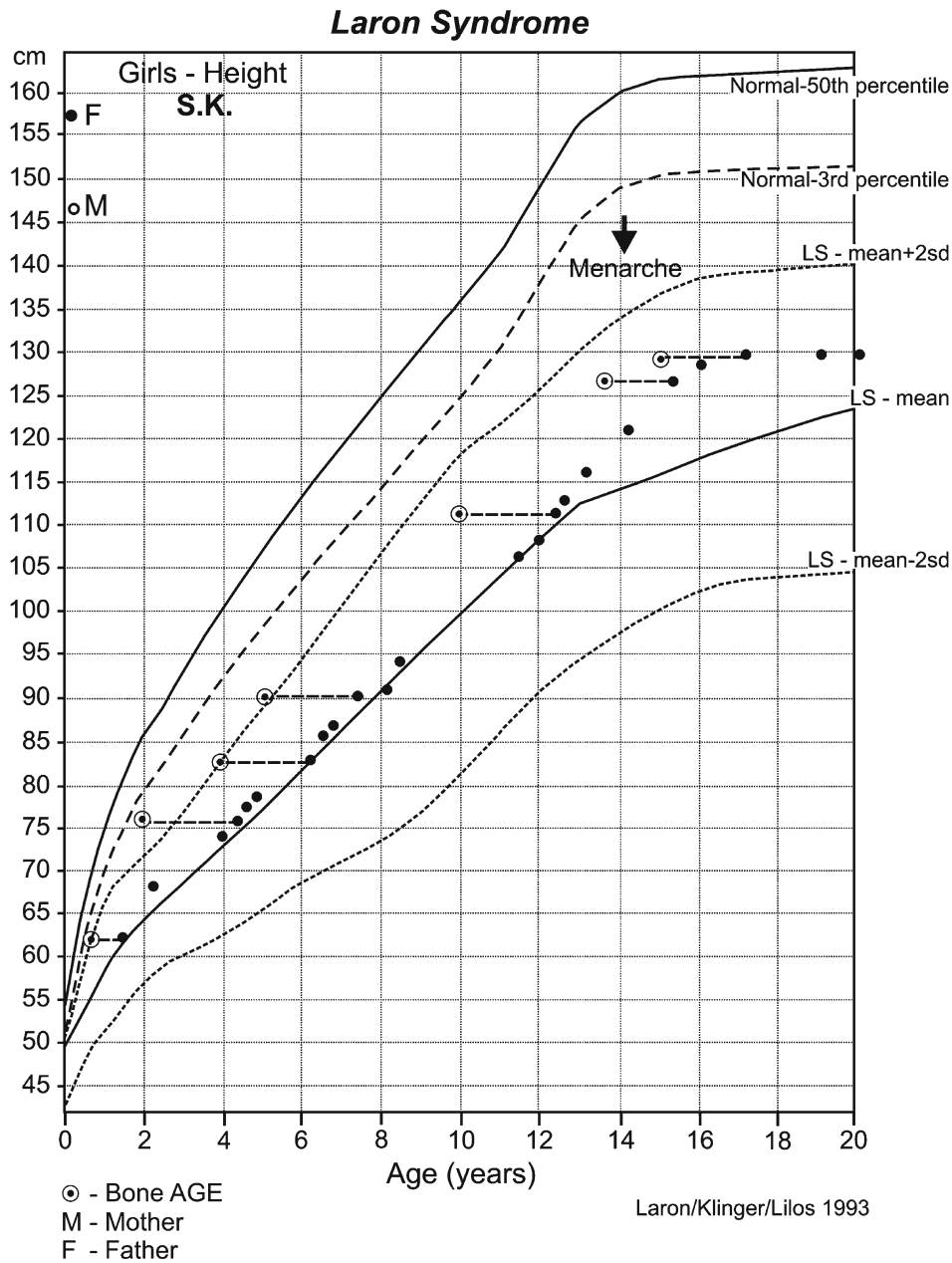

Fig. 8.19 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

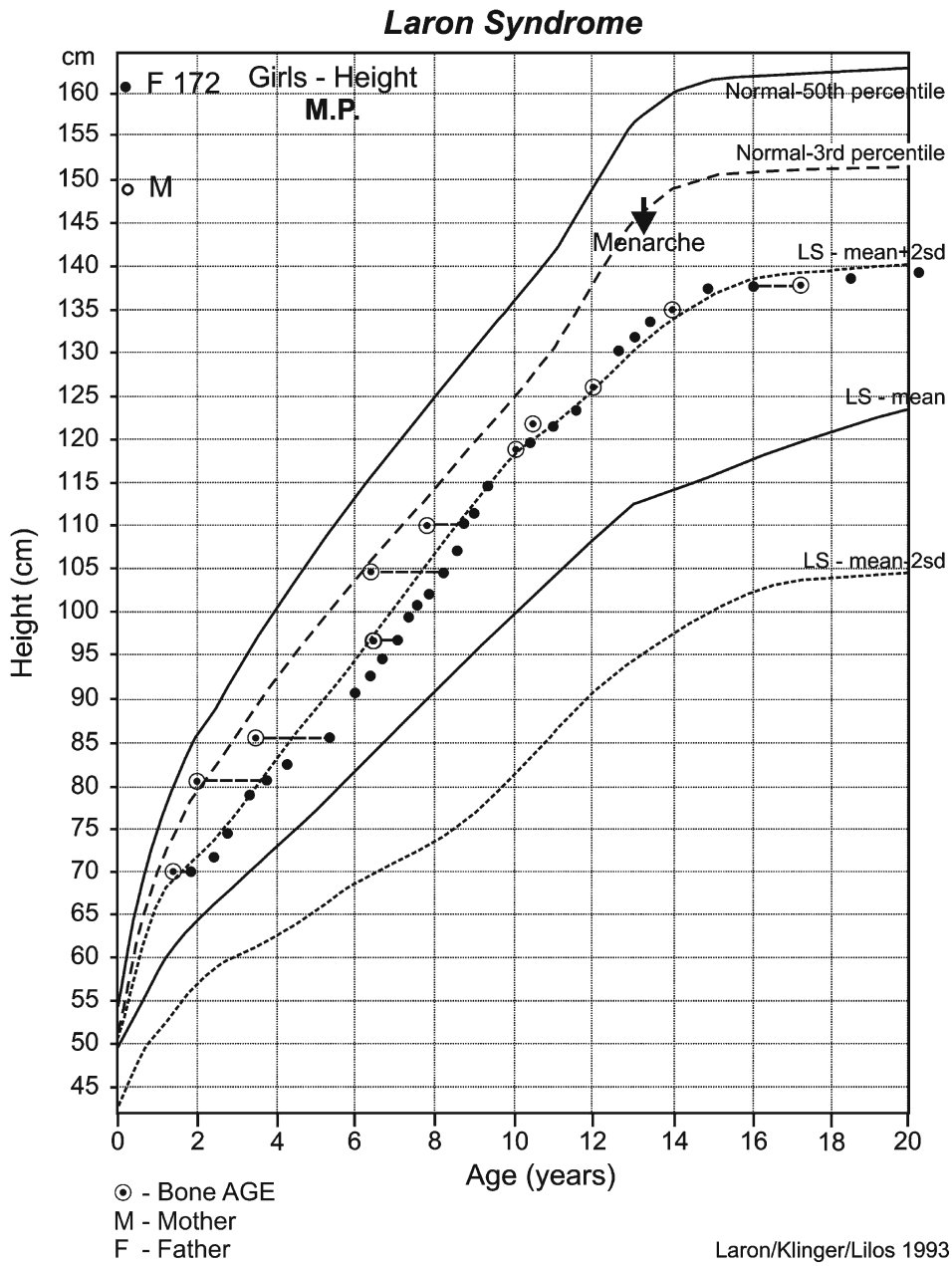

Fig. 8.20 Growth pattern of a girl with Laron syndrome drawn on the Laron syndrome specific growth charts (Laron et al. 1993)

Table 8.1 Comparison of growth deficit between GHBP-negative and GHBP-positive children with Laron syndrome

Group	Age (year) m ± SD	Height SDS m ± SEM	IGF-I (ng/mL) m ± SD
GHBP negative $n = 20$	7.9 ± 5.0	−6.7 ± 0.33	22 ± 3.8
GHBP positive $n = 13$	7.3 ± 4.1	−5.5 ± 0.24[a]	24.3 ± 2.3

[a] $p < 0.005$

Modified from Laron et al. (1997)

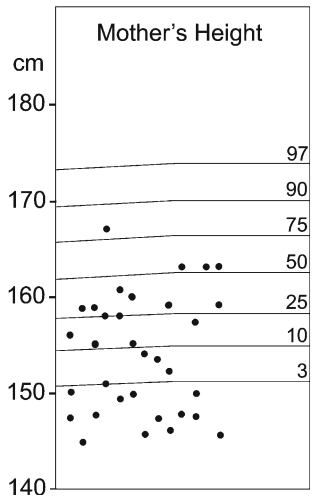

Fig. 8.21 Growth of 33 mothers of children with Laron syndrome plotted on Tanner charts (Tanner et al. 1966)

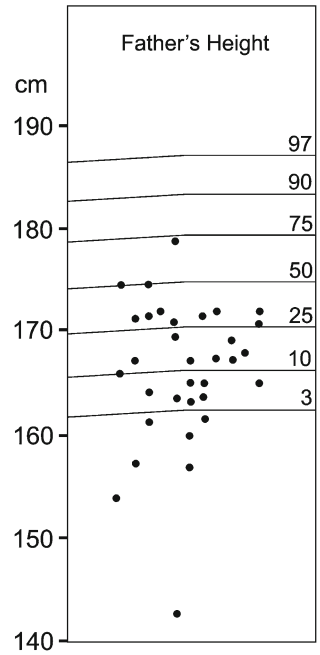

Fig. 8.22 Growth of 33 fathers of children with Laron syndrome plotted on Tanner charts (Tanner et al. 1966)

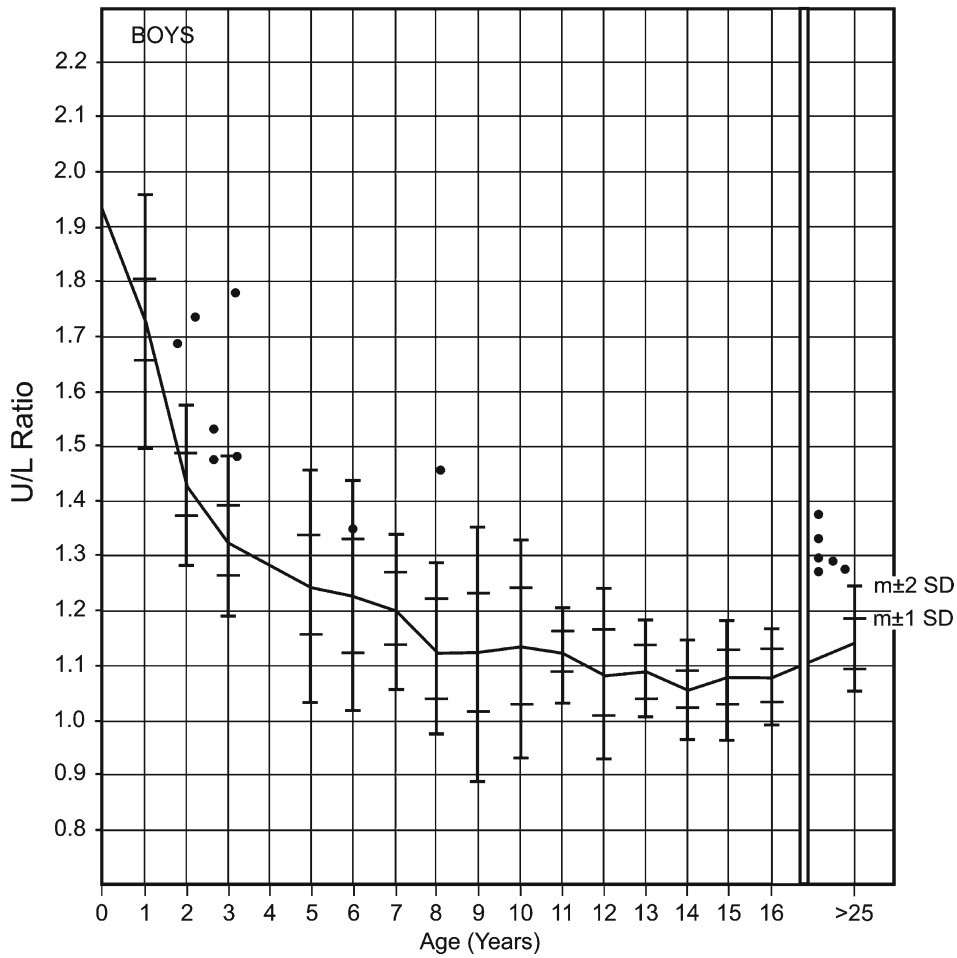

Fig. 8.23 Upper to lower body segment ratios at referral in 14 untreated male patients with Laron syndrome plotted on Arad and Laron charts (1979)

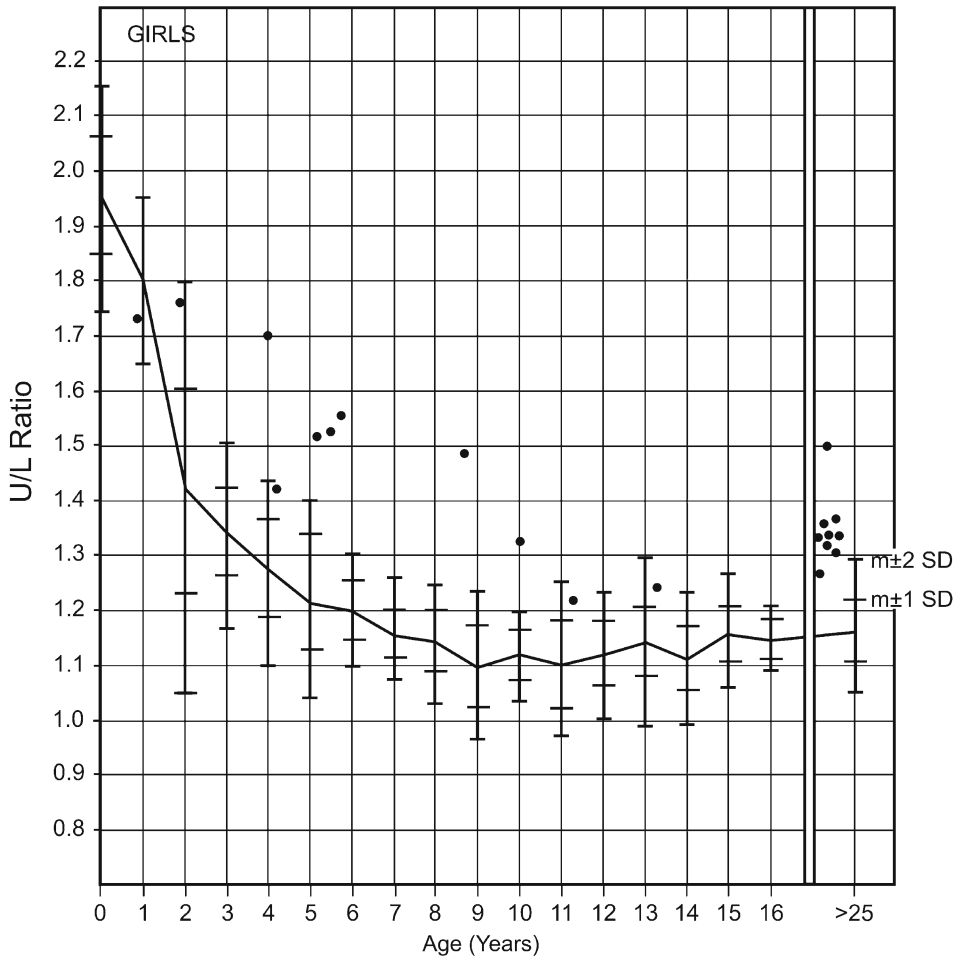

Fig. 8.24 Upper to lower body segment ratios at referral in 20 untreated female patients with Laron syndrome plotted on Arad and Laron charts (1979)

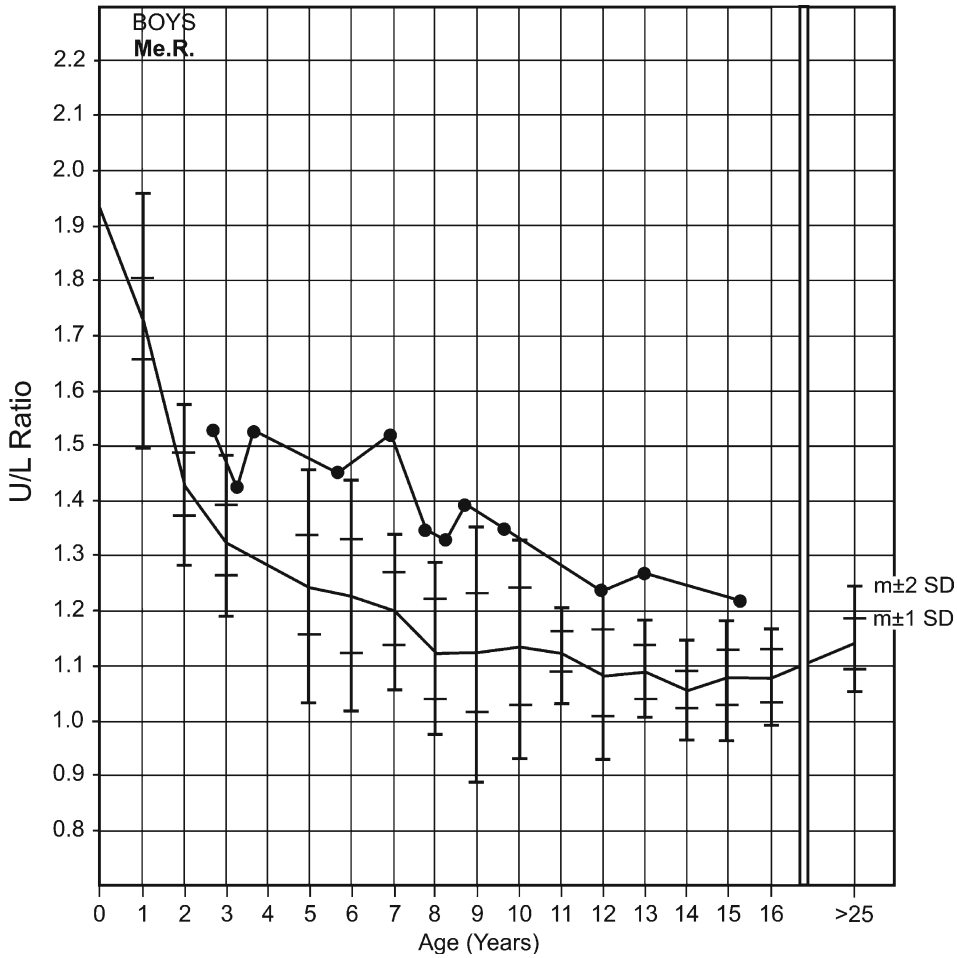

Fig. 8.25 Upper to lower body segment ratio from childhood to puberty in a boy with Laron syndrome plotted on Arad and Laron charts (1979)

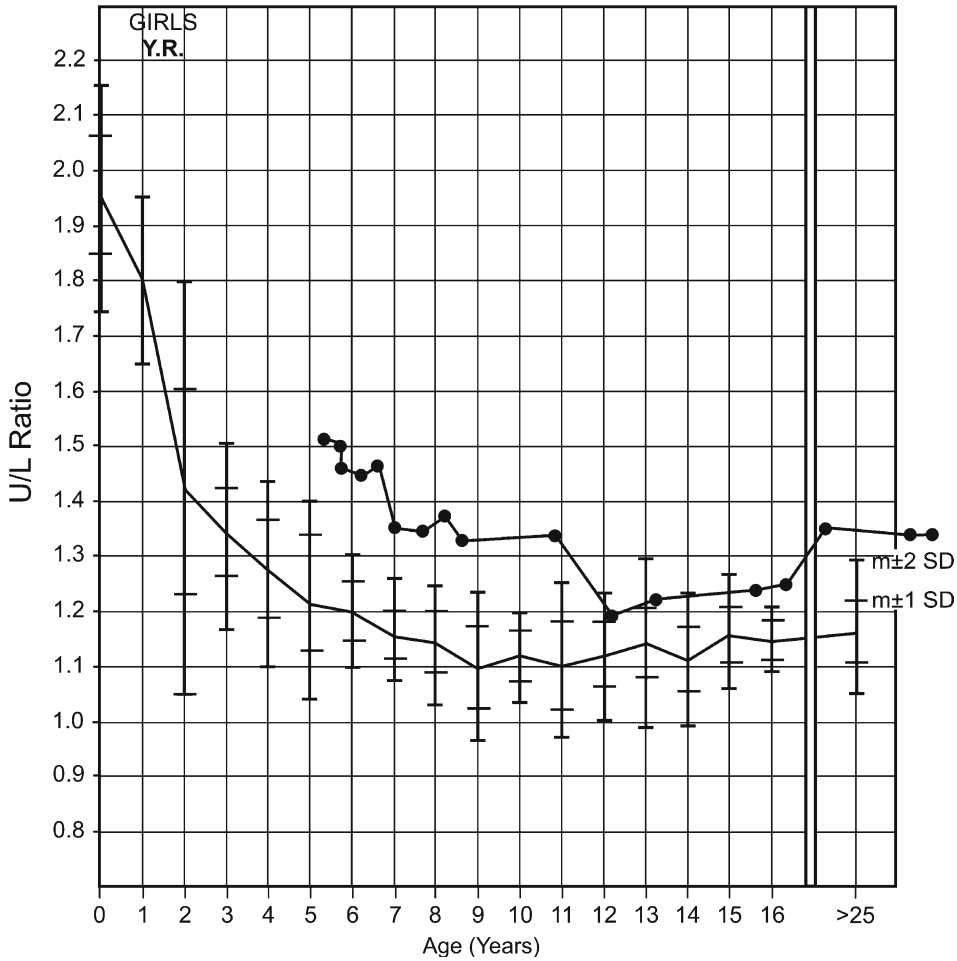

Fig. 8.26 Upper to lower body segment ratio from childhood to adult age in a female with Laron syndrome plotted on Arad and Laron charts (1979)

8.3 Conclusions

Untreated congenital IGF-I deficient patients, such as in Laron syndrome have a disproportional growth retardation proving that IGF-I deficiency impairs more limb growth than the body height (spine and head). The same was observed by us, in (the now rare) untreated congenital GH deficient patients.

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Head Shape, Size, and Growth of Untreated Patients with Laron Syndrome

9

Zvi Laron and Rivka Kauli

Core Message

› One of the characteristics of untreated Laron syndrome is organomicria, including the brain which is measured as head circumference, and the underdevelopment of the facial bones. This chapter illustrates the above in patients of both genders.

9.1 Shape

The abnormal and characteristic shape of the head was already seen in the early referred patients (Fig. 9.1). At first appearance, the head seemed large for the body, but in effect, the head circumference is, in most patients, below the normal size. There is a disproportional, underdevelopment of the facial bones leading to the typical appearance of a protruding forehead, saddle nose, and sunset look (Figs. 9.2–9.13).

This disproportional development of the cranial bones has been documented by anthropometric Konfino et al. (1975) and skull X-rays (Scharf and Laron 1972) measurements. Konfino et al. (1975) performed lateral cephalograms of 10 children with Laron syndrome, aged 9–18 years, using standardized conditions with a cephalostat and compared the measurements to normal children matched for age and sex.

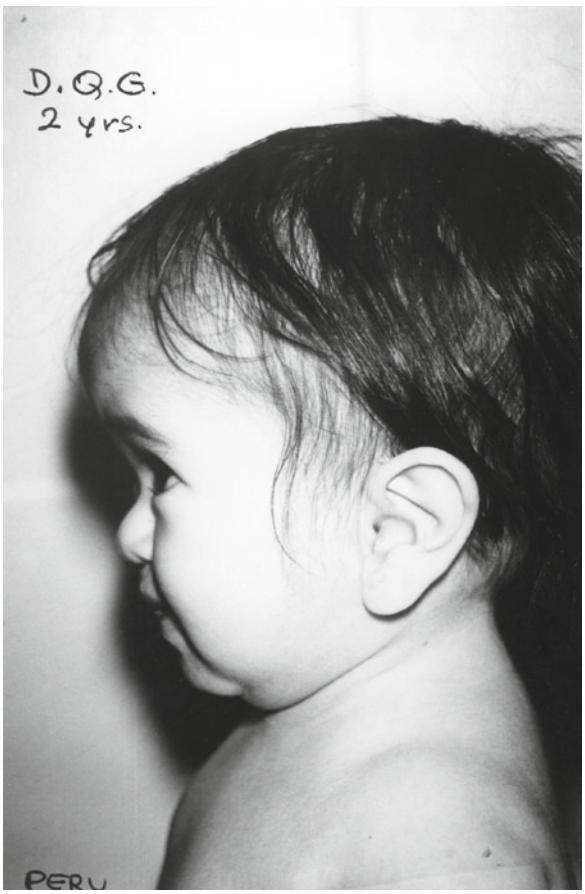

Fig. 9.1 A 2-year-old girl with Laron syndrome (QD)

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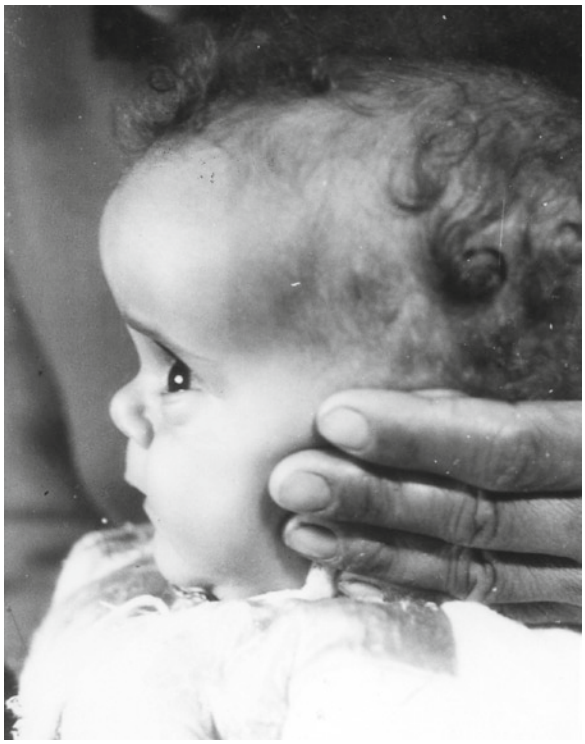

Fig. 9.2 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 1-year-old boy (SSh)

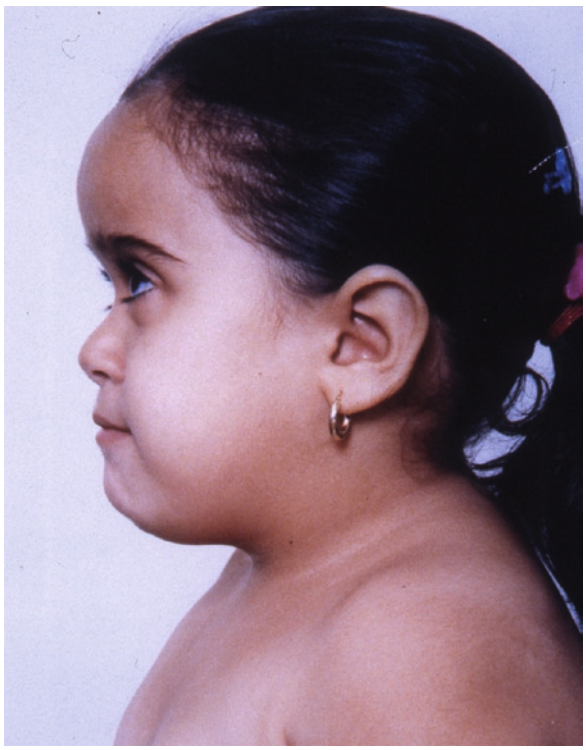

Fig. 9.3 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 2-year-old girl (SL)

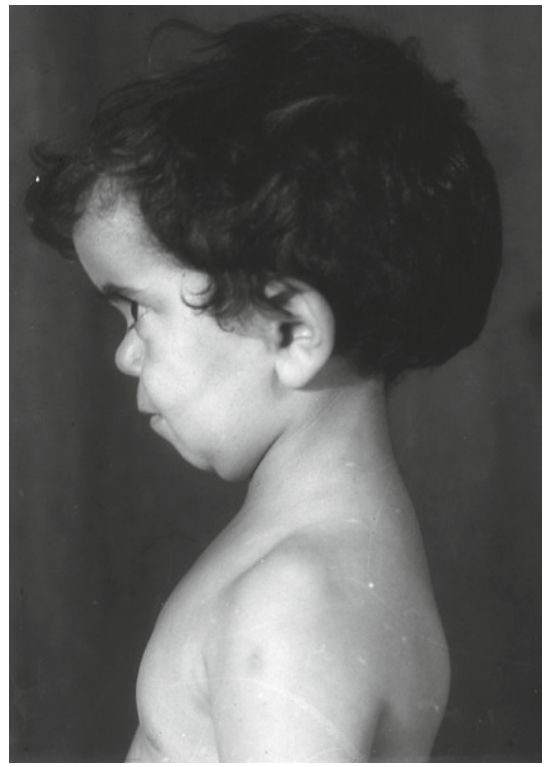

Fig. 9.4 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 5-year-old boy (SSi)

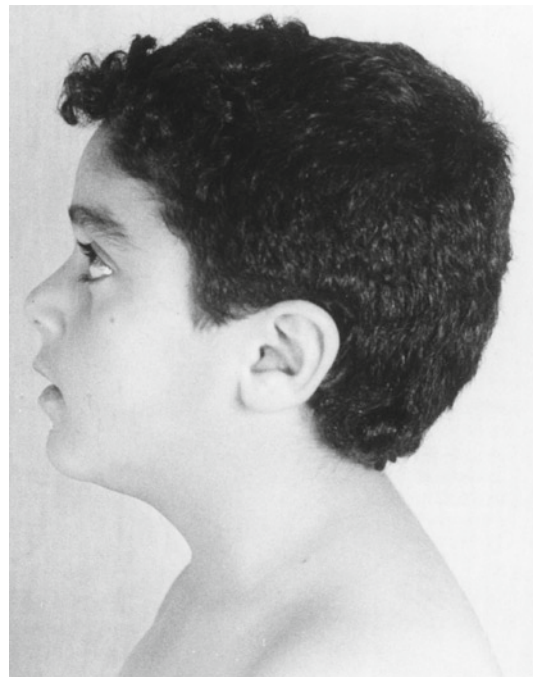

Fig. 9.5 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a $10^{6/12}$-year-old boy (SSh)

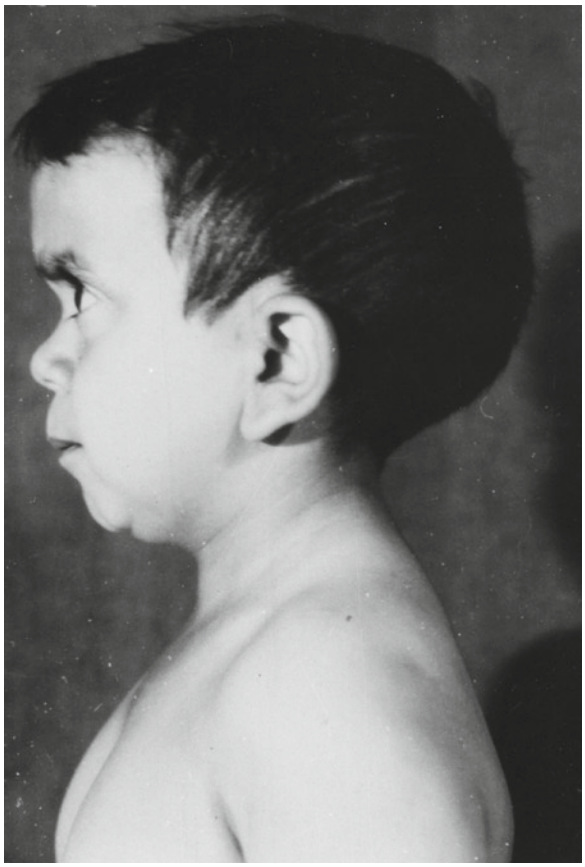

Fig. 9.6 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 14-year-old boy (LY)

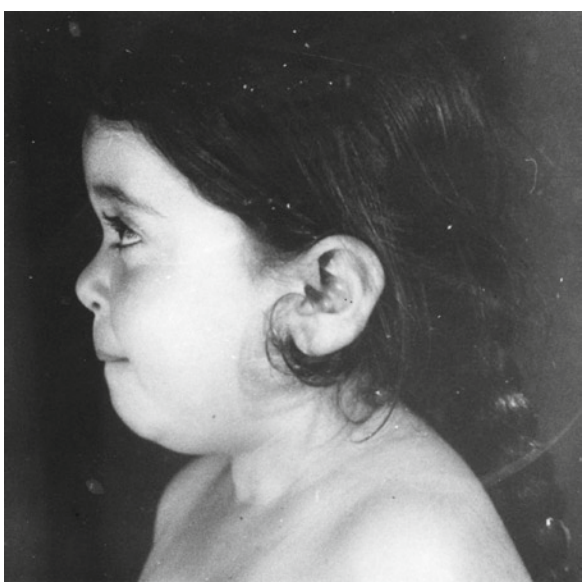

Fig. 9.7 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 7-year-old girl (MP)

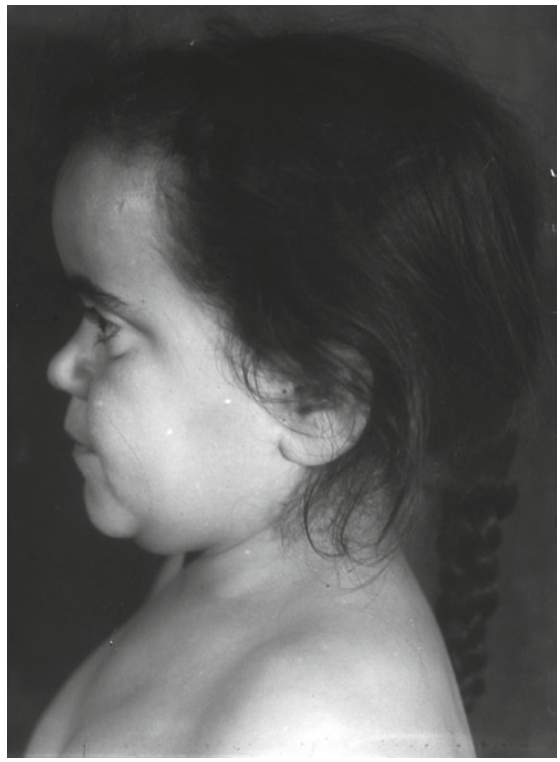

Fig. 9.8 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 12-year-old girl (SR)

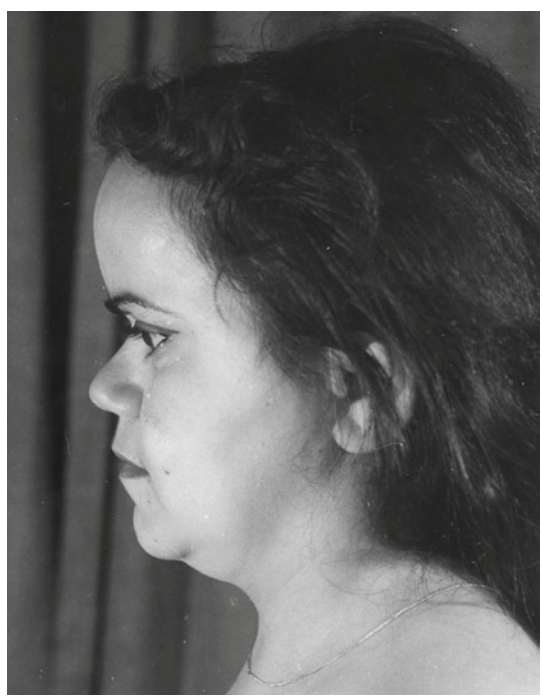

Fig. 9.9 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 17-year-old female (SR)

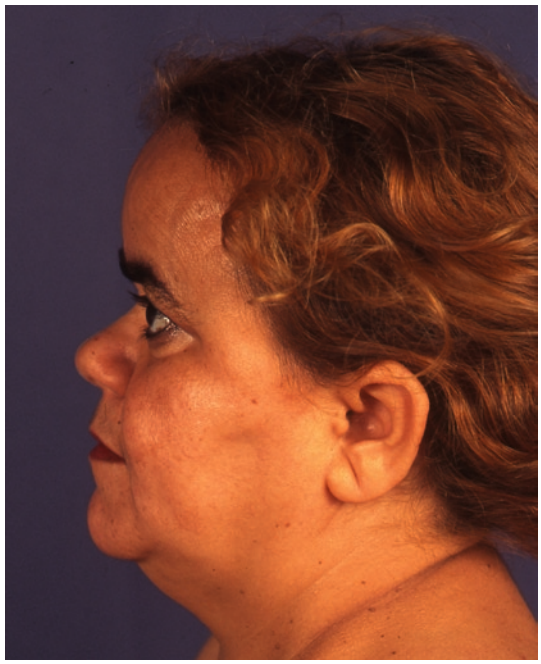

Fig. 9.10 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 39-year-old female (SR)

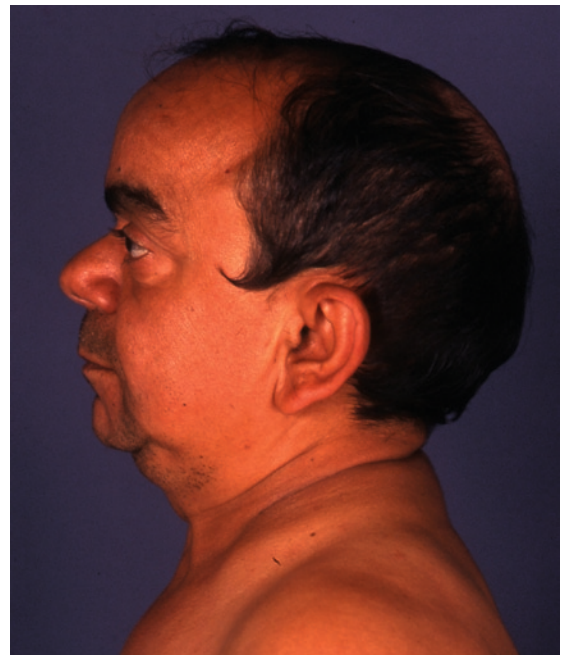

Fig. 9.12 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 42-year-old male (SSi)

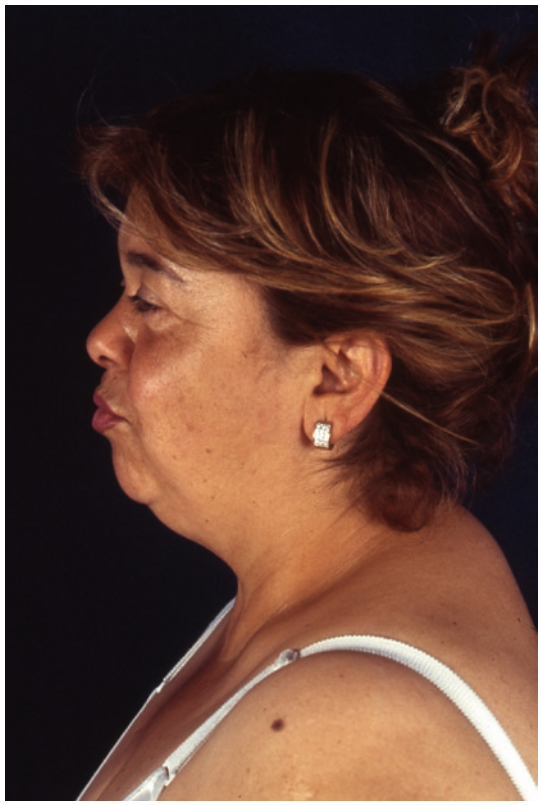

Fig. 9.11 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 43-year-old female (MP)

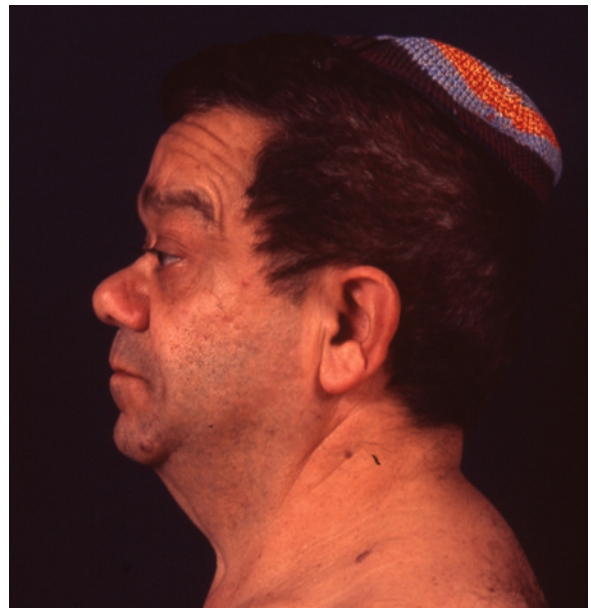

Fig. 9.13 Typical disproportional form of the head in Laron syndrome as seen on the lateral views of a 52-year-old male

The following measurements were performed:

Ba-N: Total length of the cranial base: distance between basion and nasion.

S-N: Length of the anterior cranial base: sella-nasion.

S-Ar: Length of the posterior cranial base: sella-articulare.

Ar-Pog: Total length of the mandible: articulare-pogonion.

Ar-G: Length of the ramus of the mandible: articulare-gonion.

Go-Pog: Length of the corpus of the mandible: gonion-pogonion.

ANS-PNS: Length of the maxilla: anterior nasal spine-posterior nasal spine.

A-PNS: Length of the maxillary apical base: subspinale-posterior nasal spine.

9.1.1 Angular Measurements *(Fig. 9.14)*

S-N-A (sella-nasion-subspinale): this angle measures the degree of maxillary prognathism.

S-N-B (sella-nasion-supramentale): shows the anterior limit of the mandibular basal arch in relation to the anterior cranial base.

N-S-Ar (nasion-sella-articulare): this cranial base angle indicates the degree of bending of the cranial base.

Fig. 9.14 Angular measurements of the skull

Ar-G-Gn (articulare-gonion-gnathion): this angle determines the angulation between the ramus and corpus of the mandible.

FMA (Frankfort-mandibular angle): this angle is formed between the Frankfort horizontal reference plane and the mandibular plane.

9.1.2 Results

The comparison between the measurements in the Laron syndrome patients and controls are shown in Table 9.1.

Cranial base. The total cranial base length (Ba-N) was found to be significantly smaller in the Laron syndrome patient group ($p<0.01$).

The anterior cranial base length (S-N) was smaller to the same degree in the patient group ($p<0.01$).

The posterior component of the cranial base (S-Ar) was even less developed in the patient group ($p<0.05$). The growth of this region is dependent upon the activity of the spheno-occipital synchondrosis, which is still present even after puberty.

Mandible. The total length of the mandible (Ar-Pog) was found to be significantly smaller in the patient group ($p<0.01$).

Similarly, the length of the ramus and the length of the corpus (Ar-Go) were significantly smaller in the patient group ($p<0.01$).

Maxilla. The length of the maxilla (ANS-PNS) was also smaller in the patient group, and the length of the maxillary apical base (A-PNS) was significantly smaller ($p<0.01$).

In order to evaluate the difference in the facies between the two groups, we compared a number of angular measurements.

The cranial base angle (N-S-Ar) was not statistically different in the two groups. In normal children, this angle is negatively correlated with the degree of prognathism of the maxilla and mandible.

The SNA angle, which defines the degree of prognathism of the maxilla, was found to be significantly smaller in the patient group ($p<0.01$). This means that in Laron syndrome patients, the maxilla is more retarded in comparison to healthy controls.

The SNB angle was similarly found to be smaller in the patient group ($p<0.01$), proving that the mandible is retrognathic in relation to the cranial base.

The Ar-Go-Gn angle, which shows the angulation between ramus and corpus of the mandible,

Table 9.1 Comparison of cephalometric measurements between normal control subjects and patients with Laron syndrome

Diameters (mm)	Angles (degrees)		Statistical evaluation
	Laron syndrome patients Mean (mm) ± SD	Controls Mean (mm) ± SD	
Ba-N	84.7 ± 4.52	99.0 ± 0	<0.01
S-N	58.1 ± 4.09	68.5 ± 3.00	<0.01
S-Ar	19.1 ± 2.13	32.3 ± 2.52	<0.01
Ar-Pog	75.2 ± 5.88	102.3 ± 8.26	<0.01
Ar-Go	29.2 ± 5.45	43.3 ± 5.13	<0.01
Go-Pog	52.6 ± 5.89	73.3 ± 6.29	<0.01
ANS-PNS	38.9 ± 3.57	52.4 ± 3.44	<0.01
A-PNS	36.4 ± 3.60	47.5 ± 3.05	<0.05
N-S-Ar	126.1 ± 10.69	125.3 ± 0.36	<0.01
SNA	74.4 ± 2.22	80.5 ± 0.82	<0.01
SNB	69.3 ± 3.16	77.2 ± 1.68	<0.01
Ar-Go-Gn	135.4 ± 8.93	123.3 ± 2.11	<0.01
FMA	36.0 ± 4.69	25.4 ± 1.38	<0.01

Table 9.2 The mean (±SD) bicondilar/biparietal ratios (×100) in healthy children and adolescents and in Laron syndrome patients

Group	Age (years)					
	1–3		4–8		9–20	
	Males	Females	Males	Females	Males	Females
Healthy controls						
N	18	14	16	12	22	18
Bic/bip ratio	66.3 ± 1.2	66.9 ± 1.6	70.7 ± 2.6	70.5 ± 2.3	73.7 ± 3.9	75.4 ± 2.6
Laron syndrome						
N	2	–	2	3	5	9
Bic/bip ratio	50.5 ± 2.1	–	52.5 ± 0.2	55.5 ± 2.8	63.8 ± 3	59.7 ± 2.1

was significantly wider in the patient group ($p < 0.01$).

The FMA measuring the inclination of the lower border of the mandible was greater in the patient group ($p < 0.01$), denoting that in profile, the lower border of the mandible is much steeper in Laron syndrome patients than in normal persons.

9.1.3 The "Bicondilar/Biparietal Ratio"

A simpler method to determine the underdevelopment of the facial bones is to employ anterior–posterior skull X-rays, measure the ratio between the bicondilar (bic) and biparietal (bip) width (in mm), and calculate the

$$\text{Following ratio} = \frac{\text{bic diameter}}{\text{bip diameter}} \times 100.$$

We calculated this ratio by gender and age for 100 normal control subjects and for 24 untreated patients with Laron syndrome (Scharf and Laron 1972) (Table 9.2).

The numerical expression of the ratio permits the grading of the diminution of the facial bones (sphenoid, maxilla, and mandible). Figure 9.15 exemplifies the difference in this ratio between a healthy 10-year-old boy (left) and a same-aged boy with Laron syndrome

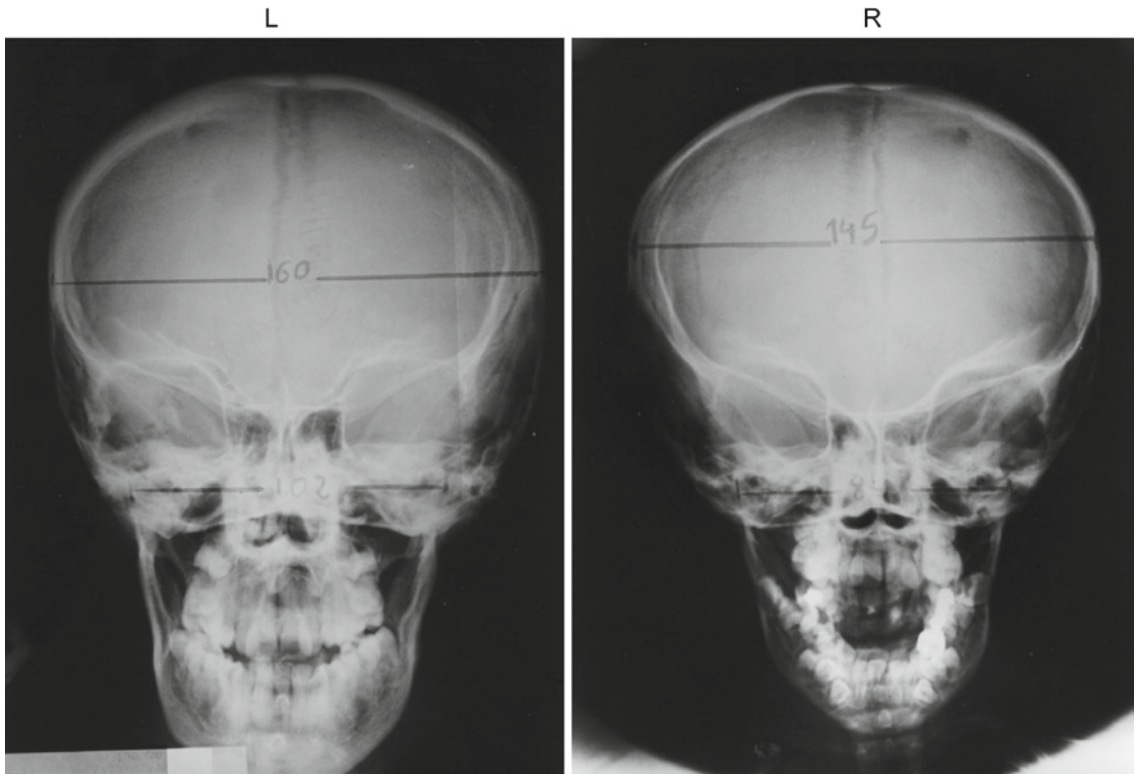

Fig. 9.15 Anterior–posterior skull X-ray of two 10-year-old boys: healthy (*left*), Laron syndrome (*right*). It is also shown how the biparietal and bicondylar diameters are measured

(right). We found similar changes in skull measurements of untreated patients with congenital isolated GH deficiency (cIGHD) (Scharf and Laron 1972).

Schaefer et al. 1994 recently reported the disproportional development of the face in 49 adult Laron syndrome patients from Ecuador by analyzing facial morphometric landmarks from AP photographs.

9.1.4 Conclusion

Both radiologic and cephalometric studies of the skull revealed that in Laron syndrome patients, the cranial base is significantly smaller than in normal subjects, so that the position of the nose root is steep and low in relation to the forehead. The face develops rearward, with both the maxilla and the mandible in retrognathic position resulting in mandibular acromicria.

Our studies also provide evidence for the major role played by GH/IGF-I in the development of the facial and cranial base bones.

9.2 Head Circumference, Growth, and Size in Untreated Patients with Laron Syndrome

Despite the impression of an enlarged head compared to the body size (Fig. 9.16), the size of the head as determined by head circumference measurements reveals that the majority of untreated patients with Laron syndrome have subnormally sized heads.

9.2.1 Methods

Head circumference was measured by passing a tape measure over the most prominent part of the occipital prominence and above the supraorbital ridges. The norms used were those of Nellhaus (1967). It is an accepted fact that the measurements of the head circumference are related to the intracranial volume and permit an estimate of brain volume and growth (Watson and Lowrey 1962).

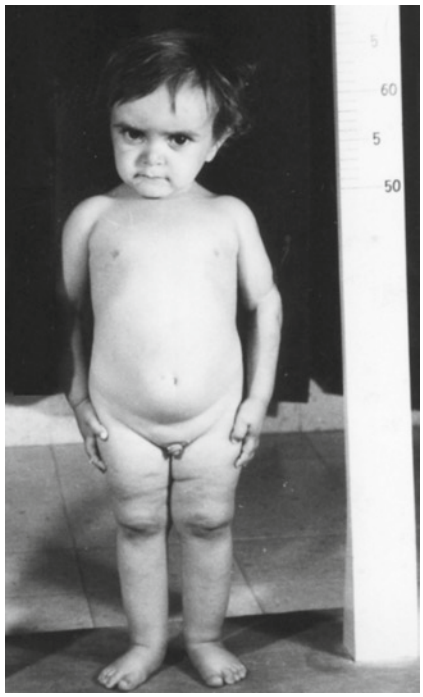

Fig. 9.16 Apparent large head compared to body size in a 4-year-old boy with Laron syndrome (YG)

With few exceptions at each visit as part of the physical examination, head circumference was measured. Thus, accurate measurements over time for all patients are available, both for untreated Laron syndrome patients as well as for those treated by IGF-I.

9.2.2 Results

The head circumference of 22 untreated adult Laron syndrome patients (12 females and 10 males) is shown in Fig. 9.17.

It is evident that with one exception, all females and all male Laron syndrome patients had a head

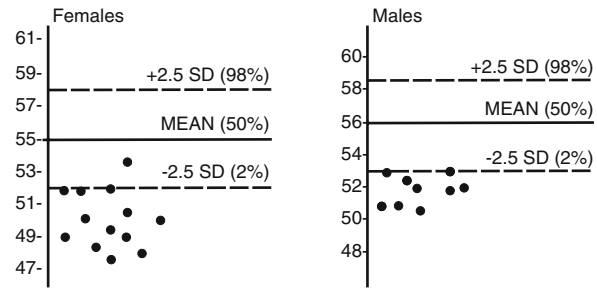

Fig. 9.17 Head circumference of 22 untreated adult patients with Laron syndrome. The *interrupted lines* denote the normal ±2.5SD limit according to Nellhaus (1967)

circumference of −2.5 SD or below. The females had lower measurements than the males ranging between 47.5 and 52.5 vs. 50.5 and 53 cm.

9.2.3 Head Growth

The longitudinal head growth as measured by head circumference is illustrated for one untreated male Laron syndrome patient in Fig. 9.18, and for 2 female patients in Figs. 9.19 and 9.20. It is seen that in untreated Laron syndrome patients, the growth is progressive but below −2 SD of the norm. The female patient who showed a lesser retardation (Fig. 9.20) reached below normal head circumference size in young adult age.

9.2.4 Conclusions

Congenital IGF-I deficient patients have a continuous below normal head circumference with very few exceptions of normal growth. A major causative factor is the subnormal brain growth in the absence of IGF-I (see response to IGF-I treatment, Chap. 43).

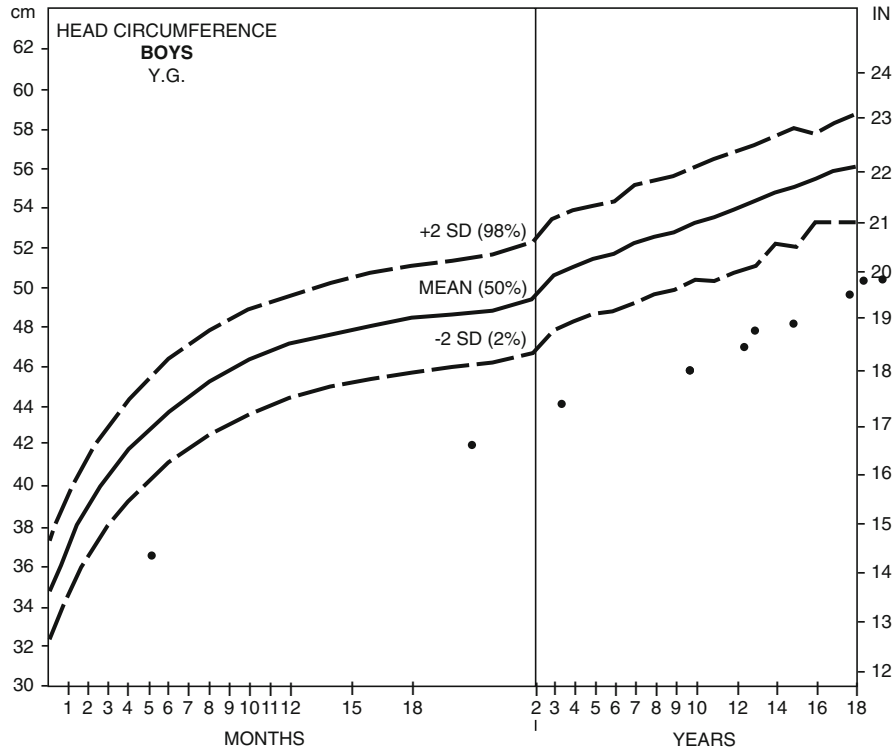

Fig. 9.18 Longitudinal head circumference growth of a male patient with Laron syndrome from the age of 5 months to 20 years. Head growth chart from (Nellhaus 1967)

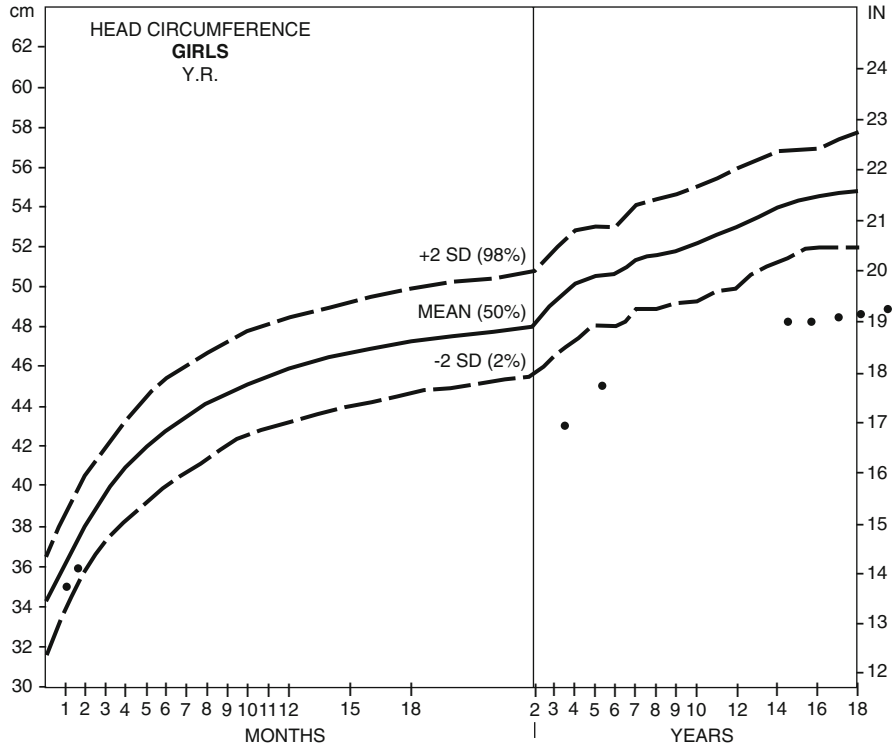

Fig. 9.19 Longitudinal head circumference growth of a female patient with Laron syndrome from the age of 1 month to 20 years. Head growth chart from (Nellhaus 1967)

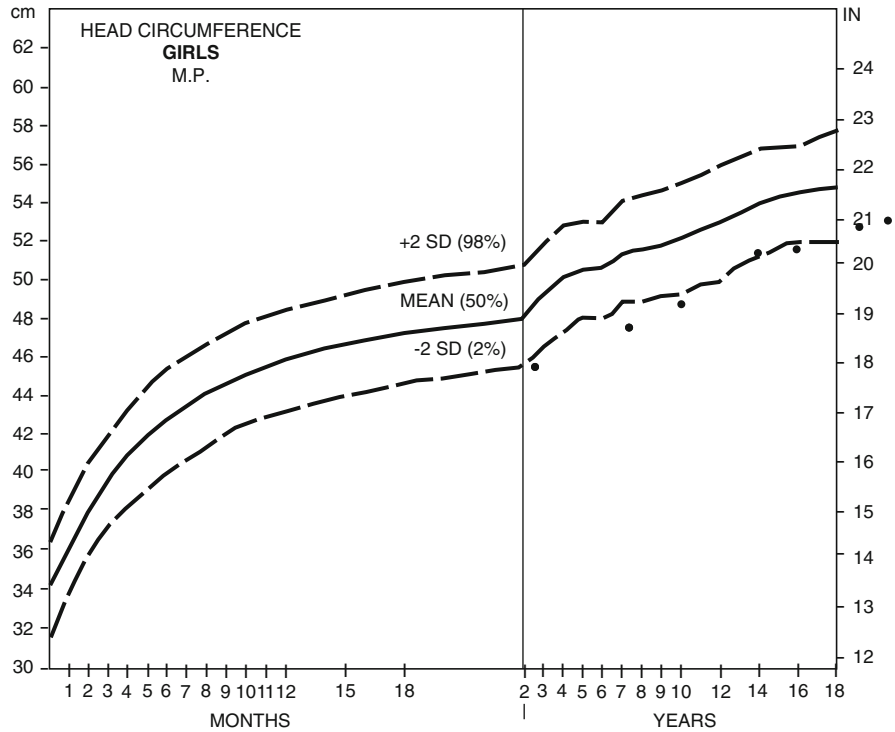

Fig. 9.20 Longitudinal head circumference growth of a female patient with Laron syndrome from the age of $2^{1/2}$ to 25 years. Head growth chart from (Nellhaus 1967)

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Sexual Development in Patients with Laron Syndrome

10

Zvi Laron and Rivka Kauli

Core Message

› The sequence of sexual development in boys and girls is described and illustrated. Despite delayed puberty mainly in boys, both genders reach full sexual development and reproductive potential.

10.1 Introduction

One of the cited characteristics of Laron syndrome is hypogenitalism and hypogonadism (Laron et al. 1968; Laron 1984, 2004).

In general terms, the size, growth, and development of the sexual organs are retarded in both genders; however, in the males, the retardation and small size of organs is more accentuated. Nonetheless, both sexes reach full sexual development.

The development of the genitalia and gonads is more documented in males than in females; as in the males, they are easily measured, and the female genitalia can only be described and graded. Ultrasonographic measurements of the ovaries and uterus during the prepubertal and pubertal periods are scant.

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10.1.1 Methods

The testicular volume was measured using an orchidometer (Prader 1966) employing the norms of Zilka and Laron (1969). Penile length was measured with a caliper during unforced stretching (Flatau et al. 1975) using the norms of Schoenfeld and Beebe (1942). Breast measurements were made with the help of a tape for measurement of the half circumference (Capraro 1977). The stages of development of the nipples and areolae (0 to ++) and genitalia (0–5+) was registered at each visit. During puberty, the girls were questioned about the appearance of menarche and the boys about their first conscious ejaculation (Laron et al. 1980a), enabling us to establish the exact age and bone (skeletal) age at which these similar milestones occurred. Bone age was assessed by hand and wrist X-ray in accordance with the Atlas of Greulich and Pyle (1959) at least once a year, and also close to menarche or the first conscious ejaculation.

10.2 Findings in Untreated Laron Syndrome Patients

10.2.1 Prepuberty

10.2.1.1 Males

Testicular Volume

In neonates and up to the age of 10 years or even beyond, the testicular volume ranged between 0.5 and 1.5 mL (Fig. 10.1).

Z. Laron and J. Kopchick (eds.), *Laron Syndrome - From Man to Mouse*,
DOI: 10.1007/978-3-642-11183-9_10, © Springer-Verlag Berlin Heidelberg 2011

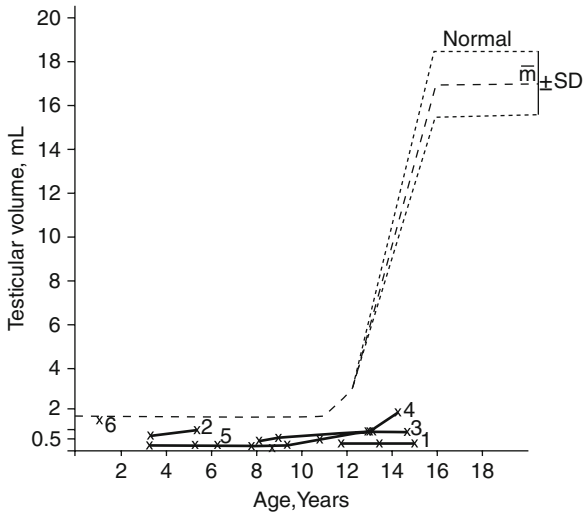


Fig. 10.1 Testicular volume in six untreated prepubertal boys with Laron syndrome. Modified from Laron and Sarel (1970)

Penile Length

Table 10.1 shows the below normal penis length in boys with Laron syndrome compared to boys with IGHD (Laron and Sarel 1970). Note the similarity.

Figure 10.2a–e illustrate the appearance of the genitalia and scrotum of a boy with Laron syndrome (YG) at ages $2^{6/12\ 6/12}$, 6, $8^{6/12}$ and of two other boys aged $10^{4/12}$ years.

10.2.1.2 Females

Genitalia

The genitalia remain infantile up to a relatively late age. Figure 10.3 illustrates the vulva and labia of a $6^{6/12}$ year-old girl with Laron syndrome.

Ovaries

The ultrasonographic appearance of the ovaries of an untreated 7-year-old girl with Laron syndrome is shown in Fig. 10.4. The size is within the normal range.

10.2.2 Puberty

Part of the physical examinations, in addition to body measurements, were the evaluation and registration of the age of appearance of the secondary sexual signs

Table 10.1 Penis length and body height of untreated prepubertal boys with Laron syndrome compared to boys with untreated congenital isolated GH deficiency (IGHD)

Patients		CA (years:months)	Height (cm)	Penis	
Diagnosis	Name			Length (cm)	Norm (cm)
Laron syndrome	MR	1	59.9	3.5	(4.5)
IGHD	WI	2:9	80.0	5.2	(5.0)
Laron syndrome	JG	3:2	62.0	2.5	(5.5)
IGHD	SA	3:5	74.6	3.5	(5.5)
Laron syndrome	BR	3:8	73.4	2.0	(5.5)
IGHD	IE	4:1	73.5	2.9	(5.5)
IGHD	AN	5:11	91.4	4.5	(6.0)
Laron syndrome	LJ	7:8	78.3	3.0	(6.0)
Laron syndrome	AbB	8	84.1	2.5	(6.0)
IGHD	DH	9:7	110.7	4.2	(6.0)
IGHD	IR	11:6	97.6	6.3	(7.0)
Laron syndrome	AlA	11:10	99.1	3.3	(7.0)
IGHD	KZ	11:11	97.6	2.3	(7.0)

Modified from Laron and Sarel (1970). Penis size norms from Schonfeld and Beebe (1942)
CA chronological age

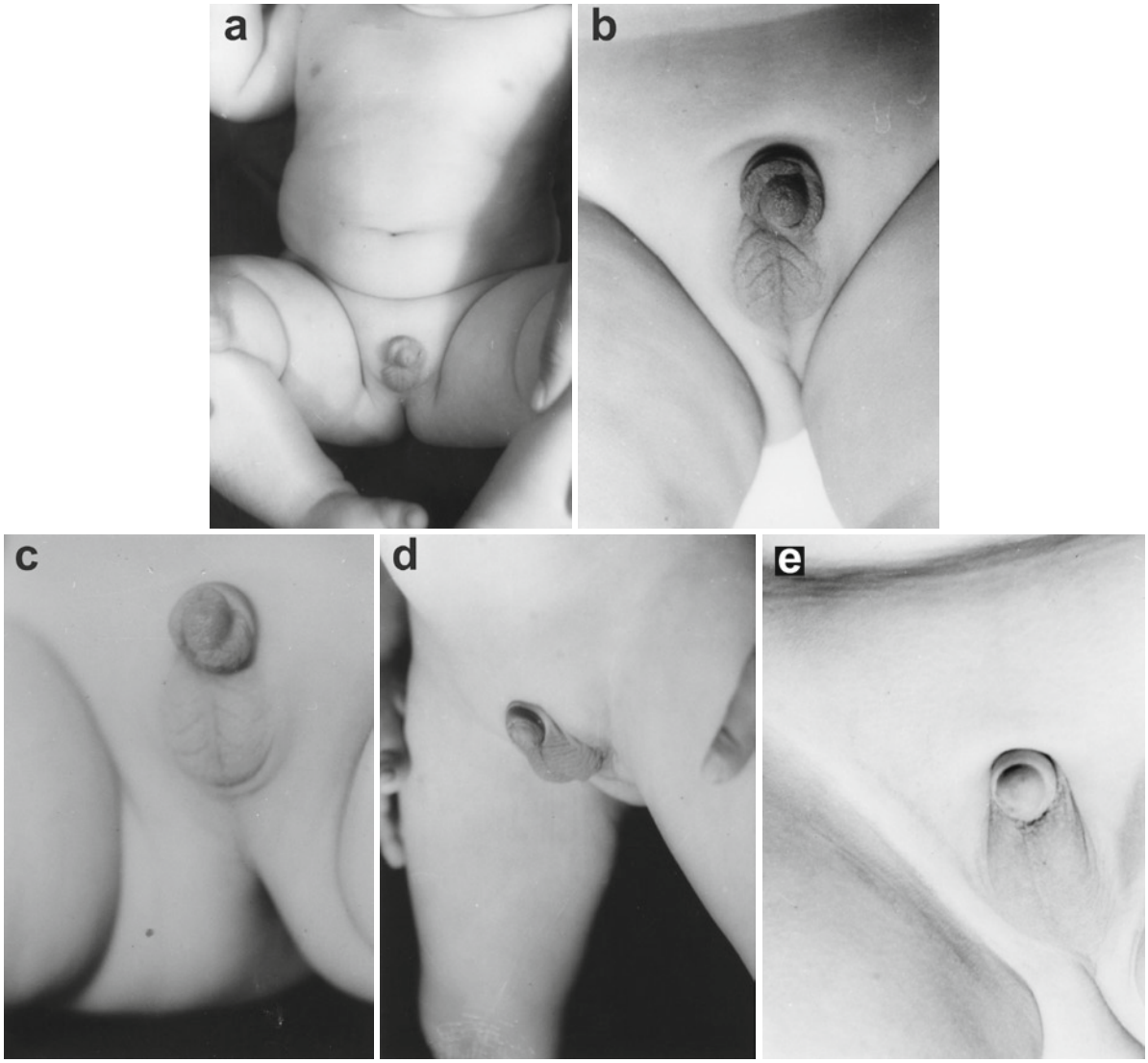


Fig. 10.2 (**a–e**) Genitalia of a prepubertal boy with Laron syndrome (YG) at ages $2^{1/2}$, 6, and $9^{9/12}$ years and of two other boys, SSh age $10^{1/2}$, and AlB age $10^{1/2}$

and developmental changes of the sex organs (Laron et al. 1980a, b) (Chap. 6, Fig. 6.1).

10.2.2.1 Subjects and Methods

Accurate parameters of puberty and/or sexual development were not available for all Laron syndrome patients.

Onset of puberty was considered when testicular enlargement in boys and breast buds in girls, or pubic hair in both sexes developed. Duration of puberty was recorded by calculating the time interval between onset and completion of puberty (stage P 5) (Tanner et al. 1966), a time when final height was also attained. Peak height velocity (PHV) during puberty was determined as the maximal yearly growth rate. Total pubertal growth (TPG) was defined as the entire growth from onset to final height. Height gain during puberty was evaluated as the difference between height SDS (Tanner et al. 1966) at onset of puberty and final height SDS.

10.2.2.2 Onset of Puberty (Gonadarche)

The sequence of appearance of pubertal signs in boys and girls with Laron syndrome in whom accurate data

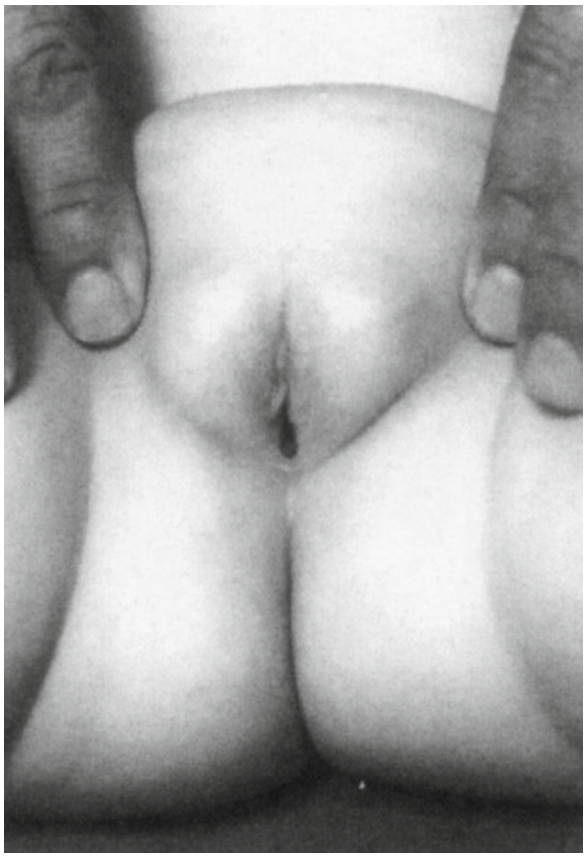


Fig. 10.3 Genitalia of a $6^{6/12}$ year-old girl with Laron syndrome (BS). Reproduced with permission from Laron (2006)

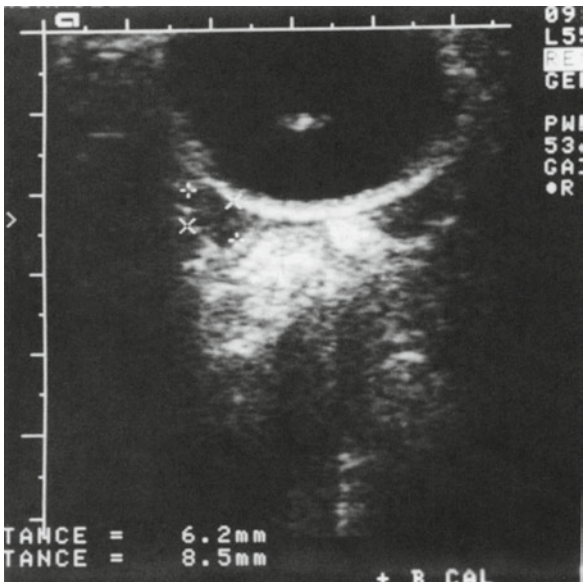


Fig. 10.4 Ultrasonographic appearance of the ovaries of an untreated 7-year-old girl with Laron syndrome. Reproduced with permission from Laron (2006)

is available are illustrated in Figs. 10.5 and 10.6. In the untreated male Laron syndrome patients, the onset of puberty was delayed (Table 10.2), mean ± SD age being 15.6 ± 6 years: a 3 years delay compared to our normal population (the range of age at the start of testicular enlargement being 11–21 years). Related to bone age, the mean onset of puberty in males was normal (m ± SD: 11 ± 1 years). In the females with Laron syndrome, the onset of puberty was less delayed (Table 10.2) (Pertzelan et al. 1993, Laron et al. 1980b).

The appearance of breast buds was registered between ages 11 and 14.5. Menarche occurred between ages 13 and 17 years. On Figs. 10.5 and 10.6, it is further seen that in patients started on IGF-I treatment in the prepubertal period (marked asterisk), the sexual signs seem to occur earlier than in the untreated boys and girls.

10.2.2.3 Duration of Puberty

In the male Laron syndrome patients, the duration of puberty was prolonged (6.2 ± 0.6 years). In the female Laron syndrome patients, the duration of puberty was close to normal (Table 10.2).

10.2.2.4 Pubertal Growth

The age at peak height velocity (PHV) occurred in the Laron syndrome males 4 years later (16.5 ± 2.6 years) than in the females (12 ± 1.2 years) (Table 10.2). Growth at PHV was low in the boys and girls with Laron syndrome (5.9 ± 1.1 and 5.6 ± 1.3 cm/year, respectively; i.e., 60% of normal. It is noteworthy that Laron syndrome patients have a slurred growth spurt (Laron et al. 1980b) (Figs. 10.7 and 10.8). The lack of growth spurt in the girls contributes to their much reduced final height. The delay in sexual development in Laron syndrome males is illustrated in Fig. 10.9a–d and that in females in Fig. 10.10a–c.

10.2.2.5 Voice

The usual change in voice during puberty in males is more protracted in Laron syndrome boys. Many Laron syndrome girls too have a high pitched voice which is not due to lack of androgen secretion, but to a narrow oropharynx (see Chap. 20) and often remains so into adult age.

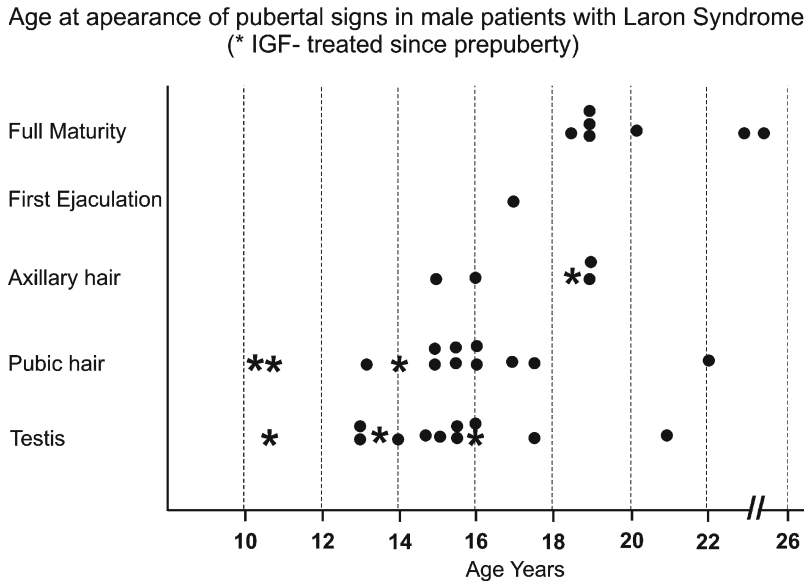


Fig. 10.5 Sequence and age of appearance of pubertal signs in boys with Laron syndrome, *asterisk* IGF-I treatment started in prepuberty

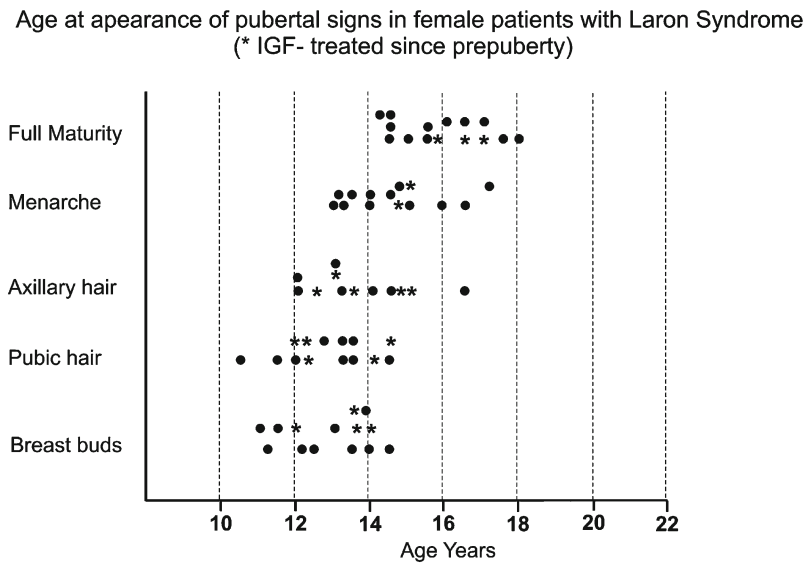


Fig. 10.6 Sequence and age of appearance of pubertal signs in girls with Laron syndrome, *asterisk* IGF-I treatment started in prepuberty

10.2.2.6 Total Pubertal Growth

TPG was reduced in the boys with Laron syndrome patients being 19.2 ± 3.4 cm compared to 28 cm in healthy boys. In the girls with Laron syndrome, the TPG was also markedly reduced, being 14.7 ± 2.7 vs. 25.5 cm in healthy girls. The reduced growth during puberty in Laron syndrome patients of both sexes increased their lag in the SDS at final height. Since the Laron syndrome boys started puberty at a later age, beyond the age of 15 years, they did not increase the height lag as much as the Laron syndrome girls (Pertzelan et al. 1993).

10.2.3 Adrenarche

The appearance of secondary sexual signs caused by adrenal androgens is also delayed in both sexes. Both girls and boys with Laron syndrome do not present with

Table 10.2 Growth during puberty in boys and girls with Laron syndrome

Group		Boys					Girls				
		Puberty (boys)					Puberty (girls)				
		Onset		Duration (years)	PHV		Onset		Duration (years)	PHV	
		CA (years)	BA (years)		CA (years)	BA (years)	CA (years)	BA (years)		CA (years)	BA (years)
Laron syndrome	Mean±SD	15.6 2.6	11.2 1.0	6.2 0.6	16.5 2.6	12.0 2.6	11.8 0.6	10.7 0.7	4.6 0.5	12/5 1.1	11.1 0.7
Normal Controls	Mean±SD	12.0 2.0	–	4.5	14.0 0.1	–	10.5 1.5	–	4.5	12.1 0.1	–

CA chronological age; *BA* bone age; *PHV* peak height velocity

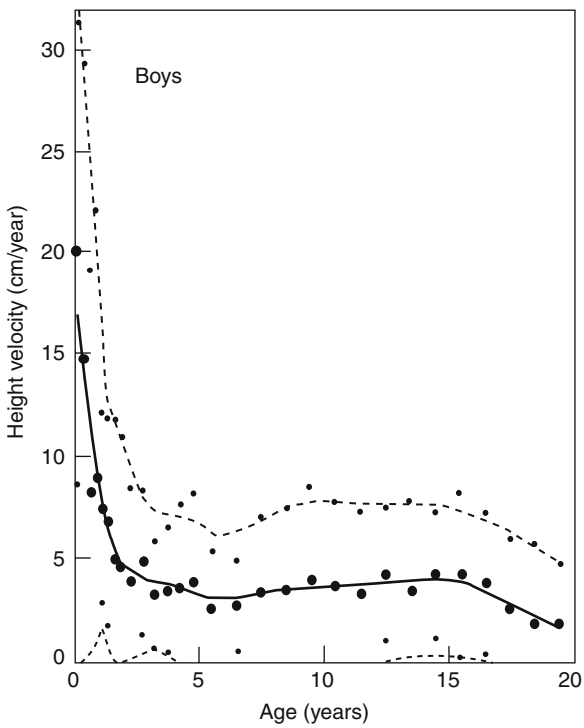


Fig. 10.7 Absence of normal growth spurt in boys with Laron syndrome. Reproduced with permission from Laron (2006)

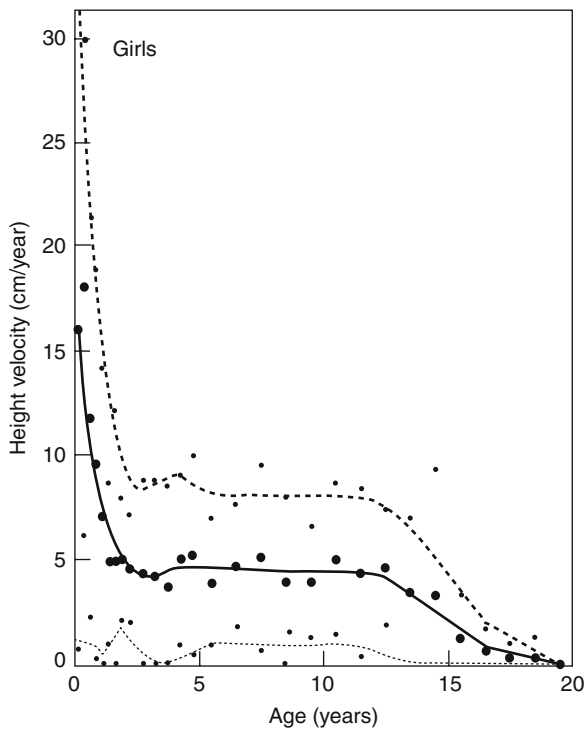


Fig. 10.8 Absence of normal growth spurt in girls with Laron syndrome. Reproduced with permission from Laron (2006)

microcomedones (preacne) or acne during puberty. This fact is considered as an evidence that the congenital IGF-I deficiency reduces the secretion of adrenal androgens, and this combined deficiency prevents the appearance of these skin lesions (Ben-Amitai and Laron 2009).

Proof for this is the appearance of severe acne as an adverse effect due to overdosage of IGF-I accompanied by elevated serum adrenal androgens (Klinger et al. 1998; Laron and Klinger 1998; Laron et al. 1998) (see Chap. 47).

10.2.3.1 Adult Age

Patients with Laron syndrome of both sexes reach full sexual development, the males (Fig. 10.11a–d) later than the females (Fig. 10.12a–e).

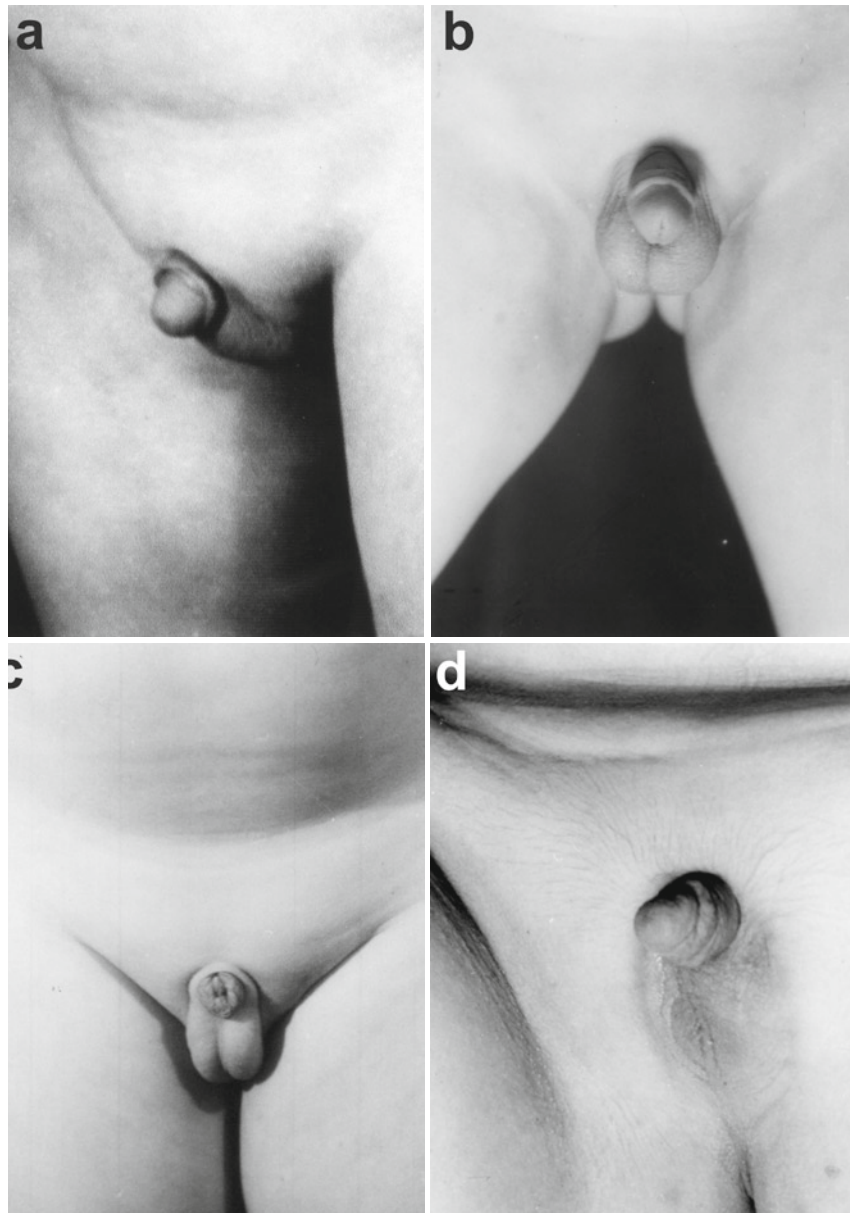


Fig. 10.9 (**a–d**) Delay in sexual development in four boys with Laron syndrome aged 13, 14, $14^{1/2}$, $15^{1/2}$ years

10.2.3.2 Males

Testes: The testicular volume in 11 fully mature adult Laron syndrome patients is illustrated in Fig. 10.13. It is seen that with one exception (patient BR), all volumes are −1 SD below the normal mean, ranging between 4 and 13 mL. One testicle (of patient ML) was dysgenetic after two orchidopexies at ages 6 and 10. The variations in the individual growth of the testes are illustrated in four Laron syndrome males (Figs. 10.14–10.17).

Penis: The penis length in ten Laron syndrome patients is illustrated in Fig. 10.18. While the penile circumference was within normal limits in five out of nine Laron syndrome patients (not shown), only 3/10 patients had a gland of normal length.

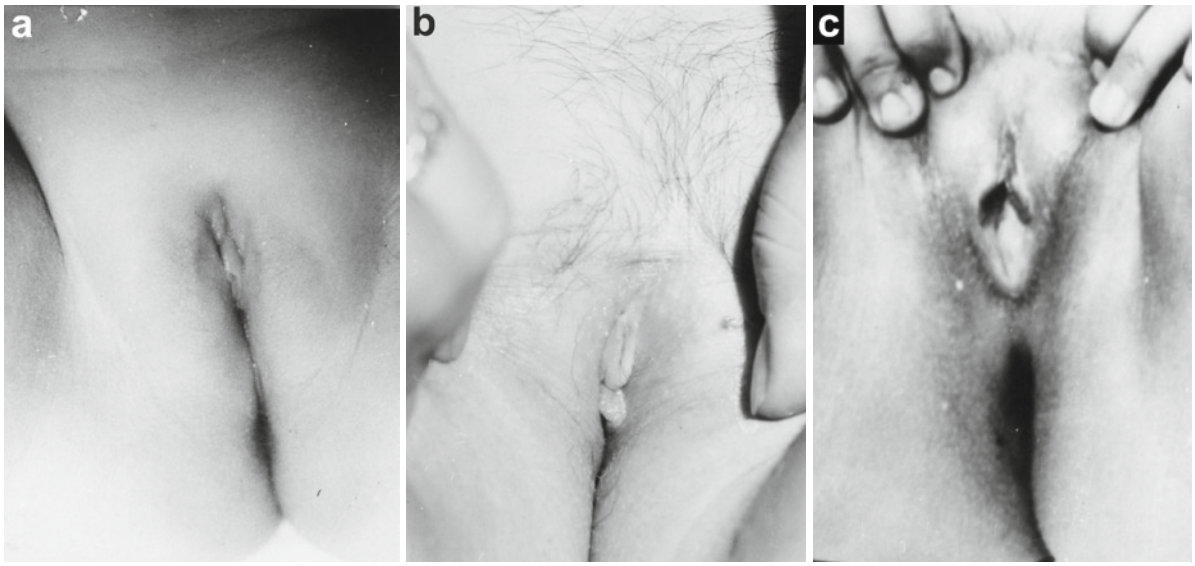


Fig. 10.10 Delay in sexual development in three girls with Laron syndrome aged 9 (**a**), 13 (**b**), and 15 (**c**) years

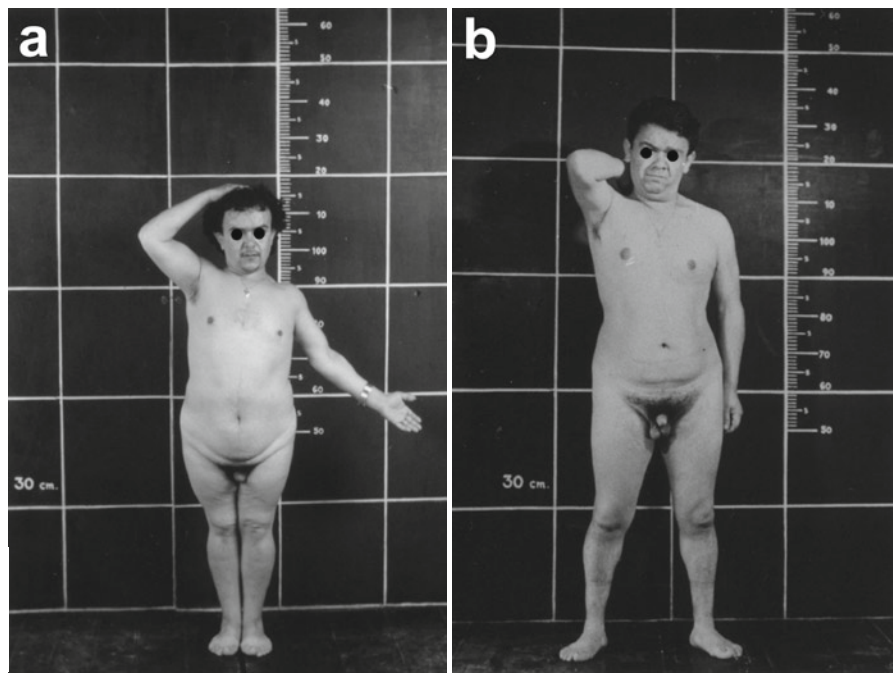
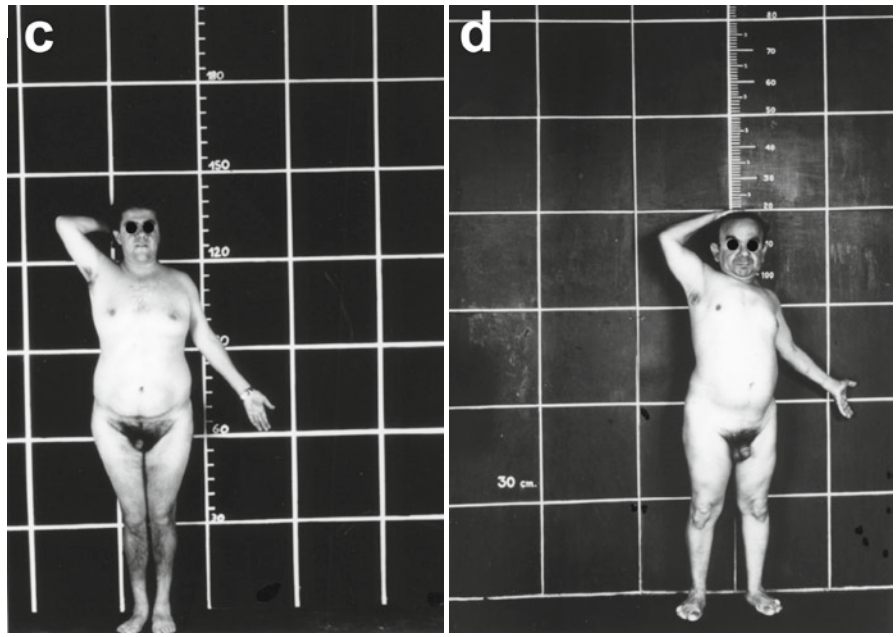


Fig. 10.11 Full sexual development in males with Laron syndrome (**a**) age 26; (**b**) age 28; (**c**) age 28; (**d**) age 42

Fig. 10.11 (continued)



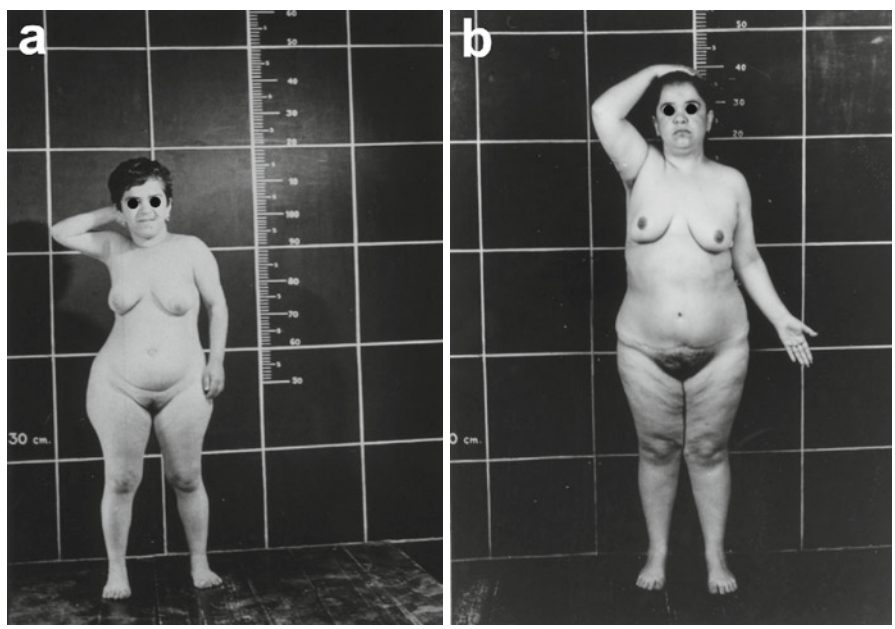


Fig. 10.12 Full sexual development in females with Laron syndrome (**a**) age 26; (**b**) age 30; (**c**) age 33; (**d**) age 43; (**e**) the genitalia of a 36-year-old Laron syndrome female

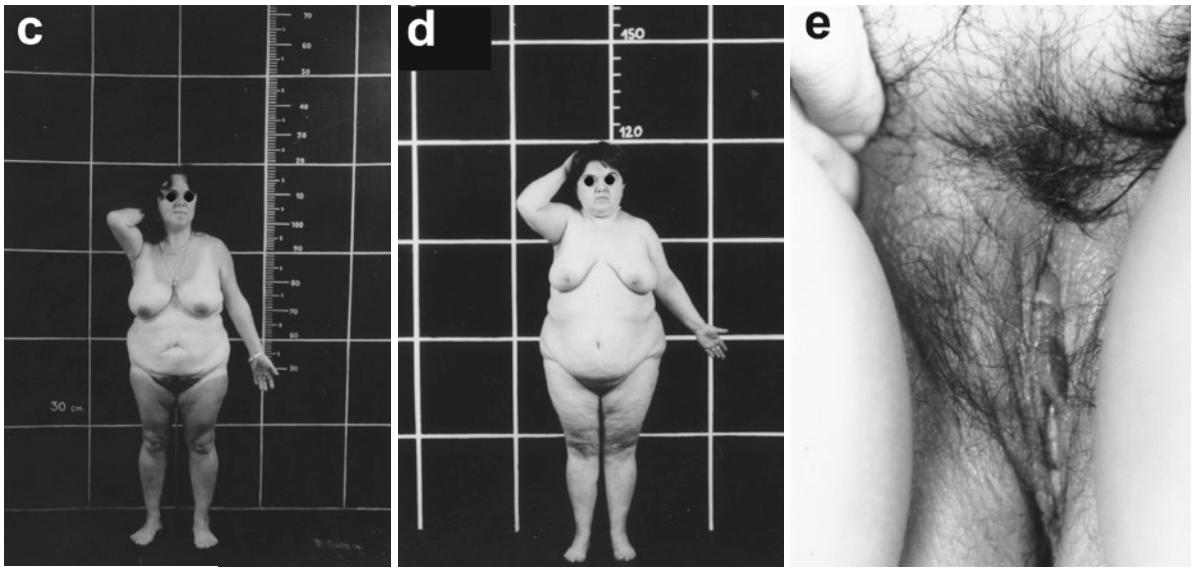


Fig. 10.12 (continued)

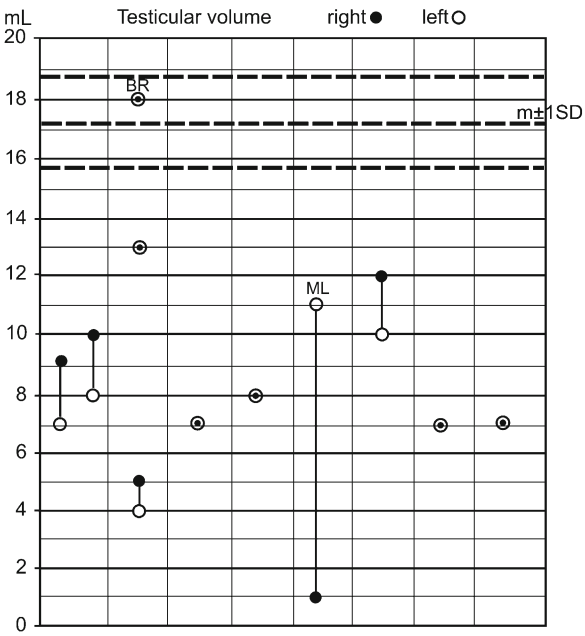


Fig. 10.13 Testicular volume in 11 fully mature adults with Laron syndrome (patient ML) has one dysgenetic testicle post-torchidopexy. Norms from Zilka and Laron (1969a, b)

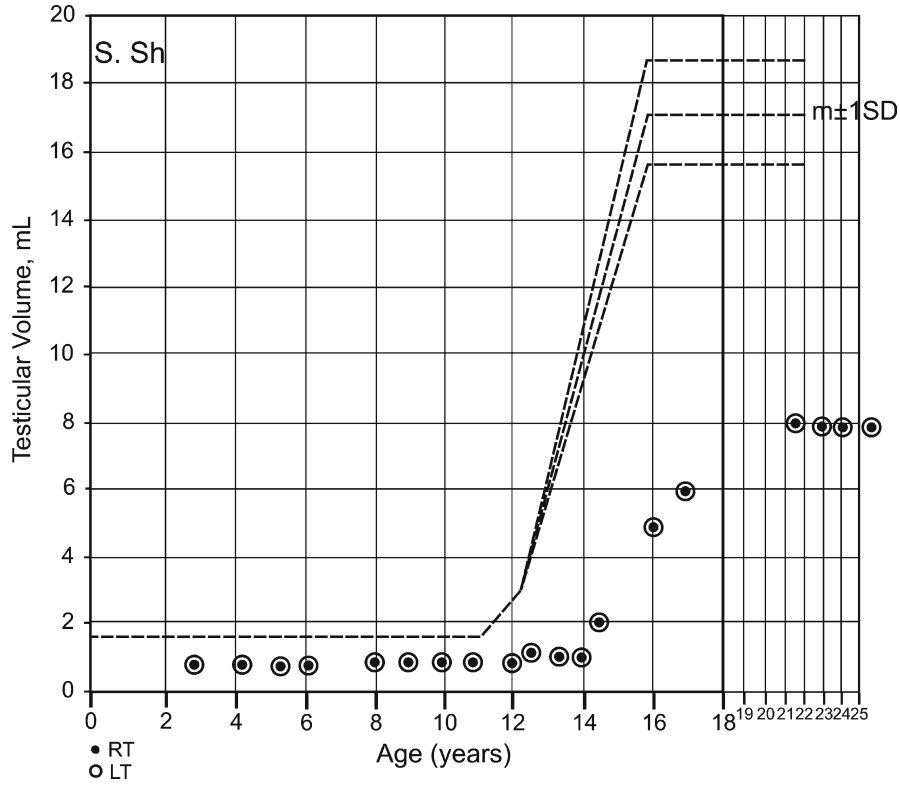


Fig. 10.14 Testicular growth in a male with Laron syndrome

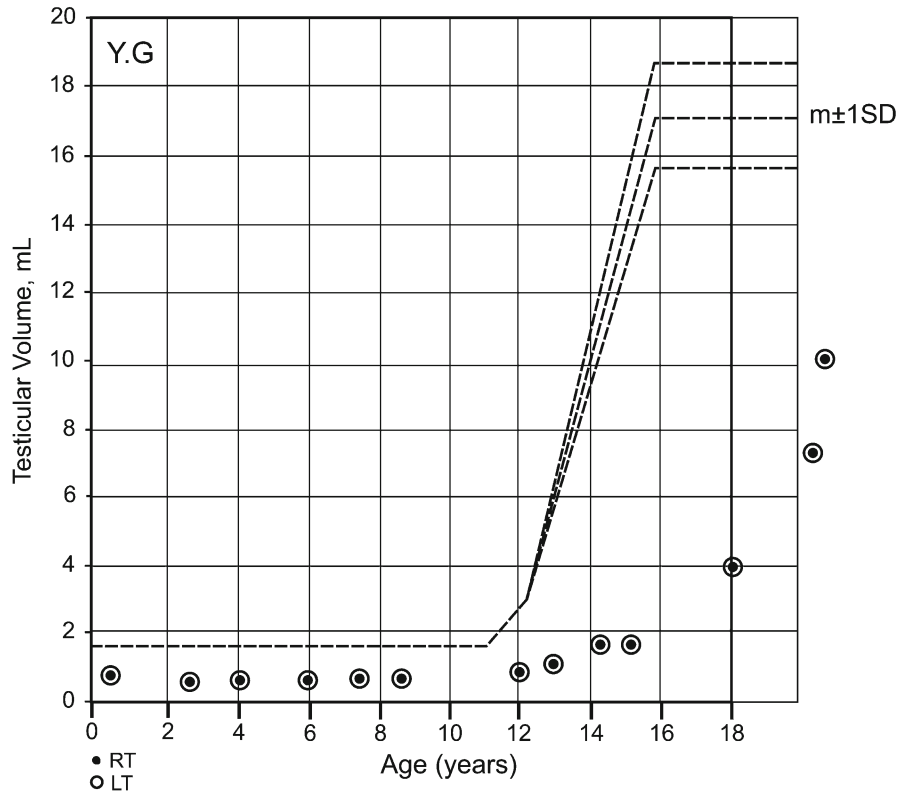


Fig. 10.15 Testicular growth in a male with Laron syndrome

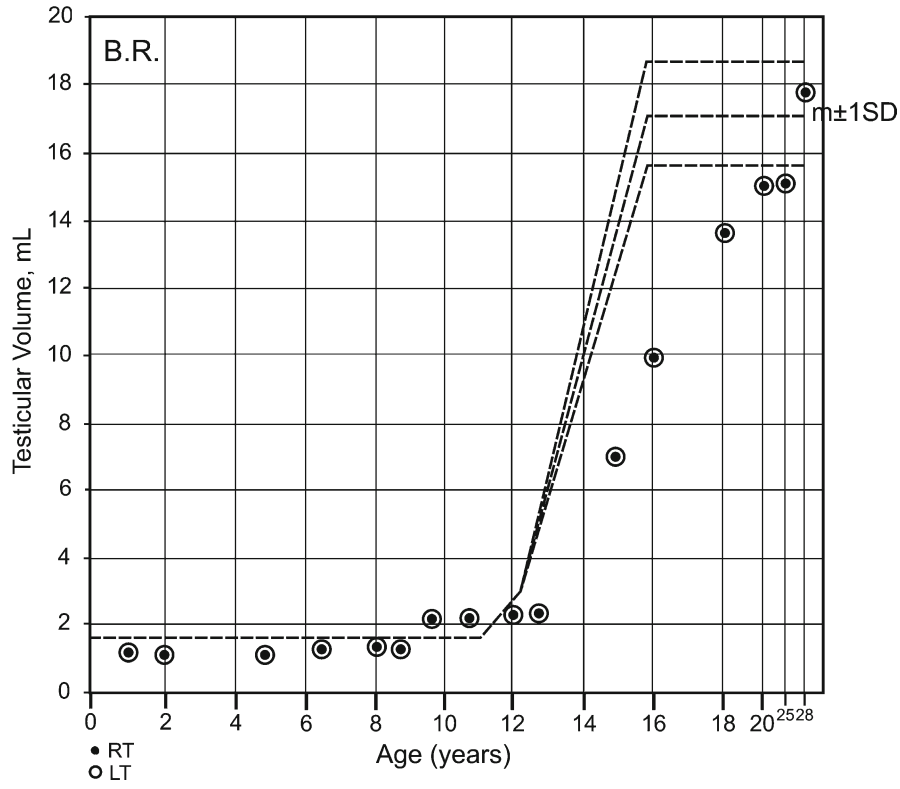


Fig. 10.16 Testicular growth in a male with Laron syndrome

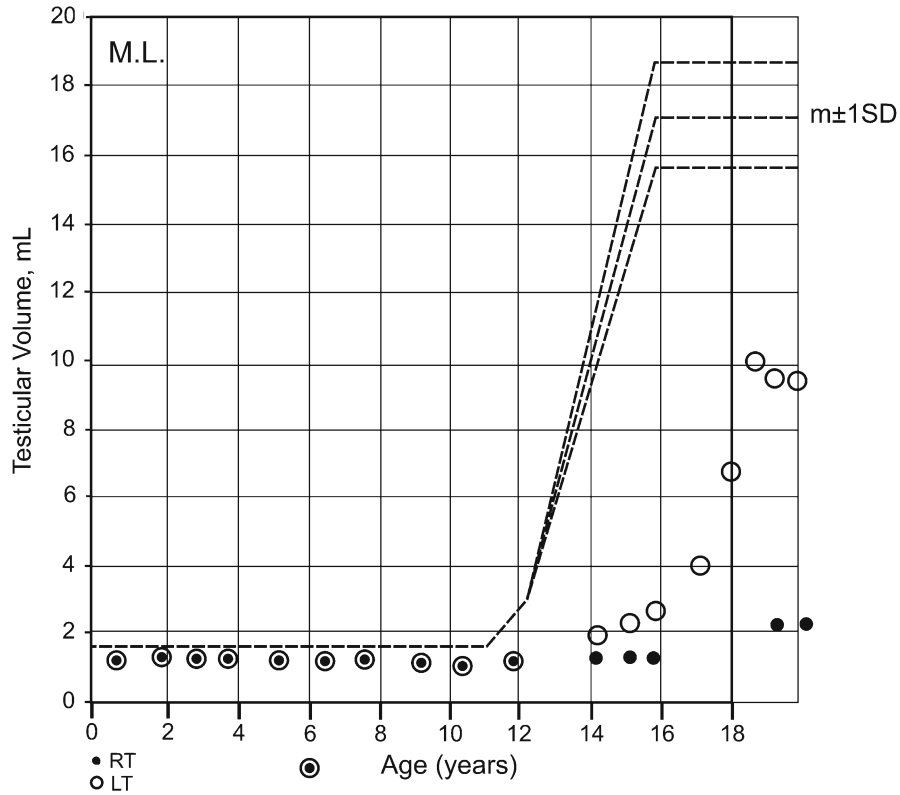


Fig. 10.17 Testicular growth in a male with Laron syndrome The right testis underwent orchidopexy at the age of 6 years because of the undescended testicle

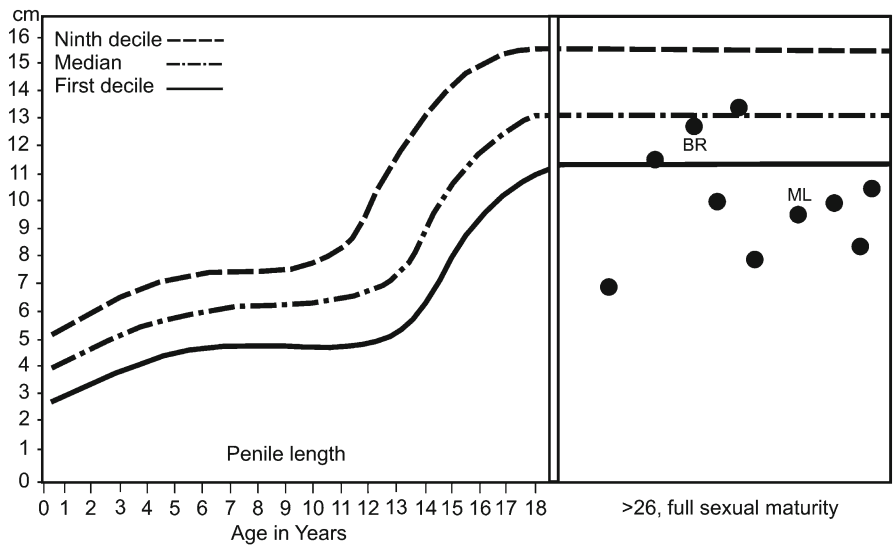


Fig. 10.18 Penile length of ten adult Laron syndrome patients with full sexual maturity. Norms from Schonfeld and Beebe (1942)

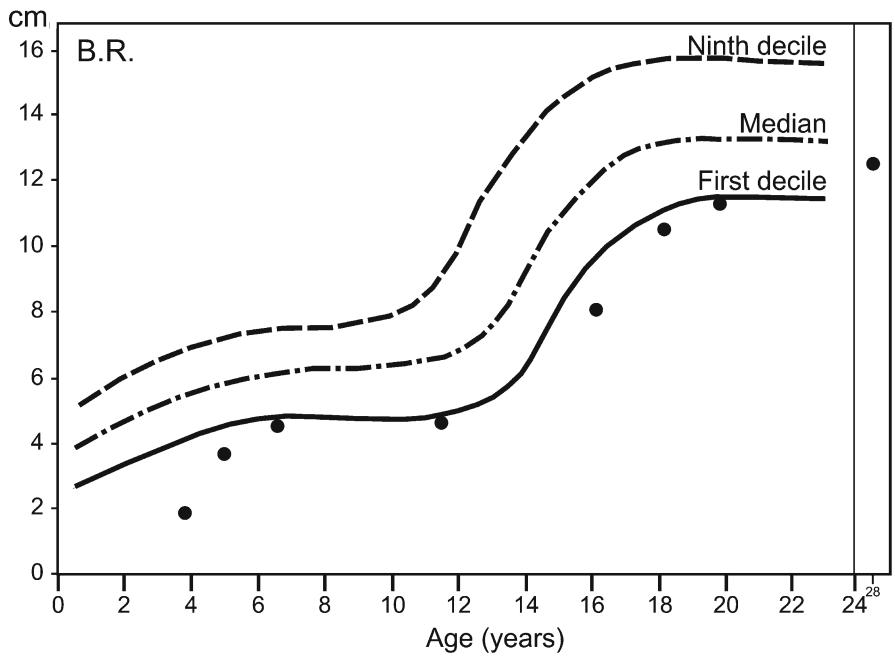


Fig. 10.19 Penile growth in length from early childhood to adult age in a male patient with Laron syndrome

Figure 10.19 illustrates the penis growth from childhood into adult age in a Laron syndrome patient who reached normal testicular size.

Conclusion

Both the penis and testes are small in the majority of Laron syndrome patients despite normal blood androgens and reproductive potential.

10.2.3.3 Females

All female patients with Laron syndrome reach full sexual maturity (Fig. 10.12). The breasts are of normal size, in some even large compared to body size (Fig. 10.20a–d) (Laron 2001, 2006). We hypothesize that this is caused by an increased prolactin secretion (Chap. 28) caused by a drift phenomenon from the somatomammotrophic cells with a high pituitary GH secretion. The menstrual cycle is regular in some, irregular in others.

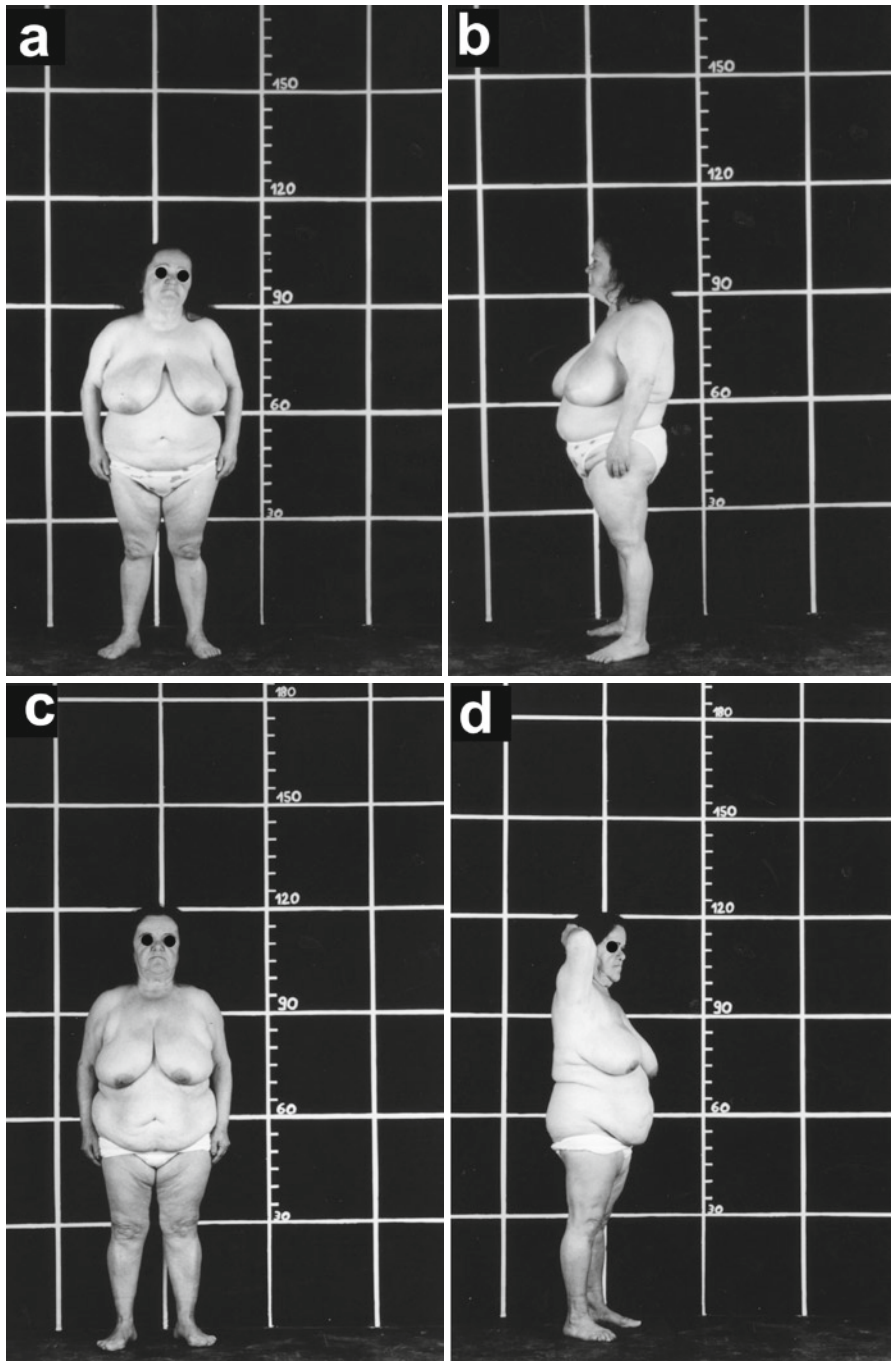


Fig. 10.20 (**a–d**) Illustrative large breasts in two unmarried female patients with Laron syndrome aged 50 and 51 years. Reproduced with permission from Laron (2006)

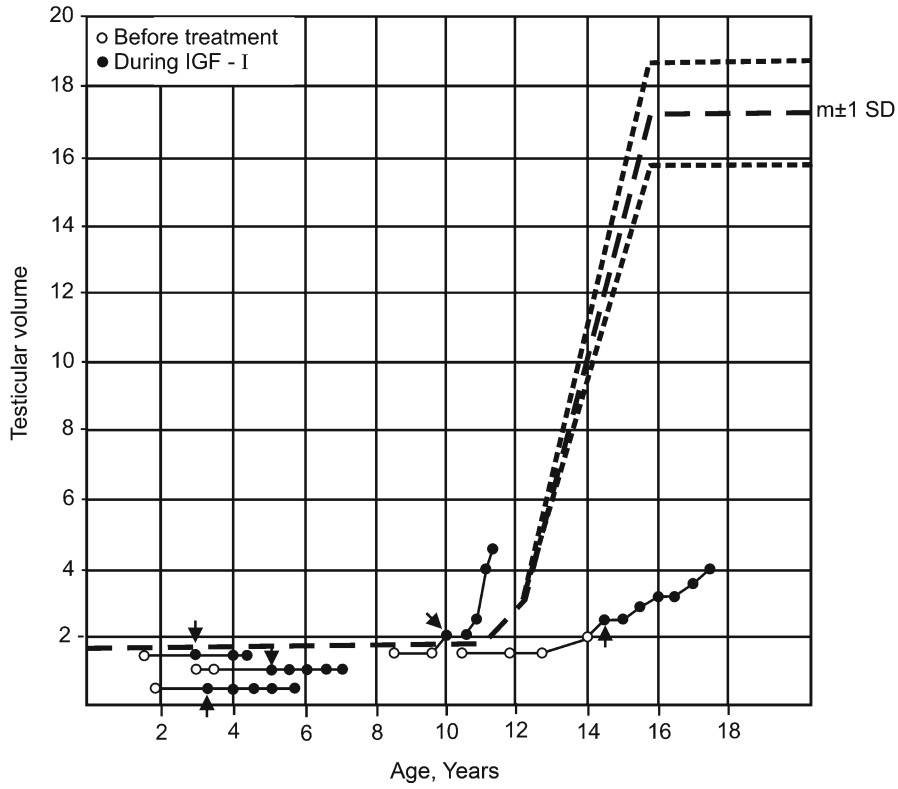


Fig. 10.21 Testicular size and growth before (*open circle*) and after the initiation (*filled circle*) of IGF-I treatment (*arrow*) in six male patients with Laron syndrome

10.3 Effect of IGF-I Treatment on Sexual Development

10.3.1 Males

Initiation of IGF-I treatment at an early age had no influence on testicular size in the prepubertal period (Fig. 10.21). Around the age of 10 years or later, a growth spurt was registered probably related to the arousal of gonadotrophins and rise in androgens. A similar effect was observed with the penile length measurements (Fig. 10.22).

10.3.2 Females

Due to the progressive obesity of the patients starting in infancy, it was difficult to differentiate between pseudogynecomastia and early development of the breasts. The impression is that early initiation of IGF-I treatment enhances the pubertal processes.

At present, IGF-I treatment is stopped when the patients reach final height.

10.4 Sexual Relations and Reproduction

Because of the longstanding relations between the patients and the treating team, we are confident that the information received is reliable. However, not all adult patients confided in us, partially due to their religious and/or oriental origin.

Seven adult female patients and 12 of the male patients reported gratifying sexual relations. Five females and five males are married and have a total of 20 children. One female has a permanent partner (YR), another (TAK.) has less stable partners. Detailed information is shown in Table 10.3.

It is seen that the females started their sexual life late; the information in three of the married males and seven additional males who claim to have sexual relations was inexact.

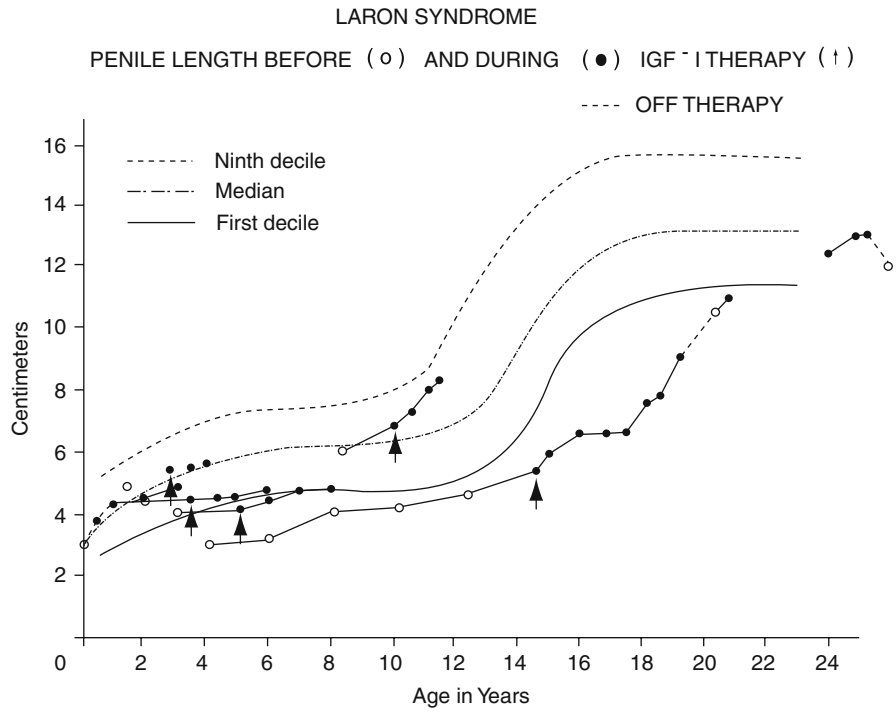


Fig. 10.22 Penile length before (*open circle*) and after the initiation of IGF-I treatment (*filled circle,arrow*) in six male patients with Laron syndrome

We have the impression that some of the very short males (AN and ES) did not have full sexual relations, whereas SSh, height 118 cm, claims to have successful relations. We are certain that the majority of female Laron syndrome patients have sexual relations in their late twenties and early thirties, whereas patients with low IQ or handicapped patients such as LJ and LM or very short females (BS 108 cm) never had.

10.4.1 Gestation

All except one of the pregnancies were spontaneous, without medication assistance and their gestations were uneventful (Table 10.3). One pregnancy (ShR) was IVF induced at the age of 40 years due to three previous spontaneous abortions. It is noteworthy that despite very low IGF-I levels in her serum and follicular fluid (less than 20 ng/mL), the patient developed

Table 10.3 Sexual activity, marital status, and reproduction of adult patients with Laron syndrome

	Patient	Age[a] (years:months)	Height (cm)	Sexual activity since age (years)	Married at age (years:months)	Children[b]	Abortions
Females	MP	46:11	137	25	33:10	2	3
	JM	42:4	132.7	27	37:8	3	–
	TAK	31:7	136	23	–	–	–
	YR	53:5	112.5	35	–	–	1
	SR	52:2	129.5	33	36	3	–
	ShR	59:2	114.5	30	35:10	1	–
	CS	57:4	123.5	24	24	2	–
Males	BR	45:10	138	27	40	1	
	DaM	45:9	135.8	+?	35	2	
	MeR	40:10	140.5	+?	+?	3	
	MeS	Died at 75	142.2	+?	28	3	
	YG	Died at 49:6	116	+?	47:2	–	
				+7 patients[c]			

[a]Age on January 2010
[b]All were delivered by cesarean section at term
[c] Exact details not known
? age not known

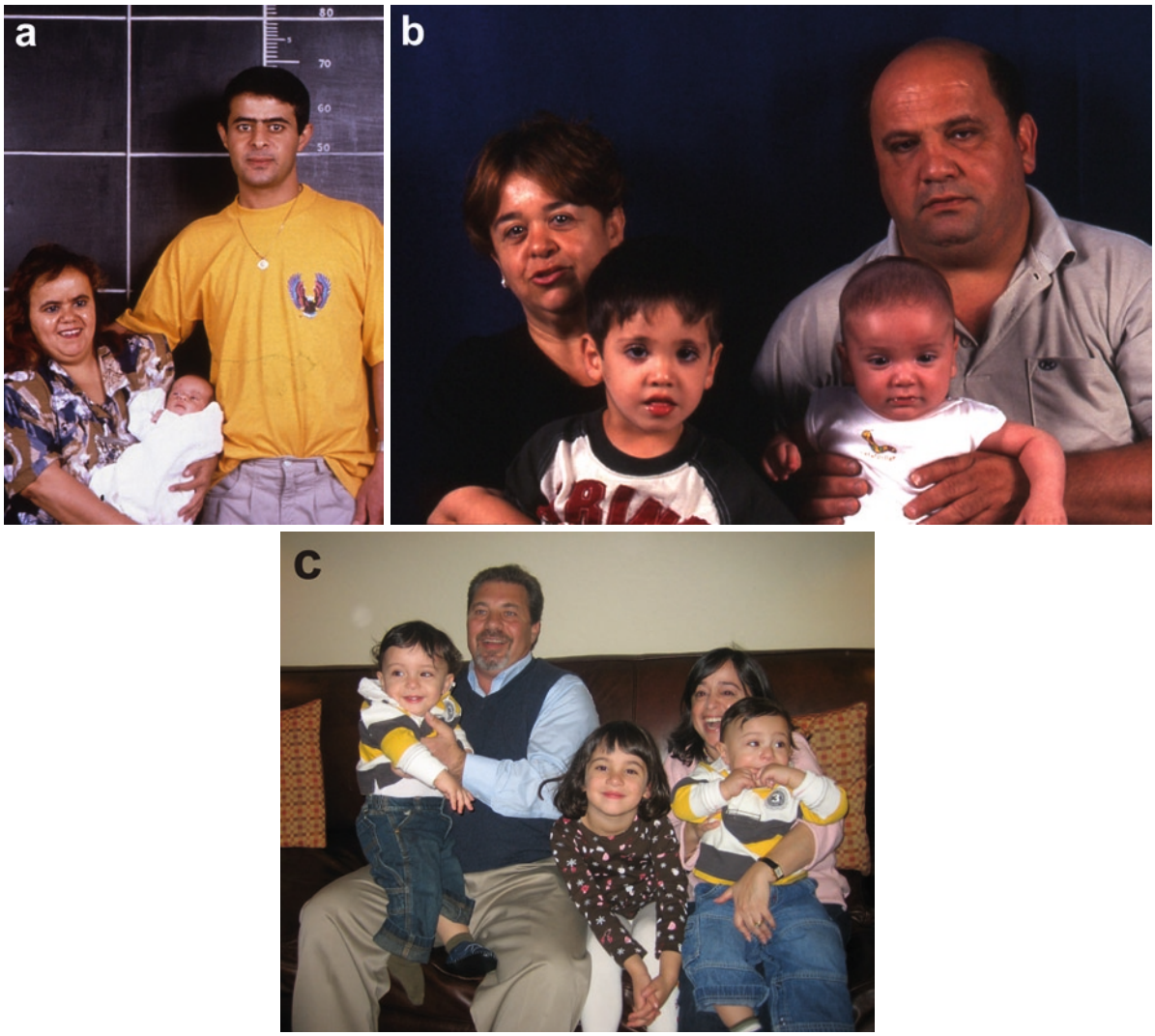


Fig. 10.23 (**a–c**) Three of the happy mothers with Laron syndrome with their husbands and children, ((**a**) SR; (**b**) MP; (**c**) JM)

normal ovarian follicles (Dor et al. 1992). All deliveries were at term by elective cesarean section, due to small pelvis. Lactation was also normal. Two of the married female patients reported natural abortions.

Figure 10.23a–c show three mothers with Laron syndrome (SR, MP, JM) with their husbands and children.

10.4.2 Children

As all patients married nonrelated and non Laron syndrome-affected partners, some even of normal stature, the height of all the children is within normal centiles, even in the five tested and found to be heterozygotes for their mothers' GH-R mutation.

10.4.3 Menopause

Reliable data could be obtained from six untreated adult females with Laron syndrome. In all, the monthly periods had stopped between ages $48^{6/12}$ to 51 years.

10.4.4 Conclusions

Despite slow development of the sexual organs and delayed puberty and marked obesity, all patients with Laron syndrome reach full sexual development, and even if not treated, the reproductive potential is intact. This shows that IGF-I has only a modulatory role on

the sexual reproduction axis. Whether the abortions in the two females were related to IGF-I deficiency is uncertain.

It can be further inferred that despite the knowledge that IGF-I receptors have been found on granulose cells (Adashi et al. 1988) and that IGF-I is of importance in early folliculogenesis (Monget and Bondy 2000), congenital IGF-I deficiency may not be essential for ovarian follicular or sperm development.

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11 Sex Hormone Binding Proteins and Sex Hormones in Untreated and IGF-I Treated Patients with Laron Syndrome

Zvi Laron and Rivka Kauli

Core Message

› The serum levels of sex hormone binding proteins in untreated and IGF-I treated patients are shown and their meaning discussed.

11.1 Sex Hormone Binding Proteins

Zvi Laron

11.1.1 Introduction

Sex hormone binding globulin (SHBG) is a glycoprotein produced by the liver and secreted into the circulation (Rosner 1990). It participates in the regulation of estrogen and testosterone by affecting free hormone availability (Damassa et al. 1991) and uptake by the target organs (Nakhla et al. 1990). However, there is still no clear-cut information concerning the main factors that control its synthesis, release, and blood clearance. High amounts of estrogens increase, while androgens decrease, the levels of SHBG, but their role at physiological levels of sex hormones was questioned (von Schoulz and Carlstrom 1989).

The role of IGF-I in regulating SHBG had not been investigated. An approach to answer this question was presented by the study of untreated and IGF-I treated patients with Laron syndrome (LS).

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11.1.2 Subjects and Methods

The following investigations were performed (Gafny et al. 1994)

1. Short-term IGF-I treatment of 15 LS patients: 8 prepubertal children, 5 males, and 3 females (mean age of 7±5 years) and 7 adult patients: 2 males (aged 27 and 35 years) and 5 females (mean age of 32±4 years). Each subject was administered IGF-I (FK-780, Fujisawa Pharmaceutical Co., Ltd., Japan), 120–150 μg/once daily for 7 days. Blood samples were drawn after an overnight fast prior to the first injection, and 24 h after 1, 3, and 7 s.c. injections. An additional sample was drawn 7 days after cessation of treatment (day 15).
2. Five-month IGF-I treatment of 9 Laron syndrome patients. The participants were 1 adult male (aged 28), 4 adult females (mean age 34±5 years), and 4 children, all prepubertal: 3 males aged 3, 5, and 15 and one 14-year-old female. IGF-I was administered s.c. once daily for 5 months, at the same dose levels as for the short-term treatment. Blood samples were drawn prior to the treatment and monthly thereafter.

Serum levels of SHBG were measured by IRMA Count, Diagnostic Products Corporation, Los Angeles, CA, USA. Sera were diluted so as to ensure that the amount of SHBG in the sample fell in the mid range of the standard curve (1–90 nmol/l). All blood samples were assayed on the same occasion, to avoid interassay variations. Concomitantly with the serum SHBG determinations serum, IGF-I and insulin were measured.

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11.1.3 Results

The normal ranges of serum levels of SHBG change with age (Table 11.1). Basal serum SHBG levels were within normal range in all Laron syndrome patients. IGF-I treatment increased significantly ($p<0.01$) the SHBG both during short-term (Fig. 11.1) and long-term IGF-I administration ($p<0.01$) (Fig. 11.2).

In the 7-day trial of IGF-I, it was seen that the rise of SHBG was progressive, the greatest change being evident in adult Laron syndrome female patients. Seven days after stopping IGF-I administration, serum SHBG returned to close to the pretreatment levels. During the 5-month IGF-I administration, the raised SHBG levels were maintained at about 150% of the pretreatment level.

Table 11.1 Normal ranges of serum SHBG in our laboratory

	Healthy subjects SHBG (nmol/L)
Prepubertal children	109–276
Adult females	16–120
Adult males	10–73

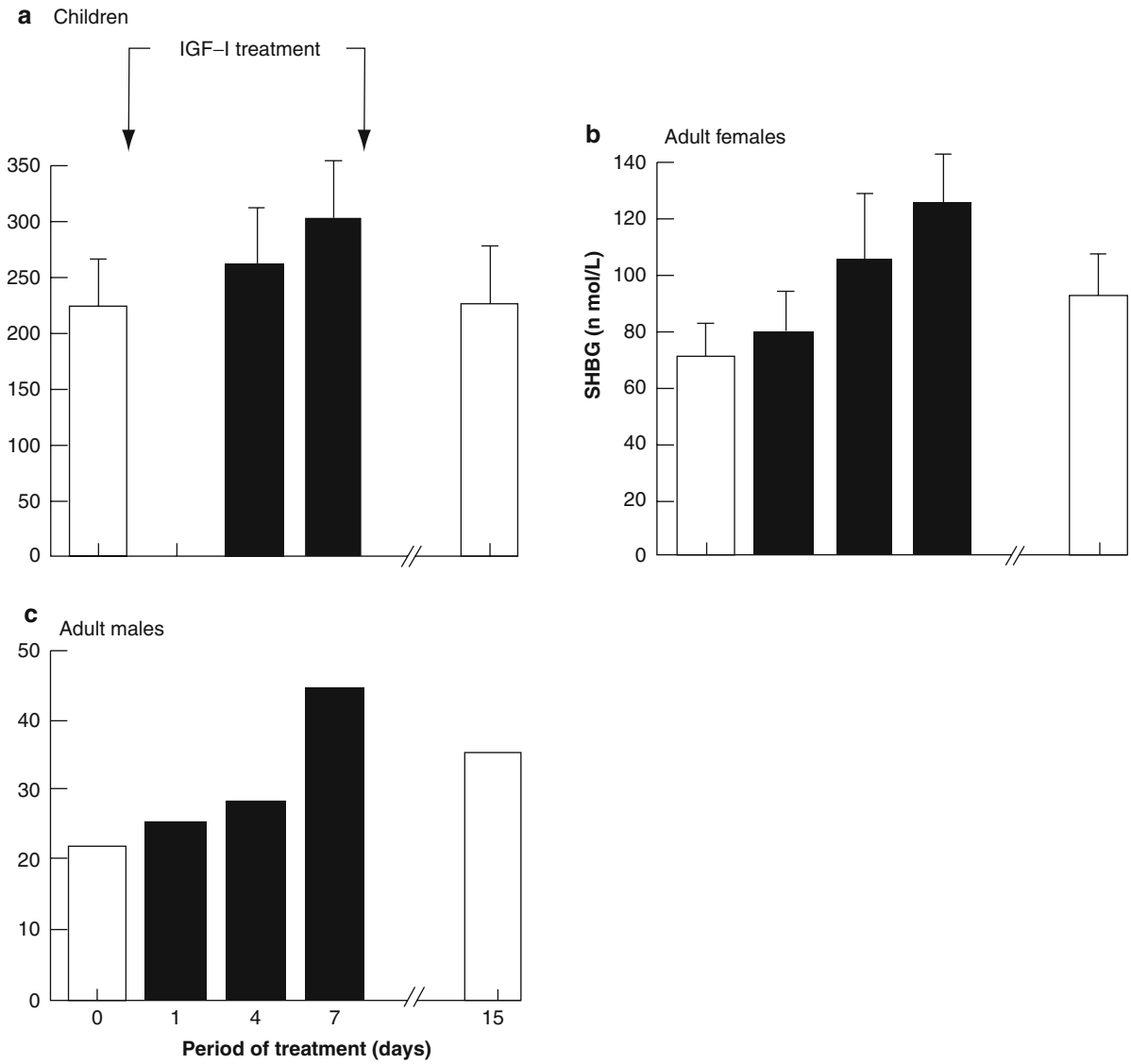

Fig 11.1 Effect of 7-day IGF-I treatment (120–150 μg/kg/day) on serum levels of SHBG in 15 Laron syndrome patients. The bars represent the levels of SHBG (mean±SD). Because of the large differences in the basal levels, separate panels were drawn for children ($n=8$), adult females ($n=5$), and adult males ($n=2$). *Open bar,* no treatment; *filled bar,* IGF-I treatment. Modified from Gafny et al. (1994)

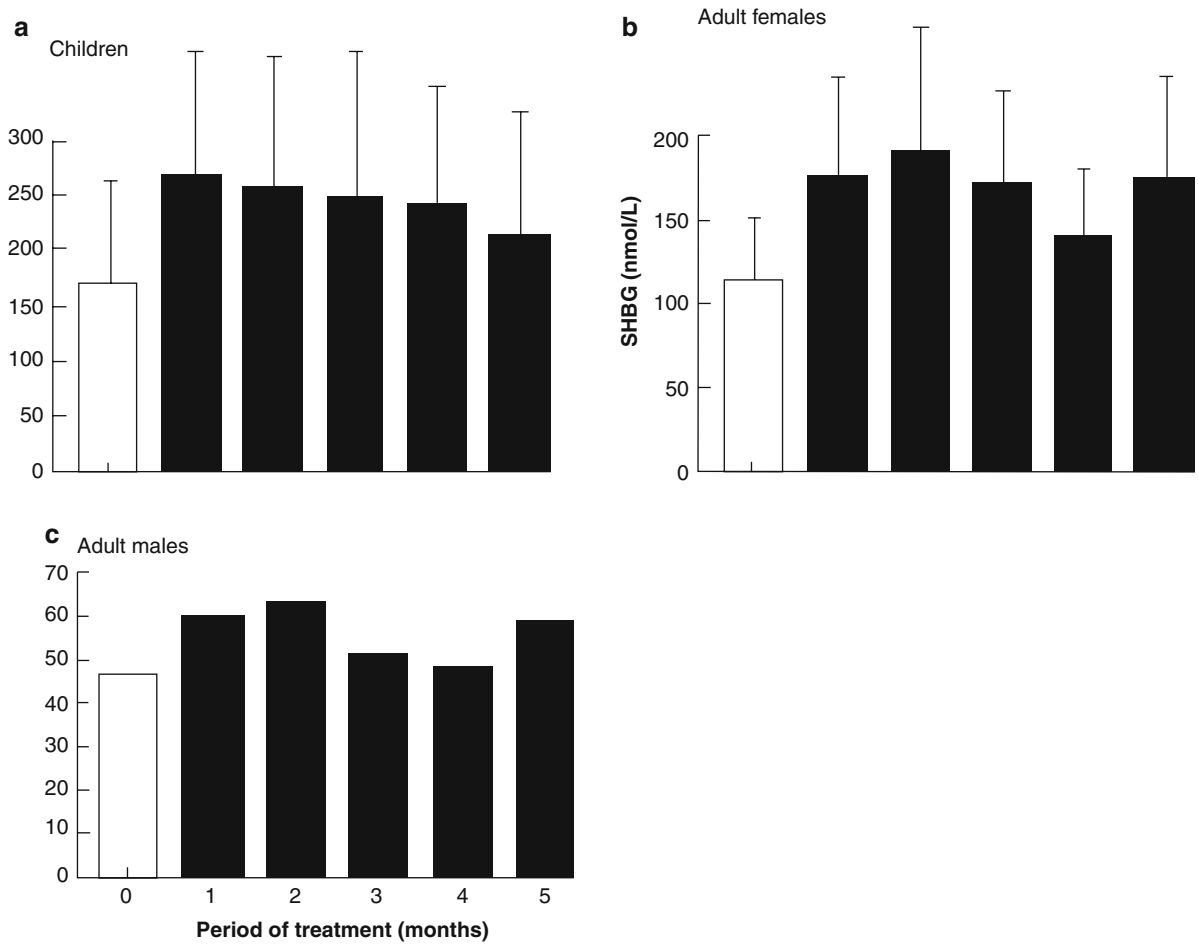

Fig 11.2 Effect of 5-month IGF-I treatment (120–150 μg/kg/day) on serum levels of SHBG in Laron syndrome patients Children ($n=4$), adult females ($n=4$), and adult males ($n=1$). *Open bar,* no treatment; *filled bar,* IGF-I treatment. Modified from Gafny et al. (1994)

Concomitantly with the IGF-I induced rise in SHBG, there was a significant decrease ($p<0.01$) in serum insulin levels presenting an opposite effect to that observed during the administration of hGH (human growth hormone) (Gafny et al. 1994) (not shown).

11.1.4 Conclusions

To what degree the SHBG levels affect sex hormone action and the mechanism of sexual development of the untreated or treated Laron syndrome patients requires further studies. It can be speculated that the suppression of IGBP-1 by IGF-I increases the amount of free IGF-I on one hand stimulating sex hormone secretion but on the other hand, the increase in serum SHGB reduces the availability of free sex hormones, their active state.

11.2 Sex Hormones in Untreated Patients with Laron Syndrome

Zvi Laron and Rivka Kauli

As the Laron syndrome patients have delayed puberty and reach full sexual maturation, sex hormone determinations were performed only occasionally in adult patients.

11.2.1 Results

The summary of available sex hormone determinations in untreated Laron syndrome patients is shown in Table 11.2. The serum testosterone levels are illustrated in Fig. 11.3. All the values were within the normal ranges once sexual maturation was complete.

Table 11.2 Serum sex hormone concentrations in untreated adult patients with Laron syndrome

	No. of patients	Age (years)	No. of Samples	Serum level		Reference values[a]	
				Median	Range	Median	Range
Females							
Estradiol (pg/mL)	12	22–46	47	68	25–203	120	9–281
DHEAS (ng/dL)	11	23–48[3]	38	135	34–380	170	35–430
Androstenedione (ng/dL)	11	23–48[3]	40	147	32–384	152	46–258
Males							
Testosterone (ng/dL)	8	20[5]–61	41	525.5	125–1250	630	262–1593
DHEAS (ng/dL)	5	20[5]–43	11	287	218–438.48	280	80–560
Androstenedione (ng/dL)	5	20[5]–43[7]	13	144.58	128–184	171	94–247

[a]Reference range according to our laboratory

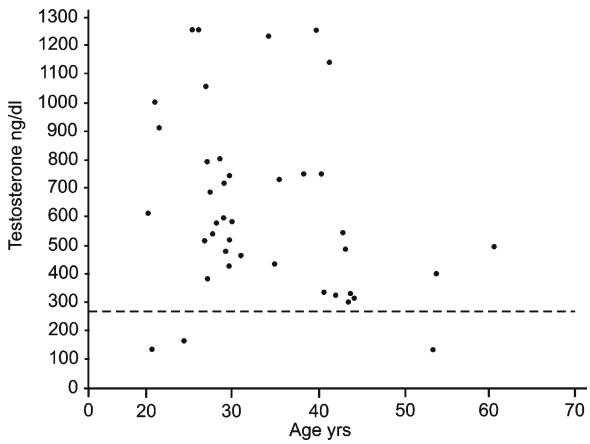

Fig. 11.3 Serum testosterone concentrations in untreated male patients with Laron syndrome aged 20 years or above. Interrupted line indicates lower limit of normal

In certain instances, gonadotropins and sex hormones were determined during IGF-I treatment, such as during the appearance of adverse effects of hyperandrogenism (see Chaps. 24 and 47); the data evidenced that IGF-I administration stimulated the secretion of gonadotropins and sex hormones in both males and females (Klinger et al. 1998; Laron et al. 1998; Laron and Klinger 1998).

11.2.2 Conclusions

The observation in our cohort of Laron syndrome patients adds further evidence to the interrelationship and interaction between IGF-I and the sex hormones.

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The Adipose Tissue in Patients with Laron Syndrome

12

Zvi Laron

Core Message

› The obesity of patients with Laron syndrome is evident already in early infancy and increases progressively as shown by illustrations, subcutaneous skinfold measurements and body composition studies.

12.1 The Obesity of Patients with Laron Syndrome

The second major clinical characteristic after dwarfism in patients with Laron syndrome is obesity, already evident at birth (Fig. 12.1) and infancy (Fig. 12.2) and progressing constantly with advancing age (Laron and Klinger 1993).

It involves the overall subcutaneous and visceral fat and is evidently the result of the congenital deficiency of GH and IGF-I.

Following are demonstrations of the progressing obesity in untreated Laron syndrome patients. Also note the typical facial features and growth retardation. Figures 12.2–12.10 illustrate the above in several untreated patients with Laron syndrome. The increasing degree of adiposity has severe consequences and is associated with abnormal metabolic changes to be reviewed in the following chapters.

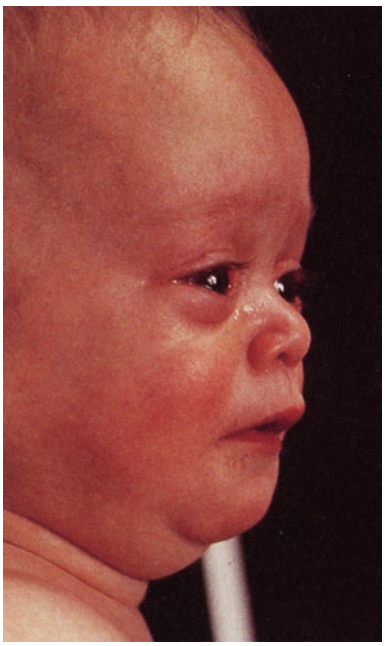

Fig. 12.1 Double chin in an infant boy with Laron syndrome

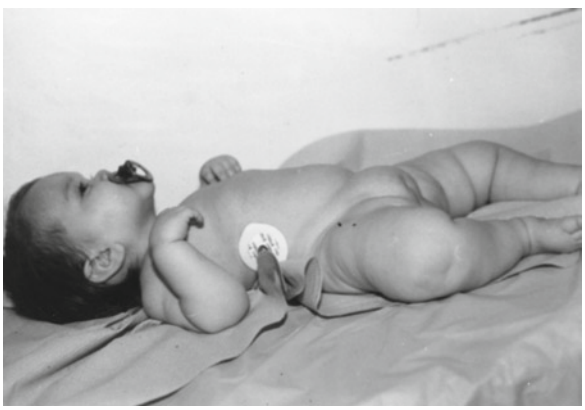

Fig. 12.2 Obesity in a one and half-year-old girl with Laron syndrome

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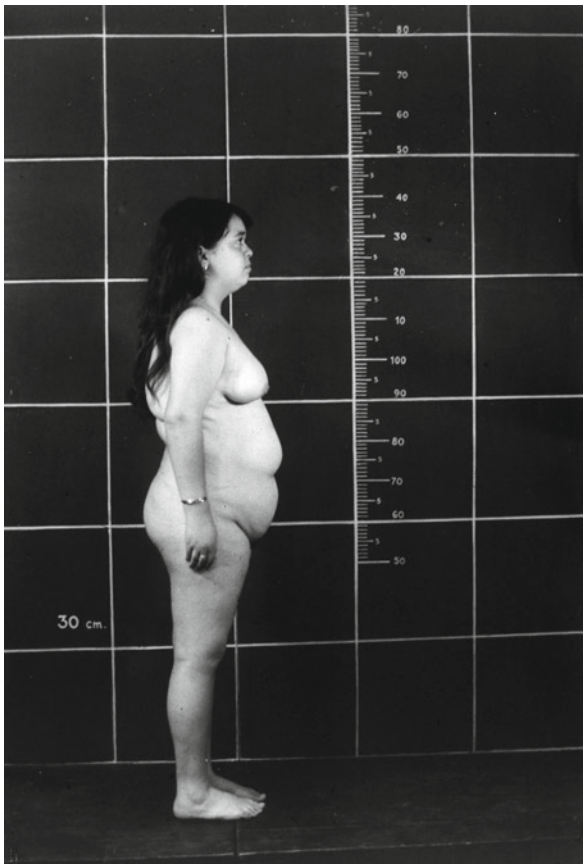

Fig. 12.3 Obesity in a 16-year-old untreated girl with Laron syndrome

To determine the degree of adiposity and to elucidate the pathology in the mechanism of the adipose tissue in the absence of GH and IGF-I, we performed a series of investigations.

12.1.1 Body Mass Index (BMI)

The BMI values calculated as body weight divided by height^2 for 8 male and 9 female Laron syndrome patients during long-term follow-up are shown in Figs. 12.11 and 12.12. It is evident that in the patients of both sexes, almost all values are below 30. This demonstrated that BMI does not truly reflect the degree of adiposity in the Laron syndrome patients. This is due to the slender bones (see Chap. 20) and underdeveloped muscular system (Brat et al. 1997) (Chap. 17) caused by the long-standing IGF-I deficiency. Not being a reliable index of obesity in this syndrome, underestimating the degree of adiposity, we have learned not to use it.

12.1.2 Skinfold Thickness

Usually, we measure the skinfolds as a measure of the subcutaneous fat tissue, using Harpenden calipers in three places: in the anterior iliac line, triceps, and subscapular regions. In the very obese Laron syndrome patients, either the iliac and triceps regions were too thick for the caliper and/or it was difficult to find the identical site on repeated measurements. Therefore, we found the only reliable site to be that of the subscapular skinfold thickness consisting of dermis + subcutaneous tissue. The measurements made in 18 untreated Laron syndrome patients followed since early childhood into adult age are shown in Fig. 12.13. It is seen that there is a steep increase starting from the age of 3 years, until puberty, followed by a plateau until around the age of 30 years, when an additional progressive rise takes place.

To get a more precise and scientific determination of the degree of obesity in the Laron syndrome patients, we performed body composition studies.

12.2 Body Composition of Patients with Laron Syndrome

12.2.1 Subjects

Eleven untreated adult Laron syndrome patients (7 females, mean age 43 ± 9 years, and 4 males, mean age 43 ± 10 years) agreed to be studied and the results were compared with healthy controls (Laron et al. 2006).

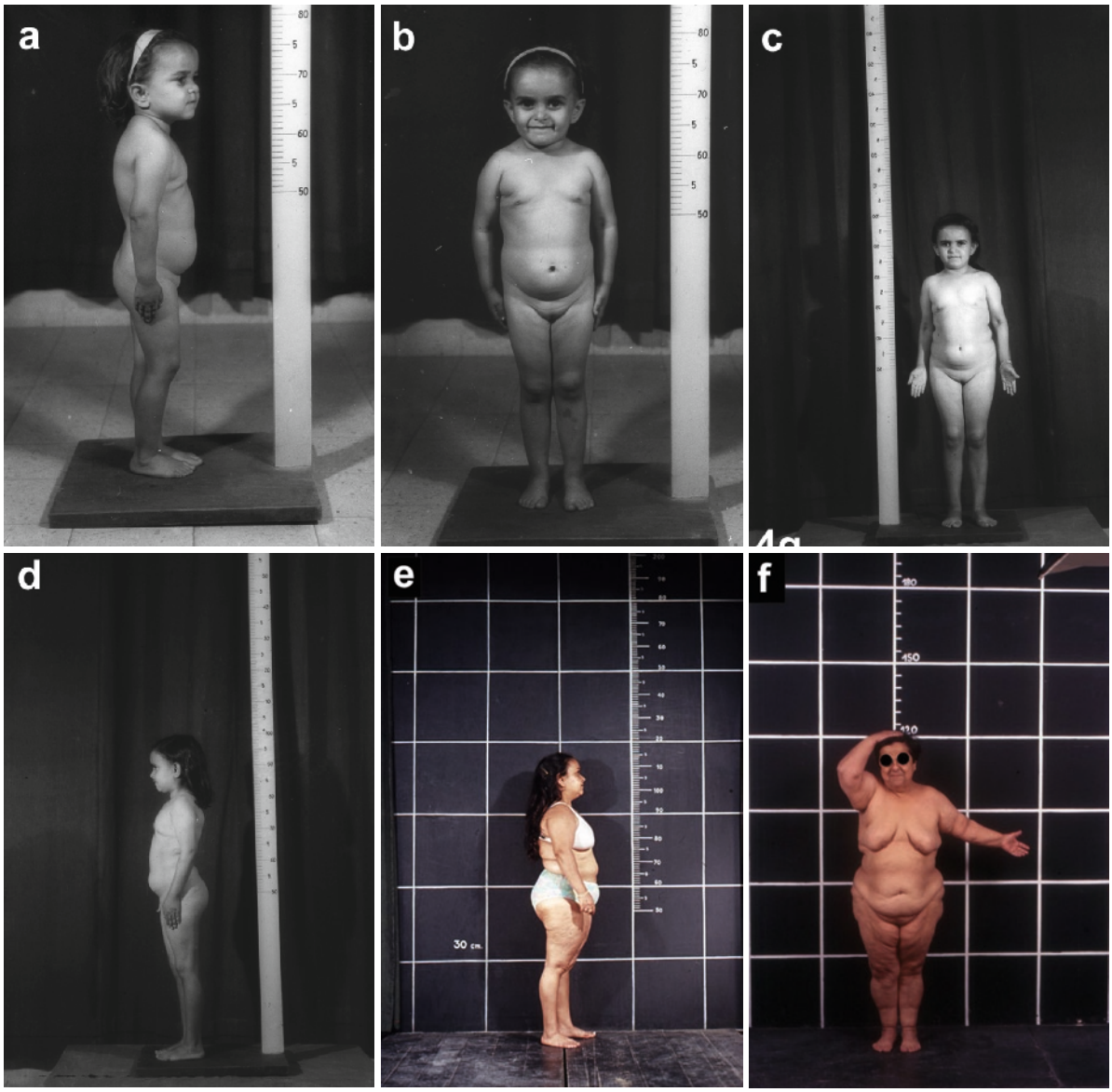

Fig. 12.4 Progressive obesity in an untreated female patient with Laron syndrome followed since early childhood illustrated as Lat and AP at ages 7 (**a**, **b**), 13 (**c**, **d**), 16 (**e**), 45 (**f**, **g**), 48 (**h**, **i**), and 51 (**j**, **k**)

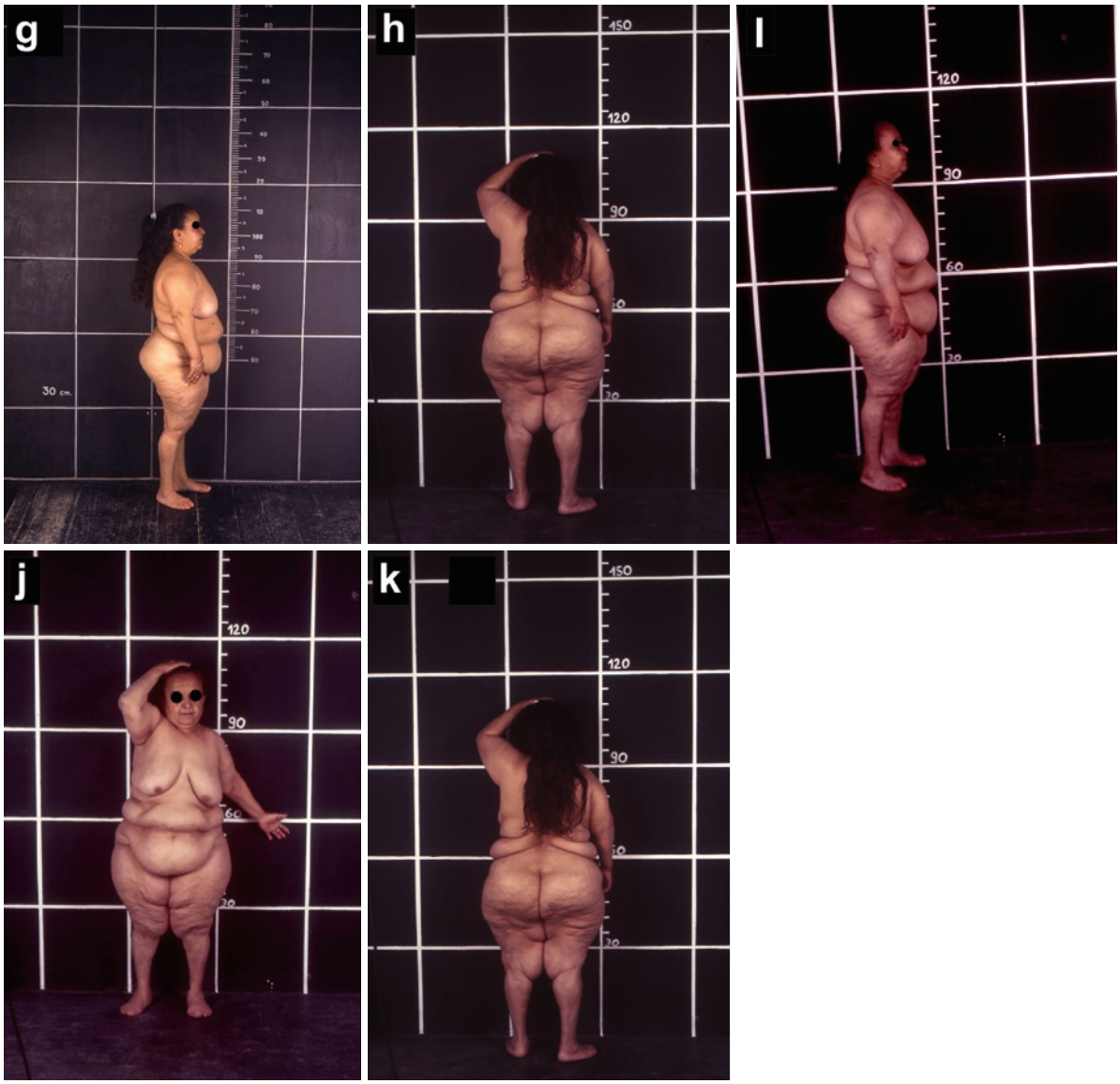

Fig. 12.4 (continued)

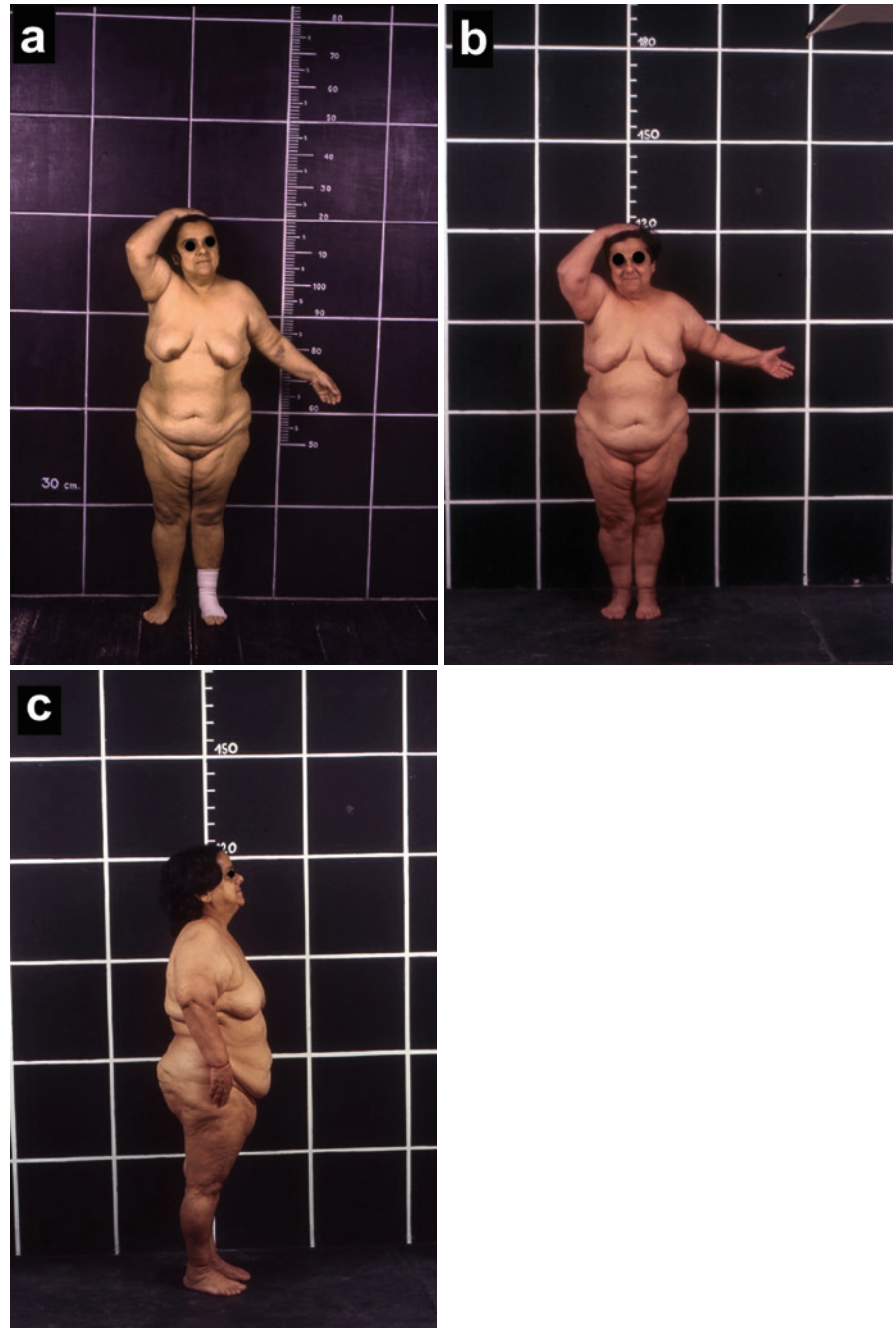

Fig. 12.5 (**a**–**c**) Frontal and lateral views of a female with Laron syndrome (CS) at ages 39 and 52.5 (AP and Lat)

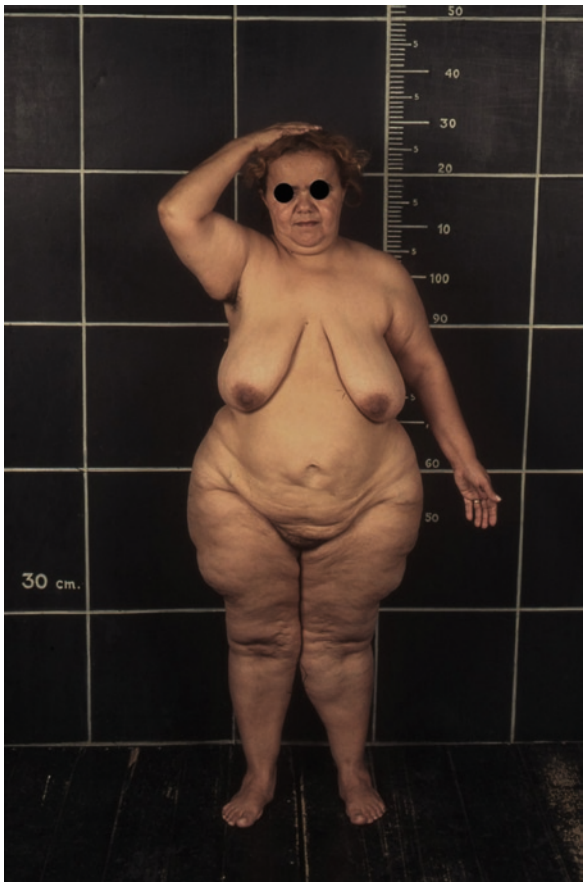

Fig. 12.6 Frontal view of an untreated female with Laron syndrome at the age of 39 years

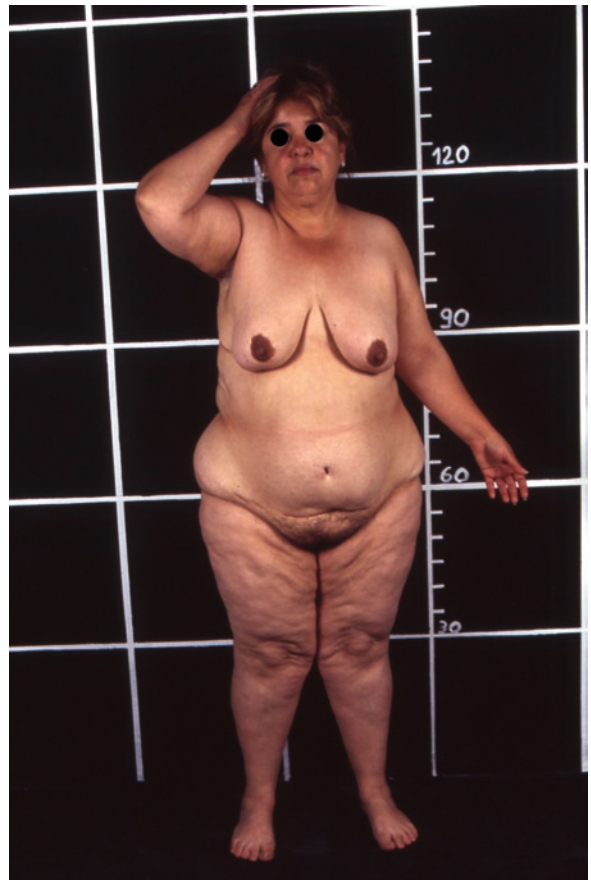

Fig. 12.7 Frontal view of an untreated female with Laron syndrome at the age of 43 years

12.2.2 Methods

Body composition of total body, trunk, and upper and lower extremities was determined using dual-energy X-ray absorptiometry (DEXA; Model DPX-IQ8565-A, Lunar Radiation Corp. Madison, Wise, USA). This method does not measure muscle mass. Body weight was determined by an electronic scale. Skinfolds were measured using a Harpenden caliper. In some of the patients, the subcutaneous skinfold thickness exceeded that possible to measure by the caliper.

Statistical analysis was performed by analysis of variance (ANOVA) separately for males and females or by the Student's t-test when indicated.

12.2.3 Results

The individual pertinent clinical data and percent body fat of the total body, trunk, arms, and legs are shown in Table 12.1, and the mean (±SD) values of 6 female and 7 male controls are shown in Table 12.2. It is seen that the male and female patients with Laron syndrome have a significantly higher amount of body fat than the healthy controls. The degree of obesity of the Laron syndrome patients is even more evident in the female patients, who have a body fat content around 60% of the total body composition compared to a mean of 29% in the healthy controls. The percentage body fat of the arms in the female patients is higher

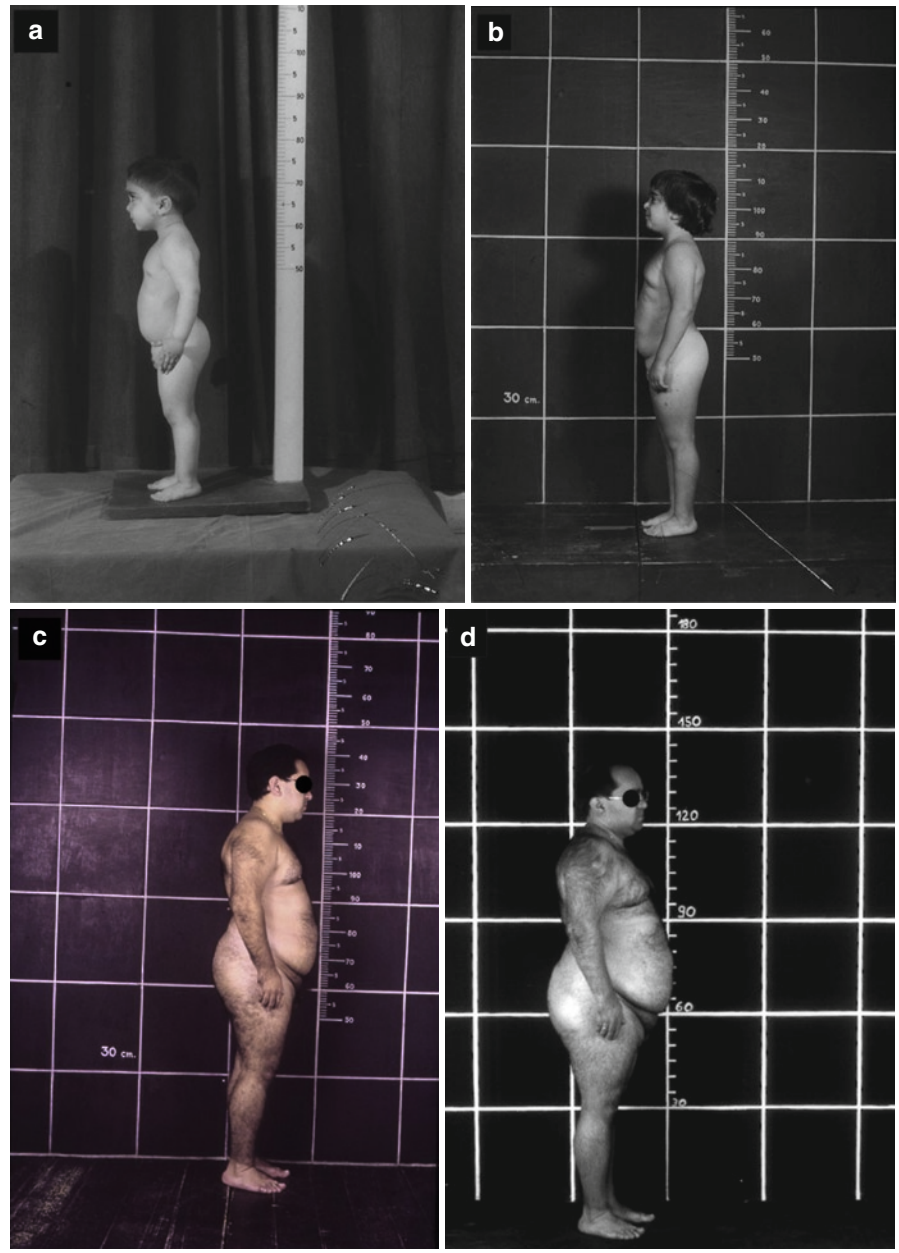

Fig. 12.8 Progressive obesity of an untreated male patient with Laron syndrome followed since early childhood and illustrated at ages 5,11, 28, and 44.5 (BR)

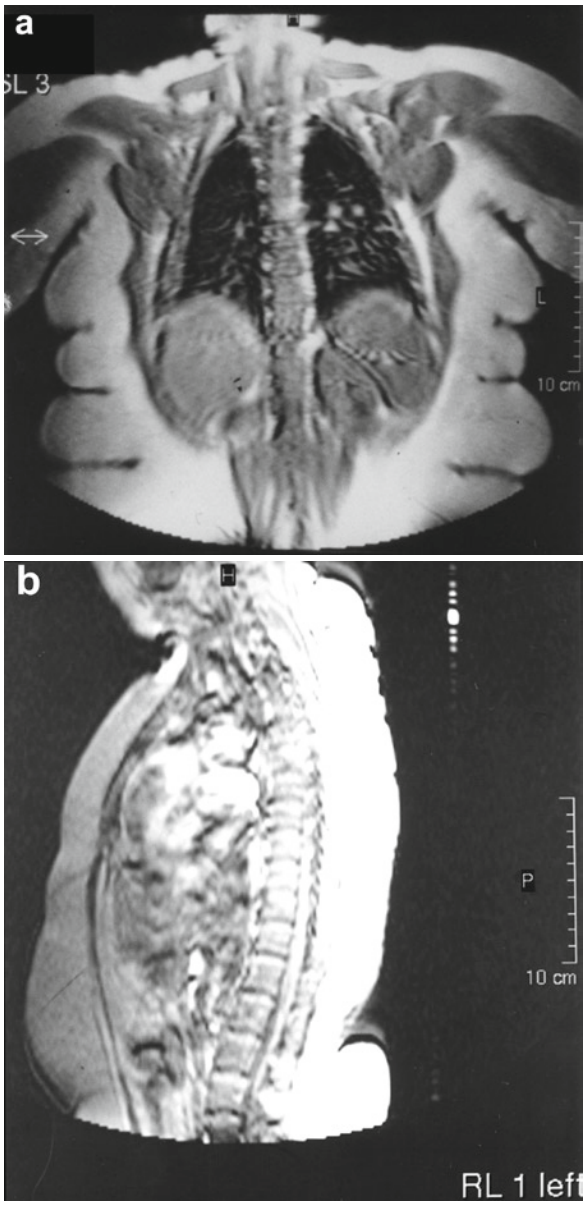

Fig. 12.9 (**a**, **b**) Frontal and lateral MRI of an untreated 41-year-old male with Laron syndrome illustrating the degree of subcutaneous and visceral adipose tissue (SSh)

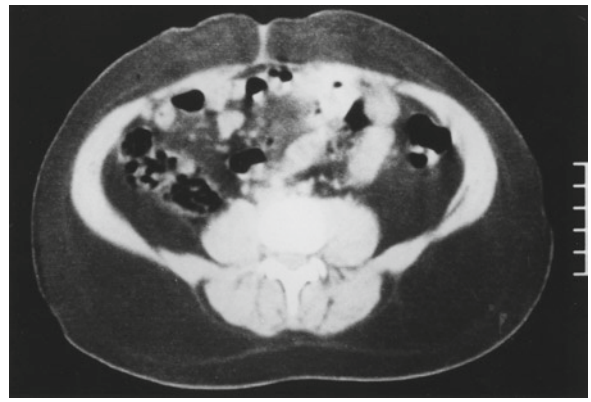

Fig. 12.10 Computerized tomography (CT) of the lower abdomen of a 27-year-old untreated female with Laron syndrome

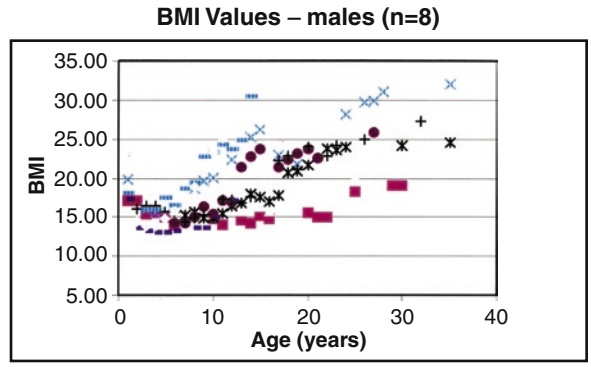

Fig. 12.11 Body mass index values for 8 males with Laron syndrome followed since early childhood into adult age. From Laron Z and Ginsberg Z (unpublished)

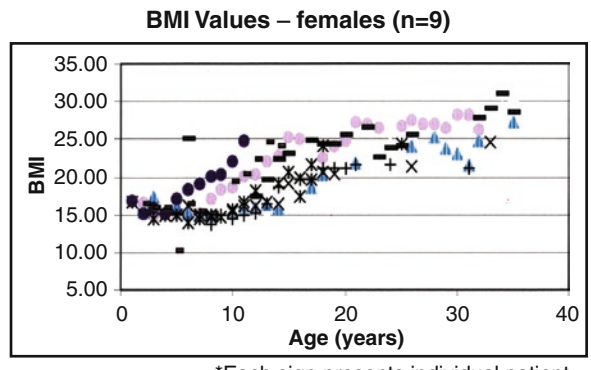

Fig. 12.12 Body mass index values for 9 females with Laron syndrome followed since early childhood into adult age. From Laron Z and Ginsberg Z (unpublished)

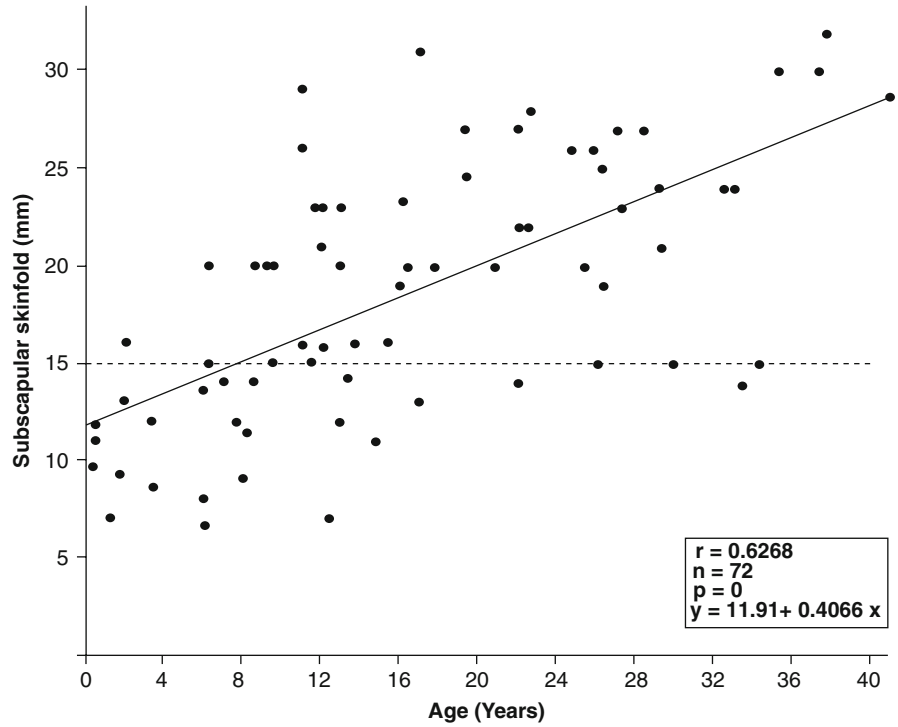

Fig. 12.13 Subscapular skinfold measurements of 18 untreated Laron syndrome patients of both genders followed since early childhood into adult age

Table 12.1 Body adiposity of adult patients with Laron syndrome

				Body fat % (DEXA)			
Patient	Age (years)	Height (cm)	Weight (kg)	Total body	Trunk	Arms	Legs
Females							
CS	53	122	48	58.5	53.4	70.4	56.1
YR	49	112	38	64.5	58.2	74.4	60.5
SR	48	130	62	65.1	58.5	72.9	64.8
BS	43	120	44	60.1	54.8	72.6	56.6
MP	43	136	60	61.0	55.8	69.9	59.1
MeY	38	128	36	51.3	50.4	64.1	44.6
TaK	27	129	55	56.1	54.5	64.5	54.0
Mean ± SD	*43 ± 9*	*125 ± 8*	*49 ± 10*	*59 ± 5*	*55 ± 3*	*70 ± 4*	*56 ± 6*
Males							
LY	50	131	42	31.6	33.0	30.5	29.8
SSh	45	116	28	36.4	36.2	49.1	31.2
BR	41	140	66	44.7	42.3	52.5	44.1
ML	28	140	45	43.7	45.1	47.1	41.0
Mean ± SD	*43 ± 10*	*132 ± 11*	*45 ± 16*	*39 ± 6*	*39 ± 5*	*45 ± 10*	*36 ± 76*
Females vs. Males							
P	*0.7388*	*0.36*	*0.6872*	*0.0023*	*0.0064*	*0.0115*	*0.0039*

Modified from Laron et al. (2006)

Table 12.2 Body adiposity of 13 healthy controls

					Body fat % DEXA			
	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Total body	Trunk	Arms	Legs
Females								
Mean ± SD	32 ± 12	167 ± 9	55 ± 3	20 ± 3	29 ± 7	27 ± 7	33 ± 11	30 ± 9
Males								
Mean ± SD	40 ± 15	176 ± 6	75 ± 11	24 ± 3	26 ± 8	26 ± 9	27 ± 12	25 ± 12

than that of the trunk and legs, less so in the males. Comparing all four parameters for percentage fat between LS and controls, for males and females separately, resulted in a higher significant difference for the females ($P < 0.001$ for all four parameters) than in the males ($P < 0.05$).

Two-way ANOVA was used to compare the ranks of the distribution in the arms, legs, and trunk between male and female patients and between both genders in controls. Both patient groups had significantly ($P < 0.0001$) more fat in their trunks compared to the controls (55 ± 3% in female patients vs. 27 ± 7% in controls and 39 ± 5 vs. 26 ± 9% in male patients and controls); significant differences were also found in the arms of female patients (70 ± 4 vs. 33 ± 11%) and also in their legs (56 ± 6 vs. 30 ± 9%) compared to controls. In the males, the difference was less evident.

Comment: To the best of our knowledge, there is only one other study in the literature that measured body composition in Laron syndrome patients (Bachrach et al. 1998). Our investigations determined quantitatively not only total body fat but also its regional distribution. It is evident that patients of both sexes are obese, with the females exceeding the males. The distribution between trunk and extremities revealed an increase in adiposity in the arms of both sexes.

We did not get permission to perform CTs or MRIs to demonstrate and quantify the amount of visceral fat in a group of Laron syndrome patients.

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13 Investigations to Determine the Cause of Obesity in Patients with Laron Syndrome

Zvi Laron, Shira Ginsberg, and Nahum Vaisman

Core Message

› This chapter describes attempts to clarify the cause of obesity of the patients with Laron syndrome. Determinations of nutritional intake and measurement of resting energy expenditure by indirect calorimetry proved that the obesity is not due to an increased caloric intake or reduced energy expenditure.

Considering the health risks and lifestyle handicaps related to the excessive obesity of the Laron syndrome patients, we initiated a series of investigations to detect the etiology of the increase in adiposity, in order to design a therapeutic approach.

The first question to be answered was to find out whether the Laron syndrome patients consume an increased amount of calories, the primary cause of simple obesity (Jelalian and RicG 2008).

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13.1 Nutrition Intake

13.1.1 Methods

Two studies were performed 17 years apart (1989 and 2006) with identical methodology:

The patients and their parents in the case of children were interviewed and asked to complete a questionnaire that includes personal details of their lifestyle and their daily agenda, sleeping hours, stool habits, sport activities and their duration, number of hours of watching TV, smoking habits, and the consumption of medication. In addition, all patients and parents of children and control subjects received a Food Intake Diary and were requested to register everything they ate or drank for 7 days, noting the exact time and quantity of ingestion.

The nutritionist in the first study was Gila Faiman, BSc, in the second Shira Ginsberg, BSc, MD.

13.1.2 First Nutritional Study

13.1.2.1 Subjects

The first study comprised 10 untreated patients with Laron syndrome (4 males and 6 females) aged 9–38 years, and as controls served 6 healthy adults (3 males and 3 females) aged 27–38 and 3 healthy prepubertal children (1 boy and 2 girls) aged 9–10 years. The pertinent clinical data of the Laron syndrome patients are presented in Table 13.1.

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Table 13.1 Nutritional Study I – pertinent clinical data of 13 untreated patients with Laron syndrome

No.	Name	Sex	Age y:m	Weight kg	Height cm	Pubertal stage	Skinfolds[a] mm
1	DS	F	9:2	23	104	1	35-35-20
2	ML	M	12:6	16.4	99	1	14-16-15
3	TAK	F	11:10	24	103	1	17-18-16
4	LY	M	35:3	40	128	5	18-6-21
5	ES	M	27	42	125	5	35-26-25
6	YR	F	33:7	38	113	5	34-26-33
7	SSh	M	34:3	31	118	5	17-13-16
8	SR	F	32	48	129	5	18-27-24
9	MP	F	28	50	137	5	33-23-27
10	CS	F	38	45	121	5	30-22-30

[a]Skinfolds: anterior iliac – triceps – subscapular

Table 13.2 Study I – nutritional intake of 3 children with Laron syndrome compared to 3 healthy controls

	Laron syndrome m ± SD	Controls m ± SD	DRI
Kcal consumed	66 ± 8.2	70 ± 8.8	65 ± 12.2
Protein g/kg consumed	1.9 ± 0.5	2.8 ± 0.5	12 ± 0.1
Protein %	14 ± 0.8	17 ± 1.2	20
Fat %	29 ± 2.3	34 ± 4.6	30
CHO %	57 ± 2.5	49 ± 3.5	50
Cholesterol g/day	174 ± 60	366 ± 69.2	
P/S ratio	1.7 ± 0.4	1.5 ± 0.1	
No. of meals	5 ± 0.8	6 ± 0.8	

DRI daily recommended intake

Table 13.3 Study I – nutritional intake of untreated adult Laron syndrome patients compared to healthy adult controls

	Laron syndrome $n=7$ m ± SD	Controls $n=6$ m ± SD	DRI
Kcal consumed	35 ± 9.9	21.1 ± 5.8	31.0 ± 0
Protein g/kg consumed	1.3 ± 0.5	0.8 ± 0.4	0.8 ± 0
Protein %	14 ± 2.8	19 ± 6.1	20
Fat %	34 ± 13.2	27 ± 6.9	30
CHO %	52 ± 15.5	54 ± 9.1	50
Cholesterol g/day	240 ± 120.4	241 ± 160.9	
P/S ratio	1.8 ± 0.9	1.7 ± 0.7	
No. of meals	4 ± 1.04	5.5 ± 0.5	

DRI daily recommended intake

13.1.2.2 Results

The results are shown in Tables 13.2 and 13.3 and reveal that Laron syndrome children did not consume more calories per day than the controls, but ate more carbohydrates and less cholesterol. The adult Laron syndrome patients consumed more calories resulting from more fat intake and less meals than the controls.

13.1.2.3 Comment

We assume that the difference in energy sources between the Laron syndrome patients and controls resulted in part to a difference in the socioeconomic class; the patients coming from families with a lower income at that time.

13.1.3 Second Nutritional Study

This study was more sophisticated measuring not only the nutritional intake by analyzing 84 food intake diaries, habits, and physical activity, but also resting energy expenditure (REE) (Ginsberg et al. 2009).

13.1.3.1 Subjects

Nine untreated adult Laron syndrome patients (6 females and 3 males) and 4 girls treated with IGF-I (FK780, Fujisawa Pharmaceutical Co., Ltd, Osaka, Japan) in doses of 120–150 μg/kg s.c. once daily were studied. As age-matched controls served healthy subjects from the Unit of Clinical Nutrition, Sourasky Medical Center, Tel Aviv – Prof. Nahum Vaisman.

13.1.3.2 Methods

The nutrition analysis was identical with that of the first study, but only 3 out of the 4 girls participated in some additional studies. The 84 Food Intake Diaries were reviewed and analyzed by S.G. according to the Food Composition Tables published in Israel Ministry of Health (Nutritional Tables, H. Meir, A. Reshef, Jerusalem 1996). The analysis included daily intake of Kcal, carbohydrates (g), fats (g), proteins (g), and cholesterol (mg). The results were compared to the DRI (daily recommended intake). Due to the excessive obesity, short stature, and ensuing orthopedic problems, the physical activity of these patients was irregular, reduced, and could not be estimated accurately.

13.1.3.3 Results

The pertinent clinical data including body composition of 9 untreated adult Laron syndrome patients and 3 IGF-I treated girls are shown in Table 13.4. The quantitative and qualitative nutritional intake is summarized in Table 13.5 and show the analysis of the caloric intake from the diary that each patient completed for 7 days. The mean caloric intake/day of the adult patients is 972 which leads to the value of 21.72 Kcal/kg/day. The DRI recommendation for caloric/kg intake is 31 for adults. The percent Kcal from fat (35.95%) is

Table 13.4 Study II – pertinent clinical data of Laron syndrome patients

Patients No	Name	Age (years)	Gender (F/M)	Weight (kg)	Height (cm)	BF (%)	LBM (kg)
Untreated adult patients							
1	CS	53	F	48	122	58.5	19.05
2	YR	49	F	42	112	62.4	15.9
3	SR	48	F	62	130	65.1	20.68
4	SSh	45	M	27.9	116	36.4	17.07
5	BS	43	F	44	120	60	18.68
6	MP	43	F	60	136	61	23.45
7	BR	41	M	66	139	44.2	35.3
8	MeY	38	F	36	128	51	
9	ML	28	M	57	141	46.6	29.5
IGF-I treated girls							
10	HoS	18	F	73	140	63.9	25.9
11	MaM	15	F	40	123	54.2	18.4
12	PR	7	F	20	105	46.9	10.6
13	HuN	6	F	13.2	94	25.0	10.0

BF body fat; *LBM* lean body mass

Table 13.5 Caloric intake of patients with Laron Syndrome

Patient No.	Total Kcal	Kcal/kg	% Kcal from Carbohydrates	% Kcal from fats[a]	% Kcal from proteins[a]	Protein/kg[a]
			Untreated adults			
1	764.30	15.92	45.01 (45–65%)	29.91 (20–35%)	25.12 (10–30%)	1.00 (0.8)
2	1,250.10	32.90	57.79 (45–65%)	26.85 (20–35%)	15.36 (10–30%)	1.26 (0.8)
3	1,122.20	18.10	43.31 (45–65%)	37.61 (20–35%)	19.03 (10–30%)	0.86 (0.8)
4	1,002.00	35.91	43.19 (45–65%)	42.49 (20–35%)	14.13 (10–30%)	1.27 (0.8)
5	840.70	19.11	51.96 (45–65%)	31.69 (20–35%)	16.22 (10–30%)	0.78 (0.8)
6	1,040.40	17.34	42.75 (45–65%)	40.66 (20–35%)	16.49 (10–30%)	0.72 (0.8)
7	1,089.90	16.57	45.91 (45–65%)	36.83 (20–35%)	17.29 (10–30%)	0.71 (0.8)
8	588.40	16.34	51.94 (45–65%)	32.58 (20–35%)	15.77 (10–30%)	0.64 (0.8)
9	1,049.30	23.32	44.22 (45–65%)	42.89 (20–35%)	12.96 (10–30%)	0.76 (0.8)
Mean	*971.92*	*21.72 (31)*	*47.29 (45–65%)*	*35.95 (20–35%)*	*16.74 (10–30%)*	*0.86 (0.8)*
			IGF-I Treated children			
10	522.70	8.17	62.83 (45–65%)	26.86 (20–35%)	10.25 (10–30%)	0.21 (1.0)
11	1,243.30	62.17	45.14 (45–65%)	38.00 (20–35%)	16.95 (10–30%)	2.64 (1.3)
12	1,136.00	74.74	52.46 (45–65%)	31.69 (20–35%)	15.85 (10–30%)	2.96 (1.5)
Range	*522.7–1,243.3*	*8.17–74.74*	*45.14–62.83*	*26.86–38*	*10.25–16.95*	*0.21–2.96*

[a]Mean values from a 7 day dairy analysis. In parenthesis the DRI recommendations

Table 13.6 Comparison of caloric intake per square meter between patients with Laron syndrome and healthy subjects of average height

Patients	Caloric intake (Kcal)	Mean height (m)	Caloric intake per one square meter
Males – LS	1,047	1.32	793
Males – DRI	2,080[a]	1.73	1,202
Females – LS	934	1.25	747
Females – DRI	1,762[a]	1.6	1,101

[a]Recommended caloric intake (Kcal) according to the DRI for calories/day for adults of average height and weight

higher than the DRI recommendation (<30%). The fat intake compensates for the carbohydrate intake (47.29%) which is lower than the DRI recommendation (55–60%). The mean protein intake is within the limits of recommendation. The distribution of energy sources in the 3 IGF-I treated girls revealed a relatively large variability in the data mainly related to one girl in whom reliability could not be ascertained, but who claimed to have reported correctly. The data shows a tendency of a high fat intake.

As our patients are significantly shorter than the normal population, we also compared the nutritional intake per 1 m^2 body surface assuming that the normal adult male height is 1.73 m^2 and 1.6 m^2 for females (using Tanner's charts) and calculating the surface area of our patient with a nomogram. As seen in Table 13.6, their food intake was less than that recommended for healthy controls. Table 13.7 presents the daily cholesterol intake compared to the serum cholesterol and triglycerides measured at the end of the registry as well as the daily iron intake compared to serum hemoglobin and the calcium and sodium intake.

There was a wide range in the cholesterol intake among the patients (mean 138 mg/day), but all values were within the normal DRI and American Heart Association recommended range (<300 mg/day). Three patients with high cholesterol levels were on Atorvastatin treatment. The serum triglyceride values of all the patients were within normal limits. The iron intake in all patients is below the recommended one and explains the low Hb values in some patients. The probable explanation is less consumption of red meat due to its greater cost than that of poultry. Those

Table 13.7 Daily cholesterol consumption compared to serum, total cholesterol and triglycerides; daily iron intake compared to blood hemoglobin as well as calcium and sodium daily intakes in Laron syndrome patients

Patient No.	Intake cholesterol mg	Serum cholesterol (mg/dL)	Serum triglycerides (mg/dL)	Fe-mg	Serum Hgb (g/dl)	Ca-mg[a]	Na-mg
1	256.1 (<300)	225[a]	80	5.4 (12)	11.7	351.3 (800)	1,266.1 (<2,400)
2	114.6 (<300)	250	88	10.5 (12)	13.1	360 (800)	3,055 (<2,400)
3	141.4 (<300)	188	83	6.8 (12)		410 (800)	2,027.6 (<2,400)
4	203.3 (<300)	244	48	6.6 (15)	14.9	143.6 (800)	1,397.1 (<2,400)
5	132.6 (<300)	221	136	4.2 (12)	15.6	298 (800)	1,429.7 (<2,400)
6	147.4 (<300)	270[a]	117	6.3 (12)	13.1	261.6 (800)	2,280.9 (<2,400)
7	116.5 (<300)	195[a]	148	6 (15)	16.4	394.7 (800)	2,115.3 (<2,400)
8	39.9 (<300)	187	46	2.2 (12)		388.6 (800)	1,224.4 (<2,400)
9	86.4 (<300)	180	61	4.4 (15)	13.9	148.9 (800)	1,060 (<2,400)
Mean	*137.58 (<300)*	*220*	*89.7*	*5.8 (12–15)*		*306.3 (800)*	*1,761.79 (<2,400)*
10	91.4 (<300)			4.85 (10)		799.7 (800)	911.5 (<2,400)
11	368.4 (<300)	185	84	5.5 (10)	13.6	695.7 (800)	1,680.3 (<2,400)
12	55.3 (<300)	185	92	1.8 (12)	12.5	158.4 (800)	660.7 (<2,400)
13	222.5 (<300)			10.3 (12)		511.4 (800)	2,656.1 (<2,400)
Range	*55.3–368.4*			*1.8–10.3*		*158.4–799.7*	*660.7–1,680.3*

Mean values of a 7 day dairy analysis
[a]On Atorvastatin treatment. In parenthesis DRI recommendations for daily intake

patients were prescribed iron preparations. The calcium intake is also low and may bear on the bone mineral density (BMD) results by DEXA (see Chap. 18). The sodium intake is within normal limits.

13.2 Conclusions

The results of the two nutritional studies prove that "the obesity of the Laron syndrome patients cannot be explained by an increased caloric intake," but it is of note that in both nutritional studies, the daily fat-derived calories were above the DRI recommendation.

13.3 Indirect Calorimetry Studies

Having found that the cause of obesity in the Laron syndrome patients is not caused by excessive nutritional intake, we wished to test whether it is due to increased energy output.

13.3.1 Resting Energy Expenditure (REE)

13.3.1.1 Subjects

Nine of the 13 patients underwent measurements of REE by indirect calorimetry (Ginsberg et al. 2009).

13.3.1.2 Methods

Resting energy expenditure (REE) and body composition [fat mass as a percentage of body weight (BF%) and lean body mass (LBM)] (muscle and bones) were measured in the Unit of Clinical Nutrition of the Tel Aviv Sourasky Medical Center. The REE was measured by an open-circuit indirect calorimeter (Deltatrac, Helsinki, Finland). Patients fasted (drinking water was allowed) from 20.00 h the night before the test until the next morning. Patients lay supine for 20 min prior to commencing the study at 08.00 h. After calibration with standardized

Table 13.8 Resting energy expenditure (REE) in 9 untreated adult patients with Laron syndrome

Patient No.	Name	Daily caloric intake (kcal)	REE measured (kcal/d)	REE predicted based on H-B kcal/d	REEPP (%)	REE predicted based on LBM Kcal/d.lbm[a]	REEPP (lbm) (%)	REE/LBM kcal/d.kg (LBM)	RQ
1	CS	764	810	1,090	74.3	816.5	99	42.6	0.84
2	YR	1,250	1,020	1,241	82.2	748.9	136.2	52.8	0.79
3	SR	1,122							
4	SSh	1,002							
5	BS	841	860	900.5	95.5	808.6	106.4	46.0	0.86
6	MP	1,040							
7	BR	1,090	1,380	1,370	101	1,165.9	118.4	39.1	0.79
8	MeY	588							
9	ML	1,049	1,213	1,348	90	1,041.2	116.5	41.1	0.82

[a]Based on Flatt (1995)
REE resting energy expenditure; *REEPP* percent of predicted REE; *RQ* respiratory quotient; *H–B* Harris–Benedict equation
Modified from Ginsberg et al. (2009)

Table 13.9 Resting energy expenditure (REE) in 4 IGF-I treated girls with Laron syndrome

Patient No.	Name	Daily caloric intake (kcal)	REE (kcal/d)	REEPP (%)	REE/LBM kcal/d.kg (LBM)[a]	RQ
1	Hos	522.7	1,170	74.3	45.2	0.84
2	NaM	1,190	940	79	51.1	0.79
3	PR	1,136	730	87.0	68.9	0.88
4	HuM	1,729	690	91.4	69.0	0.81

[a]Based on Flatt (1995)
REE resting energy expenditure; *REEPP* percent of predicted REE; *RQ* respiratory quotient
Modified from Ginsberg et al. (2009)

oxygen and carbon dioxide gas concentrations (95% O_2 5% CO_2), a plastic canopy was placed over the patient's head and REE was measured for 1 h. There was a 10-min washout period before starting data collection. The interindividual coefficient of variation in our laboratory is <3%. The respiratory quotient (RQ) was determined based on the above measurements (RQ value of 1 indicated exclusive carbohydrate utilization while RQ of 0.7 indicated exclusive lipid utilization) (Ferrannini 1988). The average RQ of healthy controls in our laboratory is 0.83 ± 0.04. Fat and lean body mass were determined by a dual energy X-ray absorptiometry (DEXA) machine (Lukaski 1987).

13.3.1.3 Results

The results for the adult LS patients are shown in Table 13.8 and those of the four girls in Table 13.9. REE ranged also when expressed as a percentage of the predicted based on the Harris–Benedict (H–B) equations. It was either lower or in the range of prediction (90–110% of the predicted) (Harris and Benedict 1919). When measured REE was calculated as a percentage of that predicted based on the LBM, the results were in the normal or above the normal range (Wang et al. 2000). REE per kg of LBM (REE/LBM) was higher in the very young IGF-I treated girls (No. 3 and 4 in Table 13.9) and was lower in the adult subjects. The ratio of REE/LBM

Table 13.10 Adipocyte size and glucose metabolism in isolated human adipose cells

		s.c. adipocytes		Omental adipocytes	
	Units	Laron syndrome	Controls[a]	Laron syndrome	Controls[b]
Cell size (m ± SEM)	μm	68 ± 3	115 ± 5	58 ± 2	78 ± 22
(range)	μm		82 – 150		52–137
Cellular D-glucose					
Uptake					
Basal	fmol/cell/ 30 min	69 ± 3	–	54 ± 5	–
Insulin (50 nM, 30 min)	fmol/cell/30 min	207 ± 10	–	150 ± 7	–
Insulin responsiveness (%)		300	~400	280	~200

[a]Karnieli et al. (1989)
[b]Armoni et al. (1987)

was significantly higher in the patients as compared to the norm in our laboratory for healthy age-matched subjects. The RQ ranged between 0.79 and 0.88.

13.3.1.4 Discussion

The abnormal body composition in the Laron syndrome patients was found to significantly affect REE, with the LBM being the main contributor to REE and the fat mass contributing to a much lesser degree (Cunningham 1980). The excessive obesity and the altered ratio of fat mass to LBM can easily explain why the H–B equations are not applicable to our patients.

13.4 Conclusion

Our studies indicate that the marked obesity and altered body composition of patients with Laron syndrome is not caused by hyperphagia or hypometabolism having lower than expected REE.

13.5 Daily Physical Activity

Because of the excessive obesity, short stature, and ensuing orthopedic limitations, the physical activity varied. In general, it was reduced and irregular, being mainly walking. It could not be estimated accurately. One can assume that the reduced physical activity in these patients contributes to their adiposity.

13.6 Metabolic Investigation of Adipose Cells from a Laron Syndrome Patient

During one of the Caesarian sections of one of our patients (MP at age 38 years), we had the opportunity to collect subcutaneous and omental adipose tissue. Investigated at the Molecular Endocrinology Unit, Rambam Hospital (Dr. Michal Armoni and Prof. E. Karnieli), it revealed that the size of both the subcutaneous and omental adipocytes was smaller than that of control subjects. The basal and insulin-stimulated glucose uptake of both types of adipocytes was normal (Armoni et al. 1987; Karnieli et al. 1989) (Table 13.10).

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Nonalcoholic Fatty Liver Disease (NAFLD) in Patients with Laron Syndrome

14

Zvi Laron

Core Message

› Some obese patients with Laron syndrome develop NAFLD (non-alcoholic fatty liver disease). No clear metabolic correlation could be established.

14.1 Introduction

Nonalcoholic fatty liver disease (NAFLD) is considered a liver manifestation of the metabolic syndrome characterized by obesity, mainly visceral, type 2 diabetes (T2DM), hyperlipidemia, and insulin resistance, which may develop into nonalcoholic liver steatosis (NASH) and fibrosis (Perlemuter et al. 2007; Dixon et al. 2001; Knobler et al. 1999; Stefan et al. 2008).

The pathogenesis of the fatty liver is assumed to be linked to ineffective suppression of glucose production via increased gluconeogenesis in the liver and impaired glucose uptake by muscle and adipose tissue. Lipid metabolism is also impaired, and steatosis can result from increased levels of free fatty acids originating in the adipose tissue and increase in "de novo" liver lipogenesis (Begriche et al. 2006).

One of the active hormones involved in these mechanisms is adiponectin, produced by adipocytes, which decreases hepatic glucose production and increases muscle glucose and fatty acid utilization (Trujillo and Scherer 2006). It has been reported that adiponectin levels are low in obese patients and inversely correlated with the hepatic fat content (Bugianesi et al. 2005); however, we have reported that obese patients with Laron syndrome have high adiponectin levels (Laron et al. 2007; Kanety et al. 2009). To advance knowledge on the above controversial issue, we looked for the presence of NAFLD in patients with Laron syndrome.

14.2 Subjects

Eleven untreated adult patients with Laron syndrome (5 males, 6 females) and 5 girls with Laron syndrome on long-term treatment by IGF-I were studied (Laron et al. 2008). None of the patients had a history of hepatitis nor consumed alcohol. None agreed to undergo a liver biopsy. All untreated and even treated patients with Laron syndrome are short and obese.

14.3 Methods

Quantification of liver steatosis was assessed introducing a new index using ultrasonography (HITACHI 6500, Japan). The cut-off point for fatty liver was a ratio of ≥1.5 (normal ~1) based on the difference between the echo densities of the liver and the right kidney (Webb et al. 2006). Four patients underwent sonography twice at 1-year interval.

Body fat was measured by dual-energy absorptiometry (DEXA) (Laron et al. 2006a). Blood cholesterol and liver enzymes were determined with the use of a Hitachi autoanalyzer (Boehringer, Mannheim, Germany). Insulin resistance was estimated by the homeostatic model assessment (HOMA-IR) (Matthews et al. 1985).

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Table 14.1 Presence of fatty liver (NAFLD), degree of adiposity, liver enzymes, and insulin resistance in untreated and IGF-I-treated patients with Laron syndrome

	Percent body fat by DEXA[a]	Cholesterol (mg/dL)	Liver[b] index	NAFLD ±	GPT (U/L)	GGT (U/L)	LDH (U/L)	GOT (U/L)	HOMA-IR
Untreated adult females									
1[c]	58.5	218[c]	0.9	–	14	14	457	16.8	0.42
2	64.5	220	1.5	+	17	21	292	16	0.45
3	65.1	216	0.9	–	19	21	408	21	2.06
4	60.1	221	1.1	–	25	37	397	24.4	1.04
5	61.0	270	1.6	+	11	11	296	15	2.16
6	56.1	217	1.1	–	15	12	395	14	1.48
Mean	**60.9**	**228.8**	**1.18**		**17**	**19.3**	**374.2**	**17.9**	**1.26**
S.D	**3.5**	**23.1**	**0.3**		**4.96**	**9.7**	**66.1**	**4**	**0.76**
Untreated adult males									
7	36.4	244	1.1	–	34	22	602	36	0.51
8[c]	43.7	166[c]	1.5	+	79	111	308	44	1.23
9	44.7	195[c]	1.5	+	22	97	314	19	0.69
10	31.6	248	1.75	+	44	30	409	31	0.95
11	-	197	2.35	+	25	24	295	20	0.48
Mean	**39.1**	**213.75**	**1.64**		**41**	**56.8**	**385.6**	**30**	**0.77**
S.D	**6.21**	**39.4**	**0.45**		**23**	**43.5**	**129.1**	**10.7**	**0.32**
Mean F±M	**52.17**	**222.11**	**1.39**		**27.77**	**36.36**	**379.36**	**23.38**	**1.04**
S.D F+M	**12.08**	**30.19**	**0.43**		**19.49**	**34.43**	**94.29**	**9.67**	**0.63**
IGF – treated girls									
12	62.8	171	1.65	+	27	21	398	25	3.54
13	49.1	177	1.1	–	12	10.7	465	19.4	2.79
14	47.0	183	1.7	+	17	7	523	31	0.37
15	48.7	203	1.18	–	20	–	–	14	0.44
16	25.0	157	1.08	–	15	10	533	29.2	0.42
Mean	**53.53**	**178.20**	**1.34**		**18**	**12.2**	**479.75**	**23.72**	**1.51**
S.D	**8.02**	**16.89**	**0.30**		**5**	**6.1**	**62.2**	**7.03**	**1.53**
			Norms for liver enzymes:		**<41**	**<60**	**<436**	**<37**	**<2**

[a]From Laron et al. 2006a, b; Modified from Laron et al. (2008)
[b]Liver index ≥1.5 = Fatty liver
[c]On Atorvastatin treatment

14.4 Results

The presence or absence of NAFLD and the relationship to percent body fat, and serum cholesterol, liver enzymes, and HOMA-IR are shown in Table 14.1. It is evident that out of 11 adult patients with Laron syndrome, 6 (4 males, 2 females) have fatty liver despite the fact that the females are more obese than the males. Three males had one or two abnormal liver enzymes. Out of the five IGF-I treated girls with Laron syndrome, two have a fatty liver, and two have one abnormal liver enzyme. The fatty liver in one adult male Laron syndrome patient is illustrated in Fig 14.1 and one of the IGF-I-treated girls in Fig 14.2. It is evident that long-term IGF-I replacement treatment did not prevent the development of fatty liver steatosis.

All patients with Laron syndrome are very obese including marked visceral adiposity (Laron 2004; Laron and Klinger 1993; Laron et al. 2006a), but not all seem to have a fatty liver. All Laron syndrome patients also develop progressive hypercholesterolemia

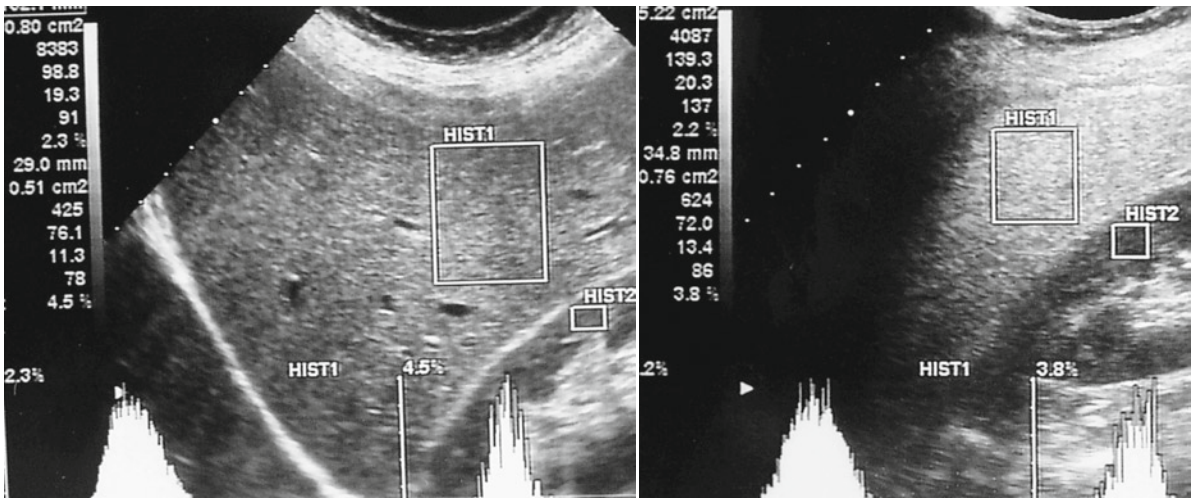

Fig 14.1 Abdominal ultrasound using histogram. *Left*: normal nonfatty liver. *Right*: hyperechoic fatty liver of a 50-year-old male with Laron syndrome

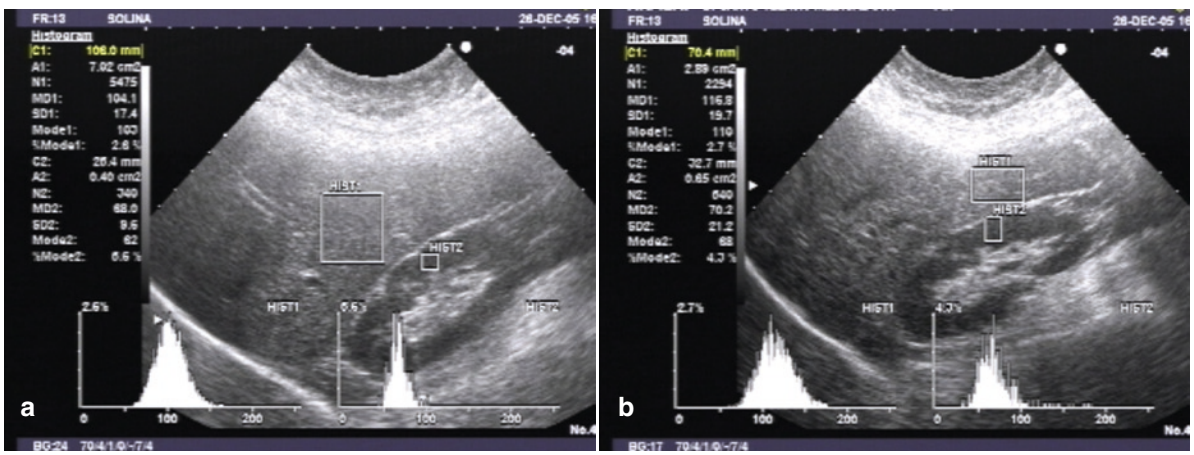

Fig. 14.2 (**a**–**f**) Fatty liver in a 15-year-old girl with Laron syndrome (HoS) treated with IGF-I since early childhood

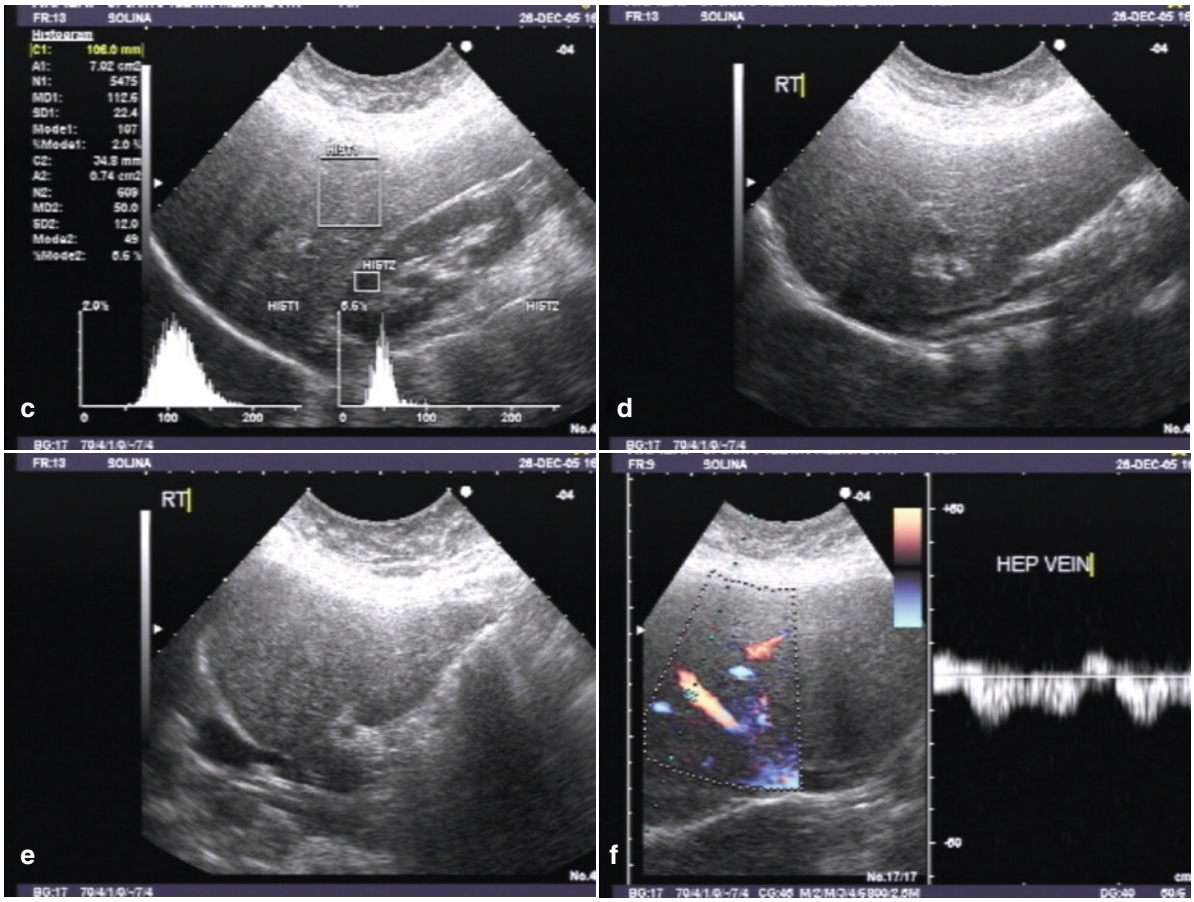

Fig. 14.2 (continued)

(Laron 2004; Laron and Klinger 1993), but three of the male Laron syndrome patients with NAFLD had cholesterol levels within normal levels during Atorvastatin treatment. The triglyceride levels (not shown) were also within normal limits; HOMA-IR was abnormal (>2) in only two adult females with Laron syndrome and in one IGF-I-treated girl and normal in the others (Laron et al. 2006b).

Elevated serum liver enzymes often associated with NAFLD (Bugianesi et al. 2005) were elevated in five patients. It is of note that the presence or absence of NAFLD did not correlate with age, sex, degree of obesity, blood lipids, HOMA-IR and therapy by statins or IGF-I replacement treatment, thus not fitting with the present theories of the development of fatty liver. Similar findings were reported by us in treated or untreated patients with congenital hGH-1 gene deletion (Laron et al. 1985).

14.5 Conclusions

The obese patients with Laron syndrome develop NAFLD even during long-term IGF-I replacement treatment.

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Serum Lipids in Patients with Laron Syndrome

15

Zvi Laron

Core Message

› This chapter describes the serum concentrations of blood lipids (FFA, cholesterol and lipoprotein [a]) along age in patients with Laron syndrome. The main observation is the progressive increase of serum cholesterol necessitating pharmacotherapy.

15.1 Introduction

Hyperlipidemia, especially hypercholesterolemia, is a widespread adverse effect of modern life, being of primary or secondary origins (Durrington 2007). Hypopituitarism has been reported to be associated with elevated cholesterol levels (Cohen and Shamir 2007), but to a lesser degree than in hypothyroidism (Kissebah and Krakower 1999; Summers et al. 1967). Furthermore, low density lipoprotein (LDL) and VLDL cholesterol levels have been found raised (Merimee et al. 1972) in growth hormone deficiency, and in acromegaly, LDL levels tend to be low (Nikkilä and Pelkonen 1975).

In no instance has a differentiation between GH and IGF-I been made. Laron syndrome with both GH and IGF-I deficiency presents an ideal model to study the effects of this double deficiency. We performed investigations of various components of blood lipids at various stages of follow-up and during IGF-I treatment. All the sampling was done after a 12–14 h overnight fast.

15.2 Free Fatty Acids (FFA)

Plasma FFA were determined according to Dole and Meinertz (1960). Figure 15.1 shows that more than half of the determinations performed were above normal concomitantly with low blood glucose levels (Fig. 15.2). To what degree did hypoglycemia or the GH/IGF-I deficiency determine the FFA levels was not established at that time (Laron et al. 1968).

15.3 Serum Cholesterol

At a later stage (Laron and Klinger 1993), serum total cholesterol (measured by Hitachi autoanalyzer) was determined in 37 Laron syndrome patients. As seen in Fig. 15.3, part of the children with Laron syndrome and many adult patients had elevated levels.

Recently, Laron and Karmon (2010) reviewed the levels of total LDL, and HDL cholesterol levels in the whole cohort of 64 Laron syndrome patients. The analysis is based on overnight fasting measurements made using a Hitachi autoanalyzer (Roche Diagnostics, Basel, Switzerland) for serum total and HDL cholesterol and computer calculated values for LDL cholesterol and triglycerides. The sampling was done during routine follow-up visits.

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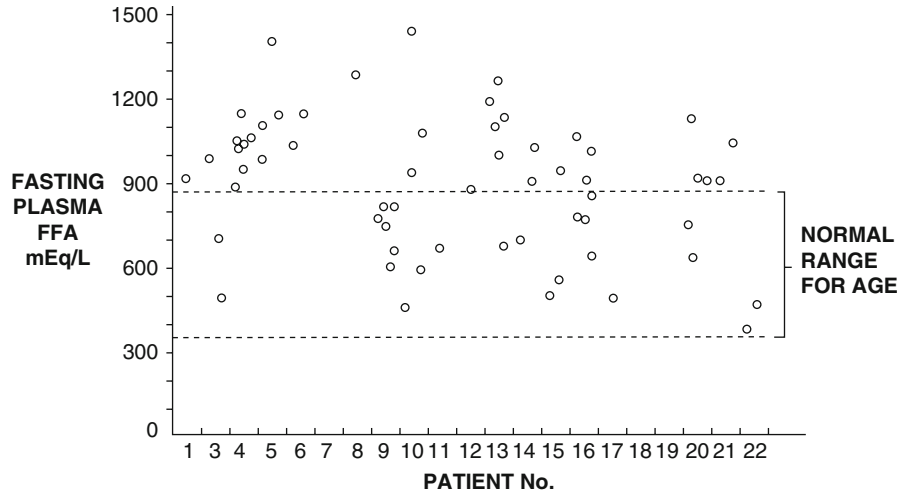

Fig. 15.1 Fasting plasma free fatty acids (FFA) in 19 patients with Laron syndrome. Reproduced with permission from Laron (1984)

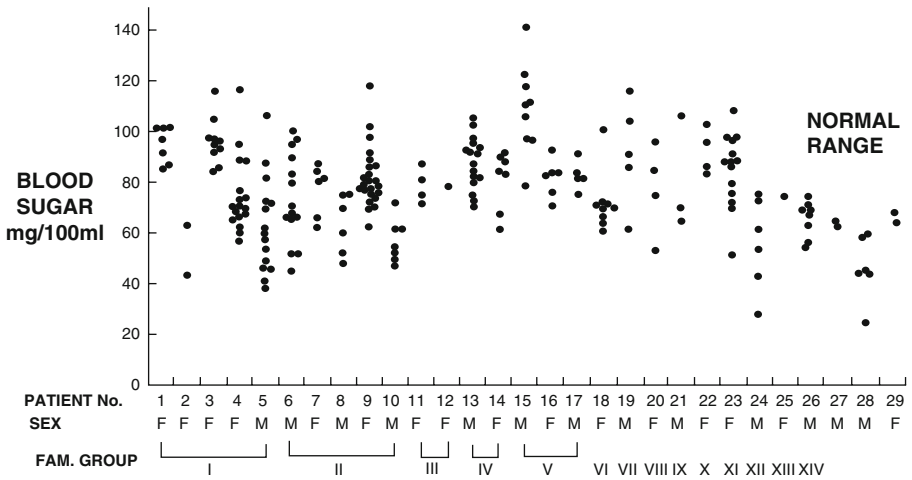

Fig. 15.2 Repeated fasting glucose as total reducing substances measurements (Rappaport and Eichhorn 1950) in 29 patients with Laron syndrome drawn concomitantly to those for FFA determinations. Reproduced with permission from Laron (1984)

15.3.1 Results

The serum levels of total LDL and HDL cholesterol of 30 untreated females with Laron syndrome plotted by age are illustrated in Figs. 15.4–15.6 for females and for 33 males in Figs. 15.7–15.9. It is evident that the majority of samples of total and LDL cholesterol are high even in young age in Laron syndrome patients.

The LDL serum concentrations begin to be high around the age of 20 years in the females and around the age of 30 years in the males. The continuously high levels demonstrate that treatment was irregular or not observed in some patients. The HDL cholesterol levels were within normal limits.

15.4 Triglycerides (TG)

Repeated serum TG determinations in the untreated patients with Laron syndrome revealed only few high levels, more so in the male patients, who had higher levels above 150 mg/dL (Figs. 15.10 and 15.11).

15.5 Lipoprotein (a)

Lipoprotein (a) [Lp(a)], a specific class of lipoprotein particles made up of a single copy of apolipoprotein B-100 linked to apoprotein (a) component (Berg 1963),

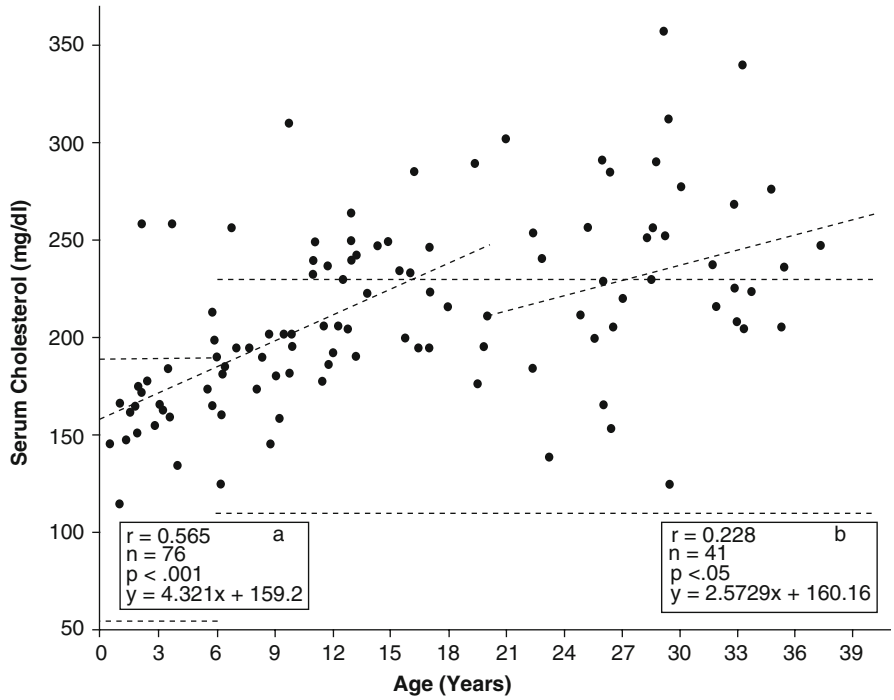

Fig. 15.3 Repeated measurements of serum total cholesterol in 37 untreated patients with Laron syndrome of both sexes. The interrupted lines denote the upper and lower limits of normal values by age groups

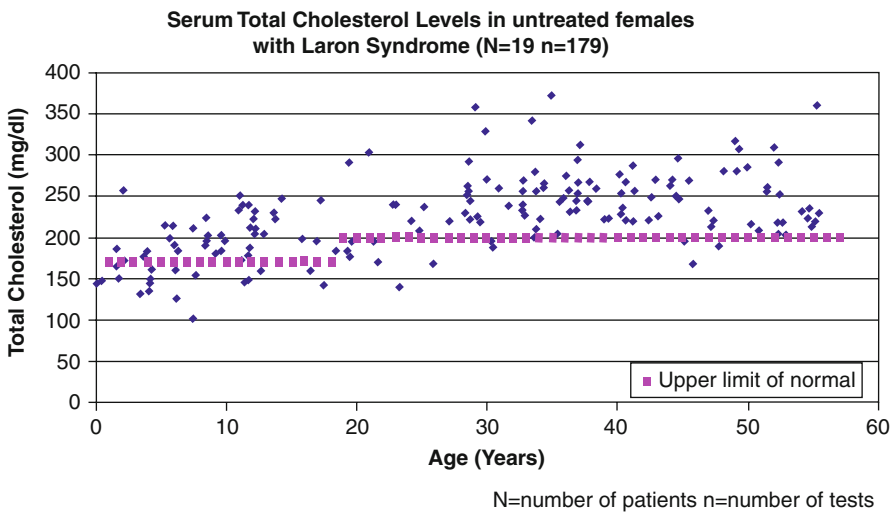

Fig. 15.4 Serum total cholesterol levels in 19 untreated female Laron syndrome patients along age, n = number of samples

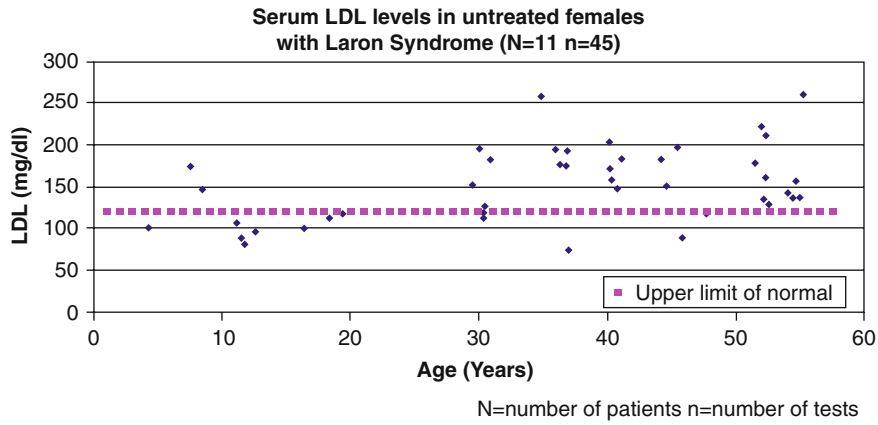

Fig. 15.5 Serum LDL cholesterol levels in 11 untreated female Laron syndrome patients along age, n = number of samples

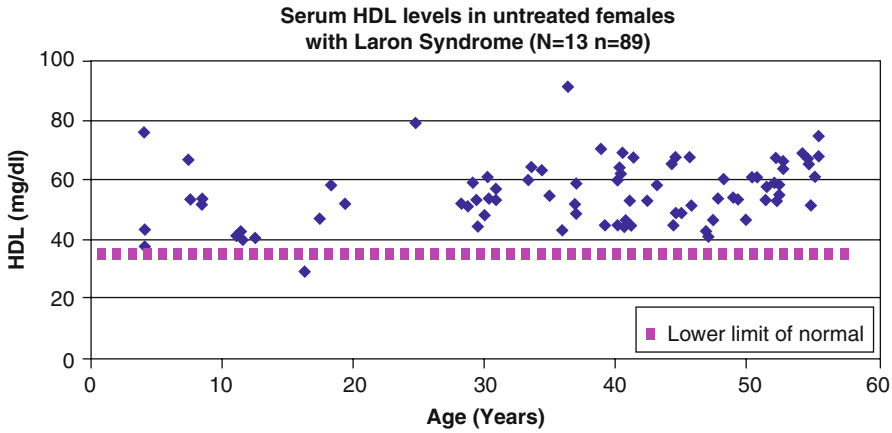

Fig. 15.6 Serum HDL cholesterol levels in 13 untreated female Laron syndrome patients, n = number of samples

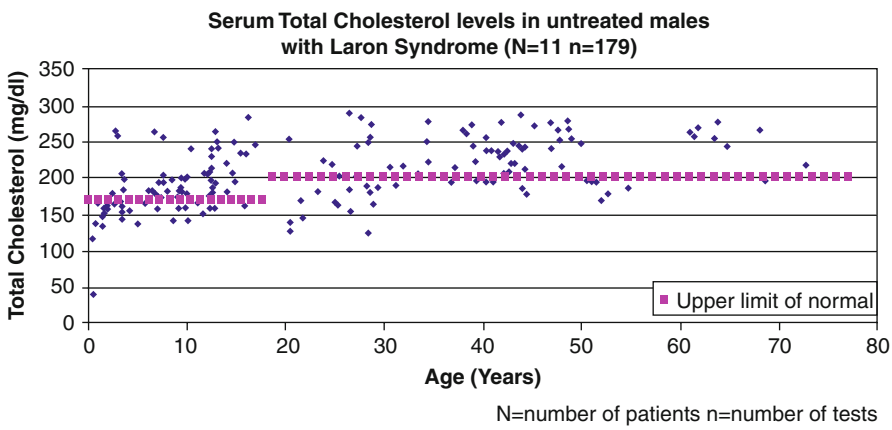

Fig. 15.7 Total cholesterol levels in 11 untreated male Laron syndrome patients along age, n = number of samples

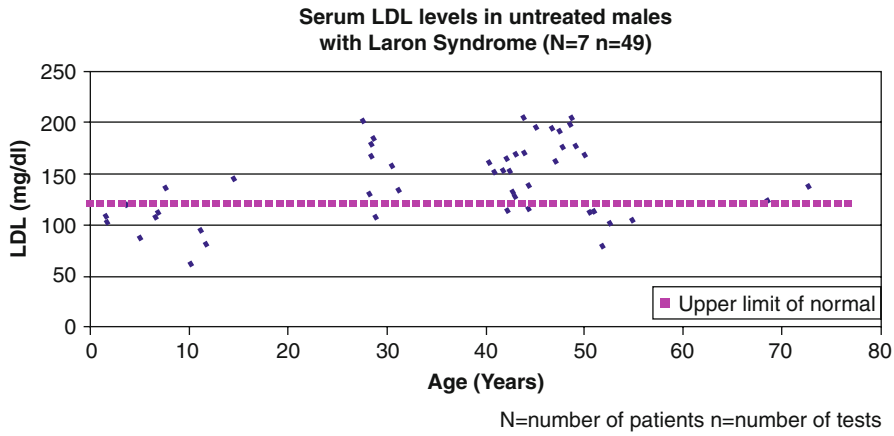

Fig. 15.8 Serum LDL cholesterol levels in seven untreated male Laron syndrome patients, n = number of samples

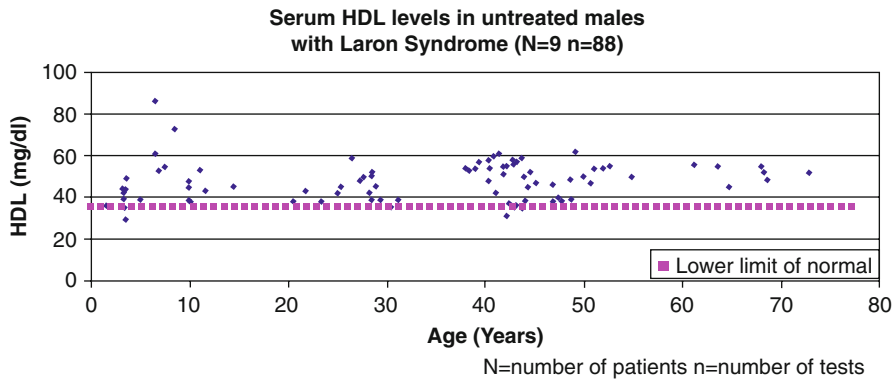

Fig. 15.9 Serum HDL cholesterol levels in nine untreated male Laron syndrome patients, n = number of samples

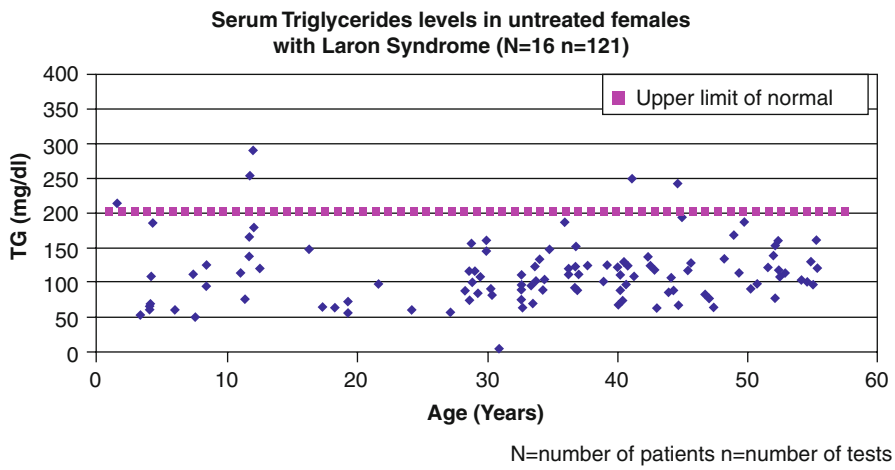

Fig. 15.10 Serum triglyceride levels in 16 untreated female Laron syndrome patients, n = number of samples

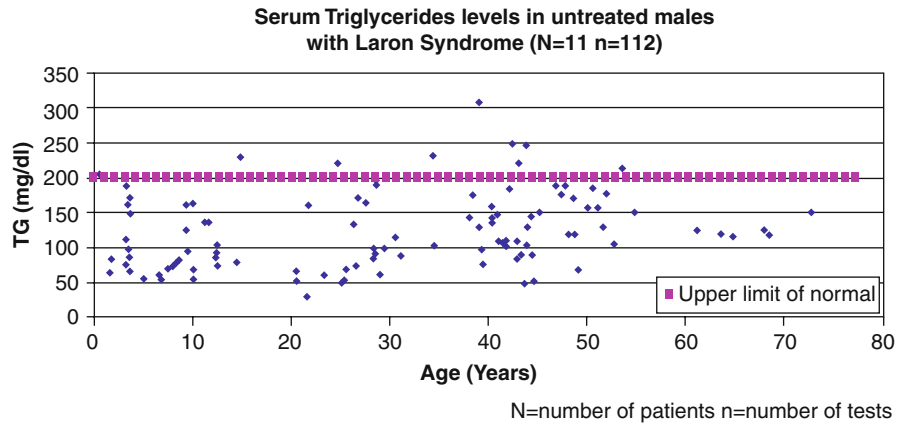

Fig. 15.11 Serum triglyceride levels in 11 untreated male Laron syndrome patients, n = number of samples

differs from LDL cholesterol by this apolipoprotein component (Lackner et al. 1993) and has a structural homology with plasminogen (McLean et al. 1987). Elevated Lp(a) levels have been found to be an independent risk factor for cardiovascular disease (Suk Danik et al. 2006).

15.5.1 Investigation

15.5.1.1 Subjects

Ten untreated patients with Laron syndrome, 4 prepubertal (3 boys, 1 girl), 1 early pubertal boy, and 5 adults (4 females, 1 male, aged 29–39 years), were studied. The same patients were studied again after 9–12 months IGF-I treatment (120–150 μg/kg once daily) (Laron et al. 1996).

15.5.1.2 Method

After an overnight fast, Lp(a) blood was sampled and the serum stored at –20°C until analyzed by an Elisa method (Wang et al. 1992).

15.5.1.3 Results

In all the untreated patients, the Lp(a) serum levels were within the normal range (<25 mg/dL for children and <35 mg/dL for adults), 7.3 ± 2.1 mg/dL (m ± SD) in the Laron syndrome children and 7.9 ± 2.2 mg/dL (m ± SD) in the adult patients. IGF-I treatment reduced the Lp(a) serum levels in a dose-dependent manner (see Chap. 44), the m ± SD reduction being 65 ± 15% (Laron et al. 1997). This decrease paralleled that of insulin. The latter study (Laron et al. 1997) also demonstrated that the effects of IGF-I on serum Lp(a) and insulin were opposed to those of hGH which increased both Lp(a) and insulin in patients with IGHD and Turner's syndrome, a finding confirmed by others (Edén et al. 1993).

15.6 Therapeutic Attempts

(a) All patients were interrogated and counseled by a nutritionist.
(b) *Cholesterol lowering drugs*: 4 adult Laron syndrome patients (CS, BS, BR and LY), 2 females and 2 males, have been on Atorvastatin 20 mg/day since ages 52, 45, 41, and 50, respectively as their serum total cholesterols ranged between 244 and 309 mg/dL.
(c) Metformin has been prescribed to several patients, but most complained of gastrointestinal upsets and stopped taking the drug.
(d) One patient (MP) underwent liposuction which reduced her fat but left ugly scars (Fig. 15.12a, b).
(e) The effect of IGF-I treatment on the serum total LDL and HDL cholesterol levels in Laron syndrome patients is described in Chap. 44.

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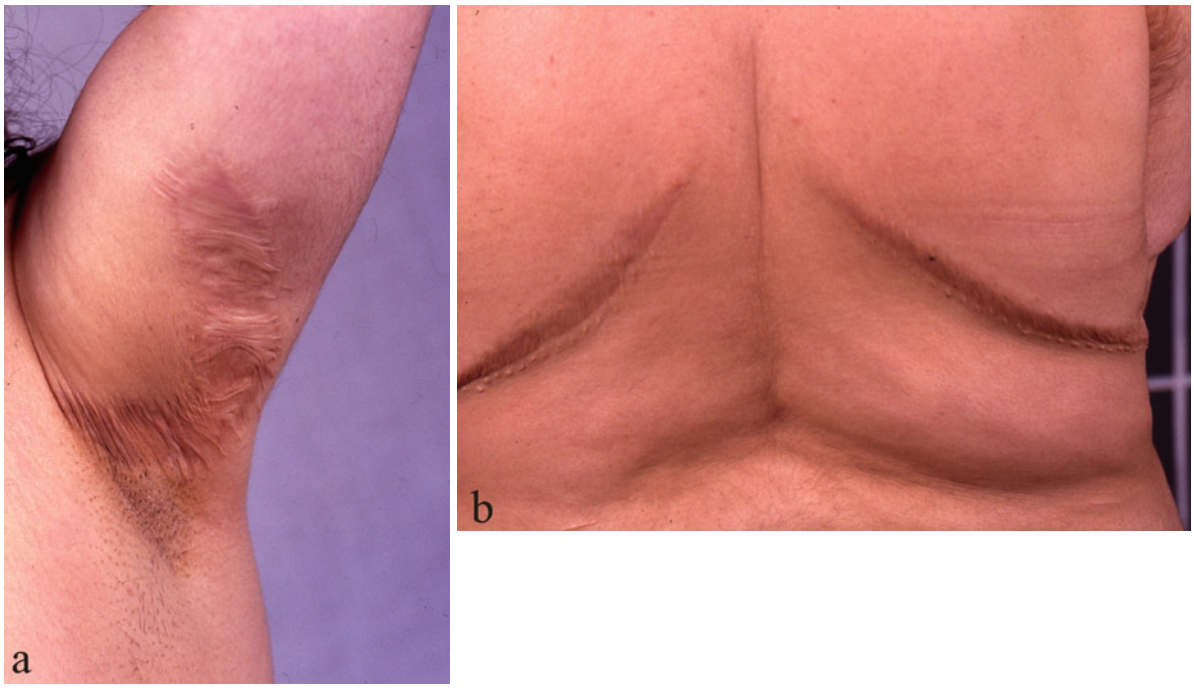

Fig. 15.12 Scars at the sites of liposuction in patient MP (**a**) upper arm; (**b**) back

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Adiponectin and Leptin in Laron Syndrome

16

Zvi Laron and Hannah Kanety

Core Message

› Serum total and high molecular adiponectin (AN) measurement in the obese Laron syndrome patients unexpectedly revealed high levels. In simple obesity the AN levels are low. Serum leptin levels were elevated as expected.

As the degree of obesity in Laron syndrome patients was found not to be caused by overeating nor by reduced energy output (Chap. 14), further studies were performed to disclose the etiology of obesity in these patients (Laron et al. 2007; Kanety et al. 2009). Thus, we measured the serum levels of adiponectin and leptin, two important factors involved in adipose tissue metabolism (Kadowaki and Yamauchi 2005; Trujillo and Scherer 2006).

16.1 Subjects

Nine untreated adult Laron syndrome patients aged 29–53 years (5 males, 4 females) and 6 girls aged 6–16.5 years receiving one daily injection of IGF-1 (120–180 μg/kg s.c.; Fujisawa, Japan) for various lengths of time (2–10 years) were investigated. The pertinent clinical data of the patients, arranged by gender and age, are shown in Table 16.1.

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16.2 Methods

Blood for adiponectin, leptin, insulin, and general blood chemistry was drawn after an overnight (12–14 h) fast. Total adiponectin and leptin were determined by RIA (Millipore, USA) as described by Modan-Moses et al. (2007) and the high molecular weight (HMW) adiponectin was measured by ELISA according to Komura et al. (2008). The interassay coefficients of variation for leptin, total adiponectin, and HMW-adiponectin were <6.2, <9.3, and <10%, respectively. Intra-assay CVs for each of the three were <8.3, <6.5, and <10%, respectively. The distribution of adiponectin multimers in serum samples was analyzed under nonreducing and nondenaturing conditions as described previously (Modan-Moses et al. 2007; Waki et al. 2003). Abundance of adiponectin multimers was determined by densitometry. Insulin was determined by a solid-phase two-site chemiluminescent immunometric assay (Immulite 2000, Siemens, USA) and blood chemistry by a Hitachi autoanalyzer. Insulin resistance was estimated by the homeostatic model assessment (HOMA-IR) (Matthews et al. 1985). Body fat was determined using dual-energy by X-ray absorptiometry (DEXA: Model DPX-IQ 8565-A, Lunar Radiation Corp., Madison, Wisc., USA) (Laron et al. 2006).

16.3 Statistical Analysis

Statistical analyses were performed using Student's *t* test for two-group comparisons. Pearson or Spearman correlation analysis was used when applicable to examine bivariate relationships.

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Table 16.1 Pertinent clinical data of Laron syndrome patients studied

	Adults		Children IGF-I treated
	Males	Females	Females
	$n=5$	$n=4$	$n=6$
Age (years)	39.4 ± 8.2 (29–51)	48.8 ± 5.1 (43–53)	10.4 ± 3.7 (5–16.6)
Height (cm)	130 ± 10 (116–140)	125 ± 10 (112–136)	115 ± 15 (89–141)
Weight (kg)	46.4 ± 13.4 (27.9–66)	47.5 ± 9.3 (44–60)	33.8 ± 18.1 (12.3–70.1)
Body fat (%) by DEXA	40.6 ± 8.1 (31.6–49.7)	61.0 ± 2.5* (58.5–64.5)	46.5 ± 13.5** (25.0–62.8)

n number of patients
Data are means ± SD, ranges in parentheses
*$P<0.05$ females vs. male LS patients
**$P<0.05$ girls vs. female LS patients

16.4 Results

Forty-one sera from 15 patients with Laron syndrome tested repeatedly (9 untreated adults and 6 IGF-1 treated children), obtained during long-term follow-up were analyzed. The mean levels of total and HMW-adiponectin, leptin, cholesterol, insulin, glucose, and HOMA-IR are shown in Table 16.2.

16.5 Adult Laron Syndrome Patients

In contrast to simple obese subjects who have low adiponectin and high leptin levels (Klein et al. 1996; Kern et al. 2003), we found that Laron syndrome patients with excessive obesity have high both total and HMW serum adiponectin. The leptin levels were high as expected (Laron et al. 1998). Both total adiponectin

Table 16.2 Serum adiponectin and leptin concentrations in 15 patients with Laron syndrome

	Adults		Children IGF-I treated
	Males	Females	Females
	$n=5$, $x=9$	$n=4$, $x=6$	$n=6$, $x=26$
Total adiponectin (μg/mL)	10.2 ± 4.6 (6.5–21.1)	21.4 ± 3.5* (15.9–25.1)	22.9 ± 7.4 (15–32.5)
HMW adiponectin (μg/mL)	5.3 ± 3.4 (1.7–12.5)	15.4 ± 2.3* (13.0–17.6)	19.2 ± 4.9 (13.7–26.8)
Leptin (ng/mL)	11.3 ± 4.9 (6.1–19.7)	27.9 ± 2.7* (25–31.8)	27.3 ± 5.3 (9.2–63.4)
Insulin (μIU/mL)	3.8 ± 1.4 (2–5.2)	5.0 ± 3.8 (2–10.1)	5.1 ± 4.2 (2–11.9)
Glucose (mg/dL)	84.2 ± 12.2 (74–99)	82.3 ± 5.7 (74–87)	80.1 ± 7.4 (69–87)
HOMA-IR	0.77 ± 0.31 (0.49–1.23)	1.02 ± 0.65 (0.42–2.16)	1.12 ± 0.97 (0.34–2.82)
Total Cholesterol (mg/dL)	146.8 ± 28.9 (195–248)	232.3 ± 25.2 (218–270)	170.5 ± 22.8** (129–205)

Data are means ± SD, ranges in parentheses
n number of patients in the indicated group; *x* number of samples
*$P<0.05$ females vs. male LS patients
**$P<0.05$ girls vs. female LS patients

and leptin were significantly higher in female patients compared with male Laron syndrome patients (adiponectin 21.4 ± 3.5 vs. 10.2 ± 4.6 μg/mL, $P < 0.001$ and leptin 27.9 ± 2.5 vs. 11.3 ± 4.9 ng/mL, $P < 0.001$), so were the HMW adiponectin levels (Table 16.2).

The total serum adiponectin concentrations of both female and male Laron syndrome patients were 2–3 fold higher compared to reported data for obese subjects of similar age, gender, and with an equivalent degree of adiposity, whereas the leptin levels were comparable (Kern et al. 2003; Ryan et al. 2003; Bullo et al. 2002; Vilarrasa et al. 2007). Even when the total adiponectin levels in Laron syndrome females were normalized for individual body fat percent, they were higher (0.36 ± 0.06) than those reported for simple obese women (0.17–0.21) (Shand et al. 2003; Polak et al. 2008).

Also, the HMW adiponectin isoform, which is markedly reduced in obese subjects (Wang et al. 2008; Schraw et al. 2008), was threefold higher ($P = 0.01$) in the severely obese Laron syndrome females.

No correlation was observed between adiponectin levels and serum cholesterol, insulin and HOMA-IR levels.

16.6 IGF-I Treatment

In the IGF-I treated Laron syndrome girls, both the serum total and HMW adiponectin were elevated similar to that in the adult untreated female patients, so were also the leptin levels.

16.7 Conclusions

The unexpected findings in our study was that in contradistinction to many observations that in obesity, adiponectin levels are low (Arita et al. 1999; Stefan et al. 2002; Kern et al. 2003; Ryan et al. 2003), in patients with Laron syndrome, both serum total and HMW adiponectin are high. Our findings are supported by similar observations in the GH receptor KO mice (Laron mice) (Berryman et al. 2004; Nilsson et al. 2005) (Chap. 51). The hypersecretion of adiponectin from adipocytes, with absent GH receptor function, could be attributed to loss of direct suppression of synthesis and/or secretion by the absence of hGH activity, IGF-I, or low IGFBP-3, all characteristic for this syndrome (Laron 2004). As high adiponectin is associated with a lower risk for cancer development (Wolf et al. 2006; Barb et al. 2007), one may speculate whether the high adiponectin levels contribute to the mechanism that protects Laron syndrome patients from malignancies (Shevah and Laron, 2007) (see Chap. 40).

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17 Muscle Force and Endurance in Untreated Adult and IGF-I Treated Children with Laron Syndrome

Zvi Laron

Core Message

› Both children with Laron syndrome (LS) and those with congenital isolated GH deficiency (IGHD) were found to have reduced muscle force and endurance. Replacement treatment with IGF-I of LS patients was less effective than that with hGH in IGHD patients in restoring muscle force.

17.1 Introduction

Growth hormone (GH) and insulin-like growth factor-I (IGF-I) are important contributors to the development and function of the muscular system (Berne and Levy 1988). A limited number of studies have been performed on muscular force in adults with GH deficiency and on the effect of GH replacement therapy on the muscular system (Rutherford et al. 1995; Sartorio et al. 1995). In the literature search, we found no previous studies on the muscle force in Laron syndrome patients, nor of lean body mass determination. We have performed two studies to investigate the above parameters.

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17.2 Subjects

Eight untreated Laron syndrome adult patients (3 males and 5 females) and 5 children (3 males and 2 females) tested 3–4 years after initiation of IGF-I replacement therapy (FK780, Fujisawa Pharmaceuticals Ltd., Osaka, Japan), 150 μg/kg once daily s.c., were enrolled (Brat et al. 1997). As controls for each patient served 3 age- and sex-matched healthy subjects as well as a group of 4 boys with isolated GH deficiency (IGHD) who were examined before and after 1–2 years of hGH replacement therapy. The pertinent clinical data of the 3 groups of patients are shown in Tables 17.1–17.3.

17.3 Methods

17.3.1 Evaluation of Motor Performance

A computerized myometric system (developed in the Motor Assessment Laboratory, Neurology Department, Beilinson Medical Center, Israel) (Ziv et al. 1992; Ziv et al. 1993) was used. This system performs rapid, online measurement of isometric force from all major muscle groups and comprises a mechanical subunit for stabilization of the examined limb in the required standard position. Force was measured by an electron strain gauge (Tedea Inc., Israel), attached by a strap to the examined limb, and data were transferred to a computer for online analysis. The force/time curve was plotted, and two parameters were calculated: (a) *peak force* and (b) *endurance*, defined as the ability of the patient to maintain force through time (Ziv et al.

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Table 17.1 Pertinent clinical data, muscle force, and endurance of untreated adults with Laron syndrome

Pt No.	Age (years)	Sex	Height cm	Muscle force[a] Upper limb	Muscle force[a] Lower limb	Endurance[a] Upper limb	Endurance[a] Lower limb
1	62	Male	142	43	42	63	66
2	45	Male	120	38	31	67	61
3	42	Female	120	35	38	49	46
4	39	Male	120	43	42	55	54
5	38	Female	129	57	57	49	58
6	38	Female	112	56	50	54	42
7	36	Female	137	46	61	55	42
8	33	Female	117	38	39	42	42
	Mean			44.5	45	54	52.8
	±SD			18.1	10.1	7.9	7.5

[a]As % of healthy matched controls
Modified from Brat et al. (1997)

Table 17.2 Pertinent clinical data, muscle force, and endurance in children with Laron syndrome during IGF-I treatment

Pt No.	Age (years)	Sex	Pubertal stage	Height cm	Height SDS	IGF-I treatment Years	Muscle force[a] Upper limb	Muscle force[a] Lower limb	Endurance[a] Upper limb	Endurance[a] Lower limb
1	6	Male	P1	87	−5.1	3.5	54	55	89	87
2	8	Male	P1	95	−5.2	3.5	69	61	78	85
3	13	Female	P3	135	−3.8	4	77	44	89	87
4	16	Male	P2	129	−6.8	4	53	69	75	83
5	17	Female	P4	135	−3.8	4	98	95	97	90
	Mean ±SD						70.2 18.5	64.8 19.1	85.6 8.9	86.4 2.0

[a]As % of healthy matched controls
Modified from Brat et al. (1997)

Table 17.3 Muscle force of four boys with IGHD before and after 24 months of hGH treatment

Pt No	Age (years)	Pubertal stage	Sex	Muscle force before treatment[a] Upper limb	Muscle force before treatment[a] Lower limb	24 months of treatment Pubertal stage	24 months of treatment Upper limb	24 months of treatment Lower limb
1	5	P1	Male	48	48	P1	71	88
2	11	P1	Male	83	78	P3	96	78
3	13	P1	Male	49	80	P3	100	114
4	13	P2	Male	43	43	P4	79	90
Mean ± SD				55.75	62.25		86.5	92.5

[a]As % of healthy controls
Modified from Brat et al. (1997)

1992). It was calculated according to the following formula:

$$\text{Endurance (\%)} = \frac{\text{area under curve}}{\text{peak force (kg) x time(s)}} \text{ x } 100$$

to provide measurement of endurance without dependence of peak force exerted.

17.3.2 Study Protocol

Following stabilization of the performance in each patient, sequential examination of eight muscle groups was carried out: biceps, triceps, hamstrings, and quadriceps bilaterally. For each parameter examined, the average of the 2 best trials out of 5 performed was calculated and stored. The final test result was defined as the average value obtained from the examined muscle groups. Patients were instructed to activate the examined muscle with maximal speed and force on the appearance of a "go" light sign, and to maintain this activity throughout the trial. All tests were performed by the same person (O.Brat).

17.4 Results

Table 17.1 shows the muscle force and endurance of 8 untreated adult patients with Laron syndrome. Muscle force and endurance in the upper and lower limbs revealed a marked deficit in these patients (approximately 50% of the normal matched controls). Table 17.2 shows the muscle force and endurance of 5 children and adolescents with Laron syndrome after 3.5–4 years of IGF-I treatment (150 μg/kg/day). It is evident that in the treated Laron syndrome patients, muscle force varied from 44 to 77% of normal and in the oldest patient with a puberty stage, P4 muscle force was normal. The mean achievement was 60–86% of normal.

Table 17.3 shows the muscle force in the four children with IGHD (isolated GH deficiency due to GH-1 gene deletion) before, and after 10 and 24 months of hGH treatment, at a dose of 0.6 U/kg/week s.c. (Norditropin, Novo/Nordisk, Denmark or Biotropin, Biotechnology, Israel).

17.5 Discussion

It was seen that the mean muscle force in untreated adult Laron syndrome patients was lower than that in the untreated IGHD children and that the 3–4 year IGF-I treated children had a muscle force similar to that in IGHD children treated for 10 months. However, even after 3–4 years of IGF-I treatment, the muscle force did not reach the level registered after 2 years hGH treatment (see also IGF-I effect on growth) (Chap. 45).

The percent of "endurance" in the untreated adult patients was higher than that of untreated Laron syndrome children, but lower than that of the IGF-I treated children.

17.6 Conclusions

Congenital IGF-I deficiency, whether primary or secondary due to hGH deficiency, causes a lowering in muscle force and performance.

The findings of similar but less striking results in five children with Laron syndrome, treated with IGF-I for 3–4 years compared to IGHD children treated by hGH, may be explained by the more severe muscular underdevelopment of the system in this disease (Laron 1993; Laron 1995), which also contributes to the late motor development in infancy in Laron syndrome children.

Another explanation may be a slower action of IGF-I than of hGH on the muscular tissue or even the need of a synergistic effect. Our findings complement the reports of Christiansen et al. (1990), Cuneo et al. (1991) Rutherford et al. (1995), and Cuneo and Wallace (2005) who studied muscle strength and performance in adults with GH deficiency, mostly of acquired origin, and found that GH treatment improved the low exercise performance. Our findings explain the complaints of untreated Laron syndrome patients of weakness, fatigue, and impaired physical performance (Shurka and Laron 1975).

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18 Bone Mineral Density in Untreated and IGF-I or Alendronate-Treated Patients with Laron Syndrome

Zvi Laron

Core Message

› Bone mineral density (BMD) of the lumbar area and femoral neck measured by DEXA showed reduced values in untreated patients with Laron syndrome, however calculation of the volumetric density (BMAD) revealed normal values. IGF-I and alendronate treatment improved the low BMD values. Whether low BMD in IGF-I and hGH deficient patients should be treated remains controversial.

18.1 Introduction

Longitudinal bone growth ceases after final height is achieved. Bone mineral density (BMD), however, continues to increase until peak bone mass (PBM) is reached around the third decade. Even after PBM is achieved, remodeling of bone continues throughout life. Growth hormone and IGF-I together with the sex hormones not only cause bone growth but also act to maintain the health of the skeletal and mineral homeostasis (Glorieux et al. 2003).

Reduced BMD has been described in adult patients with GH deficiency (Holmes et al. 1994; Ohlsson et al. 1998), and long-term GH treatment increases the BMD in hypopituitary patients (Kann et al. 1998; Sneppen et al. 2002). However, these results are debatable since GH exerts biphasic effects on bone (Vandeweghe et al. 1993). It was therefore of interest to investigate the BMD in our patients with Laron syndrome with congenital IGF-I deficiency.

We performed three studies.

18.2 Study 1

18.2.1 Subjects and Methods

Five adult Laron syndrome patients (one male, four females) aged 28–40 years participated in this study. Bone densitometry was performed using a Lunar DPX Densitometer. BMD was evaluated before and after 9 months of IGF-I treatment (FK-780; 120 μg/kg/once daily s.c. (Laron and Klinger 1994).

18.2.2 Results

The mean (±) SD lumbar (L2-L4) BMD of 0.88 ± 0.08 fell below the normal range (Figs. 18.1 and 18.2). No significant change was observed during the 9 months IGF-I treatment, despite a rise in serum phosphor procollagens and alkaline phosphatase (see Chap. 42).

Having interpreted the above findings as osteopenia, we performed a second study.

18.3 Study 2

Twelve untreated adult patients with Laron syndrome (seven females and five males) were enrolled (Benbassat et al. 2003). As controls served ten healthy subjects

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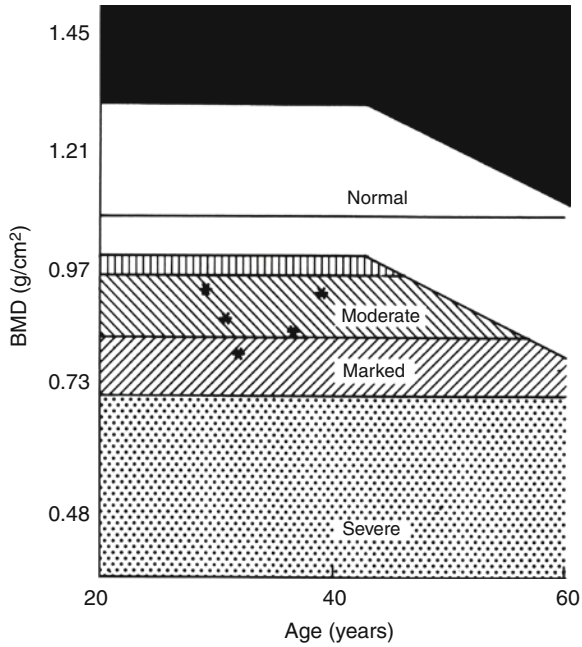

Fig. 18.1 Bone mineral density (BMD) by DEXA of the lumbar spine (L2-L4) in five adult patients with Laron syndrome. Reproduced with permission from Laron (1999)

matched for age and sex. The clinical characteristics of the two groups are shown in Table 18.1. In addition, we investigated three young Laron syndrome patients (age 22.3 ± 2.1 years) who had been previously treated by IGF-I for a mean period of 3 years until 6 years before the study.

18.3.1 Methods

Bone mineral content (BMC) and BMD were measured using DEXA (Lunar DPX, Lunar Radiation Corp., Madison, WI) at the lumbar spine (L2-L4) and femoral neck, in the posteroanterior projection. Age-and gender-specific BMD, Z scores, and T scores were provided by Lunar. Estimated volumetric density (bone mineral apparent density [BMAD] was calculated as reported by Katzman et al. (1991), using the following formula:

Lumbar spine BMAD = BMC/area$^{3/2}$

Femoral neck BMAD = BMC/area^{2}

Values are expressed as mean ± SD. Statistical analysis was performed using the *t* test.

18.3.2 Results

Comparison between the groups yielded significantly lower BMD density values in the patients with Laron syndrome than in the control subjects for both the

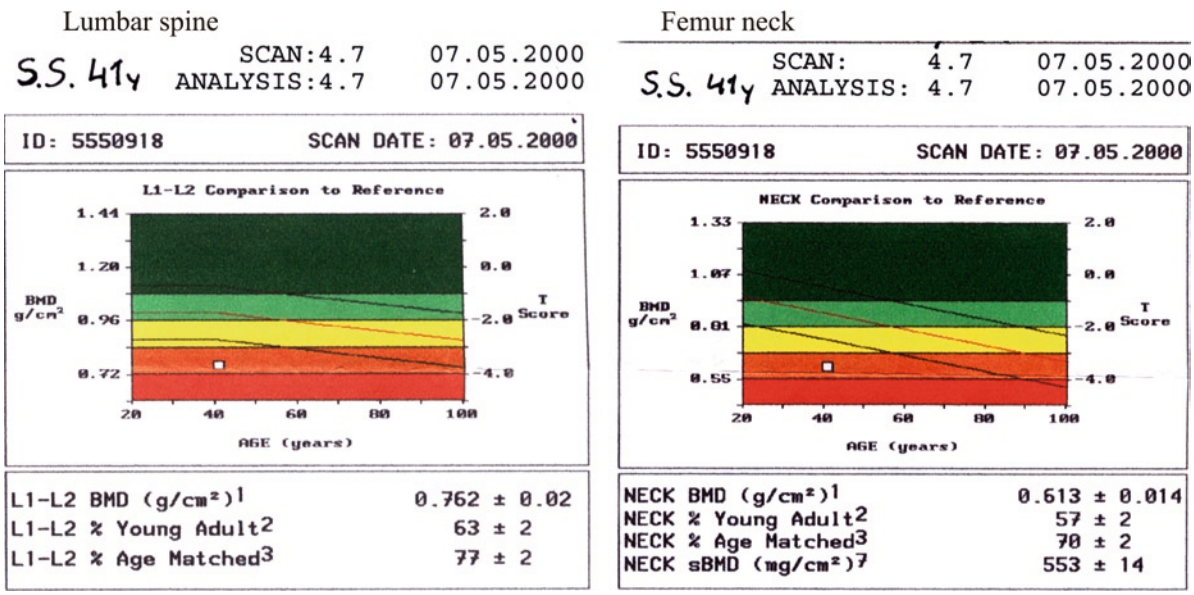

Fig. 18.2 BMD of the lumbar spine and femur neck of a 41-year-old male with Laron syndrome. Reproduced with permission from Laron (1999)

Table 18.1 Pertinent clinical data of patients with Laron syndrome compared to healthy control subjects

	Laron syndrome ($n=12$)	Control ($n=10$)
Age (year)	43.9 ± 8.5	44.8 ± 9.2
Height (cm)	123.7 ± 11.0	165.5 ± 8.3
Weight (kg)	44.7 ± 14.7	71.7 ± 12.5

Table 18.2 Area BMD (g/cm^2) and bone mineral content (BMC) assessed by DEXA in patients with Laron syndrome compared to healthy control subjects

	Laron syndrome group	Healthy controls
Lumbar spine		
BMD	0.968 ± 0.201[a]	1.166 ± 0.182
BMC (g)	23.43 ± 10.8[b]	52.3 ± 7.70
Area (cm^2)	23.34 ± 5.47[b]	42.48 ± 4.73
T score	−2.07 ± 1.64[a]	−0.33 ± 1.48
Z score	−1.14 ± 1.53[a]	0.17 ± 0.78
Femoral neck		
BMD	0.729 ± 0.120[b]	0.963 ± 0.126
BMC (g)	2.77 ± 0.88[b]	5.01 ± 0.79
Area (cm^2)	3.75 ± 0.83[b]	5.05 ± 0.41
T score	−2.33 ± 0.89[b]	−0.35 ± 0.88
Z score	−1.79 ± 0.85[b]	0.14 ± 0.50

Mean ± SD
[a]$p<0.02$ vs. control group
[b]$p<0.01$ vs. control group
Modified from Benbassat et al. (2003)

Table 18.3 Calculated volumetric bone mineral apparent density (BMAD) (in g/cm^3) in patients with Laron syndrome compared to healthy control subjects

Site	Laron syndrome group $n=12$	Control group $n=10$	p
Lumbar spine	0.201 ± 0.02	0.178 ± 0.03	NS
Femoral neck	0.191 ± 0.04	0.192 ± 0.02	NS

Modified from Benbassat et al. (2003)

lumbar spine and femoral neck (Table 18.2). Differences in mean T scores between male and female patients with Laron syndrome were significant at the lumbar spine (−1.84 vs. −2.23, respectively; $p<0.5$) but not at the femoral neck (−2.35 vs. −2.32, respectively). Mean BMC and projected area at the lumbar spine were

Table 18.4 Characteristics and densitometric measurements (BMD and BMAD) in three patients with Laron syndrome previously treated with IGF-I

	Mean ± SD
Age (year)	22.3 ± 2.1
Height (cm)	139 ± 4.7
Weight (kg)	54.6 ± 7.7
BMD for lumbar spine (g/cm^2)	1.135 ± 0.162
T score	−0.57 ± 1.40
Z score	−0.10 ± 1.21
BMAD for lumbar spine (g/cm^2)	0.225 ± 0.032
BMD for femoral neck (g/cm^2)	0.932 ± 0.020
T score	−0.60 ± 0.52
Z score	−0.43 ± 0.23
BMAD for femoral neck (g/cm^2)	0.224 ± 0.001

BMAD, volumetric estimate

23.4 g and 23.3 cm^2, respectively, in Laron syndrome subjects, and 52.3 g and 42.5 cm^2 in the controls.

Estimated volumetric bone mineral density (BMAD) for the two groups is shown in Table 18.3. The Laron syndrome group had a higher BMAD than the control group at the lumbar spine, but the difference did not reach statistical significance, nor was there a difference in the femoral neck.

The three Laron syndrome patients previously treated with IGF-I for a mean period of 3 years were 15 cm taller on the average than the untreated patients (139 ± 4.7 cm) and had a normal areal BMD 6 years later (L2-4=Z score −0101.2, and femur=−0.43 ± 0.23). Their BMAD at the lumbar spine and femoral neck was also higher than that of the untreated Laron syndrome patients and the control group (lumbar spine, 0.225 vs. 0.201 and 0.189, respectively; $p<0.01$; femoral neck, 0.224 vs. 0.201 and 0.196, respectively; $p<0.01$) (Table 18.4).

18.4 Discussion

Our study showed that untreated patients with Laron syndrome have low BMD when measured by the standard DEXA technique. However, after adjustment for areal BMD using volumetric estimates derived from DEXA measurements, their BMAD was similar to that of normal subjects. These results agree with data

reported by Bachrach et al. (1998), who studied 11 patients with Laron syndrome (eight females and three males; mean age, 30 years) belonging to the Ecuadorian cohort. These authors confirmed their data by histomorphometric analysis.

On the basis of our recalculated volumetric data of BMAD and that of Bachrach et al. (1998), the possibility arises that in effect, the BMD in adult Laron syndrome patients is normal. This may be in accordance with the low fracture rate observed in our cohort of Laron syndrome patients. However, the higher BMD observed in our three previously IGF-I-treated patients compared with the untreated ones indicates an IGF-I effect on BMD as do GH-deficient patients to hGH therapy (Kann et al. 1998; Sneppen et al. 2002). It should be remembered that areal BMD (g/cm^2) as measured by DEXA is not a true density, but rather a 2D output representing the attenuation of photons passing through the body area within the image plane. The uncertainty of the theoretical calculation of BMAD led us to a third investigation to test whether bisphosphonate (alendronate) therapy improves the BMD in adult LS patients (Eshed et al. 2006).

18.5 Study 3

18.5.1 Subjects

Seven IGF-I-untreated adult Laron syndrome patients (two males, seven females) with a mean age of 40.8 ± 7.6 years (range, 26–49) and a mean height of 123 ± 0.1 cm (range, 12–18 cm) were included in this study.

18.5.2 Methods

BMD was measured at the lumbar spine and femoral neck using DEXA (Lunar DPX, Lunar Radiation Corp., Madison, WI, USA) at baseline and at 6 and 12 months of treatment. The manufacturer provided the normal reference values for BMD. Estimated volumetric density (BMAD) was calculated as reported by Katzman et al. (1991). Serum alkaline phosphatase and calcium phosphate were measured by autoanalyzer (Roche Hitachi 747, Roche Diagnostics, Basel, Switzerland).

Serum parathyroid hormone was assessed by immunoradiometric assay (Nichols Diagnostics, San Juan Capistrano, CA, USA) and $25(OH)_2D_3$ vitamin D by radioimmunoassay (Dia Sorin, Stillwater, MN, USA). A value of below 16 ng/mL is considered to denote deficiency.

Urinary deoxypyridinoline (DPD) was measured by Immulite analyzer from the first or second morning void. The normal range for adults in our laboratory for alkaline phosphatase is 37–115 U/L, and for urinary DPD, 3–7.4 nM/mMcreatinine for premenopausal women and 2.3–5.4 nM/mMcreatinine for men aged 22–55 years.

18.5.3 Treatment

Alendronate (Fosamax, MSD, USA) 70 mg once weekly was administered for 1 year. In addition, patients were prescribed 1,200 mg elemental calcium and 800 units of vitamin D per day. BMD was measured before and after 6 and 12 months Fosamax treatment.

18.5.4 Statistics

Statistical calculations were performed with the SigmaStat 2.03 (Systat Software Inc., Point Richmond, CA, USA) computerized program. Results are expressed as mean ± SD unless otherwise indicated. For comparison of results before and after intervention, the paired Student's *t*-test was used. A p value equal or less than 0.05 was considered significant.

18.5.5 Results

Considering BMD as an indicator and mean basal BMD of 0.843 ± 0.06 g/cm^2 (T score −2.9 ± 0.5) at the lumbar spine and 0.734 ± −0.11 g/cm^2 (T score −2.2 ± 0.09) at the femoral neck, the Laron syndrome patients suffered from osteopenia. Twelve months treatment with alendronate 70 mg once/weekly led to an increase of 5.3% in BMD ($p < 0.038$) at the femoral neck. There was a similar trend at the lumbar spine, but the difference was not statistically significant (2.3%, $p = 0.34$). Mean total alkaline phosphatase decreased by 14% from normal range at baseline ($p = 0.007$). Urinary DPD levels, which

Table 18.5 Serum vitamin D $25(OH)_2D_3$ levels of adult patients with Laron syndrome

Patient		Sex	Age $years^{months}$	$25(OH)_2D_3$ ng/mL
1	BS	F	40^{11}	17.0
2	BR	M	28^{7}	15.8
3	MP	F	40^{9}	20.1
4	DS	F	21^{10}	17.5
5	YR	F	46^{11}	14.7
6	CE	F	51	15.0
7	ML	M	25^{1}	15.2
8	SR	F	44^{1}	11.2
9	SSh	M	41^{9}	11.9
10	SSi	M	46^{7}	23.1
11	CS	F	49^{11}	21.2

F female; *M* male
Reference range: 10–44 ng/mL

were elevated at baseline (10 ± 2.3 nM/mMcreatinine), showed a nonsignificant change during treatment. No relevant changes were observed in serum calcium, phosphate, and parathyroid hormone levels or in kidney and liver function.

The serum vitamin D $25(OH)_2D_3$ levels for each patient before the initiation of alendronate therapy are shown in Table 18.5. It is evident that five Laron syndrome patients suffer from vitamin D deficiency; the remaining six have borderline or low normal levels. These findings were the reason for adding 800 U/day throughout the year of bisphosphonate therapy, and recommended for thereafter.

18.6 Conclusion

Considering the uncertainty that the calculated BMAD indeed expresses the real BMD and that the BMD (by DEXA) with its limitations, repeatedly showed that patients with Laron syndrome have various degrees of osteopenia, and the fact that IGF-I replacement therapy and alendronate improved the BMD, one is left with the impression that treatment for low BMD could be considered especially in adult patients with Laron syndrome as age advances after menopause and andropause. This approach is supported by the finding that in adult patients with childhood onset GH deficiency, the BMD decreased after discontinuation of hGH treatment and improved with retreatment (Benbassat et al. 1999). Whether the low serum levels of vitamin D are linked to the longstanding IGF-I deficiency or are due to the lifestyle of the patient remains to be established.

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Muscle–Bone Relationship in Patients with Laron Syndrome

19

Yoram Epstein, Amir Hadid, Zvi Laron, Daniel S. Moran, and Nahum Vaisman

Core Message

› Investigation to determine the muscle-bone relationship in Laron syndrome patients using peripheral quantitative computerized tomography (CT) and DEXA revealed reduced lean body mass (LBM) and a normal cortical bone density.

19.1 Bone Characteristics

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19.1.1 Introduction

Bones adapt their strength to the mechanical forces they undergo (Biewener 1990). Physiologically, bones are loaded by compression as well as bending (Biewener 1990; Burr et al. 1996). The bone resistance to these forces is related to bone density and bone geometry (cross sectional area and the area moment of resistance). Peripheral Quantitative Computerized Tomography (pQCT) enables to measure tissue content (bone, muscle, fat areas, and densities) and geometry. Consequently, the density-weighted area moment of resistance (strength-strain index – SSI), an index for bone strength, can be calculated (Wilhelm et al. 1999). This index is calculated for the anteroposterior axis (SSI_x) and the lateral axis (SSI_y). It follows that the greater the SSI, the greater the bone resistance for bending.

Laron syndrome is an autosomal recessive disorder characterized by an insensitivity to growth hormone (GH) and IGF-1 deficiency, caused by defects of the GH receptor. The result is a very short stature and short bones (Laron 2004).

The present study was conducted to investigate bone characteristics and to assess muscle/bone relationship of the tibia of Laron syndrome patients in comparison to normal height individuals.

19.1.2 Subjects

Four untreated adult males with Laron syndrome and one untreated male with congenital IGHD, mean age 45 ± 10 (range: 29–55 years) agreed to be studied. As the IGHD and Laron syndrome patients showed clinical and laboratory similarities, they were analyzed together.

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19.1.3 Method

The patients were examined after receiving the approval of the Human Use Committee of the Sheba Medical Center. Each participant was briefed on the procedure and voluntarily signed a consent form. The Laron syndrome patient data was compared with the values obtained from a database of healthy, normal-height subjects.

Peripheral quantitative computed tomography (Stratec/Medizintechnik XCT 2000, Pforzheim, Germany) was used to measure bone characteristics and to assess muscle/bone relationship of the tibia. Patients were positioned on a chair with their leg extended through the scanning cylinder and were asked to maintain a convenient and stable position for the duration of the procedure (11–14 min) (Fig. 19.1). An initial scout scan was conducted to identify the distal end plate of the tibia (utilizing a scan speed of 40 mm/s). Following this, the tibia was scanned (single axial slices of 2.2 mm thickness, voxel size 0.5 mm) at a speed of 30 mm/s at 4% (T-dist), 38% (T-cort), and 66% (T-mid) proximal to the distal medial malleolus. These sites have been determined to be the best sites to analyze trabecular bone, cortical bone, and muscle area, respectively, in the tibia; the T-mid level is the relevant section for the analysis in this study (Fig. 19.2). The maximum absorbed peripheral dose was approximately 5 mrem per scan (a total exposure of 15 mrem at each time data collected).

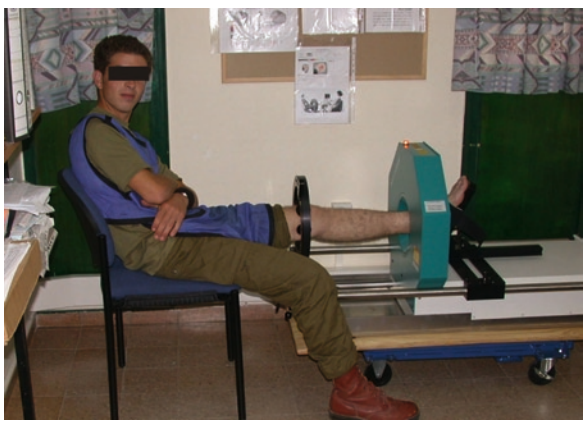

Fig. 19.1 Positioning the lower limb in the peripheral quantitative computerized tomography (pQCT) apparatus

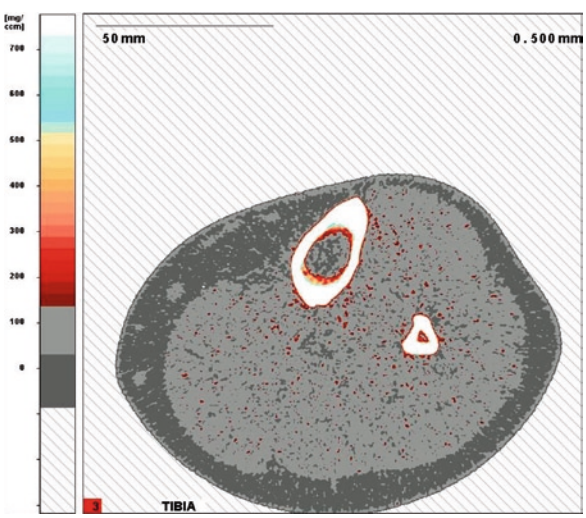

Fig. 19.2 A pQCT scan taken at 66% tibia's length proximal to the medial malleolus (T-mid). The picture illustrates normal bone cortical density and high fat to muscle area ratio

Measured parameters at each site included bone mineral content (BMC) (mg), bone area (total, cortical, and trabecular, mm^2), bone mineral density (BMD) (total, cortical, and trabecular, mg/cm^3), muscle area (mm^2), measures of bone strength (polar moment of inertia, section modulus, and SSI), and fat area.

19.1.4 Results

Table 19.1 summarizes the results, at the 66% tibia's length (T-mid), obtained in the four Laron syndrome and one IGHD patients compared to normal height individuals. The anthropometric measures depict the short stature in the patients with congenital IGF-I deficiency. Muscle area in the tibia of Laron syndrome patients was lower than in normal height individuals ($p<0.001$), but cortical bone density and all other anatomical measures were within the normal range of normal height subjects. On the other hand, the physical properties – the SSI along the *X* and *Y* axes (SSI_x and SSI_y, respectively) were significantly lower than that found in healthy subjects ($p<0.0001$).

19.1.5 Discussion

The present study enhances our understanding on the physiological and mechanical properties of the skeletal system of Laron syndrome patients. Using pQCT, it was evident that cortical bone density is within the normal range of normal-height individuals. Currently, bone density is assessed by X-ray absorptiometry

Table 19.1 Anatomic characteristics and bone physical properties of four untreated adults with Laron syndrome and one untreated IGHD patient compared to normal height individuals

	Patients	Healthy controls[a]	*p*
Age (years)	45 (10)		
Weight (kg)	47.0 (12.5)		
Height (cm)	133 (9)		
Tibia length (cm)	28.4 (4.7)	41.3 (3.4)	<0.0001
Cortical bone density (mg/cm^3)	1,119 (42)	1,100 (100)	NS
Muscle area (mm^2)	4,177 (1,199)	6,700 (1,350)	<0.001
Fat area (mm^2)	1,509 (892)	1,800 (930)	NS
Bone/muscle ratio	5.94 (1.56)	6.00 (1.00)	NS
Fat/muscle ratio	39.1 (23.60)	30.0 (12.0)	NS
SSI_x (mm^3)	940 (310)	2,270 (340)	<0.0001
SSI_y (mm^3)	740 (270)	1,690 (270)	<0.0001

[a]Young healthy Israeli population
Data presented as mean ± SD

(DEXA). Although this method is widely used in both clinical and research settings, it has some inherent limitations: (1) BD is averaged on a 2D projection, but presented per volume unit; (2) the geometric component of the bone is ignored; (3) measures are inaccurate, since they are strongly influenced by the composition of the soft tissue surrounding the bone (Russo et al. 2003). These limitations are overcome by pQCT. In the Laron syndrome patients, the normal cortical bone density values are associated with a low fracture load. These low SSI values might reflect a higher vulnerability of Laron syndrome patients to fractures. However, these values should be evaluated in relation to body dimensions. The longer tibia of individuals with normal height is inpropotionally wider than the shorter tibia of Laron syndrome patients, probably in order to make the bone more resistant to bending (Rittweger et al. 2000). In contrast, the lower body dimensions of Laron syndrome patients exert lower forces on the skeletal tissue, resulting in a narrower bone (see Chap. 20), which yields low SSI values. Thus, the low SSI values in the case of Laron syndrome patients do not necessarily seem to reflect bone pathology.

A somewhat reduced muscle mass area was noted in the Laron syndrome patients, which can be explained by the inherent IGF-I deficiency and by a relatively sedentary life style and reduced physical activity. These findings fit the data reported in Chap. 17.

19.2 Lean Body Mass (LBM) In Patients with Laron Syndrome

Zvi Laron and Nahum Vaisman

19.2.1 Subjects

The lean body mass (LBM) representing muscle and bone mass was studied in nine untreated adult (three males and six females) Laron syndrome patients and four IGF-I-treated girls. (Ginsberg et al. 2009).

19.2.2 Methods

LBM was determined by dual energy X-ray absorptiometry (DEXA) (Lukaski 1987).

19.2.3 Results

The LBM of the untreated adult Laron syndrome patients is shown in Table 19.2 and that of the four IGF-I-treated girls in Table 19.3. Duration of replacement therapy ranged between 5 and 14 years.

Despite the variations between patients, the LBM in untreated and even IGF-I-treated patients was significantly smaller than that in the age-matched normal-sized healthy controls (75–86%).

19.2.4 Discussion

The findings can be explained by the longstanding IGF-I deficiency which causes underdevelopment of the muscular and skeletal system (see Chaps. 17 and 20).

Table 19.2 Lean body mass (LBM) of nine untreated adult patients with Laron syndrome

Pt. no.	Age (years)	Gender (F/M)	Height (cm)	Weight (kg)	LBM (kg)	LBM % BWt
1	53	F	122	48	19.05	39
2	49	F	112	42	15.9	38
3	48	F	130	62	20.68	33.4
4	45	M	116	51	17.07	33.4
5	43	F	120	44	18.68	42
6	43	F	136	60	23.45	39
7	41	M	139	66	35.3	53
8	38	F	128	36	14.5	40
9	28	M	141	57	29.5	51

BWt body weight
Modified from Ginsberg et al. (2009)

Table 19.3 LBM of four IGF-I-treated girls with Laron syndrome

Pt. no.	Age (years)	Gender (F/M)	Height (cm)	Weight (kg)	LBM (kg)	LBM % BWt
1	18	F	140	73	25.9	35
2	15	F	123	40	18.4	46
3	7^{6}	F	105	20	10.6	53
4	6^{10}	F	94	13.2	7.3	55

BWt body weight

19.2.5 Conclusions

The two studies on the muscle/bone relationship demonstrated a reduction of the muscle mass and force and an increase in the fat–lean body ratio. The CTs of the lower limb revealed a normal bone trabecular structure supporting the view that Laron syndrome patients may not need alendronate or similar treatment. Of note is the improvement of LBM during IGF-I replacement therapy.

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Imaging Findings in Laron Syndrome

20

Liora Kornreich and Zvi Laron

Core Message

› The imaging findings by the various modalities (X-rays, CT, and MR) in our cohort of Laron Syndrome patients, during 50 years of follow-up, are described. The face, the orbits and the base of skull are small with underdeveloped paranasal sinuses and mastoids. The skull is relatively large for the small body. Frequently occurring is the anomaly of the dens of the os odontoideum type. Reduced dimensions of the larynx and oropharynx result in propensity for sleep apnea .The brain presents no specific pathological findings, and no abnormalities of the pituitary gland are detected. In the spine and appendicular skeleton every depicted bone is proportionally small. In the cervical spine this results in spinal stenosis. In the lumbar spine, the spinal canal is narrow as well. There is a tendency for early osteoarthritic changes. No signs of osteopenia are detected. There is marked retardation of bone age.

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20.1 Introduction

Laron syndrome (LS) constitutes a unique model permitting us to study the effects of congenital and long-term deficiency of both GH and IGF-1, particularly since nowadays GH deficiency is diagnosed and treated at an early age, whereas the diagnosis of LS is frequently delayed.

Despite the fact that there are several hundred patients diagnosed worldwide with LS, imaging data are extremely scant. In the 64 LS patients constituting the Israeli cohort, a wide range of imaging procedures were performed in the course of a 50-year period. Children routinely underwent serial X-rays of the hand for estimation of bone age, and in past years, radiographs of the skull for evaluation of the sella turcica. Many of the procedures were performed as part of the routine diagnostic evaluation of these patients for various clinical complaints, such as musculoskeletal pain, limitation of mobility, cough, and following trauma. One patient underwent intravenous pyelography for investigation of renal colic. Two patients were referred by the Emergency Department for computed tomography (CT) examination of the brain (without contrast injection) following trauma. Magnetic resonance (MR) imaging of the brain was performed in 14 patients (10 adults, 1 treated child, 3 untreated children); the initial examinations were done for investigation of mental retardation, of paresthesias, and of gait instability, but in view of the remarkable findings, additional patients were referred for screening, as well as for MR of the cervical spine (performed in ten patients).

Three patients underwent MR of the lumbar spine for the evaluation of back pain. In one patient, MR examination of both knees was available. Ultrasonography was performed only of the liver (see Chap. 14). We did not perform sonography of the abdominal organs as described by Backeljauw et al. 2001.

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20.2 Methods

All the radiographs were performed according to standard methods. MR examinations were performed on 0.5 and 1.5 T equipment, on different machines. Spin Echo T1 and T2 weighted images were obtained. No contrast medium was injected.

Herewith described are the imaging findings by the various modalities (X-rays, CT, and MR) in our cohort of patients.

20.3 Skull

In LS patients the cranium is small, particularly the face, with underdeveloped paranasal sinuses. The calvarial bone is thin with delayed closure of sutures and synchondroses.

On clinical examination, craniofacial abnormalities include subnormal head circumference (Laron 1999a) and underdevelopment of the facial bones (Konfino et al. 1975). Head size is large relative to the short stature. The physiognomy is characteristic: prominent forehead, decreased vertical dimension of the face, hypoplastic nasal bridge, shallow orbits, and small maxilla and mandible (Laron 1999a, b, 2004; Laron et al. 1968).

Lateral radiographs of the skull demonstrated this typical appearance, with frontal bossing and relatively concave nasal bridge; the calvarial vault was thin (Fig. 20.1).

Cephalometric measurements on anteroposterior (Scharf and Laron 1972) and lateral (Konfino et al. 1975) radiographs of the skull in children with IGF-1 deficiency demonstrated that the base of the skull was significantly narrower and shorter than that in normal children (Fig. 20.2) (see Chap. 9). CT studies demonstrated crowding of the vascular structures and the foramina in the skull base due to a diminished anterioposterior diameter of this region (Fig. 20.3). In this regard, cephalometric studies in treated children with GH deficiency and in acromegalics suggested that GH effects are greatest in the endochondral bone, as evidenced by an increase in cranial base length and growth of the mandibular condyle (Segal et al. 2004).

Another striking feature demonstrated by X-rays of the skull in LS is the absence or underdevelopment of the maxillary and frontal sinuses. This is best shown in the single facial bone radiograph available (Fig. 20.4).

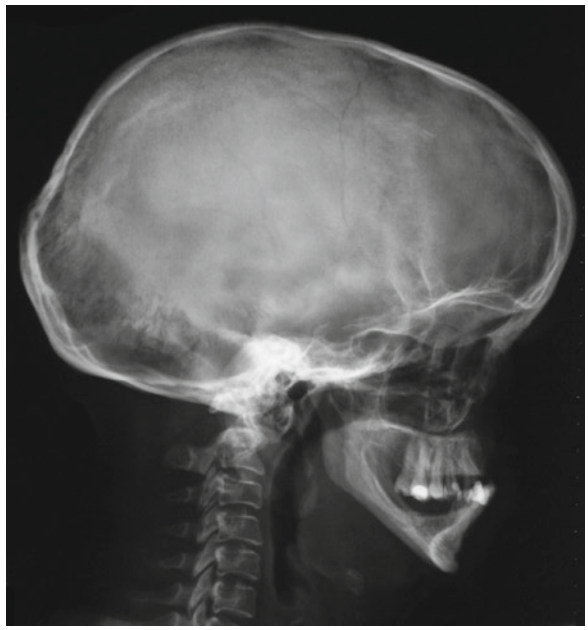

Fig. 20.1 X-ray of the skull, lateral view. A 47-year-old man with Laron Syndrome. The characteristic frontal bossing is seen. The bones of the calvarial vault are thin. The craniocaudal diameter of the face is small, relative to the size of the skull. Note the anomaly of the dens

MR imaging of nine untreated adults permitted qualitative and quantitative evaluation of the nasal sinuses. In seven of them, the frontal sinuses were absent; the maximal anteroposterior and mediolateral diameters of the maxillary sinuses were small in comparison to normal values reported in the literature (Table 20.1; Fig. 20.5). The sphenoid sinus and the mastoids were also underdeveloped (Figs. 20.3, 20.5, and 20.6). All these findings must be attributable to the IGF-I deficiency starting in utero. It is of note that in a treated child, at the age of 9 years the size of the maxillary sinus was very close to that of the adult females with LS (Kornreich et al. 2002a).

Retarded bone age is a well-known manifestation of IGF-1 deficiency. In normal individuals, sutures and synchondroses close by the third decade (Barkovich 2000). In our patients, plain films of the skull showed sutures wide for age and delayed closure of sutures and synchondroses (Figs. 20.2–20.4, 20.7, and 20.8). Moreover, in the radiograph of the facial bones the nasofrontal suture was still visible at the age of 50 years (Fig. 20.4). On the CT scan of patients aged 42 and 49 years, the sutures of the calvaria and synchondroses of

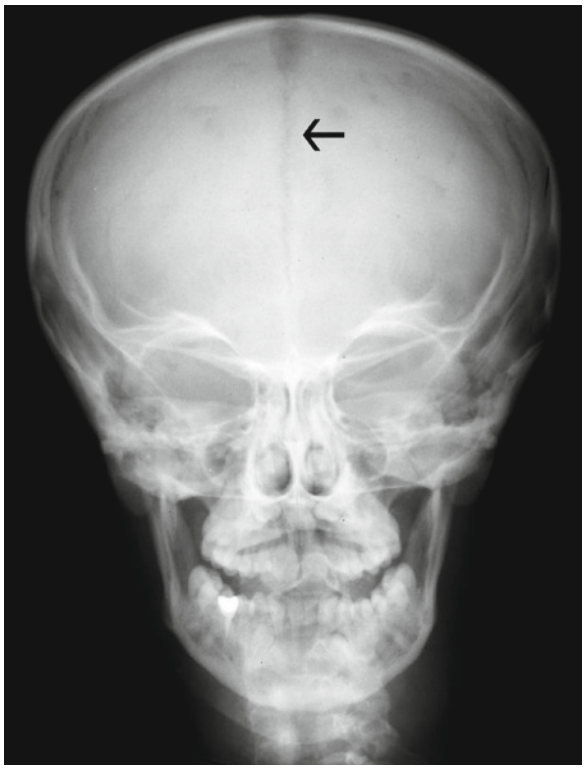

Fig. 20.2 X-ray of the skull, A-P view. A 9-year-old girl, with LS. Note the disproportion between the width of the calvarial vault and the base of skull, which is narrow. The sagittal suture is still open, as a sign of delayed bone age (*arrow*)

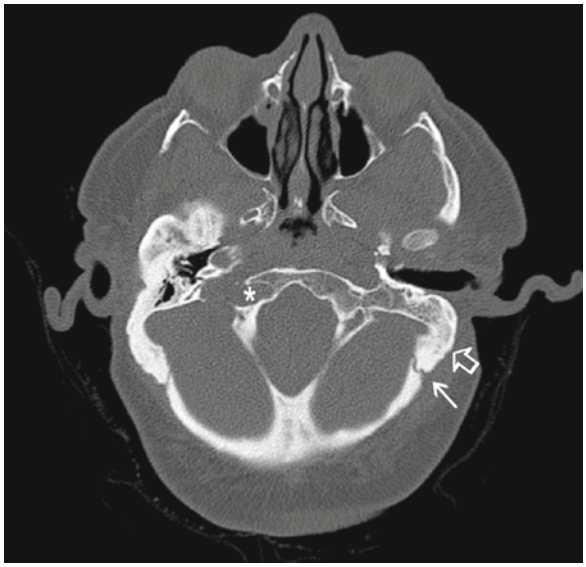

Fig. 20.3 CT of the brain, bone windows. A 49-year-old man with LS. Striking crowding of the foramina of the skull base is seen. The jugular vein causes indentation of the foramen magnum (*asterisk*) due to lack of space. Note the underdeveloped mastoids (*open arrow*), and the open lambdoid suture (*arrow*)

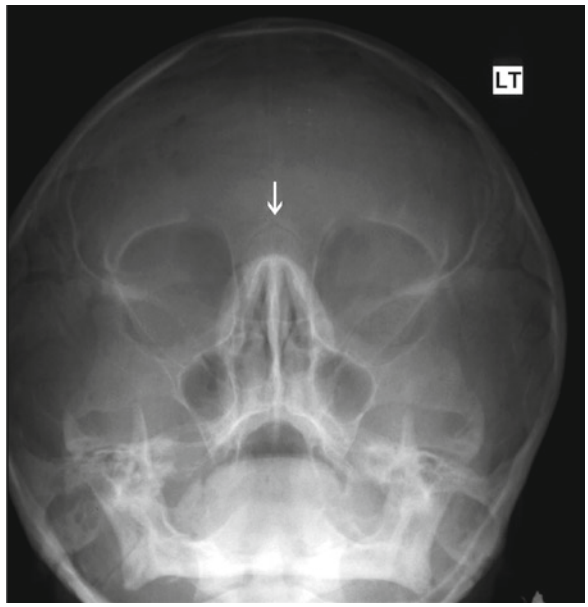

Fig. 20.4 X-ray of the facial bones. A 50-year-old woman with LS. The frontal sinuses are not developed. The maxillary sinuses are small. Note the open nasofrontal suture (*arrow*)

the base of skull were not completely closed despite the advanced age (Kornreich et al. 2002a) (Figs. 20.7 and 20.8). MR imaging showed that the sphenooccipital synchondrosis was still faintly discernible in three patients aged 36, 42, and 46 years (Fig. 20.20d) (Kornreich et al. 2002a). The vault of the skull normally develops by membranous ossification and its growth is triggered by the development of the brain (Silverman et al. 1992); as the child's brain grows, the calvarial bones are displaced and new bone is deposited in the sutural edges. If there is arrest of brain growth, the sutures close (Silverman et al. 1992). In LS, however, the microcephaly is accompanied by delayed closure of the sutures. Yet another sign of delayed maturation was a thin cranial vault (seen both on plain films and MR images), with a diploe relatively underdeveloped for this age group (Figs. 20.1 and 20.6). In the skull base as well, the fatty transformation of the bone marrow was delayed. In six patients the fatty bone marrow of the clivus and the basisphenoid were not appropriate for age (from 36 to 48 years), but were of a lower intensity, compatible with active hematopoietic marrow (Kornreich et al. 2002a) (Fig. 20.6). Overall, these findings point to a disturbance in the process of growth and maturation of the bone marrow in the skull of LS patients.

Table 20.1 Paranasal sinuses size in untreated patients with Laron syndrome

Patient No.	Sex	Age (years)	Frontal sinus		Maxillary sinus (cm)		Sphenoid sinus and mastoid
			A-P	ML	A-P	ML	
1	M	36	<1 cm		3	2	Small
2	M	41	None		3	1.5	Small
3	M	46	None		2.5	1.8	Small
4	M	68	None		2.8	1.2	Small
5	F	39	None		2.5	1.5	Small
6	F	42	<1 cm		3	1	Small
7	F	43	None		2.8	1.8	Small
8	F	44	None		2.4	1.8	Small
9	F	48	<1 cm		2	1.5	Small
Normal values							
M			1.55–1.81	2.54–3.05	3.33–4.37	2.66–3.81	
F			1.3–1.76	2.08–3.04	3.30–4.40	2.40–3.64	

Modified from Kornreich et al. 2002a
A-P anterioposterior; *ML* mediolateral; *M* male; *F* female

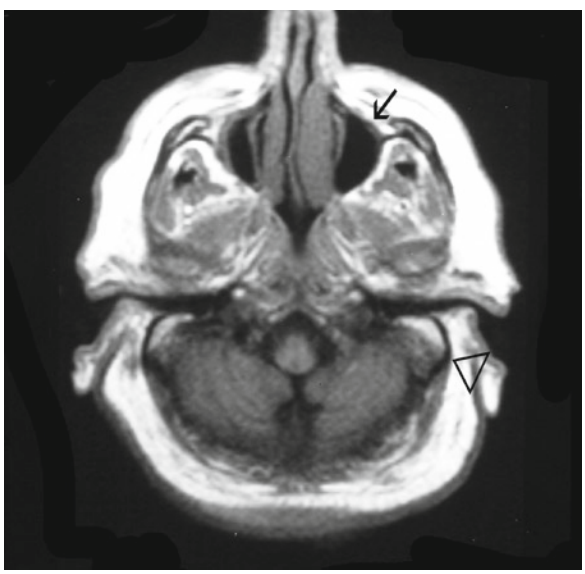

Fig. 20.5 MR axial image of the brain, T1 weighted (TR 450/TE 25). A 68-year-old man with LS. Note the small size of the maxillary sinuses (*arrow*) and the underdevelopment of the mastoids (*arrowhead*)

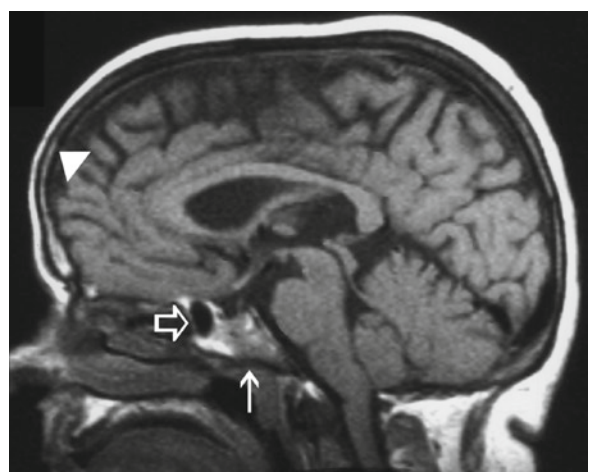

Fig. 20.6 MR sagittal image of the brain, T1 weighted (TR 450/TE 25). A 48-year-old woman with LS. The characteristic shape of the skull is seen. The diploe of the cranial vault is thin (*arrowhead*), the frontal sinuses are not developed. The sphenoid sinus is small (*open arrow*), the bone marrow in the clivus has not undergone fatty replacement (*arrow*). Note narrowing of the spinal canal at the level of the atlanto-axial joint

20.4 Orbits

In LS, the globe (see Chap. 21) and orbits are smaller, with the orbits being shallow and wide. Proptosis is present only in a minority of the patients.

MR images of the orbits were available in nine untreated adult LS patient as part of the MR examination of the brain. On the axial images, we measured several parameters and compared them to those in a group of 20 adults with no known ocular pathology

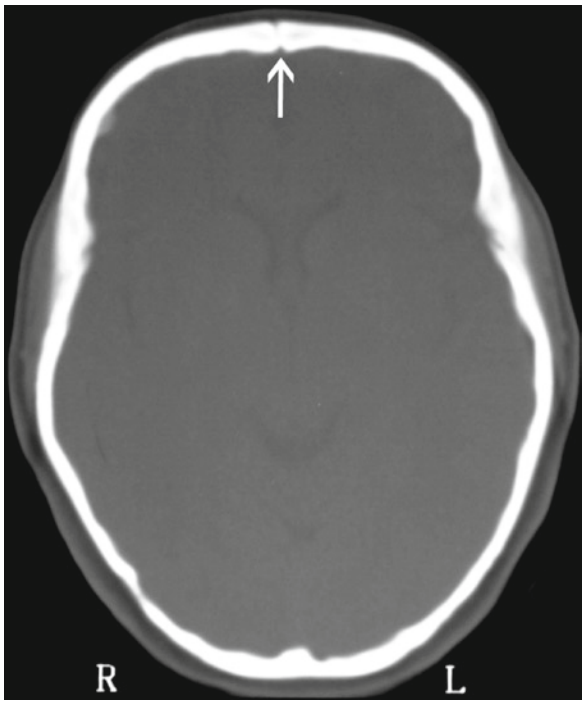

Fig. 20.7 CT of the brain, bone windows. A 48-year-old woman with LS. The sagittal suture is still open (*arrow*)

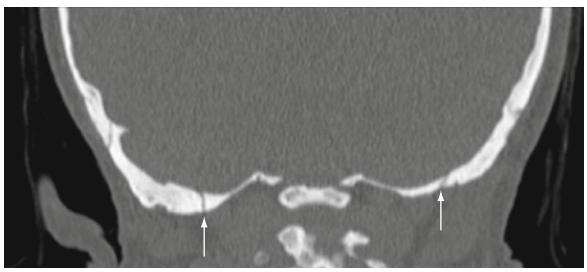

Fig. 20.8 CT of the temporal bone, coronal reconstructions. A 41-year-old man with LS. Note the open synchondroses in the skull base (*arrows*)

who had undergone MR. The various measurements are exemplified in Fig. 20.9a, b. All the measurements were taken bilaterally.

In the LS group the maximal diameter of the globe was significantly smaller. This is concordant with a recent ophthalmologic article reporting that in LS patients, the axial length of the eye and the anterior chamber were smaller than those in a control group (Bourla et al. 2006) and that children with LS treated with IGF-1 had a larger globe than untreated children. Parentin et al. (2004) found that in children with GHD the axial length of the globe was relatively short. In this regard, it is known that taller persons are more likely to have longer globes since adult height is independently related to ocular dimensions.

Measurement of the orbits showed that they are smaller and relatively shallow, but wider. The interzygomatic distance and the length of the lateral wall were smaller (Table 20.2). The medial distance between the orbits was also smaller, but the angle of the orbit was larger, and there was no significant difference in the most anterior diameter of the orbit. The ratio between the anterior diameter of the orbit and the diameter of the globe was larger. The position of the globe, as measured in relation to the anterior orbital rim (line G), was normal, but in relation to the interzygomatic line (line A) it was significantly more anterior (Fig. 20.10). It is of note that in comparison with values from the literature, in only three LS patients was the position of the globe compatible with exophthalmus (Peyster et al. 1986). As patients with GH insensitivity tend to be obese and since marked obesity may cause exophthalmus (Peyster et al. 1986), we measured the thickness of the intraorbital fat below the orbital roof, which was found to be less thick than that in the normal controls. Due to the retrospective nature of the study, we could not perform the more reliable volumetric measurements of the intraorbital fat.

Postnatal growth of the orbit is dependent upon normal growth of the globe; therefore, the proportion between globe diameter and length of the orbit does not differ significantly from normal controls. The interorbital distance, which increases with age, is smaller in LS patients and is probably also influenced by IGF-1 in the process of growth of the upper face. Normally, the ocular axis (defined as the angle between the optic nerves) diminishes slightly from childhood to adulthood (Fredrick 2005). We did not measure this angle, but found that the orbital angle (defined as the angle between the lateral wall and a line through the midline of the anterior base of skull) was larger in LS patients. It would, therefore, appear that the influence of GH and IGF-1 on the growth of the ocular globe and the orbit is multifactorial and thus differential. A possible clinical manifestation is exemplified by one of our patients who at age 36 complained that when he laughed there was prominent protrusion of his eyes: examination revealed marked proptosis and midface hypoplasia, most probably associated with hypoplastic orbits.

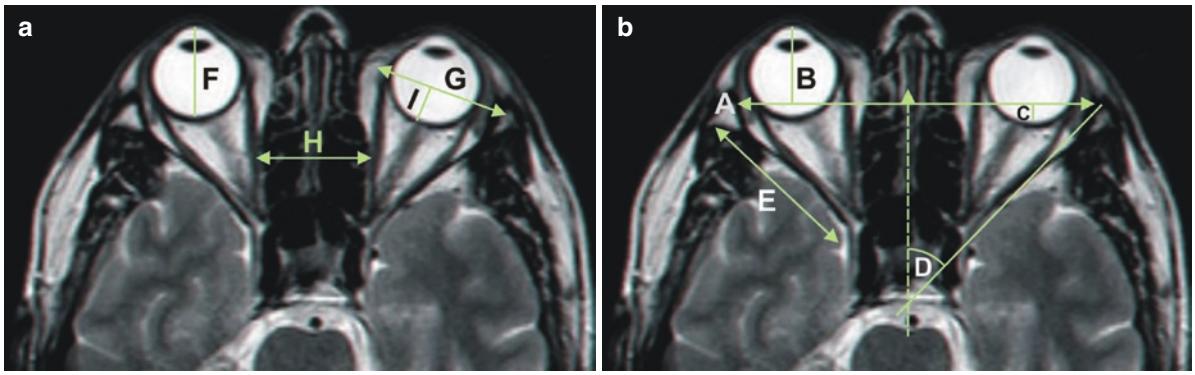

Fig. 20.9 (**a**, **b**) Axial T2-weighted images of the orbits. The measured parameters are demonstrated: *A* interzygomatic distance; *B* distance from line A to the corneal surface; *C* distance from line *A* to posterior globe surface; *D* angle of orbit; *E* length of lateral wall of the orbit; *F* diameter of the globe; *G* anterior diameter of the orbit; *H* interorbital distance; *I* distance from line *G* to posterior surface of globe

20.5 Oropharynx

In patients with LS, the dimensions of the oropharynx were small compared with normal standards (Table 20.3) (Kornreich et al. 2002b).

MR images of the oropharynx were available in eight patients. From the axial images, we obtained measurements of the minimal anteroposterior and mediolateral dimensions (at retropalatal level) (Fig. 20.11) and compared them to reference values (Schwab et al. 1995). From the sagittal images, we assessed the maximal thickness of the soft palate, comparing it with that of a control group of nine age-matched female patients referred for MR imaging for unrelated reasons (Fig. 20.12).

The minimal mediolateral diameter of the oropharynx was significantly smaller in patients with LS than

Table 20.2 Orbital measurements in patients with Laron syndrome compared to normal controls

	LS ($n=9$)			Controls ($n=20$)			
Orbital parameters	Mean	Range	SD	Mean	Range	SD	*P* value
Globe diameter (cm) (F)[a]	2.2	2–2.5	0.17	2.5	2.9–2.0	0.2	≤0.002
Interzygomatic distance (cm) (A)	7.7	8.2–7.0	0.4	9.1	8.5–97	0.3	≤0.001
Angle of orbit (degrees) (D)	55	50–60	3.5	42	33–51	4.5	≤0.001
Anterior diameter of orbit (cm) (G)	3	2.8–3.5	0.2	3	2.5–3.6	0.3	NS
Length of lateral wall (cm) (E)	3.6	4.2–3.1	0.4	3.9	3.4–4.4	0.3	≤0.06
Medial interorbital distance (cm) (H)	2.2	1.8–2.6	0.3	2.7	2.2–3.1	0.2	≤0.001
Anterior globe to A (cm) (B)	1.9	1.1–2.5	0.4	1.5	1.1–1.9	0.2	≤0.003
Posterior globe to A (cm) (C)	0.5	−0.4–2.4	0.8	1	0.5–1.2	0.3	≤0.002
Thickness of fat in superior orbit (cm)	1.7	1.3–2.6	0.3	0.8	0.4–1.3	0.3	≤0.001
Anterior diameter of orbit/globe diameter (G/F)	1.37	1.2–1.5	0.12	1.22	0.9–1.7	0.17	≤0.03
Length of lateral wall/globe diameter (E/F)	1.61	1.2–1.5	0.23	1.55	0.9–1.5	0.19	NS

[a]The capital letters in parenthesis refer to the lines in Fig. 20.9

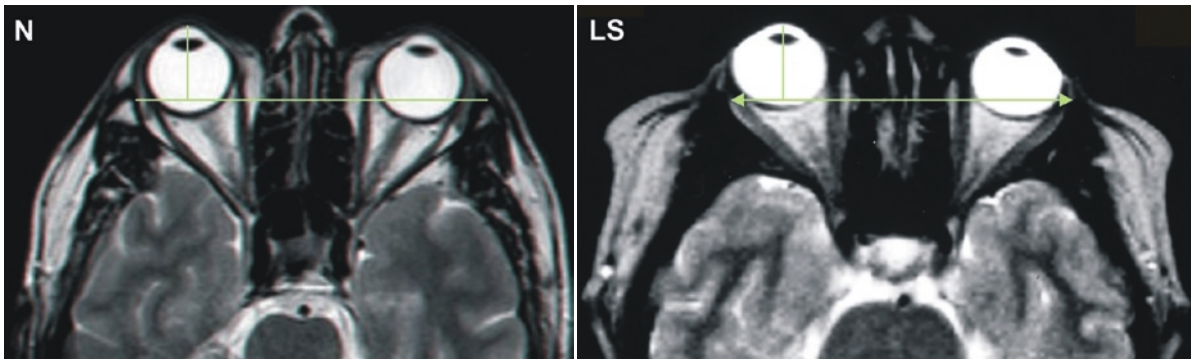

Fig. 20.10 Axial T2-weighted images of the orbits. *LS* Laron syndrome; *N* normal controls. Note that in LS the orbits are more shallow and the globe is located more anteriorly in relation to the interzygomatic line

that in the control subjects ($P \leq 0.005$) (Figs. 20.13 and 20.14) (Kornreich et al. 2002b). There was no significant difference in the anteroposterior diameter or in the thickness of the palate between the two groups (Table 20.3).

Postnatal craniofacial growth appears to be a complex multifactorial process. Currently, all the theories of growth control mechanisms are speculative. One of the most popular is the functional matrix hypothesis, which proposes that the craniofacial bone grows in response to the structures surrounding it. That is, the pharynx enlarges because of the functional demands for airway, breathing, and eating spaces concomitant with linear growth (Kozak 1998). The small size and small quantities of food ingested in combination with the IGF-1 deficiency are probably the cause of the impaired growth of the pharynx in LS patients. The characteristic high-pitched voice of adults with LS (Laron, personal communication) probably reflects the small dimensions of both the pharynx and the larynx, particularly since the pitch of the voice is inversely related to the size of the larynx. One of the most important clinical outcomes of impaired pharyngeal growth, together with the tendency toward obesity of adult LS patients, is a greater propensity for sleep apnea (Dagan et al. 2001). Early cephalometry studies in patients of normal stature suffering from apnea showed that mandibular, maxillary, and anterior cranial base length were decreased (Johnson and Braun 1998) and the

Table 20.3 Oropharynx measurements (mm) in untreated patients with Laron syndrome compared to normal controls

Patient no.	Sex	Age (year)	Transverse diameter	A-P diameter	Soft palate thickness
1	M	36	11	7.0	11
2	M	41	10	4.0	8
3	M	46	7	4.0	9
4	M	68	7	4.0	20
5	F	39	10	1.2	13
6	F	42	4	4.0	10
7	F	44	7	5.0	10
8	F	48	4	6.0	13
Mean ± SD			7.14 ± 2.6	4.45 ± 1.8	12.28±3.7
Controls mean ± SD			12.9 ± 4.0	5.8 ± 2.4	9.88±1.2
P			<0.005	NS	NS

Modified from Kornreich et al. 2002b
A-P anteroposterior; *M* male; *F* female

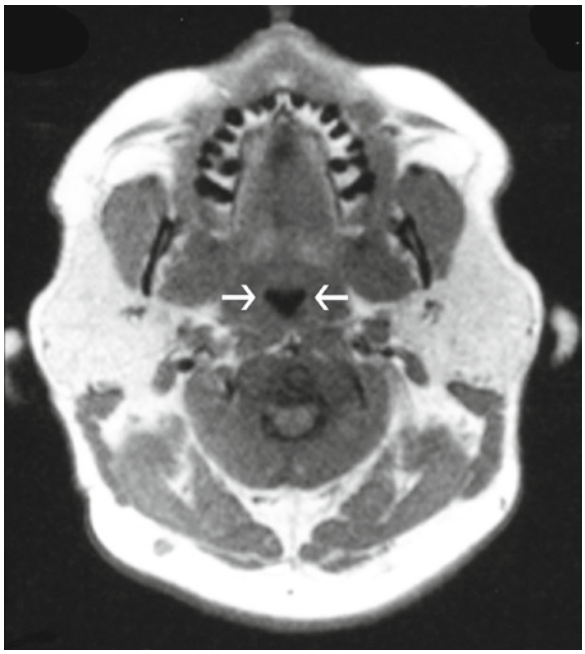

Fig. 20.11 MR axial images of the neck, T1 weighted (TR 500/TE 20). A 36-year-old man with LS. A-P and mediolateral measurements of the oropharynx were taken at retropalatal level (*arrows*). Note the abundance of fat in the subcutis

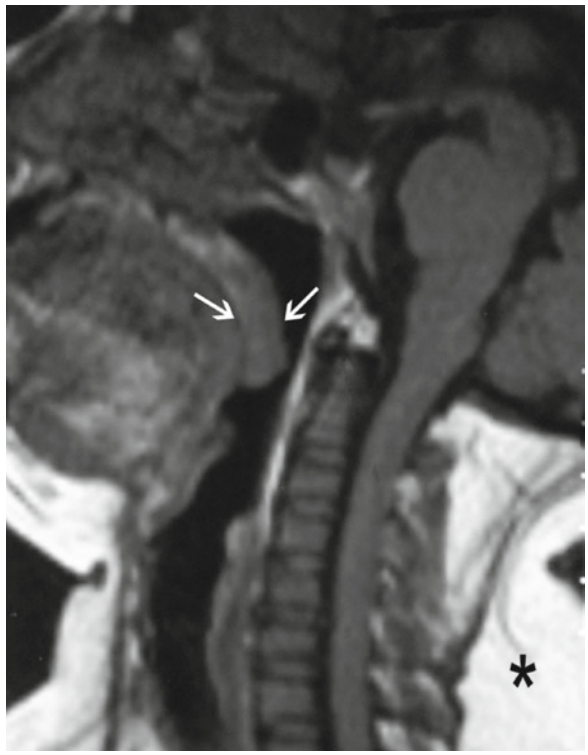

Fig. 20.12 MR sagittal images of the neck, T1 weighted (TR 460/TE 20). A 39-year-old woman with LS. The method of measurement of the soft palate is exemplified (*arrows*). Note the thickness of the subcutaneous fat (*asterisk*)

minimum airway area was significantly smaller than normal (Schwab et al. 1995; Avrahami and Englender 1995); not only size but also shape was affected with predominant narrowing of the mediolateral dimension resulting in an oval shape and with the longest axis in the anteroposterior dimension rather than the mediolateral dimension (Schwab et al. 1995). All these anatomic features, including narrowing of the airways in the transverse plane (Table 20.3), are present in patients with growth hormone insensitivity (Scharf and Laron 1972; Konfino et al. 1975). The characteristic obesity of adult LS patients, with a markedly increased fat mass (Laron 1984, 1999a; Laron et al. 2006), is an important factor in the development of sleep apnea (Schwab and Goldberg 1998). Thus, the combination of small airway dimensions and obesity means that LS patients are at high risk for developing sleep apnea.

20.6 Brain

Despite the small head circumference characteristic of patients with LS, no significant brain atrophy was demonstrated. In the nervous tissue of the brain, we

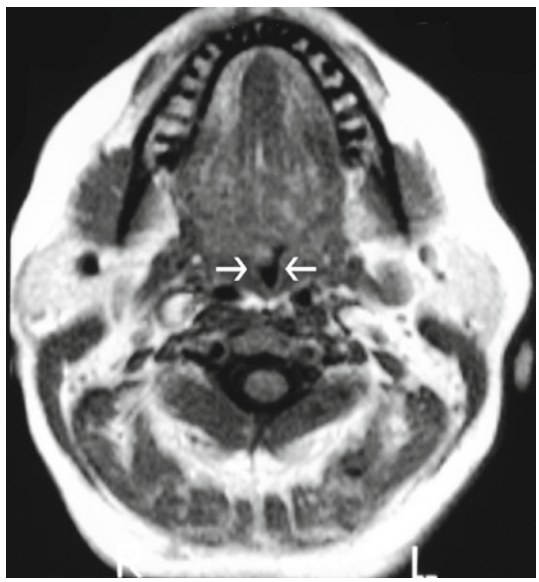

Fig. 20.13 MR axial images of the neck, T1 weighted (TR 500/TE 20). A 43-year-old woman with LS. Note the severe narrowing of the oropharynx in the mediolateral diameter (*arrows*)

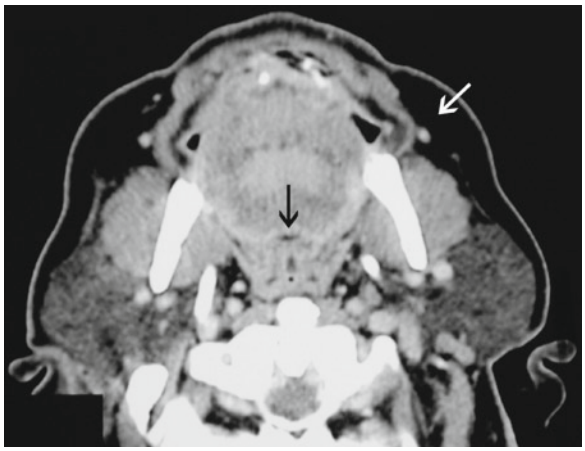

Fig. 20.14 CT of the neck. A 68-year-old man with LS. Note complete obliteration of the oropharynx (*black arrow*). A thick subcutaneous layer of fat is seen (*white arrow*)

found changes of diffuse parenchymal loss of various degrees, mostly minimal or slight (Fig. 20.15; Table 20.4) (Kornreich et al. 2002a).

Earlier research assumed that the microcrania in LS was secondary to underdevelopment of the brain tissue due to the early onset and persistence of IGF-I deficiency (Laron et al. 1993), an assumption proven by the rapid increase in head circumference in response to IGF-I treatment in children (Laron et al. 1992; Laron 2009). These findings seem to explain the wide spectrum of intellectual abilities of patients with long-term IGF-I deficiency (Galatzer et al. 1993; Shevah et al. 2005); however, whether they are directly related is not known at present. It is of interest that the patient with the most severe damage resides in an institution for the mentally impaired (see Chap. 38). In two sibling patients who suffered from mental impairment, the cerebellum was symmetrically small with enlarged foliae and fissures, compatible with cerebellar atrophy (Figs. 20.16a and 20.19). One of them had regions of posterior periventricular high signal on T2-weighted images (Fig. 20.16b) (Kornreich et al. 2002a). These pathologies do not seem specific as similar findings are found in numerous metabolic or infectious disorders that affect the cerebellum and cause cerebellar atrophy (Barkovich 2000). It should be stressed that the abnormal findings in the brain are not similar to damage, secondary to the neonatal hypoglycemia (Murakami et al. 1999) characteristic of young LS patients (Laron et al. 1968; Laron 1999a).

In three patients the ventricles were enlarged, without any evidence of a common pathogenesis (Table 20.4). One of them at the age of 46 years was found to have

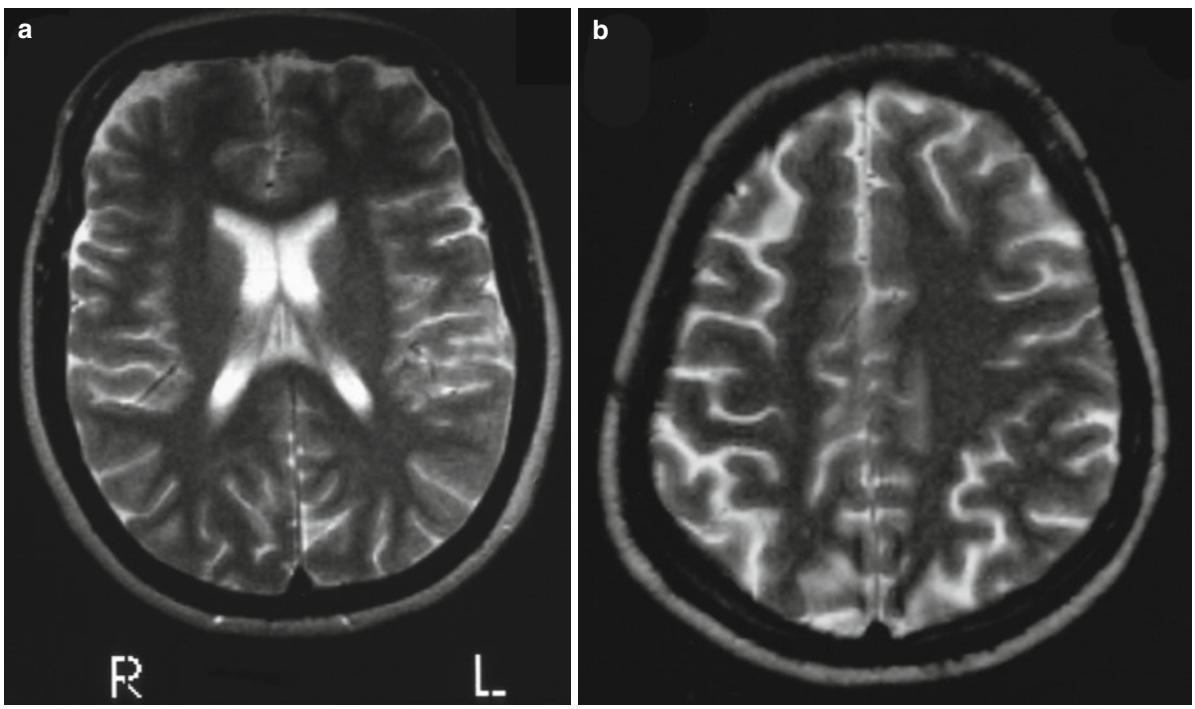

Fig. 20.15 MR axial images of the brain, T2 weighted (TR 4000/TE 100). A 26-year-old woman with LS. (**a**, **b**) Mild diffuse parenchymal loss is seen

Table 20.4 Brain abnormalities in patients with Laron syndrome

Patient no.	Sex	Age (year)	Diffuse parenchymal loss	Additional findings
Untreated adults				
1	F	26	Mild	
2	M	36	Minimal	
3	M	41	Minimal	
4	M	46	None	Atrophy of cerebellum
5	M	68	Slight	UBOs, compatible with age
6	F	39	Slight	
7	F	42	Minimal	Lacunar infarct in rt. caudate
8	F	43	Slight	Atrophy of cerebellum, T2 posterior periventricular hyperintensity
9	F	44	Minimal	
10	F	48	Minimal	
11	M	46	None	Arrested hydrocephalus
Untreated children				
1	M	16 mos.	None	Mild enlarged ventricles, T2 posterior periventricular hyperintensity
2	M	8	None	Slight ventricular enlargement
3	M	4	Minimal	

Modified from Kornreich et al. 2002a
UBOs undefined bright objects; *M* male; *F* female

marked enlargement of the lateral ventricles without any signs of increased intracranial pressure, findings compatible with arrested hydrocephalus (Fig. 20.20e) that could be related to the small foramen magnum and the pressure in the craniocervical junction. In one patient MR performed at the age of 16 months showed mild ventricular enlargement and posterior periventricular hyperintensity compatible with periventricular leukomalacia; the size of the ventricles did not change during 6 years of follow up (Fig. 20.17a, b). This patient showed no abnormalities at level C1–C2. In the third patient, an 8-year-old boy, the ventricular enlargement was slight and MR of the brain and spine showed no other abnormal findings. It is of note that in an early study based on pneumoencephalography in six children with LS, Laron et al. (1968) found the ventricles to be of normal size.

20.7 Pituitary Gland

The anatomy of the hypothalamic–pituitary region was normal in a group of 13 patients with LS. No midline anomalies were detected. To the best of our knowledge, this is the only single controlled study of the hypothalamic–pituitary region in patients with GH insensitivity (LS) (Kornreich et al. 2003).

The main findings are summarized in Table 20.5. With the exception of two male patients with a small pituitary gland, the size of the pituitary gland was within the normal range for age and gender in all adult patients (Fig. 20.18). This was also true for a 4-year-old untreated boy and a 16-month-old girl, as well as for a 9-year-old girl treated with IGF-I. The shape was either flat or concave and the contour symmetrical. There were no signs of hypertrophy and no adenomas were detected.

The somatotroph cells that secrete GH make up about 50% of the hormone-producing cells of the anterior pituitary (Thorner et al. 1998). Nagel et al. 1997 studying 91 children came to the conclusion that pituitary size reflects GH secretion. We suspected that lifelong oversecretion of GH (Keret et al. 1988; Laron 2002) could result in somatotroph hyperplasia and would be detectable by MRI as an enlargement of the pituitary gland. However, MRI of eight adults and two untreated children with LS showed a pituitary size within normal limits. This is in contrast to the enlargement of the pituitary described in patients with ectopic GH-releasing tumors (Ezzat et al. 1994) and with GH-secreting pituitary adenomas (Melmed 2001). It is of note that in a recent report, Frohman and Bonert 2007 described enlargement of the pituitary gland in two patients with acromegaly receiving pegvisomant (a synthetic antagonist of the GH receptor).

One explanation for our findings could be that patients with LS indeed secrete more GH than normal (Keret et al. 1988), but in a pulsatile manner which is

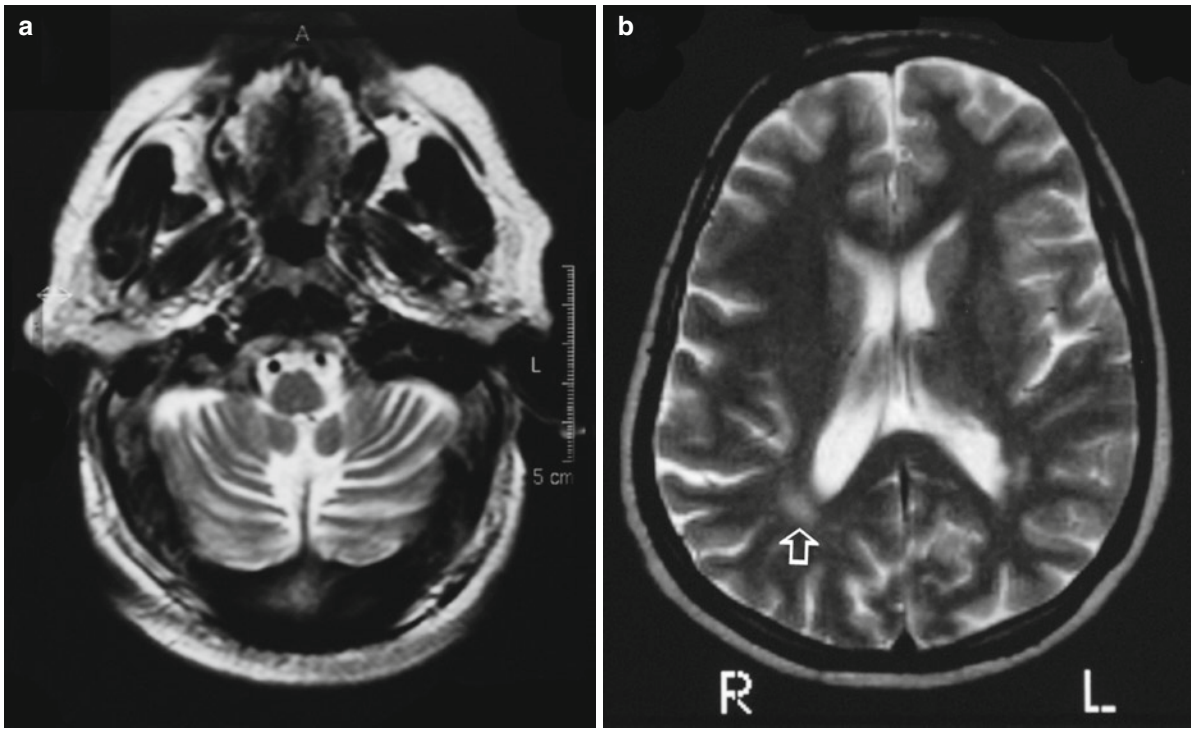

Fig. 20.16 MR axial images of the brain, T2 weighted (TR 4000/TE 100). A 43-year-old woman with LS and mental retardation. (**a**) Note the enlarged cerebellar folia, indicative of cerebellar atrophy. (**b**) Mild parenchymal atrophy and periventricular high signals are seen (*arrow*)

insufficient to produce pituitary hyperplasia. This is in contrast to patients with tumors secreting GH-releasing hormone and causing acromegaly, in whom GH secretion is constantly high. Another possibility is that the mechanism underlying the enlargement and multiplication of the somatotrophs needed for volume enlargement is GH-driven and IGF-I-dependent, and therefore, not feasible in the GH-insensitive state of LS.

In conclusion, LS is a unique abnormality of the GH axis characterized by high serum levels of GH, but with no pituitary hypertrophy despite the lifelong oversecretion of GH (see Chaps. 2 and 26).

20.8 Spine and Appendicular Skeleton

GH and IGF-1 have well-recognized and crucial effects on bone elongation and accretion during development (Maheshwari et al. 2003). A deficiency in these hormones results in linear growth failure and reduced bone size, due mostly to decreased proliferation of the growth plate chondrocytes (Maheshwari et al. 2003). It is, therefore, not surprising that patients with LS have short stature and small bones.

20.8.1 Cervical Spine

We found that untreated LS patients tend to develop cervical spinal stenosis and early osteoarthritic changes of the atlanto-axial joint, and often the syndrome is associated with os odontoideum (Kornreich et al. 2002b).

Conventional lateral radiographs as well as a number of anteroposterior open-mouth views of the dens and flexion and extension radiographs of the cervical spine were available for 15 LS patients, 4 of them children. Four LS patients underwent CT scans of C1–C2. MR imaging was done in ten patients, including one child treated with IGF-1 (Kornreich et al. 2002b). The width of the spinal canal was evaluated visually and quantitatively, with measurement of the anteroposterior diameter of the vertebra; on the

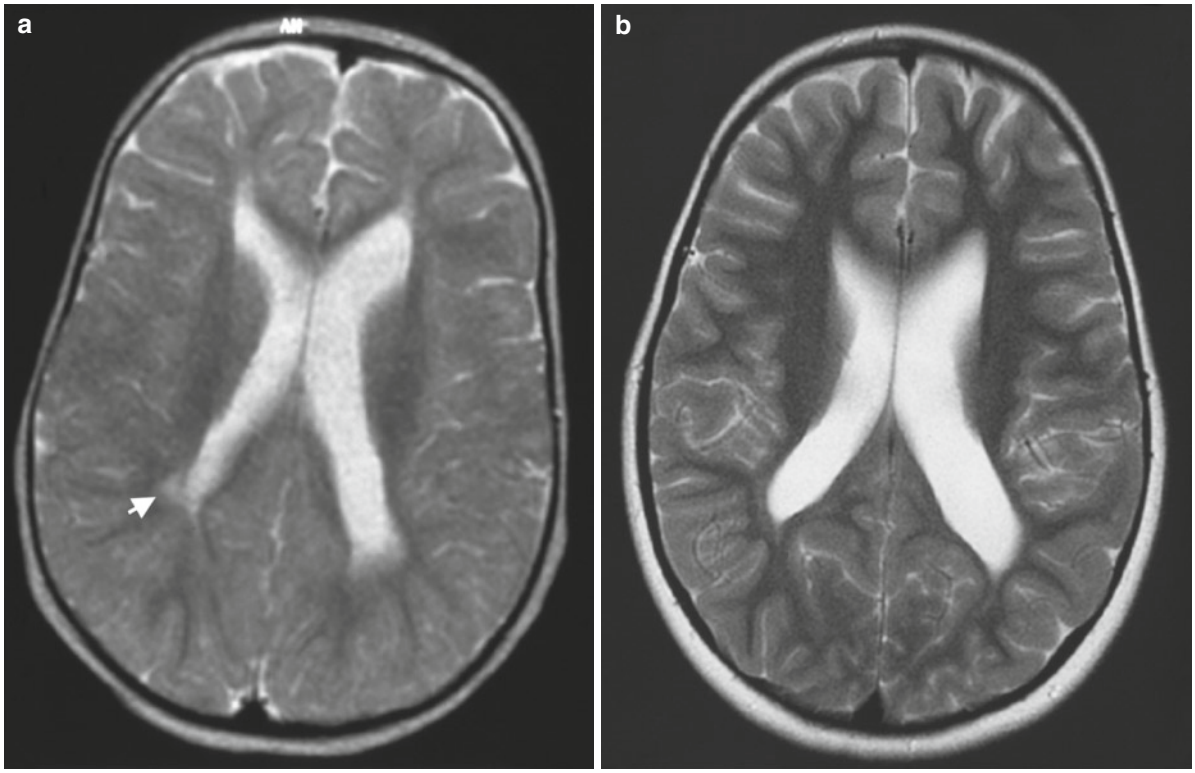

Fig. 20.17 MR axial images of the brain in LS patient. (**a**) At age 16 months, T2 weighted (TR 4000/TE 90). Mild ventricular enlargement with squaring of the occipital horns and increased periventricular high signals (*arrow*). (**b**) At age 7 years, the shape and size of the ventricles have not changed. The findings are indicative of hypoxic–ischemic damage

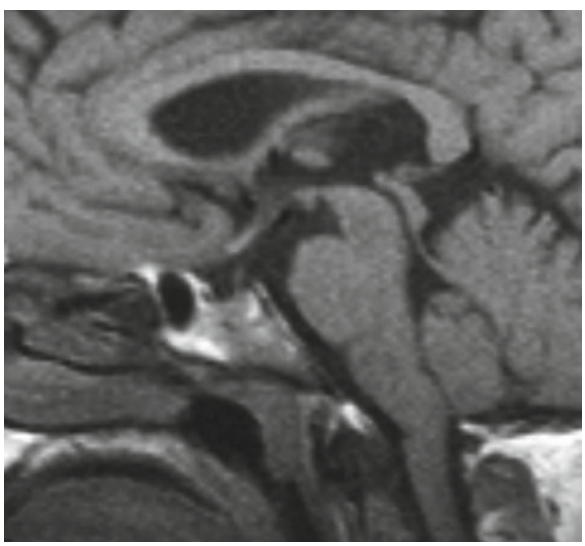

Fig. 20.18 MR midsagittal image of the pituitary gland, T1 weighted (TR 450/TE 25). A 48-year-old woman with LS. No abnormalities of the pituitary hypothalamic region are seen

sagittal images, measurements were taken at the level of C3. The ratio of the two parameters was then calculated and compared with published standard values (Remes et al. 2000). Though the reference values were obtained from conventional lateral radiographs of the cervical spine, we considered them valid also for MR sagittal images since we referred only to the ratio between the parameters.

The most common pathologic MR finding was cervical spinal stenosis, present in eight of the nine untreated patients examined, with a confidence interval of 95% (Table 20.6; Fig. 20.19). In the child receiving long-term IGF-1 treatment, the cervical spine was of normal width.

The spinal canal grows at the neurocentral synchondroses between the body and the arches of the vertebrae until ages 3–6 years, and thereafter, only by the midline posterior cartilage between the two arches until age 10 years (Silverman et al. 1992). In patients

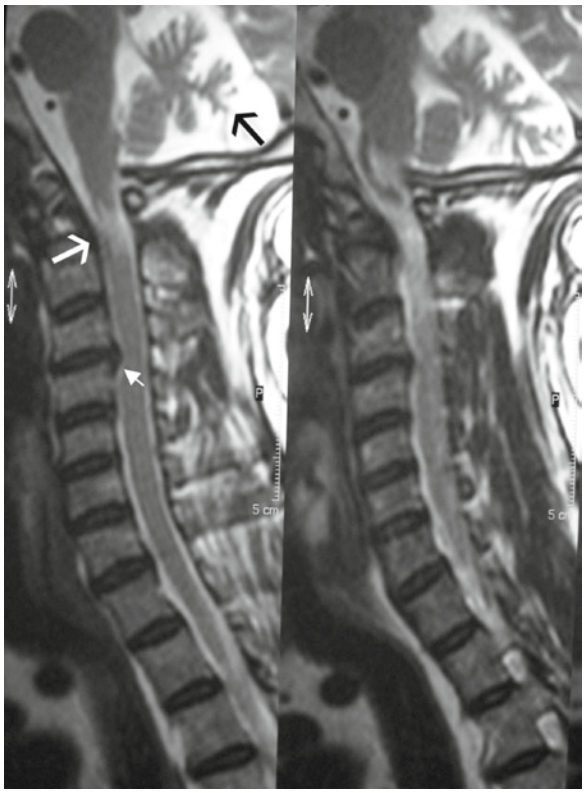

Fig. 20.19 MR sagittal images of the cervical spine, T2 weighted (TR 3500/TE 120). A 50-year-old man with LS. Note the narrow spinal canal and the disk bulge at C3–C4 causing compression of the subarachnoid space (*small arrow*). Myelomalacia of the cord is seen at the level of the os odontoideum (*white arrow*). This is one of the two patients with atrophy of the cerebellum (*black arrow*)

with growth hormone insensitivity lacking IGF-1, the capacity for growth in these regions is limited, resulting in a narrow spinal canal as seen in the above 8 untreated LS patients. This anatomic configuration increases the risk of neurologic deficit upon the occurrence of degenerative disk disease and spondylotic changes. Indeed, in two of our patients, one at age 36 and the other at age 50, there was evident disk bulge with compression of the thecal sac (Fig. 20.19).

At the C1–C2 level, there was an abnormality of the dens in four patients and in six osteoarthritic changes. The anomaly of the dens was compatible with os odontoideum: a small osseous structure was seen at the expected location of the odontoid process with a gap between it and the axis body (Fig. 20.20a, c).

This defect in the integrity of the dens may result in ligamentous incompetence, atlanto-axial instability, compromise of the spinal canal, and cord compression (Watanabe et al. 1996). Indeed, flexion and extension radiographs in these four patients showed atlanto-axial instability (Fig. 20.20b). MR images (available in three of these patients) showed focal myelomalacia at the C1 level (Figs. 20.19 and 20.20d). Clinically, two of these patients suffered from paretic episodes.

In six adult patients, lateral cervical spine radiographs and anteroposterior open-mouth views of the dens revealed an intact dens, which appeared to be bulky and sclerotic. CT scans performed for further evaluation of the MR findings in three of these patients confirmed the presence of cortical thickening of the dens and the atlas, with narrowing of the atlanto-axial joint, compatible with degenerative changes (Fig. 20.21). The MR images showed compression of the subarachnoid space at the same level.

Aplasia or hypoplasia of the odontoid process, as well as os odontoideum, are extremely rare, having been reported in association with a variety of congenital dysplasias (Lachman 1997). The etiology remains controversial: both acquired (traumatic and vascular) and congenital mechanisms have been proposed (Dai et al. 2000; Heick 1996). However, os odontoideum has not been previously associated with growth hormone deficiency or insensitivity.

Because of its function and situation, the atlanto-odontoid joint is subject to degenerative changes. These changes are similar to those commonly observed in other synovial joints and are rarely found before age 50 years (Zapletal et al. 1995; 1997). Since only one of our patients was older than 50 years, the high frequency of degenerative changes observed among these LS patients is unusual. We suggest that in LS, the disproportion between head and body size (Laron et al. 1968, Laron 1984) subjects the atlas and dens to increased stress, resulting in thickening and sclerosis at a young age. This disproportion may in fact be a common factor in the pathogenesis of both degenerative and structural changes in the C1–C2 region. The relatively large head for the short body may increase the risk of overstretching of the vascular supply of the dens in the event of trauma, resulting in avascular necrosis and a high frequency of os odontoideum, as seen in our patients.

Regardless of the etiology of the abnormal findings at the atlanto-axial joints and cervical spine, LS patients are at risk for insult to the cervical cord, and a high level of awareness to related neurological symptoms is warranted. These findings stress the need for IGF-1

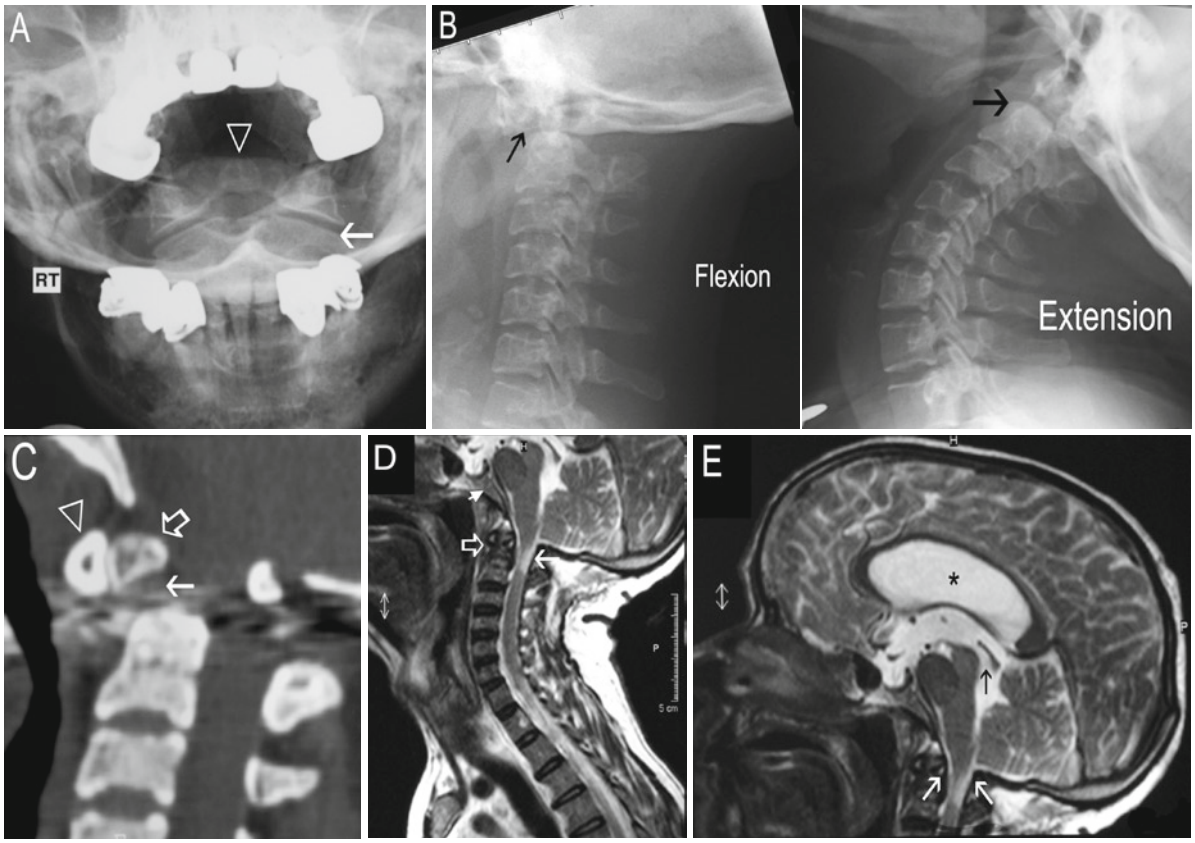

Fig. 20.20 A 46-year-old man with LS. Anomaly of C1–C2 consistent with os odontoideum. (**a**) Open-mouth view of C2. The gap between the base of the dens (*arrow*) and the os is visualized (*arrowhead*). (**b**) Lateral view of the cervical spine, on flexion and extension. C1–C2 instability is noted, with posterior shift of C2 (*arrow*) on flexion and widening of the retrodental space. (**c**) CT of the upper cervical spine, sagittal reconstruction. The characteristic findings of this malformation are clearly shown: the small os (*open arrow*), the thickened anterior ring of the atlas (*arrowhead*), and the gap between the os and the body of C2 (*arrow*). (**d**). MR sagittal T2 weighted (TR 3500/TE 120) image of the cervical spine. Spinal stenosis is seen, as well as a region of myelomalacia of the cord (*arrow*) at the level of the anomalous C2 (*open arrow*). Note that the sphenooccipital synchondrosis is still visible (*small arrow*). (**e**). MR sagittal T2 weighted (TR 3500/TE120) image of the brain. Enlargement of the lateral (*asterisk*) and third ventricles is seen, the aqueduct is patent (*black arrow*). The hydrocephalus is probably due to pressure at the craniocervical junction (*white arrows*)

replacement treatment early in childhood. MR screening of the cervical spine is recommended in patients with IGF-1 deficiency.

20.8.2 Thoracic and Lumbar Spine

In adult LS patients the thoracic and lumbar vertebrae are proportionally small, the lumbar canal is more narrow, and there is higher prevalence and greater severity of osteophytes in comparison to the normal subjects.

The only previous description of the radiographic findings in the appendicular skeleton and spine in LS is that of two untreated children found to have proportionately small bones showing an abnormal trabecular pattern and multiple growth arrest lines, with posterior wedging of the lumbar vertebral bodies (Vasil et al. 1994). Among the material available from our LS cohort, there was a single lateral radiograph of the spine in a child, and this also showed posterior wedging of the vertebrae (Fig. 20.22). In the 15 adult LS patients for whom lateral and anteroposterior (A-P) views of the thoracic and lumbar spine were available, the axial skeleton was proportionally small but the vertebrae were of normal shape without evidence of platyspondylia, wedging, or collapse, no abnormal trabeculation, and no

suggestion of osteopenia (Fig. 20.23) (Kornreich et al. 2008).

The vertebrae were evaluated both visually and quantitatively for shape and size of the vertebral body and for width of the spinal canal. In the lateral view of the lumbar spine, the A-P and craniocaudal (C-C) dimensions were measured at the center (middiameter) of the vertebral body at the level of L3 and then the ratio of the two dimensions was calculated, multiplying the two dimensions to obtain the area of the vertebral body. In the A-P view of the lumbar spine, the interpedicular distance was measured at the levels of L1 and L5 and the increment calculated. Also measured was the latero-lateral dimension of L1 with calculation of the ratio between the width of the vertebra and the interpedicular distance. The values obtained for the group of 15 adults as a whole were compared with those of a control group retrieved from the radiology files.

Table 20.5 Pituitary size and shape in patients with Laron syndrome

Patient no.	Age (year)/sex	Pituitary shape	Pituitary height (mm)	Normal values of pituitary height
1	1.4/F	Concave	3	2–6
2	4/M	Concave	3	2–6
3	36/M	Flat	5	5.40 ± 1.06
4	41/M	Flat	2	4.89 ± 0.89
5	46/M	Concave	4	4.89 ± 0.87
6	47/M	Concave	3	4.89 ± 0.87
7	68/M	Concave	6	4.78 ± 1.02
8	39/F	Flat	4	5.68 ± 1.10
9	42/F	Concave	4	5.19 ± 1.13
10	43/F	Flat	4	5.19 ± 1.13
11	44/F	Concave	5	5.19 ± 1.13
12	48/F	Flat	5	5.19 ± 1.13
IGF-1 treated	9/F	Flat	5	2–6

Modified from Kornreich et al. 2003
M male; *F* female

Comparison of the A-P and C-C dimensions and the areas of the vertebral body showed that values were significantly lower in the LS patients (Table 20.7), but there was no significant difference in the ratio of the A-P to the C-C values in the vertebral body (Table 20.7). These findings corroborated the visual impression of proportionally small vertebrae in the LS patients.

In the lateral view of the lumbar spine, the lumbar canal appeared narrow at the level of L3–L5 in all patients (Fig. 20.23). The interpedicular distance was significantly smaller in the LS patients than in the controls at both L1 and L5 (Fig. 20.24). The distance was less than 2 cm at L1 in three patients and at L5 in one patient. The increment in the width of the spinal canal between the two levels was also significantly lesser in

Table 20.6 Cervical spine measurements in patients with Laron syndrome

Patient no.	Sex	Age (year)	A-P canal diameter	Vertebral body diameter	Ratio	Normal ratio[a]
Untreated adults						
1	M	36	10.0	13.5	0.74	Males
2	M	41	10.0	13.0	0.77	0.98 ± 0.11 (n = 98)
3	M	46	10.5	15.0	0.70	95% CI 0.97–0.99
4	M	46	10.0	14.0	0.71	
5	F	39	9.5	10.5	0.90	Females
6	F	42	10.0	11.5	0.87	1.12 ± 0.14 (n = 94)
7	F	43	10.0	12.0	0.80	
8	F	44	11.0	10.0	1.10	95% CI 1.09–1.15
9	F	48	9.0	13.0	0.70	
IGF-1 treated child	F	9	13.0	10.0	1.30	1.32 ± 0.17

Modified from Kornreich et al. 2002b
A-P anteroposterior; *M* male; *F* female; *CI* confidence interval
[a]Ratio between anteroposterior diameter of the canal and the vertebral body

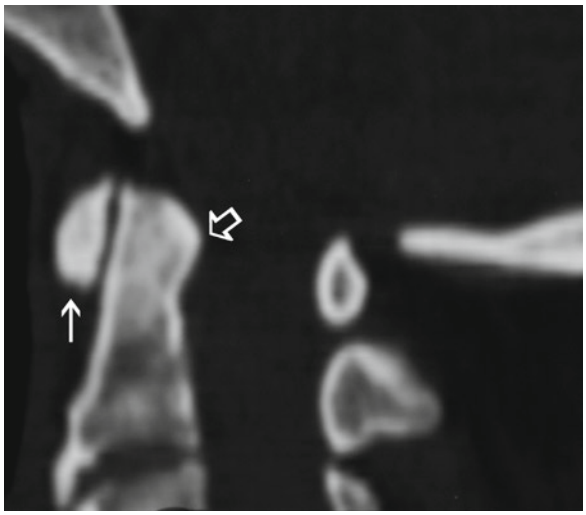

Fig. 20.21 CT of the neck. Sagittal reconstruction at the level of C1–C2. A 46-year-old woman with LS. Note cortical thickening of the dens (*open arrow*) and the atlas (*arrow*), with narrowing of the atlanto-axial joint

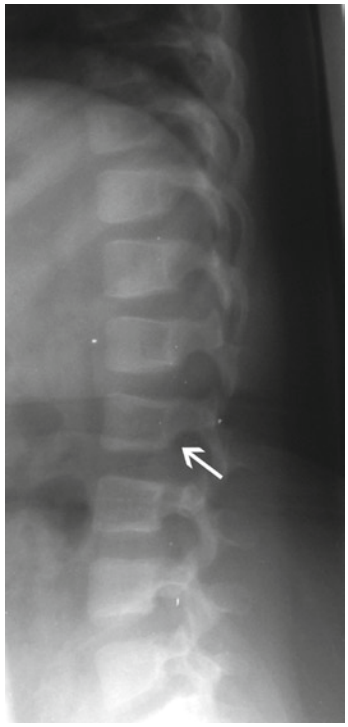

Fig. 20.22 X-ray of the lumbar spine. Lateral view. A 4-year-old girl with LS. A slight posterior wedging of the vertebral bodies is seen (*arrow*)

the LS patients (Table 20.7). Also the latero-lateral dimension of L1 was significantly smaller in the LS group. However, the ratio between the width of the vertebra and the interpedicular distance was also smaller, implying a wide canal relative to the vertebral body.

Table 20.7 Measurements of the lumbar spine in patients with Laron syndrome and healthy controls

	Laron syndrome ($n=15$)	Controls ($n=20$)	*P* value
	Mean (range)	Mean (range)	
Vertebra			
C-C	2.3 (2–2.7)	3.2 (3–3.7)	<0.001
A-P	2.6 (2–3.3)	3.7 (3–4.4)	<0.001
Vertebral area	6.0 (4.2–8.2)	12.2 (9.6–15.1)	<0.001
Vertebral C-C/A-P	0.9 (0.7–1)	0.9 (0.7–1.1)	NS
Interpedicle distance			
L1	2.0 (1.6–2.4)	2.5 (2.0–2.9)	<0.001
L5	2.2 (1.6–2.8)	3.1 (2.3–3.9)	<0.001
Difference L1–L5	0.1 (−0.4–0.8)	0.5 (−0.5–1.3)	0.01
Vertebra trans/ interpedicular	1.5	1.7	0.035

Modified from Kornreich et al. 2008
All measurements are in centimeters
C-C craniocaudal; *A-P* anteroposterior

The spinal canal grows at the neurocentral synchondroses, reaching adult size at L1–L4 in the first year of life and at L5 by age 5 years (Naidich et al. 2002). In patients with LS, the deficiency in IGF-1 results in a narrow spinal canal. On radiographs of the lumbar spine, an interpedicular distance of less than 2 cm is considered indicative of spinal stenosis (Resnick and Niwayama 1995), according to which four patients in our series had a stenotic lumbar canal, whereas in the others, the width was in the lower range of normal. It should, however, be considered that these values may not be valid for patients with LS. We found a minimal increase in the interpedicular distance from L1 to L5, but the width of the spinal canal was large relative to the width of the vertebra (Table 20.7).

The exact influence of IGF-1 on spinal cord growth is still unclear as some of the effects of GH on the central nervous system occur independently of IGF-1 (Chen et al. 1997; Laron 2009). As described above, MR imaging of the cervical region demonstrated a small bony canal relative to the cord (Kornreich et al. 2002b). The MR scans of the lumbar spine available in two children and two adults did not show spinal stenosis (Fig. 20.26). At present, none of our patients has

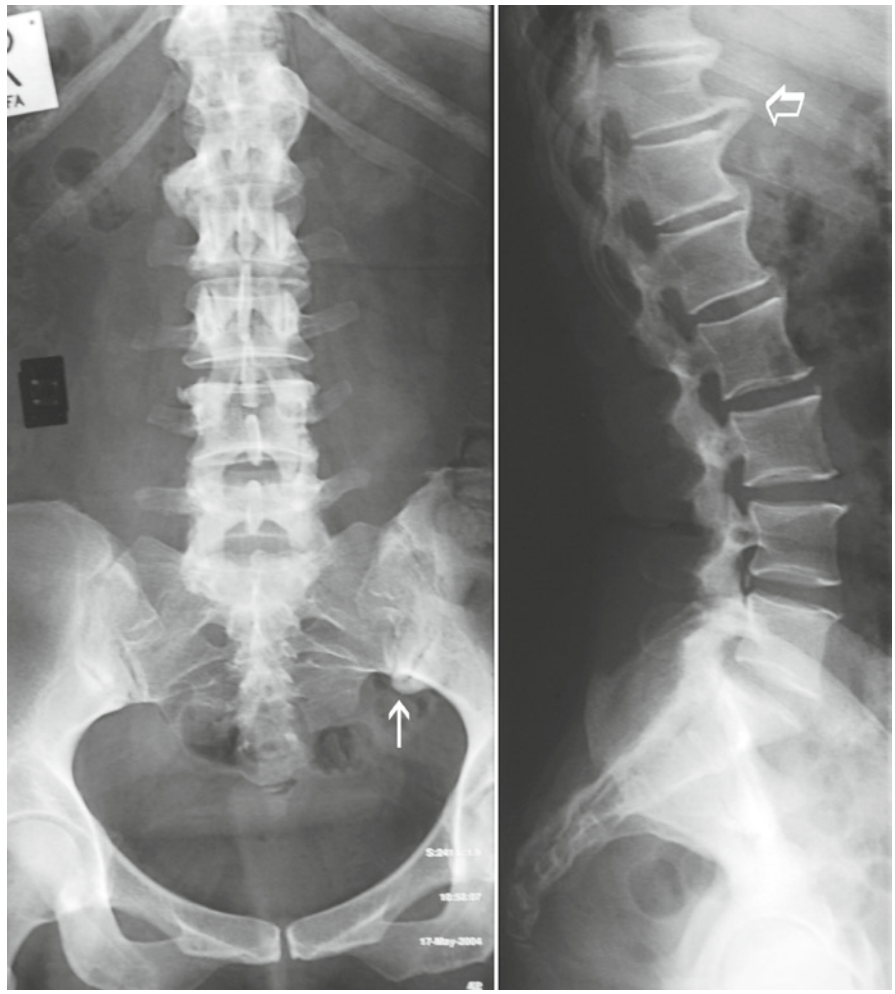

Fig. 20.23 X-rays of the lumbosacral spine, A-P and lateral views. A 38-year-old woman with LS. The vertebrae are of normal shape. Note the narrow spinal canal at the lumbar level and the marked sloping of the sacrum. Degenerative changes with anterior bone bridging are seen (*open arrow*). Note also the bone bridging of the left sacroiliac joint (*arrow*). These advanced osteoarthritic changes are unusual considering the age of the patient

complained of symptoms associated with spinal stenosis (Laron, personal communication 2007), but it is of note that all except one are younger than 51 years, and the symptoms may occur later with the development of more severe degenerative changes.

Also measured was the sacral slope, defined as the angle between the sacral plate and a horizontal line (Boulay et al. 2006). It was expected that the LS patients would show an increased sacral tilt, which can be related to a number of risk factors, including impaired stance due to obesity and decreased lean–fat mass and a narrow spinal canal or a short spinal column similar to that found in achondroplasia (Laron 2004; Sponseller 2001; Rosenbloom et al. 1997). However, although in the LS patients the sacrum was more horizontal than in the controls, the difference was not significant. Nevertheless, this parameter is influenced by the position of the pelvis, so that measurements need to be taken on X-rays obtained while the patient is in the upright position (Boulay et al. 2006), whereas ours were obtained with the patient in the recumbent position.

Degenerative changes in the lumbar spine were identified by the presence of osteophytes, graded on a 5-point scale: 0 – no osteophytes; 1 – small spinous excrescences; 2 – developed osteophytes, no bony bridge; 3 – bony bridge; and 4 – exuberant bony bridge.

There was a higher prevalence and greater severity of osteophytes in the lumbar spine in the patients with LS (100% in the age range 30–50 years) than in the control group (43% in the same age range) (Fig. 20.23; Table 20.8). In the literature, a prevalence of 36–44% of osteophytes of grade 2 or higher at the age 50–54 years, or a prevalence of 74–84% of all grades of

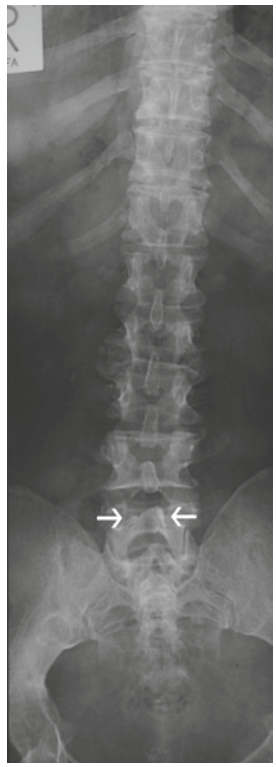

Fig. 20.24 X-ray of the lumbar spine. A-P view. A 42-year-old man with LS. Diffuse degenerative changes with bony bridging. Note the narrowing of the interpedicular distance at L5 level (distance between *arrows*)

Table 20.8 Osteophytes in 11 patients with Laron syndrome and 16 control subjects (age 30–49 years)

Grade	Laron syndrome (*n*)	Controls (*n*)
Lumbar spine		
1	5	6
2	4	1
3	1	
4	1	
Total	11 (100%)	7 (43%)
Thoracic spine		
1	4	1[a]
2	3	1[a]
3	2	
4	2	
Total	11 (100%)	

Modified from Kornreich et al. 2008
[a]Data available in only one patient

osteophytes from age 50 years and up, has been reported (O'Neill et al. 1999). Large osteophytes were present in the thoracic spine as well, but there were no controls available for comparison (Fig. 20.25).

Further studies of the spine with computed tomography or MR are needed to elucidate whether these patients are at risk of morbidity from the lumbar region in addition to the cervical symptoms. This is particularly relevant in view of a greater prevalence of osteophytes. Excessive obesity has been associated with osteophytosis (O'Neill et al. 1999) and is probably a significant risk factor for these degenerative changes in LS.

20.8.3 Chest

Lateral and A-P films of the chest were available for seven adults and two children. Every depicted bone was proportionately small. A bell-shaped chest was seen in five adults (three males, two females). This appearance of a bell-shaped thorax may be attributed to an obesity-related disproportion between the small rib cage and the large abdomen. It is of note that in the children (one infant aged 16 months and one treated 12-year-old), the shape of the thorax was normal, supporting the supposition that the bell-shaped thorax is not a primary bone anomaly (Fig. 20.27). In all LS patients, the cardiac silhouette was of normal size.

20.8.4 Pelvis

Anteroposterior views of the pelvis were available for eight patients. In all, the pelvis was small but of normal shape. In one patient the radiograph showed bilateral upward dislocation of the femoral head, with pseudoarticulation; the acetabulum was shallow and the femoral neck foreshortened with coxa vara. These findings were compatible with bilateral untreated dislocation of the femoral head (Fig. 20.28).

Avascular necrosis of the femoral head and slipped capital femoral epiphysis have both been reported to be significantly more common in children with GH deficiency than in the general population (Watkins 1996). In their study of patients with LS from Ecuador, Rosenbloom et al. (1997) reported a 25% incidence of avascular necrosis of the femoral head and a 5% incidence of slipped femoral epiphysis. However, among our patients, the appearance of the femoral head and the hip joint did not reveal any signs of either of these entities.

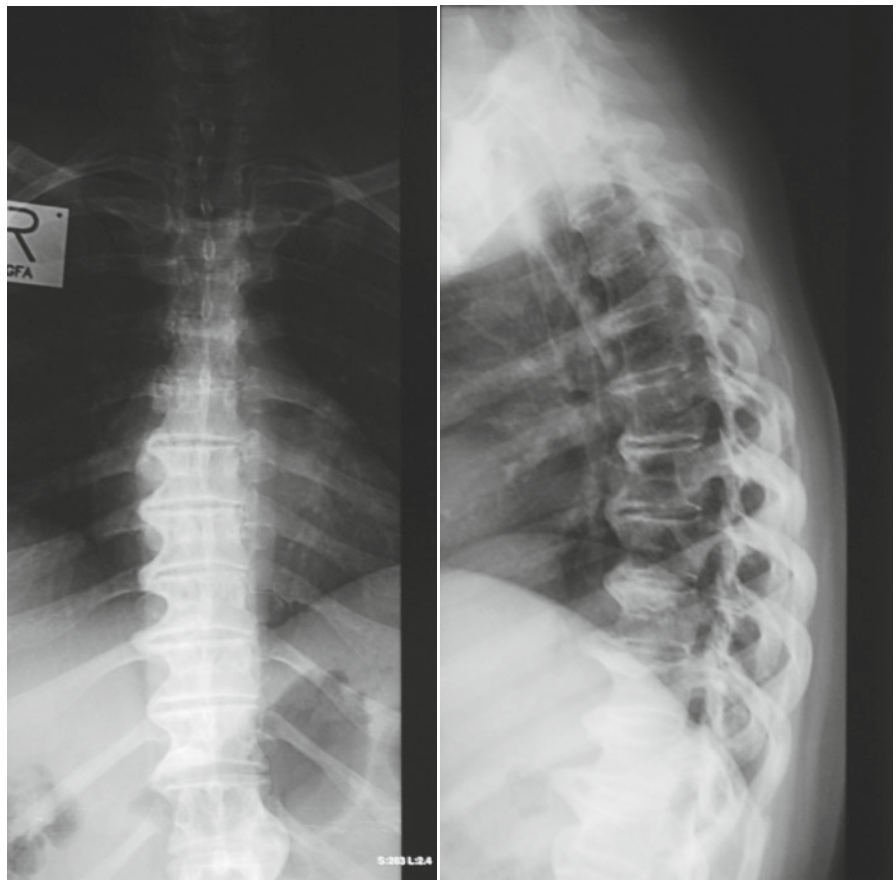

Fig. 20.25 X-rays of the dorsal spine, A-P and lateral views. A 38-year-old woman with LS. Degenerative changes with anterior bone bridging are noted

20.8.5 Extremities

The extremities in LS patients are characterized by proportionately small bones with normal or thickened cortex (see Chap. 8).

Anteroposterior views of the extremities were available for 15 patients. Of the upper extremities, the following were available: humerus in 10, elbow in 14, and radius and ulna in 13. Of the lower extremities, the following were available: femur in ten, knee in five, and tibia and fibula in ten.

Studies of the hand and foot are treated separately in Chap. 25. Radiographs of the left hand were available in 15 children; no structural anomalies were identified.

In all these patients, every depicted bone (except for the femur in two patients, one male, one female) was proportionately small (Kornreich et al. 2008). The bone structure appeared to be normal. No signs of old or recent fractures were detected except in one patient who had been involved in a motor vehicle accident.

The cortical thickness of the long bones as a marker of osseous resorption was evaluated qualitatively and quantitatively. On visual examination, the cortex was defined as thick if at the thickest cortical level the medullary cavity comprised one third or less of the diameter of the shaft. Measurements of cortical width were obtained at midfemur and upper humerus. We then calculated the cortical index, i.e., the sum of both cortices on A-P view, divided by the transverse diameter at the same level and compared it with published values (Virtama and Helela 1969).

Despite the small size of the long bones, on visual examination, the cortex appeared thickened in the upper extremities of nine patients (three males, six females) and in the lower extremities of four patients (one male, three females) (Figs. 20.29–20.30, 20.34) (see also Chap. 19). The cortical index of the humerus was

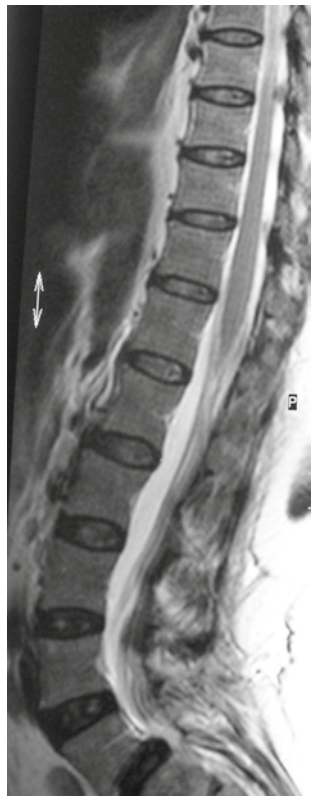

Fig. 20.26 MR sagittal T2 weighted (TR 3500/TE 120) image of the lumbar spine. A 44-year-old man with LS. The width of the spinal canal appears within normal limits

markedly greater than the mean reference value in 12 of 14 relevant radiographs. By contrast, the index of the femur was smaller than the reference value in all ten relevant radiographs (Table 20.9) (Kornreich et al. 2008).

Despite its important role in somatic growth, IGF-1 is not required for the formation of a structurally well-developed bone. In two reports of untreated patients with LS and in a study of four untreated men with deficiency of the GH-releasing hormone receptor (therefore lacking both GH and IGF-1), estimation of the volumetric bone mass indicated a normal mineral bone density (Maheshwari et al. 2003; Benbassat et al. 2003). Histomorphometry of biopsy specimens of the iliac crest from patients with LS confirmed the preservation of volumetric bone density and cortical width (Bachrach et al. 1998). These findings were corroborated by Bikle et al. (2001) in a study of IGF-I-deficient mice wherein the authors concluded that the lack of IGF-1 leads to the development of a bone structure that, although smaller, appears to be more compact. The mechanical load on the skeleton by the increased body mass characteristic of LS may also contribute to the presence of normal or thick cortical bone (Bachrach et al. 1998). (Also see Chaps. 18 and 19 and chapters in Sect. 2). However, we have no good explanation for the preferential thickening of the cortex in the upper extremities in our patients.

Several of our patients complained of severe knee or ankle pain. In one female patient aged 46 years, MR examination of both knees revealed bilateral Baker's cysts.

Radiographs of the upper extremities in the anatomic position, including both the humerus and the elbow, were available for ten patients. In six, we noted a discrepant rotation of the ulna as opposed to the anteroposterior position of the humerus (Figs. 20.29 and 20.34) (Kornreich et al. 2008). Radiographically, we did not identify any major skeletal anomaly of the

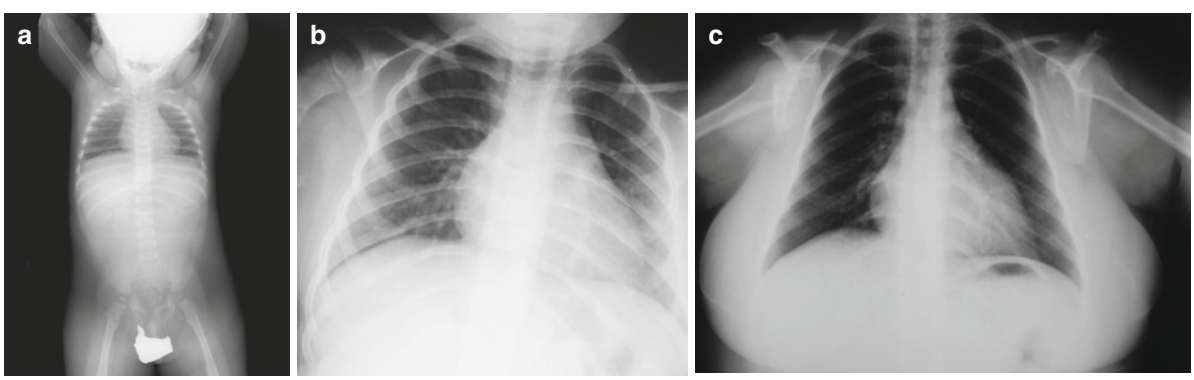

Fig. 20.27 X-ray of chest in LS patients. A-P view. A (babygram) 16-month-old girl. At this age, the chest is of normal shape. Note the prominent thickness of the subcutaneous fat. (**b**) A 12-year-old girl. The position of the diaphragm is high. The chest is of normal shape. Note the thin clavicles. (**c**) A 29-year-old woman. The chest has a bell-shaped configuration. Note the thickened soft tissues of the upper arm in contrast to the delicate humerus

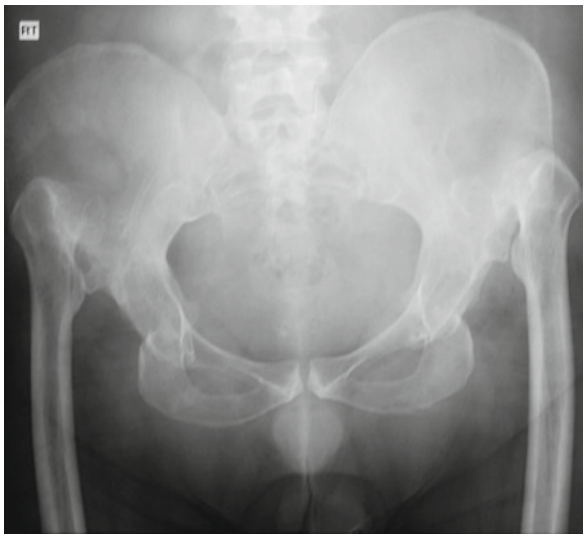

Fig. 20.28 X-ray of the pelvis. A 42-year-old man with LS. Bilateral dislocation of the femoral head is seen. Note the normal thickness of the cortical bone. The patient is ambulant

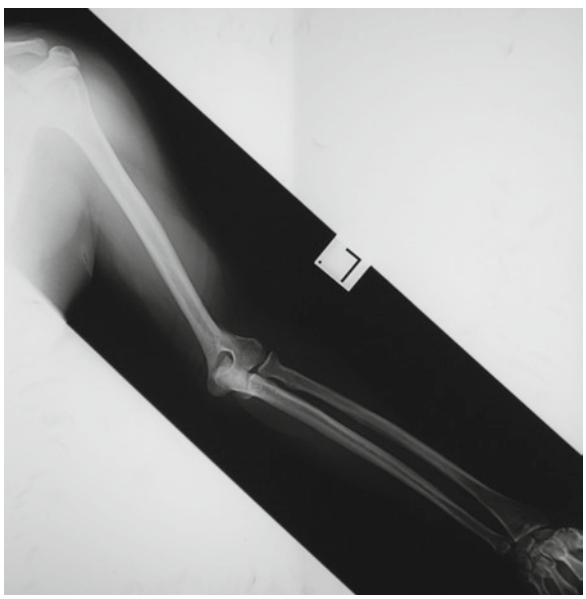

Fig. 20.29 X-ray of the upper limb, A-P view. A 46-year-old man with LS. The bones are slender, but the cortex of the radius and ulna is thickened. Note the rotation of the elbow

elbow; for three patients, lateral views were available in addition to the anteroposterior view: none showed bony abnormalities. Clinically, 85% of patients with LS have been found to have limited elbow extensibility (Rosenbloom et al. 1997). The observation in six patients of a rotated ulna in the presence of a humerus in neutral position raises the possibility of an abnormally high degree of humeral head retroversion. Normally, the humeral head is in marked retroversion in utero and at birth and derotates during childhood, reaching its final position when the individual is approximately 16 years of age (Edelson 2000). The transition to the adult angle is influenced by many factors, including the shape of the rib cage (Edelson 1999). In LS, given that both skeletal growth and the shape of the chest are abnormal, we may assume that the process of derotation is impaired, resulting in an abnormal humeral axis. During flexion and extension, the olecranon rotates around the trochlea, so that any change in their axis may limit the normal range of movement (Ericson et al. 2003), being manifested as a limitation in extension. Direct measurements of humeral head retroversion on CT may validate this supposition, but CT could not be performed for ethical reasons. We suggest that the limitation in elbow distensibility common to LS patients may be related to a marked retroversion of the humeral head (Kornreich et al. 2008).

20.9 Retarded Bone Age

LS is characterized by delayed bone age and delayed puberty (by 3–7 years) in 50% of patients, more so in boys (Laron 2004; Rosenbloom et al. 1997).

For seven patients (four male, three female), we obtained radiographs that had been taken when the patients were aged 21–25 years (lumbar spine in four, pelvis in four, chest in four, lower extremity in four, upper extremity in two). In one 22-year-old LS patient, radiograph of the hand (Greulich and Pyle) showed a bone age of 12 years and 6 months; in the spine, the ring apophyses were present but not fused (Fig. 20.31), and in the pelvis, the synchondroses of the acetabuli and the apophyses of the trochanteri were open (Fig. 20.32). In a male LS patient aged 25 years, all pelvic growth areas were closed, but the iliac crest apophyses were not completely fused (Fig. 20.33). The proximal humeral growth plate was still visible in two male LS patients aged 22 years (Fig. 20.34). In three female LS patients (aged 21–22 years), only the apophyses of the iliac crest were not completely fused (Kornreich et al. 2008).

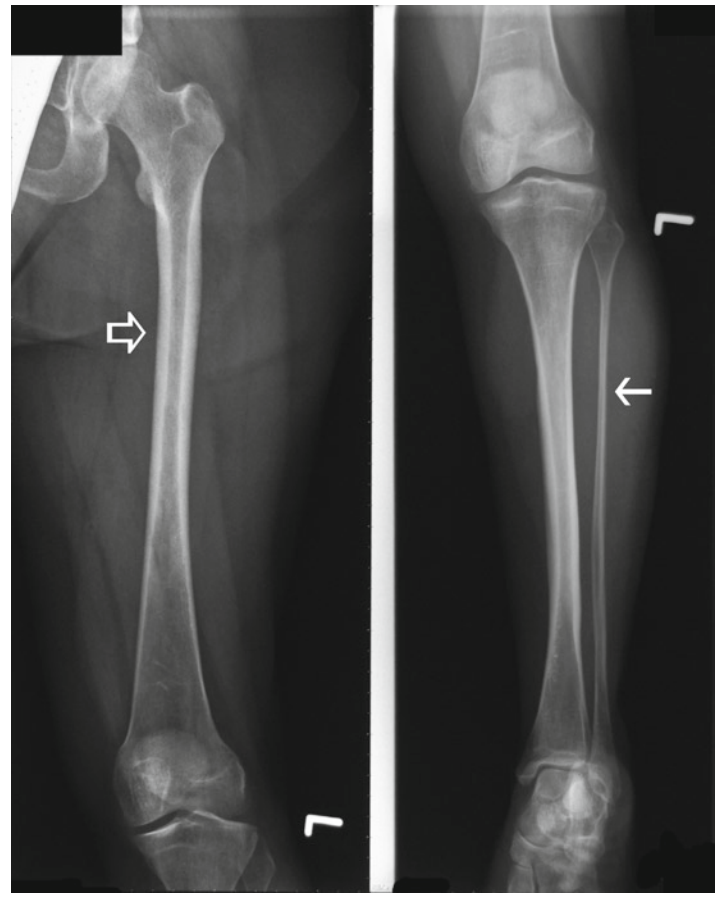

Fig. 20.30 X-ray of the left lower limb. A 47-year-old man with LS. The bones are slender (note the delicate fibula, *arrow*), but the cortex appears to be of normal width (*open arrow*)

Table 20.9 Cortical index in patients with Laron syndrome

Patient no.	Sex (M/F)	Age (year)	Femur			Humerus		
			Left	Right	Ref[a]	Left	Right	Ref[a]
1	F	21	0.52		0.56		0.64	0.39
2	F	38					0.64	0.36
3	F	40	0.52		0.64		0.64	0.36
4	F	40	0.36		0.64		0.64	0.36
5	F	45					0.57	0.38
6	F	46					0.58	0.38
7	F	49	0.4		0.61		0.53	0.37
8	F	51		0.53	0.61		0.77	0.37
9	M	26				0.47		0.43
10	M	38	0.51		0.61		0.75	0.38
11	M	41	0.55		0.61			
12	M	42		0.45	0.61		0.6	0.39
13	M	46		0.47	0.61		0.77	0.38
14	M	47					0.41	0.38
15	M	68	0.53		0.58		0.7	0.32

[a]Mean published values; *M* male; *F* female
Reprinted with permission from Kornreich et al. 2008

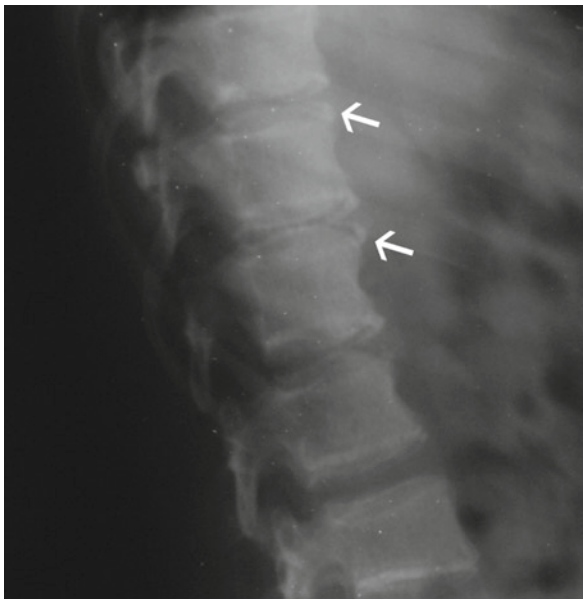

Fig. 20.31 X-ray of the lumbar spine, lateral view. A 22-year-old man with LS. The ring apophyses of the vertebrae are not fused (*arrows*), indicative of retarded bone maturation

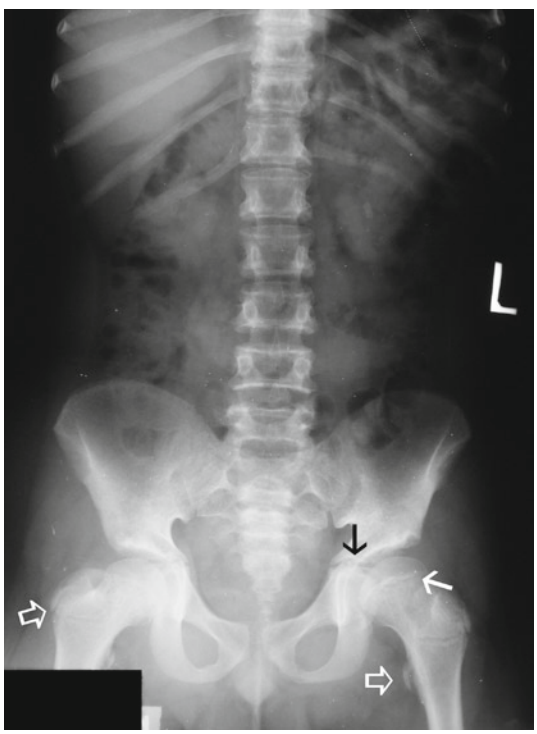

Fig. 20.32 X-ray of the abdomen. A 22-year-old man with LS. Note that in the pelvis the synchondroses of the acetabuli (*black arrow*) and the apophyses of the trochanteri (*open arrows*) are not fused. The physis of the femoral head is still visible (*white arrow*)

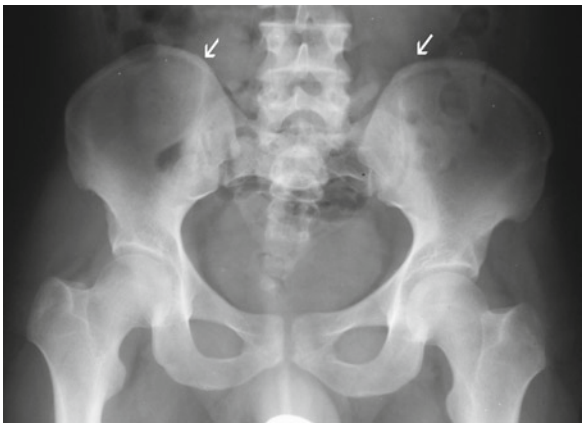

Fig. 20.33 X-ray of the pelvis. A 25-year-old man with LS. The shape of the pelvis is normal. The apophyses (*arrows*) of the iliac crests are not completely fused, indicative of retarded bone age

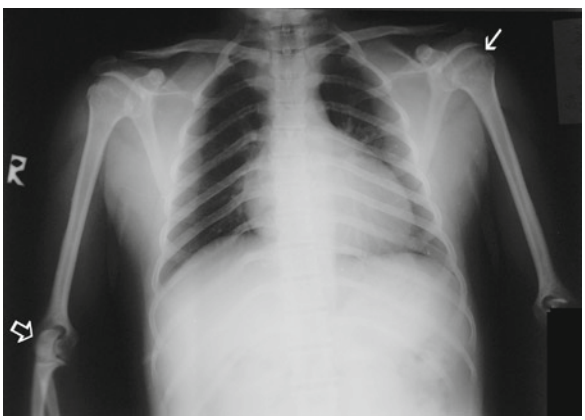

Fig. 20.34 Chest X-ray, A-P view. A 22-year-old man with LS. The chest is bell-shaped. The epiphyses of the humeral head are still not fused (*arrow*). Rotation of the elbow is demonstrated (*open arrow*). Note the thickened humeral cortex

20.9.1 Summary

The long-term consequences of GH/IGF-1 deficiency result in multisystem abnormalities. The face and the base of skull are small with underdeveloped paranasal sinuses and mastoids. The orbit, and especially the ocular globe, is small in comparison to normal controls. The skull is relatively large for the small body, but the diploe is thin. Frequently occurring is the anomaly of the dens of the os odontoideum type. A high-pitched voice and the propensity for sleep apnea are related to the reduced dimensions of the larynx and

oropharynx. The brain presents no specific pathological findings, and no abnormalities of the pituitary gland were detected. The spine and appendicular skeleton are markedly affected due to the crucial role of GH/IGF-1 on bone elongation and accretion. Every depicted bone is proportionally small. In the cervical spine this results in spinal stenosis. In the lumbar spine, the spinal canal is narrow in comparison to that in normal controls, but not so in relation to the cord width. There is a tendency for early osteoarthritic changes of the atlanto-axial joint and of the thoracic and lumbar spine. No signs of osteopenia are detected; on the contrary, the cortex of the long bones appears thickened, particularly in the humeri. Clinically, limitation of distensibility of the elbow is common in LS patients, probably due to retroversion of the humeral head. The chest is bell-shaped. There is marked retardation of bone age.

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Ocular Findings in Laron Syndrome

21

Dan H. Bourla and Dov Weinberger

Core Message

› Ophthalmological investigations of untreated Laron syndrome patients revealed retarded growth of the ocular globe. The shorter anterior length of the anterior chamber was normalized by IGF-I treatment. The retinal vascularization was reduced as were the number of tortuosities of the arteries. Two patients with diabetes mellitus developed retinal neovascularization.

21.1 Introduction

The association between ocular pathology and growth hormone (GH)/insulin-like growth factor I (IGF-I) deficiency in man has been described in a few studies and reviews (Bourla et al. 2006, 2007; Hellström et al. 2002; Harvey et al. 2007; Laron and Weinberger 2004). Current knowledge on the effects of congenital GH receptor defects on parameters of ocular development and function is limited to data found in patients with Laron syndrome (LS). Clinical studies of the cornea and lens, as well as ocular biometric studies of the retina and optic nerve in patients with LS, give us important clues to the effects of the absence of GH receptor on ocular development. Other studies of the retina in patients with defects in GH/IGF-I axis add important information regarding the effects of IGF-I on retinal vascular development (Hellström et al. 2002) and diabetic retinopathy (Laron and Weinberger 2004).

To study the effects of IGF-I on ocular growth, we measured the ocular components and refraction and performed fundus photograph and optical coherence tomography (OCT) analysis in patients with LS, some of them untreated and others treated by IGF-I (Bourla et al. 2006; Bourla unpublished data).

21.2 Subjects

Twelve patients with LS belonging to the Israeli cohort of LS patients followed up and treated at the Endocrinology and Diabetes Research Unit, Schneider Children's Hospital, Petah Tikva, Israel, were studied. Eight of these patients had no previous IGF-I therapy, and four patients were treated with IGF-I, either currently or in the past. In addition, 30 healthy age and gender-matched control subjects were examined.

21.3 Methods

From each patient and control subject examined, we obtained a history of systemic and ocular diseases, and the following ophthalmic examinations were performed: best-corrected Snellen visual acuity, applanation tonometry for intraocular pressure (IOP), biomicroscopy of the anterior segment and fundus, automated corneal keratometry and refractometry, as well as ultrasound biometry.

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OCT of the macula and optic nerve was performed and analyzed for a subset of LS patients. To be included, subjects had to have best-corrected visual acuity of 20/40 or better, spherical refraction within ±5.0 diopters and cylinder correction within ±3.0 diopters, and open angles on gonioscopy. Patients with coexisting retinal disease or glaucomatous optic neuropathy were excluded from this investigation. Twelve eyes of six patients met the inclusion criteria: ten eyes of patients who never received IGF-I treatment and two eyes of a patient who was under IGF-I therapy for 3.5 years.

The Fast Optical Disk scanning protocol was used to obtain optic nerve head (ONH) measurements with Stratus OCT. The ONH scan consists of six radial scans in a spoke like pattern centered on the ONH. The OCT interpolates between the scans to provide measurements throughout the ONH.

ONH parameters evaluated in this study were vertically integrated in their rim area (total volume of rim tissue calculated by multiplying the average of individual rim areas times the circumference of the disk), horizontally integrated rim width (estimate of total rim area calculated by multiplying the average of individual rim widths times the circumference of the disk), disk area, cup area, rim area, cup/disk area ratio (ratio of cup area to disk area), horizontal cup/disk ratio (ratio of the longest horizontal line across the cup to the longest horizontal line across the disk), and vertical cup/disk ratio (ratio of the longest vertical line across the cup to the longest vertical line across the disk). These parameters were automatically calculated by Stratus OCT software (version 3.1).

The fast retinal nerve fiber layer (RNFL) algorithm was used to obtain RNFL thickness measurements with Stratus OCT. Three images were acquired from each subject, with each image consisting of 256 A-scans along a 3.4-mm-diameter circular ring around the optic disk. A mean image was automatically created by the Stratus OCT software.

Parapapillary RNFL thickness parameters automatically calculated by existing Stratus OCT software (version 3.1) and evaluated in this study were average thickness (360° measure), temporal quadrant thickness (316–45°), superior quadrant thickness (46–135°), nasal quadrant thickness (136°–225°), and inferior quadrant thickness (226°–315°).

The Fast Macular Thickness protocol was used to obtain macular thickness measurements with Stratus OCT. The macular scans consist of six radial scans in a spoke-like pattern centered on the fovea with each radial scan spaced 30° from one to another. Macular thickness parameters automatically calculated by existing Stratus OCT software (version 3.1) and evaluated in this study were foveal thickness, superior outer macular thickness, inferior outer macular thickness, temporal outer macular thickness, nasal outer macular thickness, superior inner macular thickness, inferior inner macular thickness, temporal inner macular thickness, and nasal inner macular thickness. Average macular thickness was calculated as the weighted average of the sectoral macular thickness measurements excluding the fovea.

21.4 Results

21.4.1 Ocular Growth and Biometric Findings

Table 21.1 summarizes the pertinent clinical data for the untreated and IGF-I-treated patients.

The mean age of the untreated LS subjects was 46 years (range 28–53 year, SD±8.0), and for the IGF-I-treated patients, 15.2 years (range 8–27 year, SD±8.3).

Table 21.1 Pertinent clinical data of patients with LS

Patient number	Therapy	Age at examination (years)	Age during therapy (years)	Weight (kg)	Height (cm)
1	None	45	–	28	116
2	None	52	–	48	122
3	None	28	–	45	140
4	None	51	–	42	131
5	None	45	–	36	116
6	None	47	–	62	130
7	None	49	–	38	112
8	None	53	–	44	138
9	IGF-I	15	7–15	64	137
10	IGF-I	11	7.5–11	23	107
11	IGF-I	27	10–15	55	135
12	IGF-I	8	5–8	20	97

Table 21.2 Clinical ocular examination data, patients with LS

Patient number	Eye	Visual acuity	Refraction	AXL	ACD	LT	CC	IOP	Lens	Optic nerve CD	Macula
1	OD	6/6	+1.50	22.33	2.66	4.57	43.87	14	NS+2	0.5	Normal
	OS	6/6	+1.25	22.37	2.66	4.50	43.75	14	NS+2	0.5	Normal
2	OD	6/15	+1.25	20.97	2.20	4.38	46.10	12	Clear	0.3	Drusen
	OS	6/12	+1.25	21.00	2.20	4.40	46.11	11	Clear	0.3	Drusen
3	OD	6/6	−2.75	21.41	2.76	4.25	50.12	14	Clear	0.4	Normal
	OS	6/6	−2.50	21.42	2.68	4.22	50.37	14	Clear	0.4	Normal
4	OD	6/30	−6.50	23.05	2.56	4.71	47.62	17	Clear	0.6	Myopic
	OS	6/20	−6.50	23.00	2.60	4.78	47.50	15	Clear	0.6	Myopic
5	OD	6/12	−5.25	23.54	2.73	4.45	48.12	14	NS+1C+2	0.5	OU NPDR
	OS	6/6	−6.50	23.32	2.63	4.52	48.00	14	NS+1C+1	0.5	No CSME
6	OD	6/15	+4.75	20.97	2.53	4.71	44.12	11	Clear	0.2	Normal
	OS	6/15	+5.00	21.26	2.60	4.71	43.87	12	Clear	0.2	Normal
7	OD	6/6	+3.00	20.85	2.50	4.65	46.75	16	Clear	0.7	Normal
	OS	6/6	+1.25	20.70	2.50	4.68	46.68	38	Clear	0.9	Normal
8	OD	6/7.5	−1.50	22.47	2.52	4.66	48.50	14	NS+2C+1	0.4	OU PDR
	OS	6/7.5	−1.00	22.34	2.46	4.69	48.12	14	NS+2C+1	0.4	s/p PRP
9	OD	6/6	−2.50	22.52	3.44	3.76	47.62	10	Clear	0.3	Normal
	OS	6/6	−2.25	23.07	3.42	3.70	47.50	11	Clear	0.3	Normal
10	OD	6/6	+0.25	23.20	3.46	3.74	49.25	11	Clear	0.2	Normal
	OS	6/6	+0.50	23.33	3.40	3.72	49.00	11	Clear	0.2	Normal
11	OD	6/6	−2.75	24.00	3.65	3.90	43.37	14	NS+1	0.4	Normal
	OS	6/6	−2.50	24.04	3.60	3.85	43.87	15	NS+1	0.4	Normal
12	OD	6/6	−0.75	20.01	3.42	3.72	50.12	12	Clear	0.3	Normal
	OS	6/6	−1.00	20.05	3.46	3.76	49.87	12	Clear	0.3	Normal

OD right eye; *OS* left eye; *OU* both eyes; *AXL* axial length; *ACD* anterior chamber depth; *LT* lens thickness; *CC* corneal curvature; *IOP* intraocular pressure; *NS* nuclear sclerosis cataract; *C* cortical cataract; *CD* cup to disk ratio; *NPDR* nonproliferate diabetic retinopathy; *CSME* clinically significant macular edema; *PDR* proliferative diabetic retinopathy; *PRP* pan retinal photocoagulation

The age difference between the untreated and IGF-I-treated patients is a consequence of the availability of recombinant biosynthetic IGF-I treatment for growth stimulation only since 1986. The control group of the adult untreated patients had an average age of 45.2 years (range 25–57 year, SD±8.2), matching the untreated LS group. We were unable to obtain age-matched controls for the younger IGF-I-treated LS patients due to lack of consent. Table 21.2 summarizes the visual acuity, refraction, biometry, keratometry, IOP, anterior segment, and fundus biomicroscopy for all the LS patients whether untreated or treated. The average axial length (AXL) of the eyes in the untreated LS patients was 21.94 mm (SD±0.81), and the eyes of the IGF-I-treated patients had an average AXL of 22.53 mm (SD±1.74). In the control group, the average AXL was 23.20 mm (SD±1.35). The difference in the AXLs between the untreated LS patients and the control group was statistically significant ($p<0.01$), but did not reach statistical significance between either the untreated and IGF-I-treated patients, or the IGF-I-treated LS patients and the control group. It should be kept in mind that the number of patients treated

was small and that the initiation of IGF-I treatment in the patients studied was relatively late (Table 21.1).

The mean anterior chamber depth (ACD) of eyes in the untreated LS patients was 2.55 mm (SD ± 0.26) and that of the IGF-I-treated patients was 3.48 mm (SD ± 0.09). The eyes in the control group had an average anterior chamber depth of 3.84 mm (SD ± 0.16). The differences in anterior chamber depths were statistically significant between the untreated and IGF-I-treated LS patients, as well as between untreated LS patients and the control subjects ($p < 0.001$). There was no statistically significant difference in anterior chamber depths of the eyes in IGF-I-treated LS patients and the control subjects.

The average lens thickness (LT) of the eyes in the untreated LS group was 4.56 mm (SD ± 0.36), compared to 3.77 mm (SD ± 0.23) in the treated LS patients ($p < 0.001$). The average LT of the control eyes was 3.51 mm (SD ± 0.25). There was a statistically significant difference in LT between the untreated and IGF-I-treated LS patients, as well as between the control and untreated eyes ($p < 0.001$). The LT in the IGF-I-treated LS patients and the control subjects was similar.

The mean corneal curvature (CC) of the eyes in untreated LS patients was 46.9 diopters (SD ± 2.32), similar to that of the IGF-I-treated LS patients, 47.6 diopters (SD ± 2.83). The control eyes had an average CC of 44.4 diopters (SD ± 1.5), significantly smaller than that of the untreated LS patients ($p < 0.001$) and that of the IGF-I-treated LS patients ($p < 0.05$).

21.4.2 Optic Nerve Head, Retinal Nerve Fiber Layer, and Macular Thickness

We analyzed a total of 12 eyes of six patients: five untreated LS patients and one LS patient who was treated with IGF-I since the age of 7.5 years. Table 21.3 shows the mean values of Straus OCT ONH parameters in the eyes of untreated and IGF-I-treated patients with LS. Statistically significant differences were found for all the parameters except disk area.

Table 21.4 shows the values of OCT RNFL thickness parameters for the eyes of untreated and IGF-I-treated patients with LS. No statically significant differences were found. Table 21.5 shows the mean values of Stratus OCT macular thickness parameters in the eyes of untreated and IGF-I-treated patients with LS. No statically significant differences were found in the macular thickness parameters.

Figures 21.1 and 21.2 show typical OCT findings of the macular and optic nerve regions, respectively.

Table 21.3 Mean (±SD) values of Stratus OCT ONH analysis

Parameter	Untreated Laron syndrome ($n = 10$)	IGF-I-treated Laron syndrome ($n = 2$)	p*
VIRA (mm^3)	0.13 ± 0.07	0.62 ± 0.13	<0.0001
HIRW (mm^2)	1.40 ± 0.20	2.01 ± 0.11	0.0018
Disk area (mm^2)	2.63 ± 0.22	2.28 ± 0.02	0.0532
Cup area (mm^2)	1.78 ± 0.38	0.38 ± 0.09	0.0005
Rim area (mm^2)	1.06 ± 0.24	1.90 ± 0.08	0.0009
Cup/disk area ratio	0.62 ± 0.10	0.17 ± 0.04	<0.0001
Cup/disk horizontal ratio	0.82 ± 0.05	0.46 ± 0.04	<0.0001
Cup/disk vertical ratio	0.75 ± 0.07	0.34 ± 0.05	<0.0001

*t-test

VIRA vertical integrated rim area; *HIRW* horizontal integrated rim width

Table 21.4 Mean (±SD) values of Stratus OCT retinal nerve fiber layer analysis

Parameter	Untreated Laron syndrome ($n = 10$)	IGF-I-treated Laron syndrome ($n = 2$)	p*
Temporal RNFL	60.2 ± 11.4	64.0 ± 6.0	0.665
Superior RNFL	93.5 ± 19.9	102.5 ± 0.5	0.552
Nasal RNFL	61.0 ± 11.8	83.0 ± 1.0	0.0296
Inferior RNFL	87.0 ± 19.6	164.0 ± 4.0	0.0003

*t-test

RNFL retinal nerve fiber layer

21.4.3 General Ocular Manifestations

In the cohort of 12 patients with LS, primary open angle glaucoma was present in two subjects, bilateral cortical cataract in two subjects, age-related macular degeneration in one subject, and bilateral strabismus in one subject.

Table 21.5 Mean (±SD) values of Stratus OCT macular thickness analysis

Parameter	Untreated Laron syndrome ($n=10$)	IGF-I-treated Laron syndrome ($n=2$)	p*
Foveal minimum	166.1 ± 24.3	135.0 ± 6.0	0.114
Fovea	195.2 ± 21.1	186.0 ± 5.0	0.569
Temporal inner macula	254.8 ± 27.2	271.5 ± 3.5	0.424
Superior inner macula	270.7 ± 25.2	290.0 ± 0.0	0.321
Nasal inner macula	260.1 ± 32.3	287.5 ± 0.5	0.288
Inferior inner macula	269.0 ± 23.8	286.5 ± 6.5	0.342
Temporal outer macula	225.9 ± 23.1	248.5 ± 0.5	0.213
Superior outer macula	244.7 ± 13.8	260.0 ± 1.0	0.161
Nasal outer macula	250.6 ± 31.3	286.0 ± 1.0	0.154
Inferior outer macula	240.3 ± 24.0	265.5 ± 3.5	0.185
Macular average	252.0 ± 22.5	274.4 ± 0.6	0.245

*t-test

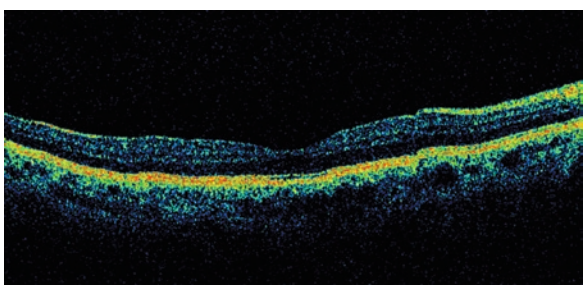

Fig. 21.1 Optical coherence tomography (OCT) of the macular region in a nondiabetic patient with Laron syndrome (LS) shows normal retinal architecture

21.4.4 Retinal Vascularization

Hellström et al. (2002) made an extensive effort to analyze the retinal vascularization with respect to the number of branching points and tortuosity of arteries and veins in thirteen individuals with genetic defects of the GH/IGF-I axis. Eleven of those patients had LS, and eight belonged to the Israeli cohort. Measurements of the retinal vessels were made by tracing each vessel (path length) from its origin on the optic disk to a reference circle with a radius of 3.0 mm from the geometric center of the optic disk. The index of tortuosity for arteries and veins was defined as the path length of the vessel divided by the linear distance from the vessel origin to the reference circle.

The patients with LS had a significantly lower number of vascular branching points (median 23, range 16–25), compared with the reference group of normal controls (median 28, range 19–40, $p<0.001$). All 13 individuals had vascular branching points below the median of the reference group (Fig. 21.3).

IGF-I or GH administration to three patients with LS and to two patients with IGF-I gene mutation did not affect the observed abnormal retinal vascularization (Hellström et al. 2002).

21.4.5 Retinal Neovascularization

The occurrence of diabetic retinopathy in patients with LS who developed diabetes and diabetic vascular complications has been investigated by Laron and Weinberger (2004). They described two adult LS patients. The findings showed that congenital IGF-I deficiency, similar to excess, causes vascular complications of diabetic retinopathy (Fig. 21.4), denoting that neovascularization may appear in the presence of congenital IGF-I deficiency.

21.5 Discussion

The values of ocular components that influence refractive error have been extensively studied in human eyes in vivo. Normal distribution of ocular components, a skewed, leptokurtic distribution of refractive errors, and a significant negative correlation between refractive error and AXL at various ages have been reported (Benjamin et al. 1957; Parentin et al. 2004; Sorsby et al. 1961; Sorsby and Leary 1969; Stenstrom 1948). The refractive error may also be the result of mismatched association among the ocular components. The components of interest are corneal power, anterior chamber depth (ACD), and AXL. The human eye

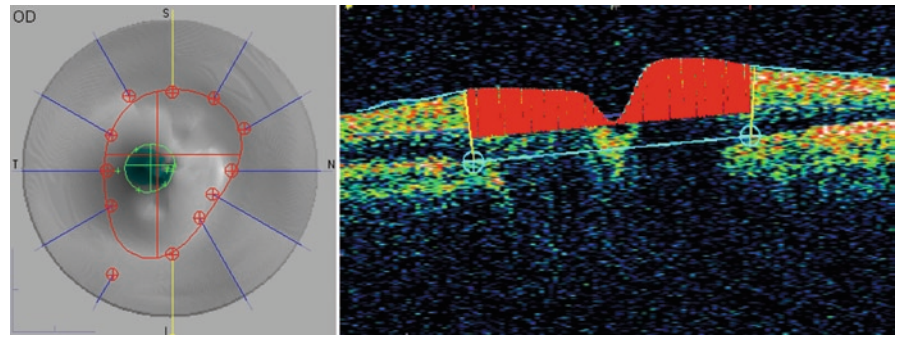

Fig. 21.2 OCT analysis of the optic nerve head (ONH) in a typical patient with LS demonstrates normal distribution of the nerve fiber layer

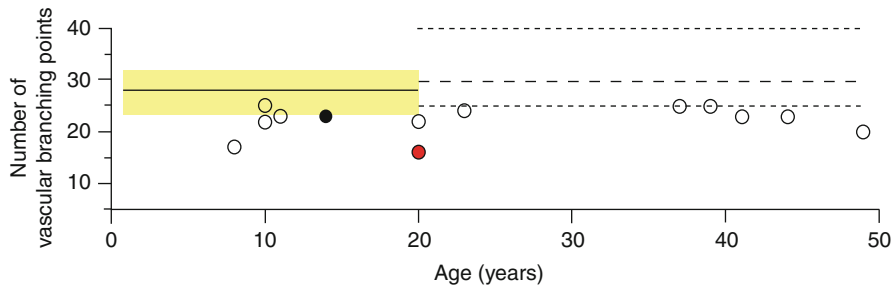

Fig. 21.3 Number of retinal vascular branching points in individuals with defects in the growth hormone (GH) (*open circle*, $n=11$), insulin-like growth factor I (IGF-I) (*filled circle*, $n=1$), or IGF-I receptor genes (*filled red circle*, $n=1$). The *shaded area* depicts the 10th to the 90th centile range, and the *center line* indicates the median for the healthy reference group of 100 controls aged 2–20 year (Hellström et al. 2002). The *lines* represent the minimum (*dotted line*), the median (*dashed line*), and the maximum (*dotted line*) of 20 healthy individuals aged 20–50 year (reported by Hellström et al. 2002)

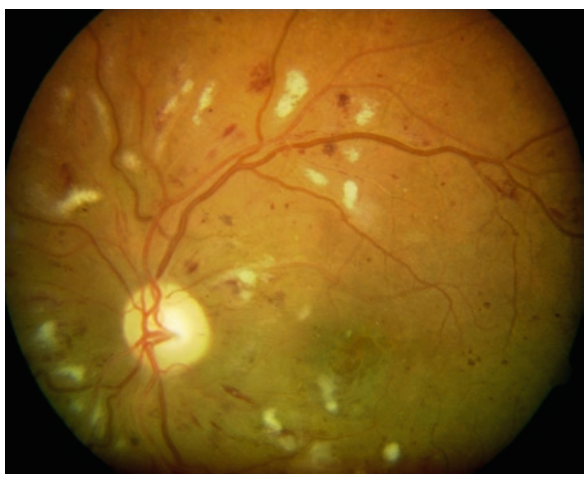

Fig. 21.4 Color fundus photograph of a 53-year-old male diabetic patient with LS. Progressive diabetic retinopathy exhibiting macular edema, retinal hemorrhages, and cotton-wool spots

achieves emmetropia in youth, despite the change in these variables. In a large cross-sectional study of ocular component data of 2,583 healthy children, Zadnik et al. (2003) showed that between the ages of 6 and 14, eyes display a general pattern of axial growth, no change in corneal power, and crystalline lens thinning. Their results show that by the age of 14, the calculated average AXL, ACD, LT, and CC are 23.59, 3.67, 3.48 mm, and 43.38 diopters, respectively.

We found that the average AXL of untreated patients with LS was significantly lower than that of aged matched controls. The patients treated with IGF-I had an average AXL higher than untreated patients, but lower than the controls. However, our IGF-I-treated patients had an average age of 15.2 years; hence we were unable to age-match them to our control group. Furthermore, two of the treated LS patients were under 14 years of age and the difference between treated patients and the control group may still change with longer periods of treatment and/or earlier initiation of IGF-I therapy.

When comparing our data to those published by Zadnik et al. (2003), we note that the average AXL of treated LS patients is shorter than the published age-matched control data. When comparing the ACD and LT values in the LS patients to those published by Zadnik, we also note that the IGF-I-treated patients have similar values to the normal age-matched data,

while the untreated LS patients have shallower anterior chambers and thicker lenses. Parentin et al. (2004) showed that children with GH deficiency have a mean hyperopic defect, related to a short AXL. In our study, we found a hyperopic tendency in untreated subjects with LS, while those who received IGF-I therapy displayed a tendency toward mild myopia (Table 21.2). This finding correlated with the increased average axial length of eyes in the IGF-I-treated group compared to the untreated group. Kusakari et al. (2001) demonstrated that IGF and other growth factors influence scleral modeling and may be involved in scleral growth by regulating the synthesis and degradation of the extracellular matrix of the sclera. Furthermore, GH/IGF-I may be possibly linked to ocular growth via its role in somatic growth. In a study investigating the relationship between height and ocular component data, Wong et al. (2002) wrote that adult height is independently related to ocular dimensions, but does not appear to influence refraction. They concluded that although taller persons are more likely to have longer globes, they also tend to have deeper anterior chambers, thinner lenses, and flatter corneas.

Our findings in LS patients showed that the CC of both treated and untreated patients with LS was significantly steeper than that of controls. Previous studies by Garner et al. (1988, 1990) showed similar values of CC as our control group. However, despite the normal values of LT and anterior chamber depth with relatively steep CC in the IGF-I-treated subjects, we only measured a mild myopic refraction. This result may be explained by a decrease in lens power, despite normal ocular proportions in the IGF-I-treated eyes. In a study of gap-junction activity in rabbit lens epithelial cells, IGF-I induced decreases in gap junctional communication (Sorsby et al. 1961; Lin et al. 2003). IGF-I overexpression in the mouse lens results in increased lens epithelial proliferation, but not in differentiation (Lang 1999; Shirke et al. 2001). IGF-I also regulates the expression of the endogenous delta 1-crystallin gene in embryonic lens cells (Alemany et al. 1990). The effect of IGF-I on lens fiber composition and gene expression may contribute to a decrease in lens power and thus may explain the reduced myopia in the IGF-I-treated LS patients.

In summary, untreated patients with LS have a significantly shorter axial length of the eye compared to IGF-I-treated patients. Furthermore, IGF-I-treated patients, though having an increasing axial length, did not reach normal values. Untreated patients with LS also have significantly shallower anterior chambers and thicker lenses compared to IGF-I-treated patients and healthy controls. All patients with LS have significantly steeper corneas compared to healthy controls. Thus, untreated IGF-I deficiency profoundly affects eye development.

Since GH may act by induction of the IGF-I system or directly through its receptor, patients with the IGF-I receptor defect have an impairment in IGF-I activity in the presence of normal GH levels and non-IGF-I-dependent action. Hence, the pathological findings in the retinal vascularization are likely caused due to low IGF-I, and not due to GH deficiency. The changes described in GH deficiency by Hellström et al. (2002) can be assumed to be IGF-I-mediated.

Our data suggest that a complex mechanism may be responsible for ocular growth. The different compartments of the eye may be influenced by a host of stimulatory and inhibitory factors. Some of these factors, such as IGF-I, have a general role in regulating tissue growth and differentiation. But, we cannot exclude paracrine factors that are produced by the eye itself, which influence the organization and growth of specific components within the eye.

21.6 Conclusions

Congenital GH receptor defects leading to IGF-I deficiency, as in LS, affect the eye in several ways. IGF-I seems to be an important regulator of ocular growth. IGF-I may also act as a critical permissive factor for normal retinal vascularization and neovascularization. IGF-I replacement therapy may have a role in normalizing ocular growth parameters in patients with LS.

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Hearing in Patients with Laron Syndrome

22

Zvi Laron, Omer Zarchi, and Joseph Attias

Core Message

› Untreated or late IGF-I treated patients with Laron syndrome have auditory defects. In very early treated children hearing is normal.

22.1 Introduction

The importance of the early detection of hearing impairment has been recognized in the last few decades, and auditory tests are now performed already in infancy.

Auditory defects are not mentioned among the pathological findings in disorders of the GH-IGF-I axis (Rosenbloom and Connor 2007).

Looking for possible defects in auditory functions in patients with Laron syndrome, we asked adult patients during their follow-up visits whether they had any hearing problems. A frequent complaint stated was sensitivity to noises, but none complained of hearing loss. It was therefore decided to investigate this problem closer in our population of Laron syndrome patients with congenital IGF-I deficiency and hGH inactivity.

22.2 Subjects

To evaluate by accurate means the influence of IGF-I deficiency and replacement therapy on their auditory functions, we examined three types of Laron syndrome patients:

1. Five untreated adult patients with Laron syndrome (three females, two males, aged 44–56 years)
2. Five (four females, one male) in whom early IGF-I treatment was initiated at chronological ages 2–3. 7 years
3. One girl treated after the age of 9.5 years and tested at the age of 15 years (Table 22.1).

22.3 Methods

Auditory tests were conducted at the Institute of Audiology and Clinical Neurophysiology, Schneider Children's Medical Center of Israel. Each patient underwent hearing tests in a sound attenuated room with calibrated GSI-16 audiometers (Grason-Stadler, Madison, WI, USA) and TDH-49 headphones. The air and bone conduction pure-tone for 0.25–8 kHz and 0.25–4 kHz were measured separately from each ear. The speech tests included speech reception threshold and speech understanding. Middle Ear function was assessed by tympanometry, and stapedial acoustic reflexes using an electroacoustic immittance instrument (AZ26, Middle Ear Analyzer, Assens,

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Table 22.1 Pertinent clinical data, the genetic defects, and the hearing status of patients with Laron syndrome

Patient	Gender	Age at testing (years)	Age at start of IGF-I treatment (years)		Mutation (del)	Hearing status of ear	
			CA	BA		Right	Left
Untreated patients							
1-BR	M	44			GH-R – W-15X (exon 2)	LTCHL	LTCHL
2-BS	F	46			GH-R – W-15X (exon 2)	HTCHL	LTCHL
3-SS	M	48			GH-R – R217X (exon 7)	FCHL	LTCHL
4-RY	F	52			GH-R – R217X (exon 7)	LTCHL	LTCHL
5-TS	F	56			GH-R – 3,5,6 exon del	LTCHL	LTCHL
Delayed treated patient							
7-MM	F	15	9.7	4.6	GH-R – R43X/ Norm (exon 4)	LTCHL	LTCHL
Early treated patients							
8-PR	F	7	2	0.5	GH-R – R43X/ Norm (exon 4)	NH	NH
9-HN	F	7	3.2	2	GH-R – R161C (exon 6)	NH	NH
10-CL	F	11	3.6	1.8	GH-R – 3,5,6 exon del	NH	NH
11-HS	F	18	3.7	2.1	GH-R – 785-1 (exon 8)	NH	NH
12-ML	M	32	3.6	1	GH-R – 230 del (exon 7)/exon 5 del	NH	NH

NH normal hearing; *LTCHL* low tone cochlear hearing loss; *HTCHL* high tone cochlear hearing loss; *FCHL* flat cochlear hearing loss

Denmark). To assess the integrity of the outer hair cells situated in the cochlea, transient evoked otoacoustic emissions (TEOAE) were recorded monaurally using an ILO92 system at the default settings (Version 1.35, Otodynamics Ltd, Hatfield, Herts, UK).

For evaluation of the auditory neural function, the auditory brain stem-evoked responses (ABR) to high level stimuli (about 80 dBHL or at a most comfortable level) were recorded from each ear (Biologic Explorer, Biologic System Corp., Mudelein, IL, USA).

To quantify loudness perception and hyperacusis, the most comfortable loudness level (MCL) and the uncomfortable loudness level (UCL) were measured for the frequencies 0.25–8 kHz of each separately. In addition, each patient was asked to complete the "Hyperacusis screening questionnaire."

22.4 Results

All untreated patients were found to have various degrees of cochlear hearing loss, (Attias et al. 2008) hyperacusis and most of them demonstrated absence of acoustic middle ear reflexes (Attias et al. 2010). In four patients, both high and low tone cochlear hearing loss was observed. Low tone cochlear hearing loss was also diagnosed in the late treated girl with Laron syndrome (Table 22.1). Figure 22.1 illustrates the audiogram of early and late or untreated patients. It is seen that the early treated patients had a significantly better, even normal, audiogram than the untreated and the late treated patients.

All five children in whom replacement treatment was initiated at chronological ages ranging from 2 to 4 years and bone age from 6 months to 2 years had

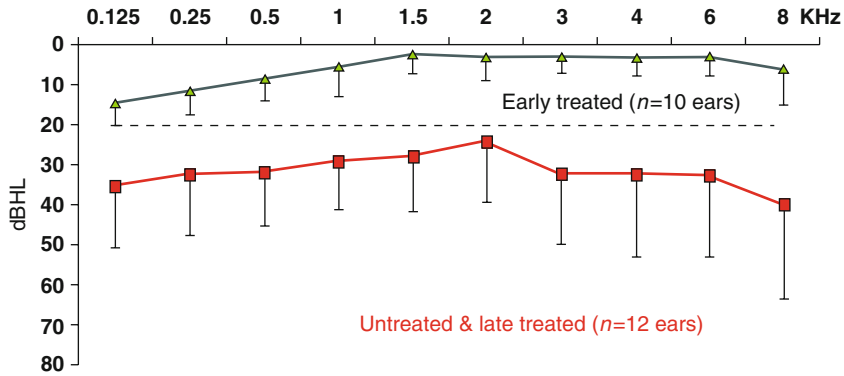

Fig. 22.1 Audiograms of the early treated and untreated or late treated Laron syndrome patients (m ± SD). The early treated patients (*in green*) had significantly better audiograms than the untreated and treated patients (*in red*)

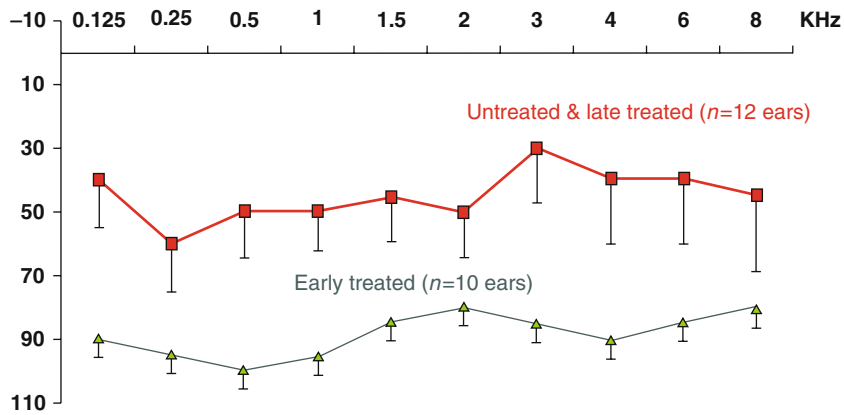

Fig. 22.2 Loudness dynamic range (Hyperacusis) of early treated and untreated or late treated Laron syndrome patients. The untreated and late treated patients (*in red*) had significantly reduced dynamic range than the early treated patients (*in green*), reflecting hyperacusis in the untreated and late treated patients

normal hearing, no hyperacusis, and, in most of them, recordable middle ear acoustic reflexes. Figure 22.2 shows the auditory response to loudness in the late or untreated Laron syndrome patients compared to the early treated ones. It is seen that the untreated patients had a significantly ($p<0.01$) reduced loudness response, that is, hyperacusis, than the early treated Laron syndrome patients.

None of the patients has ever been exposed to hazardous noises or were treated with medication that may be considered as ototoxic drugs.

22.5 Discussion

The exact mechanisms by which IGF-I interferes with the hearing loss registered in patients with Laron syndrome is unknown, but as IGF-I passes the blood brain barrier and plays an important role in the development of intracranial nervous tissue (Laron 2009), its early absence in Laron syndrome is most probably the major cause of the auditory defects. It is unlikely that the type of hearing loss found in Laron syndrome patients is caused by exogenous factors, such as noise exposures or ototoxic drugs as proven by detailed analysis of occupation history and knowing the medications of the Laron syndrome patients. Thus, the cochlear hearing loss found in our Laron syndrome patients seems to be related to their deficiency in IGF-I.

The lack of acoustic reflexes and the reported hyperacusis observed in Laron syndrome-untreated patients may also increase the susceptibility to high-tone hearing loss induced by auditory environmental sounds or low level noises.

No correlation between the type of molecular defect of the GH receptor in our Laron syndrome patients (Laron 2004; Shevah and Laron 2006) and their hearing status was found.

Moreover, it has been reported that mutations in the gene encoding human IGF-I cause syndromic hearing loss (Woods et al. 1996, Bonapace et al. 2003; Walenkamp et al. 2005). These observations are supported by findings in IGF-I KO mice that IGF-I is

involved in the inner ear cochlear vestibular ganglion neruogenesis (Sanchez-Calderon et al. 2007). Other animal experiments have demonstrated the efficacy of IGF-I in the protection of the cochlea from noise-induced hearing loss (Iwai et al. 2006), thus supporting our clinical effects of IGF-I treatment.

Chernausek et al. (2007) reported certain auditory defects, such as hypoacusis and conductive hearing loss in nine IGF-I-treated Laron syndrome patients, and explained it as complication of tonsillar/adenoidal hypertrophy during IGF-I treatment. These authors concluded that their findings did not permit to determine a link between IGF-I and hearing. The present study however offers an explanation for the auditory defects in Laron syndrome patients.

22.6 Conclusion

Based on our findings and on reports by others, it is evident that IGF-I deficiency starting in utero affects cochlear development and/or increases the susceptibility to acquired cochlear hearing loss, and to hyperacusis.

These observations are evidence of the important role IGF-I plays in the early development and function of auditory pathways. Of note are our findings that early initiation of replacement therapy normalizes the auditory deficits.

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The Teeth in Patients with Laron Syndrome

23

Zvi Laron

Core Message

› Laron syndrome patients have delayed tooth eruption, crowding, many caries and a tendency to break. Histological examination revealed defects in enamel formation.

23.1 Introduction

Knowledge on direct effects of hGH or IGF-I on dental development and structure is scant (Hamori et al. 1974; Kosowicz and Rzymski 1977); so is experimental literature. IGF-I has been shown to act on periodontal fibroblasts (Blom et al. 1992; Palioto et al. 2004), and mouse molar dentin size and shape is dependent on growth hormone (Smid et al. 2007). Laron syndrome (LS) with congenital absence of hGH and IGF-I activity is an ideal model to further our understanding on the action of these hormones on the dental structure. Forthwith our studies and findings.

23.2 Clinical Aspects

In untreated children with LS, the eruption of the primary and permanent teeth is delayed (Laron 2004). Already in infancy and prepuberty, teeth are defective with many caries (Figs. 23.1 and 23.2), probably an interplay between the IGF-I deficiency and frequent sweet food offered due to hypoglycemia in early age. Due to the underdevelopment of the facial bones (Chap. 9) (Scharf and Laron 1972; Konfino et al. 1975), there is crowding (Fig. 23.3), which again promotes caries and breakage of teeth (Fig. 23.4). Around the age of 40 years,

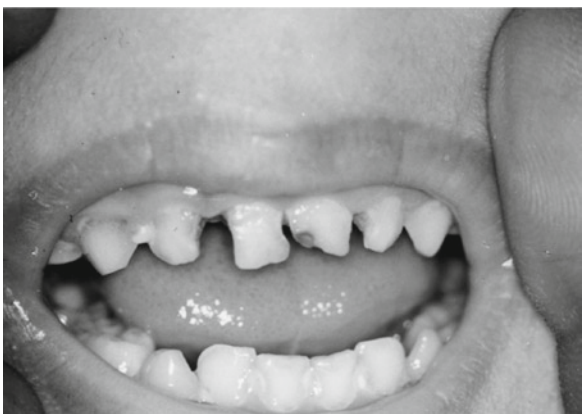

Fig. 23.1 Defective and spaced teeth and caries in a boy with Laron syndrome (LS) aged $5^{6/12}$

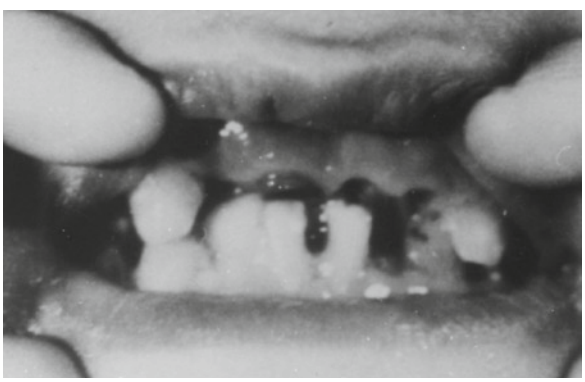

Fig. 23.2 Delayed eruption of permanent teeth and defective, spaced teeth and caries in a boy with LS aged $9^{3/12}$ years

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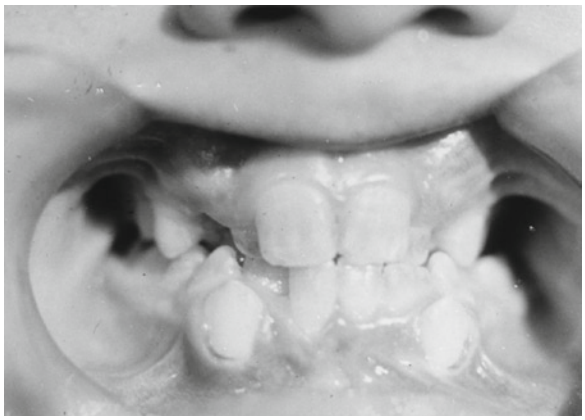

Fig. 23.3 Crowding of teeth in a $13^{8/12}$ year-old boy with LS

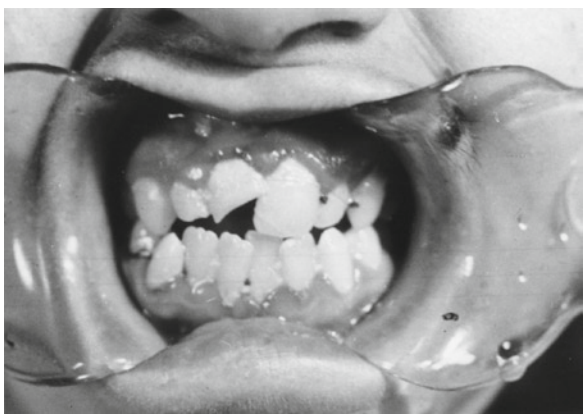

Fig. 23.4 Crowding and broken tooth in a $16^{7/12}$ year-old girl with LS

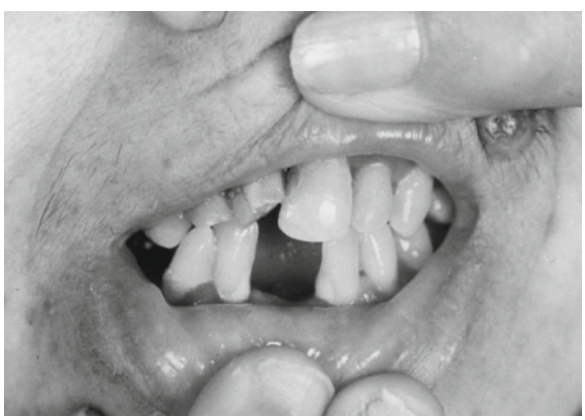

Fig. 23.5 Loss of teeth in a 42-year-old female with LS

many of the patients lose or break their teeth (Fig. 23.5) and therefore require a prosthesis at a younger age than the aging general population (Laron 1984, 2004).

23.2.1 Patient Investigations

23.2.1.1 Subjects and Methods

Detailed dental examination, accompanied by casts of the dentitions and panoramic radiographs, was performed in 13 untreated LS patients (seven males and five females). The age of six patients ranged between 2 and 18 years, and seven were adults, ages ranging from 19 to 48 years (Sarnat et al. 1988). Measurements of the arch length, arch circumference, and mesiodistal tooth dimensions were made on the plaster casts according to the methods described by Moorrees (1959) and Moyers (1973).

The dimensions for the maxilla and the mandible were analyzed separately. Panoramic radiographs were used to determine dental age and to record missing teeth, supernumerary teeth, and impacted teeth. The data were compared with 19 patients with congenital IGHD treated with hGH and healthy school children (Sarnat et al. 1988), as well as established norms (Moorrees 1959).

23.2.2 Results

The main findings were:

(a) The retardation of dental age in the untreated children with LS was less than that of the skeletal age.
(b) In the untreated LS group, 92% were without third molars compared with 37% in the treated IGHD group. All four molars were missing in 75% of the LS patients.
(c) There was a high prevalence of hypodontia in the LS patients as compared with the normal population . The high prevalence of hypodontia found only in the permanent dentition might be due to the retarded postnatal development of the maxilla and mandible.
(d) In two LS patients, a small groove-like enamel defect in the upper incisors was found.

23.2.2.1 Arch Length

Except for one male and one female LS patient, all measurements fell below the normal mean. No differences were found between the IGHD and LS groups in the maxillary arch length, but the arch length of the mandible was significantly smaller ($p<0.05$) in the untreated LS patients than in the treated IGHD patients.

23.2.2.2 Arch Circumference

According to Moorrees (1959), arch circumferences change little with age; however, the arch circumference of the mandible in patients with LS was significantly smaller than in normal subjects. These measurements complement those made by Konfino et al. (1975) (Chap. 9).

23.2.2.3 Tooth Width

Eighty percent of the LS males had a reduction in the mesiodistal width of the primary teeth; 40% of them were below 2 SD of the norm compared to 24% of female patients. The permanent teeth of 70% of the males were 2 SDs below the normal width compared to 60% of the female patients. Similar measurements are planned in IGF-I treated children.

23.3 Morphological and Histological Studies

A study on dental morphology and histology on 13 exfoliated or extracted dry primary teeth of our LS patients aged 4–13 years was performed in collaboration with the School of Dentistry of North Carolina (Wright et al. 2008).

23.3.1 Methods

Thirteen exfoliated or extracted, dry primary teeth from LS patients (aged 4–13 years) were evaluated by photography, morphometry of whole teeth, and histology using light microscopy. The findings were compared to healthy controls matched for age and sex.

23.3.2 Results

The teeth appeared grossly normal with normal crown morphology and lacking visible enamel hypoplasia or enamel discoloration suggestive of gross mineralization defects. The teeth showed a normal architecture and morphology, but reduced facial-lingual enamel thickness when compared to the total tooth width ($p<0.05$) (Table 23.1). This indicates that the enamel is significantly thinner than normal in teeth of LS patients. The facial-lingual width of some teeth was also reduced. The enamel thickness to tooth width ratios in the teeth of LS patients is significantly thinner than normal.

Table 23.1 Ratio of tooth width to enamel thickness in teeth from LS patients compared to normal controls

Tooth type	Laron syndrome	Normal controls
Primary maxillary canine	0.11	0.27
Primary mandibular second molar	0.14	0.26
Primary mandibular lat incisor	0.1	0.22
Primary mandibular central incisor	0.13	0.24
Primary mandibular first molar	0.17	0.24
Permanent mandibular central incisor	0.17	0.23

Histological evaluation showed several changes seen in some but not all teeth. The dentin in some cases showed increased interglobular dentin, while in others, there was a normal appearance of the dentinal tubules (Figs. 23.6 and 23.7). The histological examination revealed that despite a normal prismatic structure of the enamel, some patients had an accentuated neonatal line and Striae of Retzius (Fig. 23.8). The presence of interglobular dentin and accentuated Striae of Retzius are both indicative of stress on the cells responsible for mineralization of dentin and enamel respectively.

23.4 Periodontal Tissues

IGF-I deficiency affects not only the teeth but also the periodontal tissues. IGF-I is a potent modulator of periodontal regeneration stimulating cell proliferation, differentiation, and synthesis of Type I collagen and noncollagenous proteins (Blom et al. 1992). Furthermore, IGF-I and enamel matrix derivative stimulate periodontal ligament fibroblasts (Palioto et al. 2004). Thus, the IGF-I deprivation affecting the facial bones and periodontal tissues may be a contributory factor to the early loss of teeth in LS patients.

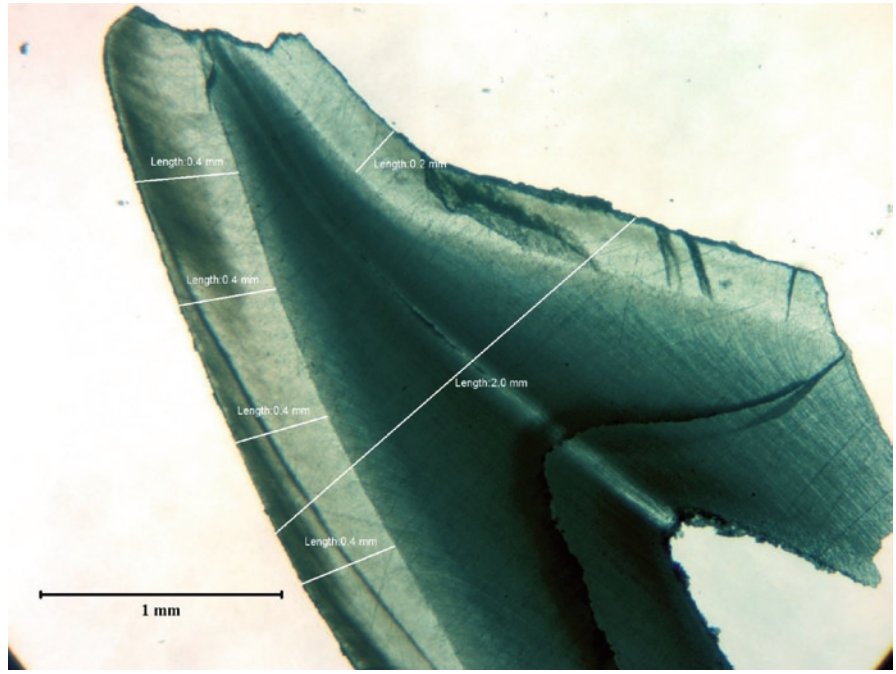

Fig. 23.6 This thin section of a primary tooth illustrates the normal tubular architecture with no evidence of mineralization defects. Many teeth showed a pronounced neonatal line

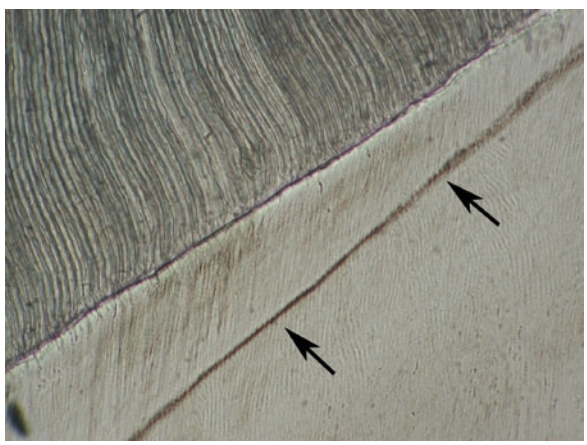

Fig. 23.7 The dentin had a normal tubular architecture with no evidence of mineralization defects. Many of the teeth showed a pronounced neonatal line (see also Fig. 23.8). The enamel prisms appeared normal in structure and course as observed in this thin section Laron syndrome

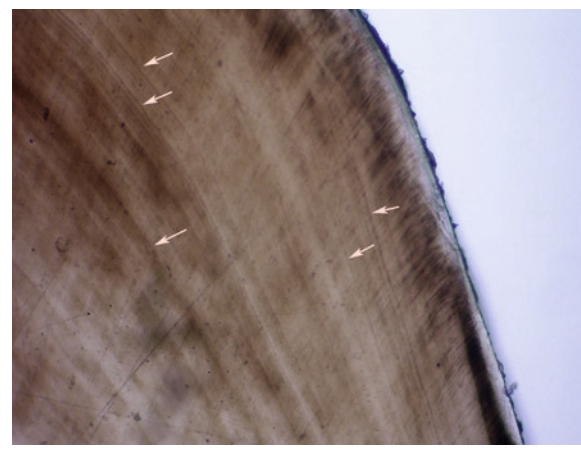

Fig. 23.8 In addition to a pronounced neonatal line, some Laron teeth had numerous pronounced Striae of Retzius (*arrows*) as seen in this thin section of enamel viewed with light microscopy. The multiple accentuated Striae of Retzius suggest a continued (albeit mild) disturbance of the enamel formation process

23.5 Conclusion

Long-term congenital IGF-I deficiency in LS patients, starting in utero, affects tooth development, size, morphology, and composition including the enamel, as well as the periodontal tissues. The defects may be due to both direct effects on the teeth as GH receptors are present during tooth bud formation (Zhang et al. 1997) and IGF-I deficiency in combination with pathological changes in the surrounding tissues. Pathological dentogenesis was also reported by Smid et al. (2007) in GH-R KO mice.

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Hair, Skin, and Nails in Patients with Laron Syndrome

24

Zvi Laron

Core Messages

- The hair of patients with Laron syndrome grows slowly and shows pathological features on electron microscopy. Young adults have sparse hair to alopecia.
- The skin is thin and on histological examination shows changes in the elastin fibers. The appearance of acne is dependent on the sex hormone concentrations and the latter on the IGF-I dose during treatment.

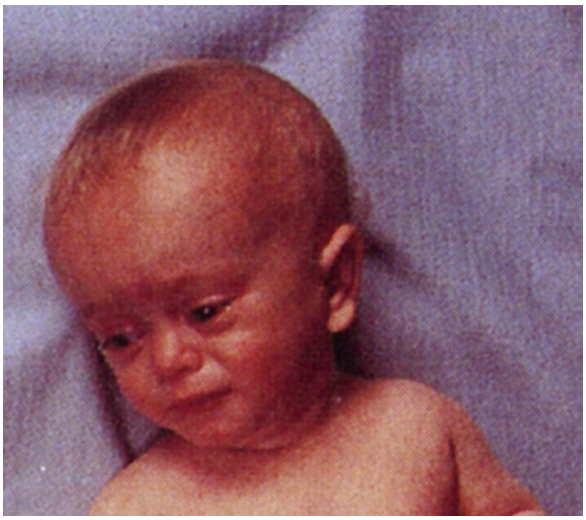

Fig. 24.1 Sparse hair in a 2-year-old boy (EM) with Laron syndrome

24.1 Hair

24.1.1 Introduction

The literature on the effects of growth hormone (GH) or IGF-I on hair is scant. Randall (2007, 2008) writes that GH is required for the full androgen response as sexual hair development is inhibited in GH deficiency. IGF-I, which has been identified as secreted by beard dermal papilla cells (Itami et al. 1995), maintains the anagen phase of hair growth in cultured scalp follicles (Philpott et al. 1994). The latter effect is blocked in IGF-I receptor KO mice resulting in abnormal patterns of hair follicle growth and differentiation (Liu et al. 1993). Other studies (Peus and Pittelkow 1996; Stenn et al. 1996) report that IGF-I plays a regulatory role in cellular proliferation and migration during the development of hair follicles.

24.1.2 Clinical Appearance

Following our large cohort of patients with Laron syndrome from early age into adulthood, we made the following observations. Already in infancy, the hair is silky and its growth is slow and sparse (Fig. 24.1), resulting in large frontal recessions (Fig. 24.2), more prominent in boys (Laron et al. 1968; Laron 2004). Slow hair growth has been described by the parents and the patients, starting

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DOI: 10.1007/978-3-642-11183-9_24, © Springer-Verlag Berlin Heidelberg 2011

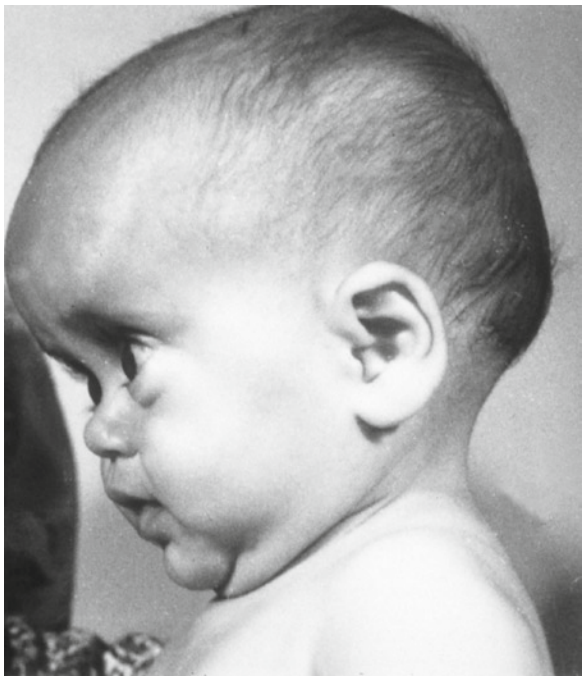

Fig. 24.2 Large frontal and temporal recession of hair in a 3-year-old boy with Laron syndrome (SSh)

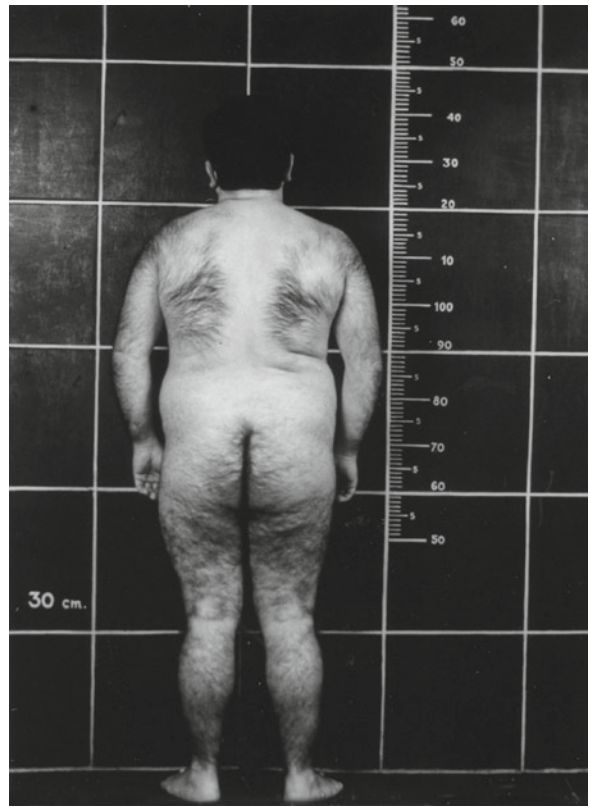

Fig. 24.4 Increased body hair in a 29-year-old male (BR) with Laron syndrome

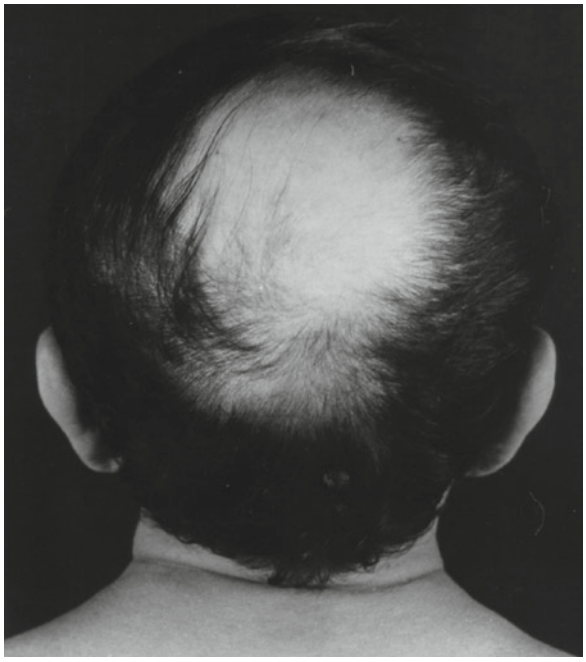

Fig. 24.3 Alopecia in a 41-year-old male (SSi) with Laron syndrome

from early childhood as judged by the need for haircuts compared to healthy siblings. Adult males develop alopecia (Fig. 24.3) and in the females the hair becomes less dense. At all ages the scalp hair is thin and easy to pluck.

The sexual hair appears late due to delayed puberty (Chap. 10).

The amount of body hair varies; one adult male is even hairy (Fig. 24.4).

24.1.3 Light and Scanning Electron Microscopy Studies of Hair Shafts

24.1.3.1 Subjects

The hair of seven untreated and four IGF-I treated patients with Laron syndrome (five children and six adults) were studied (Lurie et al. 2004).

Table 24.1 Main pathology of the hair of 11 patients with Laron syndrome as seen by electron-microscopy

Patient	Sex	Age (years)	IGF-I treatment (years)	Microscopic hairshaft defects			
				Pili torti	Pseudomonilethrix	Longitudinal grooving	Trichorrhexis nodosa
1	M	4	None	+	–	+	–
2	M	8	None	+	–	–	–
3	M	4	1[6]	–	+	+	–
4	M	13	8	–	–	–	+
5	M	15	8	–	–	–	–
6	F	23	2 (between 13[6]–15[6])	–	–	–	–
7	M	37	None	–	+	–	–
8	F	39	None	–	+	+	–
9	M	41	None	–	+	–	+
10	F	44	None	–	+	–	+
11	F	50	None	–	+	–	+

24.1.3.2 Methods

In each subject, hairs were plucked with rubber-protected forceps, together with the roots, from the frontal and occipital areas of the scalp. Hairs were also collected from each subject's comb used in daily grooming. All specimens (between age 8 and 10) were examined by light microscopy for hair shaft abnormalities. A trichogram was performed on the plucked hairs according to standard procedures (Blume-Peytavi and Orfanos 1995), and percentages of anagen, catagen and telogen, and of dysplastic, dystrophic, and fractured hair roots were evaluated. Selected hairs with obvious pathologic abnormalities were also studied by scanning electron microscopy (SEM).

24.1.3.3 Results

In seven patients, dysplastic anagen roots were more frequent than observed in healthy children, and in nine patients, the percentage of fractured hair was also higher than the normal range of 1–10%. In general, hair diameter was reduced (thin, silky, and easy to pluck).

Most of the anagen roots were lacking sheaths, indicating inherent fragility. There was no correlation between the hair findings and the different molecular defects of the GH receptor. Table 24.1 summarizes the electron microscopic hair findings. Four main hair shaft defects found were:

1. Pili torti et canaliculi (Fig. 24.5) noted in two untreated boys, in 20% of the hairs examined in one and in 25% in the other.
2. Longitudinal grooves (Fig. 24.6) were noted in one untreated and one IGF-I-treated boy (18% of hairs) and one untreated adult woman (22% of hairs).
3. Tapered hair (Pseudomonilethrix) (Fig. 24.7) was found in one treated male child and five untreated adults (two women and three men), rates ranging from 30 to 40% of examined hairs.
4. Proximal trichorrhexis nodosa (Fig. 24.8) was noted in one treated boy and three untreated adults (two women and one man) at rates of 20–30% of the hairs.

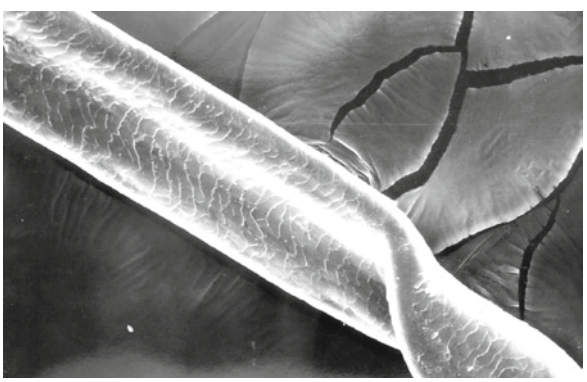

Fig. 24.5 Pili torti in an untreated child with Laron syndrome (patient no. 1). Note also flattening and grooving (SEM, original magnification ×250)

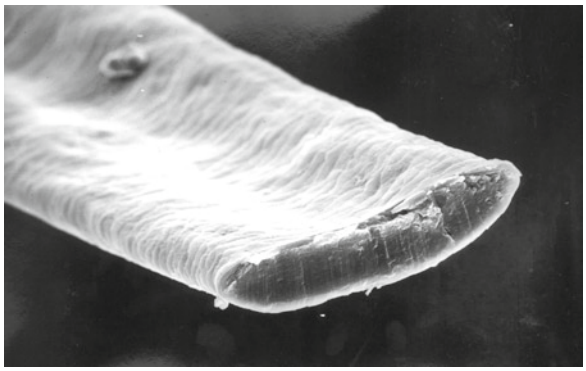

Fig. 24.6 Flattening and hair grooving (SEM, original magnification ×500)

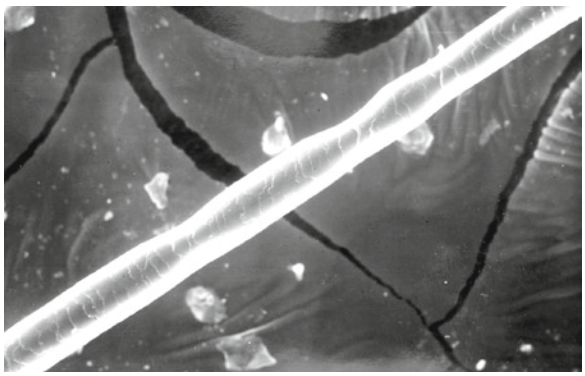

Fig. 24.7 Pseudomonilethrix in a child with Laron syndrome treated with IGF-I (patient no. 4) (SEM, original magnification ×200)

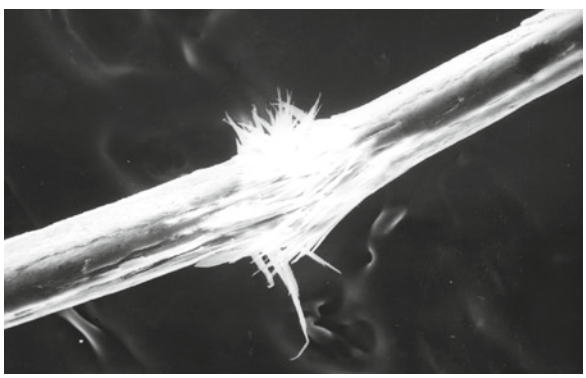

Fig. 24.8 Trichorrhexis nodosa in an untreated adult female with Laron syndrome (SEM, original magnification ×200)

24.1.3.4 Comment

Hair follicle growth involves the proliferation of the germinative matrix cells within the bulb, followed by their differentiation, keratinization, and migration into different follicle cell layers (Peus and Pittlekow 1996). Our study suggests that the proliferative action of IGF-I is important in these processes.

24.1.3.5 Conclusions

Our findings of hair shaft defects and hair fragility in patients with congenital IGF-I deficiency, whether untreated or IGF-I-treated, may be interpreted as evidence of the IGF-I involvement in hair development and quality. It further shows that IGF-I treatment did not protect from these abnormalities which may be related to late initiation of IGF-I administration or may mean that other physiological consequences of low GH signaling results in these defects that are not alleviated by IGF-I treatment.

24.2 The Skin in Patients with Laron Syndrome

24.2.1 Introduction

In the literature, the quality of the skin in hypopituitary patients, or state of GH/IGF-I deficiency, is described as being of soft texture and fine wrinkling (Laron 1969; Hogan 2006). It is also repeatedly described that in acromegaly, due to hypersecretion of GH/IGF-I, the skin texture is coarse, with thickened heel pads, attributed to glycosaminoglycan deposition and increased connective tissue collagen production (Melmed and Kleinberg 2003).

The description of the skin was part of each examination of all of our patients with Laron syndrome both in the untreated and IGF-I-treated state.

24.2.2 Clinical Appearance

Since early age the skin is smooth, and with advancing age becomes thinner and thinner in contradistinction to the subcutaneous adipose tissue which becomes thicker and thicker (Chap. 12). The thin skin and wrinkles give to some patients the appearance of early

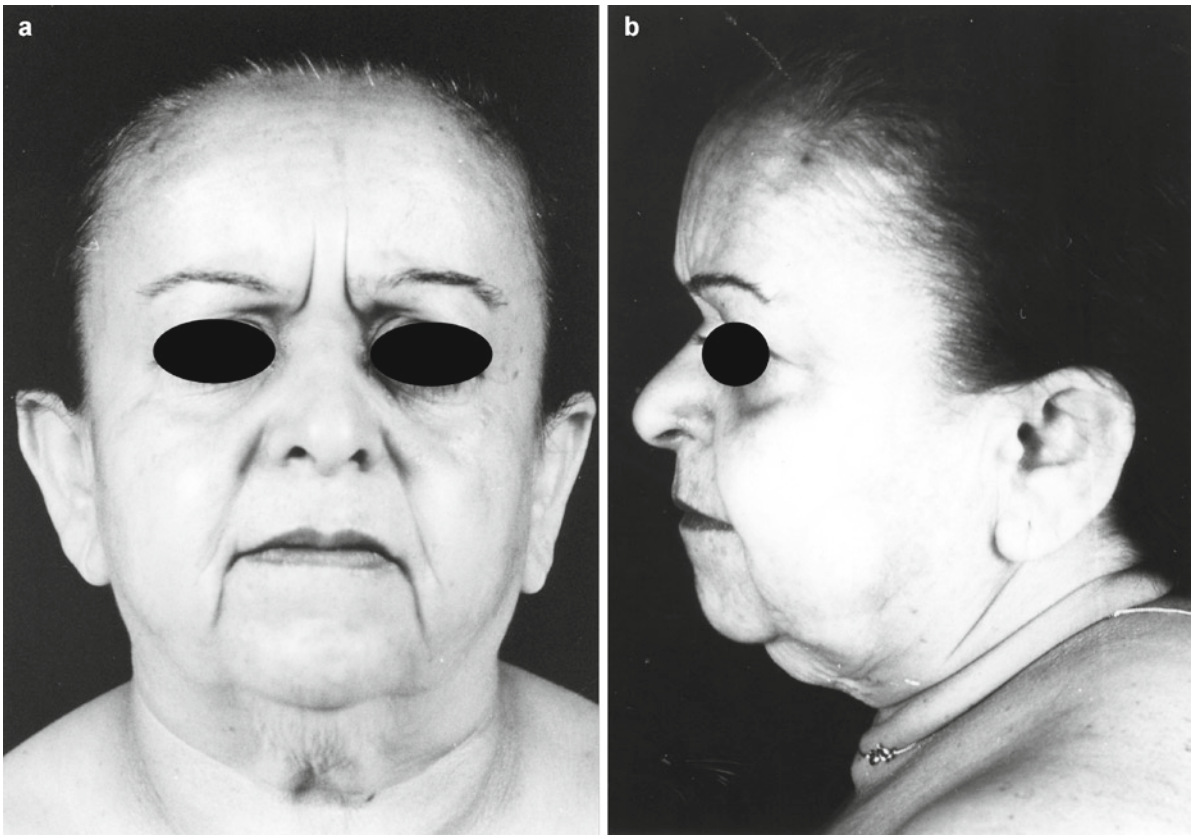

Fig. 24.9 (**a**, **b**) Early appearance of aging in a 48-year-old female patient with Laron syndrome (YR)

aging (Fig. 24.9a, b). Due to hypoglycemia in early age there is episodic increased sweating.

24.2.3 Histologic Investigations

24.2.3.1 Methods

Skin punch biopsies from the upper arm were performed under local anesthesia in six untreated Laron syndrome patients (Abramovici et al. 1983) (two females and four males; three were prepubertal and three pubertal – age 17–18). As controls served seven children with idiopathic short stature and normal hormonal state (four females and three males, aged 3–18 years) and 18 patients with isolated growth hormone deficiency (IGHD) (nine females and nine males aged 4–17 years) before initiation of hGH treatment. The study was approved by the Hospital Ethical Committee and each parent signed an informed consent form.

24.2.3.2 Results

The histological findings revealed that the patients with Laron syndrome had two distinct morphological patterns, depending on the age of the patients. In the prepubertal patients, the dermal regions appeared fairly normal, but some moderately thickened elastin fibers showing a clustering tendency could be seen in the upper dermis region (Fig. 24.10). For comparison, the normal pattern of the skin in a prepubertal boy is shown in Fig. 24.11. In pubertal Laron syndrome patients, in contrast, the upper part of the papillary layer appeared relatively empty due to a reduction in the number of elastin fibers, while the rest of the papillary layer and the reticular area contained an irregular clustering of coarse elastin fibers and collagen fibrils (Fig. 24.12). The fibrocytes and reticulin network showed no prominent changes in either age group. The low values of PIIIP (aminoterminal propeptide of Type III procollagen), the principal component of connective tissue in Laron syndrome patients (Klinger et al. 1996) probably

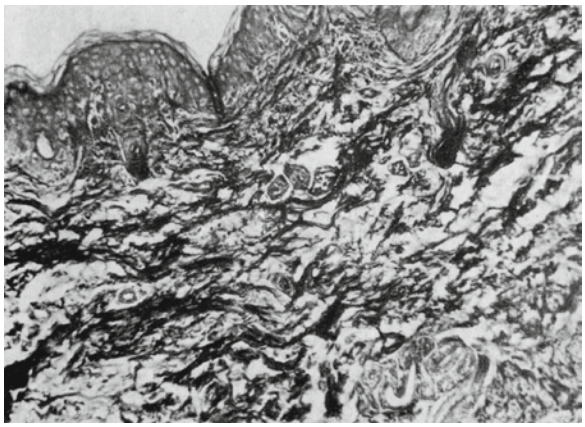

Fig. 24.10 Clustering of thickened elastin fibers in the upper dermis of a skin biopsy in a $6^{1/12}$ year-old boy with Laron syndrome. Weigert stain ×100. Reproduced with permission from Abramovici et al. (1983)

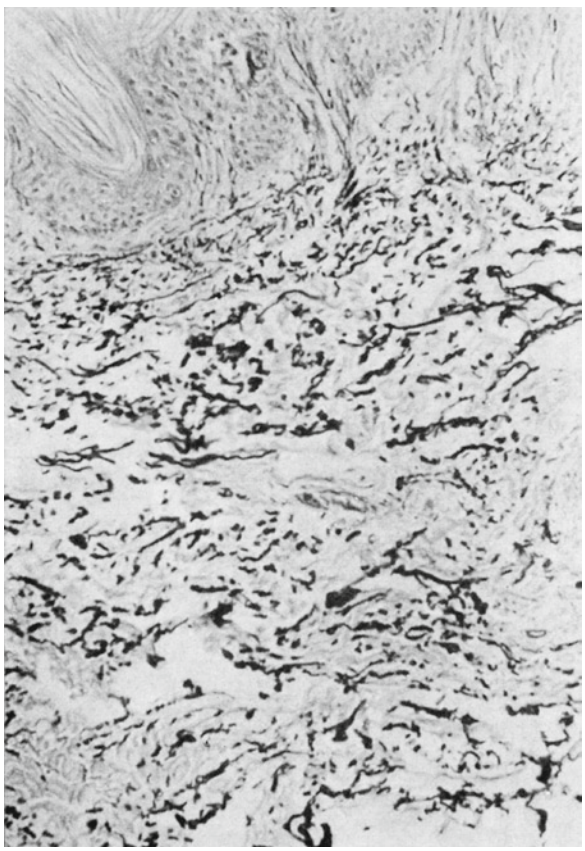

Fig. 24.11 Normal skin pattern from a $4^{6/12}$ year-old boy with idiopathic short stature. Note the meshwork of fine elastin fibers in the papillary layer and relatively thicker elastin fibers in the reticular layer. Pinkus and Hunter stain × 100. Reproduced with permission from Abramovici et al. (1983)

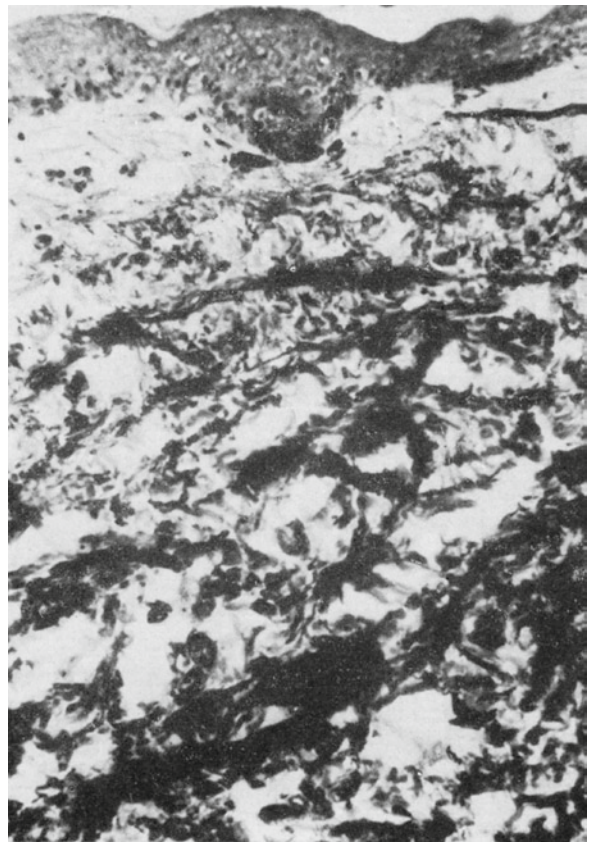

Fig. 24.12 Irregular thick bundles of coarse elastin fibers in the lower papillary and reticular layers in the skin biopsy of a $16^{1/2}$ year-old boy with Laron syndrome. Weigert stain × 100. Reproduced with permission from Abramovici et al. (1983)

contribute to the clinical changes of the skin including early wrinkling in these IGF-I-deprived subjects.

24.2.3.3 Conclusions

The main findings in the children with Laron syndrome were thickened elastin fibers, frequently arranged in irregular bundles, but the number of elastin fibers was normal in prepubertal patients and reduced in postpubertal patients.

24.2.3.4 Comment

The reduction of elastin fibers in the Laron syndrome patients was less prominent than in patients with congenital IGHD (Abramovici et al. 1983). The finding of

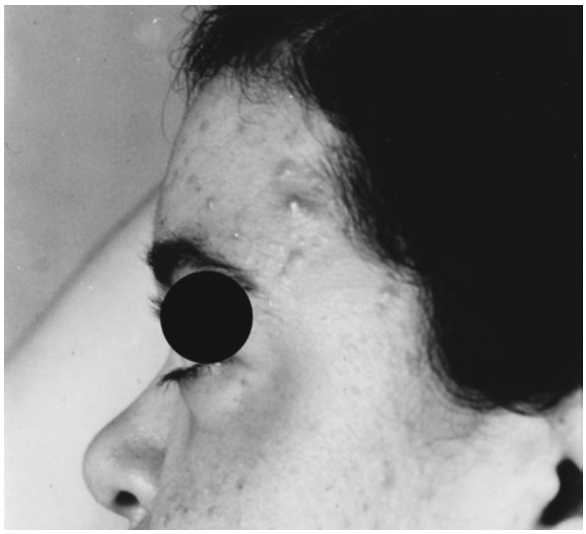

Fig. 24.13 Appearance of acne in a 30-year-old female with Laron syndrome overtreated by IGF-I (120 μg/kg once daily)

more numerous elastin fibers in Laron syndrome patients than in the IGHD patients is of interest and suggestive of a possible differential effect of IGF-I compared to hGH on skin elastogenesis.

24.3 Effect of IGF-I Treatment

IGF-I administration to children with Laron syndrome to induce linear growth increased the thickness of the skin by appearance and palpation, but no histological examinations were performed. During puberty, the skin became oily, more evident when inadvertedly an overdose of IGF-I was administered to two adult patients. Of interest was the finding that untreated pubertal Laron syndrome patients do not develop microcomedones (preacne) or acne (Ben-Amitai and Laron 2010), whereas overdosage during IGF-I treatment induced the appearance of acne as illustrated in a female patient in Fig. 24.13. The acne disappeared upon reduction of the dose (see Chap. 47).

24.3.1 Conclusions

Our observations in the Laron syndrome patients show that IGF-I has profound effects on various components of the skin. IGF-I receptor mRNA has been documented on cultured keratinocytes and GH is known to affect the growth of human fibroblasts (Tavakkol et al. 1992). The effect of IGF-I on the development of acne results from the combined action of this hormone and adrenal and/or gonadal androgens (Laron 2008).

24.4 Finger and Toe Nails

From the scant publications available (Bean 1980), nail growth is faster during childhood than in adult age. The mothers of children with Laron syndrome reported that their nails grew slower and needed trimming less often than that of their normal-sized siblings (Laron 2004). IGF-I treatment stimulated nail growth.

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Hand, Foot, and Organ Size and Growth in Untreated and IGF-I Treated Patients with Laron Syndrome

25

Zvi Laron and Aviva Silbergeld

Core Messages

- Untreated patients with Laron syndrome have acromicria, such as small hands and feet, and organomicria (i.e. small internal organs).
- IGF-I treatment stimulates the growth of hands and feet but less effective than does GH in children with IGHD.

25.1 Hand Size and Growth of Untreated and IGF-I Treated Laron Syndrome Patients

Zvi Laron

Although small hands, part of general acromicria, have been reported in the first description of the syndrome (Laron et al. 1966), no accurate measurements were performed at that time. Figure 25.1 illustrates the growth retardation of the hand in a 4-year-old boy with Laron syndrome, compared to the hand of a healthy 2-year-old boy. It is seen that the hand of the 4-year-old boy is smaller than that of the healthy 2-year-old. In order to assess both the absolute size of the hand of Laron syndrome patients and their growth during the untreated and IGF-I treated state, we performed the following study, which to the best of our knowledge was the first of its kind in this syndrome.

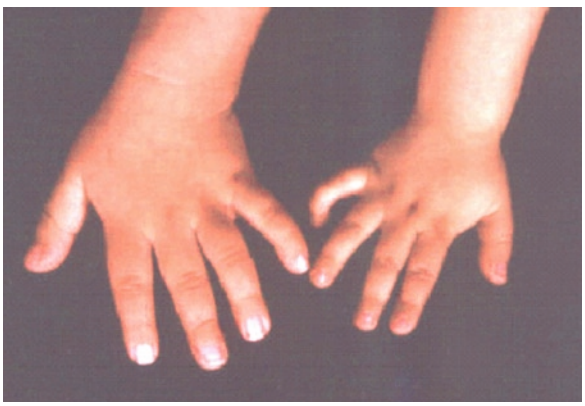

Fig. 25.1 Size of the hand of a 4-year-old boy with Laron syndrome compared with that of a 2-year-old healthy boy

25.1.1 Subjects

Two groups of patients with Laron syndrome were studied: Group I – 24 untreated patients (10 males, 14 females), age range 1–50 years; and Group II – 10 children (4 boys and 6 girls), age range 1–15 years studied before and during treatment with recombinant IGF-I (FK 780; Fujisawa Pharmaceutical Co., Osaka, Japan, 150–180 μg/kg injected once daily s.c.) (Laron 2008). The length of the treatment was up to 11 years.

25.1.2 Methods

As it is difficult to measure hand size "in vivo" in a uniform manner, we decided to use standardized hand

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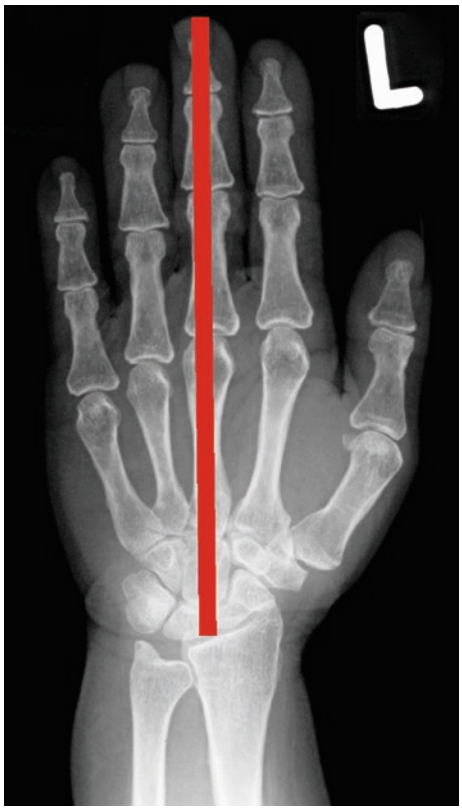

Fig. 25.2 Measurement of hand length using a standardized hand X-ray

X-rays, performed to estimate skeletal age and to measure hand size and growth (Konen et al. 2008, 2009).

Hand X-rays were taken from a standardized distance with the hand flat and stretched. The maximal length was measured from the soft tissue outline at the tip of the third finger to the soft tissue line at the base of the palm (Fig. 25.2).

The measurements were compared to normal reference values from Paediatric Morphometrics (Gerver and de Bruin 1996). SD values were calculated for each measurement (P50 – measurement/1 SD) using the above reference. Body height was expressed as SDS.

25.1.3 Statistical Analysis

The data were analyzed by the Mann–Whitney U nonparametric test using BMDP statistical software.

25.1.4 Results

We found suitable hand X-rays for size measurements in 34 patients out of a cohort of 64 patients with Laron syndrome followed in our clinic. Of these, 24 X-rays were of untreated patients (10 Males, 14 Females). Repeated hand measurements were performed in 10 IGF-I-treated patients (4 boys, 6 girls).

The deficits of hand size as mean (±SD) from normal size in seven age groups (from 1 to 50 years) of untreated patients with Laron syndrome compared to linear height SDS deficits are shown in Table 25.1 (Konen et al. 2008). It is seen that in infancy the hand size is less retarded than the body height and decreases with age, from -2.8 ± 0.7 to -7.3 ± 0.8 SD and even to -9.0 ± 3.9 SD at the age of 40–50 years, whereas the degree of severe dwarfism is already evident at a young age. With progressing age, the height deficit in the same Laron syndrome patients decreased, from -6.3 ± 0.8 to -7.3 ± 2.0 SDS. The typical slow hand growth in an untreated Laron syndrome girl is illustrated in Fig. 25.3. It is noteworthy that in young children the degree of growth retardation of height is greater than that of the hand, but in adults the hand size is more retarded.

Table 25.1 Comparison between hand size and linear growth deficits in 24 untreated patients with Laron syndrome (10 males, 14 females)

Age (years)	1–3		4–6		7–9		10–12		13–15		16–19		40–50	
	Hand	Height	Hand	Height	Hand	Height	Hand	Height	Hand	Height	Hand	Height	Hand	Height
Mean	−2.8	−6.3	−5.3	−6.5	−5.1	−4.7	−5.6	−6.5	−7.3	−8.0	−8.1	−8.3	−9.0	−7.3
SD	0.7	0.8	1.0	1.2	3.5	5.1	1.0	1.1	0.8	1.0	2.4	1.1	3.9	2.0
n	8		9		11		14		12		13		4	
N	6		6		7		8		7		9		4	

Hand size expressed as SD and height as SDS deviation from the 50th centile of norm
n number of examinations; *N* number of patients

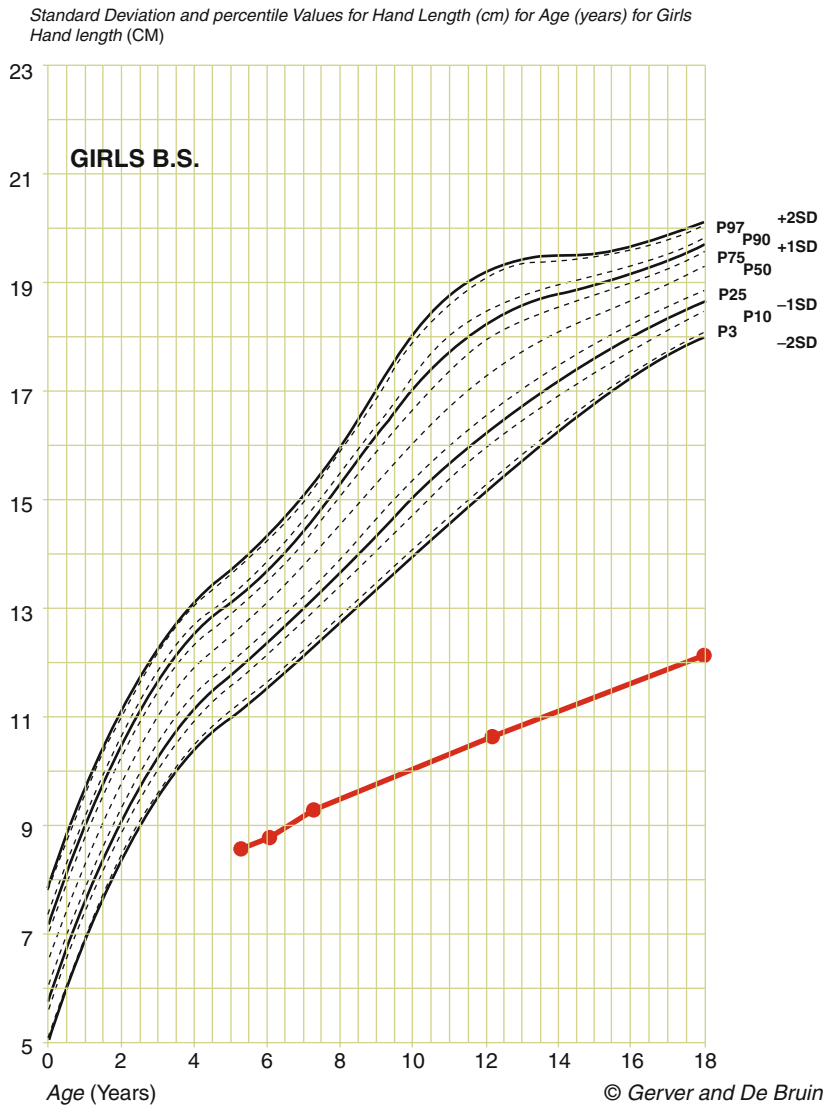

Fig. 25.3 Hand growth of an untreated girl with Laron syndrome (Growth chart of Gerver and De Bruin 1996)

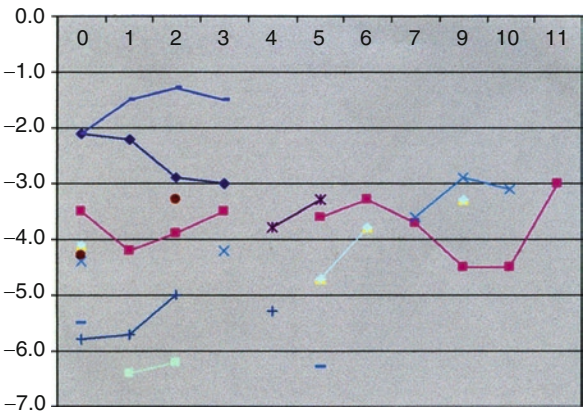

Fig. 25.4 Hand size in 11 children with Laron syndrome and growth response to IGF-I treatment in seven children (expressed as SD deviation from the 50th centile of norm)

25.1.5 Effect of IGF-I Treatment on Hand Growth

The acceleration of hand growth is shown in Fig. 25.4 and its comparison to that of linear height in ten IGF-I treated children (4 males, 6 females) in Table 25.2. The response in hand growth to IGF-I therapy varied. Whereas the mean height SDS improved from -6.2 ± 1.2 to -3.9 ± 0.5 ($p < 0.05$), the relative growth of the hand remained within the same degree of size deficit ($p = \text{NS}$). Some children showed a clear response in hand growth during puberty as illustrated in Fig. 25.5.

Table 25.2 Comparison of hand and body growth response to IGF-I treatment in patients with Laron syndrome

	IGF-1 treatment (years)							
	0		3		5		9	
	Hand	Height	Hand	Height	Hand	Height	Hand	Height
Mean	−4.0	−6.2	−3.1	−3.9	−4.5	−4.3	−3.6	−3.9
SD	1.4	1.2	1.1	1.1	1.4	0.7	0.8	0.5
n	8		4		4		3	

Hand size expressed as SD and height as SDS deviation from the 50th centile of norm
n number of examinations

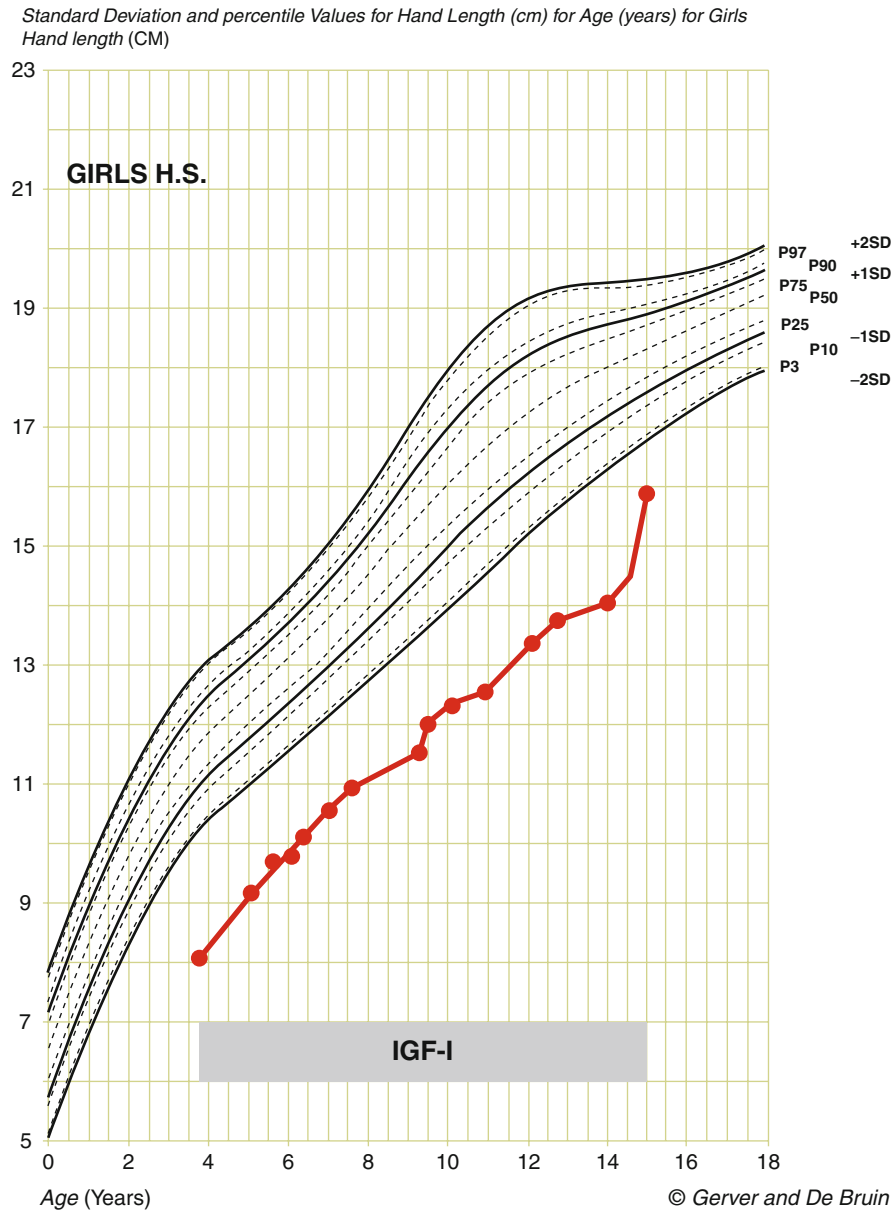

Fig. 25.5 Hand of an IGF-I treated girl with Laron syndrome (Growth chart of Gerver and De Bruin 1996)

25.1.6 Conclusions

In congenital IGF-I deficiency, such as in Laron syndrome, the hand size in early childhood is less retarded than the linear height and foot length, but with advancing age and during IGF-I treatment, the hand growth rate lags behind that of the body height and feet size (Silbergeld et al. 2007).

The reasons for these disproportional growth patterns are probably the variety of genes which control the growth of different skeletal parts of the body and even hand bones.

In order to find out whether the growth deficit of the hand resides in the palmar bones or fingers, we measured the ratio between palmar and middle finger growth using hand X-rays in 30 Laron syndrome patients from early childhood to age 20 (Zribi 2009). The analysis of the measurements and ratios revealed that the growth delay of the palm and fingers in Laron syndrome is proportional.

25.2 Foot Size and Growth in Untreated and IGF-I-Treated Patients with Laron Syndrome

Zvi Laron and Aviva Silbergeld

In children with Laron syndrome the small size of the feet becomes apparent already in infancy, and some parents reported that the only shoes that fitted were those of dolls (Shurka and Laron 1975). Though mentioned by us (Laron 2004) and others (Guevara-Aguirre et al. 1995) that Laron syndrome patients have small feet, no actual measurements had been performed before our study (Silbergeld et al. 2007).

25.2.1 Subjects

Reliable foot size measurements were available before and during IGF-I treatment of the following two Laron syndrome patient groups:

Group (a): 15 untreated adult Laron syndrome patients (8 males, 7 females), mean (±SD) age: 32 ± 5.9 years.

Group (b): 9 children (3 boys, 6 girls; age range 1.5–14.5 years) studied before and during 9 years of IGF-I (FK 780, Fujisawa Pharmaceutical Co. Ltd., Japan; 150–180 μg/kg once daily)

25.2.2 Methods

Foot length was measured with a tape from the tip of the hallux to the heel; in a few instances (10%) the shoe size was converted to foot length using the European shoe size on the Brannock scale (The Brannock Device Co. Inc., Liverpool, NY, USA). Foot length is expressed as SD for age and gender according to Anderson et al. (1956). Body height was measured by a Harpenden stadiometer.

25.2.3 Statistical Analysis

The data were analyzed by the Mann–Whitney U nonparametric test using BMDP Statistical Software (Dixon 1993). A value of $p < 0.05$ was considered significant.

25.2.4 Results

25.2.4.1 Untreated Patients

The mean (±SD) foot size deficits in the untreated adult Laron syndrome patients as compared to their mean degree of body height growth retardation (±SDS) for both genders are shown in Table 25.3.

It is seen that while the mean body growth deficits in the two genders were similar ($p = 0.41$), the male Laron syndrome patients had significantly smaller feet than the female patients: −6.2 ± 1.5 vs. −3.6 ± 1.4 SDS ($p < 0.005$).

The small foot size in a 42-year-old female Laron syndrome patient is shown in Fig. 25.6.

25.2.5 IGF-I Treatment

The response of foot growth to IGF-I treatment as compared to body growth is shown in Table 25.4. It is seen that during 12 years of IGF-I therapy, the mean foot size decreased from −4.7 ± 1.4 to −3.3 ± 1.0 compared to an improvement in height from −6.3 ± 1.5 to −3.9 ± 0.5 SDS.

Table 25.3 Growth retardation of foot length (FL) (expressed as SD from normal medians) and height retardation in untreated adult patients with Laron syndrome (LS) (expressed as SDS from normal medians)

	Age (year)	FL (SD)	Body height (SDS)
Male patients			
1	26	−4.4	−8.3
2	29	−7.6	−7.7
3	30	−4.8	−4.8
4	35	−7.4	−8.9
5	37	−4.0	−5.4
6	39	−7.4	−8.7
7	41	−7.4	−8.0
8	44	−6.5	−9.5
Mean ± SD		−6.2 ± 1.5*	−7.7 ± 1.7
Female patients			
1	25	−2.9	−6.4
2	25	−4.1	−8.9
3	26	−5.0	−8.3
4	30	−3.5	−7.4
5	32	−3.2	−5.2
6	33	−5.3	−8.5
7	42	−1.1	−4.4
Mean ± SD		−3.6 ± 1.4*	−7.0 ± 1.7

*$p = 0.005$

These results were significantly inferior ($p < 0.025$) to those registered by us in children with congenital isolated GH deficiency (IGHD) treated by hGH (Silbergeld et al. 2007). An example in two $3^{6/12}$ year-old girls, one with Laron syndrome (*filled circle*) and another with congenital IGHD (*filled triangle*), is illustrated in Fig. 25.7.

25.2.6 Conclusions

Our study showed that the foot size deficit in untreated children with Laron syndrome was similar to that in children with congenital IGHD (Silbergeld et al. 2007), but smaller than that found in children with short stature born small for gestational age (SGA) (Sas et al. 2000). Turner's syndrome (Sas et al. 1999) or Prader–Willi syndrome (Eiholzer et al. 1998) denoting the important role of IGF-I in the growth of the foot, not only on linear height, and of other area.

25.2.7 Comparison between Body, Head, Hand, and Foot Growth Deficits in Patients with Laron Syndrome

Comparison of various body measurements on the same day along progressive age was possible in 14 untreated Laron syndrome patients.

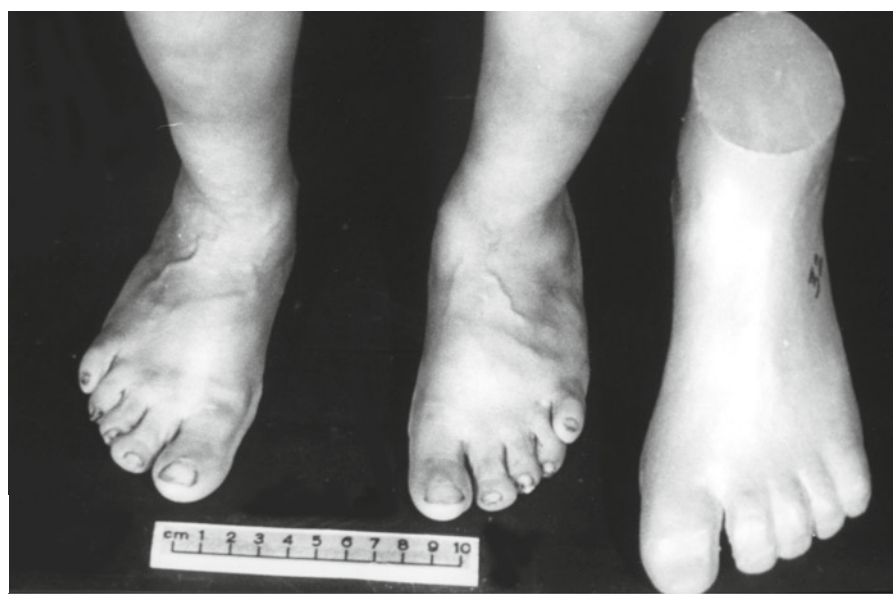

Fig. 25.6 Foot size of a 42-year-old female with Laron syndrome compared with a 37 European foot size

Table 25.4 Growth response of the foot compared to height in the same patients with Laron syndrome receiving IGF-I replacement therapy (mean ± SD)

	Foot length SD				Height SDS			
	IGF-I treatment (years)				IGF-I treatment (years)			
	0	3	5	9	0	3	5	9
Laron syndrome	−4.7 ± 1.4	−3.9 ± 1.7	−4.1 ± 1.5	−3.3 ± 1.0	−6.3 ± 1.5	−4.9 ± 1.6	−4.7 ± 0.6	−3.9 ± 0.5
n	8	8	5	3	9	9	5	3

n number of patients

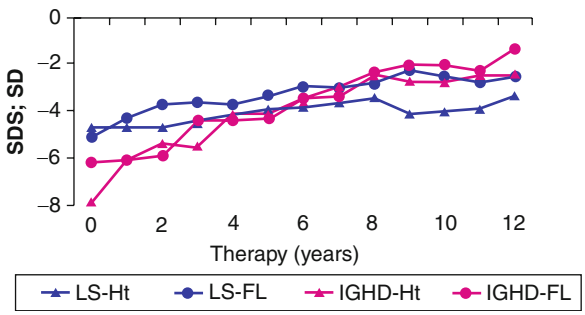

Fig 25.7 Foot size and height response to IGF-I treatment of a girl with Laron syndrome (*filled circle*) compared to that of a girl with congenital isolated GH deficiency (IGHD) (*filled triangle*) treated by hGH both since age $3^{6/12}$ for a period of 12 years. *FL* foot length; *Ht* height. IGF-I (Fujisawa, Osaka, Japan) 180 μg/kg once daily. hGH (Genotropin, Lilly, USA) 33 μg once daily

The comparative deficits (m ± SD) are shown both in Table 25.5 and Fig. 25.8. It is seen that with increasing age, the height deficit increases as well as that of the hand. The mean foot size deficit decreases to a lesser degree, whereas that of the head circumference remains rather stable. The great individual variations prevented statistical evaluations.

25.3 Organ Size

Zvi Laron

Another characteristic of congenital IGF-I deficiency is small body organs (organomicria). We have not systematically measured organ size using ultrasonography, CT, or MRI. Nevertheless, our findings of a subnormal head circumference are evidence for a small brain in the untreated patients already present at birth (Chap. 9). The echocardiographic studies of the heart revealed a small heart (cardiomicria) in untreated patients (Feinberg et al. 2000) (see Chap. 33). Backeljauw et al. (2001) reported a small spleen in Laron syndrome patients.

Table 25.5 Body height, head circumference, hand and foot lengths deficits in the same untreated Laron syndrome patients followed since childhood to adult age

Age (year)	*n*	m ± SD			
		Height	Head	Hand	Foot
1–3	8	−6.3 ± 0.8	−3.3 ± 1.5	−2.8 ± 0.7	−4.7 ± 1.5
4–6	11	−6.5 ± 1.2	−2 ± 0.9	−5.3 ± 1	−5.8 ± 0.6
7–9	12	−4.7 ± 5.1	−2.7 ± 1.7	−5.1 ± 3.5	−6.6 ± 2.3
10–12	14	−6.5 ± 1.1	−2.2 ± 1.6	−5.6 ± 1	−5.6 ± 0.8
13–15	12	−8 ± 1	−3.5 ± 1.1	−7.3 ± 0.8	−8.4 ± 1.4
16–19	13	−8.3 ± 1.1	−3.6 ± 0.8	−8.1 ± 2.4	−5.8 ± 2
35–50	4	−7.3 ± 2	−3.4 ± 1	−9 ± 3.9	−5.2 ± 2.9

Height as SDS; head, hand, and foot as SD of normal median
n number of patients

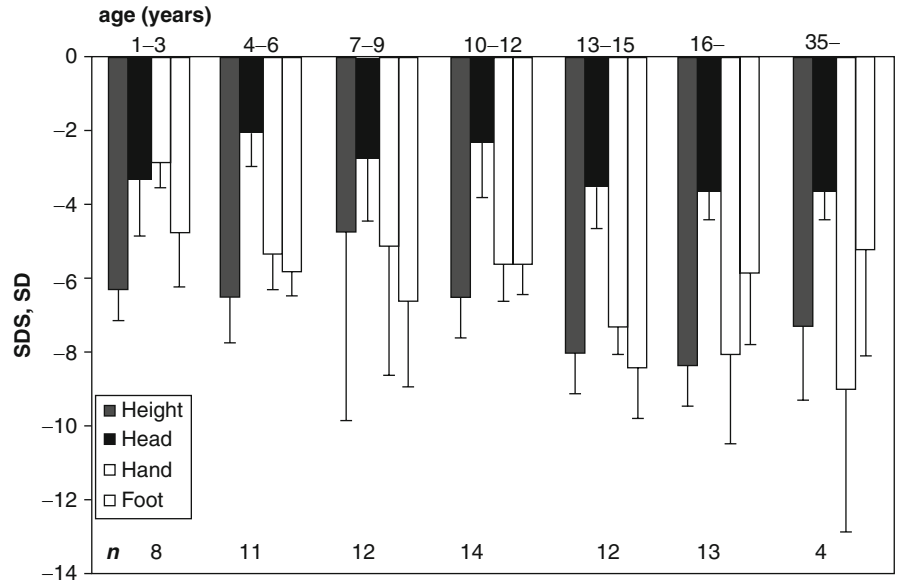

Fig 25.8 Body height, head circumference, hand and foot lengths in the same untreated Laron syndrome patients followed since childhood to adult age. Height as mean SDS (±SD) the remaining as m(±SD)

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Lifelong Serum Growth Hormone Levels in Patients with Laron Syndrome

26

Zvi Laron and Orly Efros

Core Message

› This chapter presents the overnight fasting serum GH levels from childhood to adult age in patients with Laron syndrome of both genders. The relationship between hGH and IGF-I levels is also illustrated.

26.1 Introduction

Human growth hormone (hGH) is a single chain of 191-aminoacid polypeptide containing two disulfide bonds. Under normal physiological conditions, the mature form of hGH circulates as a 22-Kd protein (Laron 2004a). The hGH genome contains a cluster of five genes (hGH-N (or I), hCS-L, hCS-A, hGH-V, and hCS-B) located on the long arm of human chromosome 17q 22–24 (Cogan and Phillips 2006).

The hGH-N gene is selectively transcribed in pituitary somatotrophs. High serum hGH is one of the diagnostic characteristics of Laron syndrome (Laron 2004b) and was indeed the "Leitmotiv" of research in this syndrome (see Chaps. 2–4) (Laron et al. 1966, 1968).

Having followed a large cohort of patients, many of them since early childhood, we were interested to know whether the increased secretion observed in childhood (Laron et al. 1968; Laron 1984) continues also in adult age, a period of life with reduced hGH secretion (Laron et al. 1970; Rudman et al. 1981; Gil-Ad et al. 1984; Zadik et al. 1985).

26.2 Subjects and Methods

The medical records of all our Laron syndrome patients were reviewed. Records on serum hGH measurements during long-term follow-up from early childhood to adult age were available from 41 patients (18 males, 23 females). Even when special tests were performed, the basal sample was drawn after an overnight fast. Serum hGH was measured by radioimmunoassay, in early years employing an in-house method (Laron and Mannheimer 1966), subsequently by commercial kits, and in recent years using the Immulite 2000 Analyzer (Diagnostics Products Corp., Los Angeles, USA). Similar procedures were used to measure IGF-I determinations.

26.3 Results

26.3.1 Serum hGH Levels in Untreated Laron Syndrome Patients

As an overview of the changes with age in morning serum hGH levels, 447 hGH measurements, 200 from 18 male Laron syndrome patients and 247 from 23 female Laron syndrome patients, are illustrated in Fig. 26.1. It is evident that up to age 30, elevated levels are encountered; with advancing age, serum hGH concentrations decline to around 20 ng/mL until age 50. A reduced number of samples from patients, age

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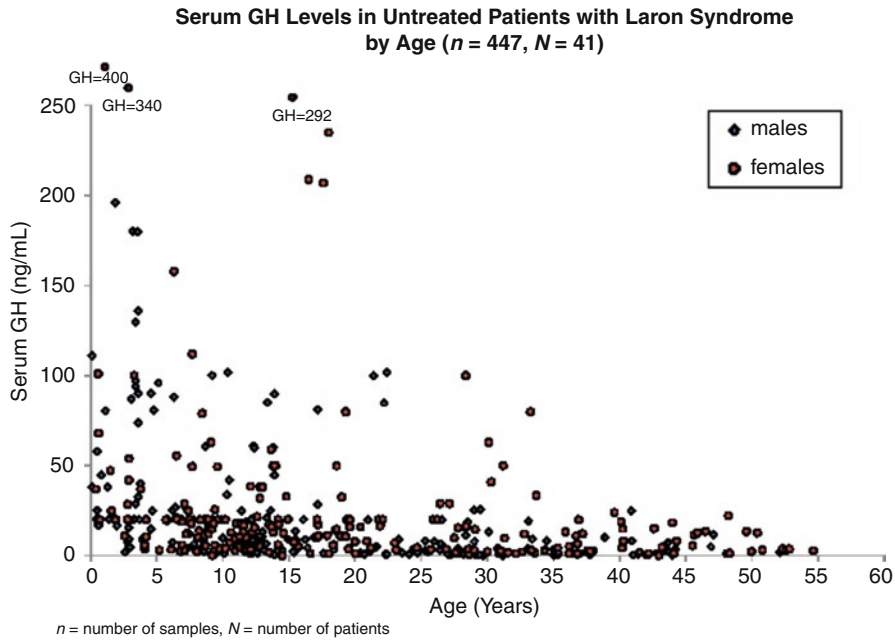

Fig. 26.1 Repeated morning serum human growth hormone (hGH) levels during long-term follow-up in 41 patients with Laron syndrome (8 males, 23 females)

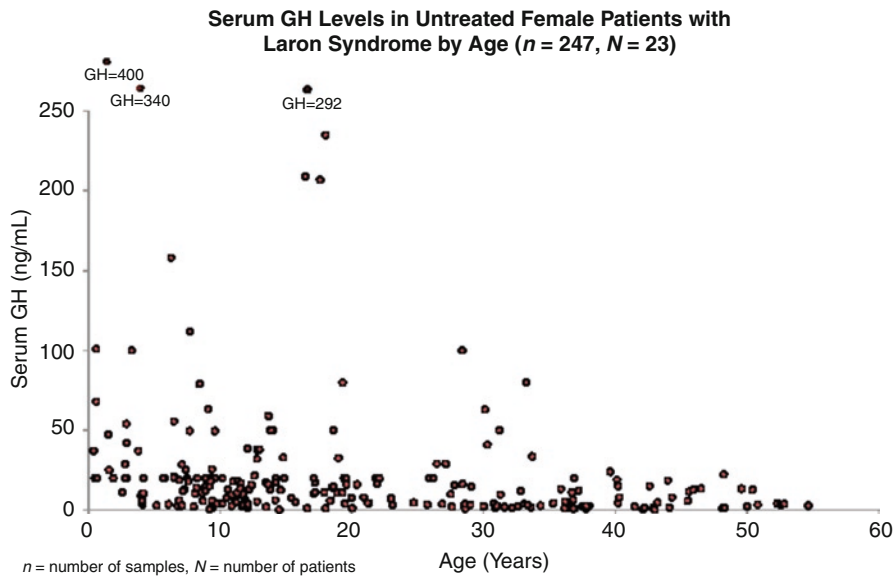

Fig. 26.2 Repeated morning serum hGH levels during long-term follow-up in 23 untreated female patients with Laron syndrome

range between 50 and 55, were available. To enable a more physiologic analysis of the data by excluding the estrogen effect in the females, serum levels for each gender are presented separately. Figure 26.2 illustrates the hGH concentrations in the female Laron syndrome patients and Fig. 26.3 in the male patients.

The displayed age pattern between the genders is similar, but the males have more elevated hGH serum values up to age 25 (the females up to age 35). Between the ages 35 and 55, the pattern is identical, the Laron syndrome patients presenting serum hGH values up to 20 ng/mL, much higher than those encountered in healthy adults of these ages (<5 ng/mL).

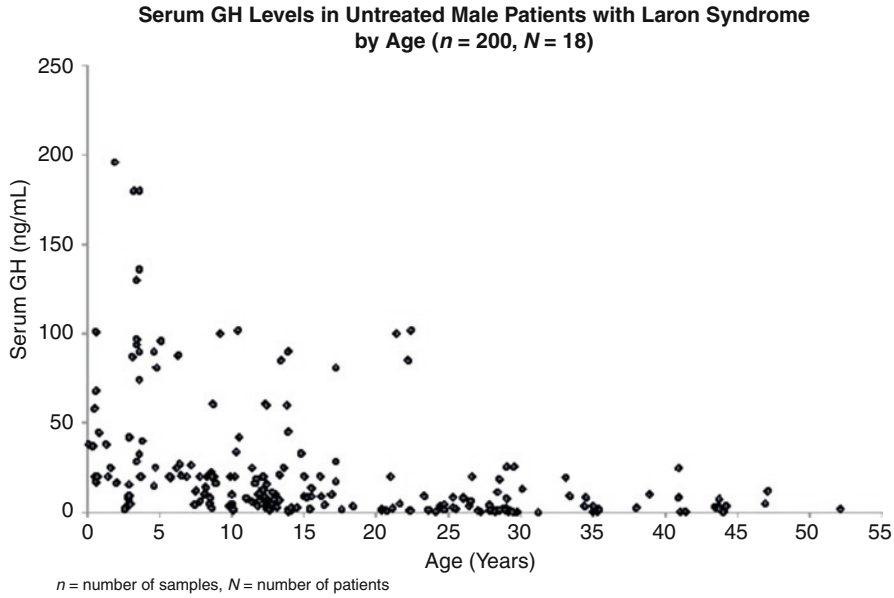

Fig. 26.3 Repeated morning serum hGH levels during long-term follow-up in 18 untreated patients with Laron syndrome

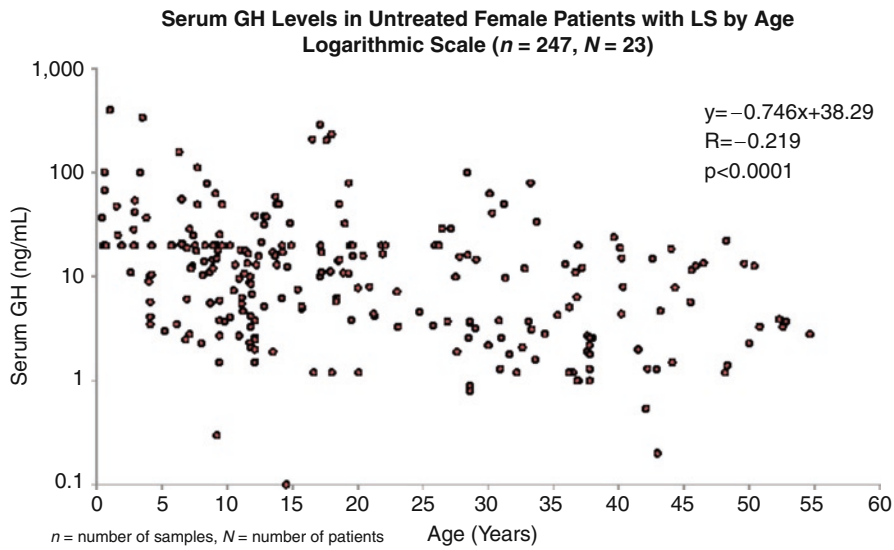

Fig. 26.4 Repeated morning serum hGH levels during long-term follow-up in 23 untreated female patients with Laron syndrome represented on a logarithmic scale

To show the dispersion of the pathologically high hGH values in a clearer way, the data have also been plotted on a logarithmic scale for female Laron syndrome patients (Fig. 26.4) and for male patients in Fig. 26.5. These presentations provide a better view of the gender differences between various serum hGH values. The tendency of more high values in the females may be explained by an estrogen effect.

The variability in hGH secretion in the same Laron syndrome patient when tested on different dates and ages is demonstrated in Fig. 26.6. This is true in both female and male patients and proves that a single determination does not always reveal the basal secretory capacity, in this case of hGH.

Our data also confirm previous data from our group that adults can secrete hGH even at an advanced age, but only when stimulated (Laron et al. 1970; Gil-Ad et al. 1984).

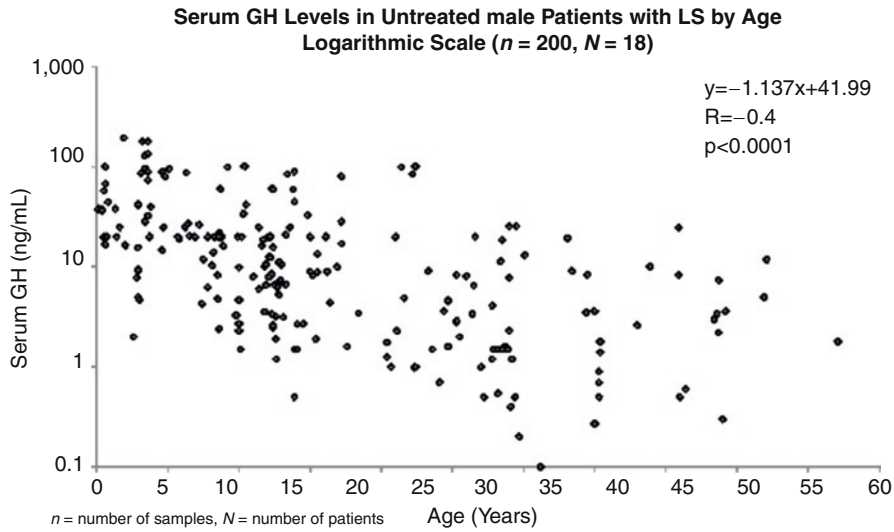

Fig. 26.5 Repeated morning serum hGH levels during long-term follow-up in 18 untreated patients with Laron syndrome represented on a logarithmic scale

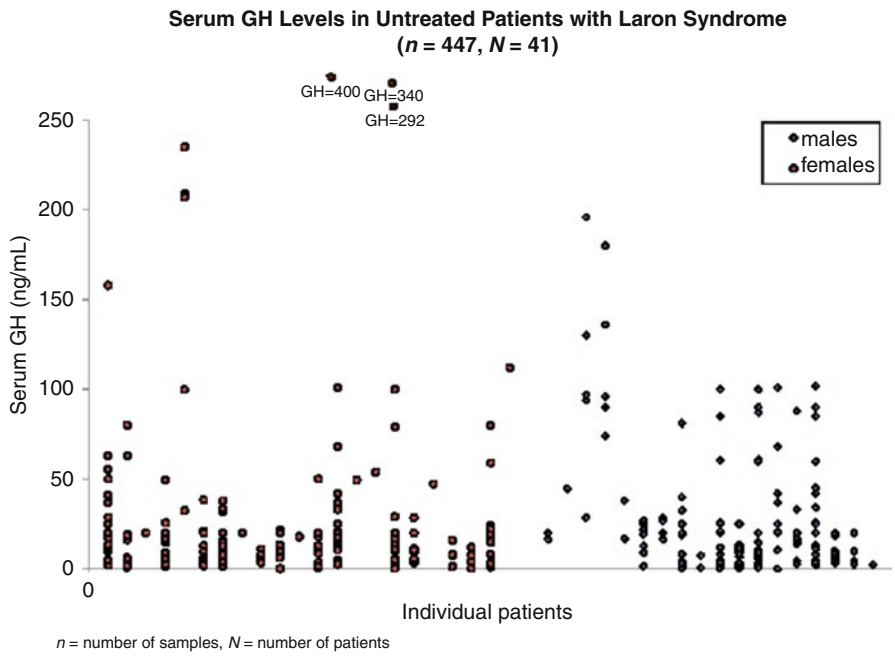

Fig. 26.6 Serum hGH values at repeated sampling on different dates and ages in untreated Laron syndrome patients

26.4 Comment

Laron syndrome patients secrete increased amounts of hGH even with advancing adult age. The increased levels in males are not estrogen dependent and seem to be due to the persistent IGF-I deficiency. A similar mechanism is observed in female patients. To test this assumption, we compared the concomitant hGH and IGF-I values in IGF-I treated patients. As demonstrated in one girl (Fig. 26.7), there is an inverse relationship between serum IGF-I and hGH levels. When the circulating IGF-I levels are elevated, they suppress the pituitary hGH secretion and vice versa. We use this correlation to ascertain whether the patients inject the

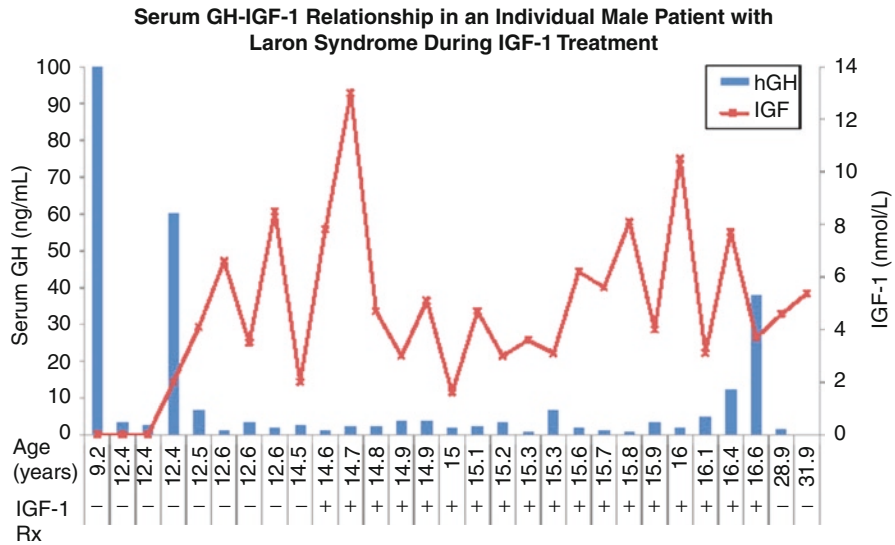

Fig. 26.7 Elevated serum IGF-I levels during IGF-I treatment in a girl with Laron syndrome suppressed the high circulating hGH levels

IGF-I, and if so, in a correct manner. This correlation also allowed us to adjust the daily dose, mainly the need to increase it in the growing child.

Acknowledgment The contribution of Orly Efros was in partial fulfillment of the MD thesis requirement of the Sackler Faculty of Medicine, Tel Aviv University

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IGF-I Binding Proteins in Laron Syndrome

27

Zvi Laron

Core Message

› Determination of serum IGF-I binding proteins revealed that in untreated patients with Laron syndrome IGFBP-1 is high and IGFBP-3 low. IGF-I replacement treatment reduces IGFBP-1 and raises IGFBP-3.

27.1 Introduction

Serum IGF-I levels in Laron syndrome patients are either very low or undetectable (Laron et al. 1991) (see Chap. 4) depending on the specificity and sensitivity of the assay used.

Virtually, all circulating and tissue IGFs are bound to specific IGF binding proteins (IGFBPs) (Bach and Rechler 1995). There are six IGFBPs, which represent a versatile and tuned mechanism for the regulation of IGF activity (Bach and Rechler 1995; Zapf 1995). The availability of free (active) IGF-I to the tissues is modulated mainly by IGFBP-1, 2, and 3. IGFBP-1 and 2 are GH independent, whereas IGFBP-3, the most abundant IGFBP in circulation, is GH dependent (Baxter and Martin 1986). IGFBP-1 levels are influenced both by insulin and IGF-I being high in deficiency states of both of these hormones. Laron syndrome characterized by IGF-I deficiency and also "de facto" GH deficiency presented an ideal model to study the state of the IGFBPs.

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27.2 IGFBP-1

27.2.1 Subjects

Fifteen Laron syndrome patients, 6 children (4 males, 2 females) and 9 adults (4 males, 5 females), as well as 40 control subjects, 20 children and 23 adults (18 males, 5 females), were included in this study. The Laron syndrome patients were investigated before and day after seven daily s.c. injections of IGF-I (FK 780 Lot 137889K, Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan) in a dose of 120–150 μg/kg/day. The controls received no treatment (Laron et al. 1991; 1992b).

27.2.2 Methods

Frozen serum for IGFBP-1 was sent to Helsinki and analyzed as described by Suikkari et al. (1987). Serum IGF-I was determined using an INCSTAR kit (Silbergeld et al. 1986) and insulin according to Heding (1972).

27.2.3 Results

Serum IGFBP-I concentration related to serum levels of IGF-I and insulin in untreated patients are shown in Table 27.1**.** It is seen that the IGFBP-1 levels in Laron syndrome both in childhood and in adult age are higher than those in the healthy subjects and correlated inversely with the serum IGF-I levels.

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Table 27.1 Pertinent clinical data and IGFBP-1, IGF-I, and insulin levels in untreated Laron syndrome patients compared to healthy controls (mean ± SD)

Group	Number of patients	Age Years	Range	Height (cm)	Weight (kg)	IGFBP-1 (μg/I)	IGF-I (nmol/I)	Insulin (pmol/I)
Laron syndrome patients								
Children	6	8.6 ± 4.8	1.8–14	90 ± 20	16.7 ± 9.1	368 ± 299.4*	4.1 ± 1.7	64.5 ± 45.2***
Adults	9	31.4 ± 3.8	25–37	122 ± 12	40.4 ± 9.3	82.4 ± 50.3**	7.2 ± 2.9	61.2 ± 37.3***
Healthy controls								
Prepubertal	9	4.5 ± 3.0	1.6–9	105 ± 15	17.0 ± 4.0	67.9 ± 38.2	18.3 ± 1.9	–
Pubertal	11	13.4 ± 2.1	10–17	156 ± 18	45.6 ± 15.0	44.7 ± 28.9	38.2 ± 7.1	–
Adults	23	30.0 ± 4.0	18–41	179 ± 3	77.0 ± 2.0	57.6 ± 12.0	–	40.2 ± 19.0

*$p < 0.02$ vs. healthy children; ** and healthy adults; ***$p < 0.05$ vs. healthy adults
Modified from Laron et al. (1992a)

27.3 Effect of IGF-I Administration to Patients with Laron Syndrome

Intravenous injections of a bolus of IGF-I (75 μg/kg) to eight Laron syndrome patients caused an increase of serum IGF-I concomitantly with a decrease in blood glucose and insulin and a slow rise in IGFBP-1 at a time when the IGF-I levels decreased (Fig. 27.1). Following s.c. injections of IGF-I for 7 days to nine Laron syndrome patients, there was a 60% rise in

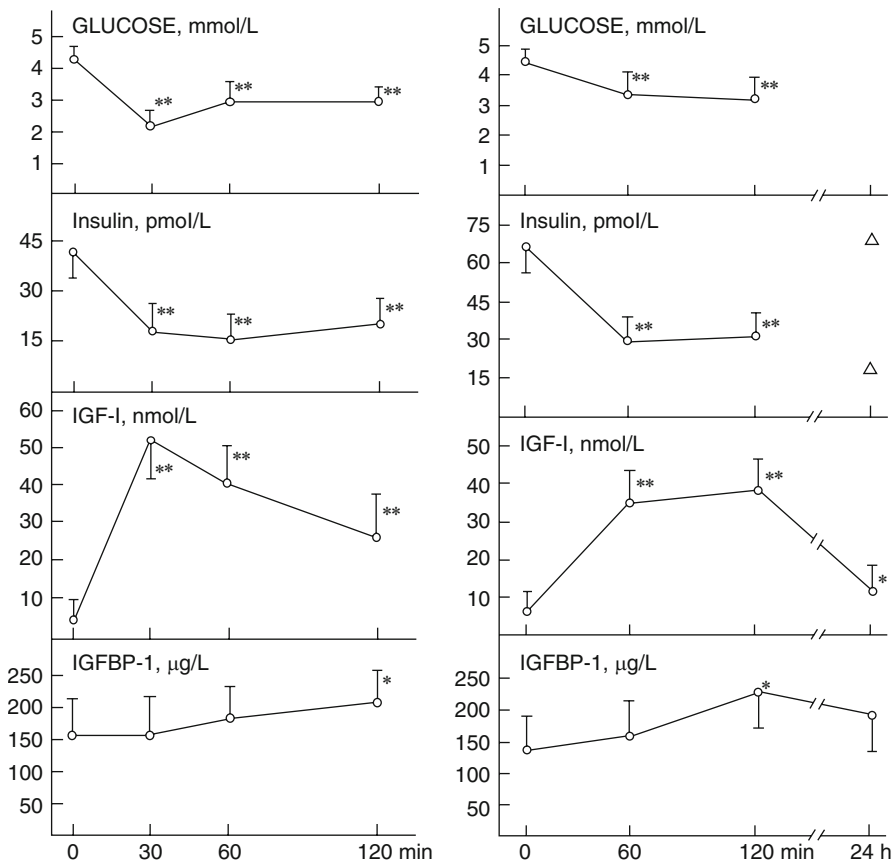

Fig. 27.1 Effect of an intravenous bolus injection of IGF-I (75 μg/kg) in eight Laron syndrome patients (three children and five adults) on serum IGFBP-I compared to levels of IGF-I and insulin. Modified from Laron et al. (1992a)

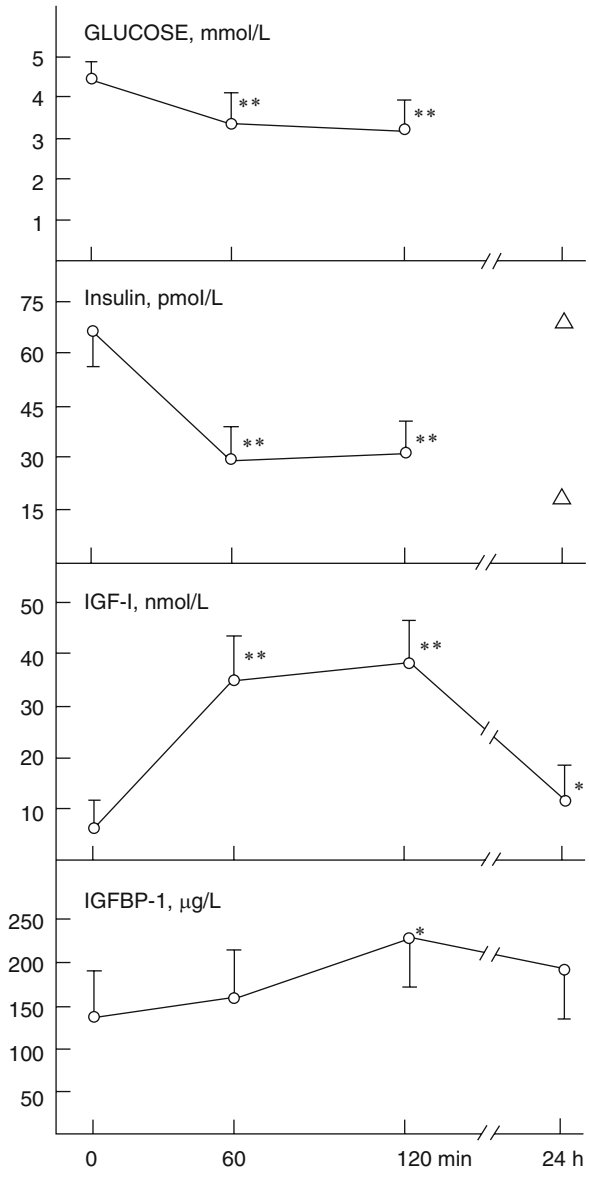

Fig. 27.2 Effect of a subcutaneous injection of IGF-I (150 µg/kg) to nine Laron syndrome patients (three children and six adults) on IGFBP-I compared to levels of IGF-I, insulin, and blood glucose

IGFBP-1 ($p<0.02$) concomitantly with a decrease in blood glucose, insulin, and IGF-I (Fig. 27.2) (Laron et al. 1992a). These findings are additional evidence for the interaction between IGF-I and insulin on the regulation of IGFBP-1 levels.

27.4 IGFBP-3

IGFBP-3 is the major IGF binding protein in human serum. It is secreted in the liver and is GH dependent (Baxter and Martin 1986; Blum et al. 1990). As patients with GH deficiency were found to have low serum IGFBP-3 values (Blum et al. 1990), we investigated serum IGFBP-3 concentrations in Laron syndrome patients both in the untreated state and during IGF-I replacement therapy (Laron et al. 1992b).

27.4.1 Study 1

27.4.1.1 Subjects and Methods

Eight Laron syndrome patients (four children, four adults) were investigated. Basal IGFBP-3 levels were low ranging from 0.294 to 1.255 mg/L (norm 2–3 mg/L). After 7 days of IGF-I administration (Laron et al. 1991), there was a further reduction (mean ± SD 30.7 ± 16.8%) ($p<0.006$) (Laron et al. 1992b). As this finding could not be attributed to the suppression of hGH secretion, it was considered due to either an influence of the decrease in serum insulin or by an acute sequestration by the injected IGF-I.

The IGFBP-3 in this investigation was determined by Dr. Blum in Tubingen, Germany (Blum et al. 1990). Later investigations during long-term IGF-I replacement treatment and using different methods yielded different results.

27.4.2 Study 2

27.4.2.1 Subjects and Methods

Six Laron syndrome patients (two children and four adults) were studied before and during 6 months of IGF-I administration (FK 780 Lot 11570K, Fujisawa Pharmaceutical Co. Ltd. Osaka, Japan) 120–150 µg/kg once daily. Serum samples were frozen until the last injection (Kanety et al. 1993). IGFBP-3 was analyzed at intervals by western ligand blotting (Hossenlopp et al. 1986).

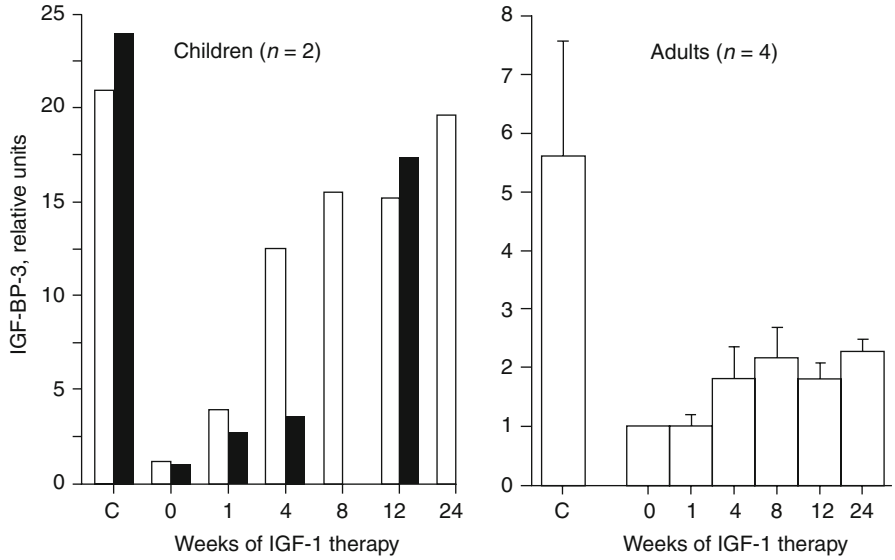

Fig. 27.3 Increase in serum IGFBP-3 after daily s.c. IGF-I injections to Laron syndrome patients (150 μg/kg to children and 120 μg/kg to adults). Reproduced with permission from Kanety et al. (1993)

27.4.2.2 Results

A steady increase in the fasting serum IGF-I levels was observed in all treated patients (from 1.8±0.7 to 17.8 nM/L). Chronic treatment with exogenous recombinant IGF-I induced a progressive rise of serum IGFBP-3 in all patients. In children, the increase was striking and after 6 months of therapy IGFBP-3 levels were almost as high as in age-matched controls. In adult Laron syndrome patients, the increase in IGFBP-3 was less dramatic and serum levels remained lower than in controls. The increase in IGFBP-3 was a slowly progressive process, and closely paralleled the increase in serum IGF-I. After 1 week of therapy, IGFBP-3 was only slightly increased in most patients, with a reduction in two patients as previously described (Laron et al. 1992b). The increase in IGFBP-3 was evident in all patients already after 4 weeks of therapy (Fig. 27.3).

27.4.3 Study 3

27.4.3.1 Subjects and Methods

In an additional study (Kanety et al. 1997), we explored the effect of IGF-I administration on the acid labile subunit (ALS) part of the IGFBP-3 complex. Three Laron syndrome patients, siblings positive for GHBP (proving that the molecular defect is not in the extracellular domain of the GH receptor), were assayed for serum IGFBP-3 and ALS concentration by radioimmunoassays (Baxter 1990; Baxter and Martin 1986) as well as by immunoblotting (Hossenlopp et al. 1986).

Sera for IGF-I, IGFBP-3, and ALS were measured before and weekly during 6 months of IGF-I treatment.

27.4.3.2 Results

In the female sibling, IGF-I treatment resulted in a progressive increase in the concentration of IGF-I, with a twofold increase after 1 week of IGF-I administration and a sixfold increase after 6 months. In the male siblings, only a small increase in IGF-I concentration was evident during long-term IGF-I treatment, with a twofold increase after 6 months of treatment. Before treatment, serum concentrations of IGFBP-3 measured by RIA were found to be 17–45% of normal values. In the female sibling, IGF-I treatment led to a gradual increase in IGFBP-3 concentrations. In the male sibling, 1 week of IGF-I treatment resulted in a decrease in IGFBP-3 concentrations, followed by a slow increase towards baseline values in the following months. Western ligand blotting confirmed the use in IGFBP-3 concentrations indicated by RIA, with a striking increase in the intensity of the 40–43 kDa doublet representing IGFBP-3. Similar results were obtained by

immunoblotting with a specific IGFBP-3 antibody. The basal serum concentrations of ALS in these children with Laron syndrome measured by RIA were low (8–40% of normal), and during IGF-I treatment, the changes in ALS were similar to those found for IGFBP-3, namely demonstrating an increase. No consistent changes in IGFBPs 2 and 4 were observed during the 6 months of IGF-I treatment in all three siblings.

27.4.4 Discussion

The discrepancies between the individual basal values of IGFBP-3 and ALS intensity changes during IGF-I treatment may be due to serum protease activity as suggested by Rosenfeld et al. (1994) or by changing the sites of IGF-I injection.

27.4.5 Conclusions

1. In the evaluation of serum IGFBP-I values, insulin concentrations need to be considered
2. In Laron syndrome, a state of both hGH and IGF-I deficiency, IGFBP-3 levels are 50% of normal
3. The rise in serum IGFBP-3 and ALS during once daily IGF-I treatment explains the progressive rise of basal serum IGF-I during long-term IGF-I therapy, leading sometimes to the necessity of reducing the IGF-I dose. These findings support the sufficiency of one daily injection of IGF-I suffices to maintain growth acceleration (see Chap. 42).

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Serum Prolactin in Untreated and IGF-I Treated Patients with Laron Syndrome

28

Zvi Laron and Orly Efros

Core Message

› Determination of serum prolactin (PRL) in untreated Laron syndrome patients revealed a tendency for higher than normal values. In both untreated and IGF-I treated patients there seemed to be a certain parallelism between hGH and PRL secretion, explained by the common origin in somato-mammotrophic cells in the patients.

28.1 Introduction

Human prolactin (PRL) is a 199 amino-acid polypeptide containing three disulfide bonds (Sinha 1995). Its gene is located on chromosome 6 (Owerbach et al. 1981) and is considered to arise from a common ancestral gene with growth hormone (GH) and placental lactogen (Cooke et al. 1980). Its receptor gene localizes on chromosome 5p13. The main factors that influence PRL gene expression and secretion include estrogens, dopamine, TRH, and thyroid hormones (Lamberts and Macleod 1990). In obesity, circulating PRL levels have been found to be reduced, compared to normal subjects (Kopelman et al. 1979; AvRuskin et al. 1985), similar to the serum levels of GH (Scacchi et al. 1999; Meistas et al. 1982; Veldhuis et al. 1991). It was also reported that in obese subjects, the PRL response to various stimuli (TRH, metoclorpramide) is blunted (Donders et al. 1985). On the contrary, Kokkoris and Pi-Sunyer (2003) found higher 24-h PRL secretion in obese premenopausal women compared to control subjects and showed it to be related to the amount of visceral fat.

As pituitary GH secretion is increased in Laron syndrome and the pituitary mammosomatotropes secrete both GH and PRL (Frawley 1989; Kovacs et al. 1989), it was of interest to study whether the serum levels of PRL are also increased in these patients and, if so, whether this results in any clinical effects. It was also of interest to learn whether IGF-I deficiency or administration affects PRL secretion. Forthwith the description and results of our studies.

28.2 Basal Serum PRL Levels of Untreated Laron Syndrome Patients Along Age

28.2.1 Subjects

At many of the routine follow up visits over many years, patients' blood was also drawn for the determination of hormone levels including PRL. The findings were recently summarized (Laron and Efros unpublished). Reliable data were found in 33 Laron syndrome patients, 16 males and 17 females.

28.2.2 Methods

Serum PRL was analyzed by radioimmunoassay using the Immulite 2000 Analyzer (Diagnostics Products. Corp, Los Angeles, USA).

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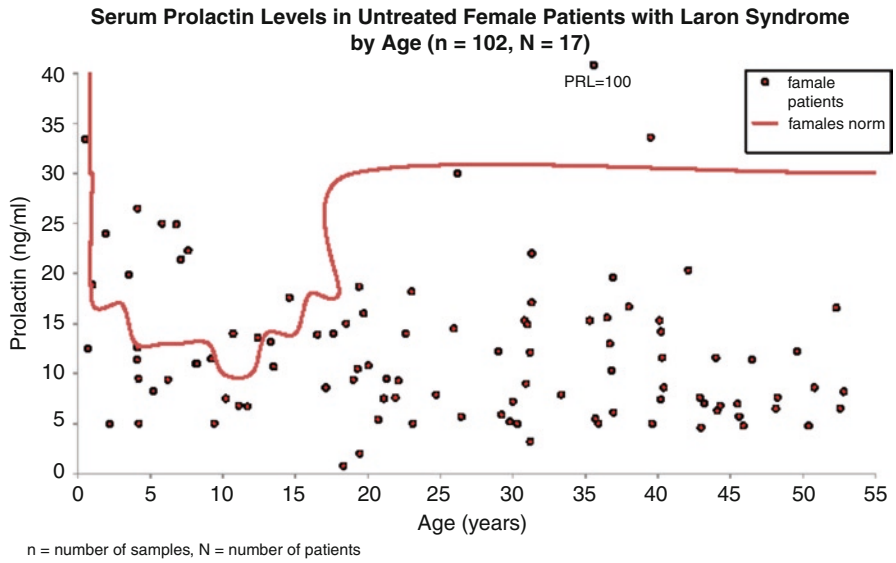

Fig. 28.1 Repeated overnight fasting serum prolactin levels of 17 untreated female patients with Laron syndrome

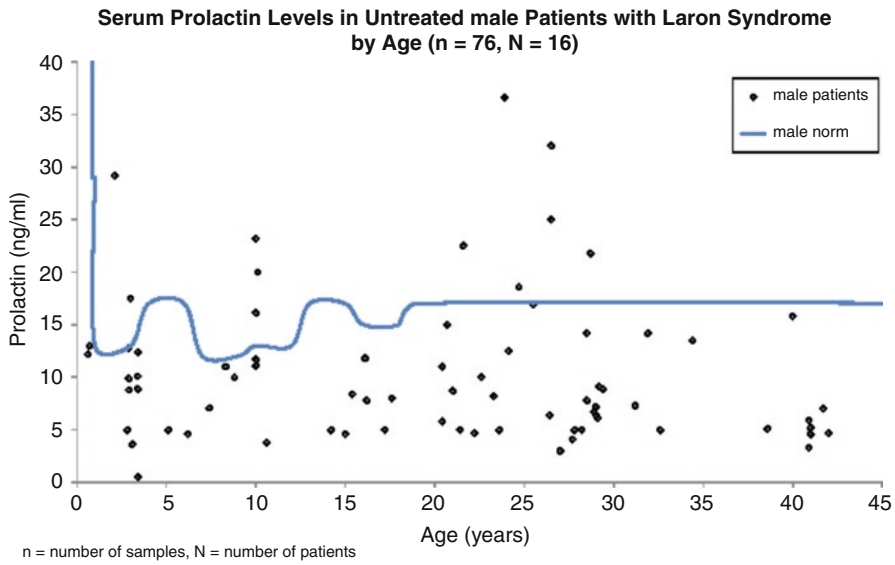

Fig. 28.2 Repeated overnight fasting serum prolactin levels of 16 untreated male patients with Laron syndrome

28.2.3 Results

Figure 28.1 presents repeated basal PRL levels in 17 untreated females with Laron syndrome along various ages. It is seen that in early childhood when no estrogen stimulation occurs PRL levels were elevated, so were incidental findings between ages 35 and 40. Figure 28.2 presents repeated PRL levels in 16 untreated male patients. Two findings are evident: (a) the female patients tend to have higher PRL levels than the males, probably due to estrogen secretion, and (b) the males have more incidences of increased levels of PRL than the females. Figure 28.3 illustrates repeated PRL determinations in individual Laron syndrome patients performed at different visits (left – females, right – males). It is seen that the serum PRL levels vary from low to normal to high in the same individual.

Considering mean normal serum PRL concentrations for young women (15–25 years) as 20.9 ng/mL and 8–10 ng/mL for women aged 55–65 years (Vekemans and Robyn 1975) and 5.2 ± 0.55 ng/mL for young adult men (Guyda and Friesen 1973), it is obvious that patients with Laron syndrome secrete increased amounts of PRL.

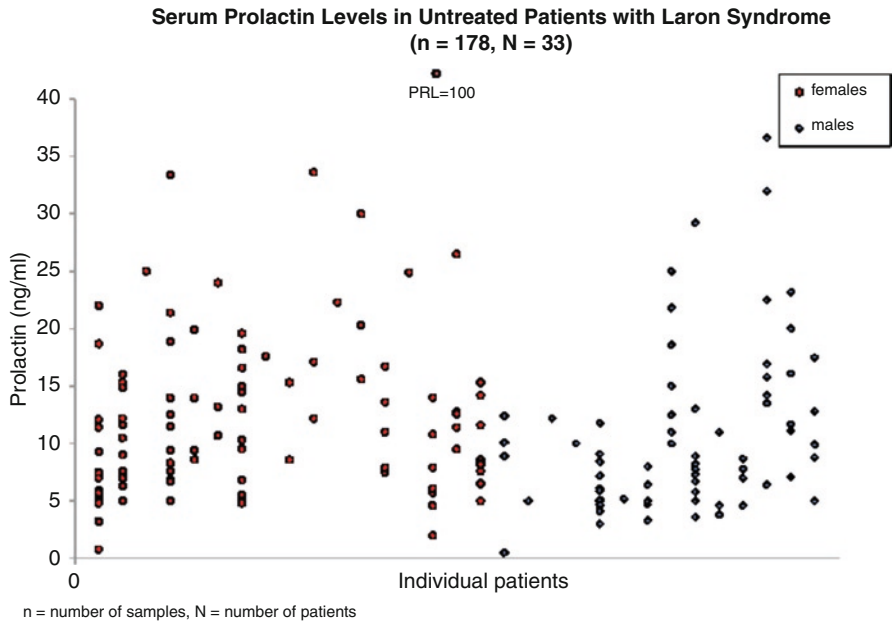

Fig. 28.3 Overnight fasting serum prolactin levels taken on different occasions in the same Laron syndrome patients

To learn the effect of IGF-I on serum PRL levels in patients we performed further investigations.

28.3 Effect of Intravenous IGF-I Injections on Serum Prolactin

28.3.1 Subjects and Methods

An intravenous (i.v.) bolus injection of IGF-I (75 μg/kg) (FK 780 Fujisawa, Osaka, Japan) was administered to ten untreated Laron syndrome patients (three children and seven adults). Three young adult volunteers and two children aged 8 and 12 served as controls. The IGF-I was injected after an overnight fast, and blood samples were drawn at 0, 2, 5, 15, 30, 60, 90, and 120 min. After the injection, the serum was separated and frozen at −20°C until assayed by RIA for PRL and human growth hormone (hGH) (Diagnostic Products Co., CA, USA).

28.3.2 Results

The basal blood glucose, and serum PRL and hGH and their responses following the i.v. bolus injection of IGF-I in ten Laron syndrome patients and five control subjects are presented in Fig. 28.4. The peak serum IGF-I level registered at 2 min after the injection was 18 ng/mL. Blood glucose fell from mean basal level of 77 mg/dL to a nadir of 39.6 mg/dL in the Laron syndrome patients vs. from 86.4 to 54 mg/dL in the controls (not shown) (Silbergeld et al. 1992).

28.3.3 Prolactin

The mean (±SD) basal PRL level in the Laron syndrome patients was significantly higher than that in the control subjects: 12.6 ± 1.9 ng/mL vs. 7.6 ± 1.2 ng/mL ($p < 0.05$). In response to the i.v. IGF-I bolus, there was a small but measurable decrease (20.55 ± 4.29%) in the PRL concentration (nadir at 2 min 9.7 ± 1.1 ng/mL); subsequently, there was a pronounced progressive rise with the mean peak values at 30–60 min being 33.3 ± 4.5 ng/mL. At 120 min, the serum PRL values were still above basal concentrations. The massive response (136 ng/mL) in one adult female is depicted separately. The control subjects also showed an immediate decrease following the i.v. IGF-I bolus followed by a rise peaking at 30–60 min, but the mean peak value was significantly lower (14.9 ± 1.9 ng/mL) than that in the Laron syndrome patients ($p < 0.05$).

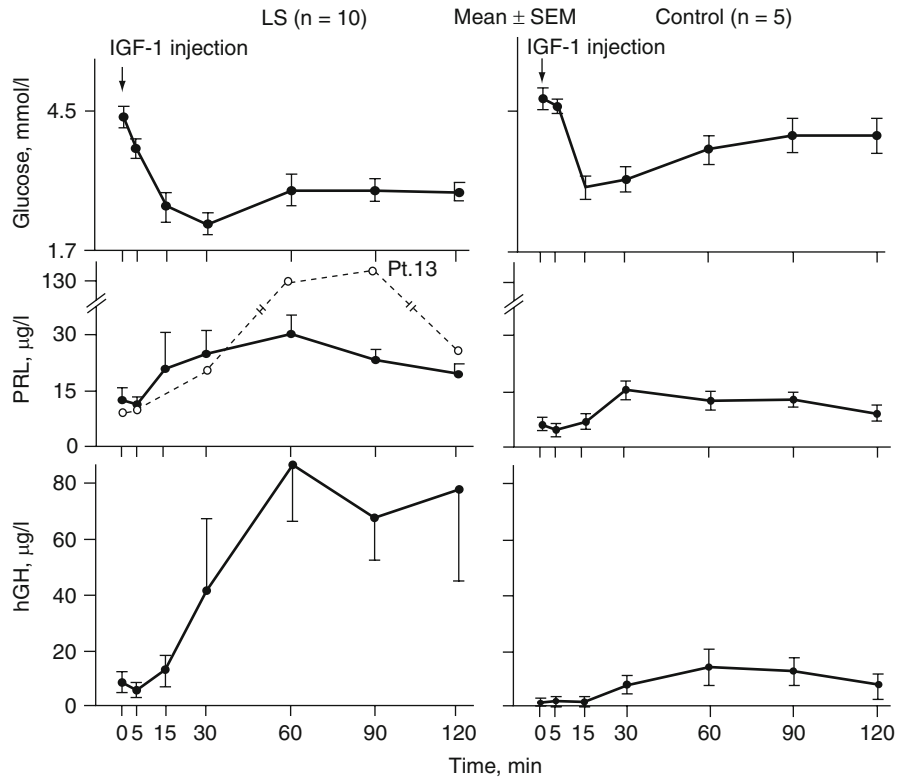

Fig. 28.4 Serum glucose, prolactin, and growth hormone response to an i.v. bolus of IGF-I (75 µg/kg)

28.3.4 GH

The basal level of hGH in the Laron syndrome patients was higher than that in the controls: 8.27 ± 2.3 ng/mL vs. 0.85 ± 0.2 ng/mL ($p = 0.01$). Following an immediate decrease to 6.4 ± 2.8 ng/mL (p = N.S.) after the i.v. IGF-I bolus, the hGH levels rose markedly to a peak of 86 ± 20 µg/L (m ± SD) rebound at 60 min. In the control subjects, the serum hGH response to IGF-I was moderate, and the mean rebound peak 14.4 ± 8.6 ng/mL was significantly lower than that in the Laron syndrome patients ($p < 0.0001$).

28.4 Effect of Long-Term IGF-I Treatment on Serum PRL in Patients with Laron Syndrome

The repeated serum PRL values during IGF-I treatment in 12 female Laron syndrome patients, children, and adults are illustrated in Fig. 28.5, and in eight treated male patients in Fig. 28.6. These plots of many determinations performed on a few patients do not permit to distinguish a clear IGF-I effect as seen by an IGF-I i.v. bolus. The only finding is the appearance of relatively more raised PRL values in the treated young boys than in the untreated pediatric Laron syndrome patients. The drawing does not permit to see whether they belong to the same or different patients.

28.4.1 Prolactin – GH Relationship

To find out whether the raised secretion of PRL is linked to the increased secretion of hGH, we compared the serum overnight fasting levels of these two hormones determined concomitantly. Figure 28.7 illustrates the hGH–PRL relationship in a girl and Figure 28.8 in an adult female with Laron syndrome. It is evident that at times, serum PRL levels tended to fluctuate in the same direction with hGH. More data on this relationship are being collected.

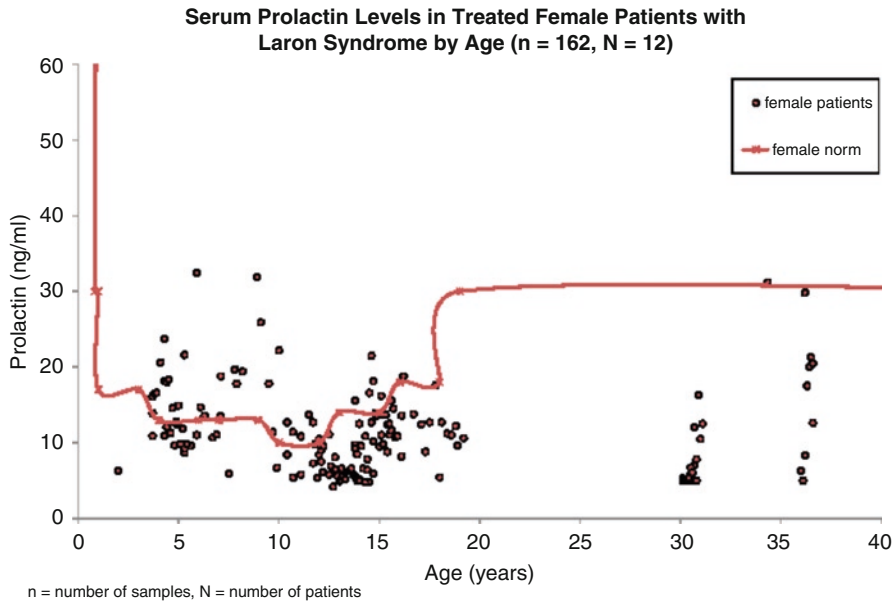

Fig. 28.5 Serum prolactin in IGF-I treated female Laron syndrome patients

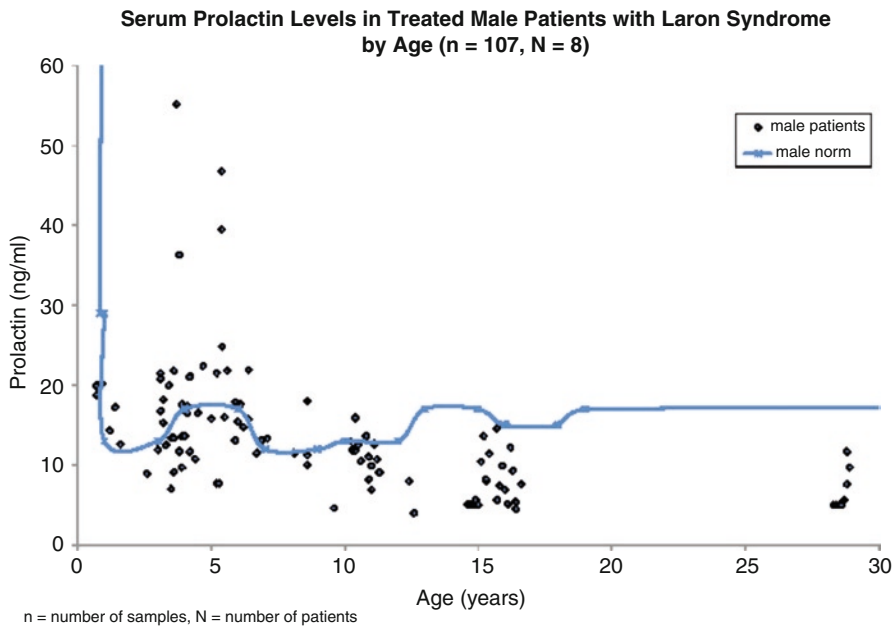

Fig. 28.6 Serum prolactin in IGF-I treated male patients with Laron syndrome

28.4.2 Response of PRL secretion to TRH Administration

As a blunted response of PRL to TRH stimulation in obese subjects had been reported (Donders et al. 1985), we wanted to find out whether the obese Laron syndrome patients present the same response.

28.4.3 Subjects and Methods

An i.v. TRH test (100 μg/m^2) (TRF Serono, Italy) was performed in 13 obese Laron syndrome patients (six prepubertal children and seven adults) in the morning after an overnight fast. Blood was withdrawn at 0, 15, 30, 60, and 90 min after injection. Sera from 20 endocrinologically

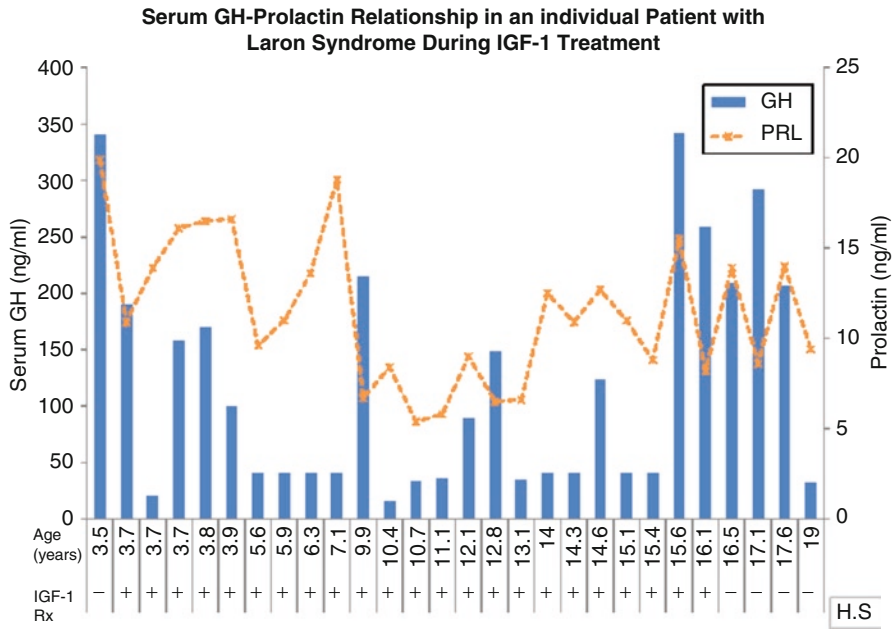

Fig. 28.7 Serum hGH–PRL relationship sampled concomitantly in a girl with Laron syndrome

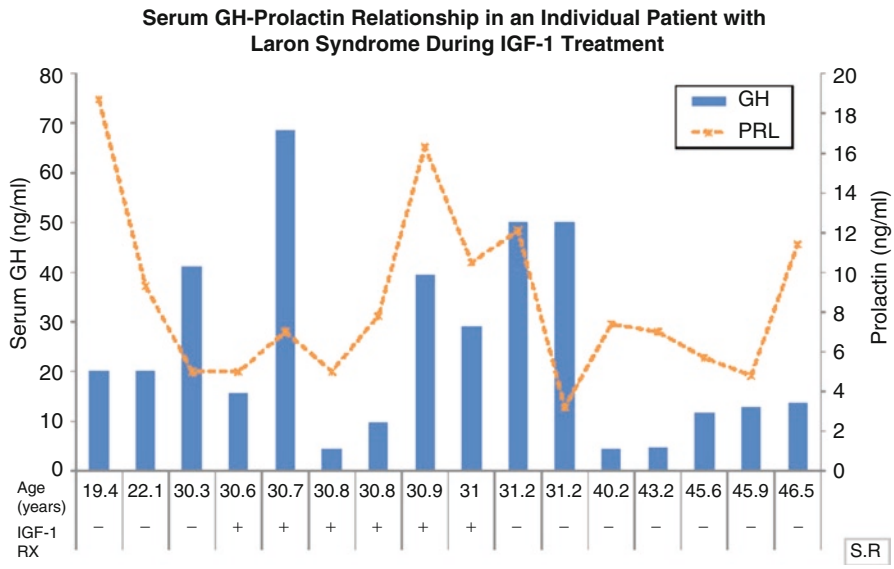

Fig. 28.8 Serum hGH–PRL relationship sampled concomitantly in an adult female with Laron syndrome

healthy subjects (11 males, 9 females) aged 16±6 years served as controls (Silbergeld et al. 1992).

28.4.4 Results

There was a marked increase of serum PRL after the i.v. TRH (peak at 20 min) in both the Laron syndrome patients and the controls, the response being similar with the exception of 1 of the 2 Laron syndrome adult males who had no response, despite a rise in TSH (data not shown). It is noteworthy that the estrogen-primed adult Laron syndrome females did not have a higher response than the prepubertal Laron syndrome children. The TSH response to TRH in all subjects was similar.

28.5 Effect of PRL Levels on Breast Size

Some of the adult female Laron syndrome patients have enlarged breasts. This is illustrated in Figure 28.9 in two unmarried sisters. We consider this to be linked to the raised PRL secretion.

28.6 Conclusions

Our studies showed that in contradistinction to other types of obesity in which lowered PRL have been reported, the obese patients of Laron syndrome tend to oversecrete PRL. The increased PRL levels tend at

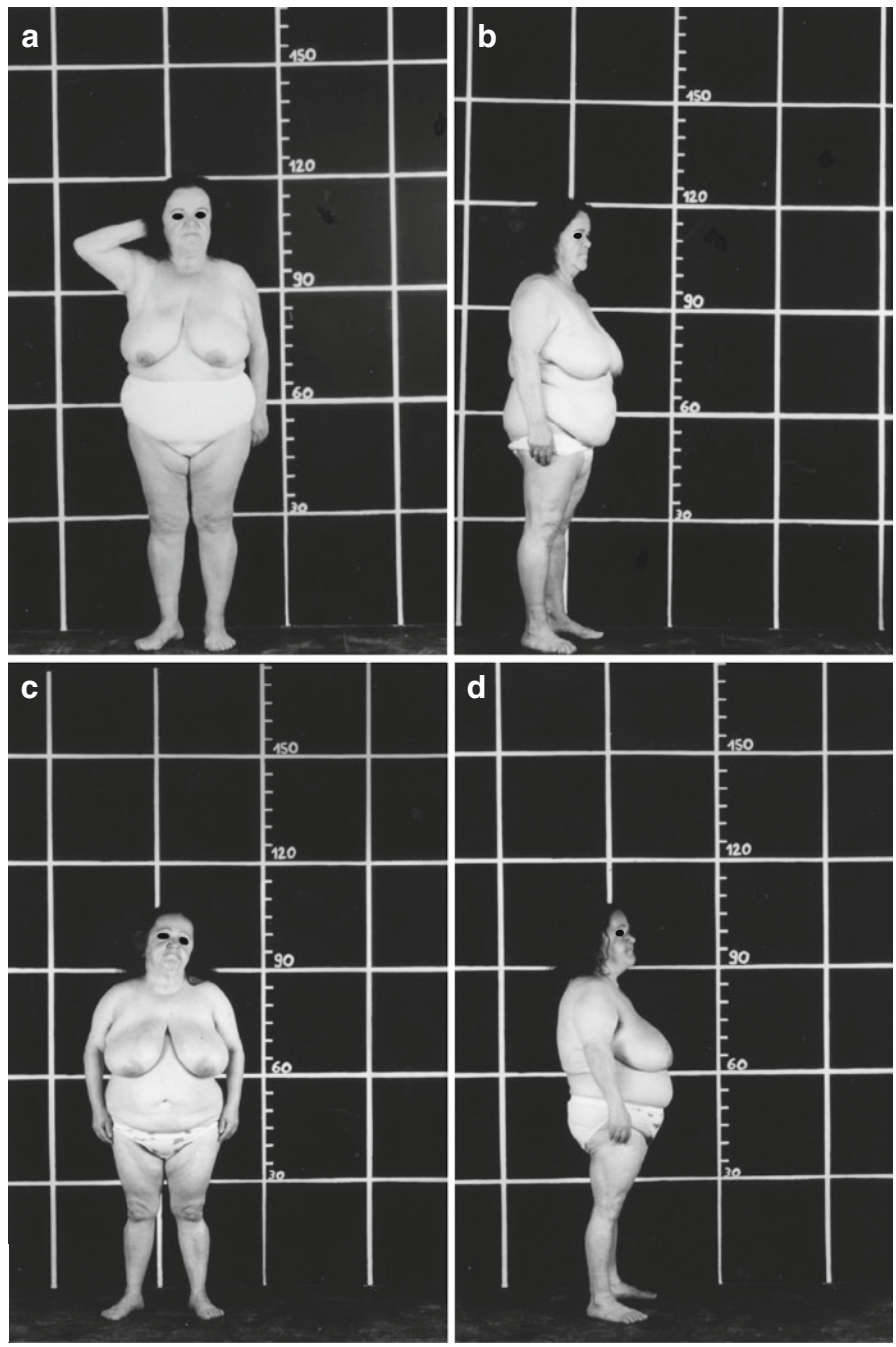

Fig. 28.9 Large breasts in two untreated sisters with Laron syndrome (anterior (**a**, **c**) and lateral (**b**, **d**) views)

times to parallel the raised hGH secretion, which results from the negative feedback to the IGF-I deficiency. Whether this is due to a drift phenomenon to the secretion of GH, as both hormones originate from common pituitary somatomammotrophic cells (Frawley, 1989), remains to be proven. The other alternative is a direct involvement of IGF-I in the regulation of PRL secretion. The elevated PRL levels in untreated prepubertal girls devoid of an estrogen effect favor the first theory. The fact that IGF-I treatment seems to suppress both the elevated hGH and, to a lesser degree, the serum may be considered as further proof.

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Thyroid Hormones in Untreated and IGF-I Treated Patients with Laron Syndrome

29

Zvi Laron and Rivka Kauli

Core Message

› The study of thyroid hormones in untreated and IGF-I treated patients with Laron syndrome revealed normal thyroid function. Administration of IGF-I decreased all serum thyroid hormone levels within normal limits. TRH administration raised serum TSH concentrations.

29.1 Background

It has been reported that administration of hGH to healthy or GH deficient patients, including children, suppressed thyroid function by variable degrees (Grunfeld et al. 1988). Some authors reported a lowering of serum T_4 and an increase of serum T_3 (Sato et al. 1977; Rezvani et al. 1981; Grunfeld et al. 1988; Jorgensen et al. 1989), a decrease in ^{131}I thyroidal uptake (Root et al. 1970), or a decrease in serum TSH (Porter et al. 1973).

29.2 Objective

The aims of our studies were to find out whether the effect of GH is direct or IGF-I mediated, by studying the state of thyroid hormones in untreated and IGF-I treated patients with Laron syndrome.

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29.3 Serum TSH and Thyroxine (T4) in Untreated Patients with Laron Syndrome

29.3.1 Subjects and Methods

The data were extracted from the routine hormone determinations performed at the first and follow-up visits. TSH, total T4, and free T4 (fT4) were measured by commercial radioimmunoassays.

29.3.2 Results

Figure 29.1 shows the serum TSH levels of 40 untreated patients with Laron syndrome along age. It is seen that with few exceptions, the values were within the normal range. The values passing the upper limit of normal were lower at repeated testing.

Figure 29.2 presenting the total T4 levels and Fig. 29.3 showing the fT4 levels reveal that all were within the normal limits.

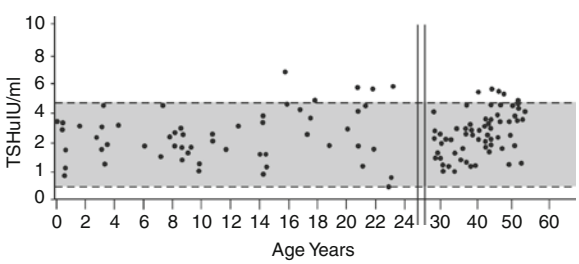

Fig. 29.1 Serum TSH levels in 40 untreated Laron syndrome patients by age

Z. Laron and J. Kopchick (eds.), *Laron Syndrome - From Man to Mouse*,
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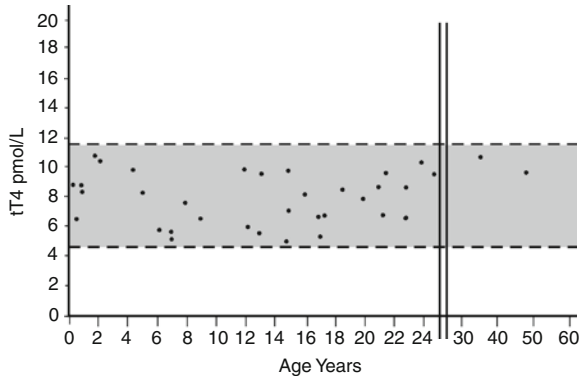

Fig. 29.2 Serum total thyroxine levels in 26 untreated Laron syndrome patients by age

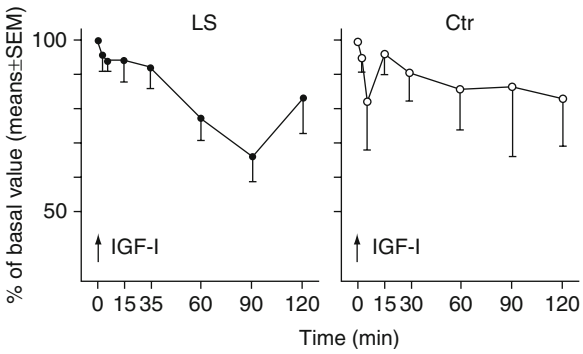

Fig. 29.4 Serum TSH response to an IGF-I bolus injection of IGF-I (75 μg/kg) to ten Laron syndrome patients as compared to eight healthy controls. Reproduced from Klinger et al. (1992) Acta Endocrinologica does not exist anymore!

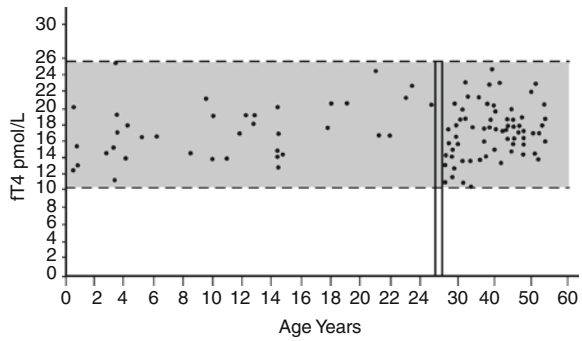

Fig. 29.3 Serum free thyroxine levels in 40 untreated Laron syndrome patients by age

29.4 Effect of IGF-I Administration on Thyroid Function in Patients with Laron Syndrome

29.4.1 Acute Effects

An intravenous bolus injection of 75 μg/kg IGF-I (FK 780 lot 115707K Fujisawa Pharmaceuticals, Osaka, Japan) was administered in the fasting state to ten Laron syndrome patients (3 children and 7 adults, 5 males, 5 females) aged 8–35 years. The IGF-I injection resulted in a significant decrease in serum TSH ($p<0.009$) from 1.72±0.19 to 1.15±0.09 mU/L in the Laron syndrome patients and from 1.18±0.22 to 0.82±0.017 mU/L in the controls ($p<0.02$) (Fig. 29.4) (Klinger et al. 1992).

29.4.2 Effect of One Subcutaneous Injection

IGF-I (150 μg/kg – same batch as above) was administered to eight Laron syndrome patients (3 males, 5 females) aged 9–35 years and three healthy children aged 9–12.5 years. The injection of IGF-I reduced significantly the mean serum TSH concentration from 2.15±0.41 to 1.12±0.24 mU/L ($p<0.005$) in the Laron syndrome patients and from 2.03±0.56 to 0.86±0.18 mI/L ($p<0.05$) in the healthy children (Fig. 29.5) (Klinger et al. 1992).

29.4.3 Effect of 7 Days' IGF-I Administration

Ten Laron syndrome patients (4 males, 6 females) aged 3.6–38 years participated in this trial. The daily dose of IGF-I injected before breakfast was 150 μg/kg up to age 20 and 120 μg/kg in older patients. Blood samples were taken before and on days 2, 4, and 8, and 7 days after the last IGF-I injection. A decrease was registered in all thyroid hormones, but the most significant effect was a decrease of fT4 ($p<0.005$) lasting even after stopping the IGF-I administration. TSH decreased for only 2 days and serum T3 showed little change (Fig. 29.6). In no instance were hypothyroid levels registered.

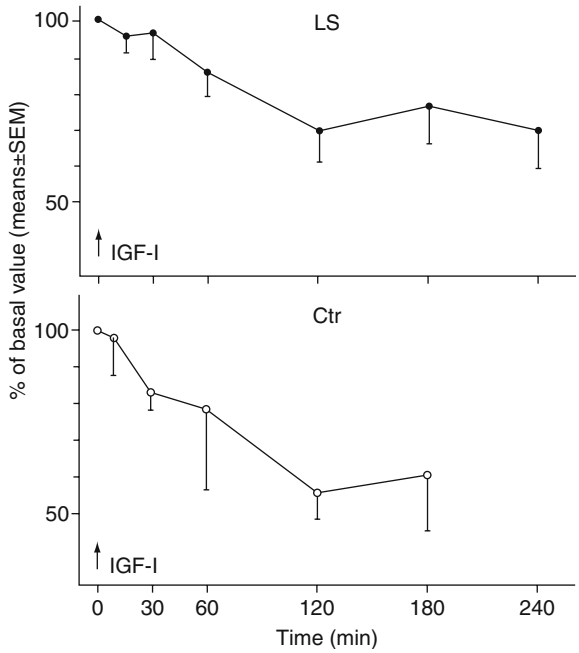

Fig. 29.5 Serum TSH response to a one s.c. IGF-I administration (150 μg/kg/day) to eight Laron syndrome patients and three healthy control subjects

29.5 Thyroid Hormones During Long-Term IGF-I Administration

During long-term administration of IGF-I to Laron syndrome patients, the levels of thyroid hormones remained within the normal limits. However, when analyzing the results in individual patients, one could observe a progressive and transitory lowering in the serum TSH values during the first year of treatment as exemplified in a girl in whom IGF-I treatment was initiated at age $3^{6/12}$ years (Fig. 29.7)

29.5.1 Thyroid Hormone Response to Intravenous TRH Administration

Performing i.v. TRH tests (100 μg/m^2 , i.v.) before and after 4 months of daily IGF-I treatment in six Laron syndrome patients, we found that IGF-I did not suppress the TSH response, rather increased it ($p<0.01$) (Fig. 29.8) (Klinger et al. 1992).

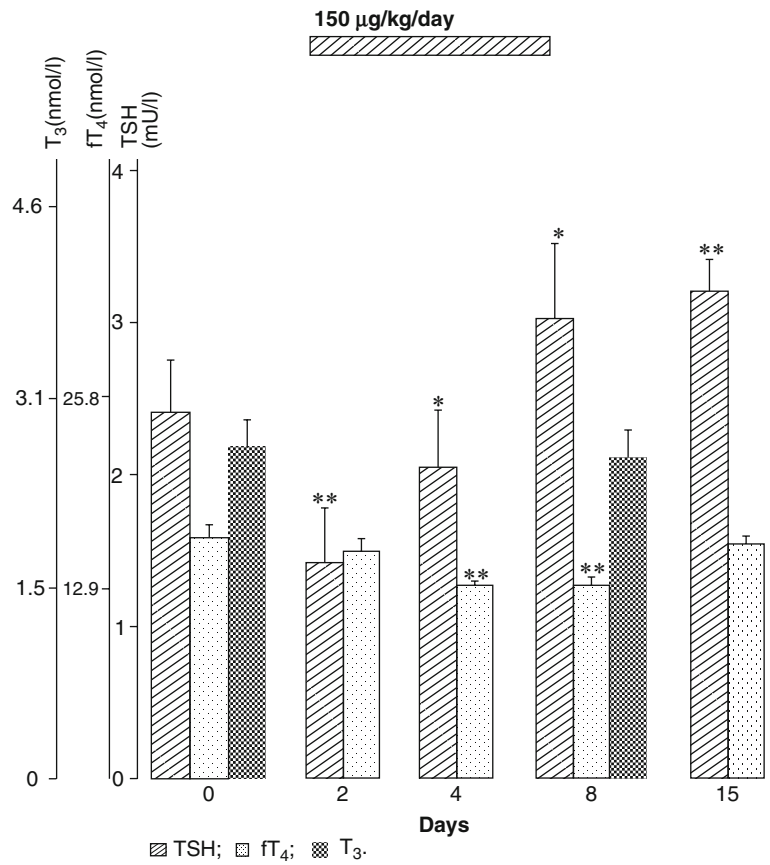

Fig. 29.6 Serum TSH, total, and free thyroxine during 7 days of IGF-I treatment of 10 patients with Laron syndrome
*$p<0.05$; **$p<0.005$

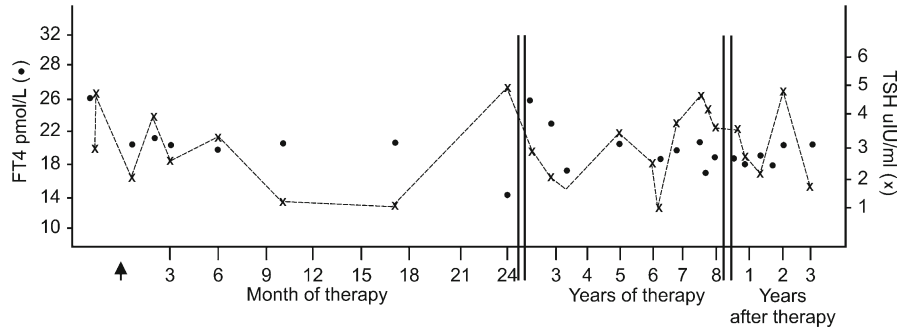

Fig. 29.7 Serum TSH and fT4 in a girl with Laron syndrome during the first year of IGF-I treatment

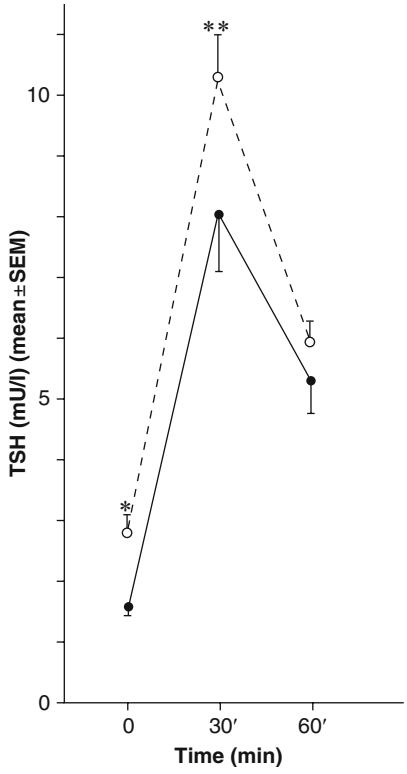

Fig. 29.8 TSH response to i.v. TRH before (*filled circle*) and after (*open circle*) 4 months of IGF-I treatment to six Laron syndrome patients $*p<0.02$; $**p<0.01$

29.5.2 Comment

The findings in our studies showed that IGF-I causes a transitory slight suppression of TSH, probably by its stimulatory effect on somatostatin (Gil-Ad et al. 1996).

Unfortunately, we have not determined the values of TBG (thyroxine binding globulin), which could have influenced the fluctuations in serum fT4 concentrations. It is of interest to know whether the TBGs behave like the sex hormone binding globulins, which increase upon IGF-I administration (Gafny et al. 1994); if so, this could also contribute to the variations in thyroid hormones observed.

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30 Insulin Secretion and Carbohydrate Metabolism in Patients with Laron Syndrome: From Hypoglycemia to Diabetes Mellitus

Zvi Laron

Core Message

› Untreated Laron syndrome patients have symptomatic and asymptomatic hypoglycemia, but with progressing age some develop relative insulin resistance, glucose intolerance, even diabetes mellitus with its complications. IGF-I treatment increases insulin sensitivity and improves the HbA1c.

30.1 Introduction

Hypoglycemia is a recognized feature of GH deficiency (Laron 1983; Wolfsdorf and Weinstein 2007). Glucose is the predominant fuel for mammalian cells. Because brain cannot synthesize glucose, nor store glycogen for more than minutes, continuous glucose supply of the brain is needed. The regulator hormones of glucose metabolism are insulin, of the same family with IGF-I and with similar receptors to those of IGF-I (Laron 2002) and glucagons. The tissues involved are the liver, muscles, and adipose tissue (Dobbins et al. 2004).

Asymptomatic and symptomatic hypoglycemia in IGF-I untreated children with Laron syndrome has been recognized as a major feature already in the first studies of these patients (Laron et al. 1966, 1968) and having serious clinical implications.

The parents of the first encountered patients (Laron et al. 1966) related that from an early age, the children wake up at night asking for sweetened drinks. Not being served or when breakfast was delayed, they sweated profusely and were subject to loss of consciousness. This story was heard again and again in the following years. We proved that these signs and symptoms were due to marked hypoglycemia reaching levels of 30–40 mg/dL.

30.2 Investigations

To understand the regulation of the low blood glucose concentrations in the early years of studying this disease, we tested the glucose responsiveness to hypoglycemia to insulin-induced hypoglycemia (insulin tolerance test (Laron 1984) (Fig. 30.1)). The dose of insulin was 0.05–0.1 U/kg i.v.

Analyzing the insulin-induced hypoglycemia responsiveness according to age groups, it is seen that in infancy and early childhood, the Laron syndrome patients present with hypoglycemia nonresponsiveness; between ages 6 and 8 years, there is a moderate responsiveness, and during early puberty, there is definite hypoglycemia responsiveness. This occurred mainly in girls whose pubertal stage was more advanced compared to the boys at that age. The normalization by compensatory mechanisms could involve the sex hormones, the glucocorticoids, and possibly glucagon (not measured concomitantly).

30.3 Fasting Blood Glucose (FBG)

Overnight FBG levels in Laron syndrome patients vary with age, but up to age 40 is lower in most patients than that of healthy same-aged controls. The variability of fasting glucose levels in three patients taken at different

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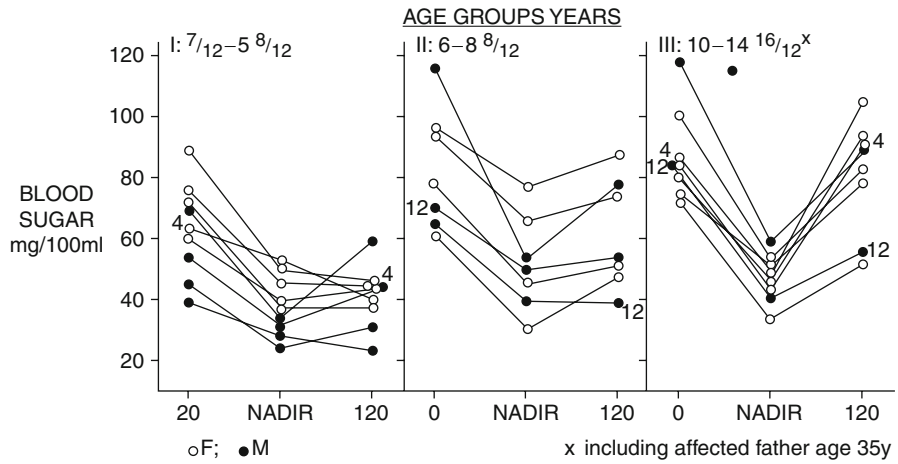


Fig. 30.1 Serum glucose response to insulin (0.05–0.1 U/kg i.v.) induced hypoglycemia in children of different age groups. Reproduced with permission from Laron (1984)

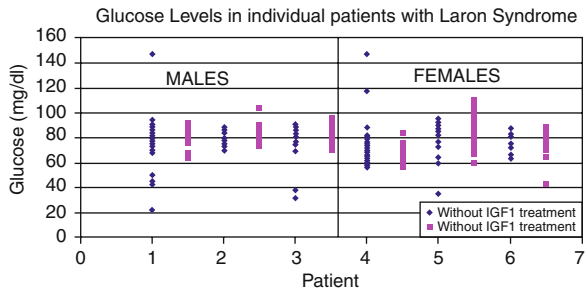


Fig. 30.2 Variations in fasting glucose in three Laron syndrome patients sampled at different dates and ages

dates and ages is shown in Fig. 30.2. The glucose concentrations in a group of 20 untreated female Laron syndrome patients followed from childhood into adult age is illustrated in Fig. 30.3. The tendency for low values is evident with the exception of five values in childhood, which were above the norm (probably not fasting), and one 52-year-old patient with elevated glucose. She subsequently developed glucose intolerance. Figure 30.4 illustrates the overnight fasting glucose levels in 11 male patients with Laron syndrome followed from infancy into adulthood. It is seen that in infancy, the glucose values are very low (25% of samples were below 30 mg/dL) and rise slowly during childhood (82.7 ± 11 mg/dL, $p < 0.05$). Around age 40 and later, hyperglycemic values are registered. These data include two male Laron syndrome patients who developed diabetes mellitus (see later). Comparing Figs. 30.3 and 30.4, it is evident that male patients with Laron syndrome have a greater tendency for hypoglycemia in early age and hyperglycemia in later age than the female patients.

30.4 Fasting Blood Glucose Concentrations During IGF-I Treatment

IGF-I treatment has been used almost exclusively in Laron syndrome children with the exception of a limited clinical trial in five young adults (see Chap. 42). In

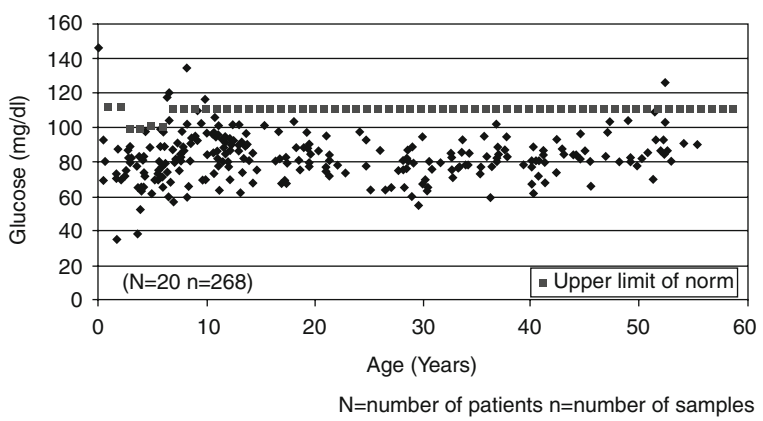


Fig. 30.3 Repeated glucose levels in untreated female patients with Laron syndrome

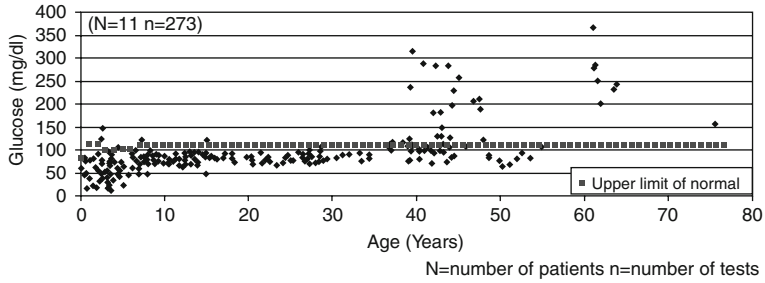


Fig. 30.4 Repeated glucose levels in untreated male patients with Laron syndrome

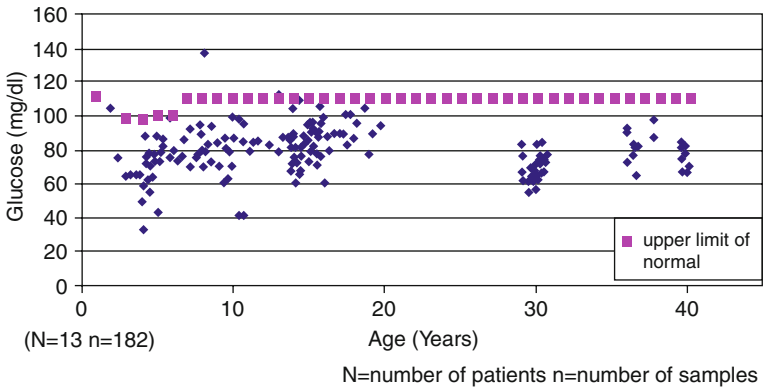


Fig. 30.5 Blood glucose in IGF-I treated female patients with Laron syndrome

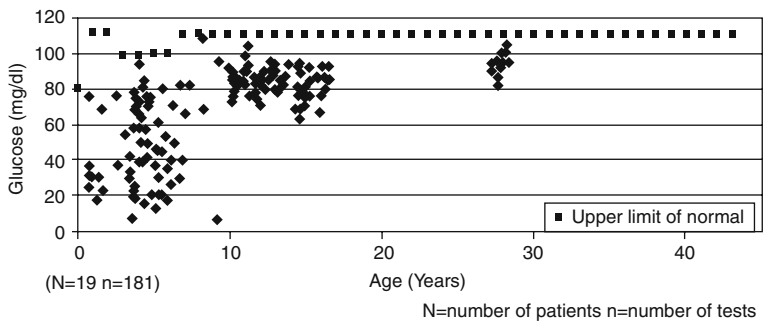


Fig. 30.6 Blood glucose in IGF-I treated male patients with Laron syndrome

most instances, the IGF-I injections were administered with dinner. The young female patients (Fig. 30.5) had fewer hypoglycemic values compared to the young boys (Fig. 30.6) who had many hypoglycemic episodes. The hypoglycemias occurred when the young children refused to eat immediately after the IGF-I injection. Therefore, the routine was changed to administer the IGF-I after the meal.

30.5 Serum Insulin Concentrations

Insulin (Laron, 2008) was determined over the years using different kits; in recent years, we use the IMMULITE 2000 analyzer (DPC, Los Angeles, USA). The fasting serum insulin concentrations in 21 untreated female Laron syndrome patients are illustrated in Fig. 30.7 and those of male Laron syndrome patients in Fig. 30.8. Three major findings are evident: (a) the majority of insulin values are high compared with the tendency for low glucose concentrations; (b) the female Laron syndrome patients have a higher incidence of high serum insulin values than the male patients. This may be explained by the greater degree of obesity in the females (Chap. 12), and (c) with advancing age, there is a decrease in the serum insulin levels. Viewing the fasting insulin levels in the same patient on different dates and ages (Fig. 30.9), it is seen that there is a great variability from normal to high in the same patient from date to date.

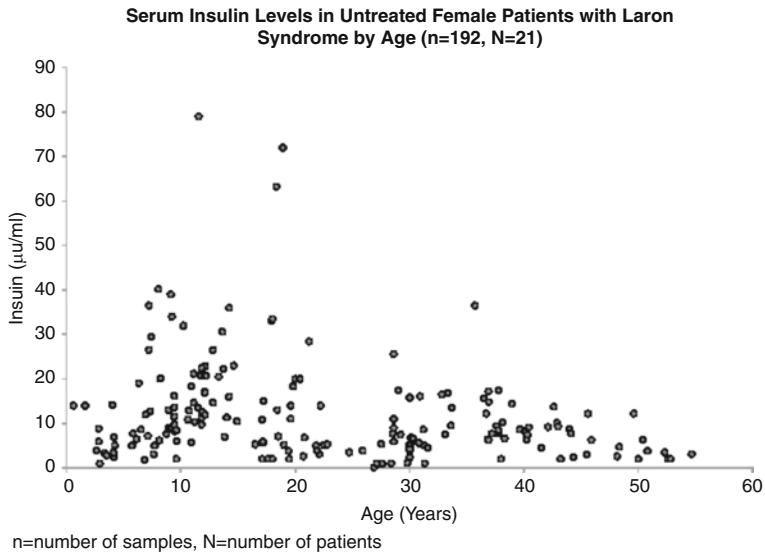


Fig. 30.7 Serum insulin levels in untreated female patients with Laron syndrome

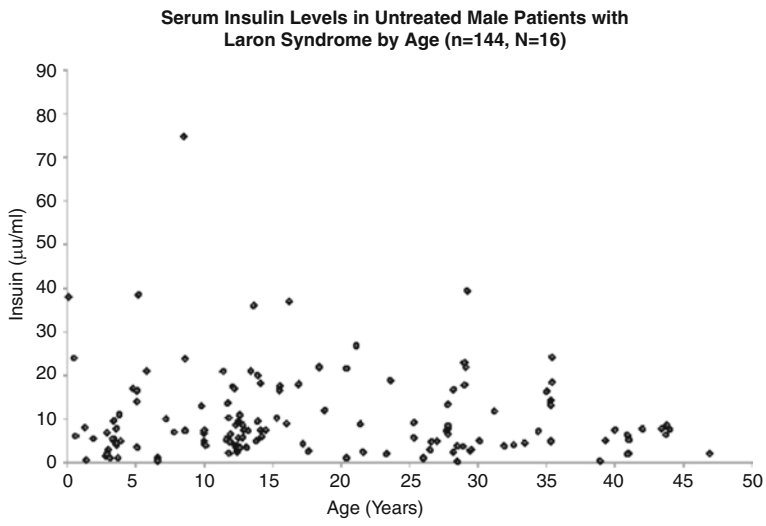


Fig. 30.8 Serum insulin levels in untreated male patients with Laron syndrome

30.6 Insulin–Glucose Relationship

To get a better understanding of the insulin and glucose metabolism in Laron syndrome, we compared fasting concentrations in both 30 Laron syndrome patients and controls grouped by age (Table 30.1). The analysis was made on the same blood samples. Figure 30.10 presents for comparison of the mean (± SD) insulin concentration by age group for the same patients as in Table 30.1. The data show the excessive insulin levels compared to healthy subjects until end of puberty. After age 23, for similar glucose levels (Table 30.1) the serum insulin levels dropped, as if the pancreatic beta cells have become tired or exhausted (Laron et al. 1995). This changing relationship is also seen by calculating the insulin/glucose ratio (Fig. 30.11), and furthermore when calculating the HOMA (Homeostasis model assessment) (fasting glucose as mM X fast insulin IU/L:22.5) (McAuley et al. 2007, Matthews et al. 1985) values for female Laron syndrome patients (Fig. 30.12) and for male patients (Fig. 30.13).Accepting a normal HOMA value of up to 2, it is evident that before age 40, many values are higher indicating insulin resistance (insulin

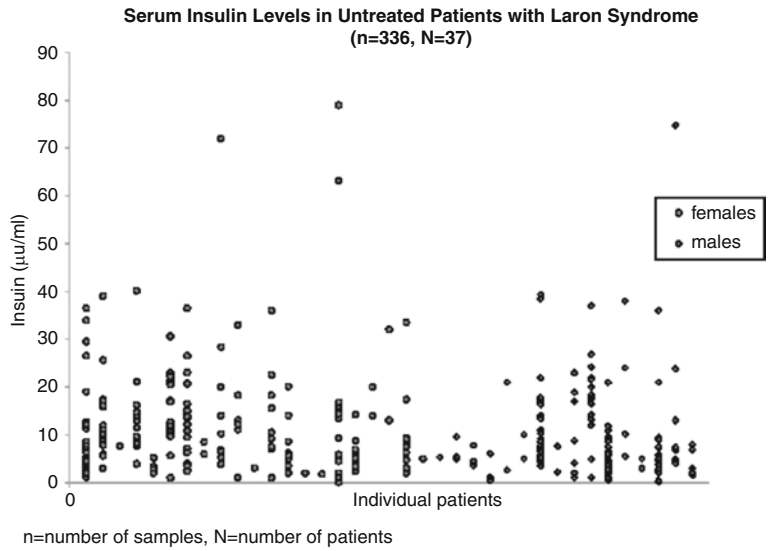


Fig. 30.9 Serum insulin in individual untreated patients with Laron syndrome sampled on different dates and ages

Table 30.1 Mean (± SD) fasting blood glucose and insulin in 30 patients with Laron syndrome by age groups

Age (year)	No. of samples	Blood glucose (mg/dL)	
		Mean ± SD	Range
0–5	51	64.5 ± 22	13–124
6–10	51	79.3 ± 14	45–109
11–22[a]	94	83.7 ± 11	48–110
23–38	43	78.4 ± 11	57–118
Age (year)	No. of samples	Serum insulin (μU/mL)	
		Mean ± SD	Range
0–5	21	10.1 ± 10.0	0.6–39
6–10	19	17.5 ± 11.4	4.0–40.2
11–22			
Total[a]	62	17.5 ± 12.0	2.0–72.1
Males[b]	25	13.8 ± 7.6	2.6–37.0
Females[c]	37	19.9 ± 13.8	2.0–72.1
23-38	33	7.6 ± 6.0	0.1–21.1

[a]Puberty in Laron syndrome patients is delayed
[b] vs. [c]: $p < 0.03$
Modified from Laron et al. (1997)

hypersecretion in response to given glucose values). This is obviously a result of the progressive obesity in Laron syndrome patients.

The female patients being more obese (Chap. 12) have also more often higher HOMA values than the male patients. The variability of HOMA values in the same patient from normal to high, mostly with advancing age, is illustrated in Fig. 30.14 for both female (left half) and male patients (right half). The abnormal glucose metabolism also became evident during the many years of follow-up when performing oral glucose tolerance tests (OGTT).

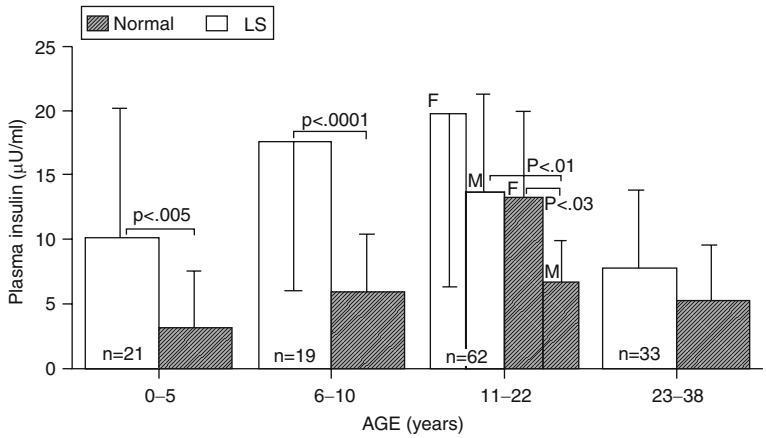


Fig. 30.10 Comparison between mean (± SD) fasting insulin levels in Laron syndrome patients (same as in Table 30.1) by age groups with healthy same-aged controls. Reproduced with permission from Laron et al. (1997)

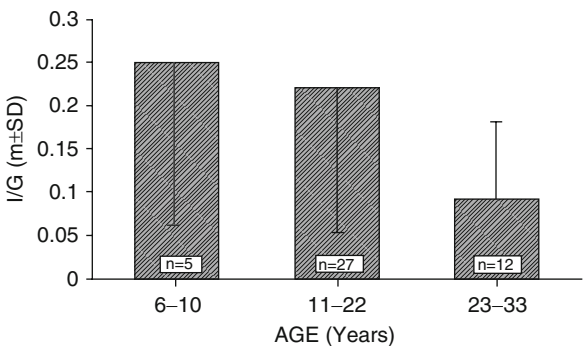


Fig. 30.11 Insulin/glucose ratio by age groups in untreated patients with Laron syndrome. Reproduced with permission from Laron et al. (1997)

30.7 Glucose Tolerance Tests in Laron Syndrome Subjects

We have performed 86 OGTTs in 27 Laron syndrome patients, 14 females and 13 males, age range 8–48 years. (Josefsberg et al. 1976, Laron and Karp, 1980).

30.7.1 Method

We advised the patients to ingest a rich carbohydrate diet 3 days before the test, unless they had been diagnosed with increased blood glucose. The oral dose of glucose in a 20% solution (1.6 g/kg = 45 g/m^2) is ingested within 5–10 min (Laron et al. 1968, 1995; Laron 1984). Glucose and insulin were always measured concomitantly half hourly between 0 and 180 min.

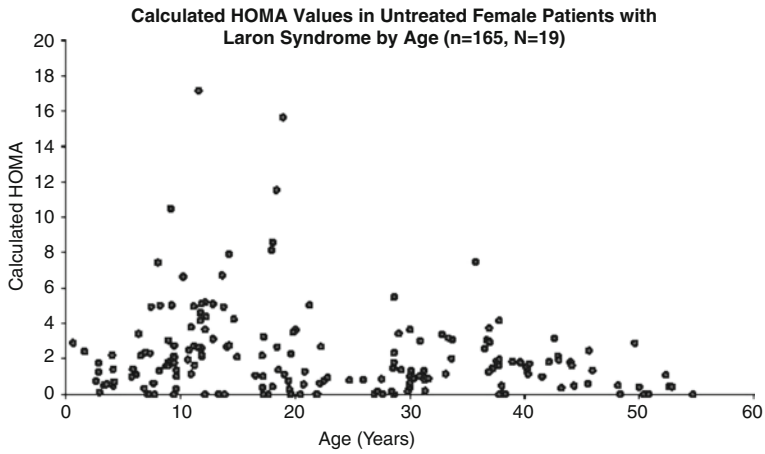
30.7.2 Results

The glucose and insulin responses varied between patients and with age in the same patients. Some patients had glucose intolerance and hyperinsulinemia already at age 13.4 (Fig. 30.15), which improved during IGF-I replacement therapy (Fig. 30.16). Figure 30.17 presents a delayed rise in glucose and insulin response during an OGTT in a 36.9-year-old female patient with Laron syndrome, possibly due to slow ingestion of the glucose. The glucose and insulin response to repeated OGTTs in two sisters with Laron syndrome is shown in Fig. 30.18. The increased insulin response in one of the sisters decreased during dietary treatment, but the insulin response was slow. She did not develop diabetes during 10 years of follow-up.

Insulin determinations during OGTT performed in two Laron syndrome children below age 5 were noninformative. The lowering of the insulin response to the oral glucose load in young adults (23–33 years) with Laron syndrome compared to the pubertal group (11–22 years) was also evident when comparing the insulin under the curve (0–180 min): 86.6±47 vs. 184±132 ($p<0.004$). Of 12 parents (heterozygotes for Laron syndrome) who underwent an OGTT, four had glucose intolerance.

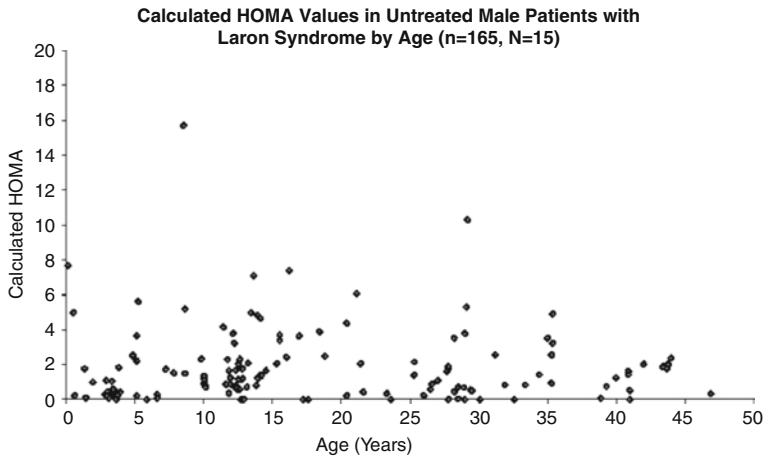
30.8 Diabetes Mellitus

One male patient aged 44 years developed delayed insulin response (Fig. 30.19) and has been advised to keep a controlled carbohydrate diet and reduce weight; he also has high cholesterol and takes Simovil



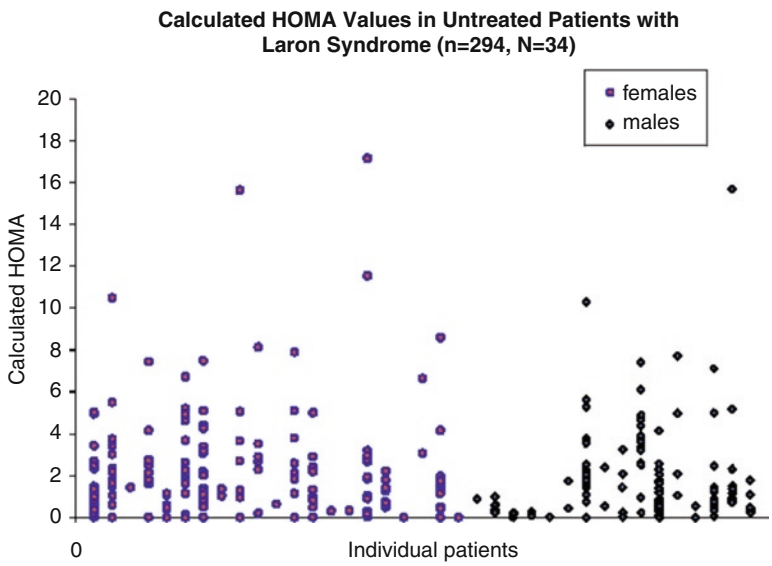
n=number of samples, N=number of patients

Fig. 30.12 Calculated HOMA values in untreated female patients with Laron syndrome



n=number of samples, N=number of patients

Fig. 30.13 Calculated HOMA values in untreated male patients with Laron syndrome



n=number of samples, N=number of patients

Fig. 30.14 Variability of calculated HOMA values in the same patients with Laron syndrome evaluated at various dates with advancing age

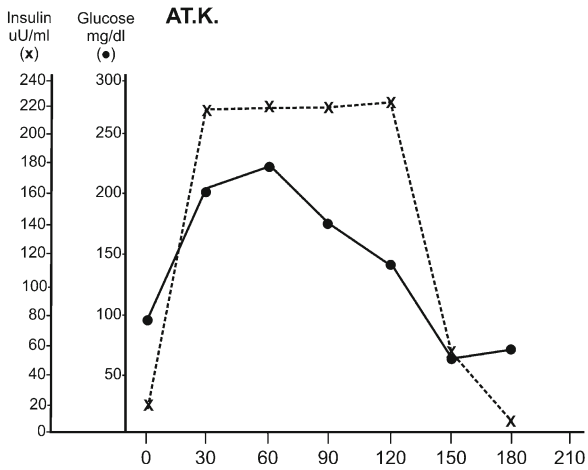


Fig. 30.15 Glucose and insulin response to an OGTT in a 13.4-year-old untreated girl (ATK) with Laron syndrome

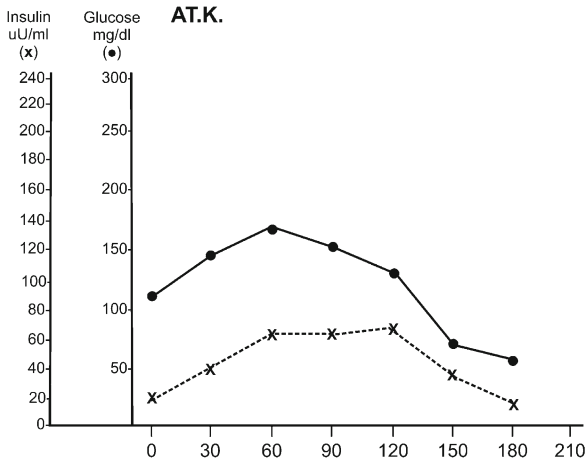


Fig. 30.16 Glucose and insulin response to an OGTT in the same girl as in Fig. 30.15 (at age 15.9 years) after 2.5 years IGF-I treatment

(Simvastatin) 10 mg/day. Four previous OGTTs at ages 8, 13, 28, and 29 were normal. In several Laron syndrome patients, both females and males, between ages 35 and 50, a decrease in insulin response during OGTT has been recorded; in some, this response is also delayed. We consider these patients as "potential diabetics" and are treated as such. Unfortunately, not all are compliant and have denied or stopped Metformin (Glucophage) treatment. Two very noncompliant patients have developed diabetes mellitus with severe cardiovascular complications, nephropathy,

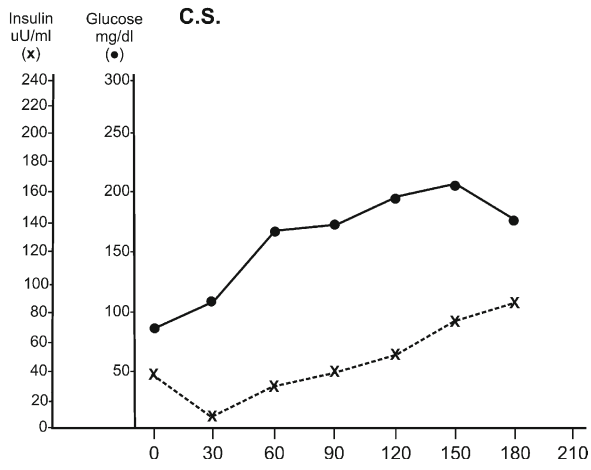


Fig. 30.17 Slow glucose and insulin response in a 35.9-year-old female Laron syndrome patient

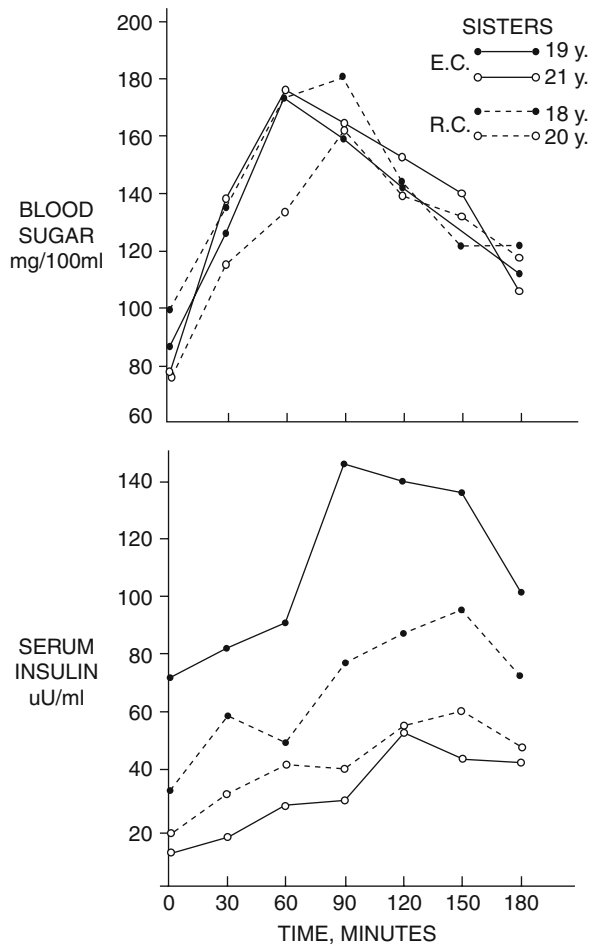


Fig. 30.18 Glucose and insulin responses during repeated OGTTs in two sisters with Laron syndrome

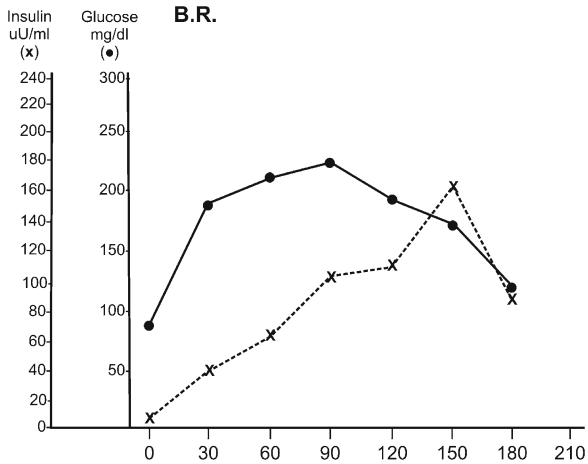


Fig. 30.19 Delayed insulin response during an OGTT of a 44-year-old Laron syndrome male

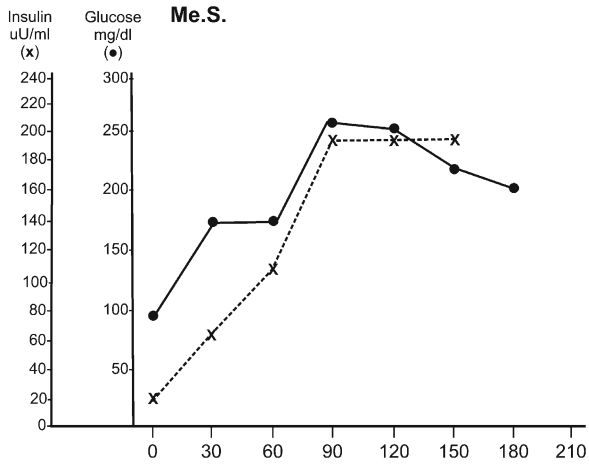


Fig. 30.20 Glucose and insulin response to an OGTT of patient MeS with Laron syndrome at age 39. Note delayed glucose and insulin response

and retinopathy (Laron and Weinberger 2004). Their detailed histories are as follows:

Patient 1 (MeS) – a male of Jewish Iraqi origin was referred to us with two of his children, both suffering from classical Laron syndrome. His adult height was 142 cm. He was obese: weight 61 kg, skinfolds: iliac 22 mm, triceps 19 mm, subscapular 28 mm, and his serum cholesterol was 278 mg/dL. DNA analysis revealed a homozygous mutation G to A at position 83 (W-15X in the signal peptide) and a homozygous mutation G to A at position 686 (R211H in exon 7). At age 39, diabetes mellitus was diagnosed (fasting glucose: 241 mg/dL (13.3 mmol/L) (Figs. 30.20 and 30.21)); oral therapy with Phenformin (DBI) 50 mg was instituted, but his metabolic control was not consistent, and at age 50, the first signs of nonproliferative diabetic retinopathy (NPDR) were detected. He refused insulin therapy; and after one year of oral antidiabetic treatment (Metformin or Glibenclamide, or Rosiglitazone), fundi examinations revealed exudates, microaneurysms, hemorrhages, and clinically significant macular edema, which was treated by grid laser macular photocoagulation. His metabolic control was bad (HbA1C:10.5–12.5%). At that stage, he accepted insulin treatment (neutral protamine Hagedorn insulin (NPH) bid and regular insulin (RI) tid). He subsequently developed neuropathy and partial arterial occlusion of the lower limbs as well as nephropathy. His blood pressure was usually 150/80 mmHg. He had smoked a few cigarettes a day but later claimed to have stopped. Table 30.2 shows the results of his repeated OGTTs. He ate a lot of everything, even at night, and increased his adiposity. He developed breathing difficulty, sleep problems, and sleep apnea (Chap. 35) necessitating oxygen supply. Due to that complication, the diabetic neuropathy and arterial occlusion of the lower limbs, he was compelled to use a motorized wheel chair equipped with an oxygen balloon. At age 75, while crossing a road, he was hit by a car resulting in many injuries including the cervical and lumbar spine. He died 2 months later in the ICU of our hospital. The family did not agree to an autopsy.

Patient 2 (YG): A male of Jewish Yemenite origin belonging to a consanguineous family was diagnosed with Laron syndrome as an infant. The molecular defect of his GH receptor was a homozygous mutation C to T in position 703 (R217X in exon 7). His adult height was 116 cm, with obesity, his skinfolds being over 25 mm. Note insufficient insulin response to a glucose load (Fig. 30.22). Table 30.2 shows results of repeated OGTTs up to age 26. At age 38, diabetes mellitus was diagnosed. Dietary management was irregular as was his metabolic control, and at age 43, he had a FBG of 283 mg/dL and an HbA1c of 10.3%. He was found to have background retinopathy including venous congestion, microaneurysms, and blot hemorrhages (Fig. 30.23) followed

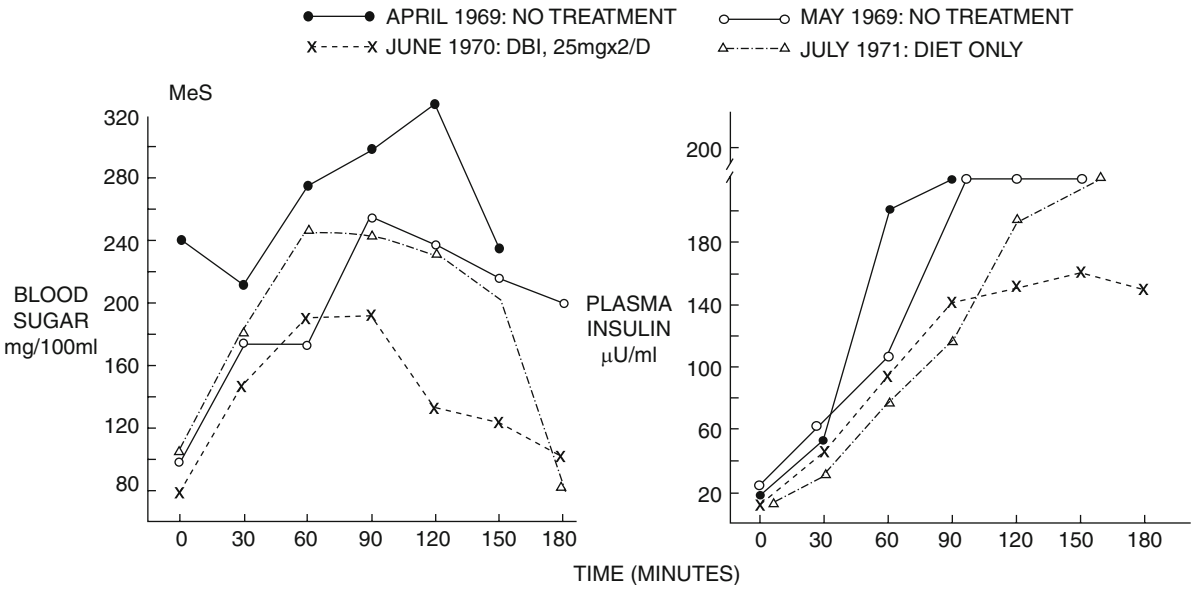


Fig. 30.21 Glucose and insulin response to OGTTs in same patient MeS at age 39.5 without treatment and 1 and 2 years on oral therapy. Whereas oral agents improved slightly the glucose levels, there was no change in the pattern of insulin response. Reproduced with permission from Laron et al. (1997)

within 2 years by clinically significant macular edema including hard exudates (Fig. 30.24). He was also diagnosed with albuminuria (25 mg/24 h) and subacute ischemic heart disease. His blood pressure varied around 140/70 mm. He smoked rarely. For a long time, he was not compliant and subsequently accepting oral and insulin treatment. He died at age 49.5, a day after a cardiac catheterization probably by a myocardial infarction. The family did not agree to an autopsy.

30.9 HbA1c

Reliable HbA1c determinations were introduced in our clinic in the years 1989–1990 and were found useful to track the state of glucose tolerance in both untreated and IGF-I treated patients. Elevated values (>6%) in untreated Laron syndrome patients were registered only in patients older than 35 years (females – 5/25 samples) and 40-year-old males. The data are illustrated in Figs. 30.25 and 30.26. The high values in the male patients belonged to those with diabetes mellitus, the high values in the female Laron syndrome to patients with glucose intolerance.

30.10 Serum Glucagon

30.10.1 Subjects and Methods

As glucagon is one of the counter-regulatory hormones in states of hypoglycemia, we tested the serum glucagon response during an arginine infusion test (L-argininge 0.5 g/kg in a 10% saline solution, pH 7.4 administered i.v. over 30 min) in six patients with Laron syndrome (1 male, 5 females) mean age 14±6.2 (SD). Glucagon was determined by the radioimmunoassay of Unger et al. (1970).

30.10.2 Results

As shown in Table 30.3, the response of glucagon in young Laron syndrome patients up to age 21 was normal. We have not performed glucagon determinations in adult patients with low or delayed insulin responses.

30.11 Discussion

Hypoglycemia and hyperinsulinemia are known sequelae of untreated GH deficiency (Laron 1983; Wolfsdorf

Table 30.2 Glucose and insulin response to repeated OGTT in two male patients with Laron syndrome

Patients	Age (years)	Glucose (mg/dL) min							Insulin (U/mL) min							HbA_1C	Therapy
		0’	30’	60’	90’	120’	150’	180’	0’	30’	60’	90’	120’	150’	180’	%	
MeS	39[3]	238	210	274	299	326	234		19.1	52.7	196	>200					
	39[5]	97	174	174	255	237	218	200	20.7	61.5	107.5	>200	>200	>200			Diet only (1,800 cal)
	40[5]	79	147	190	195	135	123	102	14.5	50	94	142	151	161	156		D.B.I.
	41[6]	100	179	244	242	230	204	82	10.5	31.5	79	119.5	197	>200	9		Diet only
	44[6]	104	141	269	287	297	263	253	29.4	71.2	>200	>200	>200	>200	>200		D.B.I.
	46[7]	112	160	206	260	250	298	262	9.7	46.6	71.8	163.3	101	87.3	117.9		Diabinese
	48	84	120	214	236	246	256	288	15.0	34.0	35.1	59.9	52.7	72.9	83.1		D.B.I.
	60															15.3	Insulin
	63[6]															10.2	Insulin
	63[9]															12.4	Insulin
	72[7]															8.9	Insulin
YG	12[2]	46	139	180	185	174	72	42	14.5	15.5	21.5	34.5	28.5	12.5	11.5		–
	15[2]	79	185	132	96	92	50	54	7.9	57.8	37.9	29.4	14	10.4	6.5		–
	26	95	213	244	230	183	95	60	<1	15.7	40.5	48.3	21.9	8.5	<1	7.6	
	39																Glucophage
	44															10.1	GliBetic
	45[2]															10.3	GliBetic
	49[4]															9.7	GliBetic

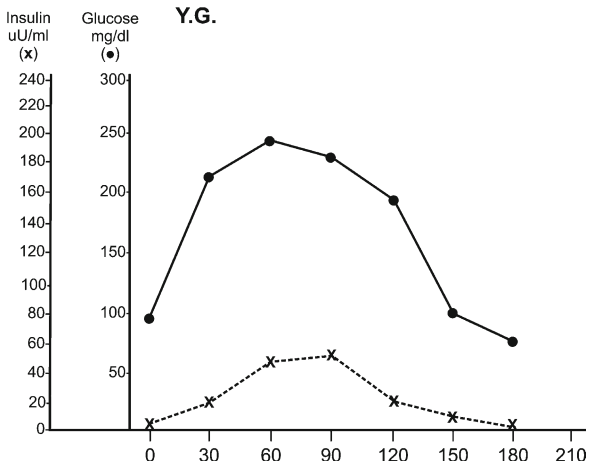


Fig. 30.22 Glucose and insulin response to an OGTT of patient YG at age 26. Note inadequate insulin response

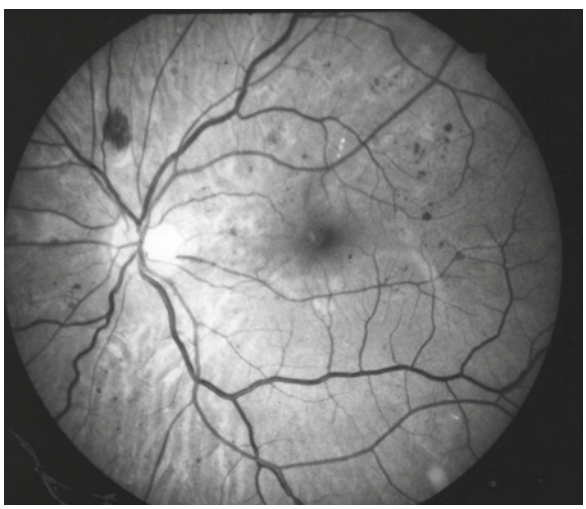


Fig. 30.23 Laron syndrome patient YG aged 45 – red free fundus photography of the left eye. Venous congestion, dot hemorrhages, and microaneurysms can be seen

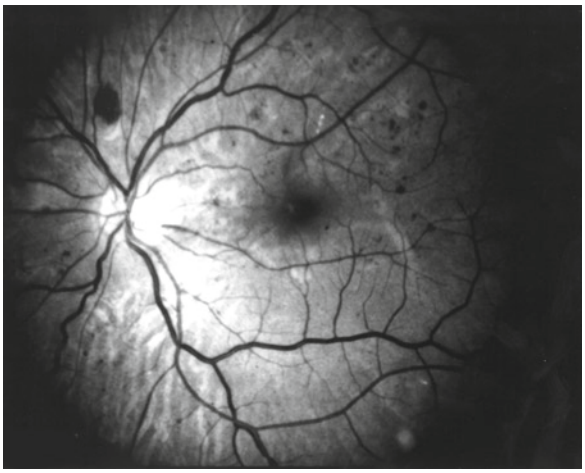


Fig. 30.24 Laron syndrome patient YG aged 45 – red free fundus photography of the left eye. A progression of the diabetic retinopathy including macular edema hard exudates can be seen

and Weinstein 2007), but diabetes and its complications have been described mainly as a consequence of hypersecretion of GH such as in acromegaly (Inokuchi et al. 1999; Krsek et al. 2002).

We found that also patients with congenital GH/IGF-I deficiency such as Laron syndrome (Laron and Weinberger 2004) not receiving IGF-I treatment or patients with GH gene 1 deletion (Laron and Weinberger 2005) not receiving hGH treatment develop diabetes mellitus and its serious vascular complications, thus another U-shaped phenomenon in biology.

30.12 Conclusions

Congenital deficiency of active GH and IGF-I such as in Laron syndrome causes symptomatic hypoglycemia in early age, followed later by asymptomatic one. With advancing age and degree of obesity, glucose intolerance develops in part of the patients. Insulin resistance already observed at an early age presents at first a hyperinsulinemic response, followed years later (mainly in the early thirties) into a slurred and late response to a glucose load that in the late thirties and early forties years of age results in insulin insufficiency and the development of glucose intolerance and type 2 diabetes mellitus.

Of importance is the fact that severe cardiovascular complications can also develop as a result of long-term GH/IGF-I deficiency similar to GH/IGF-I oversecretion. We advise that adult patients with Laron syndrome be closely followed not only for their lipidemic state but also for their state of glucose tolerance and insulin sensitivity. For further information on the role of IGF-I treatment on glucose and insulin metabolism, see Chap. 44.

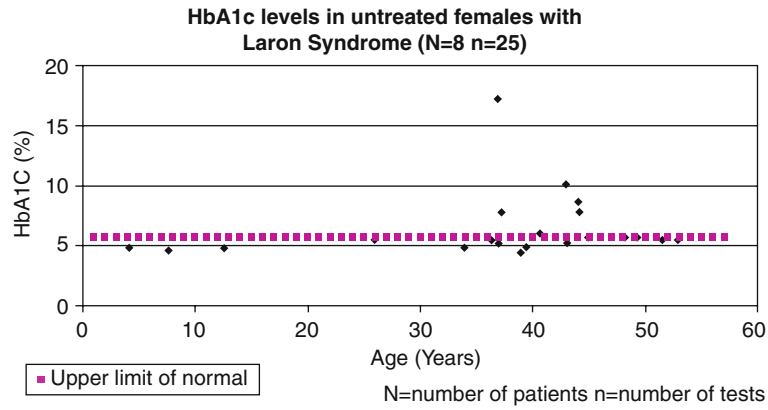


Fig. 30.25 HbA1c values in untreated Laron syndrome female patients

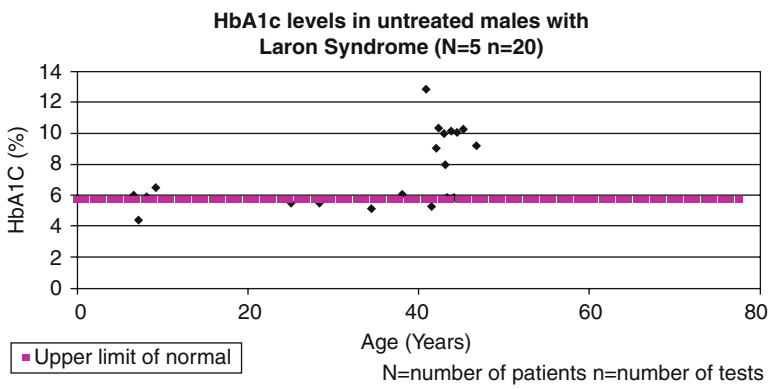


Fig. 30.26 HbA1c values in untreated Laron syndrome male patients

Table 30.3 Serum glucagon response to arginine in six young patients with Laron syndrome

Subjects	No. of patients	Mean plasma glucagon (pg/mL) ± SE at time							
		0 min	15 min	30 min	60 min	90 min	120 min	Peak conc	Δ[a]
Laron syndrome	6	121 ± 21.6	–	322 ± 60.8	199 ± 51.4	192 ± 13.5	163 ± 24.9	343 ± 50.6	222 ± 37.1
Controls	22	131 ± 12.4	303 ± 24.1	382 ± 36	201 ± 19.6	157 ± 14.7	154 ± 13.2	399 ± 34.5	268 ± 32.6

Values expressed as m ± SE

[a]Maximal increase over fasting peak concentration

Acknowledgments Part of the results are based on the Thesis of Tzvia Karmon in partial fulfillment for the MD degree, Sackler Faculty of Medicine, Tel Aviv University.

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Liver Enzymes in Patients with Laron Syndrome

31

Zvi Laron and Tsvia Karmon

Core Message

› Determination of serum liver enzyme levels revealed that with exception of the patients who had non-alcoholic fatty liver disease (NAFLD), Laron syndrome patients had variably normal levels. The Laron syndrome patients with NAFLD had slightly elevated levels. IGF-I treatment had no effect on liver enzymes.

31.1 Introduction

The liver is the central organ for metabolic homeostasis (Baumann et al. 2008), its main functions including the regulation of uptake and processing of nutrients, synthesis and biotransformation of proteins, carbohydrates and lipids, regulation of energy metabolism, and certain endocrine functions. The liver is also the organ in which the majority of IGF-I and its binding proteins are being synthesized.

The literature on liver function in diseases of the growth hormone axis is scant. Salerno et al. (2000) reported that patients with GH deficiency had normal liver and muscular enzymes before GH therapy, but during GH replacement therapy, 6 out of 78 patients (age 4–21 years) developed mild and transitory increase in serum transaminase levels, and three additional patients had a transitory increase in aspartate amino transferase (AST) and creatine phosphokinase (CPK). In acromegaly, a state of GH/IGF-I hypersecretion, enlargement of the liver (hepatomegaly), has been reported (Preisig et al. 1966), so was an increase in serum alkaline phophatase (ALP) (Hampel et al. 1990). Higham et al. (2009) reported recently that two acromegalic patients developed elevated liver transaminases (AST and ALT) during pegvisomant (a GH-R antagonist) treatment, an adverse effect that normalized on stopping the drug.

Similar findings were reported by Trainer (2003). In view of the cited literature, it was of interest to review the liver enzyme activity in patients with Laron syndrome (LS) who have both a defect in the GH receptor and a congenital IGF-I deficiency. In addition, we also learned the effects of IGF-I replacement treatment, a situation similar to GH treatment in GH deficient patients.

31.2 Subjects and Methods

All the medical charts of the 64 LS patients were reviewed. Data from 52 patients (29 males and 23 females) including follow-up data from childhood into adult age were found suitable for analysis. Liver enzyme measurements in our patients are part of routine blood chemistry tests performed after an overnight fast. Their normal values by age groups are listed in Table 31.1. The determinations were performed using a Hitachi autoanalyzer (Roche Diagnostic, Basel, Switzerland).

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Table 31.1 Upper limit of liver enzyme concentrations in serum by age (U/L)

Alkaline phosphatase (ALP)	1,107	673	644	720	448	240
Glutamate pyruvate transaminase (GPT = ALT)	33	33	29	39	24	31
γ-Glutamyl transferase (GGT)	18	18	23	17	33	32
Aspartate amino transferase (AST = GOT)	48	48	36	47	25	31
Creatine phosphokinase (CPK)	203	228	149	154	123	167
Lactate dehydrogenase (LDH)	850	850	615	580	436	480
Males						
Alkaline phosphatase (ALP)	1,107	673	644	720	936	270
Glutamate pyruvate transaminase (GPT = ALT)	33	33	29	39	27	41
γ-Glutamyl transferase (GGT)	18	18	23	17	33	32
Aspartate amino transferase (AST = GOT)	48	48	36	47	29	37
Creatine phosphokinase (CPK)	203	228	149	247	270	190
Lactate dehydrogenase (LDH)	850	850	615	764	683	480

31.3 Results

31.3.1 Liver Enzymes in Untreated and IGF-I Treated Patients with LS

In order to evaluate in a visual manner the effect of congenital IGF-I deficiency on liver enzymes and the effect of IGF-I treatment, the findings are presented in a parallel manner.

Figure 31.1 illustrates the serum GOT (AST) levels in five untreated female LS patients, and Fig. 31.2 illustrates those of 13 IGF-I treated female LS patients. It is seen that in the untreated state, five measurements in the prepubertal age and five between ages 20 and 35 were above normal. In the IGF-I treated female patients (Fig. 31.2), there were fewer abnormal values in the prepubertal period than during puberty ($n=4$) and in the early adult age ($n=6$).

The serum values of GPT (ALT) in untreated and treated female LS patients are shown in Figs. 31.3 and 31.4. It is evident that along age in untreated patients, only four measurements were above normal, and in the IGF-I treated female patients, elevated GPT values were registered almost exclusively between the ages 30 and 40.

The serum values of Glutamyl transferase (GGT) for untreated female LS patients are illustrated in Fig. 31.5, and for IGF-I treated female patients in Fig. 31.6. Only very few elevated levels were found, three of them in one IGF-I treated girl during puberty. Figures 31.7 and 31.8 show the serum Lactate dehydrogenase (LDH) values in untreated and IGF-I treated female LS patients. Only two measurements in untreated prepubertal girls were above the normal limit.

The serum levels of ALP for untreated female LS patients are presented in Fig. 31.9, and for the IGF-I treated females in Fig. 31.10. All values were in the normal range. So were the values of CPK (not shown). The same enzyme levels in untreated and IGF-I treated males with LS patients are illustrated in Figs. 31.11–31.20. The main finding is the high number of elevated γ-GGT in the untreated male patients; at an age, in the untreated female LS patients, only two slightly elevated values were registered. In the IGF-I treated LS patient, normal values were found. In the remaining liver enzymes, only occasional elevated levels were observed. This holds true also for CPK (not shown).

We have diagnosed nonalcoholic fatty liver disease (NAFLD) in several of our untreated and IGF-I treated LS patients, both in children and adults (Laron et al. 2008) (Chap. 14). Elevated serum enzymes often associated with NAFLD (Bugianesi et al. 2005) were found in five of our patients.

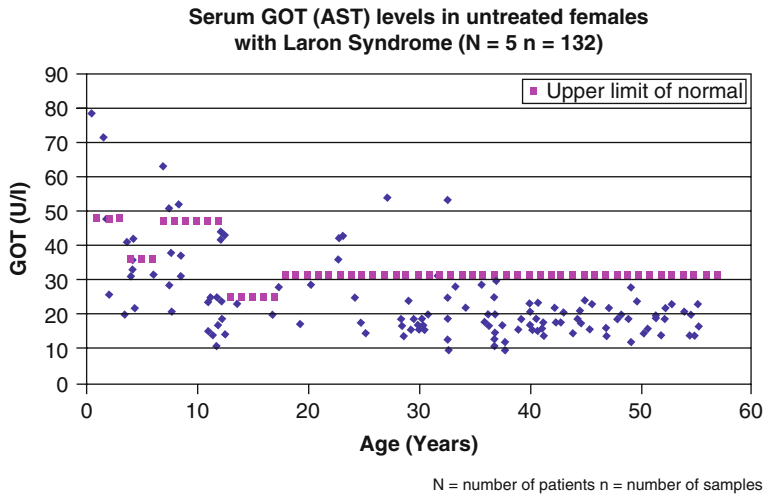

Fig. 31.1 Serum GOT (AST) levels in untreated females with Laron syndrome (LS)

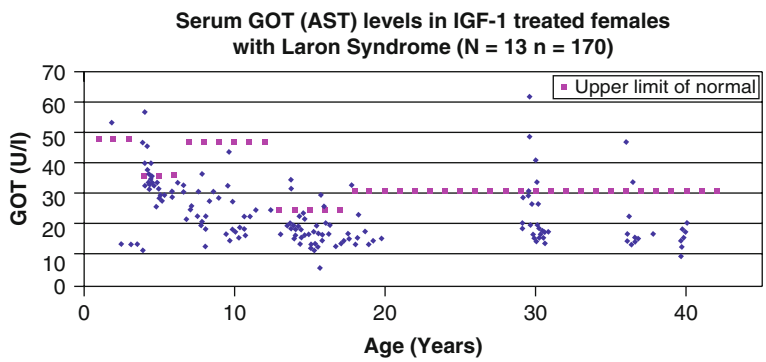

Fig. 31.2 Serum GOT (AST) levels in IGF-I treated females with LS

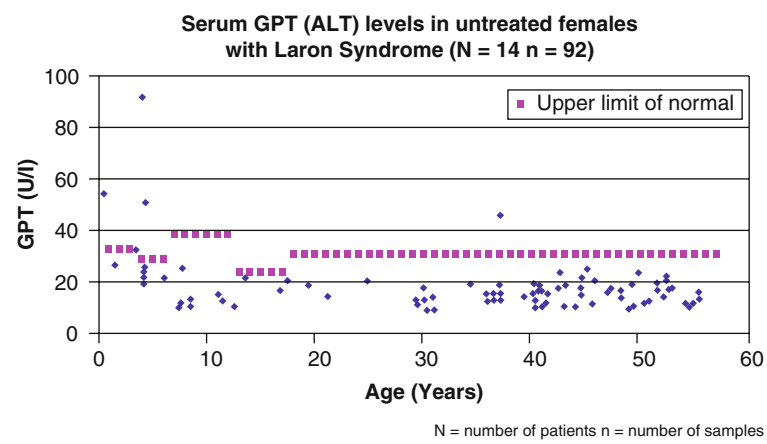

Fig. 31.3 Serum GPT (ALT) levels in untreated females with LS

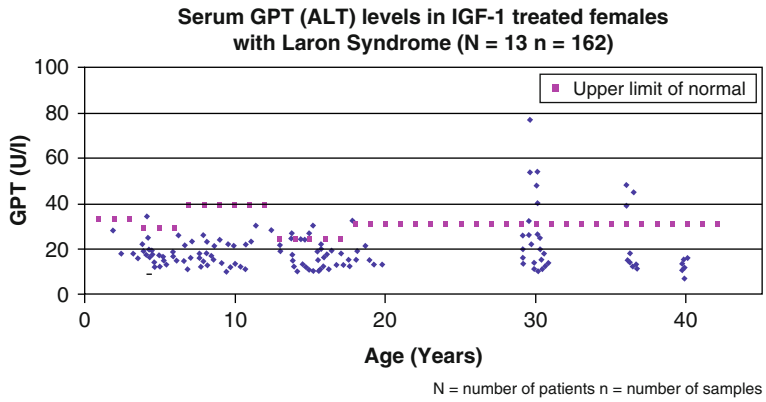

Fig. 31.4 Serum GPT (ALT) levels in IGF-I treated females with LS

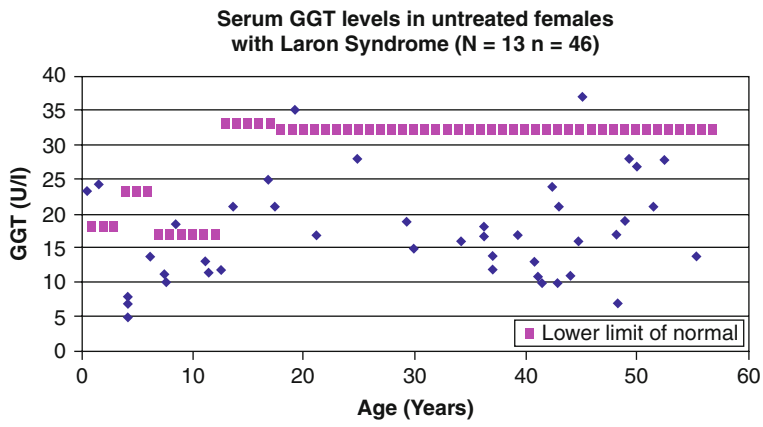

Fig. 31.5 Serum Glutamyl transferase (GGT) levels untreated females with LS

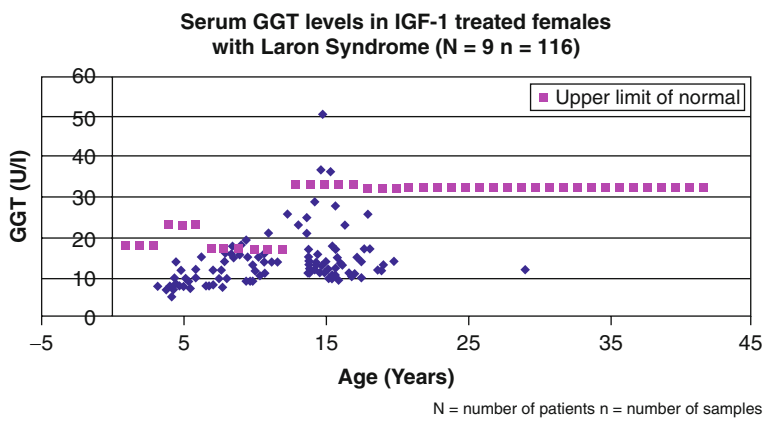

Fig. 31.6 Serum GGT levels in IGF-I treated females with LS

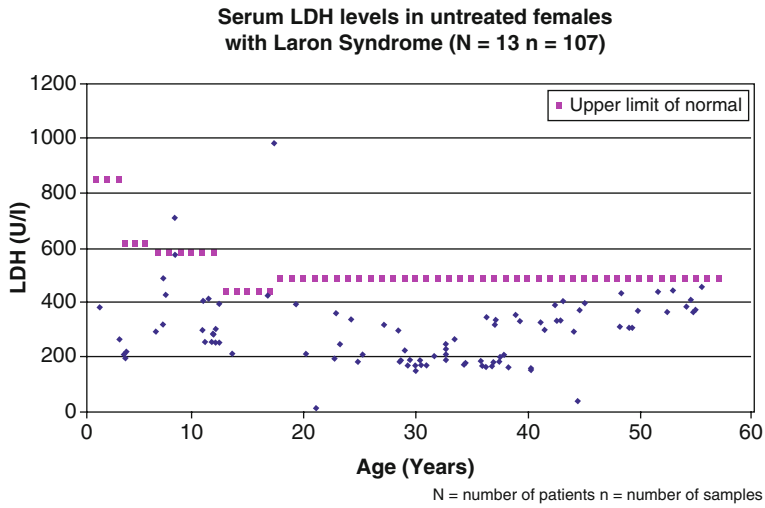

Fig. 31.7 Serum LDH levels in untreated females with LS

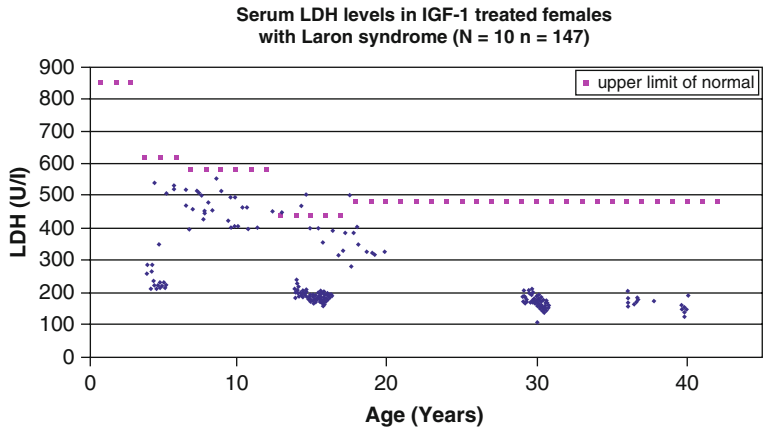

Fig. 31.8 Serum LDH levels in IGF-I treated females with LS

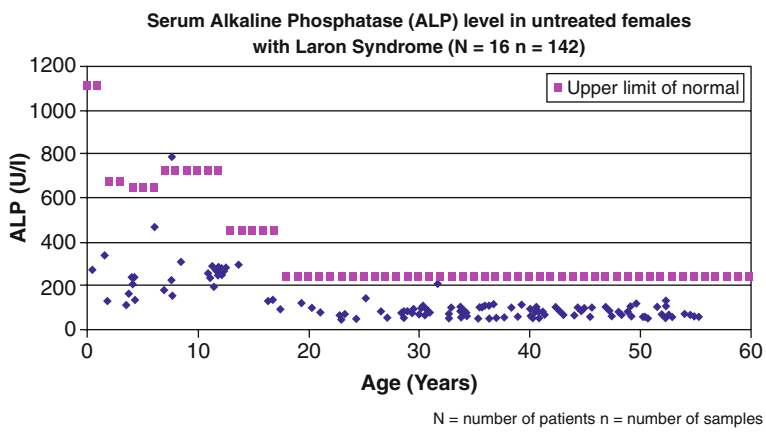

Fig. 31.9 Serum ALP levels in untreated females with LS

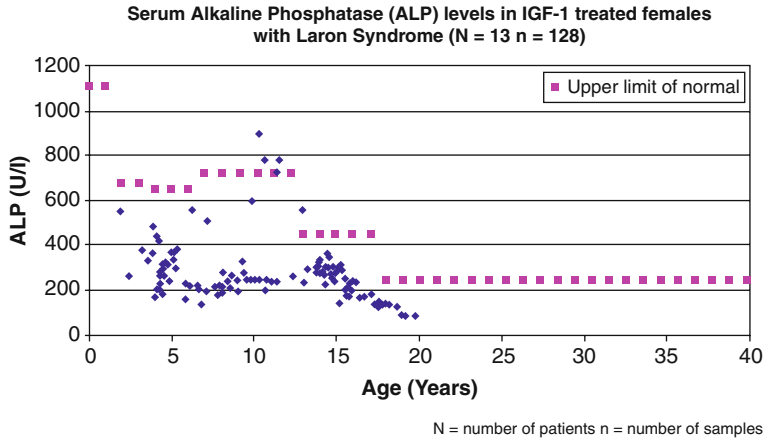

Fig. 31.10 Serum ALP levels in IGF-I treated females with LS

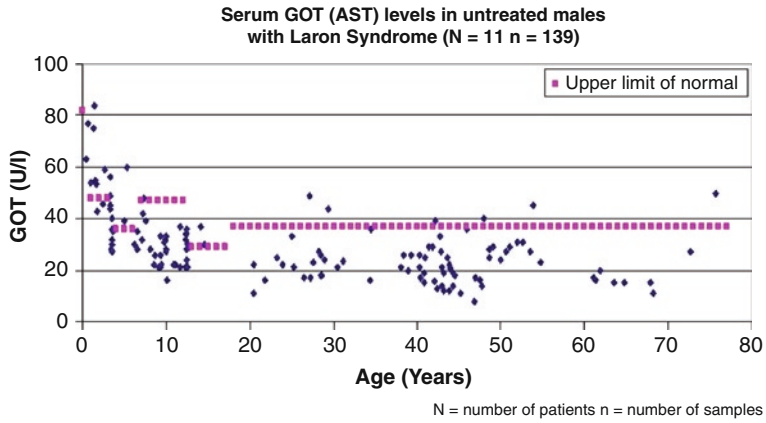

Fig. 31.11 Serum AST (GOT) levels in untreated males with LS

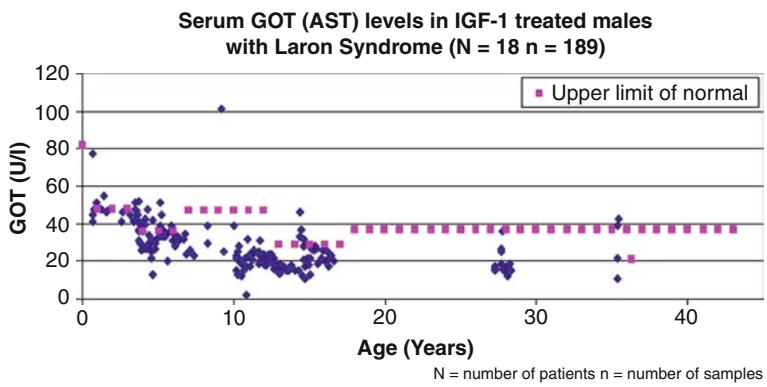

Fig. 31.12 Serum AST (GOT) levels in IGF-I treated males with LS

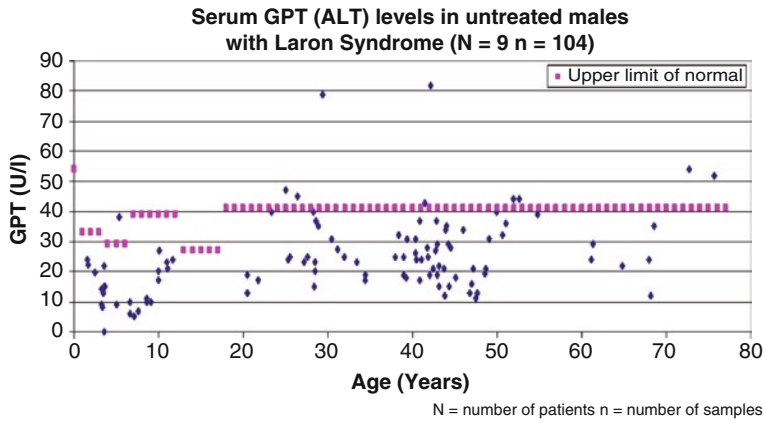

Fig. 31.13 Serum GPT (ALT) levels in untreated males with LS

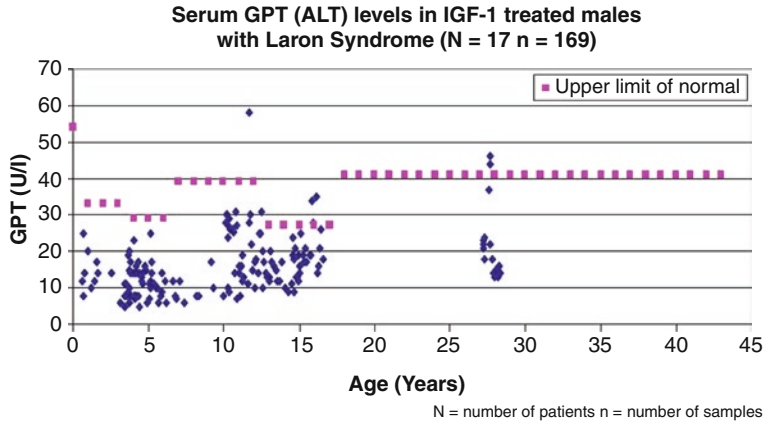

Fig. 31.14 Serum GPT (ALT) levels in IGF-I treated males with LS

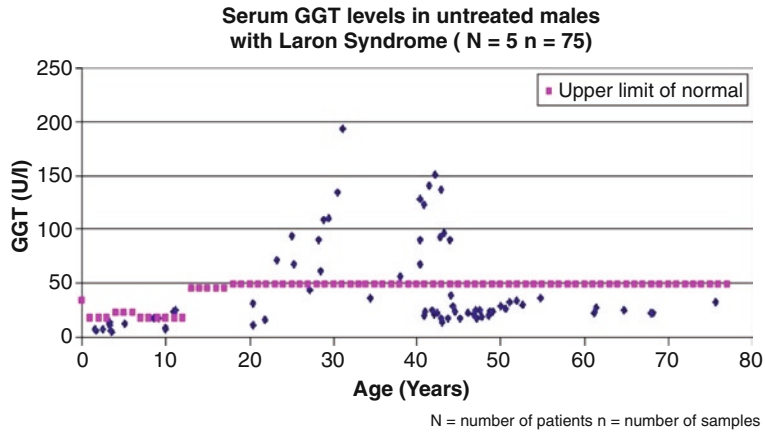

Fig. 31.15 Serum GGT levels in untreated males with LS

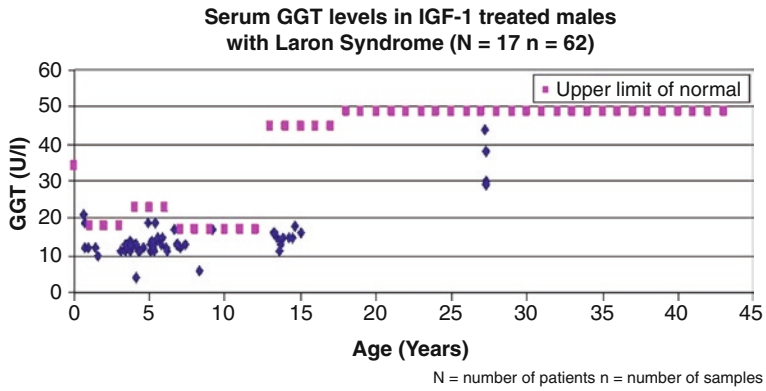

Fig. 31.16 Serum GGT levels in IGF-I treated males with LS

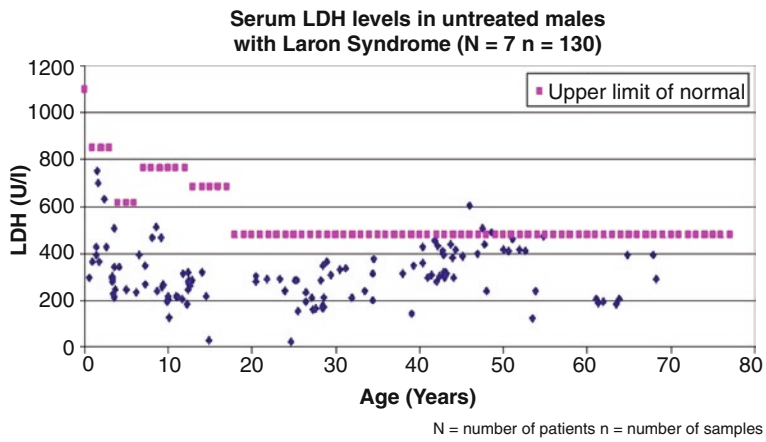

Fig. 31.17 Serum LDH levels in untreated males with LS

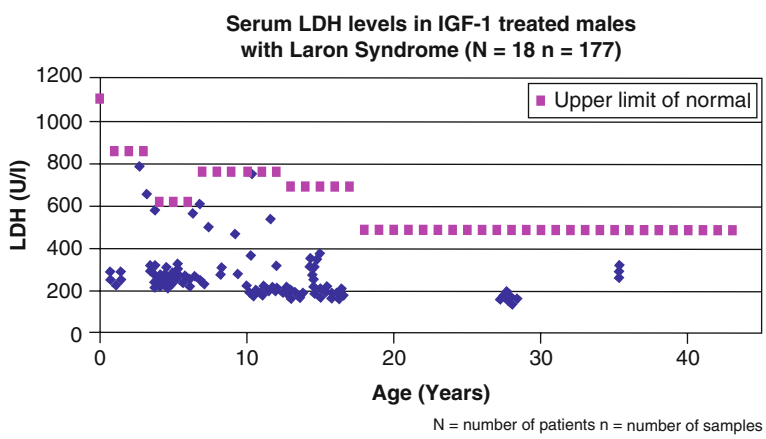

Fig. 31.18 Serum LDH levels in IGF-I treated males with LS

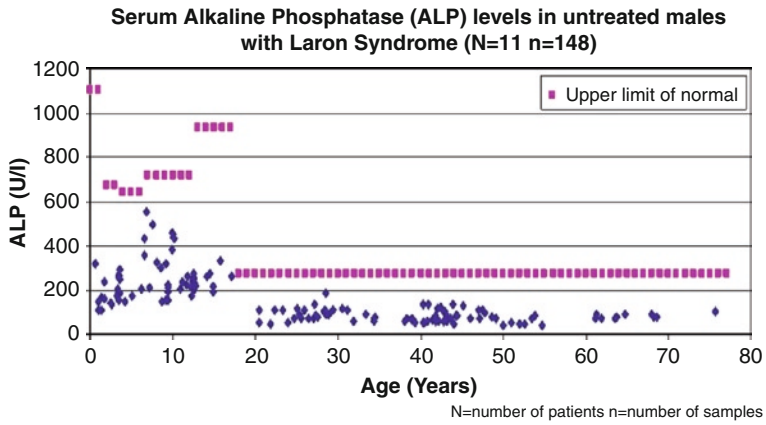

Fig. 31.19 Serum ALP levels in untreated males with LS

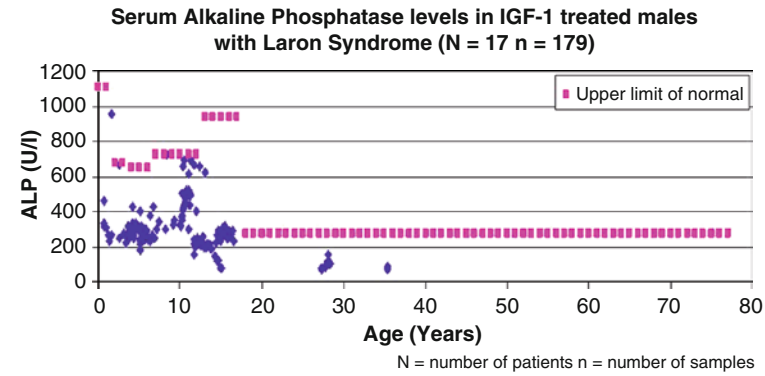

Fig. 31.20 Serum ALP levels in IGF-I treated males with LS

31.4 Conclusions

With exception of the LS patients with NAFLD, the only remarkable pathological finding was the elevated serum γ-GGT values in untreated male adult LS patients. We found no evidence that IGF-I treatment affects liver enzymes.

Acknowledgment The contribution of T. Karmon was performed as partial fulfillment of the MD Thesis at the Sackler Faculty of Medicine, Tel Aviv University.

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The Hematopoietic System in Patients with Laron Syndrome

32

Zvi Laron

Core Message

› "In vitro" and "in vivo" investigations of the effects of IGF-I deficiency and IGF-I administration on the hematopoietic system revealed a stimulatory effect of IGF-I on the red blood cell line, without a definite influence on leucocytes and lymphocytes. IGF-I had a suppressing effect on circulating monocytes, platelets and thrombopoietin.

32.1 Introduction

The availability of biosynthetic IGF-I (Niwa et al. 1986) provided by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, enabled the performance of in vitro investigations to clarify the action of this hormone on human cells including those from Laron syndrome patients. The most easily accessible cells were circulating blood cells. Forthwith are few of the studies that not only are of academic and clinical interest but also lead to advances in the knowledge of the underlying pathophysiology of Laron syndrome and IGF-I physiology (Laron 2004).

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32.2 Binding of IGF-I to Erythrocytes (Eshet et al. 1991)

32.2.1 Subjects

Heparinized blood was obtained from three groups of patients:

(a) Six untreated patients with Laron syndrome (three children and three young adults).
(b) Five untreated patients with congenital IGHD (four children and adolescents and one adult). Both these groups are characterized by IGF-I deficiency.
(c) Twenty-one healthy children and adolescents and four healthy adult volunteers who served as controls.

32.2.2 Methods

For the binding assay, iodination to a specific activity of 100–200 μCi/μg was performed using recombinant IGF-I (Chiron Co, Emerville, CA, USA). Serum IGF-I was determined using the INCSTAR kit (Stillwater, OH, USA).

32.2.3 Statistical Analysis

To evaluate the number of sites and the dissociation constant (K_d) of the IGF-I receptors on erythrocytes, we applied the nonlinear regression analysis using the BMDP3R nonlinear regression program (BMPD Statistical Software 1983, Regents of University of California) with four parameters. Comparative analysis was performed using the students' t-test.

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Table 32.1 Characteristics of IGF-I binding on erythocytes of untreated patients with Laron syndrome, congenital isolated growth hormone deficiency (IGHD), and healthy subjects

Subjects	*n*	Serum IGF-I (nmol/L) m ± SEM	Percent specific binding m ± SEM	Number of receptors[a] (sites/cell m ± SEM)
Laron syndrome and IGHD patients (Gr a + b)	11	6.01 ± 1.01	12.11 ± 1.29	7.34 ± 1.80
Healthy controls	25	26.35 ± 2.73	8.75 ± 0.62	2.84 ± 0.29
p		0.00001	0.005	0.0005

[a]High-affinity receptors only

32.2.4 Results

As the findings in Group "a" and "b," both with IGF-I deficiency, were similar, the results were combined. The findings are summarized in Table 32.1. The findings indicated that erythrocytes from untreated Laron syndrome or IGHD patients present a significantly greater number of available binding sites for IGF-I as compared to healthy subjects. These results corroborate with the findings by Morris et al. (1989) who found higher IGF-I binding to red blood cells (RBC) in normal prepubertal children than in adults, coinciding with the low IGF-I values in the prepubertal period, and the findings by Rosenfeld et al. (1979) who reported that newborns had a greater number of IGF-I receptor sites concomitantly with very low levels of plasma IGF-I as compared with those in normal adults.

32.3 Modulation of IGF-I Binding Sites on Erythrocytes of Patients with Laron Syndrome Before and During IGF-I Treatment

After characterizing IGF-I binding to RBC of Laron syndrome patients in the untreated state, it was of interest to find out what happens during IGF-I administration.

32.3.1 Subjects

Eight Laron syndrome patients (5 children (3 males, 2 females) of mean age 8.9 ± 5.0 years, and 3 adults (1 male, 2 females) of age range 26–31 years), 22 healthy children (16 males, 6 females, mean age 8.9 ± 3.0 years), and 4 healthy adults (3 males, 1 female, age range 34–55 years) were included in this study (Eshet et al. 1993a, b).

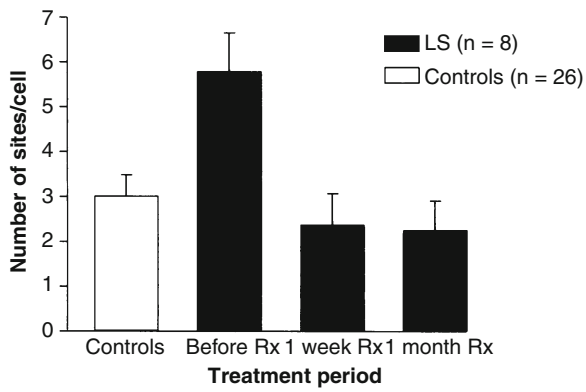

Fig. 32.1 Number of IGF-I receptor sites per red blood cell (RBC) before and after 1 week and 1 month of IGF-I daily treatment of eight patients with Laron syndrome (Laron syndrome) compared to basal findings in healthy, matched controls (21 children and 5 adults)

32.3.2 Methods

^{125} IGF-I specific binding to erythrocytes and the number of binding sites in the Laron syndrome patients were determined in the overnight fasting state before, 1 week and 1 month after daily s.c. IGF-I administration (FK 780 lot 115707K, Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) in doses of 120–150 μg/kg. In the controls, only one sample was tested (Eshet et al. 1993a). The results are summarized in Fig. 32.1 and Table 32.2.

32.3.3 Results

It is seen that during IGF-I administration, while the overnight fasting serum IGF-I increased, the number of IGF-I binding sites and percent specific binding to erythrocytes decreased. The downregulation of its own

Table 32.2 Effect of IGF-I treatment of eight patients with Laron syndrome on serum IGF-I levels, specific binding to RBC, IGF-I binding sites per red blood cell, and their affinity constant

Patients $n=8$	Serum IGF-I (ng/mL)[a]			Percent specific binding			Number of sites per cell			Dissociation constant (nM)		
	Before Rx	1 week Rx	1 month Rx	Before Rx	1 week Rx	1 month Rx	Before Rx	1 week Rx	1 month Rx	Before Rx	1 week Rx	1 month Rx
Mean	4.72	6.53	14.37	14.25	12.41	10.32	5.74	2.29	2.17	0.49	0.07	0.15
± SEM	0.84	1.58	4.56	1.39	1.64	0.26	0.86	0.64	0.53	0.27	0.02	0.05
p		0.05	0.03		N.S.	0.03		0.004	0.002		N.S.	N.S.
Controls ($n=26$)												
Mean	20.48			8.46			2.94			0.28		
SEM	2.06			0.57			0.29			0.07		

receptor by IGF-I parallels its biological activity as demonstrated by suppression of endogenous hGH and insulin, and the like (Laron et al. 1991).

We found further confirmation of this mechanism when treating constitutionally short children by human growth hormone. During the rise of serum IGF-I levels, the number of IGF-I binding sites on erythrocytes decreased together with the dissociation constant (Eshet et al. 1993c).

32.3.4 Conclusions

The above findings indicated that IGF-I receptors on erythrocytes are modulated by serum IGF-I concentrations and that this noninvasive approach may constitute an additional tool for assessing IGF-I secretion and activity in growth disorders. A relevant finding is that malignant cells have an increased number of IGF-I receptors (Yee et al. 1990; Werner et al. 1993; Werner 2009).

32.4 Binding of IGF-I to Peripheral Blood Lymphocytes (PBL)

32.4.1 Subjects and Methods

Using heparinized blood from 11 children with IGF-I deficiency (8 with Laron Syndrome and 4 IGHD) and 7 age-matched healthy controls, PBL were prepared using centrifugation over a Ficoll Hypaque gradient and RNA extracted, and the IGF-I receptor in RNA was quantified using a specific RT-PCR technique. For details, see Eshet et al. (1993b).

32.4.2 Results

Scanning densitometry showed that IGF-I receptor mRNA levels were significantly elevated in PBL from patients with Laron Syndrome and IGHD (3,108 ± 776 arbitrary units) compared to the healthy controls (567 ± 466) ($p < 0.006$).

32.4.3 Conclusion

This study showed that an increased number of IGF-I binding sites on PBL represents an increased receptor synthesis also in the lymphocytes of patients with IGF-I deficiency. It was only natural to continue these studies by learning the influence of IGF-I in vivo on the duration of IGF-I therapy, age, and sex of patients with Laron syndrome.

32.5 Hematological Findings in Untreated Laron Syndrome Patients and during Long-Term IGF-I Treatment

32.5.1 Introduction

Following a large group of untreated and IGF-I-treated patients with Laron Syndrome for many years, we had the opportunity to study the effect of congenital long-standing IGF-I deficiency as well as that of IGF-I replacement therapy on the hematopoietic system. At each visit, a complete blood count (CBC) was included in the repertoire of the examination. Forthwith, a summary of our findings in Laron Syndrome patients followed from childhood into adult age (Sivan et al. 2003).

32.5.2 Subjects

32.5.2.1 Untreated Laron syndrome patients

This group consists of 11 Laron Syndrome patients (7 males, 4 females with a mean age of 45.4 ± 9.6 years).

32.5.3 Results

32.5.3.1 Red blood cells (RBC)

The repeated hemoglobin concentrations at different ages for seven Laron syndrome patients are illustrated in Fig. 32.2 and those for 4 female Laron Syndrome patients in Fig. 32.3.

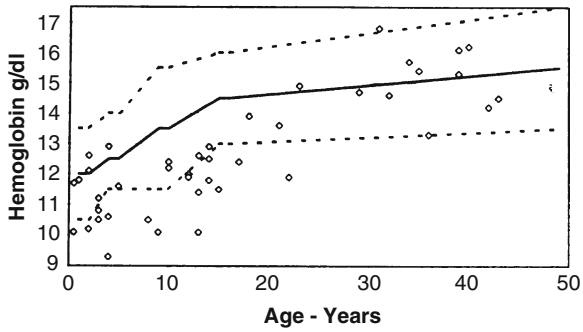

Fig. 32.2 Hemoglobin concentrations in seven untreated adult male Laron syndrome patients followed since early childhood. Normal values for age (m±2SD) are shown as continuous and *dashed line* (reproduced with permission from Sivan et al. 2003)

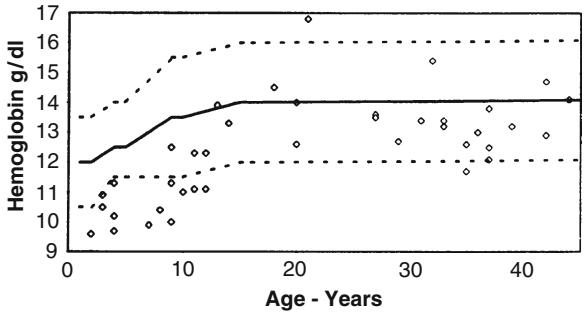

Fig. 32.3 Hemoglobin concentrations in four untreated adult female Laron syndrome patients followed since early childhood (reproduced with permission from Sivan et al. 2003)

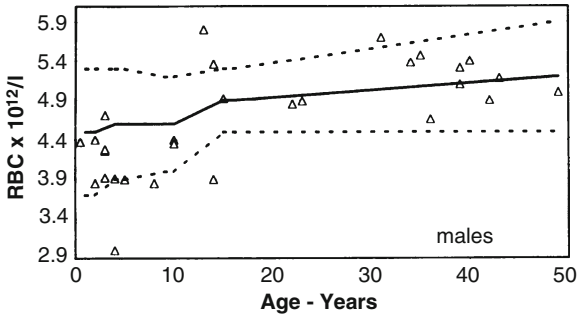

Fig. 32.4 RBC counts in seven untreated adult male Laron syndrome patients followed since early childhood (reproduced with permission from Sivan et al. 2003)

It is evident that in the young age group the majority of hemoglobin values were below the normal range. After puberty, males had normal distribution values, whereas in the female patients the hemoglobin remained below the normal mean. Similar findings are seen in the RBC count in the untreated Laron

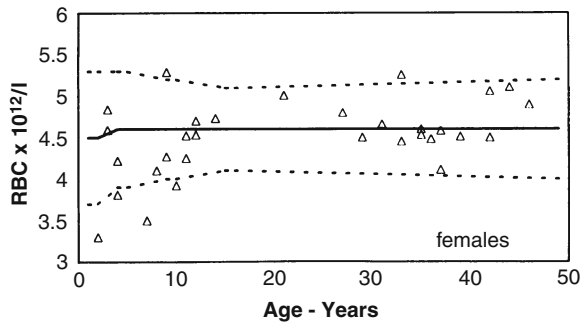

Fig. 32.5 RBC counts in four untreated adult female Laron syndrome patients followed since early childhood (reproduced with permission from Sivan et al. 2003)

syndrome males and females (Figs. 32.4 and 32.5), showing that in the young age group, most Laron Syndrome patients of both sexes had a tendency for anemia. Also, the mean corpuscular volume (MCV) of the red cells and hematocrits were low in infancy and remained in the lower range of normal after puberty. After puberty, the distribution of the RBC counts normalized. These findings could be related to the delayed puberty mainly in the male patients with Laron Syndrome (Pertzelan et al. 1993) (see Chap. 10). In infancy, the children were prescribed supplemental iron, which did not seem to have influenced the hemoglobin or RBC values. In the early years of their immigration (Laron et al. 1968), the patients belonged to a low socioeconomic class and their iron consumption was not monitored.

32.5.3.2 White Blood Cells (WBC)

Untreated State

Figure 32.6 shows the total white blood cell (WBC) count. It is seen that the WBC had a normal distribution at all ages. In contradistinction, the absolute lymphocyte count was below the normal mean at all ages in the majority of determinations (Fig. 32.7). Of note is that the monocyte count was above the normal mean (Fig. 32.8). At this stage, it was clear that long-term IGF-I deficiency affects the white blood cell lines differently from the RBC line. It is noteworthy that the Laron syndrome patients did not suffer from an excess of infectious diseases.

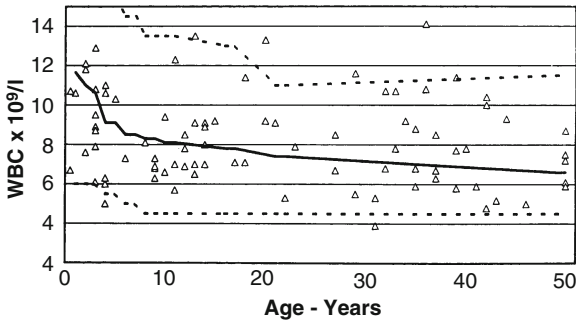

Fig. 32.6 White blood counts in 11 untreated adult Laron syndrome patients (7 males and 4 females) followed since early childhood (reproduced with permission from Sivan et al. 2003)

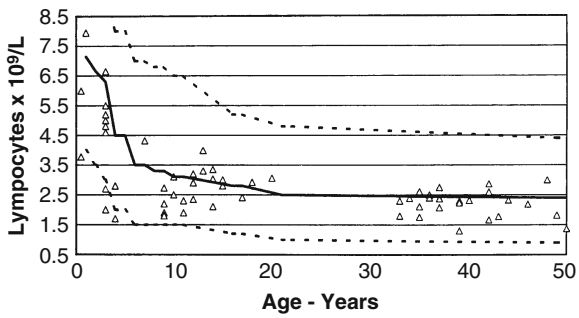

Fig. 32.7 Absolute lymphocyte count in 11 untreated adult Laron syndrome patients (7 males and 4 females) followed since early childhood (reproduced with permission from Sivan et al. 2003)

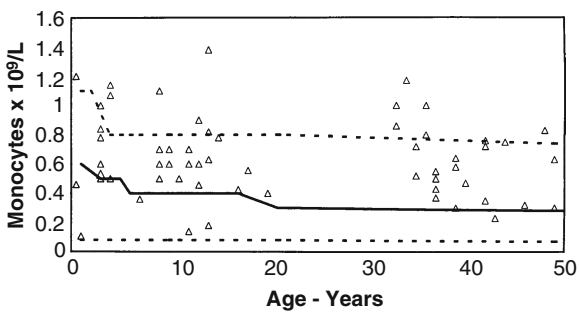

Fig. 32.8 Absolute monocyte count in 11 untreated adult Laron syndrome patients (7 males and 4 females) followed since early childhood (reproduced with permission from Sivan et al. 2003)

32.5.4 Effect of IGF-I Treatment on Blood Cells

32.5.4.1 Red Blood Cells (RBC)

The changes induced by 5 years of IGF-I treatment observed on the RBC count, hemoglobin, and MCV

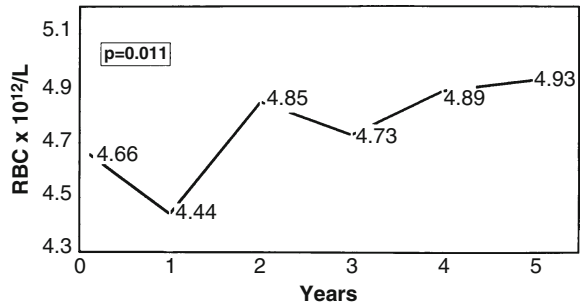

Fig. 32.9 Changes in RBC count during 5 years of IGF-I treatment of seven children with Laron syndrome (reproduced with permission from Sivan et al. 2003)

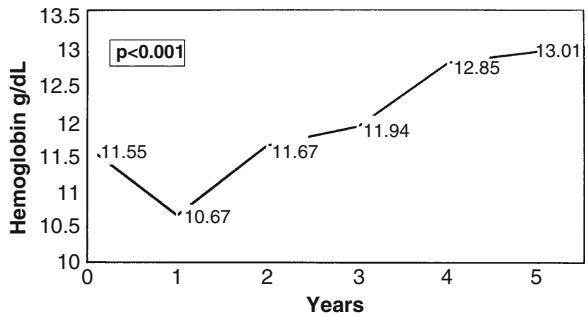

Fig. 32.10 Changes in hemoglobin concentration during 5 years of IGF-I treatment of seven children with Laron syndrome (reproduced with permission from Sivan et al. 2003)

values, as well as the hematocrit are illustrated in Figs. 32.9–32.12. The IGF-I treatment caused a temporary decrease in the mean values of hemoglobin, RBC, and hematocrit during the first year, followed by a progressive rise in all of these parameters during continuous treatment. The initial decrease is possibly due to the temporary water retention induced by IGF-I. Serum iron concentrations were in the low normal range throughout the study.

32.5.5 Discussion

The findings on erythropoiesis observed in untreated Laron syndrome patients resemble those reported in children with GH deficiency (Eugster et al. 2002) and the effects of IGF-I treatment to those during GH treatment of GH-deficient children (Vihervuori et al. 1994; Valerio et al. 1997; Bergamaschi et al. 2006). Our findings indicate that the GH effects are mediated by IGF-I.

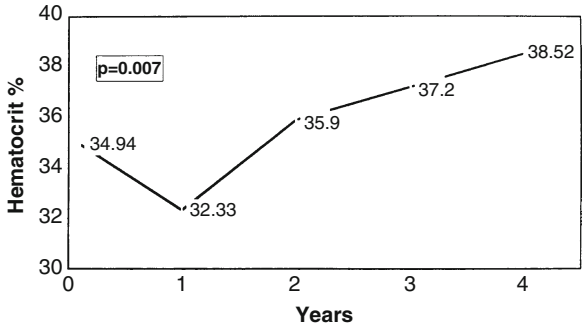

Fig. 32.11 Changes in hematocrit during 5 years of IGF-I treatment of seven children with Laron syndrome (reproduced with permission from Sivan et al. 2003)

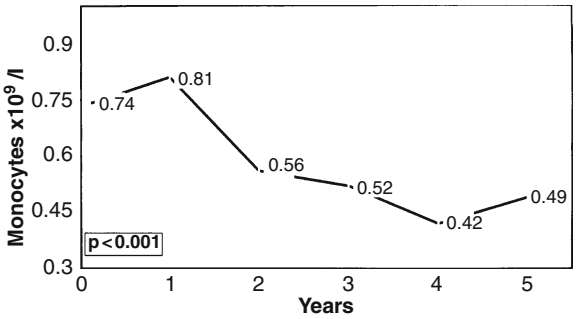

Fig. 32.13 Changes in the absolute monocyte count during 5 years of IGF-I treatment of seven children with Laron syndrome. The numbers along the curve represent the mean values (reproduced with permission from Sivan et al. 2003)

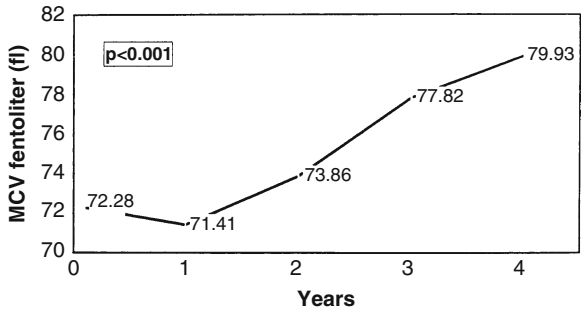

Fig. 32.12 Changes of the mean corpuscular volume of the RBC during 5 years of IGF-I treatment of seven children with Laron syndrome (reproduced with permission from Sivan et al. 2003)

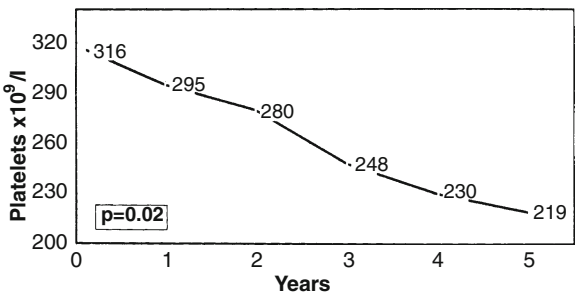

Fig. 32.14 Changes in the platelet count during 5 years of IGF-I treatment of seven children with Laron syndrome (reproduced with permission from Sivan et al. 2003)

32.5.6 White Blood Cells

IGF-I administration had no influence on the total WBC or lymphocyte counts, but caused a significant decrease to normal of the elevated monocyte count ($p<0.001$) (Fig. 32.13). The mechanism and meaning of the IGF-I effect on monocytes are so far not understood. It is of interest that we found a significantly reduced number of IGF-I binding sites on peripheral mononuclear cells (lymphocytes and monocytes) in children with acute leukemia concomitantly with a reduction in serum GHBP (Eshet et al. 2000).

32.5.7 Platelets

IGF-I treatment also caused a significant decrease in the relatively high platelet count ($p<0.02$) (Fig. 32.14).

32.5.8 Discussion

Studies in normal primates treated by IGF-I showed no rise in the peripheral WBC count, but at autopsy showed an elevation in the number of lymphocytes in the lymphatic organs (LeRoith et al. 1996). This fits the enlargement of the nasopharyngeal tissue and spleen reported by Backeljauw et al. (1996) in IGF-I-treated patients with LS (not examined in our patients). The spleen enlargement could also explain the reduction of platelets reported by us during IGF-I treatment by sequestration and destruction in the enlarged organ. It is noteworthy that Backeljauw et al. (1996) reported thrombocytopenia in one of their patients with GH gene deletion and antibodies against GH, treated with IGF-I for 25 months with a dose of 120 μg/kg b.i.d., i.e., higher than the doses used by us. None of our patients had any bleeding episodes.

32.6 Effects of IGF-I Treatment on Thrombopoietin and Erythropoietin

In order to advance the understanding of the IGF-I effect on erythropoiesis and the suppressing effect on the platelet count, we determined serum erythropoietin (Epo) and thrombopoietin (Tpo) levels in seven Laron syndrome patients (Shevah et al. 2009). Three were untreated adult patients and four were IGF-I-treated girls, in the age range of 5–15 years. Epo and Tpo were measured using ELISA kits (Quantikine, R & D Systems, Inc., Minneapolis, MN, USA). The positive results are summarized in Table 32.3. It is seen that during IGF-I treatment concomitantly with the decrease in the platelet count, there was a significant reduction in the Tpo level ($p<0.04$). No correlation between Epo levels and RBC or hemoglobin levels was registered.

32.6.1 Discussion

Whether the reduction of Tpo during IGF-I treatment is due to a direct effect of IGF-I on the liver, or whether there exists a negative feedback mechanism between the platelets and Tpo synthesis (Scheding et al. 2002), remains to be clarified. The finding that Epo levels do not correlate with the IGF-I-induced stimulation of erythropoiesis suggests that this effect is not Epo-mediated as was also shown in rats (Kling et al. 2006) and in short children (Vihervuori et al. 1996). Recently, it has been suggested that IGF-I secreted by macrophages may directly stimulate erythroblastic islands (Chasis and Mohandas 2008). This is supported by our findings that IGF-I binds strongly to RBC and thus affects their metabolism and probably action in a direct manner.

Table 32.3 Effect of IGF-I treatment on serum thrombopoietin (Tpo) levels and number of platelets

IGF-I treated Laron syndrome children			
	Before treatment	During treatment	*p* value
Tpo (pg/mL)	285 ± 189	36 ± 19	0.04
Platelets ($\times 10^9$/L)	334 ± 53	253 ± 30	0.04
Untreated Laron syndrome adults			
Tpo (pg/mL)	84 ± 60		
Platelets ($\times 10^9$/L)	240 ± 35		

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Cardiovascular Aspects in Laron Syndrome Patients

33

Mickey Scheinowitz, Micha S. Feinberg, Michael Shechter, Zvi Laron, and Rivka Kauli

Core Message

› The chapter describes the role of congenital insulin-like growth factor I (IGF-I) deficiency, such as in patients with Laron syndrome, on cardiac function, exercise capacity, endothelial function, and intimal thickening. Despite small cardiac dimensions, heart function is normal, exercise capacity is significantly reduced and endothelial function is similar to that of normal controls. Brachial artery diameter was significantly smaller than controls but with no differences in endothelial function measures. Intimal thickening in the common carotid and internal carotid arteries was slightly higher in LS compared with normal controls. The data highlight the risks for cardiovascular disease in IGF-I deficiency.

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33.1 Heart Functions in Untreated and IGF-I-Treated Patients with Laron Syndrome

Mickey Scheinowitz, Micha S. Feinberg, and Zvi Laron

33.1.1 Introduction

Despite vast research investigating the effect of growth hormone (GH) on the cardiovascular system in health and in severe GH deficiency (Climent et al. 2006; Colao et al. 2006; Colao 2008), the role of congenital insulin-like growth factor (IGF)-I deficiency, such as characterized in patients with Laron syndrome, has not been sufficiently investigated (Laron 2004; Rosenfeld et al. 1994). Several studies have indicated that low serum levels of IGF-I in non-pituitary disease patients are associated with increased risk of coronary artery disease, acute myocardial infarction, high blood pressure, impaired endothelium-dependent vasodilation, and increased all cause mortality, (Colao et al. 2008; David et al. 2005; Perticone et al. 2008; Schlueter et al. 2007). Since IGF-I plays an important role in the pathophysiology of the normal and infracted myocardium (Cittadini et al. 1998; Dean et al. 1999; Friedrich et al. 2009; Yamaguchi et al. 2008), we investigated whether the low serum IGF-I levels in children and adult Laron syndrome patients have untoward effects on the heart.

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33.1.2 Role of Insulin-Like Growth Factor in the Heart

Insulin-like growth factor (IGF)-I is known to play a major role in pre and postnatal growth and maturation of the heart. Zebrafish with IGF-I knockdown gene showed growth retardation and developmental arrest. Heart primordia failed to fuse at dorsal midline to form a heart tube (Schlueter et al. 2007). In postnatal life, IGF-I is localized and being produced by cardiac myocytes (Montessuit et al. 2006). IGF-I was recently reported to be expressed in human coronary artery smooth muscle cells (Chisalita and Arnqvist 2005), which may reflect its role on controlling vascular tone and myocardial perfusion. Overexpression of the IGF-I gene or its receptor (IGF-I receptor) was associated with cardiac myocyte hypertrophy (Laustsen et al. 2007). Other studies have shown that defect in the IGF-I signaling in postnatal life results in cardiac functional abnormalities, and IGF supplementation under such condition could reverse some of these untoward effects (Santini et al. 2007).

The following investigations were performed on patients from the Israeli cohort of Laron syndrome (LS).

33.1.3 Cardiac Dimensions and Function among Untreated Adult Laron Syndrome Patients

33.1.3.1 Subjects

Seven untreated adult Laron syndrome patients (3 males, 4 females) with a mean age of 38 ± 7 years (± SD) underwent echocardiographic assessment of left ventricular indices, as well as Doppler measurements of flows (Feinberg et al. 2000). As controls served 8 age and sex-matched controls. The findings are shown in Table 33.1. It is seen that cardiac dimensions, LV

Table 33.1 Clinical and echocardiographic and Doppler characteristics of patients with Laron syndrome

Variable	Laron syndrome, $n=8$	Control group, $n=8$	p-value
Age, years	38 ± 7	38 ± 9	0.61
Height, cm	130 ± 12	169 ± 9	<0.001
Weight, kg	45 ± 11	71 ± 15	0.006
BSA, m^2	1.23 ± 0.18	1.80 ± 0.21	<0.001
Echocardiographic variables			
VS, mm	8 ± 1	9 ± 1	<0.05
PW, mm	8 ± 1	9 ± 1	<0.05
LVDS, cm	2.4 ± 0.3	2.6 ± 0.4	0.13
LVDS, cm	3.8 ± 0.3	4.5 ± 0.4	0.002
FS, %	38 ± 5	42 ± 7	0.11
LWS, mL	18 ± 5	37 ± 9	<0.001
LWD, mL	49 ± 7	86 ± 10	<0.001
LVEF, %	63 ± 3	58 ± 7	0.12
LVM, gr	85 ± 16	141 ± 34	<0.001
Doppler variables			
LVOT D, cm	1.05 ± 0.1	1.9 ± 0.1	<0.001
LVOT TVI, cm	18 ± 2	20 ± 3	0.12
SV, mL	33 ± 3	60 ± 7	<0.001
CO, L/min	2.6 ± 0.4	4.9 ± 0.9	<0.001
MV E, cm/s	87 ± 9	77 ± 10	0.08
MV A, cm/s	62 ± 17	55 ± 11	0.29
MV E/A, ratio	1.5 ± 0.5	1.5 ± 0.4	0.61
MV E dec, ms	163 ± 31	176 ± 14	0.27

A maximal A-wave velocity; *CO* cardiac output; *E* maximal E-wave velocity; *E dec* E-wave deceleration time; *FS* fractional shortening; *LV* left ventricle; *LVDD* LV diastolic diameter; *LVM* LV mass; *LVOT D* LV outflow tract diameter; *LVOT TVI* LV outflow tract time-velocity integral; *LWD* LV end-diastolic volume; *LWS* LV end-systolic volume; *MV* mitral valve; *PW* posterior wall thickness; *SV* stroke volume; *VS* ventricular septal thickness

Table 33.2 Stress echocardiographic variables in patients with primary growth hormone insensitivity (Laron syndrome)

Variables	Laron syndrome	Control group	*p*-value
Dobutamine stress echocardiography	$n=8$	$n=7$	
Heart rate			
Base(beats/min)	78±8	71±4	0.06
Peak(beats/min)	144±9	142±11	0.71
% Max predicted	81±7	82±3	0.66
Blood pressure(mmHg)			
Base: systolic	125±11	126±15	0.82
Diastolic	68±10	75±11	0.08
Peak: systolic	144±17	142±26	0.87
Diastolic	70±9	76±13	0.20
Peak dobutamine dose infusion(μg/kg/min)	40±10	46±8	0.10
Double product	20,785±2,922	20,173±4,401	0.79
LVEF(%)			
Base	58±5	60±7	0.49
Peak	71±6	71±9	0.89
Exercise stress echocardiography	$n=4$	$n=4$	
Heart rate			
Base(beats/min)	74±8	85±10	0.21
Peak(beats/min)	156±23	162±9	0.64
% Max predicted	83±9	87±5	0.60
Blood pressure(mmHg)			
Base: systolic	95±6	133±13	0.07
Diastolic	65±13	78±17	0.19
Peak: systolic	150±10	130±8	0.18
Diastolic	70±14	73±10	0.64
Double product	17,260±3,684	21,070±1,348	0.22
Exercise duration(min)	7.7±2.5	6.9±3.3	0.51
LVEF(%)			
Base	61±5	59±4	0.56
Peak	70±3	65±5	0.09

volume, and LV wall thickness were significantly reduced in Laron syndrome patients compared with healthy controls. LV mass was 40% lower in Laron syndrome patients compared with the healthy controls even when normalized to body surface area (BSA) or to height. Left ventricular stroke volume and cardiac output were also significantly reduced ($p<0.001$ for both) compared with the healthy counterparts and were not improved after adjusting to BSA. Left ventricular (LV) ejection fraction (EF) increased significantly following dobutamine stress echocardiography and exercise stress testing from 58±5 to 71±6% and from 61±5 to 70±3%, respectively, but with no apparent differences with the control group (Table 33.2). This study showed that untreated Laron syndrome patients have reduced cardiac size (cardiomicria) and dimensions, but with preserved LV function for the given size of their body.

33.1.3.2 Exercise Tests

To test whether the reduced cardiac dimensions limit Laron syndrome patients during exercise, we performed cardiopulmonary exercise stress testing of eight untreated obese Laron syndrome patients (mean age of 36±10 years and BMI of 29±4 kg/m^2) in order to measure the individuals' maximal oxygen consumption (VO_2 max) and cardiopulmonary indices (Ben-Dov et al. 2003). Both anaerobic threshold (AT) and VO_2 max were significantly reduced (Fig. 33.1) in Laron

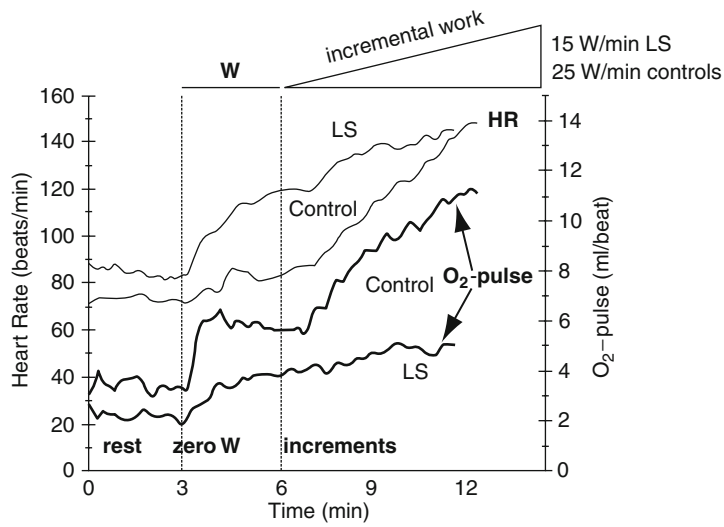

Fig. 33.1 Mean heart rate and O_2-pulse in eight adult untreated patients with Laron syndrome and in 12 control subjects, at rest, during unloaded (0 W) and during incremental cycling. Heart rate rise during unloaded pedaling is faster in the patients, while O_2-pulse rise during that phase is faster in the controls. Peak heart rate is not different. zero W = unloaded pedaling

Table 33.3 Peak exercise parameters, individual and mean data for the adult untreated patients with Laron syndrome and mean data for the control subjects

Laron syndrome patients	O_2 uptake %	Work rate %	Anaerobic threshold of predicted	Ventilation 1/min	RER	Blood pressure mmHg	Heart rate %	O_2 pulse %
1	82	78	43	54	1.14	220/110	91	91
2	33	49	26	30	1.09	150/80	87	42
3	38	45	33	22	1.04	140/90	87	46
4	77	66	36	43	1.19	180/80	83	97
5	55	–	31	15	0.92	100/70	62	71
6	59	55	35	56	1.29	140/80	85	77
7	75	73	49	33	1.05	170/80	81	94
8	39	13	27	20	0.93	–/–	79	52
Mean	*57*	*56*	*34*	*38*	*1.05*	*157/84*	*84*	*75*
±SD	*20*	*9*	*7*	*4*	*0.03*	*31/12*	*5*	*26*
Controls								
Mean	101	97	51	121	1.17	180/81	93	111
±SD	19	20	15	33	0.1	21/9	6	25

RER respiratory exchange ratio

syndrome patients compared with aged-matched healthy controls: 33 ± 9% and 51 ± 15% for AT and VO_2 max for Laron syndrome patients and 57 ± 20% and 101 ± 19% for the healthy controls, respectively. Peak heart rate was similar between Laron syndrome patients and controls (156 ± 23 vs. 162 ± 9 bpm, $p = 0.64$), reflecting similar maximal effort though with reduced oxygen delivery capacity to the working muscles. Interestingly, both resting and peak exercise blood pressure were lower in Laron syndrome patients compared with controls (see Table 33.3), though these changes were not statistically significant.

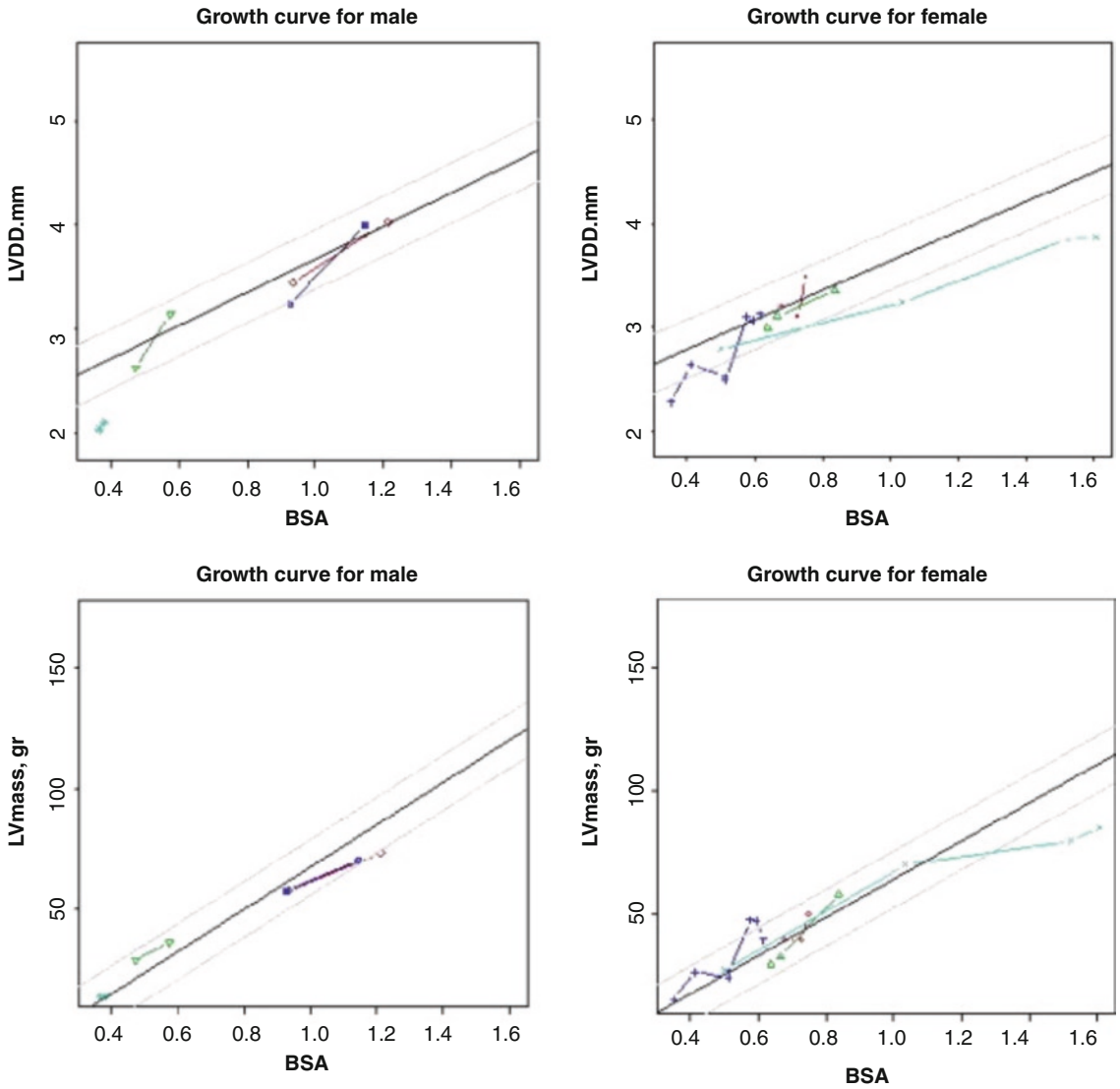

Fig. 33.2 Changes in the individual LV diastolic dimension (LVDD) and LV mass among boys and girls with Laron syndrome treated with IGF-I. Mean normal values (±SD) are illustrated for each parameter

33.1.4 Cardiac Dimensions and Function in IGF-I-Treated Laron Syndrome Children

Cardiac dimensions and function were studied by echocardiogram in young Laron syndrome children (mean ± SD age of 7.1 ± 3.6 years; range 1.6–11.6 years) treated with IGF-I (FK-780, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, 150–200 μ/kg body weight injected once daily, s.c.) for nearly 7 years (Scheinowitz et al. 2009). Left ventricular diastolic (LVDD) and systolic (LVSD) dimensions and LV mass were significantly smaller in boys (39.8 ± 21.9 gr) and girls (28.3 ± 9.9 gr) with Laron syndrome as compared with aged-matched controls (80.7 ± 49.0 gr for boys and 69.6 ± 32.2 gr for girls, $p = 0.001$) (Fig. 33.2). Fractional shortening (%) as a measure of myocardial function and intraventricular septum (IVS) thickness of children with Laron syndrome were similar to controls; 35 ± 5% vs. 38 ± 4% and 0.63 ± 0.18 mm vs. 0.66 ± 0.16 mm, respectively, p = NS. IVS thickness showed smaller values for the low BSA children, but normal above 1.4 m^2. LV mass in Laron syndrome

children was somewhat smaller especially in the low spectrum of BSA. When normalized for BSA, LVDD and LVSD of Laron syndrome patients were similar to the value of the aged-matched control children with no differences between genders. When the echocardiographic data obtained over time (during IGF-I treatment) were plotted against BSA, all measured parameters were within the normal mean ± 1 SD. This pattern was similar for both male and female Laron syndrome patients. Thus, it seems that IGF-I therapy starting at very young age ensures normal cardiac dimensions and function among growing Laron syndrome children. How IGF-I treatment acts in adult Laron syndrome patients remains to be investigated

33.1.5 Discussion

In summary, we found that adult patients with Laron syndrome have a small heart size, but with a normal LV function, both at rest and during exercise. Blood pressure of Laron syndrome patients was found to be lower at rest and during exercise, though not statistically different from healthy age-matched controls. We also found reduced functional capacity as determined by a low peak VO_2. We have previously shown that IGF-I mRNA is overexpressed in the heart of swimming exercise rats (Scheinowitz et al. 2003), being required for cardiac hypertrophy (Kim et al. 2008).

Similar effects on the heart as those found in our IGF-I-deficient and IGF-I-treated patients were reported in GH deficient and in patients receiving GH replacement treatment (Casajús et al. 2003; Hartman et al. 2008). Those effects can be assumed to be caused by the GH-induced IGF-I secretion.

In our very young Laron syndrome patients, long-term IGF-I replacement therapy was effective by maintained and restored cardiac dimensions and function within 1 SD of normal for age-matched children when corrected to BSA. Similar results were published by Backeljauw and Underwood (2001) who showed normal intracardiac anatomy and LV function following 6.5–7.5 years of IGF-I therapy in 2–12-year-old Laron syndrome patients.

In children with GH deficiency, systolic LV function was similar to healthy children, while diastolic function was significantly impaired (observed by isovolumetric relaxation time) (Szezepaniska Kostro et al. 2004).

33.1.6 Conclusions

Untreated adult patients with Laron syndrome have a small heart size, but with normal cardiac dimensions and function when normalized for BSA. Under stress (exercise), they exert reduced functional capacity with low oxygen uptake. Among young children with Laron syndrome, IGF-I therapy maintains cardiac dimensions along the normal spectrum for age. These data emphasize the role of IGF-I therapy in young age, but uphold the question whether IGF-I therapy should be continued into adulthood to balance cardiac function with the whole body physiological needs.

33.2 Endothelial Function in Adults with Laron Syndrome (Primary Growth Hormone Insensitivity)

Michael Shechter and Zvi Laron

33.2.1 Introduction

There is growing evidence that growth hormone (GH) deficiency (GHD) is associated with increased vascular risk, dyslipidemia, central adiposity, increased thrombotic tendency, and insulin resistance (Feinberg et al. 2003; Kvasnicka et al. 2000; Johansson et al. 1995).

Vascular endothelial dysfunction is also a strong predictor for cardiovascular mortality. The endothelium is an active autocrine, paracrine, and endocrine organ, which plays a central role in the regulation of vascular homeostasis by releasing paracrine factors that influence vascular tone, platelet activation, monocyte adhesion, thrombogenesis, inflammation, lipid metabolism, and vessel growth and remodeling (Cherry et al. 1983; Furchgott 1996; Celermajer et al. 1992; Corretti et al. 2003). Endothelial dysfunction is, therefore, a systemic disorder and a key variable in the pathogenesis of atherosclerosis and its complications (Bonetti et al. 2003; Shechter and Sherer 2003). In a recent review, Conti et al. (2004) showed an association between low IGF-I levels and endothelial dysfunction, endothelial and smooth muscle cell apoptosis, and atherosclerotic plaque development. In addition, GH replacement therapy in hypopituitary adults

Table 33.4 Pertinent clinical and laboratory data of the study population

Variable	Laron syndrome ($n=11$)	Control group ($n=11$)	p-value
Age (years)	43 ± 7	43 ± 7	1.0
Males	5 (45%)	5 (45%)	1.0
Weight (kg)	47 ± 11	71 ± 8	<0.01
Height (m)	1.27 ± 0.11	1.69 ± 0.07	<0.01
Total body fat males (%)	39 ± 6	26 ± 8	0.02
Females (%)	59 ± 5	29 ± 7	0.02
Total cholesterol (mg/dL)	222 ± 26	232 ± 30	0.47
LDL cholesterol (mg/dL)	142 ± 28	176 ± 12	0.03
HDL cholesterol (mg/dL)	55 ± 11	54 ± 14	0.52
Triglycerides (mg/dL)	113 ± 41	124 ± 43	0.45
Fasting blood glucose (mg/dL)	85 ± 10	88 ± 12	0.59

Values are expressed as mean ± SD; *LDL* low-density lipoprotein; *HDL* high-density lipoprotein

improved endothelial cell function (Abdu et al. 2004) and reduced vascular inflammatory markers without affecting endothelial function (McCallum et al. 2005).

Due to variations regarding endothelial function in GHD patients, we have conducted a study to explore the impact of long-term IGF-I deficiency on peripheral vascular endothelial function in adults with Laron syndrome; patients who do not generate IGF-I are obese and hyperlipidemic (Shechter et al. 2007).

33.2.2 Subjects and Methods

Following an overnight fast, we assessed percent improvement in endothelium-dependent FMD (%FMD) and endothelium-independent nitroglycerin (%NTG)-mediated vasodilation noninvasively in the brachial artery, using high resolution ultrasound (Deanfield et al. 2002), in 11 untreated adult patients with Laron syndrome without known coronary artery disease and compared them to 11 age- and sex-matched healthy controls. All subjects underwent symptom-limited exercise testing.

33.2.3 Results

Laron syndrome patients had a significantly higher body mass index (29 ± 6 vs. 25±2 kg/m^2, $p=0.04$), lower low-density lipoprotein cholesterol (LDL-C) (142 ± 28 vs. 176 ± 12 mg/dL, $p=0.03$) (Table 33.4), and a smaller mean brachial artery diameter (4.6 ± 0.7 vs. 5.7 ± 1.0 mm, $p=0.01$) compared to controls. However, brachial artery %FMD and %NTG were not significantly different between the Laron syndrome patients and controls (13.1 ± 6.2 vs. 15.4 ± 5.2%, $p=0.28$ and 22.3 ± 6.0 vs. 18.9 ± 6.2%, $p=0.30$; respectively) (Table 33.5). Cardiac performance, assessed by exercise duration time and metabolic equivalents (METs), was significantly greater in control subjects than in Laron syndrome patients (10.3 ± 2.0 vs. 6.0 ± 1.4 min, $p<0.01$ and 10.2 ± 2.0 vs. 7.2 ± 1.4 METs, $p<0.01$; respectively) (Table 33.5). Thus, FMD was found to be within normal limits in non-IGF-I-treated adult patients with Laron syndrome, despite the congenital absence of IGF-I and obesity.

33.2.4 Discussion

Endothelial dysfunction, a pathologic feature of obesity, predicts the occurrence of cardiovascular disease (Raitakari et al. 2004; Sciacqua et al. 2003; Ziccardi et al. 2002; Hamdy et al. 2003) and is felt to mediate the increased cardiovascular risk associated with a number of classic and nonclassic risk factors. Adult patients with Laron syndrome are characterized by marked obesity and elevated blood lipids. Moreover, endothelial dysfunction seen in obese individuals may be multifactorial, probably the result of comorbidities

Table 34.5 Hemodynamic and vascular parameters of the study population

Variable	Laron syndrome ($n=11$)	Control group ($n=11$)	p-value
Resting SBP (mmHg)	116 ± 26	129 ± 14	0.17
Resting DBP (mmHg)	68 ± 9	79 ± 12	0.03
Resting heart rate (bpm)	71 ± 10	67 ± 12	0.52
Peak exercise heart rate (bpm)	147 ± 28	163 ± 12	0.03
Maximal predicted heart rate (%)	89 ± 7	92 ± 5	0.43
Exercise duration (min)	6.0 ± 1.4	10.3 ± 2.0	<0.01
Metabolic equivalents	7.2 ± 1.4	10.2 ± 2.0	<0.01
Brachial artery diameter (mm)	4.63 ± 0.72	5.70 ± 1.06	0.01
%FMD	13.1 ± 6.2	15.4 ± 5.2	0.28
%NTG	22.3 ± 6.0	18.9 ± 6.2	0.30

Values are expressed as mean ± SD; *DBP* diastolic blood pressure; *SBP* systolic blood pressure; *%FMD* % change from baseline in brachial artery diameter caused by flow-mediated dilation; *%NTG* % change from baseline in brachial artery diameter caused by nitroglycerin-induced dilation. The metabolic equivalent (MET) is a unit of sitting and resting oxygen uptake (3.5 mL O_2 per kilogram body weight per minute (mL/kg/min)).

(Williams et al. 2002), such as dyslipidemia, insulin resistance, hypertension, increased inflammation, and increased oxidative stress. The unexpected finding of normal endothelial function in the Laron syndrome patients with advanced obesity is as yet unclear. It seems logical to assume that the congenital IGF-I deficiency is the major cause, but the mechanism involved remains to be clarified. One possible explanation could be related to nitric oxide levels found to be activated/released following GH administration (Boger et al. 1996; Napoli et al. 2003); however, the Laron syndrome patients are resistant to this hormone.

The Laron syndrome patients demonstrated a significantly lower cardiac performance, assessed by lower exercise duration time and METs compared to the healthy controls, although no significant exercise-induced ischemic parameters (such as chest pain or ischemic ST-T changes) were observed in either group. The lower cardiac performance in the Laron syndrome patients is obviously due to the long-standing IGF-I deficiency.

Previous studies have demonstrated that brachial artery diameter is inversely related to endothelial function (Schroeder et al. 2000). In the current study, the brachial artery diameter in the Laron syndrome patients was significantly smaller compared to the healthy volunteers, probably also due to the IGF-I deficiency. This finding may counteract the deleterious effects of obesity on endothelial function, and thus the Laron syndrome patients, despite their obesity but due to their smaller brachial artery diameter, might have an enhanced FMD and thereby endothelial function.

33.2.5 Conclusions

The long-standing IGF-I deficiency in Laron syndrome patients seems to exert a protective mechanism on the vascular endothelial function, despite the obesity of these patients.

33.3 Intimal Thickness of the Extracranial Arteries in Untreated Adult Patients with Laron Syndrome

Zvi Laron

As the long-standing IGF-I deficiency in our adult patients with Laron syndrome had negative effects on the heart (Feinberg et al. 2000; Ben-Dov et al. 2003), in addition to hyperlipidemia (Laron and Klinger 1993), excessive obesity (Laron et al. 2006), and in few patients cardiovascular complications of diabetes (Laron and Weinberger 2004), we felt the need to test for the presence of arterial elasticity as an indicator of atherosclerosis.

33.3.1 Subjects

The study group consisted of 14 untreated adult patients with Laron syndrome (9 females, 5 males) with a mean (±SD) age of 39±9 years (range 23–50 years). Ten healthy subjects (6 females, 4 males) with mean (±SD) age 32.7±6.6 years (range 24–45 years), randomly selected from the personnel of our medical center, served as controls.

33.3.2 Methods

The intima-media thickness (IMT) of the bilateral common carotid, internal carotid, and femoral arteries was measured in all patients and control subjects by Ms. Ida Zadik, Ms. Sigal Tabatchnik, and B. Bsharah, MD, in the laboratory of the Dept. for Vascular Surgery, Beilinson Hospital, using high resolution B-Mode ultrasonography (ATL 3000, 5–7 MHz). The arterial wall was studied in the longitudinal section at well-defined positions. The same two sites on each artery (common carotid, internal carotid, femoral) were measured in the two groups, and the segment-specific mean of these sites on both the right and left sides was calculated. The mean IMT at each site was compared with the identical site in the control groups. Findings were analyzed by the analysis of variance and covariance with repeated measures using BMDP software.

33.3.3 Results

The pertinent clinical data of the Laron syndrome patients and control subjects are shown in Table 33.6. Half of the patients with Laron syndrome were receiving treatment for high serum cholesterol level, and three patients had glucose intolerance or Type 2 diabetes and were being treated by drugs and/or diet. One Laron syndrome patient and one control subject had slight hypertension. Despite an apparent similar body mass index in the two groups, the patients with Laron syndrome were very obese, a finding masked by the underdevelopment of the skeleton and muscular system (Laron et al. 2006) (see Chap. 17, page 161). Table 33.7 presents the intimal thickness values of the carotid and femoral arteries of the patients and control subjects. The mean (±SD) IMT of the left common carotid and left internal carotid arteries was significantly greater in the patients with Laron syndrome than that of the control subjects ($p<0.016$ and $p<0.012$, respectively). The mean IMT of the right-sided carotid arteries in the Laron syndrome patients was also thicker than that in the healthy controls, but the difference did not reach statistical significance. The sonographic scan revealed intraluminal plaques in the left and/or right carotid artery in six patients (Fig. 33.3, Table 33.8). The IMT of the right common femoral arteries was only slightly greater in the Laron syndrome group than in the controls. The presence of associated disorder in the Laron syndrome patients with definite pathological findings are shown in Table 33.8. It is of note that none of the patients or controls had hemodynamic disturbances.

Table 33.6 Pertinent clinical characteristics of the untreated adult patients with Laron syndrome and healthy controls included in this study

	Laron syndrome patients	Control subjects	*p*-value
Number	14	10	
Sex (F/M)	9F/5M	6F/4M	
Age (years)[a]	39±9	32.7±6.6	0.776
Height (cm)[a]	128.5±12.9	165±9.9	<0.001
Weight (kg)[a]	44.6±12.2	66±17.9	0.002
BMI (kg/m^2)[a]	27.2±6.6	24.6±4	0.29
Hypertension	1/14	1/10	
Type II diabetes	3/14	None	
Smoking	4/14	1/10	
Hypercholestermia	6/14	None	

[a]Values are mean±SD

Table 33.7 Intimal media thickness (IMT) arteries in untreated adult patients with Laron syndrome and controls

Artery	Laron syndrome patients	Control subjects	*p*-value
Left common carotid (mm)	0.219 ± 0.056	0.169 ± 0.024	0.016
Left internal carotid (mm)	0.24 ± 0.066	0.179 ± 0.029	0.012
Right common carotid (mm)	0.215 ± 0.063	0.177 ± 0.024	0.084
Right internal carotid (mm)	0.215 ± 0.073	0.188 ± 0.027	0.280
Right femoral (mm)	0.215 ± 0.062	0.186 ± 0.031	0.186
Left femoral (mm)	0.217 ± 0.067	0.192 ± 0.023	0.268

All values are expressed as mean ± SD

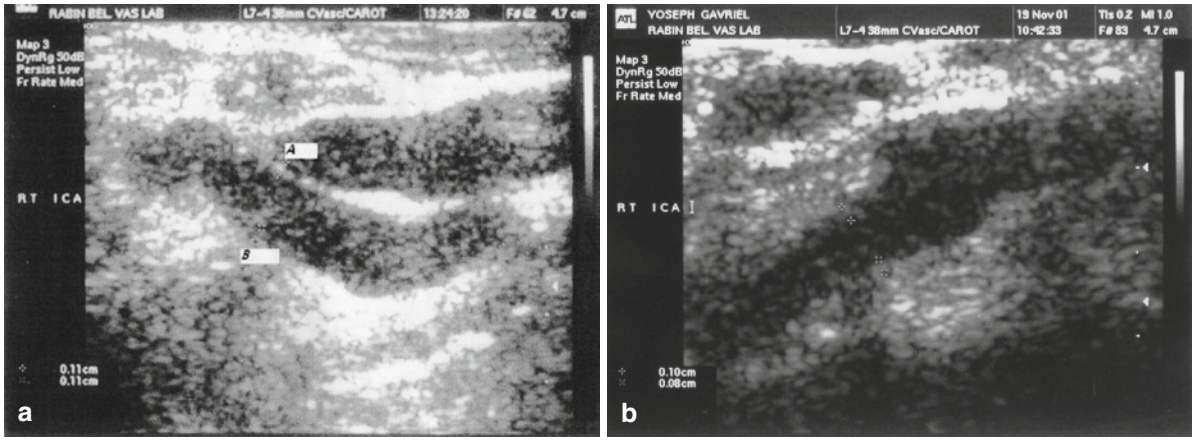

Fig. 33.3 (**a**, **b**) B-mode ultrasonography scan of right internal carotid artery in a 42-year-old male patient with Laron syndrome (YG). This patient had Type 2 diabetes, 2 years later developed cardiac vasculopathy and died at age 49.5 (see Chap. 30)

Table 33.8 Summary of pathological sonographic findings of the internal carotid arteries of untreated adult patients with Laron syndrome

Patient no.	Age (years)	Sex	T2DM	Hypercholesterolemia	Smoking	Hypertension	IMT of ICA (mm)		Plaques on ICA	
							Right	Left	Right	Left
1	50	F	+	+	–	–	0.15	0.13	+	–
2	38	M	–	+	–	–	0.15	0.16	+	–
3	42	M	+	–	+	+	0.09	0.12	+	+
4	32	M	–	–	+	–	0.1	0.11	+	+
5	45	F	+	+	–	–	0.16	0.23	+	+
6	40	F	–	–	–	–	0.18	0.14	+	+

ICA internal carotid artery; *T2DM* type 2 diabetes mellitus
+ = positive
– = negative

33.3.4 Discussion

An increased carotid IMT represents the earliest morphological change in the arterial wall in the process of atherosclerosis (Grobbee and Bots 1994) and is an accepted measure for assessing cardiovascular risk and extent of atherosclerosis and end-organ damage (Salonen and Salonen 1993). These authors also found an increased risk of 11% of acute myocardial infarction for

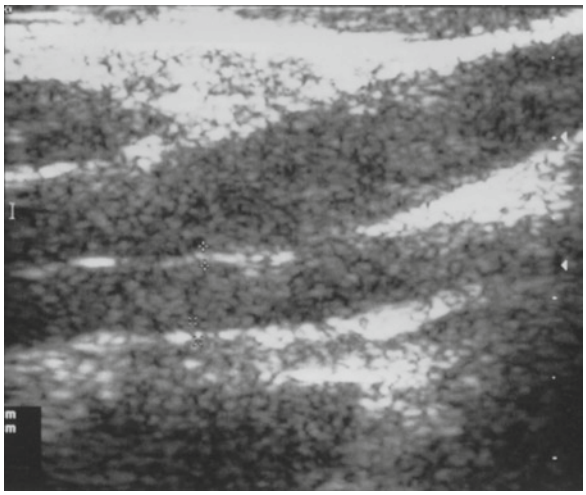

Fig. 33.4 Right internal carotid artery ultrasonography in a same-aged healthy control subject as Laron syndrome patient in Fig. 33.3

each of 0.1 mm increase in IMT, which doubled over 3 years in association with a carotid IMT above 1 mm. It is noteworthy that, indeed, the Laron syndrome patient illustrated in Fig. 33.4 had a myocardial infarct 2 years after the illustration of the carotid plaque. It should be stressed that he had Type 2 diabetes and was highly noncompliant.

The failure of the differences for controls in the right common and internal carotid arteries and the bilateral femoral arteries to reach statistical significance was probably due to the small sample size and the relative young age of the patients. Nevertheless, pathological plaques were documented in the internal carotid arteries in almost half of the Laron syndrome patients.

The early morphological and functional atherosclerotic changes in the major arteries reported in adult patients with hypopituitarism can be reversed with GH treatment (Marija et al. 1999). Regrettably, we have so far no data on the effect of IGF-I on our adult Laron syndrome patients.

33.3.5 Conclusions

Untreated Laron syndrome patients with a long-standing IGF-I deficiency have a tendency to develop an increased IMT of the internal cranial carotid and femoral arteries at a relatively early adult age.

33.4 Cardiovascular Disease

Zvi Laron and Rivka Kauli

Cardiovascular disease was encountered in six of our Laron syndrome patients, five of them males. Two have congenital heart disease, without present complaints. Three died, two due to coronary heart disease, a third with CVA following a car accident. Their detailed histories are presented in the Table 33.9.

Guevara-Aguirre et al. (2007) reported five deaths of myocardial infections in the Ecuadorian cohort of Laron syndrome patients.

Table 33.9 Pertinent data of Laron syndrome patients with cardiovascular disease

Patients no.	Name	Sex	Present age (year)	Clinical problem
1	HS	M	12	Cong. patent ductus arteriosus, operated at age 3
2	AN	M	47	Cong. patent ductus arteriosus, not operated; no hemodynamic problems
3	DaM	M	45	Endothelial dysfunction of LT brachial artery. Diagnosed during study (see Chap. 2)
4	YG	M	Deceased at age 49.5	Diabetes mellitus, (see Chap. 21) coronary heart disease diagnosed at age 41. Severe (50–80%) stenosis of coronary arteries. Died day after coronary catherization. Was a heavy smoker
5	SgA	F	Deceased at age 53.5	Coronary heart disease, diagnosed at age 47, bronchial asthma, spinal neurinoma. Myocardial infarction
6	MeS	M	Deceased at age 75 after accident	Diabetes mellitus. Hypertension. Sleep apnea. Ischemic heart disease

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34 Kidney Functions in Untreated and IGF-I Treated Patients with Laron Syndrome

Zvi Laron

Core Messages

- Renal functions were studied in children and adults with Laron syndrome in the untreated and IGF-I treated state.
- In untreated patients the glomerular filtration rate is below normal and rises during IGF-I treatment as does blood urea nitrogen and serum phosphorus. In the initial phase of treatment there is transitory water retention and hypercalciuria.

34.1 Introduction

While renal functions in GH deficient patients and the effects of GH administration have been studied soon after the purification of human GH (Henneman et al. 1960; Gershberg et al. 1967; Ogle et al. 1992a, b), only one description of the kidney size in Laron syndrome patients has been reported to the best of our knowledge (Backeljauw et al. 2001).

We investigated the kidney functions in children and adult patients before and during IGF-I treatment. Forthwith a summary of the findings in our cohort of Laron syndrome patients.

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34.2 Kidney Functions in Children with Laron Syndrome

34.2.1 Subjects and Methods

In addition to the general blood chemistry taken at most visits to the clinic, we performed kidney function tests before and during IGF-I treatment in eight children (five boys, three girls) and five adult patients (Klinger and Laron 1994).

Eight children with Laron syndrome (five males, three females) aged 3–$14^{5/12}$ years were treated with IGF-I (FK 780 Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan), 150–200 μg/kg once daily. Clinical and laboratory evaluations were performed before initiation of therapy, weekly during the first month of treatment, and monthly thereafter. At each visit, blood was drawn after an overnight fast, and in six children, 24-h urine collections were performed on the preceding day and stored at -4^0C.

The following parameters were analyzed: serum creatinine, blood urea nitrogen (BUN), electrolytes, calcium, phosphorus, and alkaline phosphatase; 24-h urine collections were performed the day before the blood analyses and volume, creatinine, nitrogen, calcium, phosphorus, and electrolyte concentrations were measured. The serum analyses were performed using a Hitachi autoanalyzer. Urinary urea nitrogen, calcium, phosphorus, and creatinine were measured using a COBBS photometer, sodium and potassium by flame photometry, and chloride by chloridometer.

The creatinine clearance was calculated (mL/min/1.73 m^2). The tubular reabsorption of phosphorus (TmP) was calculated as follows: $TmP = SPO_4 - (UPO_4 \times S.Cr.)/U.Cr.$ where SPO_4 = serum phosphate, UPO_4 = urine phosphate, S. Cr. = serum creatinine, and U. Cr. = urine creatinine.

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Table 34.1 Changes in serum electrolytes, weight, and urinary volume during the first weeks and months of IGF-I treatment of children with Laron syndrome (mean ± SEM)

	IGF-I treatment			
Period	0	1 week	1 month	5 months
Serum Na (mEq/L)	141.9 ± 1.0	143.4 ± 0.6	141.1 ± 0.7	140.0 ± 1.2
Serum K (mEq/L)	4.5 ± 0.1	4.5 ± 0.2	4.7* ± 0.1	4.7 ± 0.1
Serum Cl (mmol/L)	106.2 ± 1.0	107.6 ± 0.8	109.6* ± 1.0	105.9 ± 1.2
Weight (kg)	19.0 ± 4.1	–	19.3 ± 4.2	20.3 ± 4.4
Urinary volume (L)	0.38 ± 0.08	–	0.61 ± 0.10	0.78 ± 0.13

*$p < 0.05$ from 0

34.2.2 Results

During the first 2 weeks of IGF-I treatment, a transitory increase in mean body weight of 194 ± 15 g was recorded. Concomitantly, there was a reduction in the 24-h urinary volume of 32.0 ± 11.5% from the basal collection. Serum Na^+ increased slightly during the first week of IGF-I treatment from 141.9 ± 1.0 to 143.4 ± 0.6 mEq/L, and concomitantly, urinary Na^+ was reduced from 171.8 ± 10.0 to 123.6 ± 23.5 mEq/L. Both differences fell short of significance due to individual variations and the small number of patients, but all patients showed a uniform response. Serum K^+ increased progressively from 4.46 ± 0.1 to 4.76 ± 0.1 mEq/L ($p < 0.05$). Concomitantly, there was a significant but transitory reduction in urinary excretion of K^+ (from 73.5 ± 7.9 to 46.2 ± 4.7 mEql/L; $p < 0.05$).

There was also progressive significant increase in serum chloride ($p < 0.05$). The urinary chloride excretion was transitorily reduced in the first week of treatment, remaining thereafter in the range of the basal levels. The main findings are summarized in Table 34.1. Before and during the whole study, blood pressure measurements were normal.

The glomerular filtration rate (GFR) as measured by creatinine clearance was below normal in all untreated Laron syndrome patients, and during IGF-I treatment, while serum creatinine remained essentially unchanged, the clearance rose toward normal levels (Fig. 34.1), increasing by 73 ± 28% from the basal level ($p < 0.02$) after 2 months of therapy. Figure 34.2 illustrates the changes in BUN and urinary nitrogen excretion. BUN was initially reduced by IGF-I treatment from 5.18 ± 0.69 to 4.55 ± 0.58 mmol/L ($p < 0.01$). After 1 month of treatment BUN levels started to rise, reaching the pretreatment levels at 2 months. The urinary urea nitrogen excretion was also temporarily decreased from 3.99 ± 0.90 to 2.78 ± 0.77 g/24 h (NS), with a subsequent rise to 6.65 ± 0.95 g/24-h, which is higher than the pretreatment values. During IGF-I administration, urinary calcium excretion increased (Fig. 34.3). IGF-I also induced a rise in serum phosphorus, an increase in tubular reabsorption of phosphorus, and a rise in serum alkaline phosphatase (Fig. 34.4).

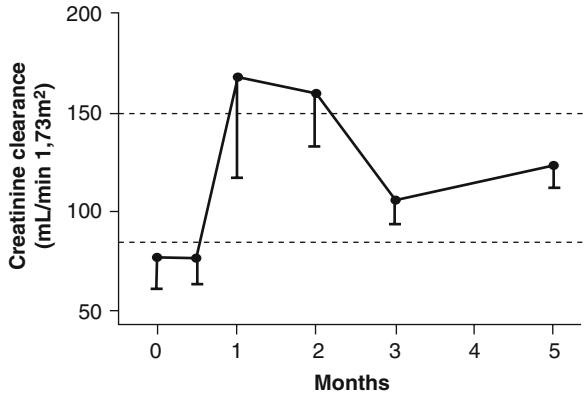

Fig. 34.1 Creatinine clearance before and during IGF-I treatment of eight children with Laron syndrome (m ± SEM) normal limits. Reproduced with permission from Klinger and Laron (1994)

34.2.3 Comment

Experimental studies have demonstrated that the kidney possesses binding sites for IGF-I, especially in the inner medulla (Matejke et al. 1992). In vitro microperfusion of the proximal convoluted tubule with IGF-I stimulated phosphate transport, an effect not observed with GH (Quigley and Baum 1991).

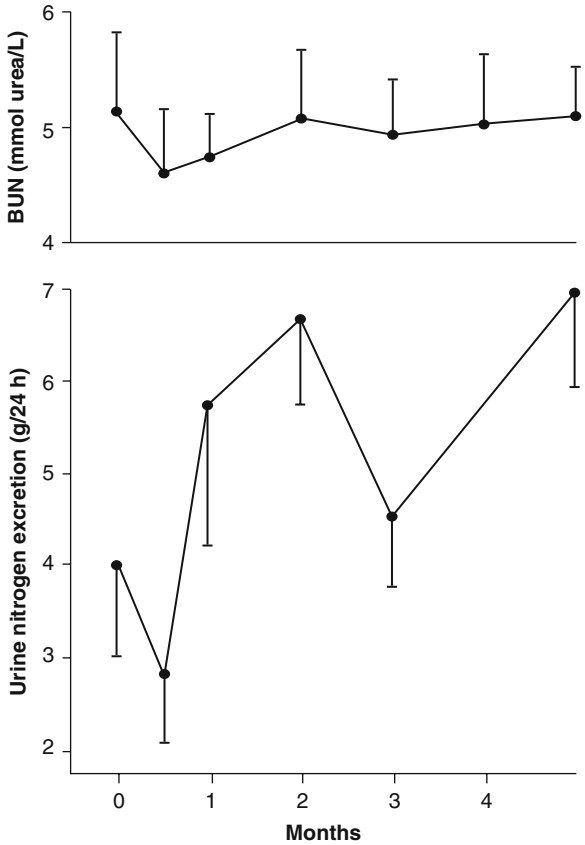

Fig. 34.2 Blood urea nitrogen (BUN) and urinary nitrogen excretion in eight children with Laron syndrome before and during IGF-I treatment (m ± SEM). Normal: 2.9–8.2 mM/L = 8–23 mg/dL. Reproduced with permission from Klinger and Laron (1994)

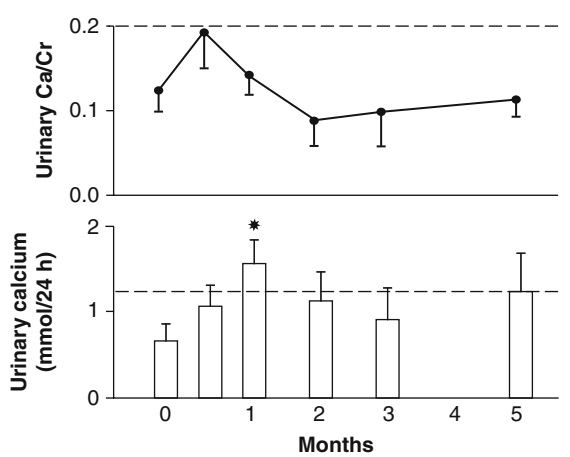

Fig. 34.3 24-hour urinary calcium excretion and urinary calcium creatinine ratio before and during IGF-I treatment of eight children with Laron syndrome. Upper limit of normal; $p < 0.03$. Modified from Klinger and Laron (1994)

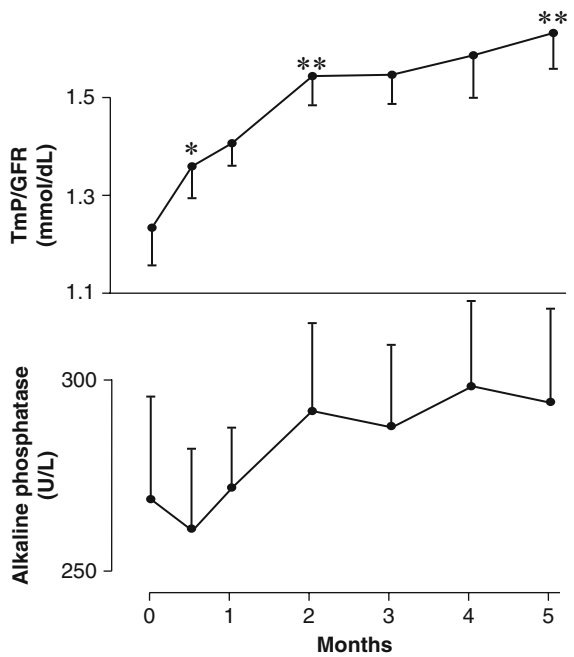

Fig. 34.4 Serum alkaline phosphatase and maximal renal tubular phosphorus reabsorption before and during IGF-I treatment of eight children with Laron syndrome ($p < 0.003$; $^{**}p < 0.005$). Modified from Klinger and Laron (1994)

34.3 Adult Patients

34.3.1 Subjects

Five adult patients with Laron syndrome (one male, four females) age range of 28–39.5 years were studied (Laron and Klinger 1994).

34.3.2 Methods

Laboratory examinations (fasting blood samples for biochemical determinations and 24-h urinary collections) were performed weekly during the first month of IGF-I treatment, and following once monthly for 9 months. IGF-I (FK 780, Lot 115707 K, Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) was administered subcutaneously in a once daily dose of 120 μg/kg.

Table 34.2 Effect of IGF-I deficiency and treatment on kidney function in 5 adult patients with Laron syndrome

	IGF-I treatment			
Months	0	3	6	9
Serum creatinine (mg/dL)	0.8±0.05	0.78±0.05	0.82±0.051	0.8±0.05
Creatinine clearance (mL/min/1.73m^2)	71.2±4.4	86.8±4.4*	61.3±1.8	57.7±1.7
BUN (mg/dL)	11.6±0.58	10.8±0.73*	8.48±0.5**	11±1.7
Urinary N (g/24 h)	3.9±0.4	4.3±0.2	3.9±0.2	4.9±0.5
Urinary Ca (mg/L)	64.4±8.4	101.2±21.2	91±9.2	103±21.2
Serum phosphorus (mg/dL)	3.3±0.18	4±0.22*	3.25±0.18	3.4±0.09
TmP/GFR (mg/dL)	2.5±0.2	3.4±0.09*	2.7±0.07	2.6±0.08

*$p<0.04$ vs. basal

**$p<0.01$ vs. basal

34.3.3 Results

The kidney functions before and during 9 months of IGF-I treatment are shown in Table 34.2. During the first week of treatment, all the patients had a slight and transitory weight gain of 700±240 g (mean±SEM), concomitant with an increase in serum sodium from 141.2±1.0 to 143±1.5 mmol/L, and a reduction in the 24-h urinary volume from 0.83±0.15 to 0.78±0.21 (Fig. 34.5) and urinary sodium excretion from 123.7+46.7 to 78±21 mEq/24 h. With the exception of

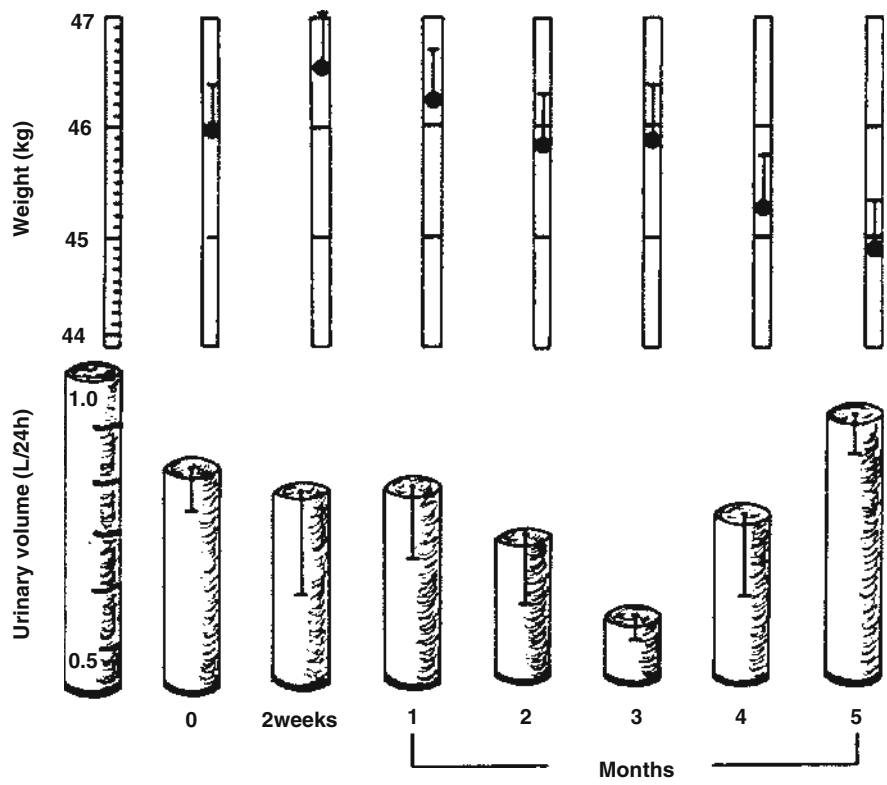

Fig. 34.5 Change in body weight and 24-h urinary volume during IGF-I treatment of five adult patients with Laron syndrome (m±SEM)

serum chloride, which showed a statistically significant increase ($p<0.04$) from 103.6 ± 0.7 to 107.8 ± 0.7 mEq/L, all differences fell short of significance. Table 34.2 shows the effects of IGF-I treatment on serum creatinine and its clearance, which serve as a measure of glomerular filtration and the renal regulation of calcium and phosphorus. The basal creatinine clearance in all the untreated adult Laron syndrome patients was below normal but increased transiently to normal values after 3 months of IGF-I treatment. The BUN fell during IGF-I therapy and returned to basal pretreatment levels after cessation of the IGF-I administration. During the first month of treatment, there was a transient reduction in urinary nitrogen excretion (not shown). There was a significant increase in serum phosphorus ($p\leq0.04$) and concomitant increase in renal tubular reabsorption of the phosphorus. There was also a tendency for urinary calcium excretion to increase.

34.4 Summary of Main Kidney Functions in Untreated and IGF-I Treated Patients with Laron Syndrome along Age

Figure 34.6 illustrates the GFR as measured by the creatinine clearance in 16 untreated female Laron syndrome patients along age. It is seen that after age 20, the majority of values are below the lower limit of normal. Similar findings are evident in untreated males (Fig. 34.7). The effects of IGF-I treatment in adult Laron syndrome have been shown before (Table 34.2). Figures 34.8 and 34.9 illustrate the effect of IGF-I treatment on the GFR in girls and boys during childhood and puberty. It is seen that the GFR is greater than in the untreated state becoming normal and even surpassing the upper normal limit with progressive age and duration of treatment.

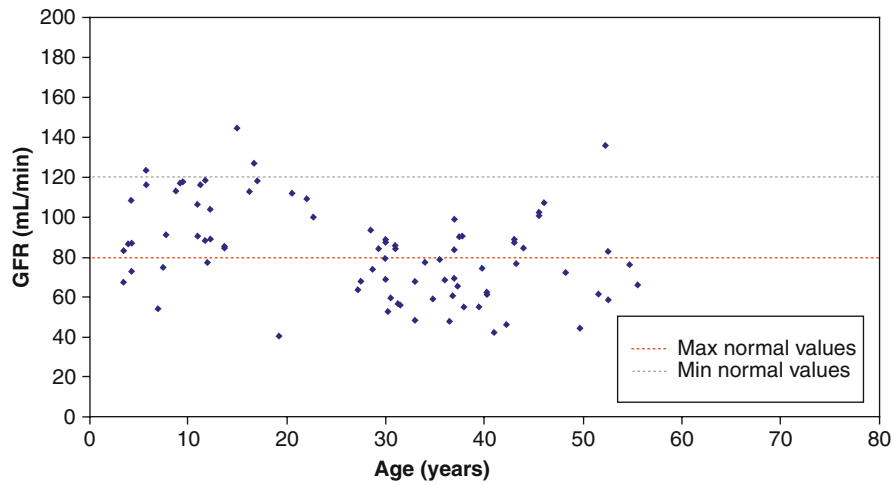

Fig. 34.6 Glomerular filtration rates (GFR) in untreated female patients with Laron syndrome ($N=16$, $n=89$). N number of patients; n number of determinations

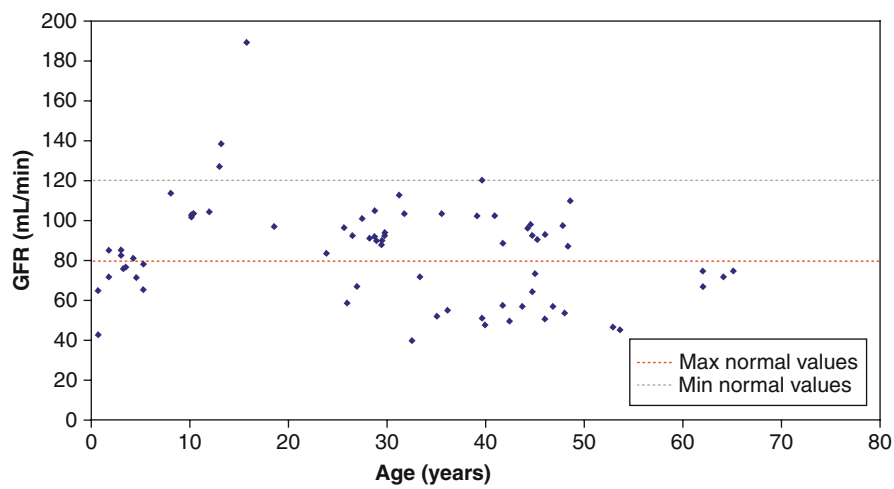

Fig. 34.7 GFR in untreated male patients with Laron syndrome ($N=18$, $n=74$). N number of patients; n number of determinations

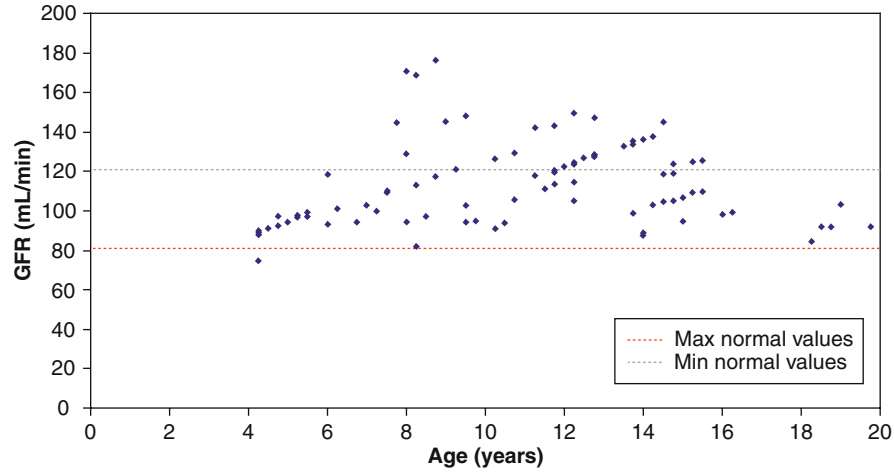

Fig. 34.8 GFR in IGF-I treated girls with Laron syndrome ($N=8$, $n=95$). *N* number of patients; *n* number of determinations

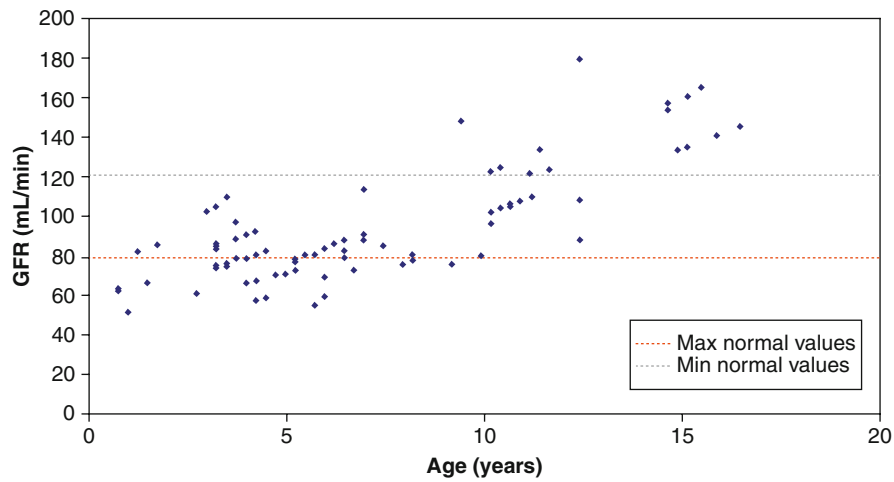

Fig. 34.9 GFR in IGF-I treated boys with Laron syndrome ($N=20$, $n=95$). *N* number of patients; *n* number of determinations

The serum phosphorus levels in the untreated state in both female and male Laron syndrome patients were normal with few exceptions of low levels in both genders (Figs. 34.10 and 34.11, respectively). IGF-I treatment increased the serum PO_4 levels in girls (Fig. 34.12) as well as in boys (Fig. 34.13). A similar effect is seen in the five adult Laron syndrome patients treated for only 9 months (Fig. 34.14). In these patients, it is clearly seen that shortly after stopping treatment, the serum PO_4 concentrations declined in four out of five Laron syndrome patients.

34.4.1 Serum Electrolytes

Serum sodium, chloride, and potassium levels were within normal levels in untreated patients with Laron syndrome, and little if any changes were observed during IGF-I treatment. A short transitory sodium retention was observed in the adult Laron syndrome patients after initiation of IGF-I treatment.

34.4.2 Kidney Size

We did not perform systematic measurements as Backeljauw et al. (2001) did but found on three occasions a smaller than normal for age kidney size.

34.4.3 Diabetic Nephropathy

Two of our patients with neglected Type 2 diabetes mellitus developed nephropathy. This complication is described in Chap. 30.

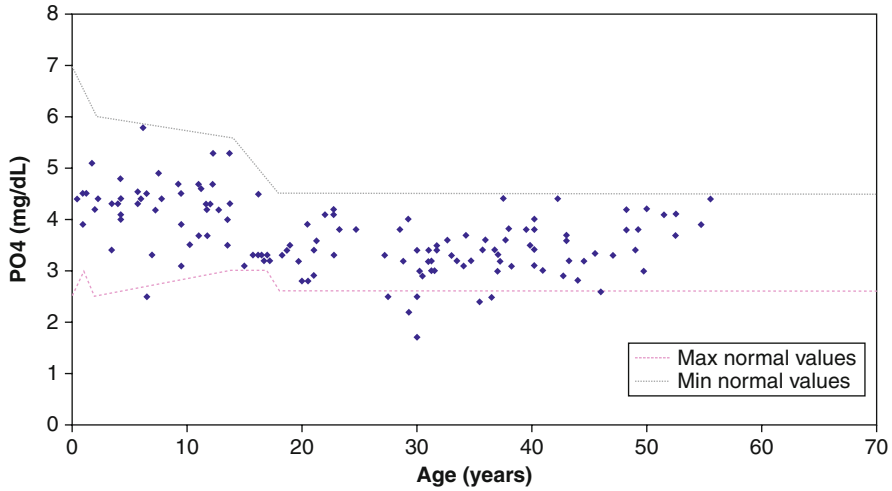

Fig. 34.10 Serum phosphorus levels in untreated female patients with Laron syndrome ($N=20$, $n=140$). N number of patients; n number of determinations

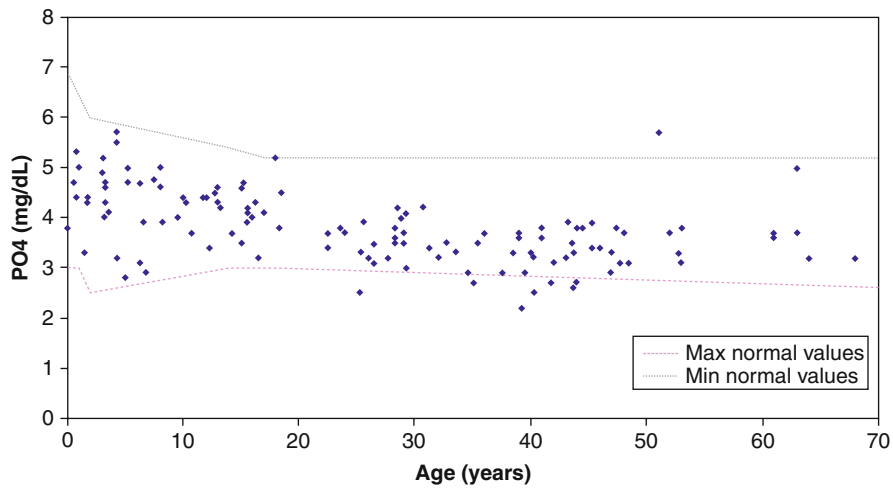

Fig. 34.11 Serum phosphorus levels in untreated male patients with Laron syndrome ($N=21$, $n=129$). N number of patients; n number of determinations

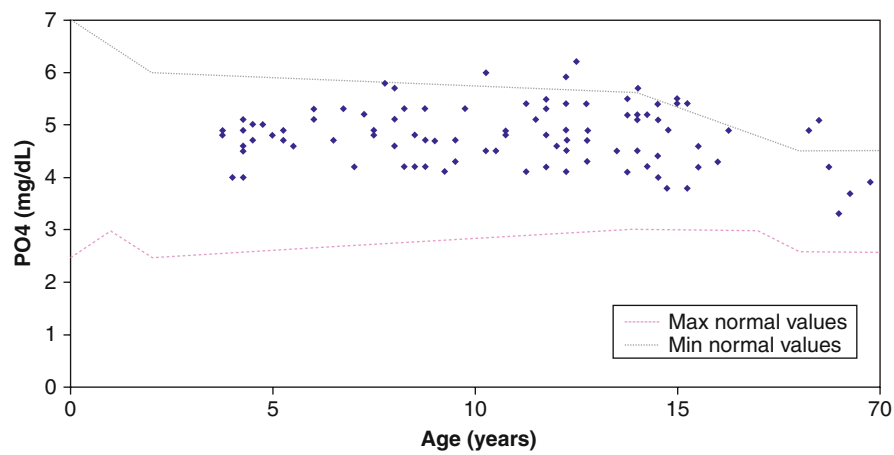

Fig. 34.12 Serum phosphorus in IGF-I treated girls with Laron syndrome ($N=20$, $n=99$). N number of patients; n number of determinations

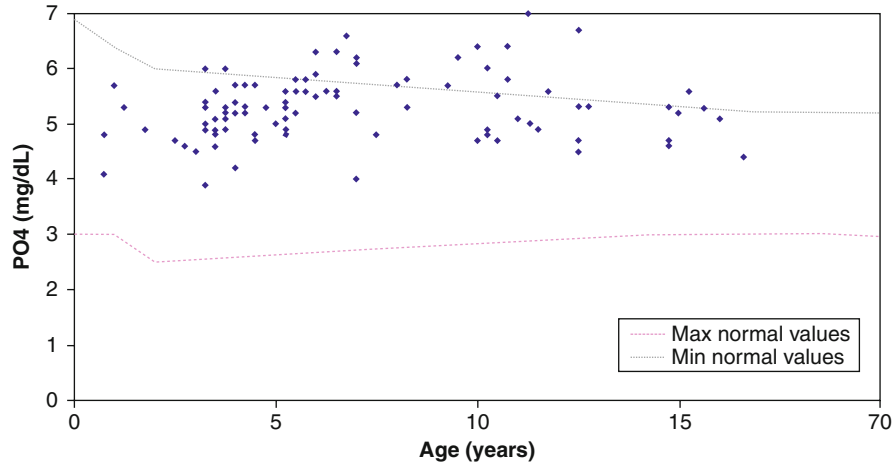

Fig. 34.13 Serum phosphorus in IGF-I treated boys with Laron syndrome ($N=7$, $n=108$). N number of patients; n number of determinations

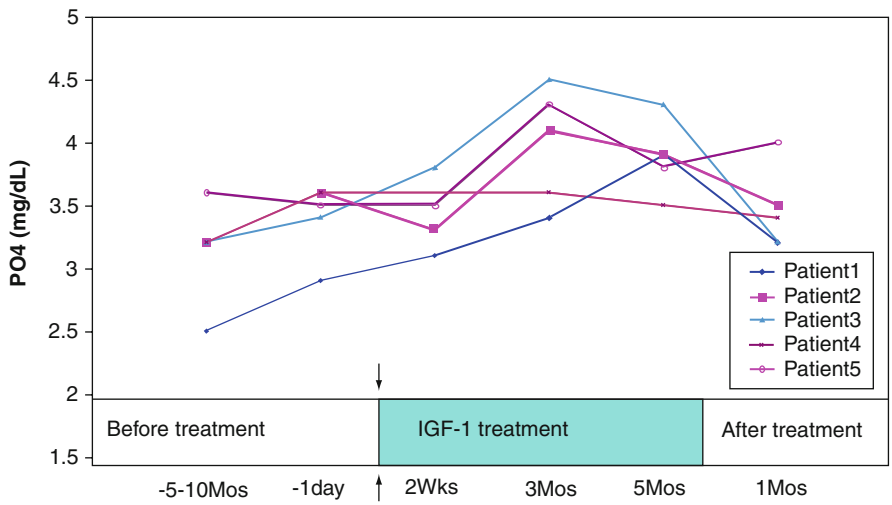

Fig. 34.14 Serum phosphorus levels in adult patients with Laron syndrome before, during, and after stopping IGF-I treatment ($N=5$, $n=29$). N number of patients; n number of determinations

34.5 Conclusions

IGF-I definitely affects kidney development and functions. Our investigations in Laron syndrome patients have shown that patients with longstanding IGF-I deficiency have a smaller kidney and a tendency for low GFR, which increases during IGF-I treatment. The serum phosphorus and tubular reabsorption of phosphorus are also increased by the IGF-I treatment but are not low in the state of deficiency. IGF-I treatment also causes transitory water retention and calciuria. These findings prove that the effects on the kidney reported for GH are IGF-I mediated.

Acknowledgment We acknowledge the assistance of Amir Yahav in the preparation of Figs. 34.6–34.14, which were performed in partial fulfillment of the MD thesis requirements of the Sackler Faculty of Medicine, Tel Aviv University

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Sleep and Sleep Disorders in Patients with Laron Syndrome

35

Zvi Laron, Rivka Kauli and Eyal Rosenzweig

Core Message

› Many patients with Laron syndrome complain of sleep disorders. Polysomnographic examination revealed obstructive sleep apnea (OSA) caused by the obesity and narrow oro-pharynx.

35.1 Sleep Quality

Zvi Laron, and Rivka Kauli

35.1.1 Introduction

The information on sleep disorders including obstructive sleep apnea (OSA) in growth hormone deficiency is scant. Vgontzas et al. (2006) write that "mild GH deficiency has been described in patients having sleep apnea," and Gislason and Almquist (1987) mention that treatment of sleep apnea appears to improve the growth hormone and IGF-I levels. On the other hand, Franco Ramos et al. (2006) reported that GH treatment to obese adults increased the severity of OSA. Sleep apnea has been observed frequently in acromegalic patients mainly over 50 years of age and linked to the macroglossia and craniofacial changes (Grunstein et al. 1991; Hochbau et al. 1999). It was therefore of interest to investigate the sleeping behavior of patients with Laron syndrome (Laron syndrome), a state of hGH inactivity and IGF-I deficiency and severe obesity. We have previously reported severe OSA in a male adult with Laron syndrome (Dagan et al. 2001). Two types of studies were performed.

35.1.2 Method

A prestructured questionnaire (Table 35.1) was constructed and offered for completion during their follow-up visits.

35.1.3 Subjects

Sixteen patients with Laron syndrome – ten females and six adult males and three girls aged 6–12 – completed the whole questionnaire. Eleven patients (six adult females and five adult males) completed the questionnaire on two occasions with at least 3 year intervals; five patients (four females and one male) completed it three times at different ages. All patients were obese.

35.1.4 Results

The summary of 31 questionnaire replies according to score categories is shown in Table 35.2. It is seen that 14 repeated questionnaires to 6 patients scored over 30

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Table 35.1 Questionnaire for the evaluation of sleep quality

Do you have difficulties falling asleep at night
Do you wake up very early in the morning and fail to fall asleep again
Do you take medications for sleep or tranquilizers
Do you fall asleep at daytime (excluding a programmed afternoon nap)
Are you tired in the morning (immediately after waking up)
Do you snore while sleeping (as far as you know) or being told by a relative
Do you wake up from time to time during your sleep
Do you have headaches after waking up from your night sleep
Do you feel tired with no apparent reason
Is your sleep restless with many movements of hands and feet
Do you have complaints of fatigue

Score for each question: 1. never, 2. very rarely, 3. rarely, 4. sometimes, 5. often, 6. very often, 7. always
Sum of scores: 10–24 (good sleep); 25–30 (suspected sleep disturbance – average); >30 (severe sleep disturbance)

Table 35.2 Analysis of responses of the questionnaire for the evaluation of sleep quality

Type of sleep	Number of Laron syndrome patients
Normal	10
Suspected sleep disturbance	7
Severe sleep disturbance	14

denoting a severe sleep disturbance. One of the patients in this category reported that he could not sleep at night and needed to sit up. Of the seven patients with a score of suspected "sleep disturbance," four had normal sleep patterns at their first test taken approximately 3 years before. The patients who reported a normal sleep pattern were the youngest age group. One of the major complaints was fatigue.

35.1.5 Conclusion

Self-reporting using a prestructuring questionnaire is useful in classifying the type of sleep and selecting those subjects who need further investigations. The patients with severe sleep disturbance accepted the advice to undergo polysomnography; those with suspected disturbance remain under observation.

35.2 Severe Obstructive Sleep Apnea (OSA)

Zvi Laron

In 2001, we reported severe OSA in a 68-year-old male obese patient with Laron syndrome (MeS) (Dagan et al. 2001). His height was 145 cm; weight, 66 kg; subscapular skinfolds, >40 mm. Since the age of 38, he suffered from Type 2 diabetes mellitus (see Chap. 30) with complications. He received insulin and blood-pressure-lowering drugs. He complained of severe sleepiness, mental fatigue, nervousness, and difficulties in memory, attention span, and concentration. He also noted a dry mouth on waking, and his wife reported that he snored heavily. His sleep was disturbed by sleep-related breathing problems, and so he agreed to undergo two consecutive nights of standard polysomnographic examinations. The tests revealed Time in bed (TIB) = 433 min, Total sleep time (TST) = 355 min, sleep efficiency = 82%, sleep onset latency (SOL) = 8 min, rapid eye movement (REM) = 11.7%, SWS = 10.5%, number of obstructive apneas = 87 (maximum duration = 76.9 s), number of central apneas = 2, number of mixed apneas = 1, number of hypopneas = 345, Apnea Hypopnea Index (AHI) = 58.3, mean overnight SaO_2 = 77.6%, and time snoring = 35.8% (for interpretation see also part III). There were no sleep-related electroencephalogram (EEG) abnormalities, periodic limb movement, and electrocardiogram (ECG) abnormalities (including bradycardia). This information led to the diagnosis of severe obstructive sleep apnea syndrome (OSAS), and he was successfully treated by CPAP (continuous positive air pressure) using a nasal mask during sleep.

35.3 Additional Polysomnographic Examinations of Patients with Laron Syndrome

Eyal Rosenzweig and Zvi Laron

35.3.1 Subjects

Five obese Laron syndrome patients (three men and two women, age range 29–56) who had a score of sleep disturbances of over 30 agreed to undergo

polysomnography at the Institute for Fatigue and Sleep Medicine, Sheba Medical Center (ER).

35.3.2 Method

On admission to the Institute, all subjects underwent standard polysomnography. Recording included EEG, electrooculogram (EOG), electromyogram (EMG); submental and anterior tibialis, ECG, nasal airflow, chest and abdominal breathing movements, snoring, position, and pulse oxymetry. Digital sleep data system (Somnologica 3.2) was used. In the following day, a Multiple Sleep Latency Test (MSLT) was performed according to a standard protocol (Carskadon et al. 1986): five 20-min sleep attempts that took place at 10:00, 12:00, 14:00, 16:00, and 18:00.

35.3.2.1 Sleep Scoring

Polysomnographic data of the nocturnal sleep recordings and MSLT were scored by trained technicians according to the International Classification of Sleep Disorders (American Sleep Disorders Association; Diagnostic Classification Steering Committee 1997).

The following parameters were calculated for the nocturnal sleep report: TST, sleep latency, REM latency, percentage of REM, percentage of SWS, number of arousals (<15 s), number of awakenings (>15 s), sleep efficiency (TST−Time awake/TST), number of apneas, number of hypoapneas, Respiratory Distress Index (RDI; apneas + hypoapneas/hour), number of oxygen desaturations ($<O_2$), and percentage of snoring. MSLT sleep report included SOL and REM latency from sleep onset.

35.3.3 Results

The findings are summarized in Table 35.3 and Fig. 35.1. Five patients completed the sleep evaluation. In one patient, there was no sleep pathology. One patient had moderate OSAS with RDI = 21 and no subjective or objective sleepiness. Sleep stage distribution was normal in this patient. Two patients with normal ESS value have an MSLT of severe objective sleepiness. In these two patients, the most prominent finding on PSG was shortening of SWS. In one of them, REM sleep was also shortened. In these Laron syndrome patients, ESS indicated no objective sleepiness. Another patient with moderate objective sleepiness had a shortened EM sleep with normal SWS. In this patient, ESS value indicated severe subjective sleepiness. Another patient had severe OSAS (RB). The use of CPAP has been recommended to him.

35.3.4 Discussion

Our findings show that not all Laron syndrome patients reporting sleep disturbances have OSAS, despite being very obese (Laron et al. 2006) and having a narrow oropharynx (Kornreich et al. 2002). Until now, we have diagnosed three Laron syndrome patients with severe OSAS;

Table 35.3 Findings at polysomnography in five adult patients with Laron syndrome

Pt.	Age (years)	Sex	S2 (%)	REM (%)	SWS (%)	MSLT	ESS	Snore (%)	$<O_2$ min	ODI	RDI/year	TBF (%)
YR	50	F	45.1	14.6	40.3	10	21	0	90	3.1	2.6	64.5
TS	54	F	70.6	8.3	21.1	2.5	9	70.2	87	0	5.2	58.5
ML	29	M	49.9	16.5	33.6	15.5	1	0.6	–	0	1.1	43.7
BR	42	M	42.2	28.3	29.5	5.1	5	0	–	0	10	44.7
SSh	46	M	44.8	20.4	34.8	15.4	4	4.6	87	1	20.9	36.4

Abbreviations: *F* female; *M* male. Units: *S2* duration of light sleep; *REM* rapid eye movement; *SWS* slow wave sleep; *MSLT* multiple sleep latency; *ESS* Epwarth sleepiness scale; $<O_2$ minimal oxygenation; *ODI* oxygen desaturation index; *RDI* respiratory distress index (apneas + hypopneas/hour); *TBF* total body fat (by DEXA)

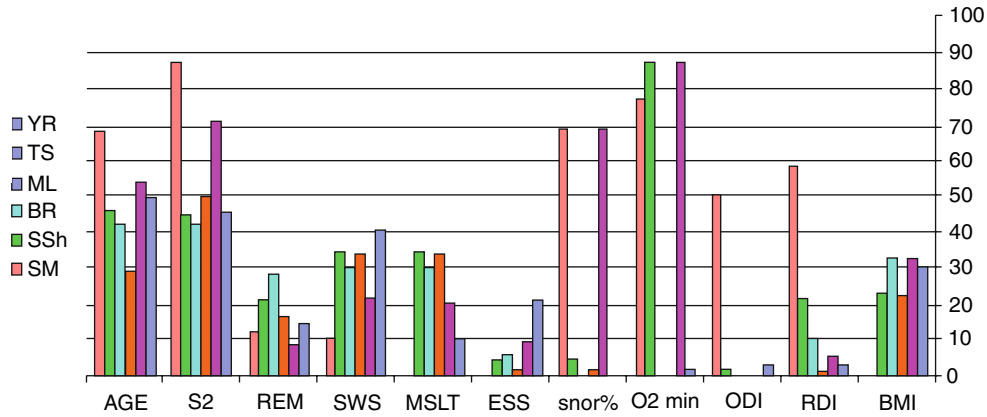

Fig. 35.1 Polysomnography of six patients with Laron syndrome. For explanation of abbreviations see Table 35.3

two of them had Type 2 diabetes mellitus, and both have died (MeS and YG). Whether the OSA has contributed to the development of their vascular complications (Yaggi et al. 2005; Bradley and Floras 2009) is speculative.

35.3.5 Conclusions

As all untreated and even treated patients with Laron syndrome are very obese and tend to have narrowing of the oro-pharynx, sleep disturbances should be looked after and polysomnography performed if indicated. As IGF-I treatment increases the lymphoid tissue, attention should be paid even to the treated young patients.

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Neurological Aspects in Laron Syndrome

36

Zvi Laron

Core Message

- The neurological findings encountered in patients with Laron syndrome were headaches, few epileptic episodes not always related to hypoglycemia, low back pain probably connected with the spinal stenosis found in few patients.
- MR imaging revealed white matter pathology in some patients, leading to varying degrees of psychomotor disturbances. Two patients with diabetes mellitus developed neuropathy.

36.1 Symptoms and Signs

The most frequent complaint in young as well as adult patients was headaches. Within the first cohort of 22 patients (Laron et al. 1968), we performed 14 EEG and found 12 to be normal; one presented asymptomatic epileptic waves, and another revealed a paroximal pattern. Subsequently, one of the male patients with a normal EEG until age 13 developed epileptic (convulsive) episodes, concomitantly with hypoglycemia. One female patient (HS) treated by IGF-I between age $3^{8/12}$ and 18 years developed progressively severe epileptic episodes and headaches culminating with a difficult to control status epilepticus after cessation of the IGF-I treatment. None of her episodes were related to hypoglycemia. Her repeated MRI was normal, and the last EEG revealed abnormal waves of low intensity in the left temporo-occipital region. Recently, she also complained of difficulties in walking. MRI of the spine did not reveal stenosis, and Doppler of the upper and lower limbs as well as of the carotis arteries revealed normal blood circulation.

Another female had a transitory petit mal episode during IGF-I treatment, which did not recur despite continuation of treatment. She too complained of headaches for years. Several of the Laron syndrome patients had CNS damage as revealed by MRI (Table 20.4 in Chap. 20), some with clinical sequelae (Chap. 38).

In 1967, at a time when CT or MRI were not available, we performed pneumoencephalography in six Laron syndrome children; all revealed normal sized ventricles (Laron et al. 1968); subsequently with a larger cohort of patients and using MRI, some patients had enlarged ventricles (Chap. 20).

One patient (LY) with cervical spinal stenosis and an anomalous CNS on MRI (Table 20.4 in Chap. 20) suffered from spastic paraparesis, oculomotor apraxia, and polyneuropathy.

The two male patients with diabetes mellitus (Chap. 30) suffered from diabetic neuropathy. One female patient developed a psychotic disorder, which necessitated transitory hospitalization, but we failed to get details. Another female patient (LM) is institutionalized with mental deficiency; she had an abnormal CNS on MRI (patient LM; Shevah et al. 2005).

36.2 Conclusions

Considering the important role GH and IGF-I play in CNS and nervous tissue development and function

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(Laron 2002, 2009), one can assume that the neurological deficits and diseases in Laron syndrome patients are linked to the GH/IGF-I deficiencies starting in utero with variable sequelae postnatally. The exact relationship needs further elucidation. It is of note the high incidence of headaches in both untreated and IGF-I treated patients, in the latter without evidence of excessive water retention or hypertension.

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Orthopedic Problems in Laron Syndrome

37

Zvi Laron and Rivka Kauli

Core Message

› Orthopedic congenital malformations were dislocation of the hip and Perthes' disease. Half of the patients had lack of elbow extension. With advancing age patients had problems with walking, related to spinal pathology. Despite lifelong IGF-I deficiency, Laron syndrome patients did not have more fractures than in the general population.

37.1 Introduction

In the literature reporting on patients with Laron syndrome from various parts of the world (Savage et al. 1993), nothing or little is mentioned on orthopedic problems. Rosenbloom et al. (1993) report from the large Ecuadorian cohort that avascular necrosis of the femoral head (Legg-Calvé-Perthes diseases) has been found in 25% of these patients. These authors also mention limited elbow extension in children and adults with Laron syndrome.

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37.2 Subjects and Methods

During 50 years of follow-up of 64 patients with Laron syndrome, we observed some congenital orthopedic defects and a series of acquired orthopedic problems.

37.3 Results

As physical complaints required roentgenological investigation, the underlying skeletal defects in our patients are described in detail in Chap. 20 on imaging. The major skeletal findings and complaints were Perthes' disease in one male patient and congenital dislocation of the hip in two male patients who were diagnosed early in infancy and childhood the patients presenting walking problems. The most frequent complaint with advancing age and degree of obesity was pains in the knees. Due to the marked obesity and reduced muscle mass, almost all Laron syndrome patients developed "genu valga" and flat feet. Many of the adult female patients complained of back pains, probably related to the spinal stenosis (Chap. 20) and osteophytes. Two of our patients, an 18-year-old girl (HoS) and a 60-year-old male (MeS) who suffered from diabetes mellitus, had serious problems with walking. Both the problems were of neurological origin. In approximately half of our patients, we observed lack of elbow extension, also described by Rosebloom et al. (1993). Judging by the shape of the humerus, this deformity may have already occurred in an ancient population with Laron syndrome (Hershkovitz et al. 2007), the cause being underdevelopement of the muscular system due to the IGF-I deficiency leading to an abnormal degree of torsion of the humerus. Three of our patients, two males

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(MeSh, SSh) suffered from road accidents, both causing multiple fractures. The second remained with some skeletal deformations. One female broke her tibia by falling. Healing of the fractures in the Laron syndrome patients was normal.

37.4 Leg Lengthening in Patients with Laron Syndrome

As short stature is a major handicap (Aran et al. 1995; Eiholzer et al. 1999; Stabler and Underwood 1994), a discussion arose as to whether surgical limb lengthening would apply to patients with Laron syndrome (Laron 1995). Considering that Laron syndrome patients do not have osteopenia and heal bone fractures in a normal manner, it is our opinion that limb lengthening should be considered either in young adults who have not been treated or in those young patients in whom IGF-I treatment does not or did not achieve a near normal height. As the muscles are underdeveloped and some patients are deficient in Vitamin D, this procedure should be supplemented by IGF-I treatment administered before and during the surgical and physical rehabilitation treatment, as well as by Vitamin D.

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Psychological Aspects in Patients with Laron Syndrome

38

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Core Message

› Patients with Laron syndrome present with a range of psychological defects from normal to mental retardation. Part of them are explained by the type of GH-receptor defect.

38.1 Introduction

There is both clinical and experimental evidence that congenital GH deficiency affects the development of the nervous system and psychological functioning (Frankel and Laron 1968; Rotnem et al. 1977; Almqvist et al. 1986; Deijen et al. 1996; Laron 2009). One anatomical proof is the smaller brain (as measured by head circumference) in children born with congenital hGH deficiency as well as the fast growth of the head (i.e., brain) (Laron et al. 1979a) with a rise in the intelligent quotient upon hGH replacement therapy (Laron et al. 1995; Laron and Galatzer 1981). Trygstad and Seig (1964) reported that one of their male patients with hereditary pituitary dwarfism had a retarded psychomotor development. Mental retardation with an IQ of approximately 50 was noted in one patient with isolated GH deficiency reported by Wilber and Odell (1965) and Roth et al. (1967) found IQs of 80 in an 8-year-old girl and a 15-year-old boy with the same disease.

Considering that IGF-I is the anabolic effector hormone of pituitary GH, we performed a series of psychological evaluations in our patients with Laron syndrome, a model of congenital IGF-I deficiency and hGH inactivity.

38.2 Background

Two facts enabled the study of various aspects of psychological qualities and social status of the patients in our cohort:

(a) The building of a psychosocial unit consisting of psychologists and social workers in our pediatric endocrinology department (Laron and Galatzer 1982, 1983; Laron et al. 1979b)
(b) The long-term follow-up of our patients from childhood into adult age (Galatzer et al. 1987)

Various tests were performed at different ages and information was obtained at follow-up visits and by telephone conversations with the parents, teachers, and old enough patients.

38.3 Early Investigations

38.3.1 Subjects

Eighteen Laron syndrome patients (7 males, 11 females) aged 4–17 years (mean age 13.3 ± 5.2 years) underwent extensive psychological examinations.

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38.3.2 Intelligence Tests

The first set of tests were performed when the first cohort of patients were young (i.e., late 1960s, early 1970s). Not all patients or their parents agreed, and so, data are not available for all the patients.

38.3.3 Methods

All subjects were administered a battery of neuropsychological tests on an individual basis. The tests were applied in the course of routine clinic follow-up and included an intellectual evaluation with standard intelligence tests consisting of one of the following: Wechsler Intelligence Scale for Children (WISC) (Wechsler 1974), Wechsler Adult Intelligence Scale (WAIS) (Wechsler 1981), Stanford-Binet Tests of Intelligence and the Cattell Infant Intelligence Scale, the Bender Visual Motor Gestalt Test, human Figure drawings, and teacher report (Frankel and Laron 1968; Kopitz 1964)

38.3.4 Results

The individual results of those Wechsler tests are shown in Table 38.1 (test I – 1968).

The findings reveal a distinct tendency to score well below the normal intelligence quotient (IQ) range, i.e., below 90–110. The presence of subnormal intellectual function seems to characterize these and later examinations: the mean Wechsler Full Scale IQ of 23 patients was 78.35, and the mean IQ of 33 patients based upon the Wechsler, Binet, and Cattell was 76.46. Two additional observations, relevant to the Wechsler performance, deserve note – a marked discrepancy between the verbal and performance IQ scores, a very marked subtest scatter that occurred in every case, as seen in the WISC test for children.

38.3.5 Teachers' Reports

The teachers' responses to a semistructured questionnaire fairly closely followed the IQ scores as reported in Table 38.1 (test I). None of the patients was reported to excel. Average work and grades were reported for those scoring average or above in IQ, whereas poor and failing work was reported for those patients whose IQs were borderline and below. Sample teacher comments on one of the boys: "In grades 1–4 he was considered a good student. Beginning with the fifth grade his work declined, especially in arithmetic. Does well in no particular subject." Of a female patient (CR): "At the end of her fourth year, her grades were good. Beginning with the fifth grade it appears she has "braked" and has begun to be a weak student. Is weak in all subjects, particularly arithmetic."

Table 38.1 Wechsler intelligence scale. Comparison of full-scale, verbal, and performance IQ distribution of patients with Laron syndrome tested twice, in adolescence (I: $n=18$) and adulthood (II: $n=12$)

	Norms	Full-Scale		Verbal		Performance	
IQ score	%	I	II	I	II	I	II
>130	2.2	–	–		–	–	–
120–129	6.7	–	8.3		8.3	–	–
110–119	16.1	–	16.7		16.7	–	8.3
100–109	25.0	22.2	–	11.1	–	16.7	16.7
90–99	25.0	27.8	16.7	22.2	25.0	16.7	16.7
80–89	16.1	27.8	16.7	33.3	16.7	33.3	–
70–79	6.7	5.5	41.6	16.7	33.3	11.1	58.3
≤69	2.2	27.8	–	16.7	–	22.2	–

I 1968 ($n=18$) II 1980 ($n=12$)
Reproduced with permission from Laron (2002)

38.3.6 Emotional Factors

Relevant to emotional factors, the teachers indicated average or above social acceptance and involvement. In only one patient (ML) did the teacher indicate that the acceptance was based upon sympathy; most teachers actually reported the subjects to be gregarious and well-liked in their own right.

38.3.7 Visuomotor Functioning

Visual-motor results as based on the Bender test (Fig. 38.1) (Kopitz 1964) are summarized in Table 38.2. It is evident from the table that the group as a whole, regardless of age, performed deficiently in the Bender test (the subject is asked to copy a drawing). Furthermore, the most prevalent form of error was "distortion," which frequently took an extreme form, as seen in Fig. 38.2. In addition, a unique phenomenon emerged in the Bender recall when, following the completion of the Bender test, the subject is instructed to produce from memory

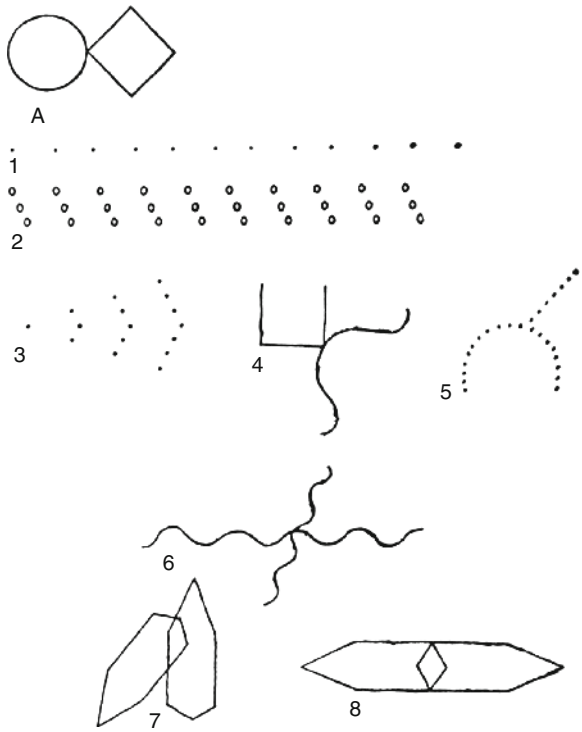
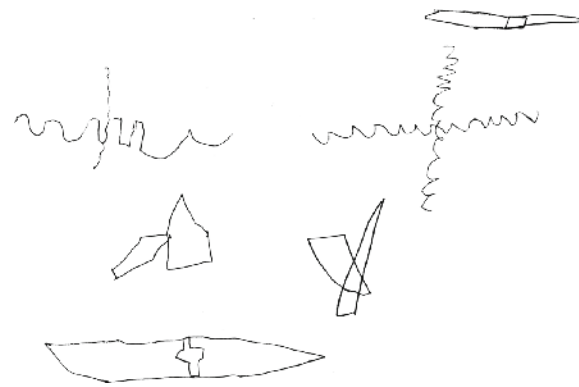


Fig 38.1 The design of the Bender Test (Kopitz 1964)

Table 38.2 Bender test in children and adolescents with Laron syndrome

Age	<11 years	>11 years	
SD above mean	Number of patients	Errors	Number of patients
Less than 1 SD	2	0	1
1 SD	3	1–3	7
2 SD	5	4–6	6
3 SD	0	7–9	1
4 SD	2	10	0
Total	12		15

Classification of errors and SDs according to Kopitz (1964)



Distortion of Bender designs 6, 7, and 8 by two 12-year-old males (no. 10 and 17). with the syndrome of pituitary dwarfism, due to inactive HGH (hereditary).

Fig 38.2 Distortion of Bender designs by two 12-year-old boys with Laron syndrome. Reproduced with permission from Frankel and Laron (1968)

the Bender designs he has just copied: here a quarter of our patients recalled the designs in bizarre fashion, producing half of the design or else combining two distinct parts of the two designs into one "original" design.

38.3.8 Investigation of Siblings

We also examined seven nonaffected siblings representing four families in our sample and compared their results with the seven affected siblings. The results are as follows: Laron syndrome patients verbal, performance, and full scale IQs were 75.2, 71.8, and 71.0, respectively; the siblings' scores were 80.6, 89.3, and 83.0, respectively. Although the IQs of the siblings are

substantially higher than those of our patients, they too are below normal, particularly the verbal and full-scale scores. As the patients investigated belong to consanguineous families, the "unaffected" siblings are obviously heterozygotes for the genetic defect. As our patient population covers a broad range of socioeconomic levels from low to high, this may be of only minor influence. We found that visuomotor functioning showed no variation with socioeconomic class.

38.3.9 Comment

The almost ubiquitous difficulty in visual-motor functioning, as based upon the Bender test and the Kopitz scoring system, raises the question whether in cases of congenital IGF-I deficiency, one finds a deficient visual-motor performance that is above and beyond the possible limitation imposed by the lower IQ. Thus, even the relatively brightest patients, in terms of full scale IQ, were sufficiently impaired in their Bender performance to warrant consideration of the existence of a specific visual-motor handicap, perhaps akin to that found by Money and Alexander (1966) in the Turner syndrome**.** Most authorities believe that at age 10–11 the average child should be able to produce the Bender designs without error; yet virtually all of the older subjects had difficulties with the Bender tests, despite conservative Kopitz scoring system.

The unique and puzzling recall phenomenon of creating a new gestalt by combining separate aspects of two original designs remains unexplained. The rarity of this occurrence is attested to by the fact that a search for similar lapses of cognitive memory in over 100 files, in a variety of local child clinics, yielded similar observations in only about 3% of the children, compared to 25% in our sample, and even then not to the extreme extent depicted (Frankel and Laron 1968)

Whether the tendency for hypoglycemia in early infancy of these patients contributed to the psychological abnormalities is not clear. Very few patients with congenital GH/IGF-I deficiencies have undergone detailed psychological testing.

38.3.10 Conclusions of the Early Tests

Laron syndrome patients were found to have a borderline or low-average intelligence. There was no difference between verbal (81.8 ± 17.24) and performance IQ (83.6 ± 17.52). Five of the 18 patients had an IQ lower than 69, which is one of the indications for mental retardation. Out of these five, one is severely retarded and lives in an institution for the mentally retarded.

38.4 Second Later Investigation

In 1980, 12 years later, we were able to retest eight of the Laron syndrome patients examined in 1968 and examine four additional Laron syndrome patients (mean age 28.9 + 8.6, age range 12–43 years) Test II (Galatzer et al. 1993)

38.4.1 Methods

The following tests were used: The Wechsler Intelligence Scale (WISC-R or WAIS-R) (Wechsler 1974, 1981)

1. Memory for stories subtest of the Wechsler Memory Scale (Wechsler 1945)
2. Associative memory subscale of the Wechsler Memory Scale (Wechsler 1945)
3. The Road Map test (Money et al. 1965)
4. Benton Visual Retention test (Benton 1974)
5. Trial Making – Part B (Reitan 1958)
6. Verbal fluency (Thurstone and Thurstone 1941)
7. Motor speed (US Department of Labor Manual l970)
8. Dot localization (Gordon 1986)

38.4.2 Results

The comparative data of the two studies are shown in Tables 38.1 and 38.3. Table 38.1 compares the full scale, verbal, and performance IQ distribution of all 12 patients, and Table 38.3, the comparative IQ scores for individual tests in 8 Laron syndrome patients tested as children and 12 years later.

The mean verbal IQ of the current group was 91.9 ± 16.91 and the performance IQ was 86.6 ± 15.5. A comparison of the distribution of these two groups reveals that in the original group (I), none of the patients reached the upper quartile of the normal distribution (IQ >110), while 3 of 12 patients of the current study (the expected 25%) scored in this range (one from the original group and two new patients). Sixty-one percent of the original group as compared to 58.3%

Table 38.3 Comparison of full-scale, verbal, and performance IQ scores in eight patients with Laron syndrome tested twice, in adolescence (I) and in adulthood (II)

Patient No.	Sex	Age years		Full-scale		Verbal IQ		Performance	
		I	II	I	II	I	II	I	II
1	M	14	36	85	80	82	84	92	76
2	M	10	28	104	91	95	92	113	92
3	F	7	36	75	73	74	74	80	74
4	F	18	43	108	121	106	121	108	115
5	F	12	30	99	78	99	82	100	78
6	F	13	31	92	82	94	91	92	75
7	F	18	36	82	76	81	79	85	74
8	F	18	36	67	72	76	75	60	70
Mean		13.7	33.2				91.9		86.6
±SD		3.5	10.4				16.9		15.5
Change (1/2 SD)		None		4		6		2	
				1		1		1	
				3		1		5	

of the recently tested group scored in the lower quartile of the IQ range (IQ<90). The fact that none of the patients in the current study had an IQ lower than 69 as compared to 5 of the original 18 patients who scored at this range is due to a selection bias as we decided not to include these patients in the current study.

Out of the 12 Laron syndrome patients who comprise the current group, 8 belong to the original group and 4 are new patients who were never tested. We decided to compare the data of those patients who had been tested twice. Table 38.2 demonstrates changes in IQ scores in these 8 patients. While there was no significant change in total and verbal IQ scores, a significant decrease in performance IQ was found. This difference was due to a significant decrease in 2 of the 3 scales tested in this area, the picture completion and the digit symbol subscales, tests which measure attention to details and immediate learning (Table 38.4).

The results of this study corroborates earlier reports from our group of a low-average intelligence and a skewed distribution toward the lower part of the curve in verbal and performance IQ in patients with Laron syndrome. The current sample (of which 2/3 of the patients belong to the original sample) achieved similar results in performance IQ and a slightly higher (not significant) verbal IQ. The higher IQ score was due to the fact that 25% (3/12) of these patients in the current sample scored in the upper quartile of the curve (as expected in the general population), while none of the patients in the original study scored with this range. Two of the three patients who scored above 110 in verbal IQ are adolescents who had not been tested before. The third subject improved her verbal IQ from 106 at the age of 18 to121 at the age of 43, a period during which she married, had a child, and successfully finished her PhD Degree in Microbiology – all these without receiving IGF-I treatment. Subsequently, further few patients reached higher education (see Chap. 39). As these Laron syndrome patients come from the same ethnic background as other members of the sample, it seems that better chances for education for youngsters in the late 1980s could easily explain part of the difference in verbal IQ. We have not, as yet, tested children with Laron syndrome treated for long-term periods starting from early life.

38.5 Correlation Between Intellectual Capacity, Molecular Defect of the GH Receptor, and CNS Findings on MRI

Later investigations with newly developed techniques clarified a series of issues related to the variability in the intellect of patients with Laron syndrome. Among

Table 38.4 Comparison of IQ scores of eight patients with Laron syndrome tested as children and 20 years later as adults (mean ± SD)

Scale	First study	Second study	*p*
Information	8.37 ± 2.82	8.25 ± 3.65	n.s.
Similarities	7.57 ± 2.07	8.43 ± 2.22	n.s.
Arithmetic	8.28 ± 1.80	7.28 ± 2.63	n.s.
Comprehension	7.43 ± 1.71	9.14 ± 4.18	n.s.
Digit-span	8.12 ± 2.80	7.12 ± 4.18	n.s.
Picture completion	8.75 ± 281	5.00 ± 2.72	0.01
Block design	9.85 ± 3.18	8.43 ± 3.35	n.s.
Digit symbol	8.25 ± 1.58	6.75 ± 2.19	0.014
Verbal IQ	88.37 ± 11.60	87.25 ± 15.15	n.s.
Performance IQ	92.60 ± 14.60	81.75 ± 14.93	0.020
Total IQ	90.00 ± 12.98	84.12 ± 16.00	n.s.

those were the identification of the genetic defects in the GH receptor of our cohort (Shevah and Laron 2006) and the abnormalities in the CNS as detected by skull MRI (Kornreich et al. 2002).

38.5.1 Subjects

We were able to analyze data from 10 Laron syndrome patients in whom comparable data, i.e., IQ scores, brain MRIs, and molecular characterization of the GH-R gene, were available (Shevah et al. 2005). All had uneventful gestation and deliveries.

38.5.2 Methods

The molecular defects of the GH-R were determined by PCR and direct sequencing of each exon of the GH-R regions as described before (Shevah et al. (2005), see also Chap. 5). IQ determinations were taken from the tests performed previously on two separate occasions at 12 or more years' interval. Norms established in Israel for the same ethnic population served as controls. All patients underwent a brain MRI. We used a MR system operating at 0.5T. The imaging protocol included axial T2-weighted images and T1-weighted axial, coronal, and sagittal images of the brain. Contrast agent was not injected. The scans were initially read independently by two radiologists, and then reviewed together until a consensus was reached (see Chap. 20). The brain was examined for congenital anomalies or parenchymal lesions. Parenchymal loss was graded visually as minimal, slight, moderate, or severe.

38.5.3 Results

Table 38.5 presents the comparative findings between molecular defect, IQ, and CNS pathology arranged according to the degree of IQ, which ranged from 121 (normal) in one patient to 67–45 (severe mental retardation) in four patients. Most severe CNS pathology and lowest IQ were evidently found in patients with 3, 5, 6 exon deletions. In these two siblings patients (nos. 9, 10), the cerebellum was symmetrically small with enlarged foliae and fissures compatible with cerebellar atrophy. A minimal to slight degree of diffuse parenchymal loss was seen in nine patients. One patient (no.8) demonstrated a lacunar infarct in the right caudate nucleus. Patient no. 6, who suffered from slight mental and motor retardation, had regions of posterior periventricular high signal on the T2-weighted image. No midline anomalies were detected. Further mutations in exons 2, 4, and 7 were found in patients with minimal or mild parenchymal loss. The patient with the E180 mutation in exon 6 had a normal IQ without any CNS pathology.

Table 38.5 Education, occupation, and comparison between molecular defect, IQ, and MRI of the brain in Laron syndrome patients

Patient No No.	Gender	Education/ years	Occupation	Molecular defect	IQ	MRI of the brain
1	F	University	MA student	E180 splice	121	Normal
2, 3	1 M[a] 1 F[a]	12 years	Bank employee Senior bank employee	W-15X+R211H[b]	99 104	Slight parenchymal loss
4	F	10 trade	Worker	R217X	82	Mild parenchymal loss
5	M	10 trade	Unemployed	R217X	85	Obstructive hydrocephalus
6	F		Child	R43X/Normal[c]	85	Periventricular hyperintensity on T2-weighted images
7	M	8 trade[d]	Unemployed	R217X	50	Minimal parenchymal loss
8	F	10 trade[d]	Worker	R217X	67	Lacunar infract Rt Caudate
9	M[a]	8 years[d]	Sheltered Workshop	3,5,6 exon deletion	64	Atrophy of cerebellum
10	F[a]	None	Institutionalized	3,5,6 exon deletion	46	Atrophy of cerebellum. Posterior periventricular hyperintensity on T2-weighted images

[a]Siblings
[b]Double homozygotes
[c]Heterozygote
[d]Learning difficulties

Comparing the education and occupation of the Laron syndrome patients with the IQ and GH-R defect as shown in Table 38.5, we see that those Laron syndrome patients with E180, W-15X combined with R211H defects evidently had the best education and occupation. The patients with the R217X defect and a lower IQ and various mild to medium brain abnormalities had an incomplete education, and they either performed manual work or were unemployed. The patients with the exon 3, 5, 6 deletions with the lowest IQ had little or no education. The lower levels of education were due to difficulties in learning rather than socioeconomic state.

38.5.4 Discussion

Our studies on the intellectual performance of patients with Laron syndrome advance the knowledge on the relationship between IGF-I deficiency and neurofunctional capacity as well as on the influence of the type of molecular defect of the GH-R on IQ and consequences. The roles that GH and IGF-I play in the development and function of the CNS have recently been reviewed (Laron 2002, 2009). Woods et al. (1997) investigating Laron syndrome patients in other parts of the world, comprising patients from North Africa and the Mediterranean region, reported that 13.5% were mentally retarded. So are reports of the few patients with IGF-I gene deletion (Murakami et al. 1999) or IGF-R gene defect (Abuzzahab et al., 2003) as was of a patient with a STAT 5b mutation (Kofoed et al. 2003). On the other hand, Kranzler et al. (1998) reported normal intelligence in a homogenous cohort with Laron syndrome in Ecuador, all of whom had the E180 mutation in exon 6 similar to our patient with a normal IQ and normal skull MRI.

How molecular defects of the GH-R gene cause various degrees of CNS damage is unclear. All patients with Laron syndrome had very low to undetectable serum IGF-I levels, so IGF-I alone cannot be implicated as the only cause of the CNS functional abnormalities. None of the patients had thyroid hormone deficiency (see Chap. 29) and the pathological changes in the CNS detected by MRI were not consistent with those reported in hypoglycemia (see Chap. 20). Although parenchymal loss may be a nonspecific finding, the additional abnormalities such as periventricular hyperintensity, lacunar infarct, and hydrocephalus seen in patients 4–7 suggest a specific insult related to IGF-I deficiency. As

the age of the adult patients ranged between 27 and 46 years, an aging effect in the MRI findings is difficult to conceive.

38.6 Conclusions

Patients with Laron syndrome with congenital GH and IGF-I deficiencies present with a range of psychological and neurobiological deficits which are partially explained by the type of GH receptor defect and neuroanatomical lesion in the CNS.

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39 Adjustment and Rehabilitation Problems of Children, Adolescents, and Adults with Laron Syndrome

Zvi Laron

Core Message

› The adjustment to the very short stature and mobility of the patients with Laron syndrome are reviewed as encountered during the long-term follow-up of the large Israeli cohort. In early childhood there are problems in finding clothing, in school there are social problems and in adult life the quality of life is affected.

39.1 Introduction

The majority of families with children with Laron syndrome are of Jewish Oriental, Arab, and Mediterranean or Mid Eastern origin, with few exceptions to a low socio-economic class. In the previous generation, many of the parents had limited education but attempted to offer a better education to their children. The problems of adjustment in early life of patients with Laron syndrome belong mostly to the past as families with this hereditary disorder do not tend to have additional children, and the newly diagnosed patients in isolated or remote areas seem to be few. Nevertheless, there still are young children with Laron syndrome; we treat five at present and counsel two more, followed by colleagues. On the other hand, there are many adult patients, part of whom have been treated by IGF-I for various lengths of time. The adjustment problems of our patients and their parents have been reviewed in several stages (Shurka and Laron 1975; Aran et al. 1995; Galatzer 1995; and present).

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39.2 Family Attitude When the Children were Young

Since the dwarfism in this syndrome is of a hereditary nature and may occur in several children, it is liable to constitute a central problem in such a family (Shurka and Laron 1975). The parents regard their own and their children's lives as ruined and full of frustration and see a bleak future for the children. In 12 such families, the parents expressed the hope that their children would marry but were skeptical as to the realization of this hope. Only one of the fathers felt that his daughter could marry and lead a normal family life, provided that special arrangements were made with respect to certain aspects of daily life. Most of the parents doubted that their children would ever be capable of leading independent lives and were only hopeful that they might be able to learn some skill and so obtain employment. At a time when IGF-I treatment was started, parents expressed the hope that the children will reach normal height.

39.3 Clothing Problems

One of the major problems encountered, even in childhood, is the finding of clothing of a suitable size. Some parents told us that they even had to buy doll's shoes. The uniforms worn by school children in Israel come in standard sizes, so that the parents had no choice but to adapt them for their children. The older patients who wished to

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dress according to current fashion found it difficult to find anything that they would like to wear. Growth during IGF-I treatment ameliorated this state, but due to the obesity, normal sizes for age and height rarely fit.

39.4 Problems of Mobility and School Problems

One of the worst handicaps from which these patients suffer is their limited physical capacity and mobility. Laron syndrome is characterized by weak muscles and a tendency to the development of hypoglycemia. Thus, even carrying books, which may often weigh more than the child, becomes a problem of major magnitude; the new bags on wheels solve this problem. Public transportation constitutes an obstacle to the very short individual, who finds it difficult to manage the steps getting on and off the bus. (Unfortunately, we are not in Curitiba.) Most families in the socioeconomic group of the Laron syndrome patients did not have cars. At school, the children with Laron syndrome performed from poor to good (Chap. 38).

39.5 Socially

Socially, school constitutes a frustrating experience. The children very often become scapegoats and negative centers of attraction. Other children tend to laugh at them and push them around, and they can neither defend themselves nor run away easily. On the other hand, they may sometimes serve as mascots for the other students, who may carry them around or embrace them. This form of behavior is encouraged by some of the dwarfed children in self protection, while others show an unpleasant negative reaction, giving their peers an additional reason for rejecting them.

With the institution of IGF-I treatment in our pediatric population at various ages since 1988 (Chap. 42) and as the treatment only ameliorated but did not normalize growth, the problems remained. The standard school furniture is often unsuitable for these children and constitutes another problem. Some schools have provided specially constructed tables and chairs, but others have refused to do so. In the early years, some school principals refused to accept children with Laron syndrome.

Explanation of the disease nature to the teachers and peers by the psycho-social team improved the state. Nevertheless, the dwarfed patients preferred to play and socialize with younger children, and parents reported that the children did not have close friends at a young age.

39.6 Other Social Problems in Childhood

One of the most serious problems in the adjustment and rehabilitation of these patients is the fact that they cannot fail to attract attention wherever they go. In the early years, most parents related that when they went out for a walk with their affected children, they felt as though they were being looked at “like animals in the zoo.” Other well-intentioned people approached the children as though they were pets, embracing and kissing them and talking to them as though they were infants, or of a much younger age.

39.7 Counseling

Our findings clearly showed the need for intensive counseling at the family and school level, an approach which we found necessary to continue throughout puberty, vocational training, and adult age. In most instances, the psycho-social team of our department helped find an occupation through their contacts with the social services of handicapped patients and encouraged the adult patients to find partners. Some of the marriages (Chap. 10) came about with our teams’ help.

39.8 Occupation

The finding of employment does not so much depend upon the size of the patient as of his intellectual and physical capabilities. Nevertheless, they encountered discrimination, and assistance was often needed. Table 39.1 lists the occupation of 29 of our adult patients. Four female Laron syndrome patients have an academic degree. One of them is a PhD working in a laboratory, two patients (brother and sister) work in a bank and have responsible positions, one female is a secretary at a newspaper, and one very short male is an actor playing

Table 39.1 Occupation of adult patients with Laron syndrome

	Total	Females	Males
White color work	10	7	3
Blue collar work	6	4	2
Housewives	4	4	–
Sheltered work	2	–	2
On social security	7	3	4

Included are three patients deceased at the time of publication

parts adjusted to his size. Ten Laron syndrome patients (6 males and 4 females) drive a car.

39.9 Quality of Life

How the GH/IGF-I deficiency affects the social adjustment and quality of life is illustrated in Fig. 39.1. Much depends on the size and intellectual capacity of the patients, the socio-economic status of the family, their religious and ethnic origins, the attitude of the surrounding society, the local health and social insurance system, and the availability of a psycho-social team.

In Israel, the entire population has a health insurance. Once the handicapped individuals, like our Laron syndrome patients, get their driving license on a car with special features for hands handling only, they can purchase such a car tax free. The married couples with children are psychologically better adjusted; two of the mothers returned to work shortly after delivery.

39.10 Conclusions

Laron syndrome patients, whether treated or untreated, whether young or adult, and their families (parents, siblings, teachers or children, employers, and spouses in adult age) need counseling by an expert multidisciplinary team as established in our pediatric endocrine clinic as early as the late 1960s (Frankel and Laron 1968).

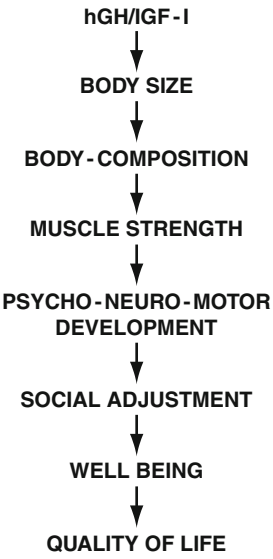

Fig. 39.1 How growth hormone and IGF-I deficits affect social adjustment and quality of life

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40 Laron Syndrome Patients with Congenital IGF-I Deficiency Seem Protected from Malignant Diseases

Zvi Laron, Rachel Steuerman, and Orit Shevah

Core Message

› Reviewing the prevalence of malignancies in our cohort of 64 Laron syndrome patients and of 102 of their first degree relatives we registered none in the homozygous patients with LS, but 11 instances of cancer in the heterozygous family members.

40.1 Introduction

Overexpression of insulin-like growth factor (IGF-I) or its receptor has been found in malignancies in children, such as osteosarcoma (Kappel et al. 1994), Ewing's sarcoma (Minniti et al. 1994), Wilms tumor, neuroblastoma, etc. (Singleton et al. 1996) and in tumors of the breast, ovaries, prostate, and colon in adults (Werner et al. 1993; Werner 2009). Epidemiological studies have indicated that patients with increased circulating levels of IGF-I, as well as those with insulin resistance and obesity, are at an increased risk for cancer (Calle and Kaaks 2004).

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40.2 Subjects and Methods

As oversecretion of GH and IGF-I is linked to a risk for malignancy, Laron syndrome with congenital deficiency of both hormones presents a unique model to examine the effect of long-term IGF-I deficiency starting in utero on the development of cancer. For this purpose, a detailed questionnaire containing information on the diagnosis of malignancies was constructed. The information on the Israeli cohort of patients was collected by personal interviews and their medical charts. Questionnaires were also completed for as many as possible first degree family members.

40.3 Results

Results were recorded until summer 2009. A summary is presented in Table 40.1. We found that out of the 64 patients with Laron syndrome from the Israeli cohort, none had any type of cancer, whereas among the 102 first degree relatives (all or the majority heterozygotes for the GH receptor defect) reported 11 instances (10.8%) of malignancy (Table 40.2). Eight of our adult patients (three females and five males) acknowledge smoking.

40.4 Comment

Our data collected from physicians treating patients in other countries have been partially published (Shevah and Laron 2007). Upon our initiative, Dr. Guevara Aguirre et al. (2007) investigated the large Ecuadorian cohort and confirmed our findings that Laron syndrome patients were protected from cancer. Among 1,032 relatives in Ecuador studied, 80 had died of cancer.

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Table 40.1 Prevalence of malignancy in the Israeli cohort of Laron syndrome patients and their first degree family members

		Total number	Malignancy
Patients	*N* M:F Age range	64 33:31 2–75 years	0
First degree relatives	*N* M:F Age range	102 44:58 6–96 years	11 (10.8%) 8:3 25–76

Table 40.2 Type of malignancies in first degree relatives of patients with Laron syndrome

Patient number	Cancer type	Age at diagnosis
1	Prostate cancer	63
2	Prostate cancer	76
3	Thyroid cancer	25
4	Lung cancer	48
5	Breast cancer	68
6	Squamous cell carcinoma, nose	65
7	Prostate cancer	80
8	Prostate cancer + testis cancer	Unknown (before 40)
9	Gastric cancer	Unknown
10	Uterus Ca	48
11	Breast cancer	75

40.5 Conclusions

The finding that patients with congenital IGF-I deficiency seem to be protected from the development of cancer raises issues with many practical clinical implications:

(a) Justification of ongoing programs to develop GH and IGF-I receptor blocking drugs as an adjunct to cancer therapy (Kopchick et al. 2002; Pollak 2004).

(b) Indication that a careful selection of adult patients programmed for either hGH or IGF-I treatment should be performed, and patients or subjects with a familial incidence may have to be excluded from these two and GHRH alike hormone treatment.

Acknowledgment The contribution of Rachel Steuerman was performed in partial fulfillment of the MD Thesis requirements of the Sackler Faculty of Medicine, Tel Aviv University.

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Lifespan and Mortality of Patients with Laron Syndrome

41

Zvi Laron

Core Message

› Once diagnosed in early childhood and severe hypoglycemia is prevented as well as diabetes adequately treated in later life, patients with Laron syndrome have a normal lifespan.

41.1 Introduction

The question whether GH and/or IGF-I influence the lifespan has occupied the mind of many scientists (Katic and Kahn 2005; Rincon et al. 2005). Of special interest was the question whether the physiological decrease of GH/IGF-I with advancing age shortens the lifespan (Laron 2005, 2008; Sherlock and Toogood 2007). Untreated patients with Laron syndrome having severe and long-term IGF-I deficiency are a unique model to test the above hypothesis, but patients with Laron syndrome are spread worldwide and with the exception of three cohorts are dispersed among many physicians and if not treated as children are lost to follow-up. Only our and the Ecuadorian cohorts are large and have been followed carefully. Forthwith the findings in the Israeli cohort.

41.2 Subjects

Figure 41.1 shows the present age (2009) of the Israeli cohort. It is seen that eight female and two male patients approach the age of 60.

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41.3 Mortality

In our cohort of 64 patients, we registered four deaths (their details are shown in Table 41.1). One was a $3^{9/12}$-year-old girl, a sister of one of our patients, who died of a CNS infection. She died before 1958. One male patient aged 50 with neglected diabetes and with complications, including cardiovascular disease, died 1 day after a cardiac catheterization. Another male patient with diabetes and vascular complications died at age 75 after a car accident with severe damage to his cervical spine. One female patient aged 54 died presumably of coronary heart disease. She was seen by us only once.

41.4 Discussion

Data reported by Guevara Aguirre et al. (2007) revealed 75 living Laron syndrome patients in Ecuador, the oldest aged 76 years. Guevara Aguirre et al. (2007) also reported that 22 children with Laron syndrome died at an early age apparently from infection and/or hypoglycemia. Six adult patients of the originally reported Ecuadorian cohort with Laron syndrome have died, five of myocardial infarction (four males aged 33, 41, 52, and 54 and one female aged 67) and one in an accident. No further information on death of Laron syndrome patients was found in the literature, but one can assume that a number of undiagnosed and untreated patients in remote locations have died.

Animal data to be reported separately (see Chap. 50) show that congenital IGF-I deficiency prolongs rather than shortens the lifespan (Bartke 2005).

Once IGF-I replacement therapy for adult patients with Laron syndrome will be approved, similar to hGH in hGH deficient patients, it will be of interest to find out whether it influences the lifespan of these patients.

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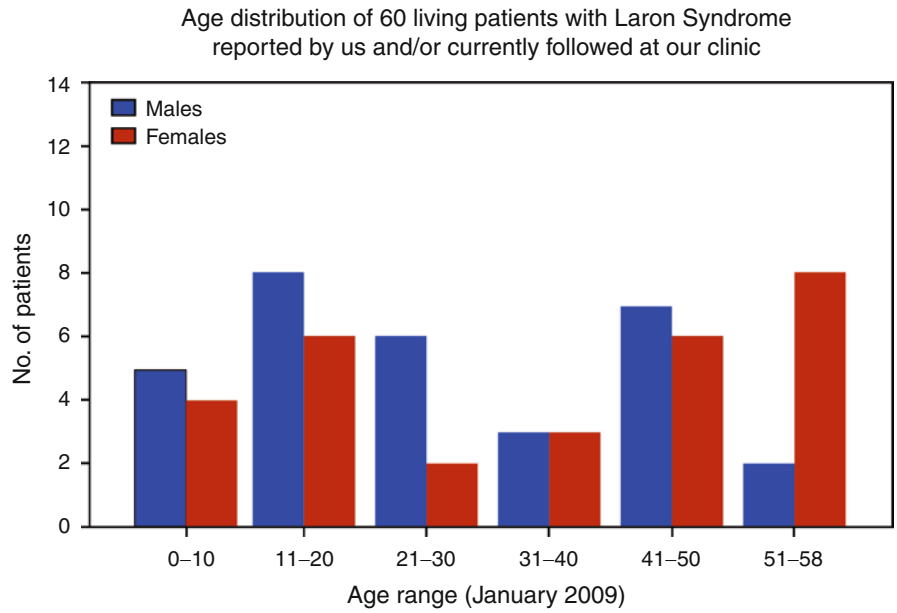

Fig. 41.1 Age and sex distribution of 60 living patients with Laron syndrome currently followed at our clinic (2009)

Table 41.1 Age and cause of death of Laron syndrome patients in the Israeli cohort

Patient name	Sex	Age at death (years)	Cause of death
Sh. Z.[a]	Female	$3^{9/12}$	Encephalitis suspected
Y.G.	Male	$49^{6/12}$	Coronary heart disease (postcatheterization)
Sg. A.	Female	54	Coronary heart disease (acute MI suspected)
Me.S.	Male	75	Road accident

[a]This patient, sister of patient ShR, died in the year 1958 before our clinic existed

41.5 Conclusion

Present knowledge shows that lifelong deficiency of IGF-I in untreated patients with Laron syndrome does not seem to markedly influence their lifespan unless they neglect to treat in time the metabolic and cardiovascular complications, such as in diabetes mellitus and the hyperlipidemia. The protection from cancer (Shevah and Laron 2007) (Chap. 40) may also positively influence their life longevity.

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IGF-I Treatment of Patients with Laron Syndrome

42

Zvi Laron

Core Message

› In this chapter we describe pharmacokinetic studies with IGF-I in children, once biosynthetic IGF-I became available. The biochemical and hormonal effects upon short-term administration and growth promoting effects upon long-term treatment are summarized. It was found that IGF-I is a potent anabolic and growth stimulating hormone. Our results are compared with those obtained by other investigators.

42.1 Background

Insulin-like growth factor I (IGF-I) is the anabolic effector hormone of the growth hormone releasing hormone (GHRH)-somatostatin-GH axis (Merimee and Laron 1996) (Fig. 42.1). IGF-I and IGF-2 are members of a large family of insulin-related peptides, which include proinsulin, insulin, and relaxin that exhibit approximately 50% amino acid homology (Blundell and Humbel 1980). The amino acid sequence of IGF-I and IGF-2 in humans and several animal species has been determined. IGF-I consists of 70 amino acids, and IGF-2 consists of 67 amino acids. The molecular weight of IGF-I is 7,649 Da and that of IGF-2 is 7,471 Da. There is 62% sequence homology between the two (Rinderknecht and Humbel 1978). "They have specific receptors, which account for their different biological activities." The gene for pre-pro-IGF-I is encoded on the long arm of chromosome 12, whereas the gene for IGF-2 is encoded on chromosome 11. *The biological half-life of IGF-I is the longest among the hormones of the GH axis* (*8–16 h*). This is due to its interaction with its binding proteins (BPs) (Jones and Clemmons 1995; Zapf et al. 1996). There are six IGFBPs, and their levels vary from one IGFBP to another and are dependent on age and physiological or pathological conditions. The most important IGFBP is IGFBP-3. In serum, most of this IGFBP circulates as a 150 Kd complex that consists of IGF-I (or IGF-2) plus IGF-BP3 and a component termed acid-liable subunit (ALS). In contrast to IGFBP-1 and -2, IGFBP-3 is regulated by pituitary GH and to some degree by IGF-I itself (Kanety et al. 1997). This property of IGF-I to stimulate the generation of its own specific major carrier (binding) protein is supported by findings of Zapf et al. (1989) who showed that IGF-I increased IGFBP-3 in hypophysectomized rats and by "in vitro" studies using hepatocytes (Uchijima et al. 1995; Scharf et al. 1996). It is unlikely that IGFBP-1 plays a role in prolonging the IGF-I bioavailability as its levels are suppressed by IGF-I (Laron et al. 1992a, b).

The IGFs are present in the circulation and throughout the extracellular space almost entirely bound to their BPs. All the BPs share structural homology with each other and specifically bind the IGFs. Their functions are to regulate the availability of free IGF-I and its biological functions (see also Chap. 27). IGF-I acts via its own receptor, which resembles that of insulin. A hybrid receptor between IGF-I and insulin has also been reported (Werner et al. 2008). These types of receptors belong to a family of tyrosine kinase receptors. The genes for these receptors encode single polypeptides that are cleaved into α and β subunits, which form a dimer

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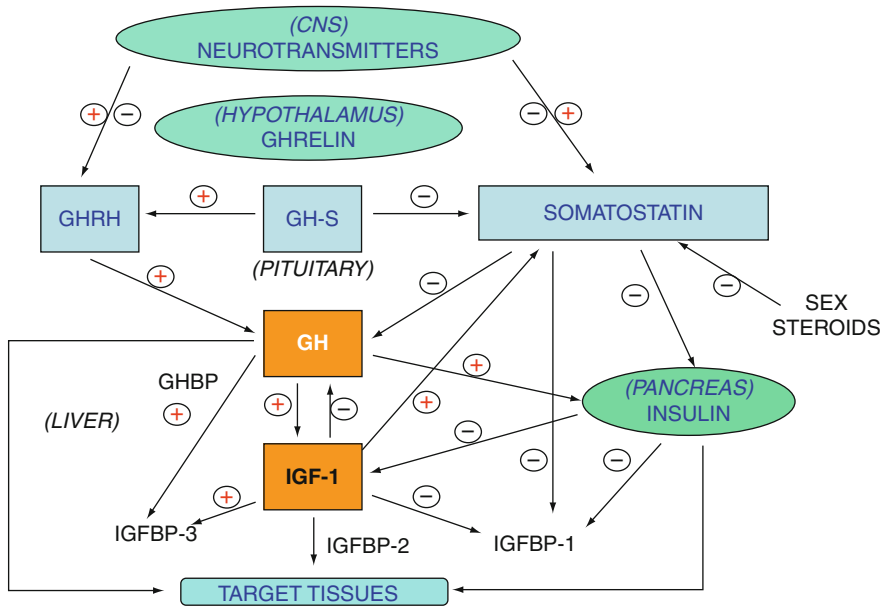

Fig. 42.1 The GHRH-GH-IGF-I axis

via disulphide bonds into heterotetrameric (α_2 and β_2) mature receptors (LeRoith et al. 1996). Generation of IGF-I in the liver is controlled mainly by GH, but insulin also has some stimulating effect. Figure 42.1 illustrates the complex regulatory interactions of and with IGF-I.

The biosynthesis of recombinant IGF-I (Somatomedin-1 or -C) (Niwa et al. 1986) enabled the design of specific radioimmunoassays and thus the quantitative determination of IGF-I levels in the circulation and tissues, as well as allowing pharmacological trials in animals and humans. IGF-I treatment is uncontestably indicated as replacement therapy in patients with primary GH insensitivity (Laron syndrome) and IGF-I gene defects who do not synthesize IGF-I, and in patients with GH gene deletion who developed antibodies when treated by recombinant biosynthetic hGH.

42.2 Introduction

Our experience with IGF-I started soon after its biosynthesis and we were the first to test its pharmacology and biological effects in children with Laron syndrome, having been shown previously to be safe and active in healthy adults (Guler et al. 1987).

As there was no previous experience in children when we received recombinant biosynthetic IGF-I from Fujisawa Pharmaceutical Co., Osaka, Japan in 1989, we had to start from scratch to study its pharmacology and effects in young patients, and later in older ones. Our studies were progressive, and the Laron syndrome patients, their families, and certainly our team had great expectations from what we hoped would cure all defects of this disease causing severe growth retardation and many other physical and metabolic abnormalities.

The publications of our findings did not follow the order of the studies, but the summary will. We used recombinant biosynthetic IGF-I in all our studies reported in this book (Niwa et al. 1986). Mecasermin provided by Fujisawa labeled FK-780. The preparations had an identical amino acid sequence to that of human IGF-I. The purity of the preparation was stated to be 99% and that of Met 59 (0)-IGF-I 2.1%. The batch numbers will be mentioned in the various studies when indicated.

FK-780 was available in 10 mL vials containing 10 mg of lyophilized h-IGF-I, which remains stable for very long periods of time if kept refrigerated.

All investigations and treatments were approved by the Hospital Ethical Committee, and the parents of patients or subjects signed an informed consent form. Since summer 2009, we started to use IGF-I (Increlex by Tercica-IPSEN, USA). This preparation comes in 4 mL vials containing 40 mg IGF-I in solution. The manufacturers recommend that the vial be used within

1 month of being opened. It is the same preparation as marketed previously by Genentech and Kabi.

Following is our progressive experience using IGF-I in patients with Laron syndrome and healthy controls when possible. The stages of use of IGF-I since 1989 in our group were: (a) pharmacokinetic and biochemical effects; (b) as clinical trials; (c) as an approved orphan drug; and in recent years (d) as an approved drug with specific indications, the cost being covered by the National Insurance policy.

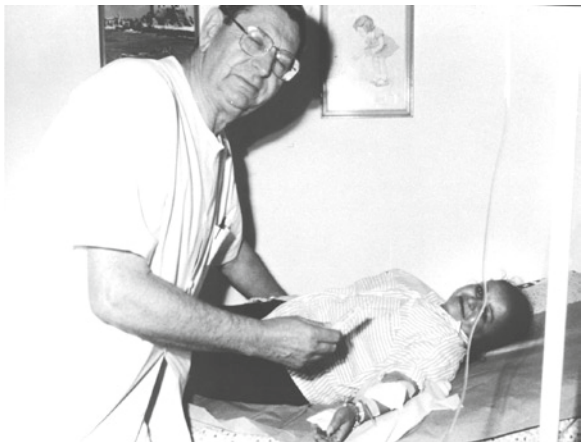

Fig. 42.2 Zvi Laron administers the first injection to YR

42.3 Initial Clinical Trials with IGF-I

42.3.1 Pharmacokinetic Studies

42.3.1.1 Subjects and Methods

Freshly dissolved IGF-I (FK-780 Lot 144770K) in a dose of 75 μg/kg was injected i.v. as one bolus to Laron syndrome patients of both sexes (3 children aged 9–11 years and 7 adults (mean age 30±3.5 years)) as well as to 6 healthy controls (3 children aged 8–13 years and 3 adults aged 28–40 years) (Klinger et al. 1990) (Fig. 42.2).

Blood samples were drawn through an indwelling catheter into nonheparinized tubes at −30, 0, 2, 15, 30, 60, 90, 120, and 180 min and at 6, 8, 10, and 24 h. IGF-I was determined after acid extraction using the Incstar kit (USA) (Silbergeld et al. 1988). As the rIGF-I was twice as potent in RIA as the native hormone, values after the injection of the exogenous hormone were corrected accordingly. *The values obtained were higher than presently used standards*. The apparent distribution rate constant ($K_{e\alpha}$) and the elimination rate constant ($K_{e\beta}$) were estimated by the negative slope regression line fitted by the method of least squares (slope = K_e/2.303). The elimination half-life ($t_{1/2\beta}$) was calculated using the formula $0.693K_{e\beta}$ (Gibaldi and Perrier 1975).

42.3.1.2 Results

The pharmacokinetic parameters expressed as mean ± SD for the two groups are shown in Table 42.1. It is seen that the basal endogenous serum IGF-I levels in the Laron syndrome patients were significantly lower (39.8 ± 21 ng/mL) than those of the control subjects (206.7 ± 71 ng/mL) ($p < 0.01$). The distribution half-life ($K_{e\alpha}$) was similar in the two groups, but $K_{e\beta}$ and the estimated half-time of the injected IGF-I were significantly shorter in the Laron syndrome patients: 2.57 ± 0.67 vs. 4.43 ± 0.52 min ($p < 0.01$). The values after 24 h were similar to the basal levels.

The faster elimination rate of injected IGF-I in the Laron syndrome patients compared to the healthy subjects (Fig. 42.3) is due to the deficiency of IGFBP-3. Comparable findings were reported by Zapf et al. (1986) in hypophysectomized rats. *The biological half-life of IGF-I in healthy adult subjects was found to be 8–16 h* (Guler et al. 1989).

Table 42.1 Pharmacokinetic parameters of an intravenously injected insulin-like growth factor I (IGF-I) bolus (75 μg/kg) to patients with Laron syndrome and matched controls

Group	*n*	Endogenous IGF-I (ng/mL)	$K_{e\alpha}$ (min^{-1})	$K_{e\beta}$ (min^{-1})	$T_{1/2\beta}$ (min)
Laron syndrome	10	39.82 ± 21.2	0.306 ± 0.01	0.0045 ± 0.0011	2.57 ± 0.67
Controls	6	206.72 ± 71.4	0.377 ± 0.02	0.0026 ± 0.0087	4.43 ± 0.52
p		0.01	N.S.	0.01	0.01

Data as mean ± SD
Modified from Klinger et al. (1990)

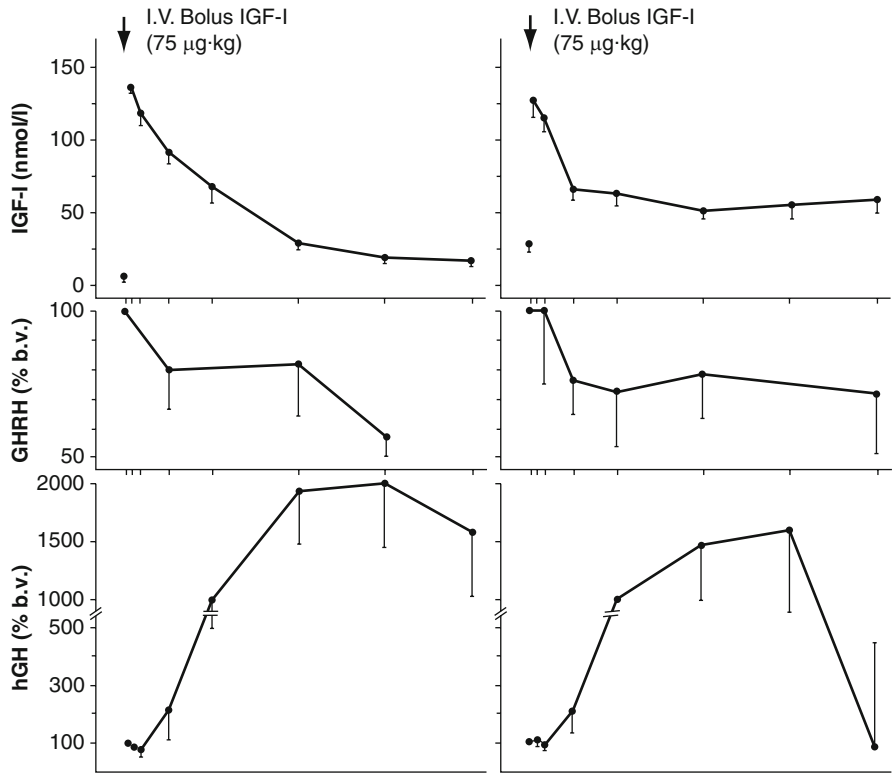

Fig. 42.3 Serum insulin-like growth factor I (IGF-I), growth hormone releasing hormone (GHRH), and hGH levels after a bolus injections of 75 μg/kg i.v. to ten patients with Laron syndrome and six healthy controls. Modified from Klinger et al. (1990)

42.3.2 Acute Biochemical and Endocrine Effects of Intravenously Injected IGF-I

An i.v. bolus of IGF-I (75 μg/kg) was injected to 9 patients with Laron syndrome (2 children aged 11 years and 7 adults aged from 25 to 34.8 years). The mean (±SEM) basal values of glucose were 75.6 ± 3.6 mg/dL, insulin: 5.3 ± 0.8 mU/mL and hGH 7.5 ± 2.1 ng/mL. The IGF-I injection resulted in a rapid onset of hypoglycemia (−50% of basal level) concomitantly with a reduction in plasma insulin (−55% of basal level) (Laron et al. 1988) (Fig. 42.3) as was also found in healthy patients by Guler et al. (1987). The reduction in insulin by IGF-I denotes that the induced hypoglycemia was an independent effect of IGF-I and not insulin mediated showing that IGF-I deserves its name. We showed that the insulin-suppressing effect of IGF-I is caused by the IGF-I stimulation of hypothalamic somatostatin secretion (Gil-Ad et al. 1996).

The concomitant determination of serum hGH, GHRH, and TSH (Laron et al. 1990) revealed also an acute reduction of hGH followed by a rebound more pronounced in the seven adult patients than in the two young Laron syndrome patients (Fig. 42.4). We found a similar rebound after somatostatin infusion (Laron et al. 1977) (see Chap. 2, Fig. 2.7). The lowering effect of serum hGH by IGF-I is explained by the suppression of serum GHRH (Laron et al. 1990) and the stimulation of somatostatin (Gil-Ad et al. 1996) by IGF-I. Somatostatin on the other hand inhibits the secretion of insulin, GH, and TSH explaining the indirect effects of IGF-I (see Chap. 29).

42.3.3 Seven Days Subcutaneous IGF-I Administration

42.3.3.1 Subjects

Ten patients with Laron syndrome, 5 children (3 boys aged 3.6–14.5 years and 2 girls aged 9.3–11.10) and 5 adults (1 male and 4 females, aged 28.6–37.9 years), were enrolled in this study.

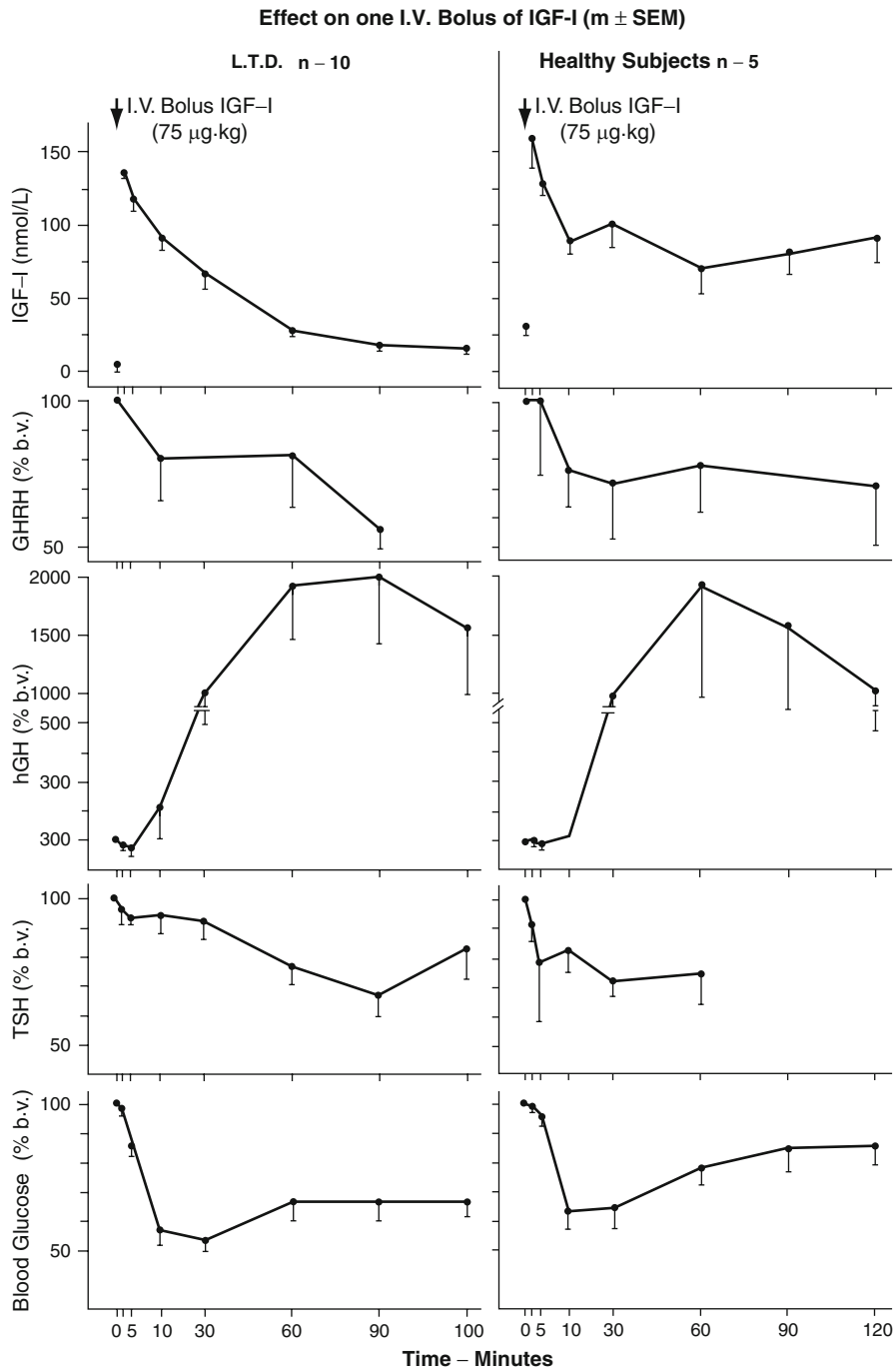

Fig. 42.4 hGH and TSH response to an intravenous bolus of 75 μg IGF-I to ten Laron syndrome patients and five control subjects. Reprinted with permission from Laron et al. (1990)

42.3.3.2 Methods

IGF-I (FK 780, lot 137889K) was administered s.c. every morning with breakfast. The dose was 150 μg/kg once daily to 7 patients (5 children and 2 adults) and 120 μg/kg/day to 3 adult patients. Blood samples were drawn before (day 0) and after 1 and 3 injections (days 2 and 4), and after 7 injections (day 8), and 7 days after the last injection (day 15) (Laron et al. 1991).

The following parameters were analyzed: hematological (red blood cells, RBC, Hb, WBC, thrombocytes) by Blood Counter Cell Dryer; blood chemistry (glucose cholesterol, triglycerides, proteins, Ca, PO_4, alkaline phosphates, creatinine, BUN, electrolytes, SGOT, LDH) by Hitachi autoanalyzer; serum type III procollagen (PIIINP) by RIA (in the laboratory of Dr. L.T. Jensen, Copenhagen).

Plasma hGH was determined by a modification of a double antibody RIA (Laron and Mannheimer 1966), which used hGH-RP-1 as standard (donated by the National Pituitary Agency, Baltimore, USA). Plasma insulin was measured by a double antibody RIA (Heding 1972) with an insulin standard donated by Novo Research Institute (Denmark). Plasma TSH was determined by immunoradiometric assay (Magnetic solid phase, Serono). Free T4 was determined by a standard RIA. Statistical analysis was performed using Student's paired and unpaired *t* test.

42.3.3.3 Results

The drug was well tolerated by the children, even by the 3½-year-old patient, who had previously experienced asymptomatic hypoglycemia from time to time as checked by the mother with a glucometer and was always craving for sweet food or drinks before initiation of the IGF-I injections. Upon repeated questioning, five adults complained of occasional headache during the trial but there was no evidence of hypoglycemia. Transitory water retention was suspected.

Basal blood glucose levels fluctuated between 73 and 92 mg/dL.

Growth hormone: The elevated basal serum hGH levels (up to 148 μg/mL) were suppressed and remained so during the whole week of treatment. One week after termination, they increased again (Fig. 42.5).

Insulin: Basal serum insulin was elevated in one child and two adults, probably related to the marked obesity in these patients. Insulin was significantly suppressed ($p<0.03$) already after 3 days of IGF-I treatment and in most patients returned to pretreatment values after further 4 days of treatment (Fig. 42.5).

Procollagen: A striking finding was the rise in type III procollagen, which, increased significantly from 4.2 ± 0.9 to 7.35 ± 1.9 ng/mL (mean ± SD) ($p<0.0003$) (Fig. 42.6). No estimation was performed after stopping the administration of IGF-I.

Lipids: Basal serum total cholesterol was above normal or at the high level of normal for age in all but the youngest patients (227 ± 88 mg/dL) (mean ± SD). IGF-I caused a slight but statistically significant reduction ($p<0.004$), which in six patients was still maintained 1 week after discontinuation of the IGF-I (Fig. 42.7).

The mean basal values of serum triglycerides were 144 mg/dL and showed little variation during or after IGF-I administration.

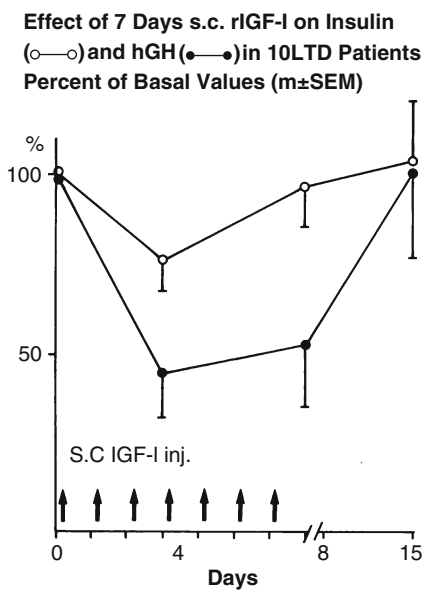

Fig. 42.5 Effect of 7 days of s.c. administration on serum hGH (*closed circle*) and insulin (*open circle*) in ten patients with Laron syndrome. As percent of basal values (mean ± SD). Reproduced with permission from Laron et al. (1991)

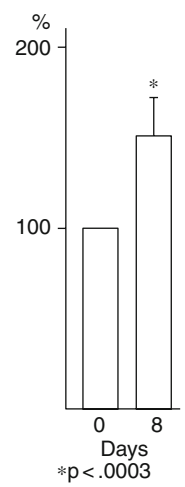

Fig. 42.6 Effect of 7 days of s.c. administration of IGF-I on serum type III procollagen (PIIINP) in eight patients with Laron syndrome. As percent of basal values (mean ± SEM), *$p<0.0003$

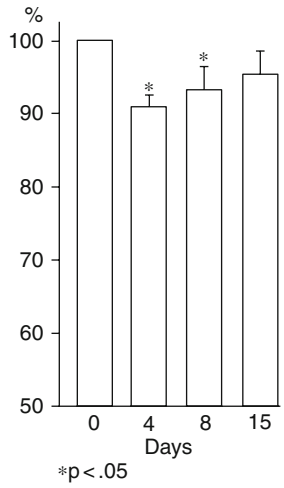

Fig. 42.7 Effect of 7 days of s.c. administration of IGF-I on serum total cholesterol in ten patients with Laron syndrome. As percent of basal values (mean ± SEM), *$p < 0.004$

Liver function tests: Four out of nine patients had marginally elevated values (39–42 U/L) of GOT considering 35 U/L as the upper normal limit. IGF-I administration induced a significant decrease ($p < 0.01$) of GOT levels after 3 days of administration in all the seven patients for whom pretreatment values were available. After further administration, the levels rose to higher than basal level (up to 50 U/L) to return to pretreatment values 7 days after discontinuation of IGF-I.

The basal LDH levels were elevated or at the upper limit of normal – 305–311 U/L (norm < 100–225 U/L) – in 7/10 patients, and especially high levels were registered in the youngest patient (505 U/L). In 9/10 patients, IGF-I administration led to a significant decrease in these values ($p < 0.045$) after 3 and 7 days of treatment, respectively, whereas stopping IGF-I administration led to a return to pretreatment values in the majority of patients (see also Chap. 31).

Thyroid functions: Plasma TSH and fT4 were significantly reduced ($p < 0.005$) but within the normal range after 3 days of IGF-I administration and returned to pretreatment values during continuation of the treatment (plasma TSH) or upon discontinuation of the IGF-I (plasma fT4) (see also Chap. 29). No effect on blood electrolytes and proteins were registered.

42.3.3.4 Comment

Despite its hypoglycemic effect when injected as a bolus, IGF-I did not cause hypoglycemia when administered s.c. with a meal. The causal factor is assumed to be the IGF-I-induced reduction in insulin secretion (see long-term treatment).

42.3.3.5 Discussion

Walker et al. (1991) reported the effects of a continuous 6 days of i.v. infusion of IGF-I (Genentech, USA) 16–24 μg/kg/h to an 8.9-year-old boy with Laron syndrome. The main effects were a 56% decrease in serum urea nitrogen, a twofold increase in urinary calcium excretion, a 30% decrease in the urinary phosphate excretion, an increased urinary sodium excretion, and a creatinine clearance, without change in serum N or K. The above results resembled those registered during administration of hGH (Dahms et al. 1989; Harbison and Gertner 1990). Of note are the appearance of urinary ketones in Walker's patient and that 8/32 urine samples tested positive for glucose 1–4 h after the most recent meal; as were episodes of hyperglycemia during the IGF-I infusion period, explained by our finding that IGF-I inhibits insulin secretion (Laron et al. 1988) and the large doses of IGF-I administered by these investigators.

Table 42.2 summarizes the hormonal changes induced by IGF-I administration, compared to the state in IGF-I deficiency.

Table 42.2 Hormone levels in patients with Laron syndrome

Hormone	Laron syndrome patients	
	Untreated	IGF-I treated
Somatostatin	↓	↑
GHRH	↑	↓
hGH	↑↑	↓
GHBP	↓ or N or ↑ depending on GH-R mutation	↓
Insulin	INS/GLUC ↑	↓
IGF-I	↓	↑
IGFBP-1	↑	↓
IGFBP-3	↓	±↑
IGFBP-2	N	?
TSH	N	±↓ temporary
T4	N	±↓ temporary
PRL	N or ±↑	↓
FSH	N	N
LH	N	±↑
Androgens	N	±↑
Sex hormone BG	N	↓

N normal; ↓ low; ↑ high ? not known

42.3.4 Study of the Modulation of IGF-I Binding Sites and Affinity to Red Blood Cells (RBC) During Daily IGF-I Administration

42.3.4.1 Subjects

Eight patients with Laron syndrome, 3 adults (1 male, 2 females, age range 26–31 years) and 5 children (3 males, 2 females, mean age 8.9 ± 5.0 years (mean ± SD)), were included in this study (Eshet et al. 1993). As controls served 26 healthy subjects, 4 adults (3 males, 1 female, age range 34–55 years) and 22 children (16 males, 6 females, mean age 8.9 ± 3.0 years (mean ± SD)).

42.3.4.2 Methods

In the Laron syndrome patients, blood was sampled 3 times, before starting the study, and 1 week and 1 month after initiation of IGF-I administration. In the controls, only one blood sample was taken. IGF-I (rIGF-I FK 780, Lot 115707K, Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) 120–150 μg/kg/day was administered s.c. Blood samples (20 mL heparinized blood) were drawn after an overnight fast (10–12 h). Red cell binding studies were performed immediately, and plasma aliquots were frozen at −20°C until assayed. For detailed laboratory methods, see Chap. 2 and Eshet et al. (1993).

42.3.4.3 Results

The individual findings in the eight Laron syndrome patients and the mean ± SEM values of the controls are shown in Table 42.3. It is seen that basal fasting serum IGF-I averaged 156 ± 16 ng/mL (mean ± SEM) in the control group compared to 36 ± 6.4 ng/mL in the eight Laron syndrome patients ($p = 0.0001$). After 1 week of IGF-I treatment serum, IGF-I levels increased to 50 ± 12 ng/mL ($p < 0.05$) and after 1 month of treatment to 109 ± 35 ng/mL ($p < 0.03$). Concomitantly, we found a significant decrease in the number of high affinity IGF-I binding sites from 5.74 ± 0.86 sites/cell (mean ± SEM) in the nontreated state to 2.29 ± 0.64 sites/cell and after 1 week to 2.17 ± 0.53 sites/cell ($p < 0.004$), and after 1 month of IGF-I treatment ($p < 0.002$), values similar to those were found in the control group. These data demonstrate that replacement treatment of Laron syndrome patients with IGF-I downregulate its specific receptors.

42.3.4.4 Comment

We assume that all tissues with IGF-I receptors react in the same way as the RBC.

42.3.5 Finding the Optimal IGF-I Dose

In order to determine the optimal dose, which is active and devoid of adverse effects and the optimal frequency of injection, we measured the serum IGF-I concentrations of two doses of IGF-I (FK 780 Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) 75 and 150 μg/kg injected s.c. to nine prepubertal Laron syndrome children (Laron et al. 1999). Blood samples were taken before the injection (in the morning before breakfast, and then after 15, 30, 60 min, 1, 2, 3, 4, and 24 h). During this time, the patients followed the regular activity and meals. The mean ± SEM serum IGF-I concentrations in the nine prepubertal children are shown in Fig. 42.8. It is seen that already at 1 h after injection, serum levels of over 250 ng/mL were reached with a dose of 150 μg/kg and maintained for 4 h or more. After 24 h, a mean serum concentration 100 ng/mL was found. With the 75 μg/kg dose, lower serum IGF-I concentrations were measured. We therefore decided to use in children doses ranging between 150 and 200 μg/kg/day s.c.

We use a 150–180 μg/kg/ as the initial dose in any new child with Laron syndrome to test its sensitivity to IGF-I.

An adequate dose is expected to yield a fasting overnight IGF-I level of 150–250 μg/mL or more, a suppressed serum hGH level and stimulate body length.

A high serum hGH, low IGF-I, and less than expected growth velocity (for year of treatment) are interpreted as an inadequate IGF-I dose or wrong injection. This was encountered in very young children necessitating small doses, the use of too-small needles, and injection into the adipose tissue rather than s.c. We encountered this recently in small children using the very concentrated INCRELEX (Tercica-IPSEN) preparation. It also occurred by lack of rotating the injection

Table 42.3 Effect of IGF-I treatment on serum IGF-I levels, specific binding to red blood cells (RBC), IGF-I binding sites per red blood cell, and their affinity constant in eight patients with Laron syndrome

Laron syndrome No.	Age	Sex	Serum IGF-I (ng/mL)[a]			Percent specific binding			Number of sites per cell			Dissociation constant (nM)		
			Before Rx	1 week Rx	1 month Rx	Before Rx	1 week Rx	1 month Rx	Before Rx	1 week Rx	1 month Rx	Before Rx	1 week Rx	1 month Rx
1	3	M	21	18	–	18.7	16.0	–	5.6	3.0	–	2.34	0.05	–
2	4[3]	F	9	20	38	11.1	8.5	9.8	4.8	1.1	0.7	0.15	0.03	0.01
3	10[1]	M	20	15	30	15.2	14.3	10.5	5.0	2.0	1.7	0.17	0.12	0.31
4	13[2]	F	53	74	65	17.0	8.0	9.9	7.0	2.7	2.8	0.29	0.15	0.07
5	14	M	38	42	40	8.6	11.7	11.3	6.5	6.3	1.0	0.39	0.14	0.03
6	26	M	58	67	286	13.8	9.7	9.2	4.2	1.3	3.8	0.09	0.05	0.29
7	30	F	53	112	108	10.5	9.5	11.1	2.9	0.5	0.7	0.04	0.004	0.01[a]
8	31	F	33	51	198	19.1	21.6	10.5	10.5	1.2	4.3	0.48	0.01	0.34
Mean			36	50	109	14.25	12.41	10.32	5.74	2.29	2.17	0.49	0.07	0.15
± SEM			6.4	12	35	1.39	1.64	0.26	0.86	0.64	0.53	0.27	0.02	0.05
p				0.05	0.03		N.S.	0.03		0.004	0.002		N.S.	N.S.
Controls ($n=26$)														
Mean			156			8.46			2.94			0.28		
± SEM			16			0.57			0.29			0.07		

p = by paired *t* test, as compared to Laron syndrome before treatment[a]The overnight fasting IGF-I levels do not mirror the peak levels obtained 4–6 h after injection
Modified from Eshet et al. (1993)

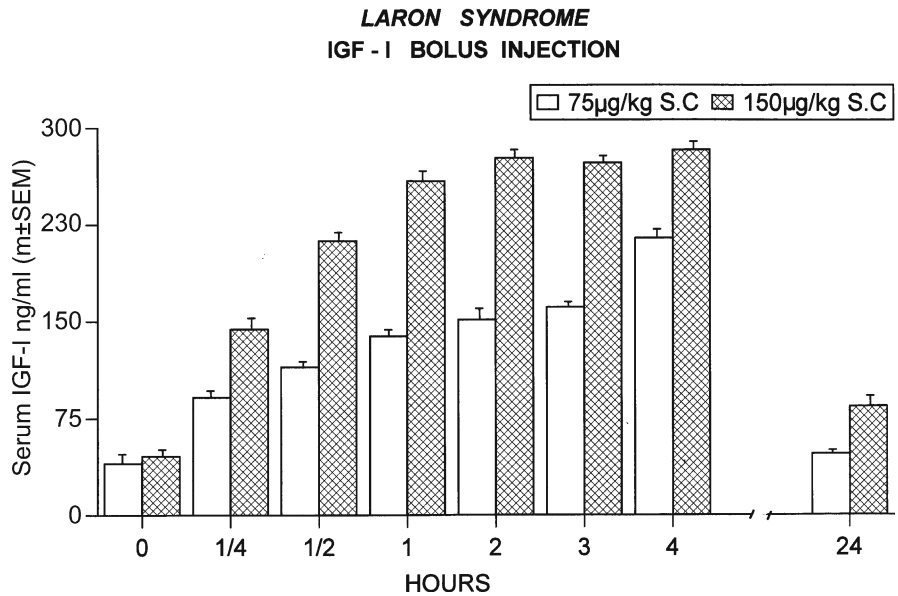

Fig. 42.8 Serum IGF-I levels after one s.c. injection of 75 or 150 µg IGF-I to nine children with Laron syndrome

site causing lipohypertrophy and injecting into such a site. In very small children, we advised that injection of the IGF-I be administered with dinner rather than with breakfast to shorten the daily half-life. Figures 42.9 and 42.10 show the progressive rise of overnight fasting and 4-h postinjection IGF-I levels during the first years of treatment of prepubertal children with Laron syndrome. It is evident that mean basal levels of IGF-I rose progressively during the first year of treatment from 24 ± 6 to 76 ± 13.4 ng/mL and remained subsequently at a mean serum concentration of 66 ng/mL. The 4-h postinjection serum IGF-I levels also rose from a mean of 237.1 ± 26.6 to 365 ± 15 ng/mL at 12 months of treatment and to 346 ± 15 ng/mL after 3 years of IGF-I treatment.

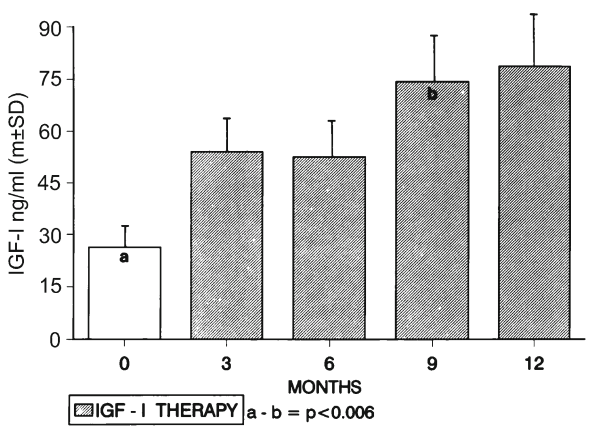

Fig. 42.9 Overnight fasting serum IGF-I in eight prepubertal children with Laron syndrome during their first year of IGF-I treatment (the injection was administered 24 h before)

42.3.5.1 Comment

Considering that the half-life of IGF-I may be too short to cover 24-h activity, other investigators in Europe (Ranke et al. 1995, 1999) and in the US (Backeljauw and Underwood 1996; Backeljauw et al. 2001) have decided to use two daily s.c. injections of IGF-I.

42.4 Short- and Long-Term IGF-I Treatment of Children with Laron Syndrome

42.4.1 Effects of 3–10 Months of IGF-I Treatment

42.4.1.1 Patients and Methods

We studied three boys and two girls with Laron syndrome, all of whom were of prepubertal age (3.3–14.5 years) (Laron et al. 1992a, b; Laron 1993). IGF-I (150 µg/kg FK 780 Lot 115707K) was injected s.c. once daily with breakfast. The dose was adjusted

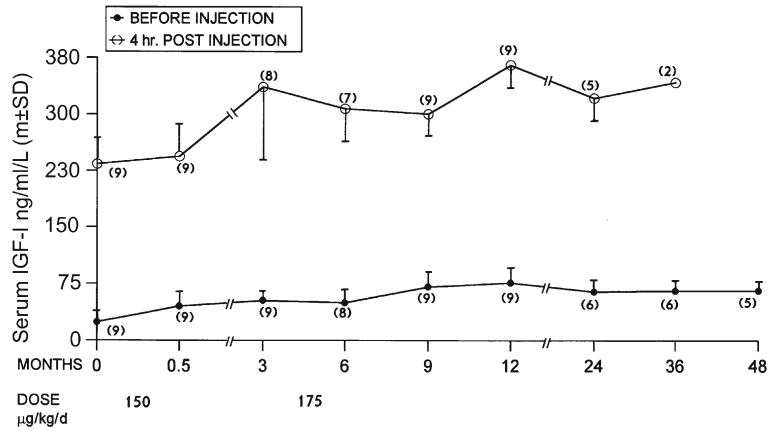

Fig. 42.10 Basal serum IGF-I 4 h before and after the daily s.c. injection of IGF-I in children with Laron syndrome (values as mean ± SEM; *n* number of children). Modified from Laron et al. (1999)

to reach a serum concentration of about 190 ng/mL 4 h after the injection. Harpenden stadiometers were used by the same two nurses for monthly recumbent and standing anthropometric measurements; the investigators also measured head circumference with a tape measure, finger span and suprailiac, triceps, and subscapular skinfolds with a Harpenden caliper. Bone age was estimated by Greulich and Pyle (1959), from a hand and wrist radiograph, with separate readings for carpal and phalangeal bones, and an average taken. Height was plotted on Laron syndrome growth charts (Laron et al. 1993) and head circumference on Nellhaus (1967) charts. Monthly blood samples taken after a 12-h overnight fast were analyzed for complete blood chemistry (SMA) by a Hitachi autoanalyzer and insulin by using the method of Heding (1972).

42.4.1.2 Results

IGF-I administration was associated with a striking growth-promoting effect, as shown by the change in linear growth velocity (Table 42.4). This effect could already be observed 1 month after the initiation of IGF-I treatment and was greater in younger children (Laron et al. 1992a, b).

Growth was not confined to the limbs but also included the trunk as measured by sitting height. There was also a striking increase in head circumference, even in children aged 13½ and 14½ years old (see also Chap. 43). This finding was similar to that described in young GH deficient children treated by hGH (Laron et al. 1979).

Despite the observed weight gains (in all except one patient), the skinfold thickness (fat tissue) was reduced in four obese patients. Fasting blood concentrations of total and HDL cholesterol, blood urea nitrogen, and creatinine showed little change during this period of treatment. In three patients, plasma insulin showed a striking fall (by over 50%) during IGF-I administration; the patients older than 10 years had blood glucose concentrations of over 110 mg/dL during routine home testing in the first month of treatment. During the second month, glucose concentrations were stable in the normal range and HbA1 values were normal. Two patients aged 3.5–5 years who the parents reported to have had persistent hypoglycemia before treatment, related that during IGF-I treatment, the young children had less hunger and less awakening episodes during the night. IGF-I administration was well tolerated by all patients.

Following these encouraging results, we engaged in long-term treatment in as many children as possible.

42.5 Long-Term IGF-I Treatment of Children with Laron Syndrome

42.5.1 Linear Growth

42.5.1.1 Subjects

We have treated 18 children with IGF-I for long periods of time, the longest being 15 years. The age of initiation varied depending upon the age at referral; eight were below age 3.5 years (Table 42.5).

Table 42.4 Effect of 3–10 months of IGF-I administration on linear growth, body weight, and skinfold thickness in children with Laron syndrome

Pt. no.	Sex	Before treatment							At last examination						
		CA (years)	BA (years)	Height (cm)	Growth velocity[a] (cm/year)	Sitting height (cm)	Weight (kg)	Subscap. skinfold (mm)	IGF-I treatment (months)	Height (cm)	Height gain[b] (cm)	Growth velocity (cm/year)	Sitting height (cm)	Weight (kg)	Suscap. skinfold (mm)
4	M	3^{3}	1^{5}	69.0	2.8	41.7	6.8	9	3	72.4	3.7	13.6	44.0	7.6	10.0
3	M	5^{1}	1^{8}	70.3	3.1	41.5	6.7	13	4	74.0	3.7	11.1	45.0	7.8	8.8
5	F	11^{6}	11	118.8	4.3	68.0	34.6	30	3	121.4	2.6	10.4	69.0	36.5	23.0
1	F	13^{7}	11	112.5	4.5	67.0	33.3	30	10	121.8	9.3	11.2	70.0	36.7	24.0
2	M	14^{5}	9	110.3	5.8	62.0	25.5	21	10	117.6	7.3	8.8	67.5	29.1	14.6

CA chronological age; *BA* bone age; *Subscap* subscapular
[a]Calculated for 1 year before start of treatment
[b]Calculated for period of treatment
Modified from Laron et al. (1992a, b)

Table 42.5 Children with Laron syndrome of the Israeli cohort treated with IGF-I

Pt	Name	Sex	Started therapy			Stopped therapy			Dose of IGF-I (μg/kg/day)	Present CA years December 2009
			CA (years)	BA (years)	Pubertal stage (tanner)	CA (years)	BA (years)	Pubertal stage (tanner)		
1	AlS	M	7/12	3/12	1	[a]9	4 9/12	1	75 → 100 → 120 → 150 → ?	18
2	AlA	M	1 7/12	6/12	1	[a]3 6/12	1 6/12	1	1.0 → 1.2 μg/day → ?	12 6/12
3	PR	F	1 7/12		1				130 →	9 5/12
4	CO	M	1 10/12	9/12	1				150 → 225 →	3
5	ArL	M	2 6/12		1				120 → 80 → 120 → ?	20 2/12
6	AbJ	M	3 1/12	2	1	[a]4 9/12	~3	1	150 → 175 → ?	21 1/12
7	HuN	F	3 2/12	2 6/12	1				162.5 →	8 11/12
8	AlB	M	3 5/12	1 6/12	1	[a]12 4/12	10	1	150 → 175 → ?	21 9/12
9	CLH	F	3 6/12	1	1				160 →	13 6/12
10	HoS	F	3 8/12	2 6/12	1	17 2/12	15 9/12	4–5	150 → 175 → 180	20 1/12
11	AbN	F	4 3/12	3 6/12	1	[a]5 11/12	~5	1	150 → 175 → ?	22 3/12
12	AlG	M	5 1/12	1 9/12	1	[a]14 7/12	11 6/12	2	150 → 175 → 190 → 200 → 190 → ?	22 6/12
13	SaL	F	6 10/12		1	[b]			160 → 180 → ?	13 11/12
14	MaM	F	9 7/12	4 6/12	1	[a]15 4/12	13 6/12	4	200	17 5/12
15	AbB	M	10 2/12	7	1	[a]11 10/12	~9	2	150 → 175 → ?	28 2/12
16	DS	F	11 8/12	11	1	14 10/12	14	5	150 → 175 →	29 10/12
17	ATK	F	13 9/12	11	1	21 2/12	~18	5	150 → 80 → 50 → 150	32 6/12
18	ML	M	14 6/12	9	1	20 7/12	17	4–5	150 → 175 → 200	33 3/12

[a]Data of patients lost for further follow-up
[b]Treated abroad with our counsel
? not known

42.5.1.2 Methods

IGF-I until 2008, Mecasermin (FK-780 Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) and recently INCRELEX (Tercica-IPSEN) was injected s.c. by the parents and by a nurse in two instances in doses of 150–200 μg/kg once daily.

42.5.1.3 Results

We have reported previously the growth-promoting effects of IGF-I in children with Laron syndrome after 1 year (Laron 1993), 3 years (Klinger and Laron 1995), and up to 9 years of treatment (Laron 2008). Analyzing the growth velocity every 6 months in eight children with Laron syndrome (Fig. 42.11), it is evident that after increasing from a mean of 4.6 ± 1.3 cm/year before initiation of treatment to 8.2 ± 0.8 cm/year ($p < 0.0004$), the growth velocity decreased to 6.0 ± 1.3 cm/year in the second year and returned to 4.8 ± 1.3 cm/year in the third year that is a similar velocity to that in the pre-treatment period (Klinger and Laron 1995), meaning that the main gain is in the first year of treatment.

Duration of Treatment

Treatment was stopped when reaching final height and closure of the cartilage epiphyses of the hand and long bones. In seven Laron syndrome children, the parents interrupted treatment under various pretexts despite the fact that the drug was supplied at no cost. Six children (1 boy and 5 girls) continue IGF-I treatment.

42.5.2 Effect of IGF-I Treatment on Skeletal (Bone) Age

Bone age was estimated using Greulich and Pyle (1959) from a hand and wrist X-ray performed at least once a year. The reading was done by either Zvi Laron, Athalia Pertzelan or Beatrice Klinger, usually by two of the above.

Table 42.6 presents the advancement in bone age along years of IGF-I treatment as the ratio between chronological and bone age. It is evident that from marked bone age retardation (high ratio) before initiation of treatment, the ratio decreases progressively

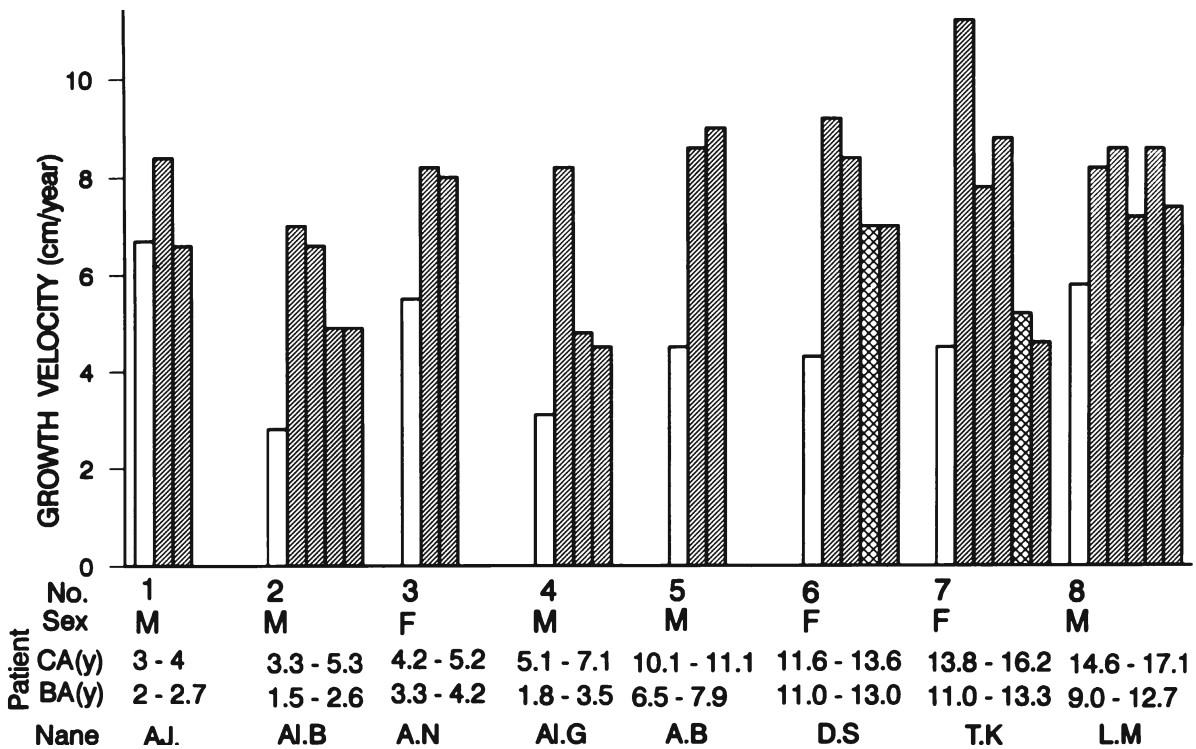

Fig. 42.11 Changes in linear growth velocity (cm/year) calculated every 6 months after initiation of IGF-I treatment in eight children with Laron syndrome

Table 42.6 Changes in skeletal maturation during long-term IGF-I treatment of 11 children with Laron syndrome expressed as chronological age/bone age ratio (CA/BA)

Pt	Name	Sex	CA at start of IGF-I	BA at start of IGF-I	Duration of IGF-I therapy (years)												
					0	1	2	3	4	5	6	7	8	9	10	11	12
1	PR	F	1 $^{7}/_{12}$			1.29	1.52	1.61	1.46	1.5	1.29						
2	HoN	F	3 $^{2}/_{12}$	2 $^{6}/_{12}$	1.26			1.02	1.04								
3	CLH	F	3 $^{6}/_{12}$	1	1.6				1.26	1.21	1.21	1.09					
4	HoS	F	3 $^{8}/_{12}$	2 $^{6}/_{12}$	1.46	1.69	1.61	1.58	1.08		1.30	1.31	1.17	1.06		1.04	1.03
5	MaM	F	9 $^{7}/_{12}$	4 $^{6}/_{12}$	2.12	1.45	1.66	1.26	1.23	1.31	1.14						
6	DS	F	11 $^{8}/_{12}$	11	1.06	1.05	1.05	1.047									
7	ATK	F	13 $^{9}/_{12}$	11	1.25	1.22	1.23	1.19	1.17	1.19							
8	AlS	M	$^{7}/_{12}$	$^{3}/_{12}$	2.32	1.9	2.06	2.04	1.80	1.88	1.88	1.80					
9	AlB	M	3 $^{5}/_{12}$	1 $^{6}/_{12}$	2.27	1.73	2.0			1.27	1.25			1.23			
10	AlG	M	5 $^{1}/_{12}$	1 $^{9}/_{12}$	2.9	2.43	2.0	1.9	1.65	1.44	1.48	1.27		1.22			
11	ML	M	14 $^{6}/_{12}$	9	1.62	1.63	1.41	1.34	1.36	1.29	1.20						

denoting that IGF-I advances not only growth but also skeletal maturation, leading with advancing puberty to closure of the epiphyses of the long bones. This effect is more pronounced in the girls as the boys have delayed and slurred puberty.

The linear growth-promoting effect of IGF-I is illustrated in Figs. 42.12–42.19 in eight girls with Laron syndrome arranged according to age of initiation of treatment. In two patients, attempts were made to slow the advancement of puberty by concomitant administration of a GnRH analog or cyproterone acetate.

Figures 42.20–42.27 present similar data for boys with Laron syndrome.

42.5.3 Effect of IGF-I Treatment on Upper to Lower Body Segment Ratio

Having previously described that untreated patients with Laron syndrome have an abnormal high ratio denoting short limb compared to body size (Chap. 8), figures 42.28 and 42.29 show that IGF-I treatment improves and even normalizes this body proportion.

42.5.4 Comparison Between One and Two Daily IGF-I Injections on Long-Term Growth Stimulation

Comparing the growth-promoting effect of our regime using one daily injection of IGF-I with that of other investigators using two daily IGF-I injections per day (Ranke et al. 1995; Backeljauw et al. 2001, etc.), we found that the results are identical (Laron 2008). As shown in Table 42.7, it is seen that during the first year, the growth velocity doubles to 8–9 cm/year and progressively decreases to 4–5 cm/year (i.e., prepubertal rate) within 4–5 years, findings true for both therapeutic regimens.

42.5.4.1 Comment

IGF-I is a potent linear growth-stimulating hormone. To achieve this effect, one daily injection suffices. Two daily injections or increasing the dose do not improve the achievements, while increasing the incidence of adverse effects (see Chap. 47).

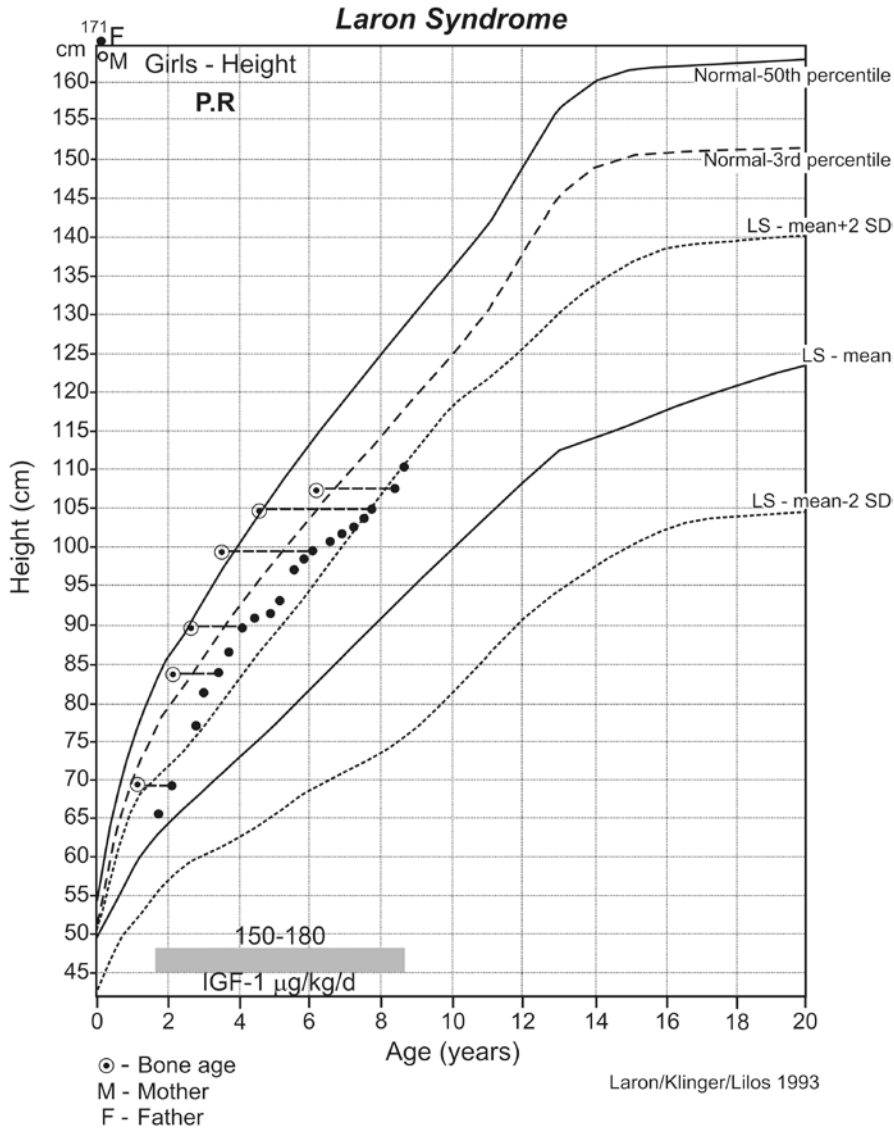

Fig. 42.12 Linear growth of one girl with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

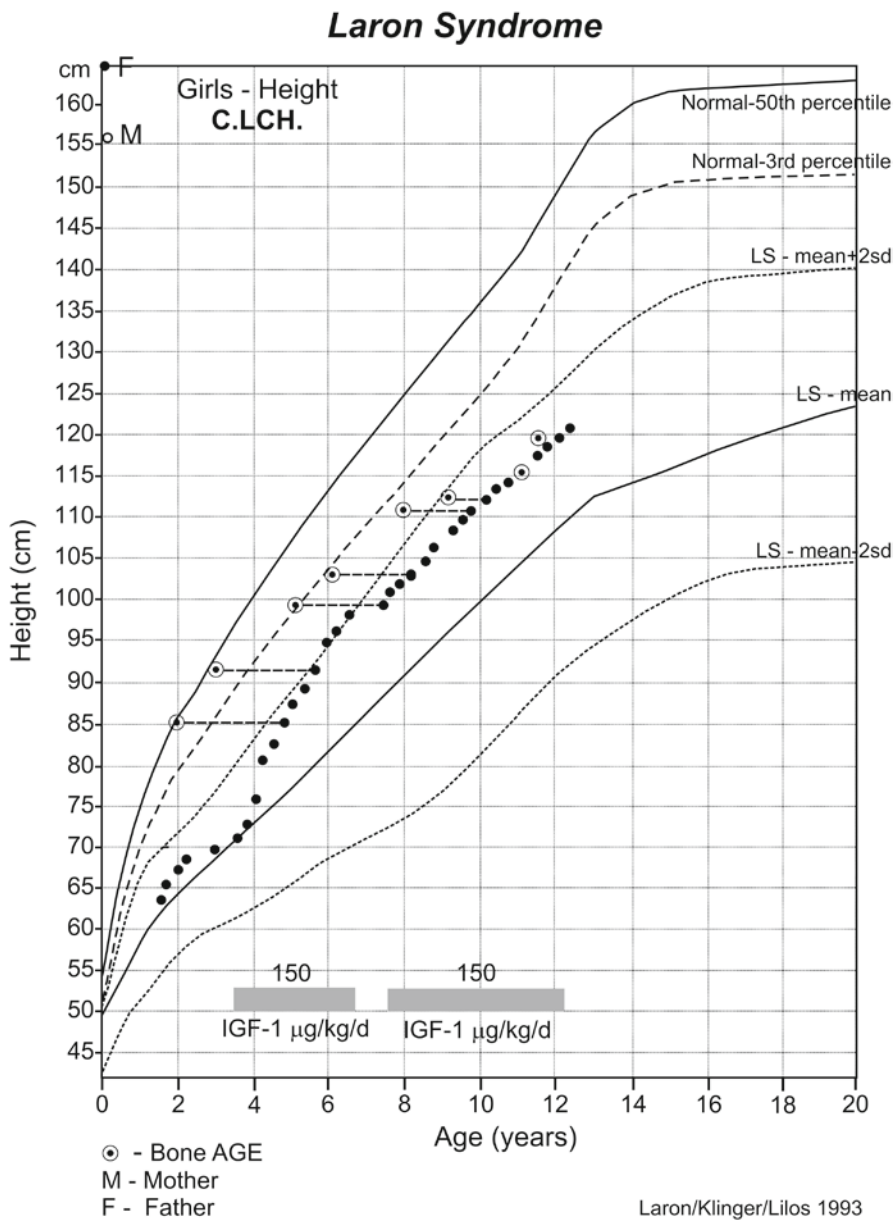

Fig. 42.13 Linear growth of one girl with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

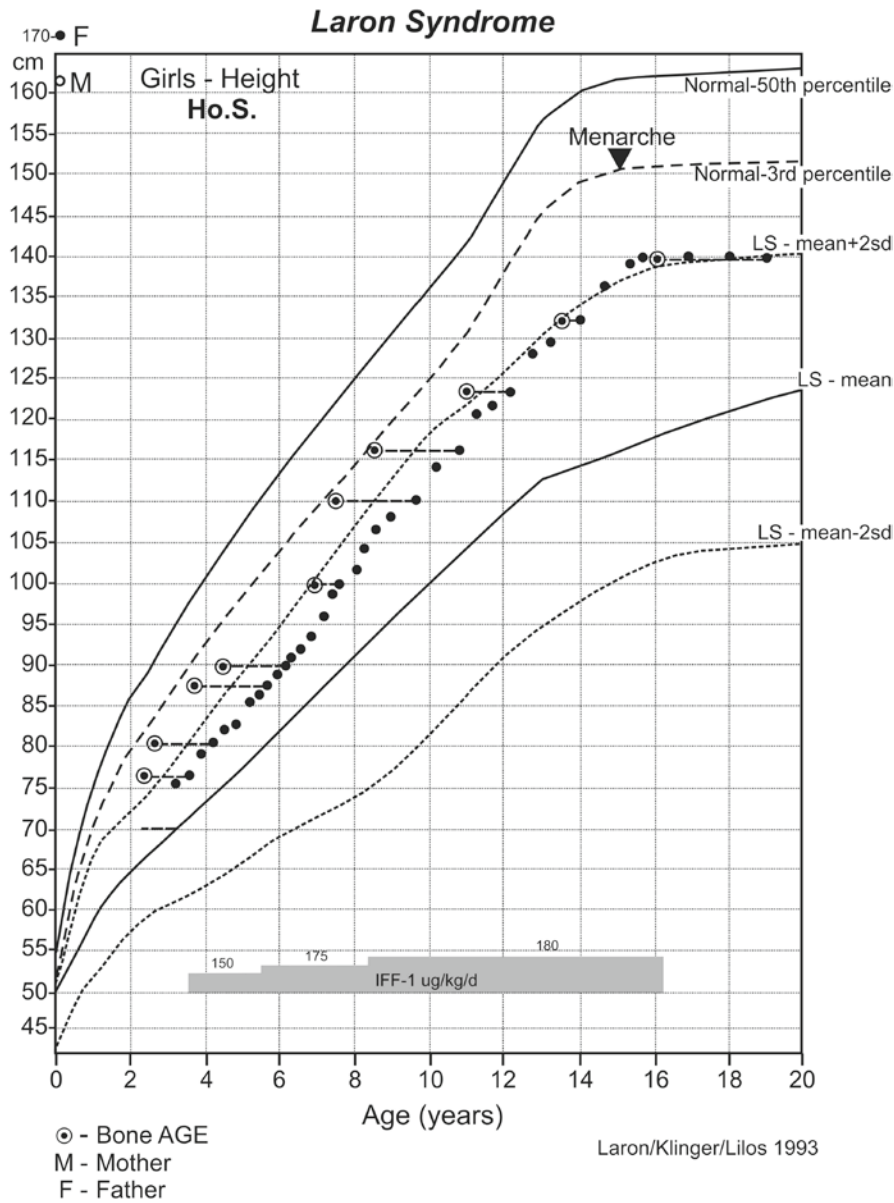

Fig. 42.14 Linear growth of one girl with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

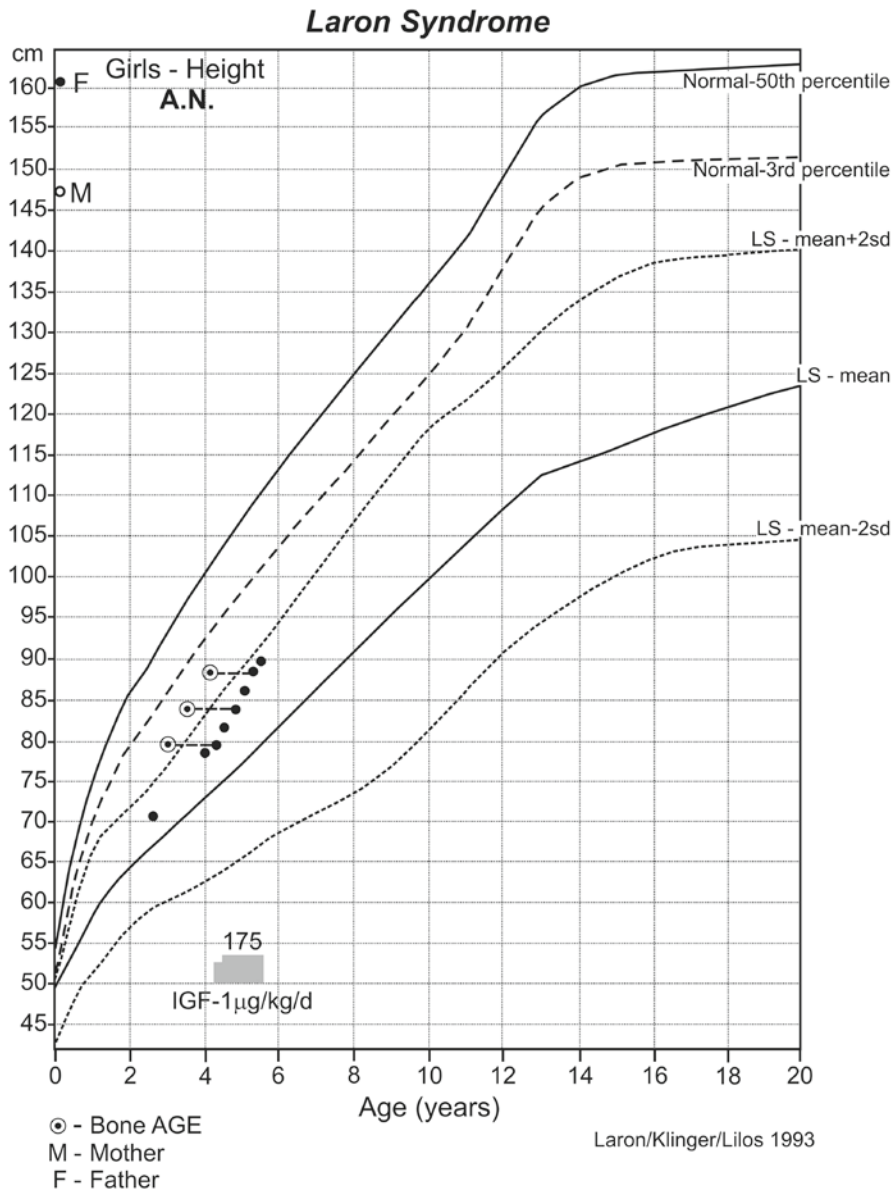

Fig. 42.15 Linear growth of one girl with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

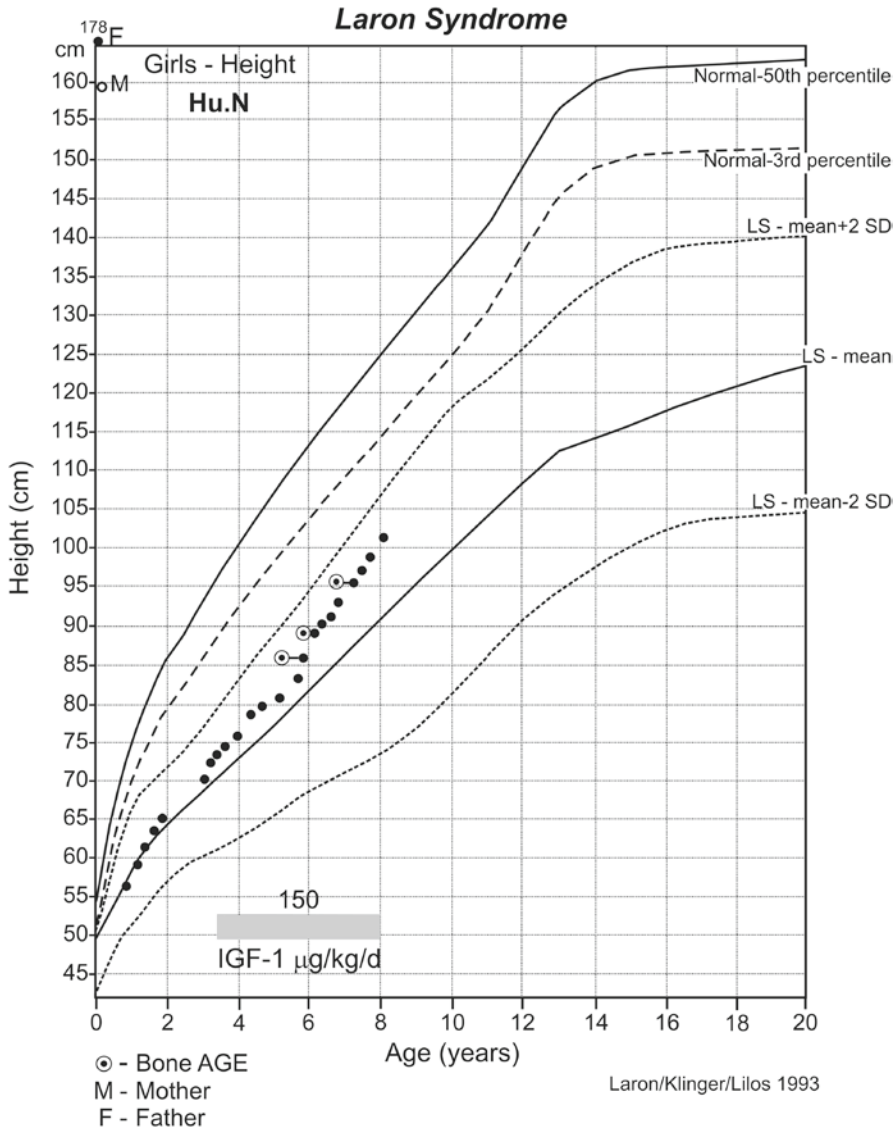

Fig. 42.16 Linear growth of one girl with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

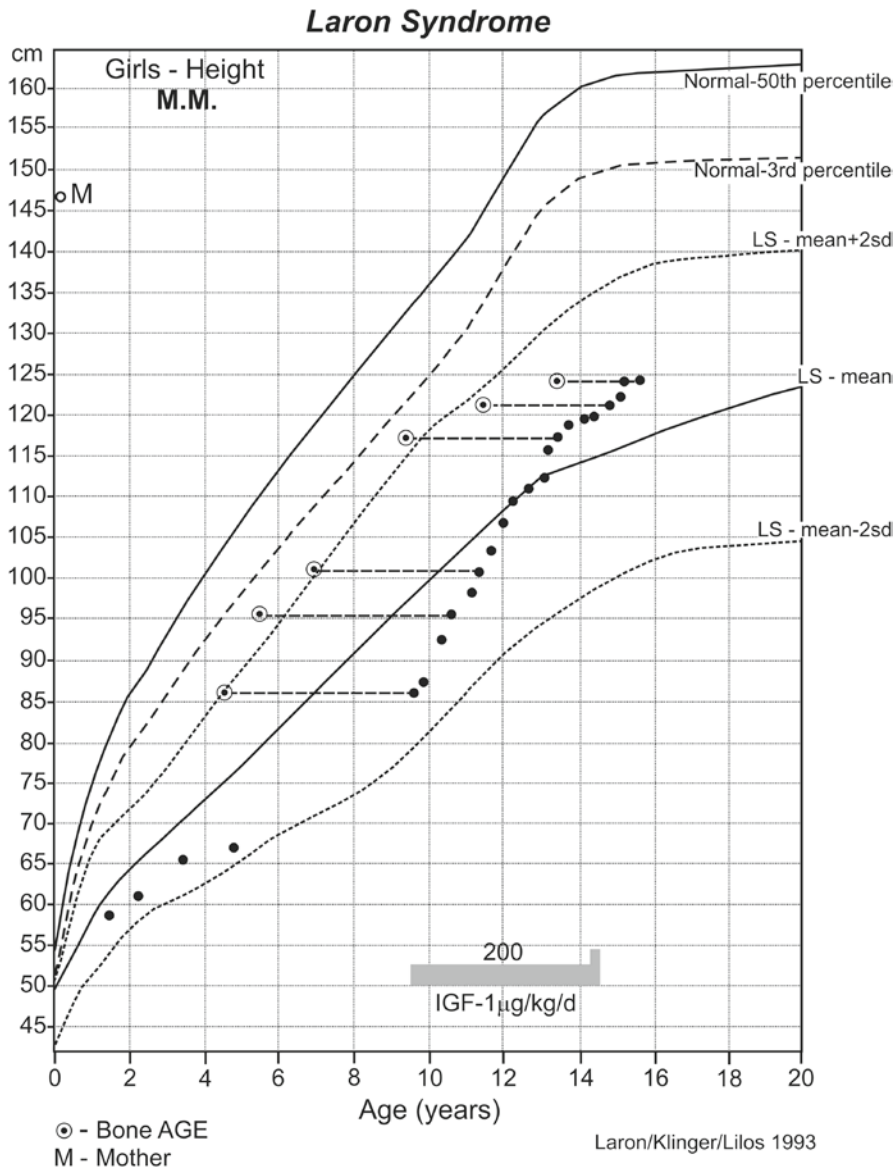

Fig. 42.17 Linear growth of one girl with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

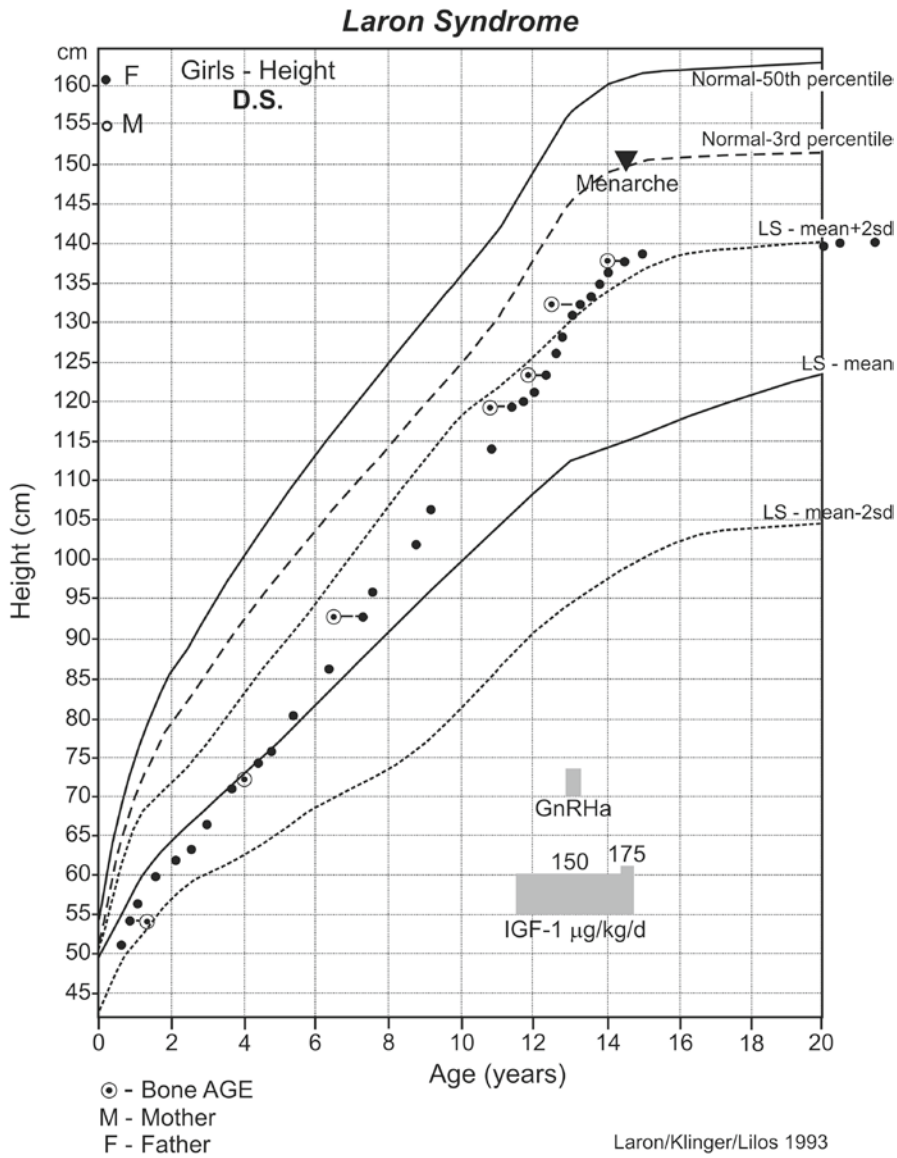

Fig. 42.18 Linear growth of one girl with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

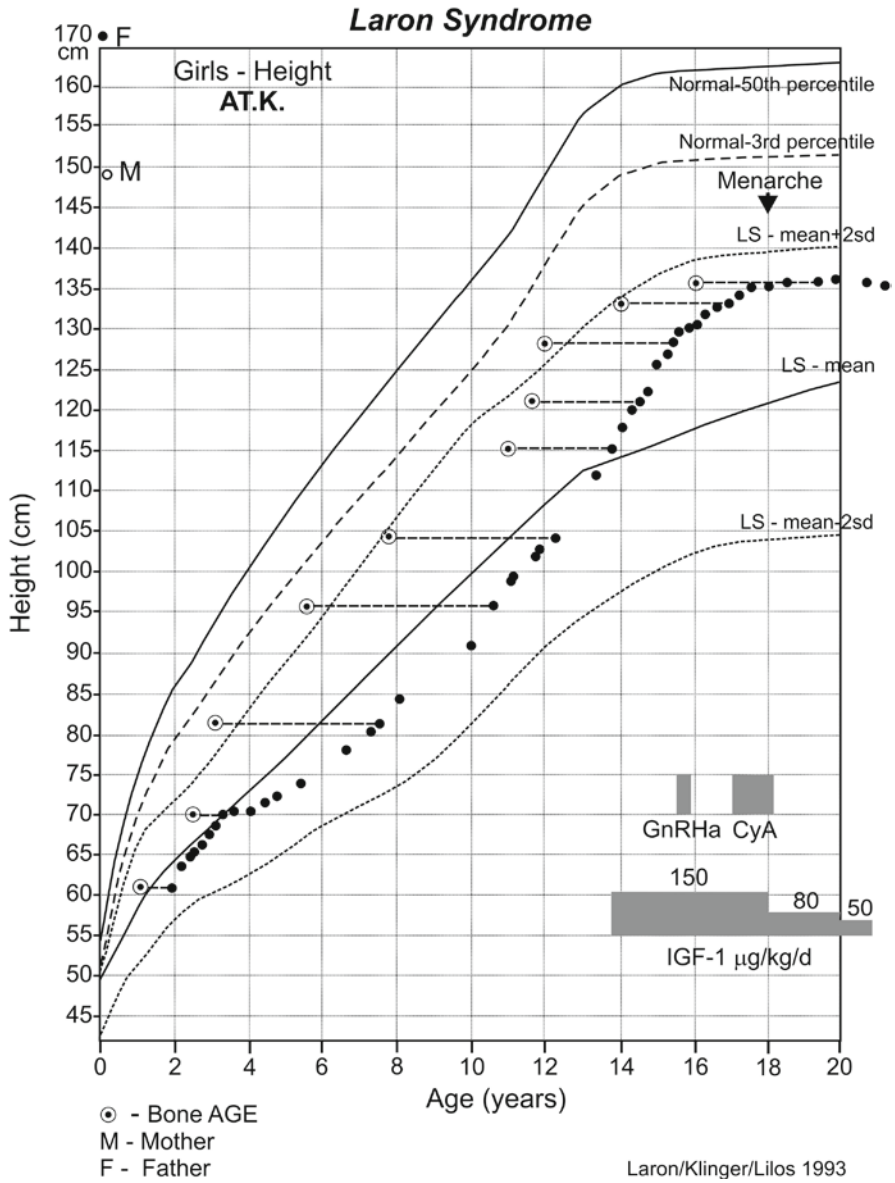

Fig. 42.19 Linear growth of one girl with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

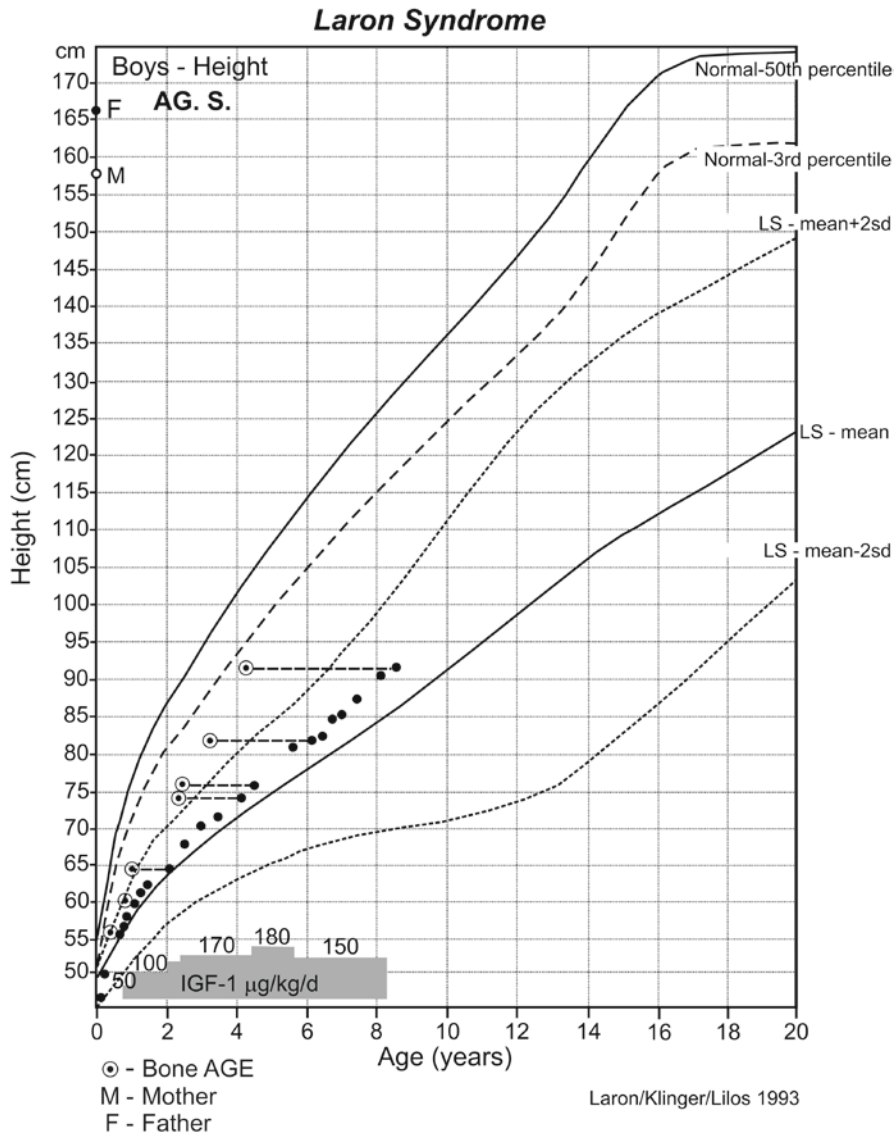

Fig. 42.20 Linear growth of one boy with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

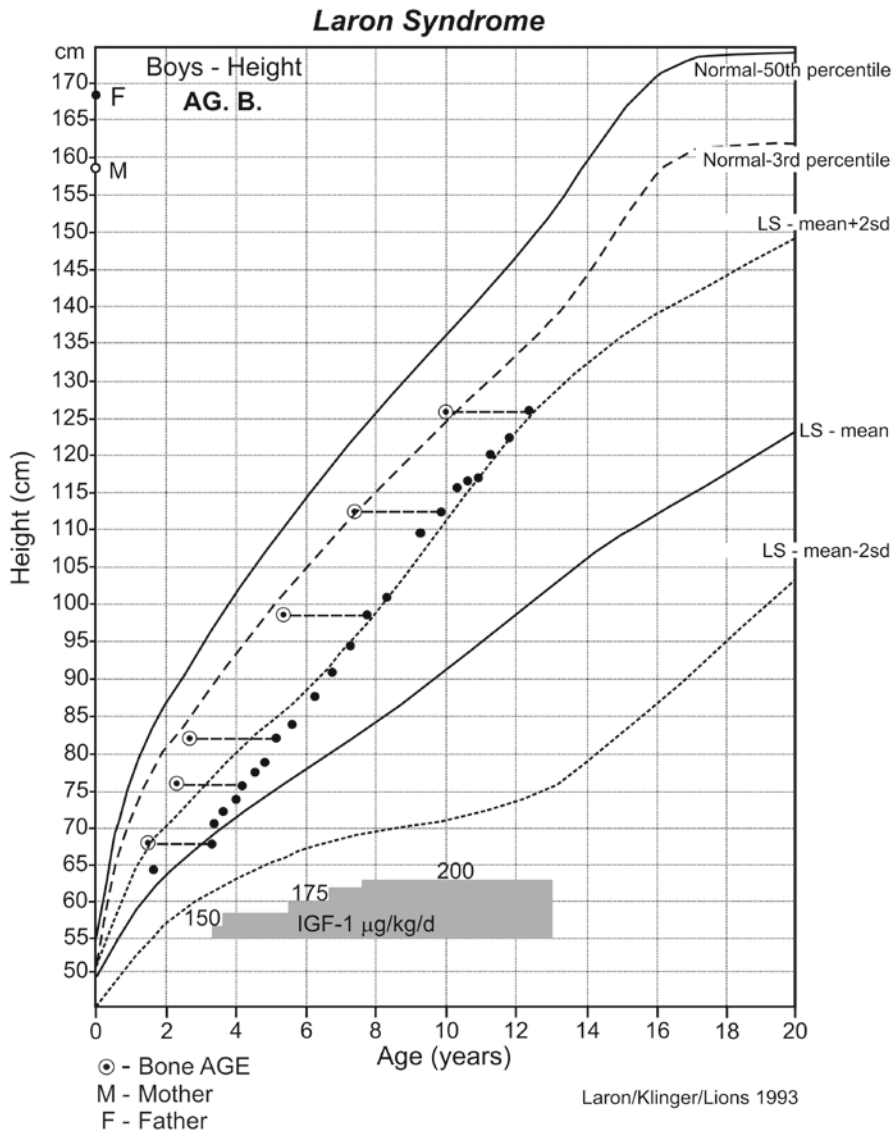

Fig. 42.21 Linear growth of one boy with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

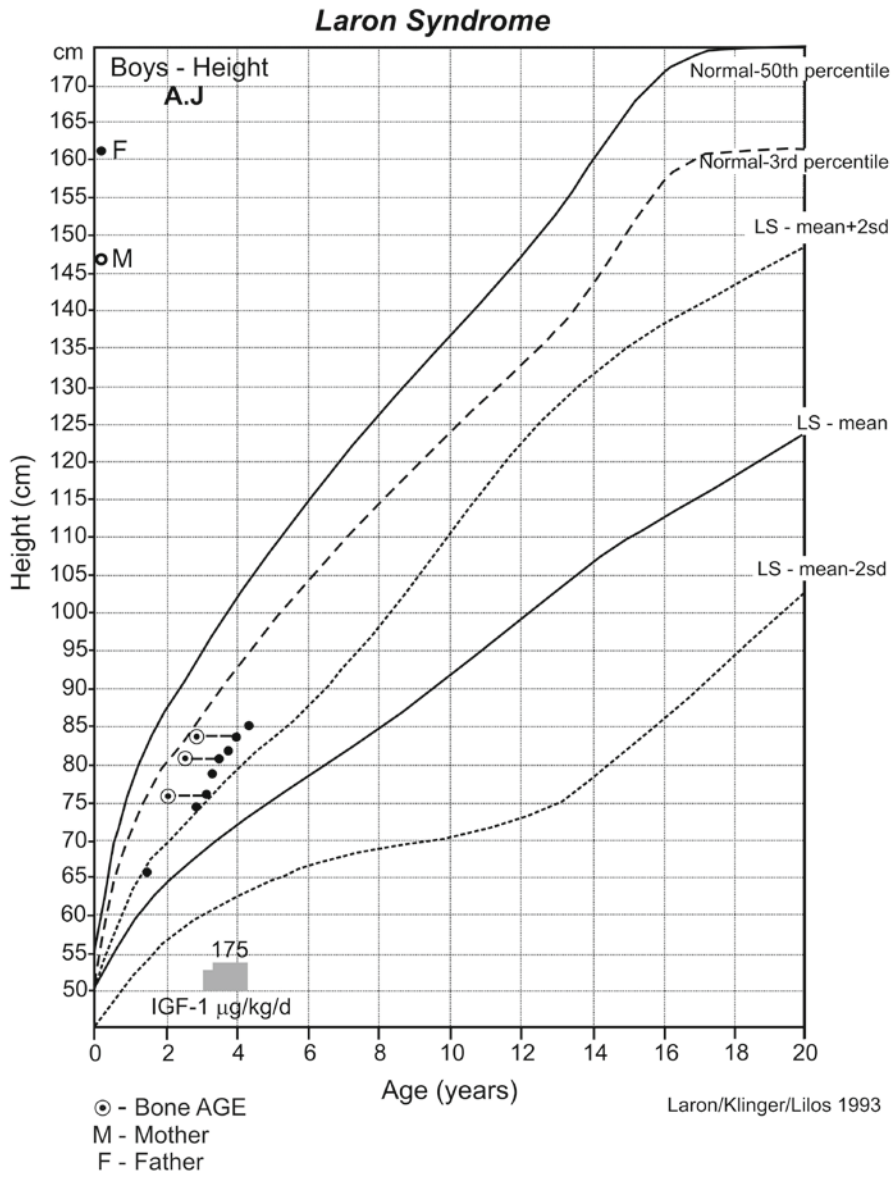

Fig. 42.22 Linear growth of one boy with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

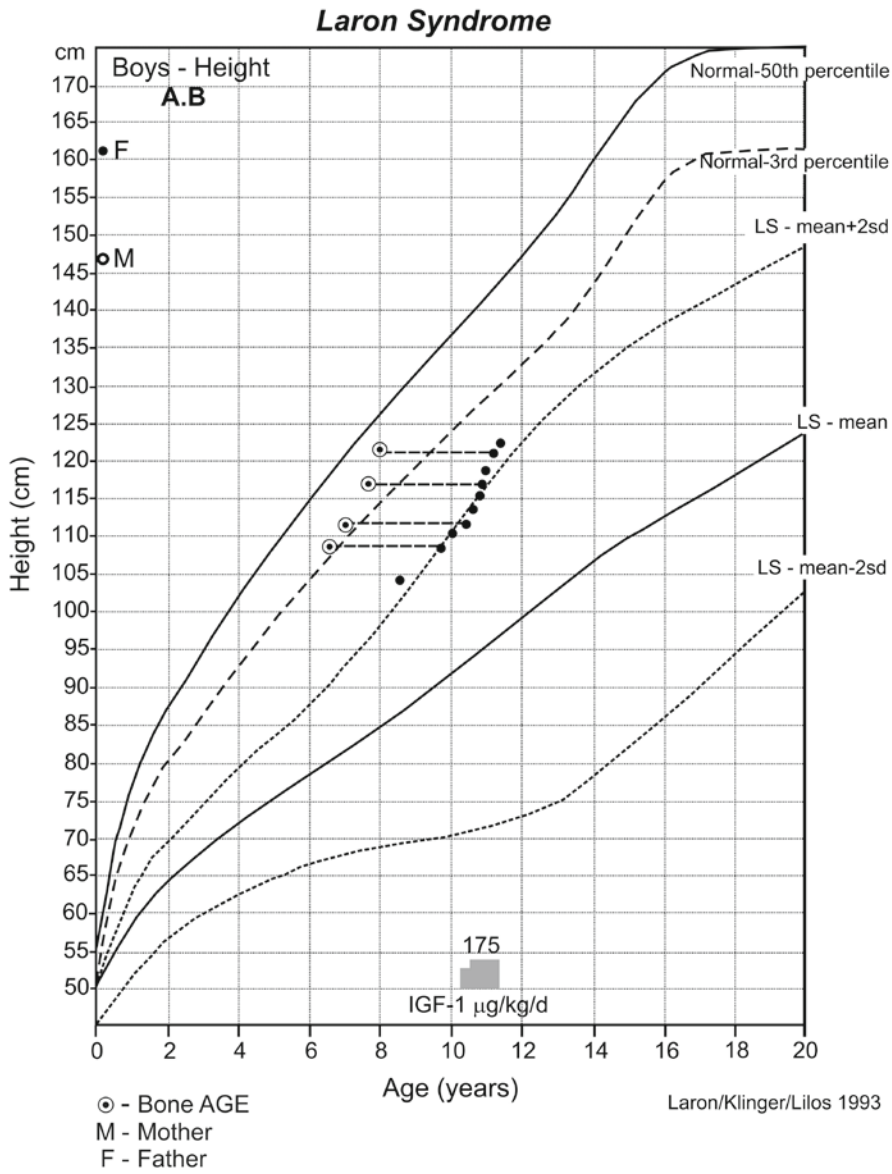

Fig. 42.23 Linear growth of one boy with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

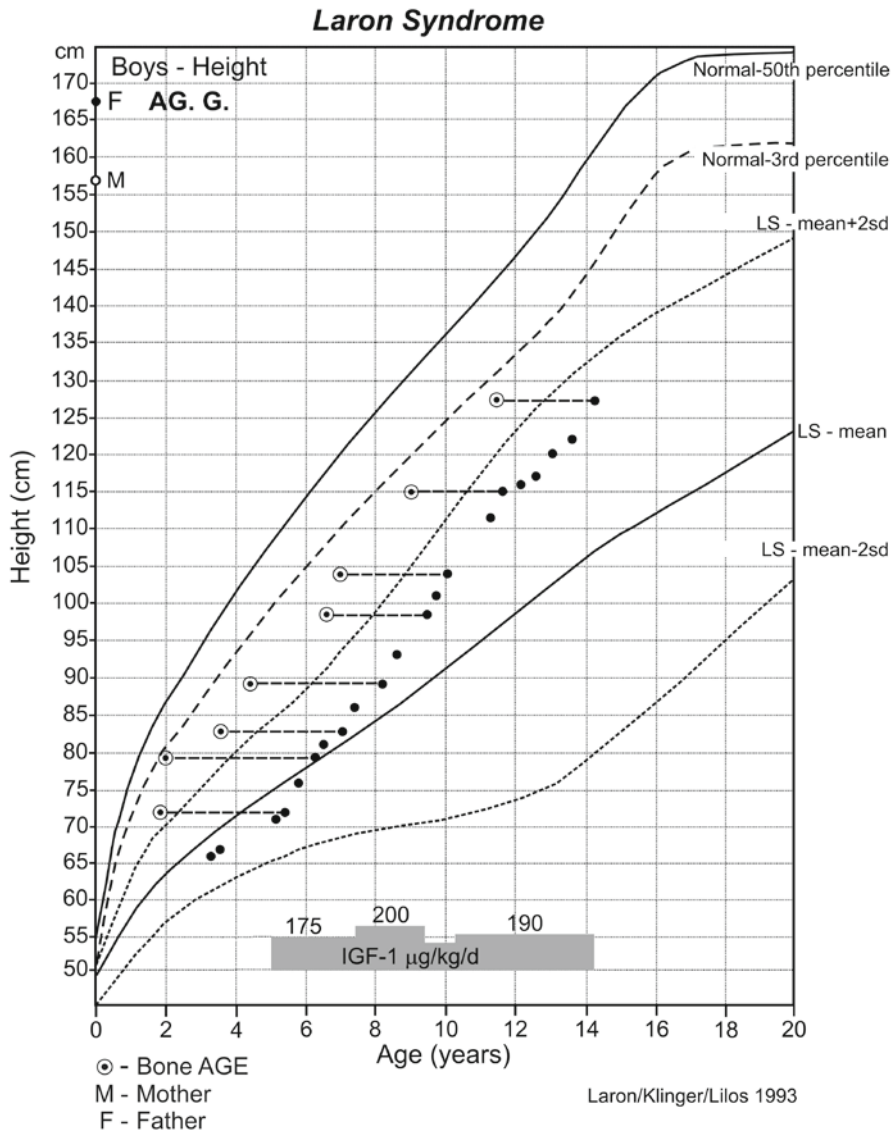

Fig. 42.24 Linear growth of one boy with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

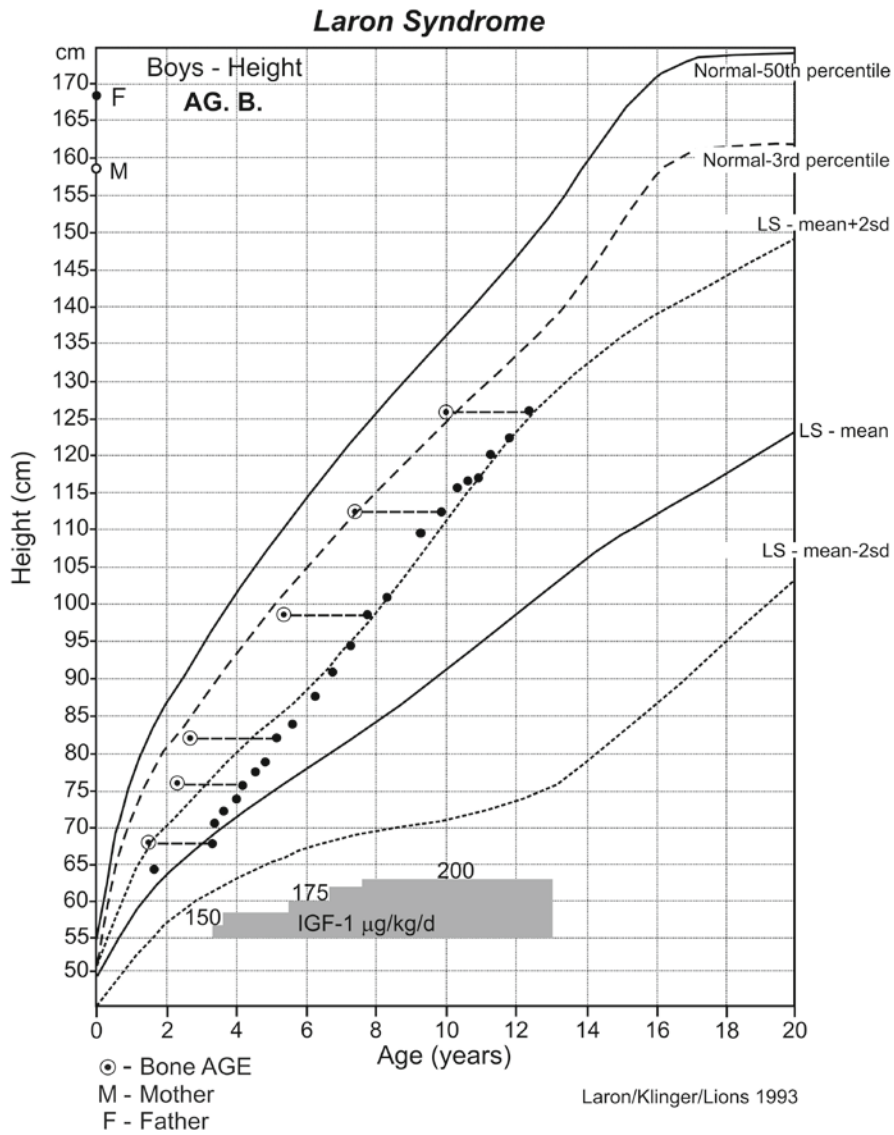

Fig. 42.25 Linear growth of one boy with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

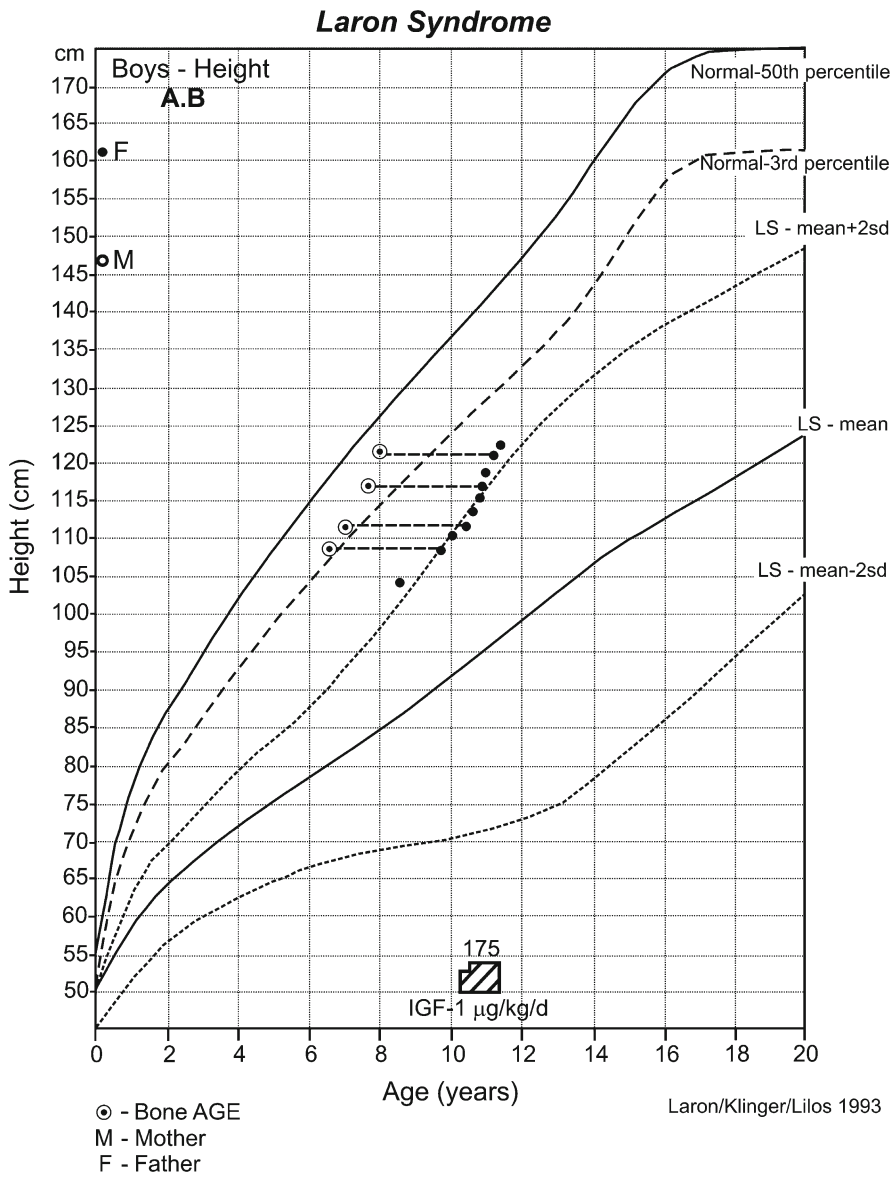

Fig. 42.26 Linear growth of one boy with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

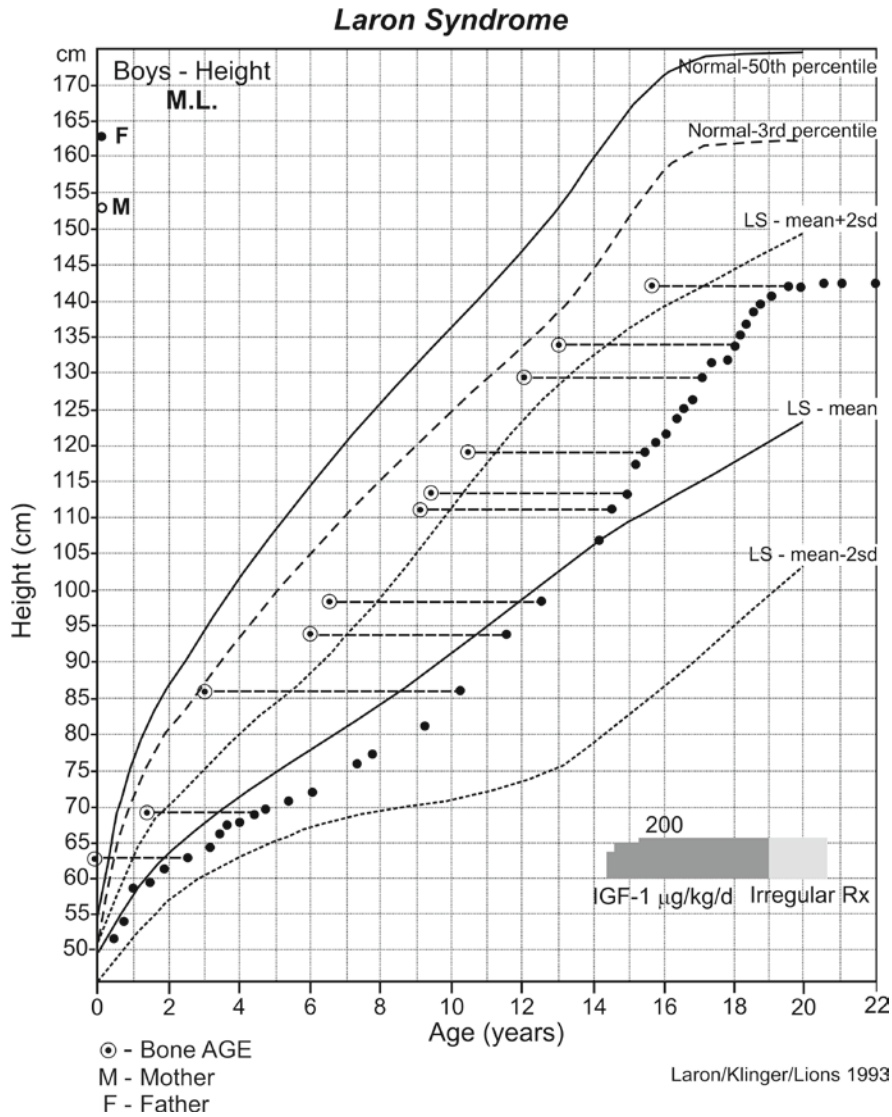

Fig. 42.27 Linear growth of one boy with Laron syndrome treated by IGF-I plotted on Laron syndrome specific growth charts. The third percentile is that from Tanner et al. (1966) chart

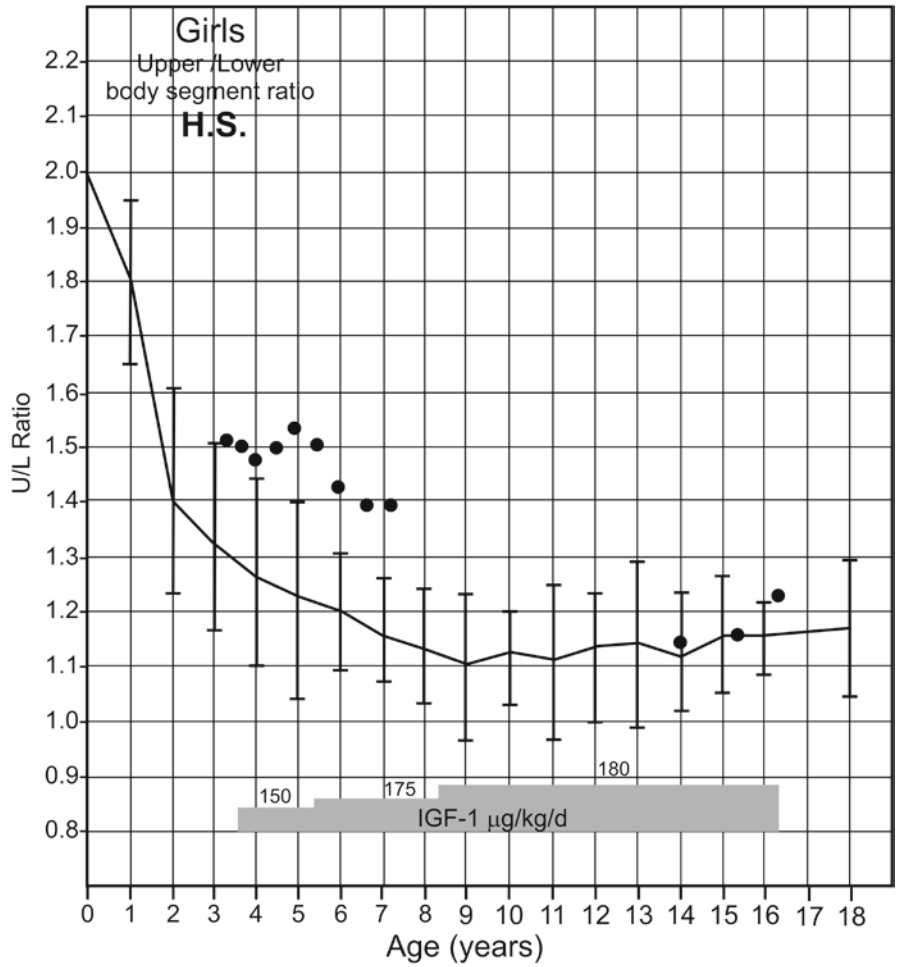

Fig. 42.28 Upper/lower segment ratio during IGF-I treatment in a girl with Laron syndrome drawn on the charts by Arad and Laron (1979)

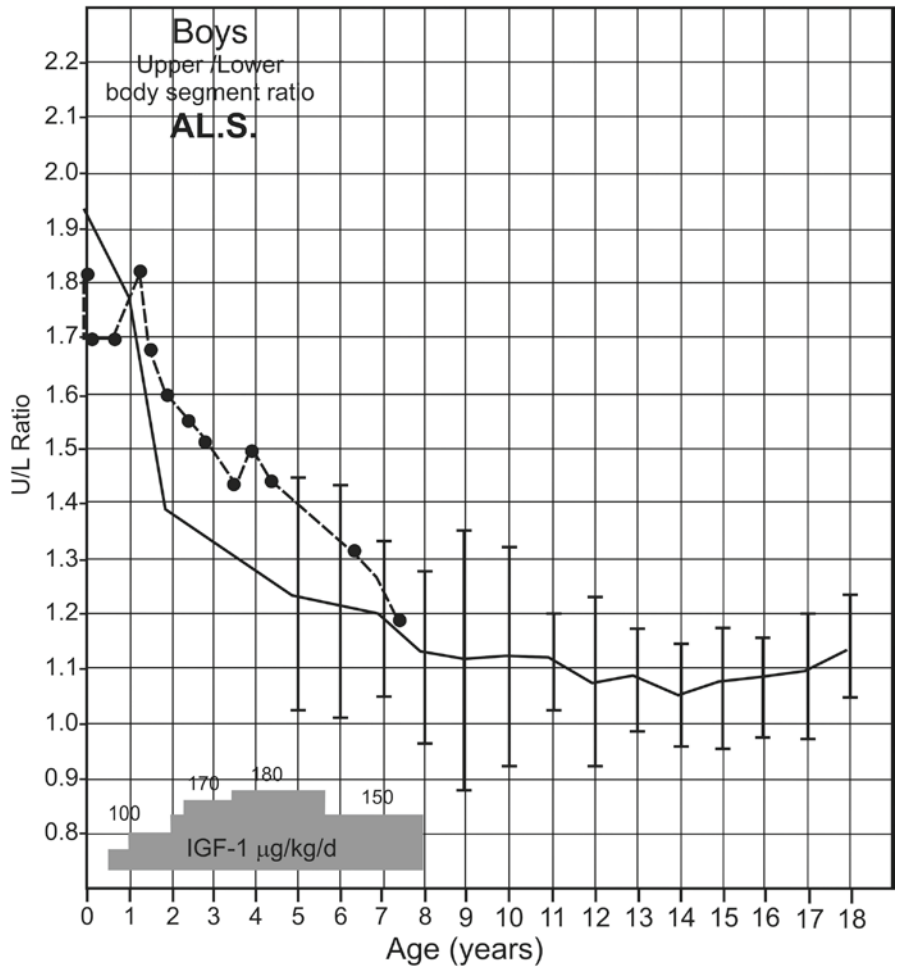

Fig. 42.29 Upper/lower segment ratio during IGF-I treatment in a boy with Laron syndrome drawn on the charts by Arad and Laron (1979)

Table 42.7 Linear growth response to IGF-I treatment of children with Laron syndrome

References	*n*	Parameters at start of treatment				Growth velocity (cm/year) as mean (SD) Length of treatment (years)						
		Age range (years, unless otherwise specified)	Bone age (years)	Height SDS (m)	IGF-I dose (μg/kg)	0	1	2	3	4	5	8–9
Klinger and Laron (1995)	9	0.5–14	0.2–11	−5.6	150–200 once daily	4.7 (1.3)[a]	8.2 (0.8)	6 (1.3) (*n* = 6)	4.8 (1.3) (*n* = 5)			
Silbergeld et al. (2007)	9	1.5–14	0.2–11	−6.3 (0.5)	120–200 once daily	4.7 (1.5)	8.2 (1.8)	6.1 (1.6)	5.7 (2.1)	6.2 (1.9) (*n* = 7)	4.4 (1.5) (*n* = 5)	5.3 (0.7) (*n* = 3)
Ranke et al. (1995)	18	3.7–16.7	1.8–12	−6.4 (1.7)	40–120 twice daily		8.6 (1.7)	6.4 (2.2)				
Ranke et al. (1999)	31	3.7–19	1.8–13.3	−6.9 (3.2)	40–120 twice daily	3.9 (1.8)	8.5 (2.1) (*n* = 26)	6.4 (2.2) (*n* = 18)	(*n* = 17)			4.9 (1.9) (*n* = 6)
Backeljauw and Underwood (1996)	5	2–11	0.3–6.8	−5.6	80–120 twice daily	4.0	9.3	6.2	6.2 (*n* = 1)			
Backeljauw et al. (2001)[a]	5	2–11	0.3–6.8	−5.6	80–120 twice daily	4.0	9.3	6.2	5.4	5.5	5.2	4.8
Frane et al. (2006)	19				80–120 twice daily	2.8 (1.3)	8.7 (1.7)	6.1 (1.6)				
Underwood et al. (2004)[b]	54	6.5 (3.7)		−6.7 (1.7)	80–120 twice daily	2.6 (1.6)	8.0 (2.3) (*n* = 54)	6.0 (0.5) (*n* = 42)	5.0 (1.0) (*n* = 24)	4.5 (*n* = 23)	4.3 (1.0) (*n* = 23)	4 (1.0) (*n* = 12)
Guevara-Aguirre et al. (1997)	15	1.7–3.1	4.5–9.3		120 twice daily	3.4 (1.4)	8.8 (11)	6.4 (1.1)	5.7 (1.4) (*n* = 6)			
Guevara-Aguirre et al. (1997)	8				80 twice daily	3.0 (1.8)	9.1 (2.2)	5.6 (2.1)				
Heinrichs et al. (1993)	2			−6.1, −6.8	40 twice daily	3.8, 4.3	6.3, 6.6					
Krzisnik and Battelino (1997)	1	9.3	8.5	−4.5	120 twice daily	4.0	10.0	8.5	7.5	8.0	6.0	

Table 42.7 (continued)

References	*n*	Parameters at start of treatment				Growth velocity (cm/year) as mean (SD) Length of treatment (years)						
Walker et al. (1998)	1	11	7.8	−6.3	80 twice daily	4.7	9.2	7.3	7.1			
Besson et al. (2004)	1	7 months		−6.1	80 twice daily	3.0	8.9	6.3			5.6	
Azcona et al. (1999)	11	2.5–11.7	2.3–9.1	−5.6 (1.6)	80 twice daily	3.1 (1.1)	7.7 (1.6)	7.0 (3.4)				
Zucchini et al. (2005)	1	7	4.7	−7.3	80–120 twice daily		7.1	6.0	5.5	3.9		4.0
Kaji et al. (1997)	1	9	3	−8	150 once daily	3.2					5.4	
Savage et al. (2006)	8	2–18			2 mg once daily[c]	2.0	8.3					

Comparison between 1 and 2 injections of IGF-I per day as the data reported by Chernausek et al. (2007) include the patients reported by Frane et al. (2006) and Backeljauw et al. (2001), that data are not reproduced again

IGF-I insulin-like growth factor; *rhIGF-I* recombinant human IGF-I; *rhIGFBP-3* rhIGF binding protein-3; *SDS* standard deviation score

[a]Results of first study (1996) are included

[b]Includes six children reported by Backeljauw and Underwood (1996)

[c]Combined rhIGF-I/rhIGFBP-3 preparation

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IGF-I Stimulation of Head Growth in Patients with Laron Syndrome

43

Zvi Laron

Core Message

› Long-term IGF-I treatment changed the craniofacial morphology and increased head circumference (i.e. brain growth) to normal values.

43.1 Introduction

Having previously shown that human growth hormone (hGH) replacement therapy increased the head circumference in children with GH deficiency (Laron et al. 1979) and that lack of IGF-I replacement treatment causes a subnormal head development (Chap. 9), we investigated the effect of IGF-I treatment on growth circumference (i.e., brain growth), changes in the bicondylar/biparietal ratio, and craniofacial morphology.

43.2 Subjects and Methods

At every visit in the clinic, the head circumference was measured before and during the IGF-I (Mecasermin FK 780, Fujisawa, Osaka, Japan) treatment (150–200 μg/kg/once daily). The head circumference was measured with a tape, the bicondylar and bilateral diameters measured from anterior X-rays from the skull, and the bicondylar/biparietal ratio was calculated (Scharf and Laron 1972) (see also Chap. 9).

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43.3 Results

43.3.1 Effect of IGF-I on Head Growth

Already in the first months of IGF-I administration, there was a striking increase in head circumference, even in children over 10 years of age (Laron et al. 1992). From subnormal sizes typical of IGF-I deficiency (Chap. 9), head circumferences normalized rapidly in a spurt greater than that of linear growth. The head circumference growth response to IGF-I for 4 girls is illustrated in Figs. 43.1–43.4, and for 3 boys in Figs. 43.5–43.7.

43.3.2 Effect of IGF-I on the Bicondylar/Biparietal Ratio of the Skull

The changes in the bicondylar/biparietal ratio during the first 2 year of IGF-I treatment of 3 children with Laron syndrome (LS) are shown in Table 43.1. It is seen that IGF-I increased the ratio denoting a change in the skull proportions. During continuing treatment, there were further increases in the growth rate but none reached the normal values of 70.7 ± 26 for healthy males and 75.4 ± 2.6 for healthy females (Table 43.2) (Scharf and Laron 1972).

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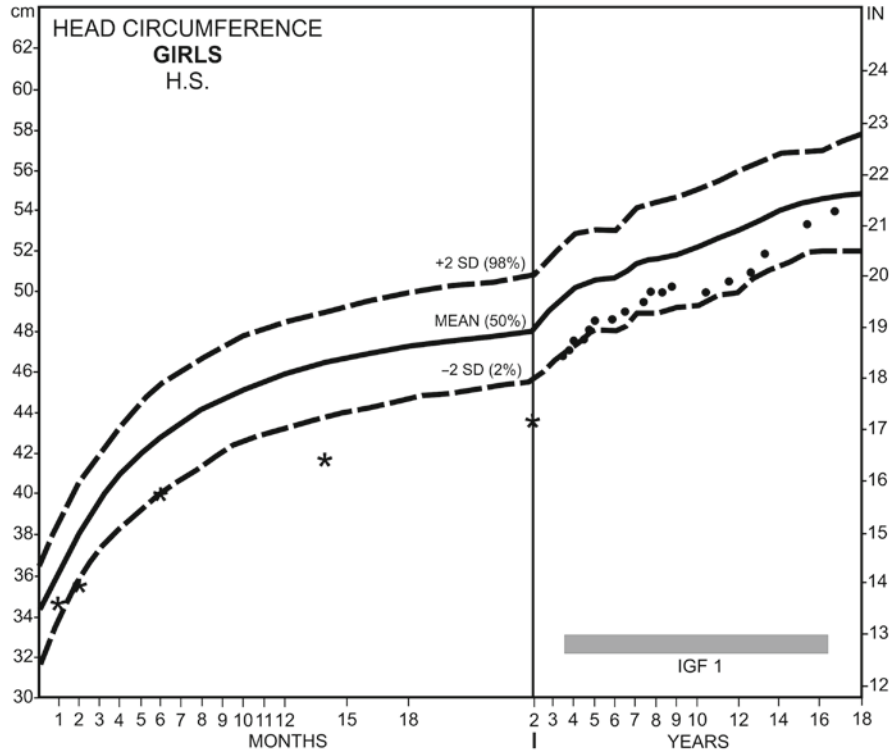

Fig. 43.1 Head circumference growth before and in response to IGF-I treatment in a girl with Laron syndrome (LS) (growth chart by Nellhaus 1967)

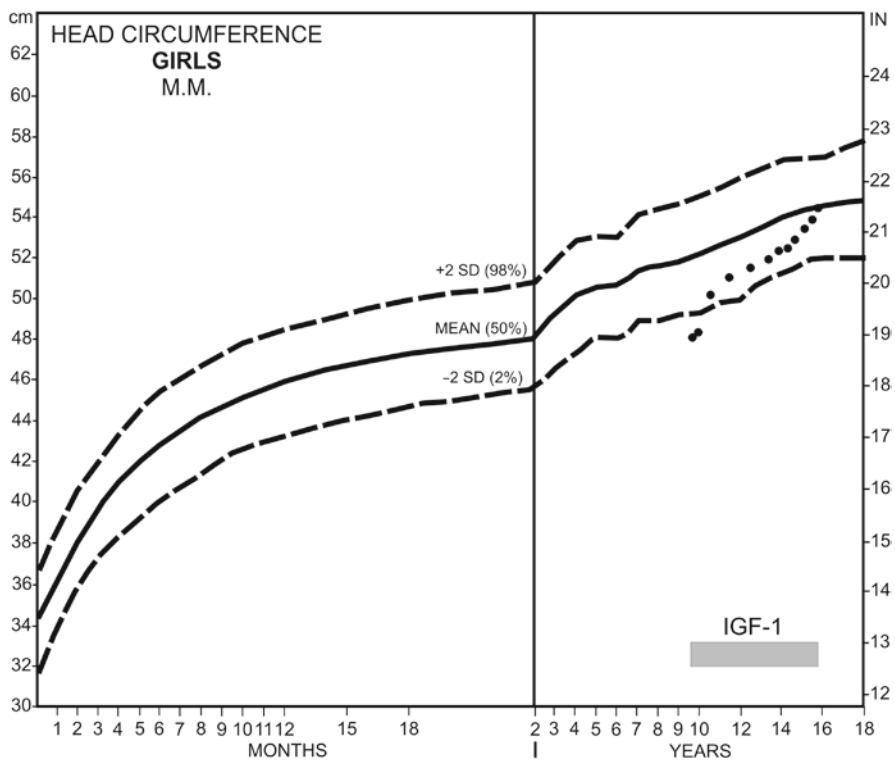

Fig. 43.2 Head circumference growth before and in response to IGF-I treatment in a girl with LS (growth chart by Nellhaus 1967)

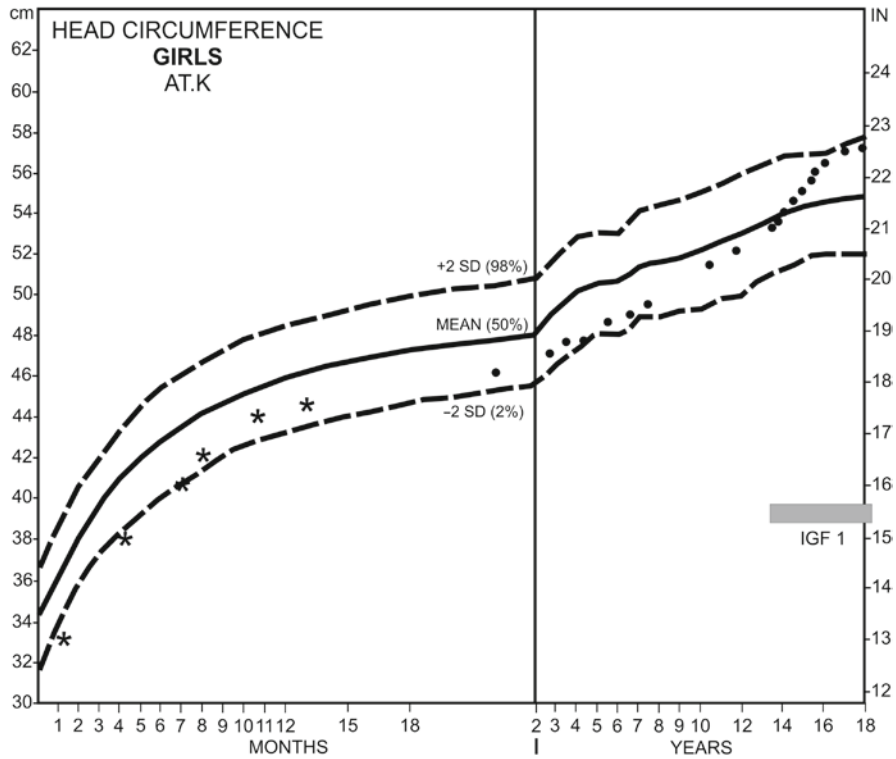

Fig. 43.3 Head circumference growth before and in response to IGF-I treatment in a girl with LS (growth chart by Nellhaus 1967)

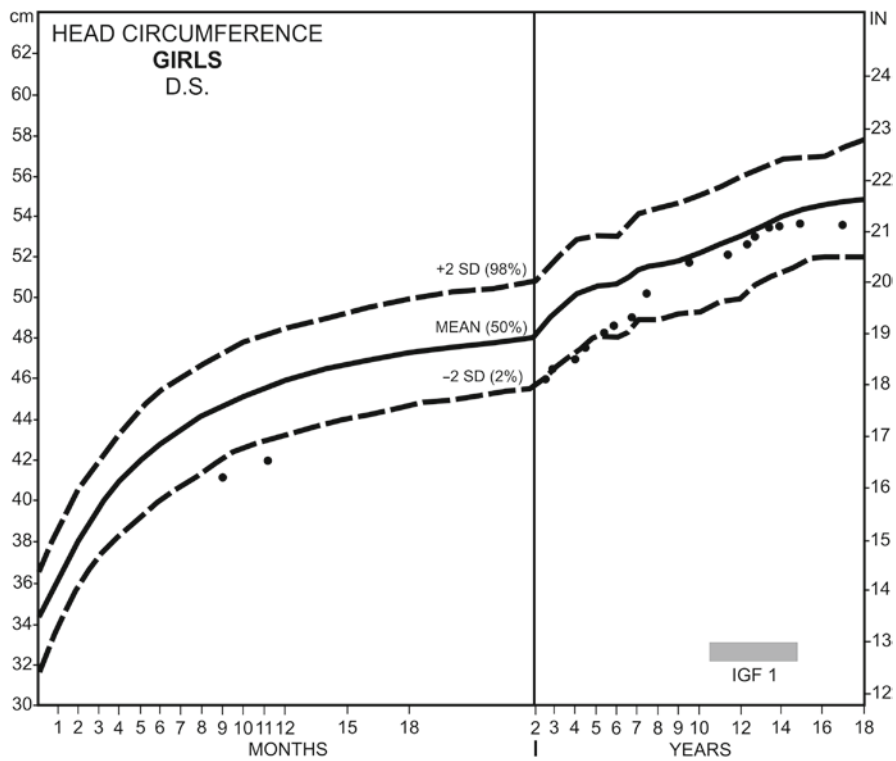

Fig. 43.4 Head circumference growth before and in response to IGF-I treatment in a girl with LS (growth chart by Nellhaus 1967)

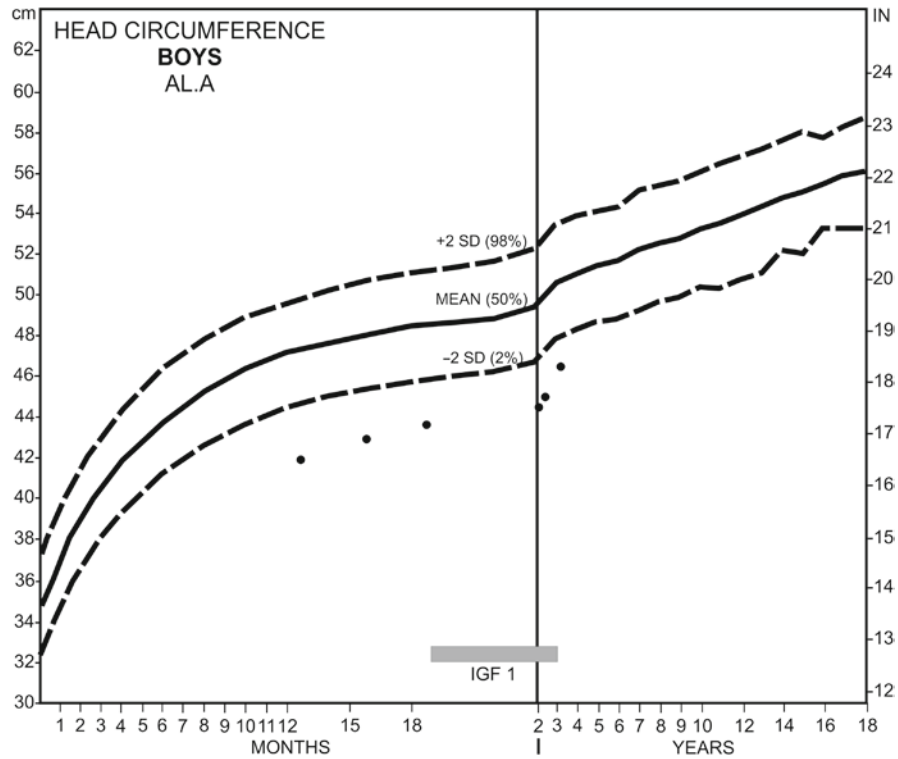

Fig. 43.5 Head circumference growth before and in response to IGF-I treatment in a boy with LS (growth chart by Nellhaus 1967)

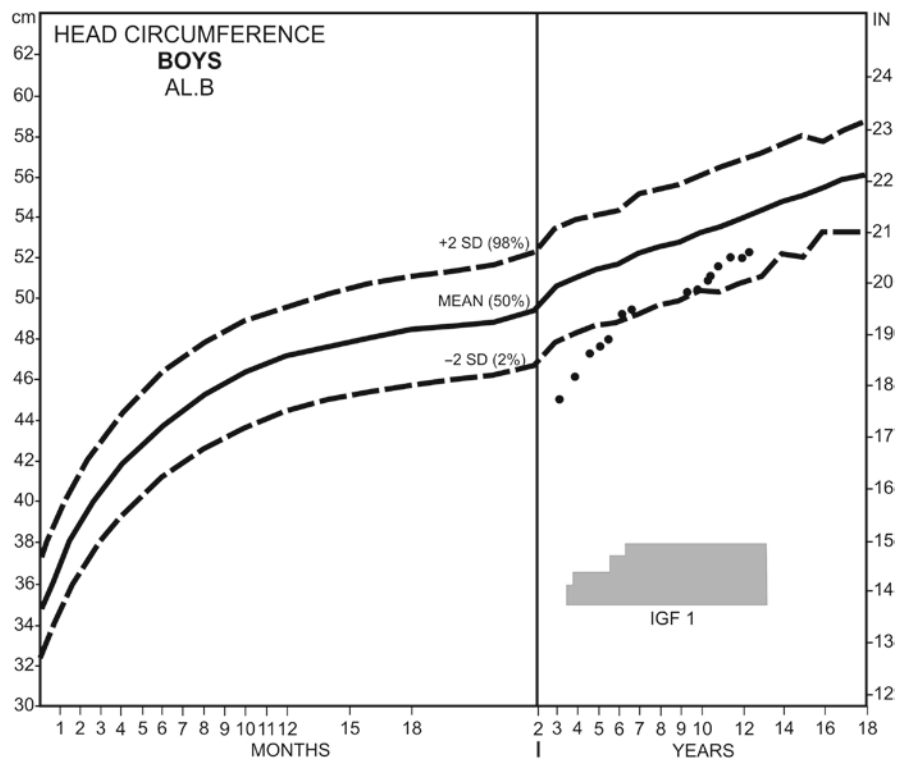

Fig. 43.6 Head circumference growth before and in response to IGF-I treatment in a boy with LS (growth chart by Nellhaus 1967)

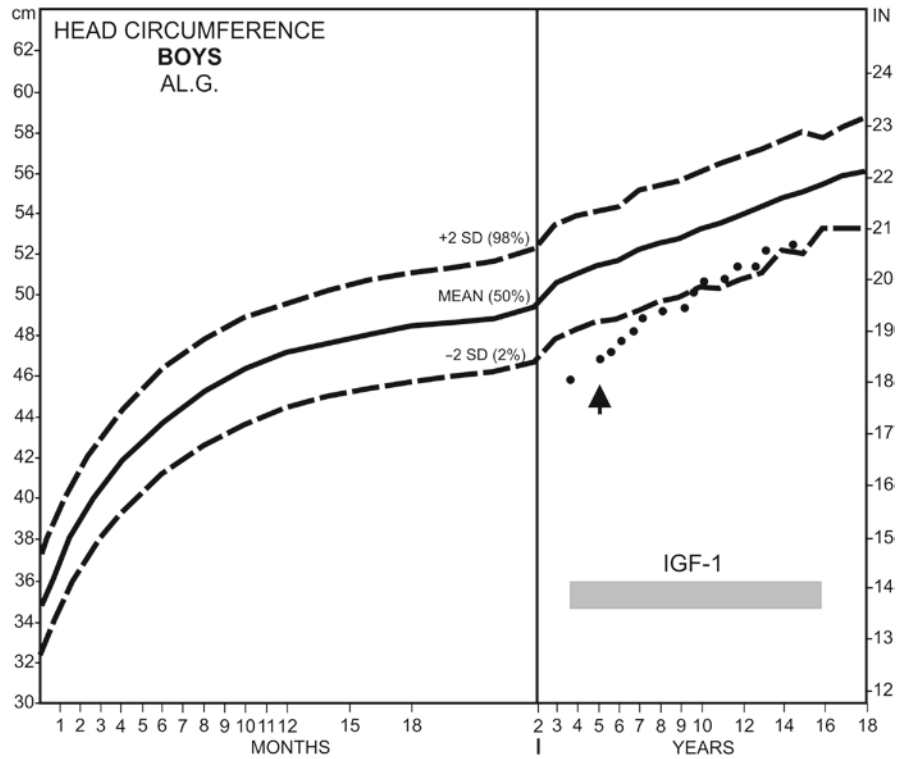

Fig. 43.7 Head circumference growth before and in response to IGF-I treatment in a boy with LS (growth chart by Nellhaus 1967)

Table 43.1 Effect of IGF-I treatment on the bicondylar/biparietal skull ratio in 3 children with LS

Patient	Number	Sex	CA years	BA years	Bicondylar/biparietal ratio × 100		
					Before treatment	IGF-I treatment	
						1 year	2 years
TAK	1	Female	10	6	55.17	59.74	63.75
AlG	2	Male	5	1.8	51.7	57.4	–
AlB	3	Male	3	1.5	50.7	54.0	–

CA chronological age; *BA* bone age

Table 43.2 Bicondylar/biparietal ratio × 100 in groups of normal and IGF-I treated LS patients

Group	Number of patients	Sex	Age (years)	Ratio
Normal control	18	Female	8–20	75.4 ± 2.6
Normal control	16	Male	3–8	70.7 ± 2.6
LS patients	9	Female	8–20	59.7 ± 2.1
LS patients	2	Male	3–8	52.5 ± 0.2

From Scharf and Laron (1972)

43.3.3 Effect of IGF-I Treatment on Craniofacial Morphology

Having described the typical appearance of the head and skull during long-term IGF-I deprivation (Chap. 9) characterized by small facial bones resulting from a small mandible and chin (acromicria) a saddle nose and a "sunset look," we observed definite changes in the appearance of the face in the children treated more than 3–4 and up to 15 years.

Fig. 43.8 Lateral view of the head of a $6^{3/12}$-year-old girl with LS treated by IGF-I for 3 years

Fig. 43.9 Lateral view of the head of a 6-year-old girl with LS treated by IGF-I for 4 years

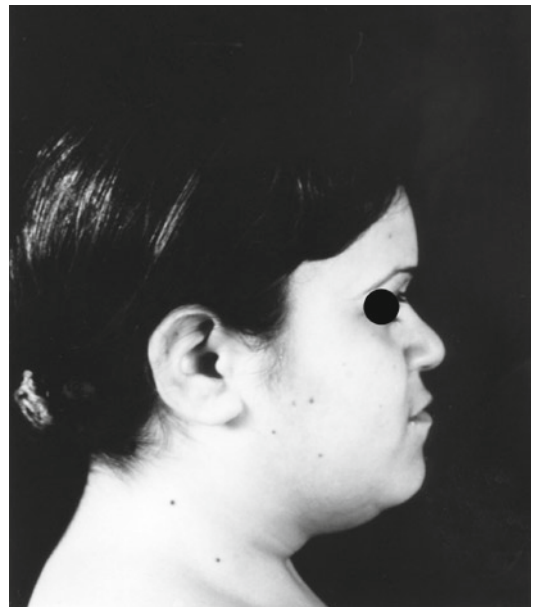

Fig. 43.10 Lateral view of the head of a 17-year-old girl with LS treated by IGF-I for 14 years

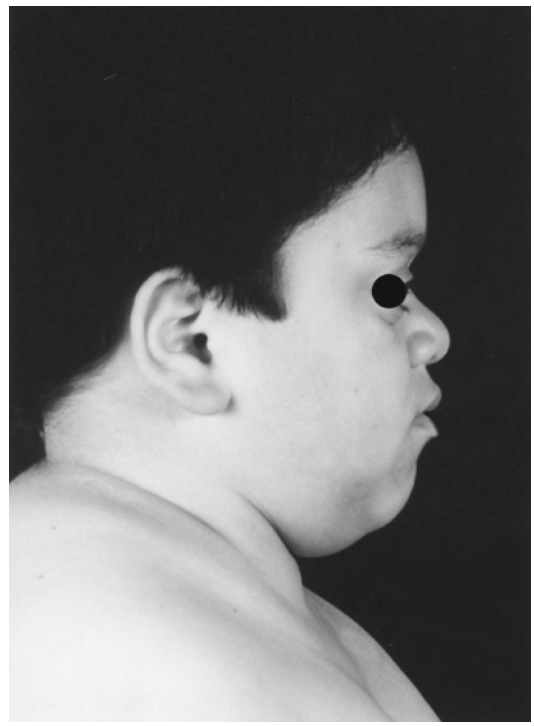

Fig. 43.11 Lateral view of the head of a 12-year-old boy with LS treated by IGF-I for 9 years

Figures 43.8–43.10 illustrate the lateral view of 3 IGF-I treated girls with LS and Figs. 43.11 and 43.12 the appearance of 2 IGF-I treated boys. With the exception of the youngest girl (Fig. 43.8) treated for only 3 years and in whom the frontal bossing, retracted hair line, and saddle nose are still visible, in all other girls and boys treated for longer periods of time, the facial appearance is normal.

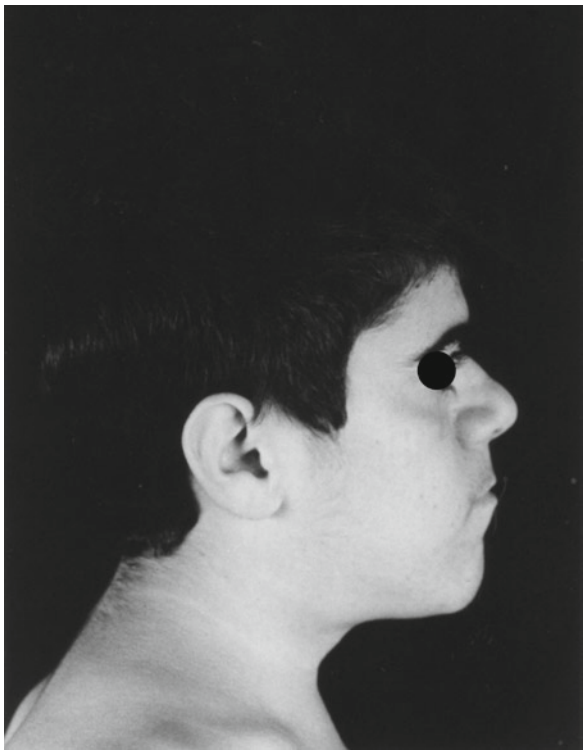

Fig. 43.12 Lateral view of the head of a 20-year-old male with LS treated by IGF-I for 10 years

43.4 Comment

Also Leonard et al. (1994) measured auxological parameters in 5 patients with LS and described changes in facial morphology during IGF-I treatment.

43.5 Conclusions

IGF-I treatment of children with LS induces a rapid and dramatic growth of the skull circumference, an indirect measure of brain growth, and less rapid changes in the facial morphology. In view of the effect on the brain, the diagnosis of IGF-I deficiency should be made at the earliest age possible, and replacement therapy initiated.

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44 Effects of Long-Term Administration of IGF-I on the Adipose Tissue and Carbohydrate Metabolism in Children with Laron Syndrome

Zvi Laron

Core Messages

- Long-term treatment with IGF-I of children with Laron syndrome caused a transitory reduction in serum cholesterol and subcutaneous skinfolds but subsequently the values of cholesterol started to rise and so the degree of adipose tissue.
- The effects on the carbohydrate metabolism were a reduction in serum insulin concentration resulting in a relative rise of glucose, preventing hypoglycemia.

44.1 Introduction

Having described the progressive and marked obesity in untreated children and adults with Laron syndrome (Laron and Klinger 1993; Laron 2004; Ginsberg et al. 2009) (see also Chap. 12), we hoped that IGF-I replacement therapy would reverse or at least decrease this overdevelopment of the adipose tissue. We also expected that the progressive development of hypercholesterolemia (Laron and Klinger 1993) would be reversed. A summary of the short- and long-term effects in our cohort of patients is described.

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44.1.1 Local Effects of IGF-I

Using the IGF-I (FK 780 by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) in all our patients until summer 2008, a lyophilized preparation, which we could dilute according to the dose to be administered, we have observed only in one instance lipohypertrophy in a small girl, because the mother did not rotate the site of injection and used a too-short needle. We have more problems with the use of INCRELEX (Tercica-IPSEN, USA) as the preparation is very concentrated, and in small children, the injection is obviously injected into the fat tissue rather than subcutaneously. So far, we have observed lipohypertrophy in a 2-year-old boy weighing 6 kg with a well-developed adipose tissue. The dose of IGF-I we administer to children is 150–200 μg/kg.

44.1.2 Changes in Skinfold Thickness During Long-Term Treatment

As the standardized measurement of subscapular skinfold thickness is a more reliable measure of body adiposity than measurements at other sites, we shall summarize only this measurement taken under the scapula with a Harpenden caliper (Laron et al. 1992). Table 44.1 shows that 6 months of IGF-I treatment of six Laron syndrome children (four boys, two girls) with a molecular defect in the extracellular domain of the GH-R and serum negative for GHBP as well as four children (two boys, two girls) with a defect in the transmembranal region and serum positive for GHBP had a reduced subscapular skinfold thickness.

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Table 44.1 Changes in subscapular skinfold thickness during 6 months of IGF-I treatment of Laron syndrome patients

Laron syndrome patients	Number	Age range (years)	Serum GHBP	Subscapular skinfold (m + SD)		Serum insulin (μU/mL)	
				At start (mm)	6 months IGF-I (mm)	Basal	6 months IGF-I
Group 1 (2F, 4M)	6	0.7–14	Negative	19.3 ± 3.7	14 ± 2.4[a]	12.3 ± 2.6	7.1 ± 1.7[b]
Group 2 (2F, 2M)	4	3–10	Positive	10.7–0.6	9.4 ± 1.3	3.4 ± 0.7	5.3 ± 1.3

[a] $p < 0.01$

[b] $p < 0.03$

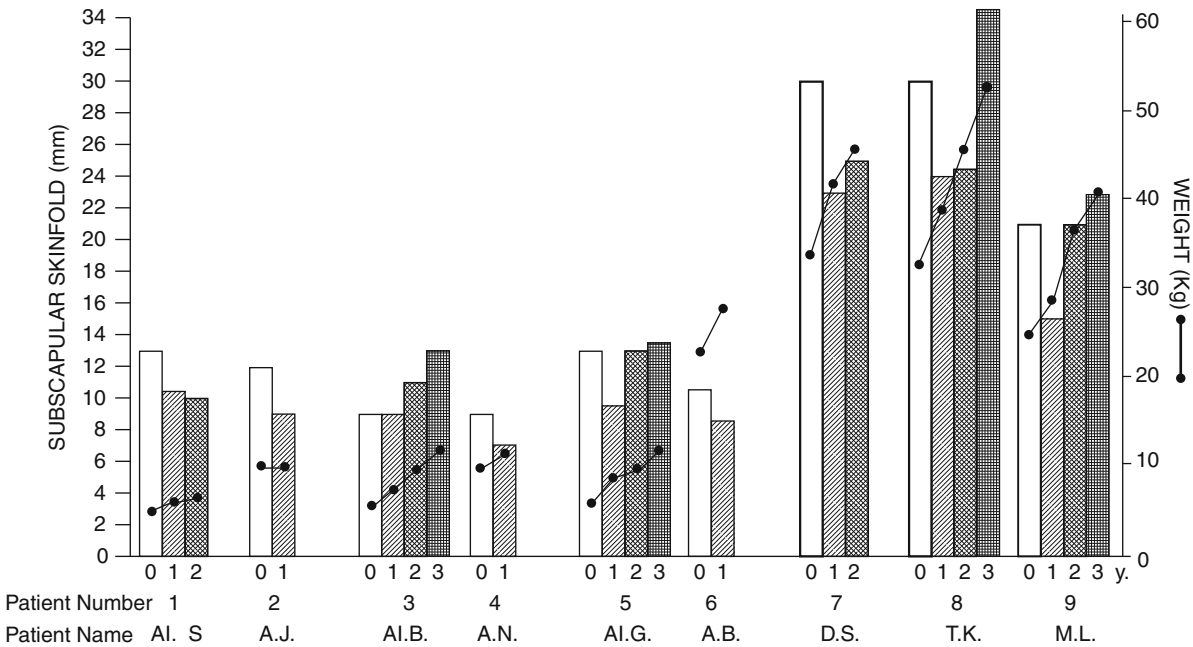

Fig. 44.1 Individual changes in subscapular skinfold thickness and body weight during IGF-I treatment for 1–2 years in nine children with Laron syndrome reproduced with permission from Klinger and Laron (1995)

It is of note that the children with a positive GHBP were less obese than those with a negative GHBP and also their decrease in skinfold thickness was less and not significant. The same group was also less retarded in height (Chap. 8).

Correlating individual changes in subscapular skinfold thickness with the changes in body weight in nine children with Laron syndrome 1 year before initiation of treatment and during 1–2 years of IGF-I administration (Klinger and Laron 1995) (Fig. 44.1), we see that while body weight increased progressively, the skinfold thickness decreased in the first year of treatment but increased again in the second year in most children.

Table 44.2 summarizes the changes in subscapular skinfold thickness in 11 children treated for more than 5 years. It is seen that there is a progressive increase in adiposity as we have reported previously (Laron et al. 2006). Forthwith are illustrations of the progressive obesity of three girls with Laron syndrome during long-term IGF-I treatment. Figures 44.2–44.7 show the anterior and lateral appearance of a girl before treatment at age $2^{7/12}$ years and the lateral appearance at ages $4^{7/12}$, $5^{6/12}$, $6^{6/12}$, and $7^{4/12}$ after 5 years of IGF-I treatment. Figures 44.8–44.15 illustrate the anterior and lateral view of a girl before initiation of treatment at age $9^{6/12}$ and during IGF-I treatment at ages $10^{7/12}$, $13^{6/12}$, $14^{3/12}$, and $15^{3/12}$ years.

Figures 44.16–44.22 illustrate the anterior view of a girl at age 5 after 1 year of IGF-I treatment and at ages 7, 11, 13, 15, 17, and $18^{4/12}$ when treatment was stopped.

Table 44.2 Subscapular skinfold thickness of patients with Laron syndrome during long-term IGF-I treatment

During IGF-I therapy (years)																After IGF-I
Patient	Name	Sex	CA at start IGF-I	0	1	2	3	4	5	6	7	8	9	10	11	12	1 year
1	PR	F	$1^{7/12}$	13	17	20	17	20	16^{5}	28	26						
2	HuN	F	$3^{2/12}$				7^{5}	5^{5}	11								
3	CLh	F	$3^{6/12}$					17	17	22	20	23	27				
4	HoS	F	$3^{8/12}$	13	16	14	12	16	23	28	36	32	35	38	42	43	33
5	MaM	F	$9^{7/12}$	17	14	20	29	31	31	33							
6	DS	F	$11^{8/12}$	30	23	23	25	33									30
7	ATK	F	$13^{9/12}$	30	24	24^{4}	34^{5}	35		35	39						31
8	AlS	M	7	13	11	11		9	16	15	20						
9	AlB	M	$3^{5/12}$	9	8	11	15	18	26	29	37						
10	AlG	M	$5^{1/12}$	13	9^{4}	13	13^{5}	15	21	24	27	35	44				
11	ML	M	$14^{11/12}$	22.6	16	21	23^{5}	19	15	15							12

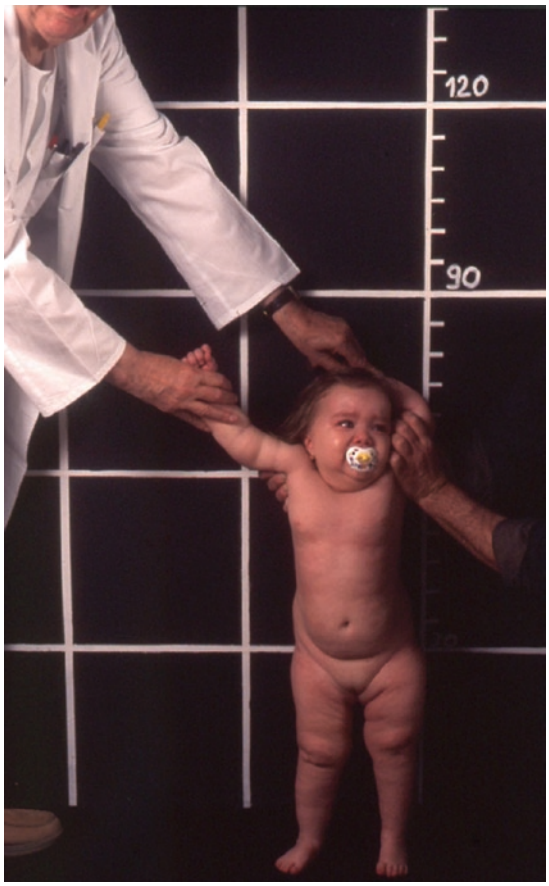

Fig. 44.2 Obesity of a $2^{7/12}$-year-old girl with Laron syndrome (PR) before initiation of IGF-I treatment

Fig. 44.3 Lateral view of same girl as Fig. 44.2 at the same age

The lateral view of the same girl is shown in Figs. 44.23–44.29. The first view at age $3^{6/12}$ years is before IGF-I treatment followed during treatment at ages 7, 10, 14, 16, 17, and $18^{6/12}$. We have previously described the unexpected lipotropic (adipogenic) effect of IGF-I during long-term treatment (Laron et al. 2006).

44.1.3 Comment

It is obvious that long-term IGF-I treatment increases the degree of adiposity despite a reduction in insulin levels (Table 44.1) and a reduction in serum Ghrelin levels (Uckun-Kitapci et al. 2008) (see Chap. 30). It is possible that IGF-I activates the insulin receptors, but at no time, the serum insulin levels are excessive. Increased adiposity during IGF-I treatment has also been reported by Backeljauw et al. (2001).

44.2 Serum Lipids During Long-Term IGF-I Treatment

Despite an initial slightly suppressing effect on total and LDL cholesterol, this effect waned, and Laron syndrome patients treated for years with IGF-I showed elevated blood lipid levels, which necessitated the use of lipid-lowering drugs even at a relatively young age.

Figures 44.30–44.35 illustrate the serum levels of total, LDL, and HDL cholesterol in both genders. It is evident that Laron syndrome patients had elevated values even before puberty despite the IGF-I treatment. Figures 44.36 and 44.37 show the serum triglyceride levels during IGF-I treatment along age. It is seen that above normal values were encountered more during puberty in girls.

We showed that the obesity and hyperlipidemia are not associated with excessive nutritional intake (Ginsberg et al. 2009).

Fig. 44.4 Lateral view of same girl as Fig. 44.2 at age $4^{7/12}$ after 2 years of IGF-I treatment

Fig. 44.5 Lateral view of same girl as Fig. 44.2 at age $5^{6/12}$ after 3 years of IGF-I treatment

44.2.1 Conclusions

Long-term IGF-I treatment increases the mass of adipose tissue and does not prevent the progressively developing hyperlipidemia in patients with Laron syndrome.

44.3 Effect of IGF-I Treatment of Children with Laron Syndrome on Insulin, Blood Glucose, and HbA1c

44.3.1 Introduction

Insulin, the important regulator of glucose homeostasis, is insufficient to maintain the energy needs of the brain metabolism, which depends largely on circulating fuel supplies, especially from carbohydrates (Evans and Amiel 1998). The body also uses for this purpose a series of counter regulatory hormones such as glucagons, cortisol, and adrenalin (Bolli 1998).

We had the opportunity to expand the knowledge on the role that GH and IGF-I deficiencies play in the carbohydrate metabolism (Laron 2004 and Chap. 30) but also to study the effects of IGF-I when administered to Laron syndrome patients. Forthwith our experience in children (for effects in adult patients, see Chap. 46).

44.3.2 Results

44.3.2.1 Intravenous Bolus Injection of IGF-I

The bolus injection of 75 μg/kg IGF-I after an overnight fast to two children aged 11 and $11^{7/12}$ years (Laron et al. 1988) resulted in a reduction of serum glucose (nadir at 15 min) and a concomitant decrease of

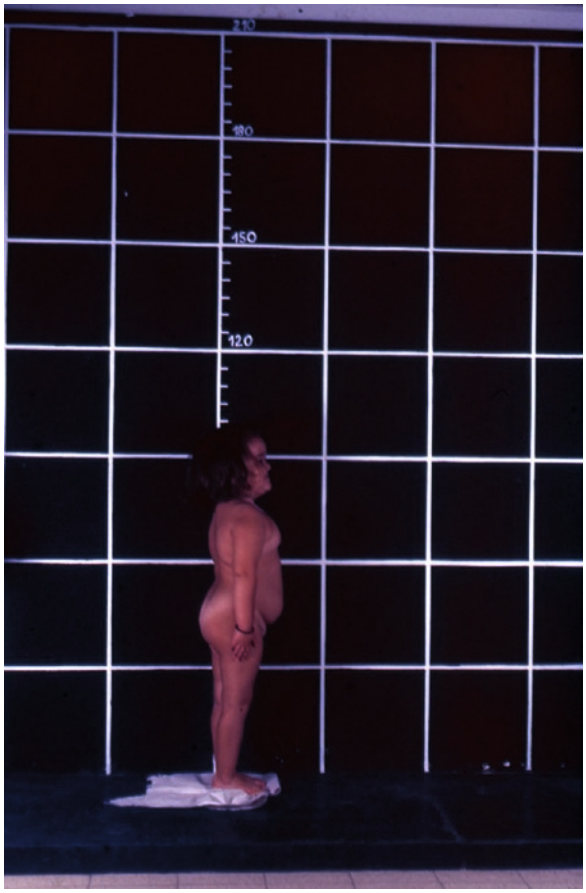

Fig. 44.6 Lateral view of same girl as Fig. 44.2 at age $6^{6/12}$ after 4 years of IGF-I treatment

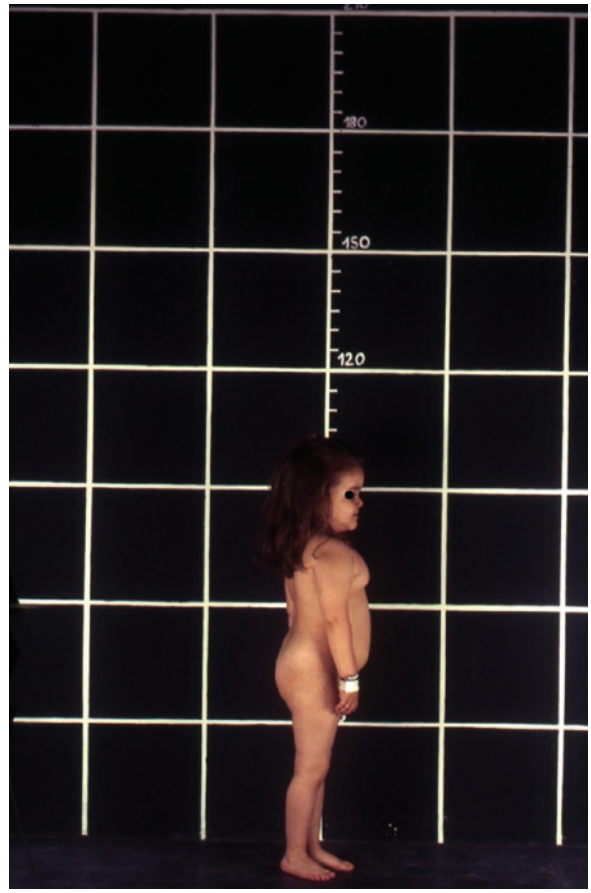

Fig. 44.7 Lateral view of same girl as Fig. 44.2 at age $7^{4/12}$ after 5 years of IGF-I treatment

serum insulin (Table 44.3) denoting that the hypoglycemia induced by IGF-I was independent of insulin.

44.3.2.2 Effect of Continuous IGF-I Treatment on Serum Insulin, Glucose, and HbA1c

Daily subcutaneous treatment of IGF-I (one injection per day) resulted in reduction of the insulin levels during the first 2 months of treatment followed by small fluctuations thereafter (Fig. 44.38).

The reduction in insulin levels resulted in a rise in blood glucose as expressed in the HbA1c levels (Fig. 44.39) so that the net effects were less (if any) complaints of hypoglycemia.

Table 44.4 summarizes the overnight fasting serum levels of glucose, insulin, and HbA1c in six children with Laron syndrome before and during 2 years of IGF-I treatment.

IGF-I treatment also persistently affected the glucose and insulin response during OGTT. In three out of five adult Laron syndrome patients, long-term IGF-I

Fig. 44.8 Anterior view of a $9^{6/12}$-year-old girl with Laron syndrome (MM) before initiation of IGF-I treatment

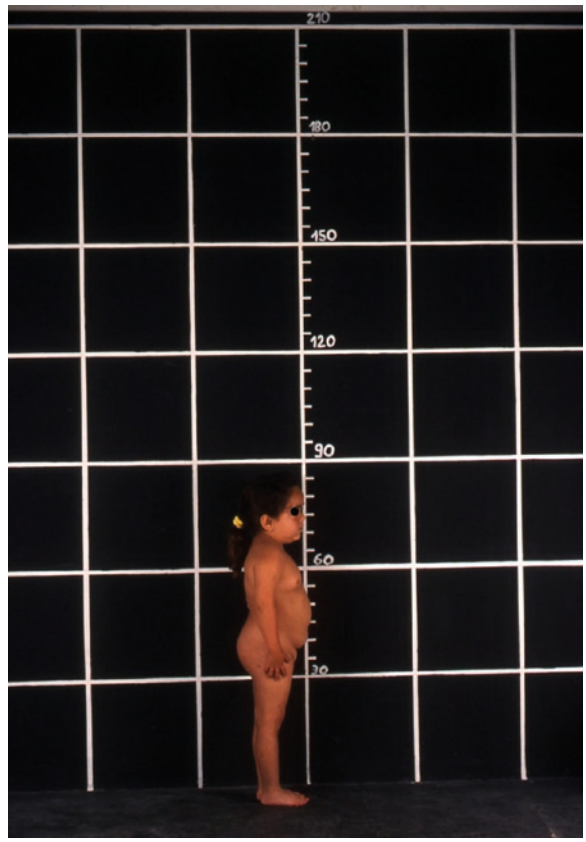

Fig. 44.9 Lateral view of same girl as Fig. 44.8 at the same age

treatment decreased the insulin response and improved the glucose response. The effect of IGF-I treatment in a 13-year-old girl with Laron syndrome is shown in Fig. 44.40 (Laron et al. 1995).

44.3.3 Comment

Despite the beneficial effects of IGF-I treatment on carbohydrate metabolism, which resulted in both subjective and objective improvement of blood glucose and an improved insulin sensitivity (Dunger et al. 1997; Moses 1997), Laron syndrome patients on long-term IGF-I treatment had hypoglycemia occasionally (most often subclinical) as evidenced in our patients (Figs. 30.5 and 30.6). The hypoglycemia episodes have been reduced also by keeping regular meal times. The HbA1c levels along age in our IGF-I treated female and male Laron syndrome patients are shown in Figs. 44.41 and 44.42.

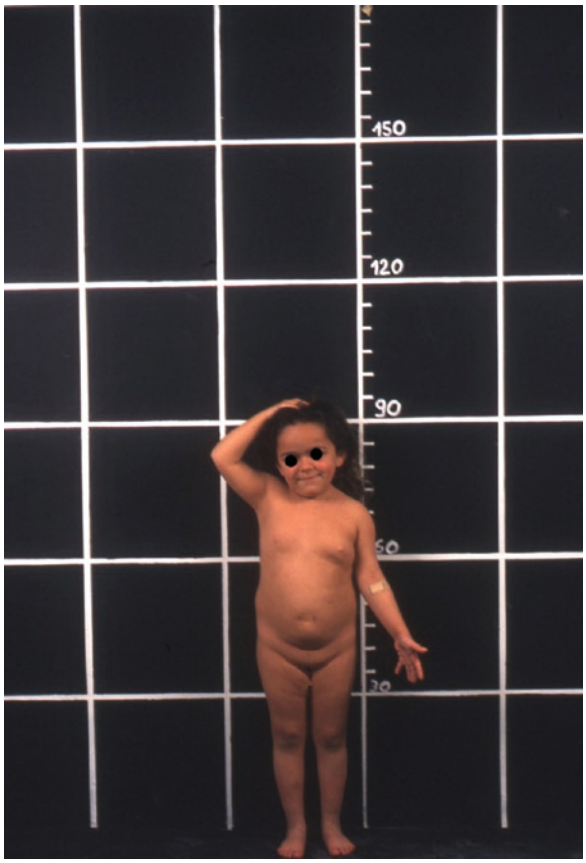

Fig. 44.10 Anterior view of same girl as Fig. 44.8 at age $10^{7/12}$, 1 year after IGF-I treatment

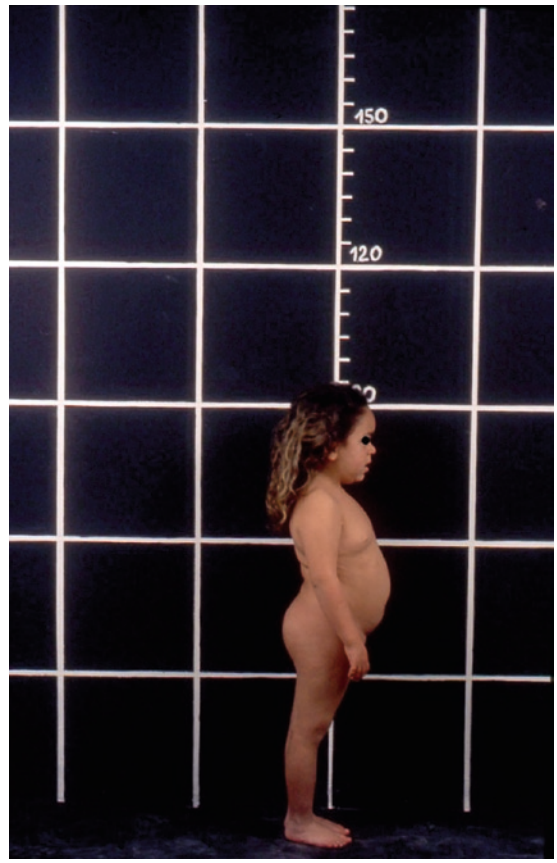

Fig. 44.11 Lateral view of same girl as Fig. 44.10 at age $7^{6/12}$ after 5 years of IGF-I treatment

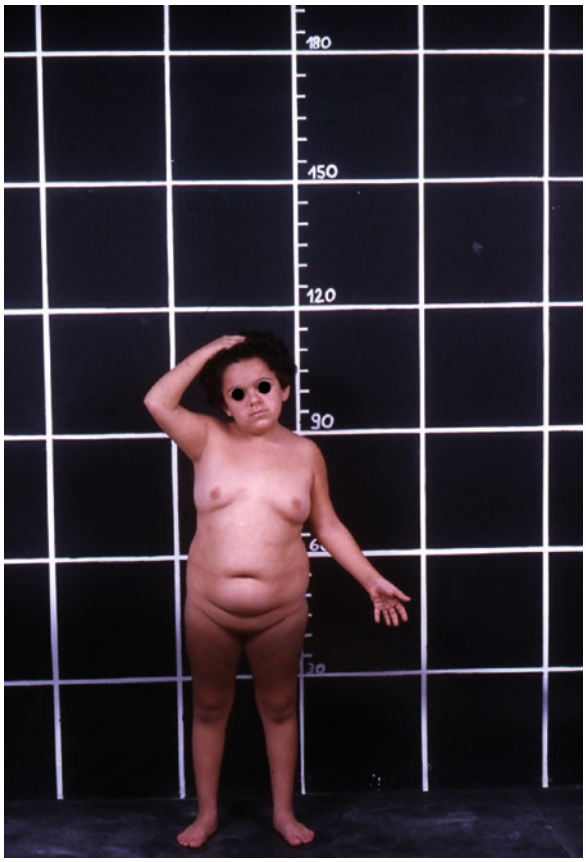

Fig. 44.12 Anterior view of same girl at age $13^{6/12}$, 4 years after initiating IGF-I treatment

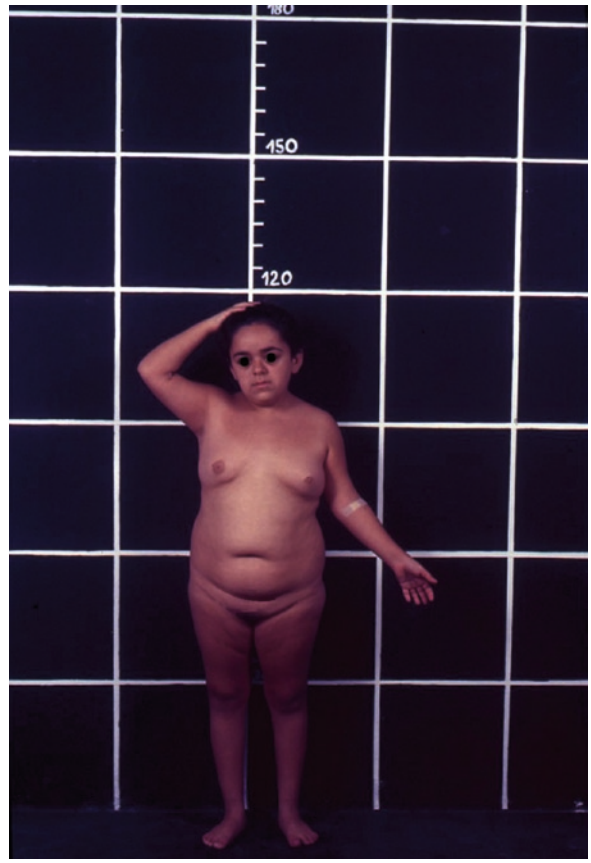

Fig. 44.13 Anterior view of same girl at age $14^{3/12}$, almost 5 years after initiating IGF-I treatment

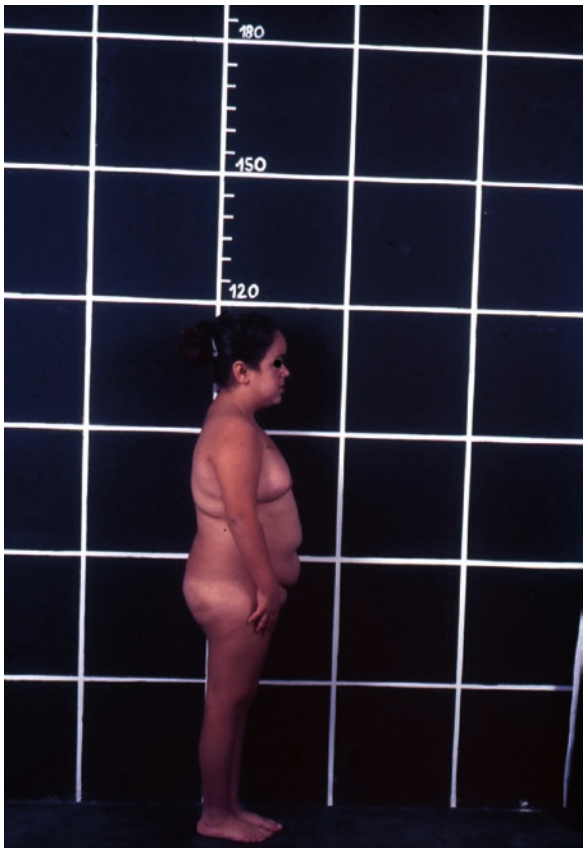

Fig. 44.14 Lateral view of same girl at age $14^{3/12}$, almost 5 years after initiating IGF-I treatment

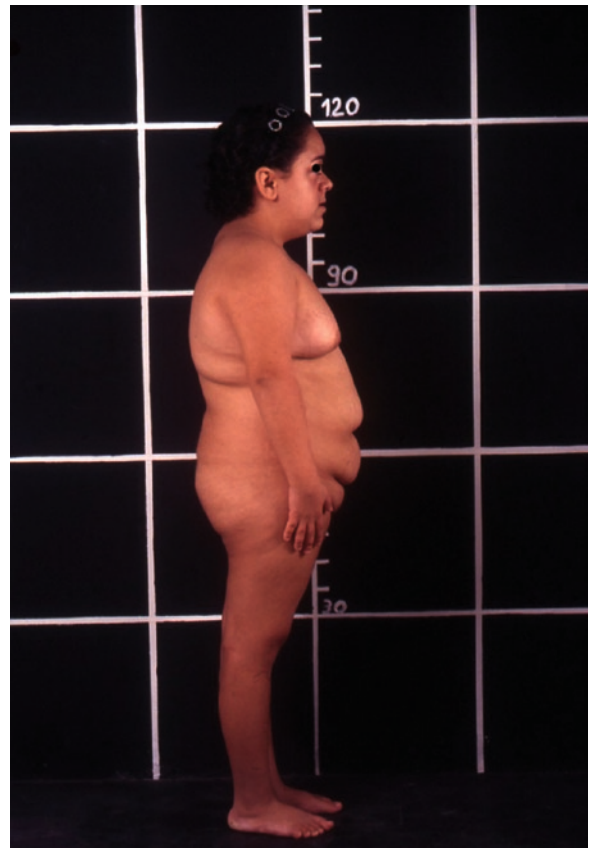

Fig. 44.15 Lateral view of same girl at age $15^{3/12}$ after 6 years of IGF-I treatment

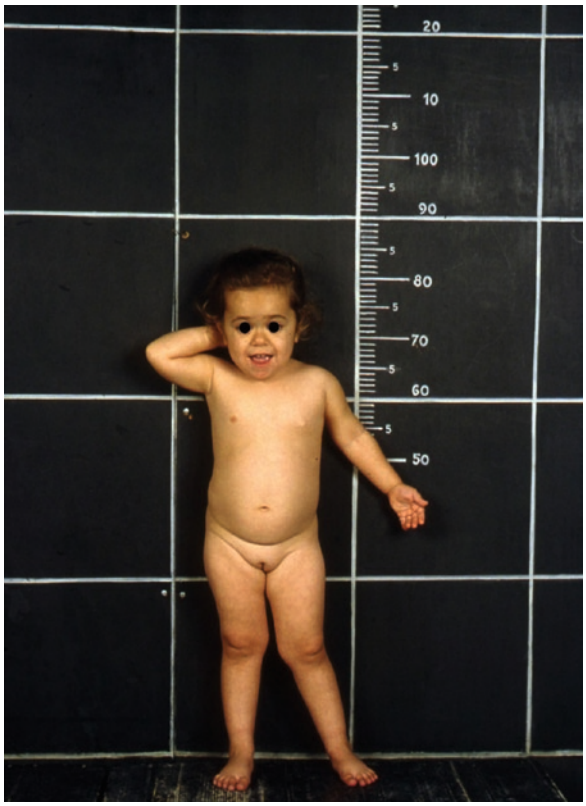

Fig. 44.16 Anterior view of a girl with Laron syndrome at age 5 (HS), 1 year after initiating IGF-I treatment

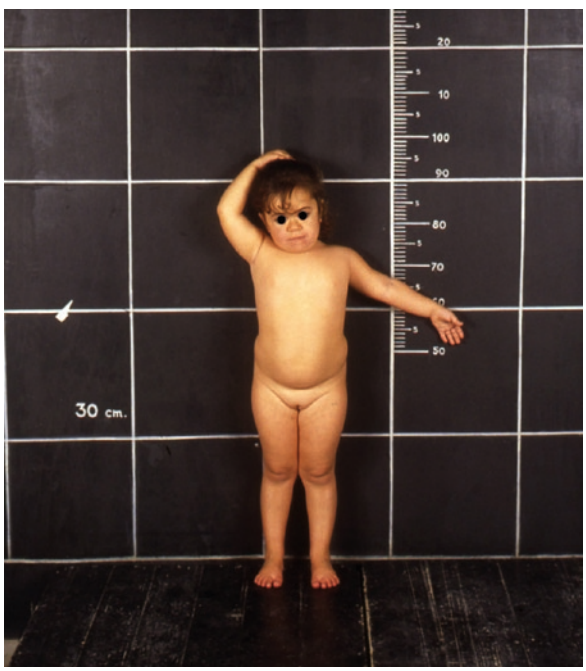

Fig. 44.17 Anterior view of same girl at age 7, 3 years after IGF-I treatment

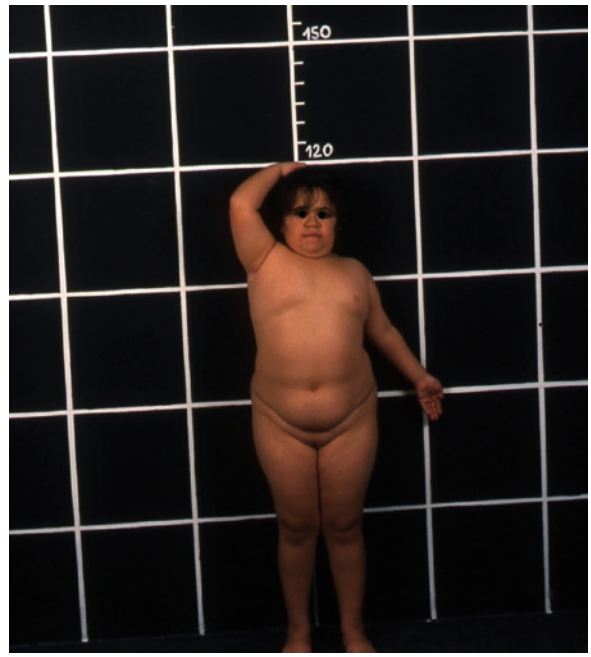

Fig. 44.18 Anterior view of same girl at age 11 during 7 years of IGF-I treatment

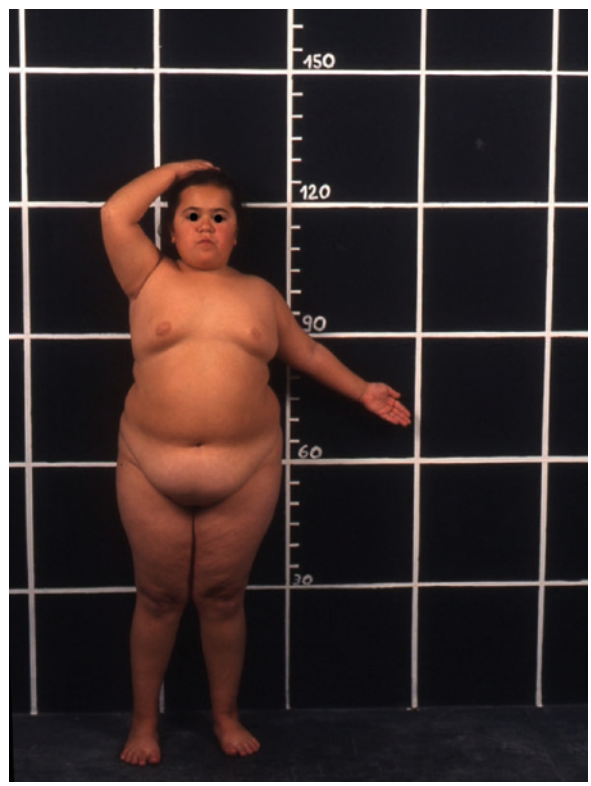

Fig. 44.19 Anterior view of same girl at age 13 during 9 years of IGF-I treatment

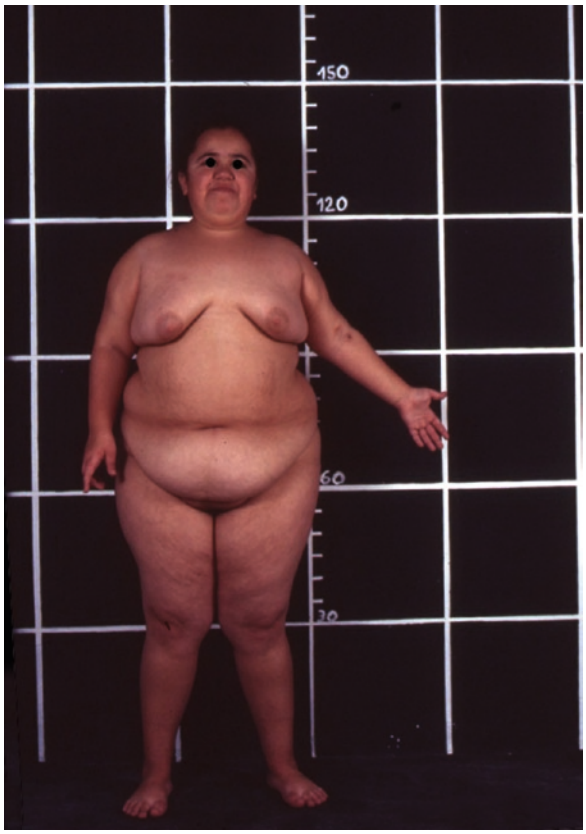

Fig. 44.20 Anterior view of same girl at age 15 during 11 years of IGF-I treatment

Fig. 44.21 Anterior view of same girl at age 17 during 13 years of IGF-I treatment

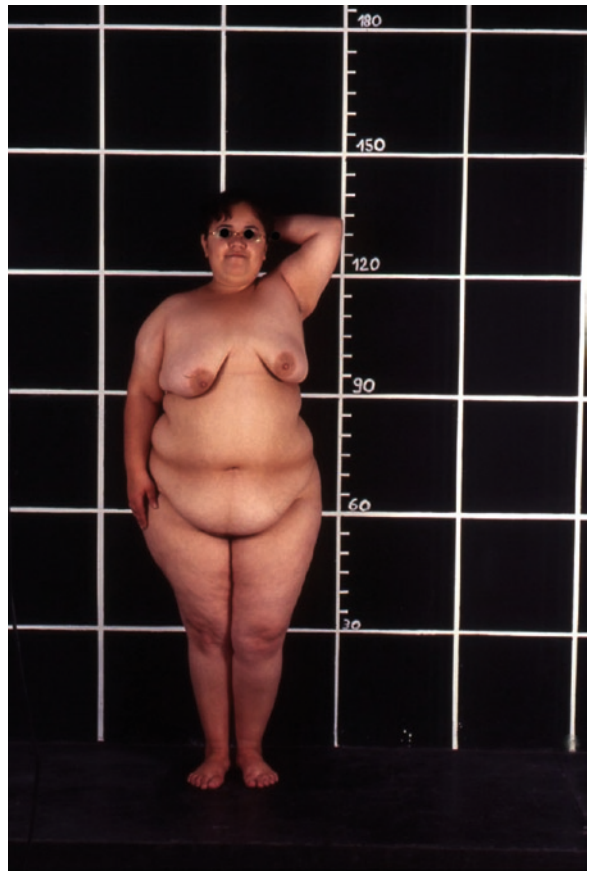

Fig. 44.22 Anterior view of same girl at age 18 in the last year of IGF-I treatment

Fig. 44.23 Lateral view of HS at age $3^{7/12}$ years before initiation of IGF-I treatment

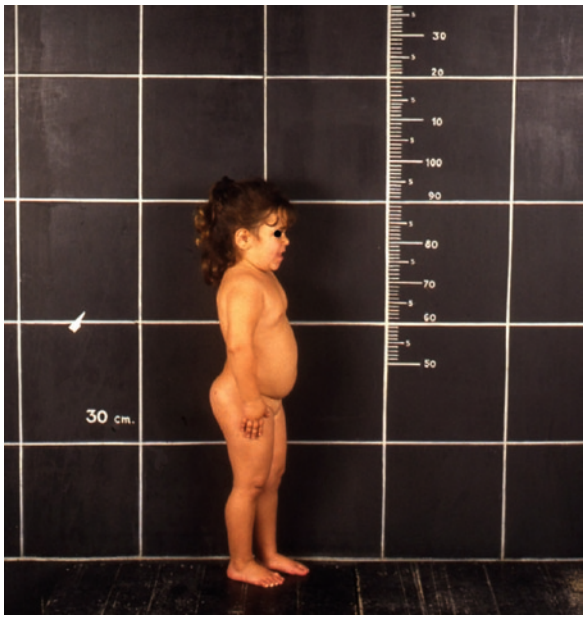

Fig. 44.24 Lateral view of same girl at age 7, $3^{1/2}$ years after starting IGF-I treatment

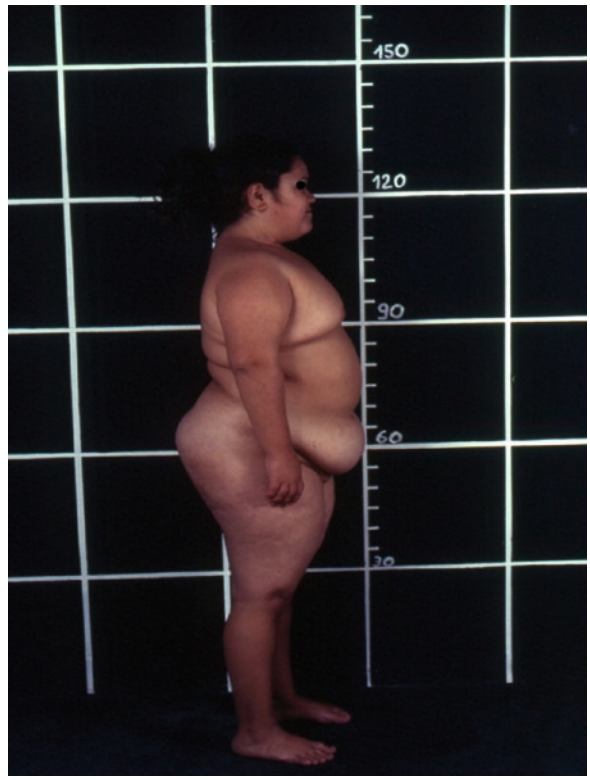

Fig. 44.26 Lateral view of same girl at age 14 during IGF-I treatment

Fig. 44.25 Lateral view of same girl at age 10 during IGF-I treatment

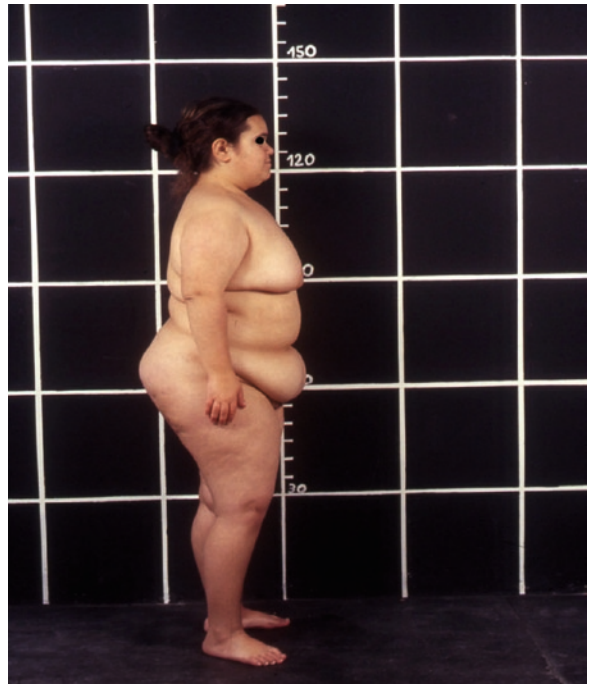

Fig. 44.27 Lateral view of same girl at age 16 during IGF-I treatment

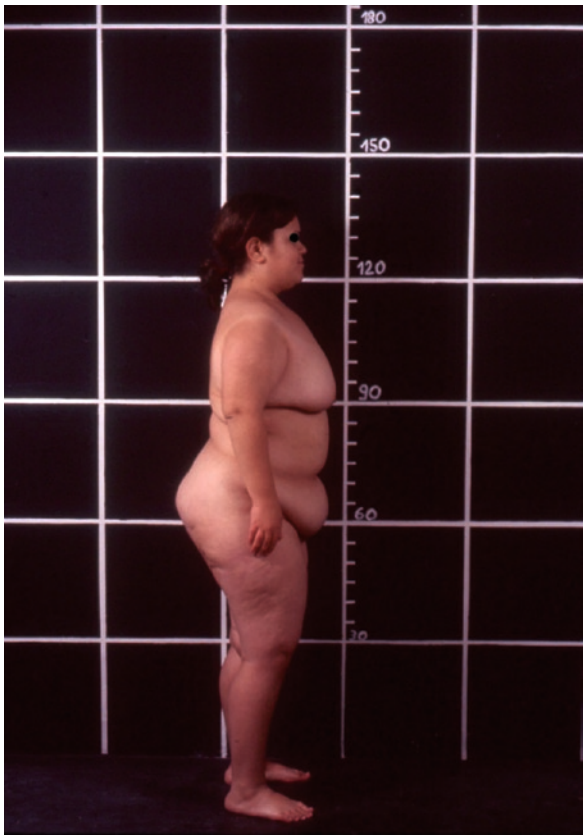

Fig. 44.28 Lateral view of same girl at age 17 during IGF-I treatment

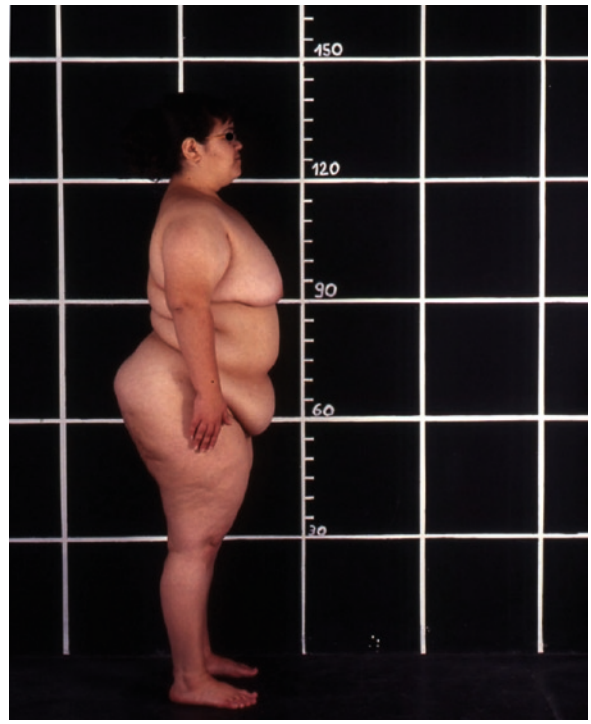

Fig. 44.29 Lateral view of same girl at age $18^{6/12}$ when IGF-I treatment was stopped after 15 years administration

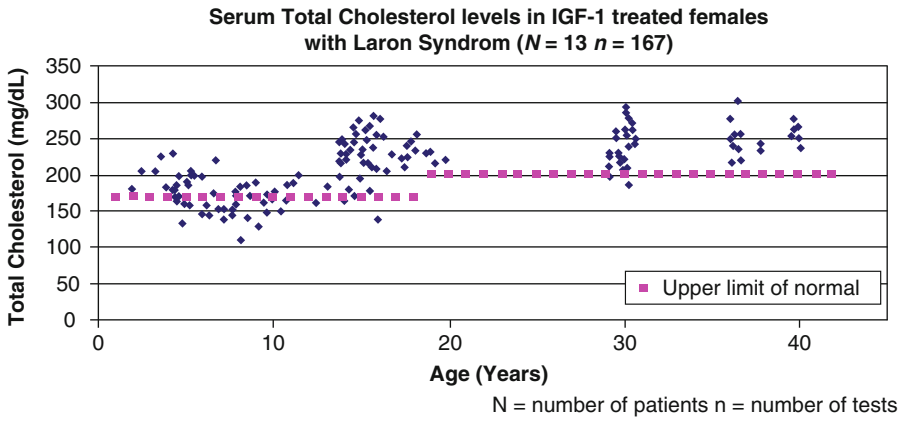

Fig. 44.30 Serum total cholesterol in female Laron syndrome patients during IGF-I treatment along age

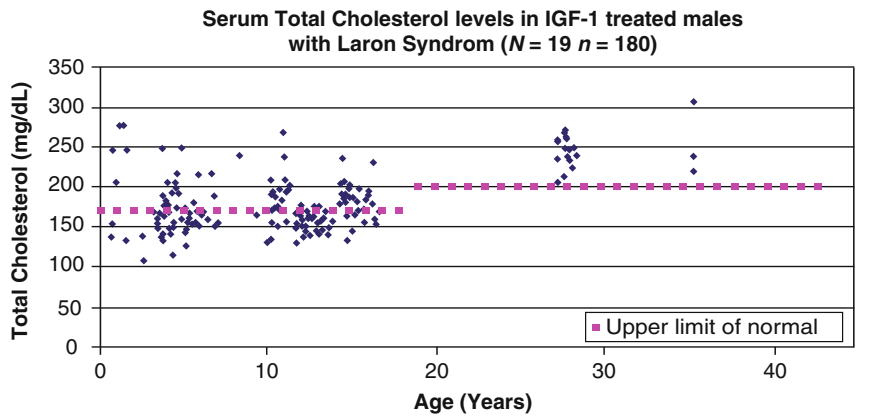

Fig. 44.31 Serum total cholesterol in male Laron syndrome patients during IGF-I treatment along age

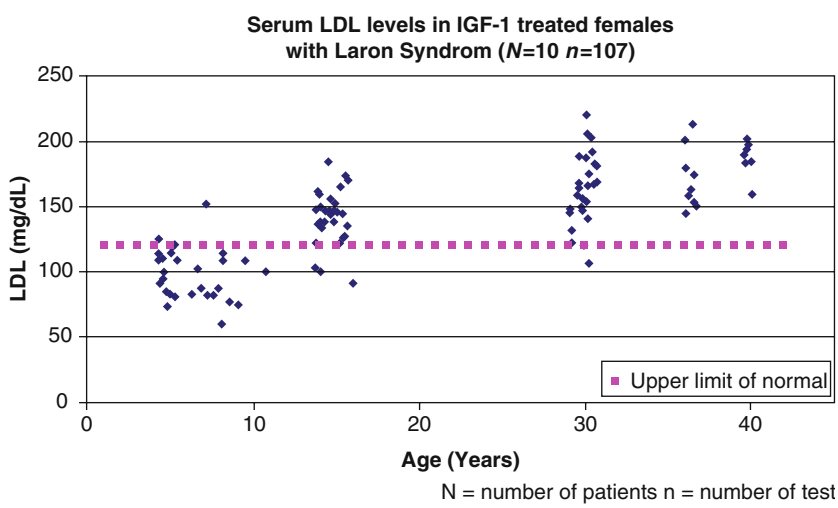

Fig. 44.32 Serum LDL cholesterol in female Laron syndrome patients during IGF-I treatment along age

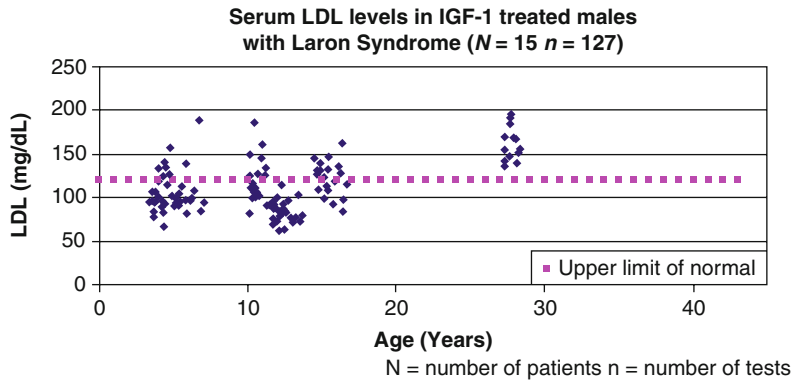

Fig. 44.33 Serum LDL cholesterol in male Laron syndrome patients during IGF-I treatment along age

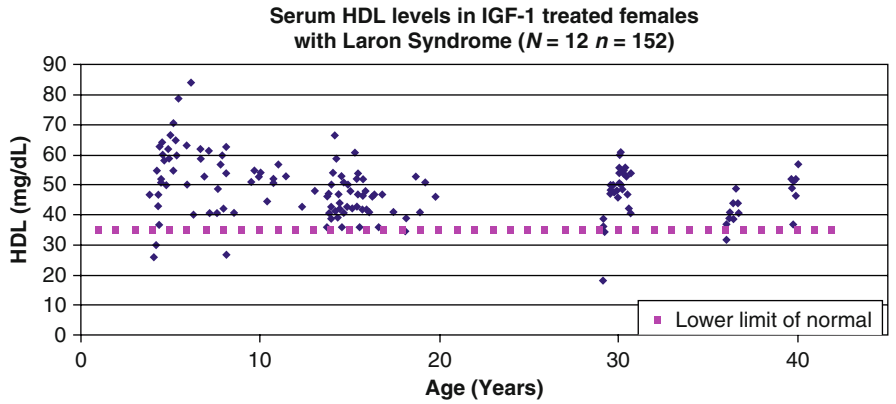

Fig. 44.34 Serum HDL cholesterol in female Laron syndrome patients during IGF-I treatment along age

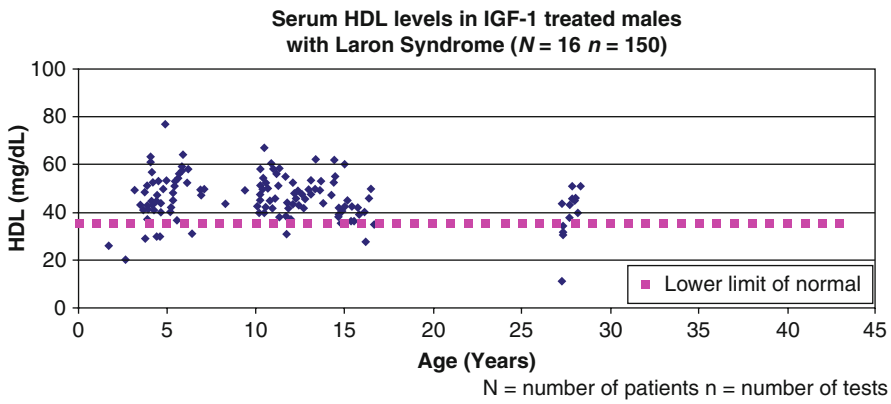

Fig. 44.35 Serum HDL cholesterol in male Laron syndrome patients during IGF-I treatment along age

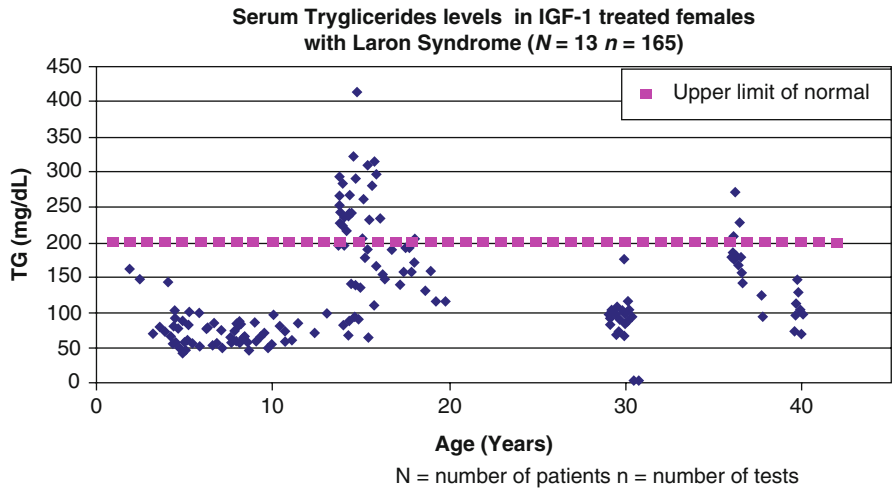

Fig. 44.36 Serum triglyceride levels in female Laron syndrome patients during IGF-I treatment

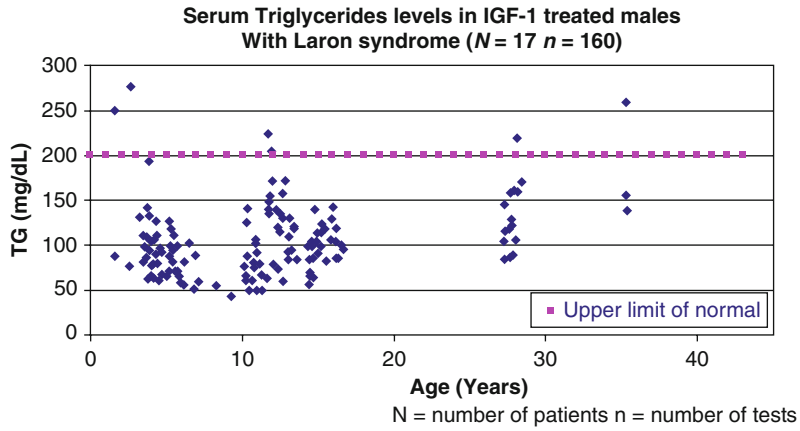

Fig. 44.37 Serum triglyceride levels in male Laron syndrome patients during IGF-I treatment

Table 44.3 Glucose and insulin response to an intravenous bolus of IGF-I injection (75 μg/kg) administered to two children with Laron syndrome

		Time (min)													
		Glucose (mg/dL)							Insulin (mU/mL)						
Minutes	Sex	0	2	5	15	30	60	120	0	2	5	15	30	60	120
Patient															
1	Girl	77.4	79.2	73.8	52.2	57.6	82.8	73.8	5.7	5.3	3.8	2.3	2.1	1.8	1.8
2	Boy	66/6	68.4	59.4	37.8	–	50.4	52.2	2.2	2.1	1.8	1.6	1.4	1.5	1.4

Modified from Laron et al. (1988)

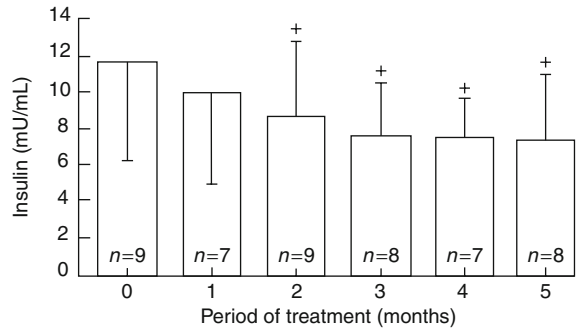

Fig. 44.38 Serum insulin levels during 5 months of IGF-I treatment to patients with Laron syndrome reproduced with permission from Gafny et al. (1994)

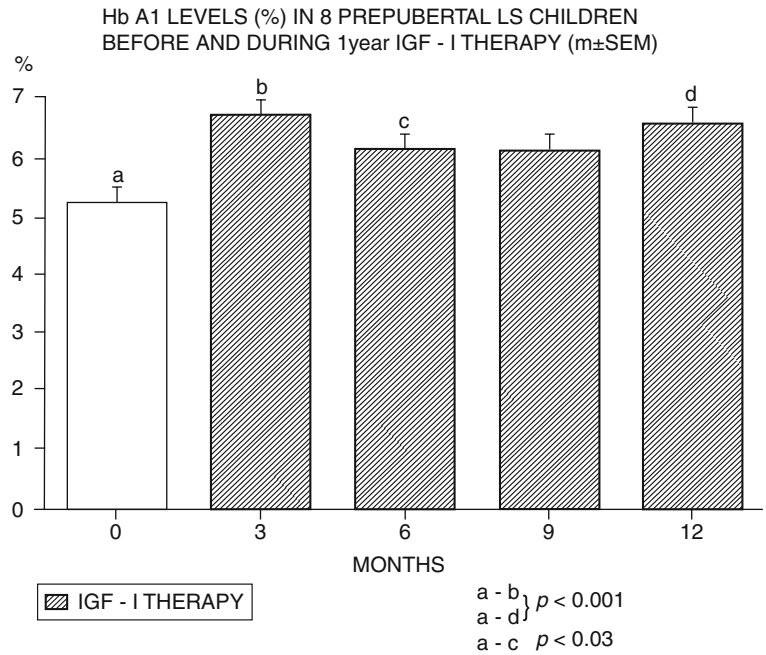

Fig. 44.39 Blood hemoglobin A1c levels in eight prepubertal children with Laron syndrome before and during IGF-I treatment (m ± SEM)

Table 44.4 Glucose, HbA1c, and insulin levels before and during 2 years of IGF-I treatment of children with Laron syndrome

	0	1 year	2 years	3 years
Glucose (mg/dL)	64.8 ± 7.2	68.4 ± 9	72 ± 9	77.4 ± 3.6
HbA1c (%)	5.35 ± 0.21 (6)	6.01 ± 0.3 (9)	5.65 ± 0.24 (6)	6.99 ± 0.11 (4)
Insulin (mU/mL)	9.63 ± 2.35 (9)	6.08 ± 1.45[a] (9)	7.06 ± 2.82 (6)	

The number within the parentheses indicates the number of patients
As m ± SD
[a] $p < 0.0.04$ vs. basal

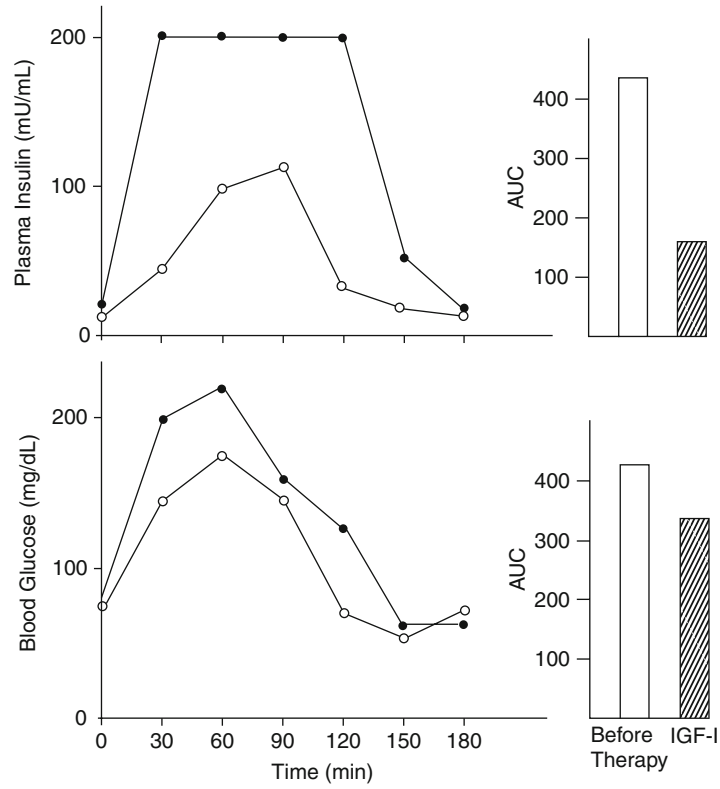

Fig. 44.40 Glucose and insulin response to an oral glucose tolerance test in a 13-year-old girl with Laron syndrome before (*closed circles*) and during IGF-I treatment (*open circles*) reproduced with permission from Laron et al. (1997)

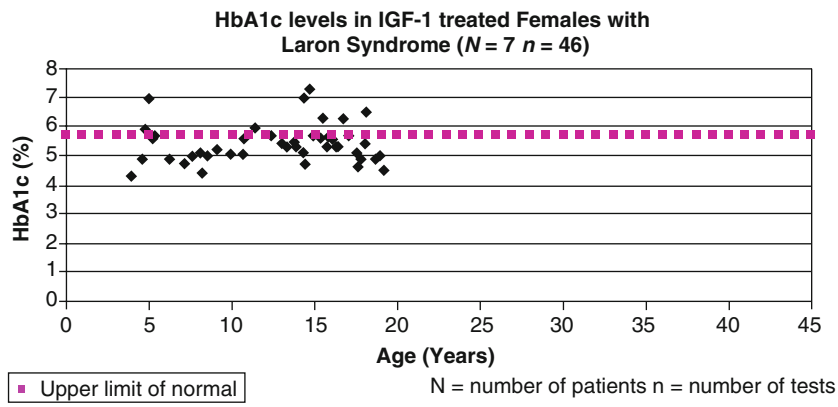

Fig. 44.41 HbA1c in IGF-I treated female patients along age

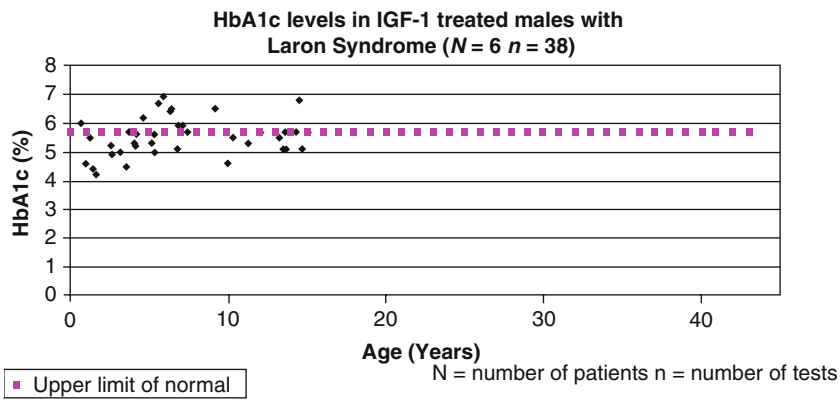

Fig. 44.42 HbA1c in IGF-I treated male patients along age

44.3.4 Conclusions

IGF-I treatment has beneficial effects on the carbohydrate metabolism in patients with IGF-I deficiency and also seems indicated in conditions of severe insulin resistance.

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45 Comparison of the Growth Promoting Response of IGF-I in Children with Laron Syndrome with that of hGH in Children with Isolated GH Deficiency

Zvi Laron

Core Message

› Comparing the growth promoting effect of IGF-I in children with Laron syndrome to that of hGH in children with isolated GH deficiency (IGHD) we found that hGH has a superior effect to that of IGF-I.

45.1 Background

Having found in previous studies that young children with isolated congenital GH deficiency (IGHD) respond better to hGH treatment than older ones (Laron et al. 1987), we wanted to know whether this holds also for IGF-I.

45.2 Subjects

Four young children with cIGHD (2 females and 2 males) aged 0.9–3.0 years and 5 children with Laron syndrome (2 females and 3 males) aged 0.6–3.6 years were studied (Laron and Klinger 2000).

45.3 Methods

Body length was measured before and every 3 months during treatment with a Harpenden anthropometer. Head circumference was measured by a tape. The Tanner growth charts (1966a, b) were used to calculate SDS height. Head circumference was plotted on Nellhaus charts (1967).

IGF-I was administered in doses of 150–200 μg/kg once daily with one exception of a young male who was started on a dose of 50 μg/kg due to previous hypoglycemias. The dose was progressively increased to 150 μg/kg as the hypoglycemias ceased. The dose of hGH was 0.07 U/kg once daily.

45.4 Results

45.4.1 Body Length

Growth velocity was calculated for the years preceding treatment in both groups and during 3 years of treatment. Before the treatment was initiated, the children with Laron syndrome had a decreased height up to −6 or more SDS height and those with IGHD to −5.7 SDS height. During 3 years of IGF-I treatment, the Laron syndrome children gained 1.4 SDS height compared to the IGHD children who gained between 1.2 and 2.4 SDS height during the same length of treatment period.

45.4.2 Head Circumference

Before the initiation of treatment, the head circumference of the Laron syndrome children was below the norm and increased rapidly to the lower limit of norm, i.e., −2.5 SD, with IGF-I administration (Fig. 45.1). The IGHD children had a low normal head circumference before treatment and reached almost mean normal values during hGH administration (Fig. 45.2).

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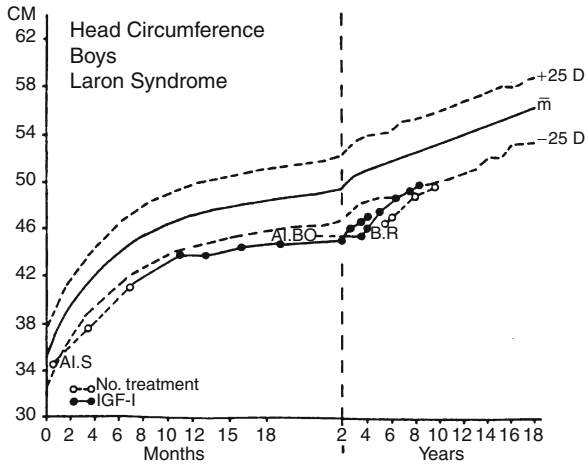

Fig. 45.1 Head circumference of 3 young boys with Laron syndrome before (*open circle*) and during (*filled circle*) IGF-I treatment

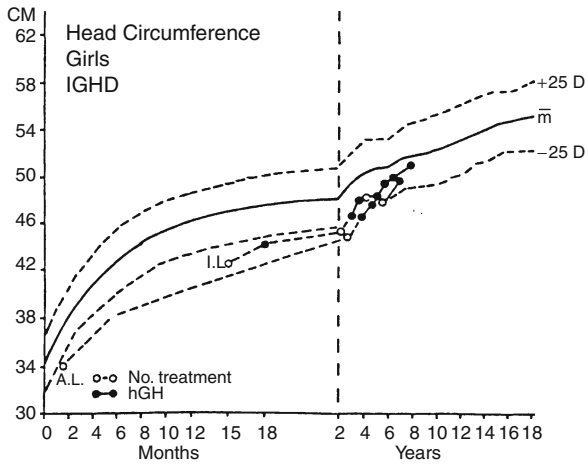

Fig. 45.2 Head circumference of 2 young girls with IGHD before (*open circle*) and during (*filled circle*) hGH treatment

45.4.3 Height and Foot Length

Comparing the height and foot growth response to IGF-I in children with Laron syndrome with that to hGH in children with IGHD (Silbergeld et al. 2007), we found (Table 45.1) that, despite an identical growth deficit at the initiation of treatment during 5 and even 9 years of therapy, both body height and the feet in the children with IGHD treated by hGH grew faster than those in children with Laron syndrome treated by IGF-I.

Table 45.1 Mean growth retardation in height and foot length in untreated adult patients with Laron syndrome (expressed as SDS from normal medians)

	Age range (years)	Body height (SDS)	Foot length (SDS)
Male patients			
1	26	−8.3	−4.4
2	29	−7.7	−7.6
3	30	−4.8	−4.8
4	35	−8.9	−7.4
5	37	−5.4	−4.0
6	39	−8.7	−7.4
7	41	−8.0	−7.4
8	44	−9.5	−6.5
Mean ± SD		−7.7 ± 1.7	−6.2 ± 1.5*
Female patients			
9	25	−6.4	−2.9
10	25	−8.9	−4.1
11	26	−8.3	−5.0
12	30	−7.4	−3.5
13	32	−5.2	−3.2
14	33	−8.5	−5.3
15	42	−4.4	−1.1
Mean ± SD		−7.0 ± 1.7	−3.6 ± 1.4*

Reproduced with permission from Silbergeld et al. (2007)
*$p = 0.005$

45.5 Discussion

Despite both being the "growth hormones" and most probably hGH acting via IGF-I, the present replacement therapies regime gives a certain advantage to the hGH replacement therapy. This is exemplified in Fig. 45.3 representing the growth of 2 girls, one with Laron syndrome and one with congenital IGHD in whom treatment with IGF-I or hGH was initiated at age $3^{6/12}$ and $4^{6/12}$ years, respectively, and who were treated until final height was achieved. It is seen that the catch-up speed and final height were greater in the hGH-treated IGHD girl.

Whether these differences in response are due to genetic or in utero epigenetic factors or failure to initiate the normal growth physiology by the presently applied IGF-I regime remains to be established.

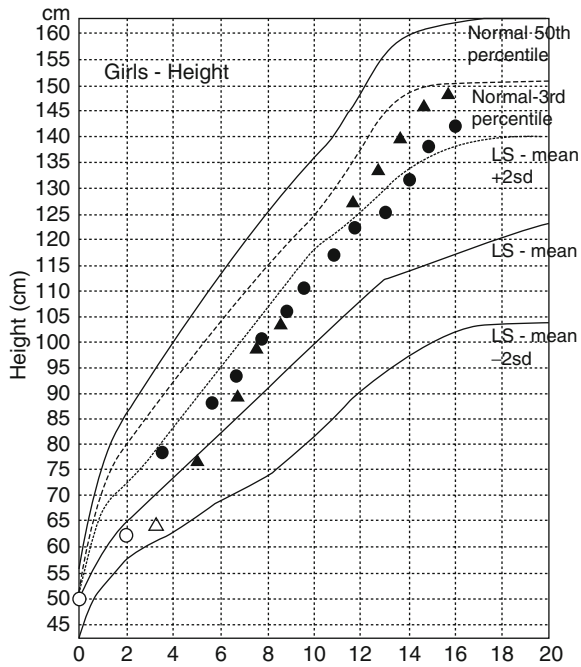

Fig. 45.3 Comparative growth response between a girl with Laron syndrome (*open circle*) treated by IGF-I (*filled circle*) and a girl with congenital isolated IGF-I deficiency (*open triangle*) treated by hGH (*filled triangle*). The growth chart is that for Laron syndrome (Laron et al. 1993). Reproduced with permission from Laron (2008)

Even if the concomitant action effect of GH is needed to evoke full IGF-I activity, this would be of no avail in those with Laron syndrome who are insensitive to hGH.

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IGF-I Treatment of Adult Patients with Laron Syndrome

46

Zvi Laron

Core Message

› IGF-I treatment of adult Laron syndrome patients for a period of 9 months had beneficial metabolic effects on protein and fat metabolism. An improvement in kidney function was also registered.

46.1 Introduction

In recent years, evidence has accumulated that adult patients with GH deficiency benefit from replacement therapy with hGH (Laron and Butenandt 1993; Juul and Jorgensen 2000; Abs et al. 2005; Jorgensen and Christiansen 2005) and that their quality of life is improved (Koltowska-Häggström et al. 2006).

Untreated adult patients with Laron syndrome also suffer from many biochemical abnormalities and an abnormal body composition (Laron 1996, 2001, 2004; Laron et al. 2006. As all are consequences of the long-standing IGF-I deficiency, it seemed logical that they too deserve replacement therapy. We received permission to treat a small group of adult Laron syndrome patients for a period of 9 months. To the best of our knowledge, this is the only long-term trial performed so far (Klinger et al. 1993; Laron and Klinger 1994).

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Following is a summary of our experience with the administration of IGF-I to adult patients with Laron syndrome.

46.2 Effect of One IV Bolus Injection of IGF-I

46.2.1 Subjects and Methods

Seven adult Laron syndrome patients (3 females and 4 males) aged 25–34$^{8/12}$ years were administered 75 μg/kg IGF-I (FK 780, Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) intravenously after an overnight fast (Laron et al. 1988).

46.2.2 Results

The mean (±SD) responses of glucose, insulin, and hGH are shown in Table 46.1. It is seen that IGF-I induced a marked hypoglycemia (nadir at 30 min) in the presence of reduced serum insulin. The hGH levels suppressed at 2 min developed a marked rebound of more than ten times the basal values.

46.3 Long-Term IGF-I Administration

46.3.1 Subjects and Methods

Five adult Laron syndrome patients (1 male, 4 females) belonging to the group followed by us since childhood (Laron et al. 1968) were included in the study (Laron and

Z. Laron and J. Kopchick (eds.), *Laron Syndrome - From Man to Mouse*,
DOI: 10.1007/978-3-642-11183-9_46,

Table 46.1 Glucose, insulin, and hGH response to an IV injection of IGF-I to 7 adults with Laron syndrome (m ± SD)

Time (min)	Glucose (mg/dL)	Insulin (μU/mL)	hGH (ng/mL)
0	77.4 ± 3.6	5.75 ± 0.8	4.4 ± 2.1
5	64.2 ± 3.6	4.58 ± 0.7	5.2 ± 4.8
15	43.2 ± 5.4	2.56 ± 0.4	9.4 ± 5.6
30	35.7 ± 3.6	2.33 ± 0.2	25.6 ± 9.6
60	46.0 ± 5.3	2.17 ± 0.3	70.7 ± 10
120	49.1 ± 3.5	2.62 ± 0.7	52.3 ± 8.7
180	128.6 ± 7.2	16.6 ± 3.6	

Serum hGH at 2 min = 2.76 ng/mL

Table 46.2 Pertinent clinical data of 5 adult Laron syndrome patients at the start of IGF-I treatment

Patient number	Sex	Age (years)	Height		Weight (kg)
			cm	SDS	
1	Male	$28^{1/12}$	138.6	−5.4	58.7
2	Female	30	137.0	−5.5	54.6
3	Female	$30^{3/12}$	118.0	−7.4	33.4
4	Female	$35^{9/12}$	112.3	−8.0	39.0
5	Female	$39^{7/12}$	121.3	−7.0	44.0
Mean			125.4	−6.7	45.9
SD			5.2	0.5	4.7

SDS Standard Deviation Score

Klinger 1994). Their pertinent clinical data are shown in Table 46.2. Figure 46.1 shows Dr. Zvi Laron with one of the adult Laron syndrome patients. The drug used was synthetic recombinant IGF-I (FK780, Lot 11570K, Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan).

Knowing that adult patients with GH deficiency become sensitive to hGH, we tried the same with low and increasing doses of IGF-I in the adult patients from 50 to 150 μg/kg. We found that a dose of 120 μg/kg once daily is the optimal dose for metabolic activity and well-tolerated by 3 patients. In 2 females the dose had to be reduced to 50 and 75 μg/kg/day.

Blood chemistry determinations were made in the laboratory of our hospital.

Serum procollagen I (PICP) and III (PIIINP) were determined by RIA in the laboratory of Dr. L.T. Jensen, Copenhagen, Denmark.

The main findings are summarized below. Certain specific data have been incorporated in previous chapters and will not be repeated.

46.3.2 Results

In the 5 adult Laron syndrome patients, the mean fasting IGF-I level before initiation of IGF-I treatment was 31.16 ± 5.3 ng/mL, and after 9 months of IGF-I treatment with 120 μg/kg/ injected s.c. once daily, it rose to 122.4 ± 29 ng/mL. At 4 h after the first IGF-I injection, the mean serum level was 183.2 ± 44 ng/mL and rose to 507.7 ± 117 ng/mL after 9-month-IGF-I treatment (Laron et al. 1999).

46.3.3 Adiposity

Body weight fluctuated and did not yield conclusions. Subscapular skinfold thickness decreased from 27.5 ± 1.4 (SD) to 23.3 ± 2.6 mm after 3 and 6 months of IGF-I treatment ($p < 0.002$), but thereafter, the subcutaneous fat increased again with a further increase

Fig. 46.1 Dr. Laron with one of the adult patients with Laron syndrome enrolled in the clinical trial

after stopping the IGF-I administration at 9 months (Laron and Klinger 1994). Thus, the reduction in body fat induced by IGF-I is only of temporary duration.

46.3.4 Blood Lipids

Table 46.3 shows the effects of IGF-I administration on serum cholesterol and triglycerides. IGF-I caused a progressive significant reduction in total and LDL cholesterol ($p<0.01$) with a return to pretreatment values upon its discontinuation. There were no significant changes in the serum values of HDL cholesterol and triglycerides.

Serum lipoprotein a [Lp(a)], an independent cardiovascular risk factor (Assmann et al. 1996), was studied in all 5 adult patients before and after 9 monthly IGF-I administration. A marked reduction (−66% change; $p<0.001$) was observed during IGF-I treatment (Table 46.4) concomitantly with a decrease in serum insulin ($p<0.05$) (Laron et al. 1996, 1997a).

Our results have been replicated by Bianda and Schmid (1998) and Olivecrona et al. (1995) using 1-week-IGF-I administration to GH-deficient adults. *It is noteworthy that we (Laron et al. 1997b) as well as Oscarsson et al. (1994) found that hGH administration to GH-deficient patients had an opposing effect to that of IGF-I, i.e., increased the serum Lp(a) level, as well as those of serum insulin.* These contrasting actions of IGF-I and GH are one of the major differences between these two anabolic growth hormones.

The effects of IGF-I on leptin and adiponectin have been described in detail separately in Chap. 16. The major and unexpected findings were elevated serum adiponectin levels in the presence of extreme obesity (Laron et al. 2007; Kanety et al. 2009).

Table 46.3 Effect of 9-month-IGF-I administration on serum cholesterol in 5 adult patients with Laron syndrome

	Before IGF-I	9 Months IGF-I	*p*
Total cholesterol mg/dL	262.2 ± 10.8	228.1 ± 13.9	<0.01
LDL cholesterol mg/dL	187.9 ± 8.9	145.4 ± 13.5	<0.01

Table 46.4 Serum lipotropin (a) concentrations in patients with Laron syndrome before and after 9-month-IGF-I administration

Patient number	Sex	Age (years)	IGF-I treatment	
			Before	9 Months
1	Male	$28^{1/12}$	70	37
2	Female	30	153	92
3	Female	$30^{3/12}$	103	50
4	Female	$35^{9/12}$	35	5
5	Female	$39^{7/12}$	35	10

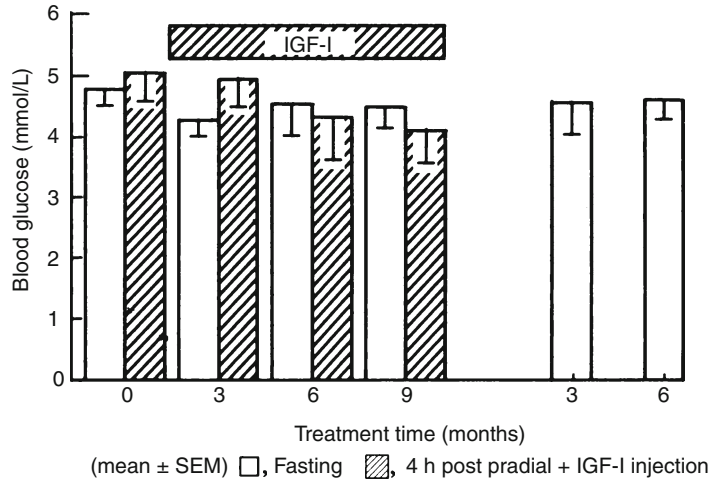

Fig. 46.2 Fasting and 4 h postprandial blood glucose levels before and during IGF-I treatment of 5 adult Laron syndrome patients. Reproduced with permission from Laron and Klinger (1994)

Table 46.5 Mean IGF-I and serum procollagen levels before, during, and after 9 months of IGF-I treatment in 5 adults with Laron syndrome

Procollagens (μg/L)	IGF-I treatment (months)						
	0	3	6	9	3 Months off treatment	*p*	Norm
PIINP	2.7	8.4	6.02	6.6	3.22	<0.001	2.0–4.2
PICP	43.4	135.8	128.6	134.4	87.2	<0.001	100–200
ICTP	3.6	–	5.5	4.9	3.7	<0.001	1–4.5

46.3.5 Carbohydrate Metabolism

The mean ± SD fasting basal and postprandial (4 h after the IGF-I injection and breakfast) blood glucose levels throughout the whole 9-month-period of study are shown in Fig. 46.2.

Fasting glucose levels were within the low normal range most of the times, during the 9-month-IGF-I therapy. In the first 2 weeks, there was an initial and transitory reduction (from 85.76 ± 4.86 to 71.5 ± 5.58 mg/dL (m ± SD). Postprandial glucose levels appeared to show a slight decrease during treatment, but this was statistically not significant. After discontinuation of the treatment, basal glucose levels rose again. Fasting insulin levels were at the upper limit of the normal range before therapy (11.26 ± 2.4 μU/L) and fluctuated slightly during the IGF-I administration. The mean ± SD serum insulin levels after 5-month-IGF-I treatment were 7.1 ± 2 μU/L).

46.3.6 Serum Procollagens

The basal levels of serum PICP, the principal component of the bone matrix, were below the normal range for age. Both PICP, procollagen III (PIIINP) and the pyridinoline cross-linked carboxyterminal telopeptide of Type I collagen (ICTP), increased significantly soon after the initiation of treatment (Klinger et al. 1996). The findings are summarized in Table 46.5.

Bone mineral density and kidney functions have been described and discussed in the respective Chaps. 18 and 34.

46.4 Discussion

Our experience with IGF-I treatment of adult Laron syndrome patients is so far the longest. Vaccarello

et al. (1993) administered 40 μg/kg b.i.d. to 6 adults with Laron syndrome from Ecuador for 7 days. They described an increase of IGFBP2 and found with the above dose no significant changes either in the IGF-I half life (8.2 ± 1.5 to 9.7 ± 1.9 h) or in the metabolic clearance of the hormone. Mauras et al. (2000) administered IGF-I 60 μg/kg s.c. bid to 10 adult Laron syndrome patients from Ecuador over a period of 8 weeks. While the plasma IGF-I increased from 9.3 ± 1.53 to 153 ± 23 ng/mL, these authors observed no significant change in weight, but a significant decrease in percent body fat, an increase in lean body mass, and an increase in the rate of protein turnover.

46.5 Conclusion

IGF-I treatment to adult patients with Laron syndrome has positive metabolic effects on protein and fat metabolism, even if of limited duration of the latter. Kidney functions are improved as is procollagen and lipoprotein(a) metabolism. Considering the seemingly encouraging effects of hGH administration on adults with GH deficiency, it seems indicated to restart long-term trials with IGF-I treatment on adult patients with Laron syndrome, may be even not discontinue treatment with a low dose after growth has stopped. The disappointing part of this treatment is the adipogenic effect of IGF-I evident during long-term administration, a fact which necessitates finding an appropriate obesity lowering drug.

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47 Adverse Effects Encountered During IGF-I Treatment of Patients with Laron Syndrome

Zvi Laron

Core Message

› Following IGF-I administration the following adverse effects have been observed in our cohort: hypoglycemia, headaches, hyperandrogenism, obesity, snoring and sleep apnea. These and further adverse effects have been reported by the investigators using two IGF-I injections a day. Most are probably linked to IGF-I over dosage.

Acute pain at the injection site has been a complaint at the initiation of treatment. Overt hypoglycemia has been encountered in small children until the parents learned to cover the immediate IGF-I effect by an adequate carbohydrate intake.

Few patients complained of headaches, but this was true also for the untreated state. Thus, we cannot relay it to the IGF-I administration.

The most severe adverse effect observed during long-term IGF-I treatment was progressive obesity (Laron et al. 2006) (see also Chap. 12), a complication that led to snoring, sleep apnea (Dagan et al. 2001) and mobility difficulties in the older patients, and fatty liver (Laron et al. 2008).

Seemingly overdosage of IGF-I in 2 prepubertal girls aged 11 and 13.8 years (150 μg/kg once daily?) accelerated the appearance of puberty and induced a progressive rise in serum androgens with clinical virilization resembling PCOS (Laron et al. 1998). Temporary interruption of IGF-I administration and resumption of treatment starting with smaller doses (50 μg/kg once daily) reversed the virilization and permitted normal feminine pubertal development.

Development of hyperandrogenism during IGF-I treatment was also encountered in two adult female Laron syndrome patients aged 30, their daily dose being 120 μg/kg (Klinger et al. 1998). In both patients the 4 h postinjection IGF-I levels rose to 790 ng/mL (our aim is no more than 200 ng/mL), which led to a rise in serum androgen levels, a rise in the LH/FSH ratio from 0.6 to 5.8, facial acne, swelling and widening of the nose and face (Figs. 47.1–47.3), and amenorrhea in one and irregular menses in the second. Ultrasonography in one of these female patients revealed small ovarian cysts and hypoechographic areas typical of PCOS.

Progressive reduction of the IGF-I dose led to decrease in the androgenization and interruption of IGF-I administration after 9-month-therapy and to a normal appearance (Fig. 47.4) and monthly cycle. Table 47.1 illustrates the relationship between microcomedones (preacne) and serum acne androgen levels.

During our 50 years experience in the treatment of Laron syndrome patients with IGF-I, we have not encountered many of the adverse effects reported by other investigators (Ranke and Wilton 1994; Guevara-Aguirre et al. 1995; Camacho-Hubner et al. 2006; Savage et al. 2006a, b; Chernausek et al. 2007, etc.), such as papilledema, facial nerve paralysis or the need for tonsillectomy or adenoidectomy, intracranial hypertension, hypoglycemia, swelling of lymphoid tissue, and thromcytopenia (Table 47.2).

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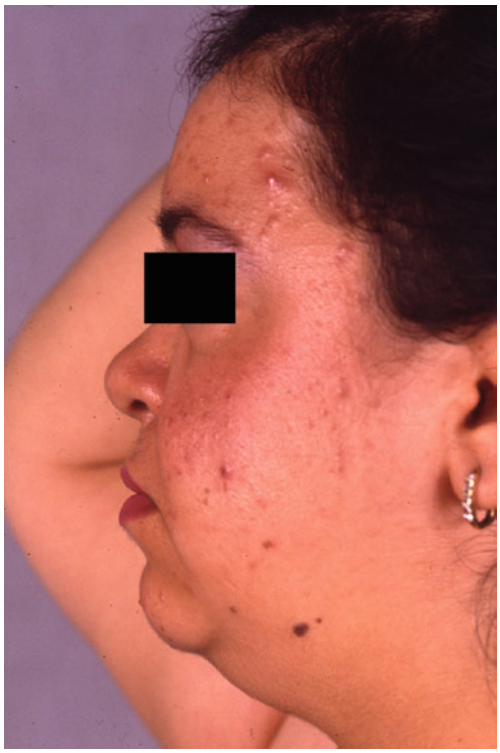

Fig. 47.1 Facial acne in a 30-year-old-female with Laron syndrome treated by once daily IGF-I (120 μg/kg)

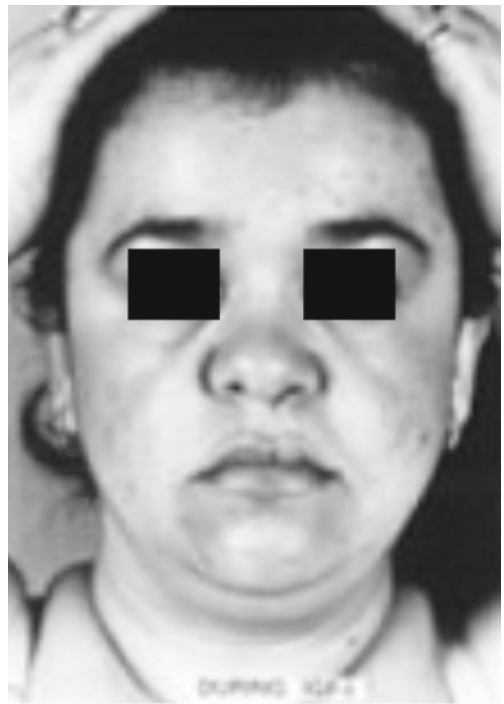

Fig. 47.3 Facial and nasal swelling and acne in a 30-year-old-female with Laron syndrome treated with once daily IGF-I (120 μg/kg/day)

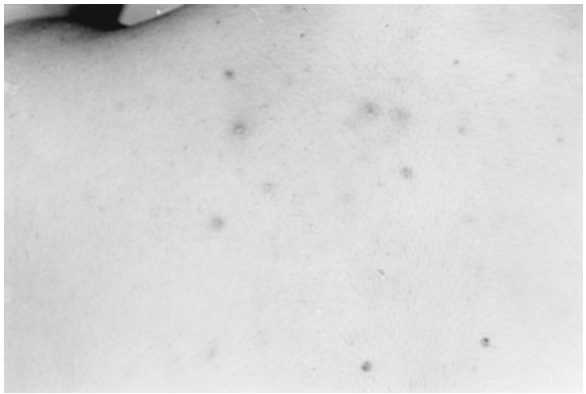

Fig. 47.2 Acne on the back of the same patient as in Fig. 47.1

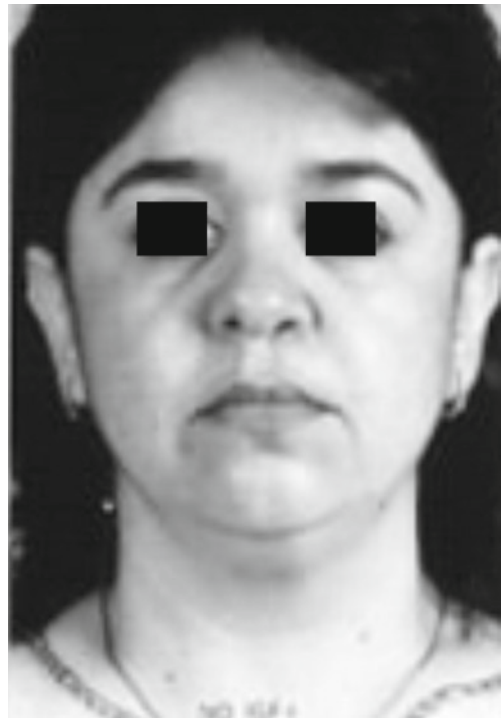

Fig. 47.4 Normal facial appearance after stopping IGF-I treatment in Laron syndrome patient shown in Fig. 47.3

47.1 Comment

We have recently summarized all the reported adverse effects we could find in the literature (Laron 2008) (Table 47.2). It is apparent that our group using one IGF-I injection per day has less adverse effects than the investigators using either two daily injections of IGF-I. The necessary use of one large

Table 47.1 Relationship between gonadotropin and androgen levels and microcomedones (preacne) and acne in IGF-I-treated patients with Laron Syndrome patients (modified from Ben-Amitai and Laron (2009))

Laron syndrome patients	IGF-I treatment		LH (mIU/mL)		FSH (mIU/mL)		Testosterone (nmol/L)		Delta 4-androstendione (nmol/L)		IGF-I treatment	
	Period of treatment (age-years)	IGF-I dose (μg/kg/day)	Basal	IGF-I	Basal	IGF-I	Basal	IGF-I	Basal	IGF-I	Micro-comedones	Acne
Males												
1	10–11.5	150–175	>0.3	1.5	0.9	1.7	>0.7	5.8	2.7	2.3	None	None
2	14.5–20.5	170–200	>0.3	1.0	0.8	1.8	0.9	3.8	4.2	1.9	Few	None
3	28–29	170–200	0.3	6.0	3.0	7.3	18.6	28.0	6.8	2.8	Few	None
Females												
1	11–14	150	0.1	11.9	2.8	4.4	0.7	3.5	2.2	8.0	None	None
2	3.5–16	150	1.5	16.6	6.8	7.2	0.7	3.2	2.8	9.0	None	*Yes*
3	30–31	120	6.7	38.6	11.9	6.7	1.0	4.7	7.1	9.8	None	*Yes*
4	30–31	120	2.4	29.8	8.9	7.0	0.8	3.7	3.5	10.0	None	*Yes*
5	36–37	120	4.4	6.2	4.9	6.5	1.1	2.1	4.6	6.6	None	None
6	34	120	2.1	4.6	6.9	8.0	0.8	1.7	4.0	5.6	None	None

Metz et al. (2009)

Table 47.2 Adverse effects reported during insulin-like growth factor-1 treatment of children with Laron syndromeModified from Laron (2008)

Acute	References
Pain at injection site	Ranke and Wilton (1994) Ranke et al. (1995) Savage et al. (2006a, b)
Hypoglycemia	Backeljauw et al. (2001) Ranke et al. (1995) Walker et al. (1992)
Nausea	Chernausek et al. (2004) Ranke and Wilton (1994) Ranke et al. (1995)
Headache	Azcona et al. (1999) Backeljauw and Underwood (1996) Lordereau-Richard et al. (1994) Ranke and Wilton (1994) Ranke et al. (1995) Savage et al. (2006a, b)
Intracranial hypertension	Azcona et al. (1999) Backeljauw and Underwood (1996) Ranke et al. (1995) Ranke et al. (1999)
Transient papilledema	Azcona et al. (1999) Backeljauw et al. (2001) Guevara-Aguirre et al. (1997) Ranke et al. (1995) Wollman and Ranke (1994)
Lowering of serum K (rare)	Backeljauw and Underwood (1996) Ranke et al. (1995)
Transient thrombocytopenia	Backeljauw and Underwood (1996)
Chronic	
Hypoglycemia	Azcona et al. (1999) Backeljauw and Underwood (1996) Backeljauw et al. (2001) Besson et al. (2004) Camacho-Hubner et al. (2006) Frane et al. (2006) Guevara-Aguirre et al. (1997)
Progressive obesity	Krzisnik and Battelino (1997) Laron et al. (2006) Ranke et al. (1995)
Lipohypertrophy (at injection site)	Azcona et al. (1999) Accumulation of adipose tissue Backeljauw and Underwood (1996) Backeljauw et al. (2001) Besson et al. (2004) Frane et al. (2006) Krzisnik and Battelino (1997) Laron et al. (1999) Ranke et al. (1995) Walker et al. (1998)

(*continued*)

Table 47.2 (continued)

Acute	References
Snoring	Backeljauw et al. (2001) Frane et al. (2006) Ranke and Wilton (1994)
Sleep apnea	Backeljauw et al. (2001) Dagan et al. (2001)
Swelling of lymphoid tissue	Azcona et al. (1999) Backeljauw et al. (2001) Camacho-Hubner et al. (2006) Chernausek et al. (2004); Frane et al. (2006) Ranke et al. (1995) Savage et al. (2006a, b)
Swelling of facial soft tissue	Backeljauw and Underwood (1996) Besson et al. (2004) Krzisnik and Battelino (1997) Walker et al. (1998) Wollman and Ranke (1994)
Paresis of facial nerve	Guevara-Aguirre et al. (1997) Ranke et al. (1999) Wollman and Ranke (1994)
Abnormal tympanometry	Azcona et al. (1999) Chernausek et al. (2004) Frane et al. (2006)
Enlarged spleen	Azcona et al. (1999)
Hypoacusis	Frane et al. (2006)
Tachycardia	Ranke and Wilton (1994) Vasconez et al. (1994)
Hyperandrogenism	Laron et al. (1998)
Hair growth	Camacho-Hubner et al. (2006)

Modified from Laron (2008)

dose of the combined IGF-I/BP-3 drug (Camacho-Hubner et al. 2006) also caused many and severe complications.

While the majority of adverse effects can probably be avoided by adjusting the dose of IGF-I and administration with a meal, the progressive and marked obesity poses a so far major unresolved problem.

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Summary of the Clinical History: The Laron Syndrome Clock

48

Zvi Laron

We hope that clinical grade IGF-I, nowadays available in unlimited quantities, will be accessible to all patients with Laron syndrome to avoid many of the consequences due to lack of treatment (Laron 1996, 2001; Laron and Weinberger 2004; Laron et al. 2008).

Figure 48.1 illustrates schematically our contribution to the history of the Laron syndrome.

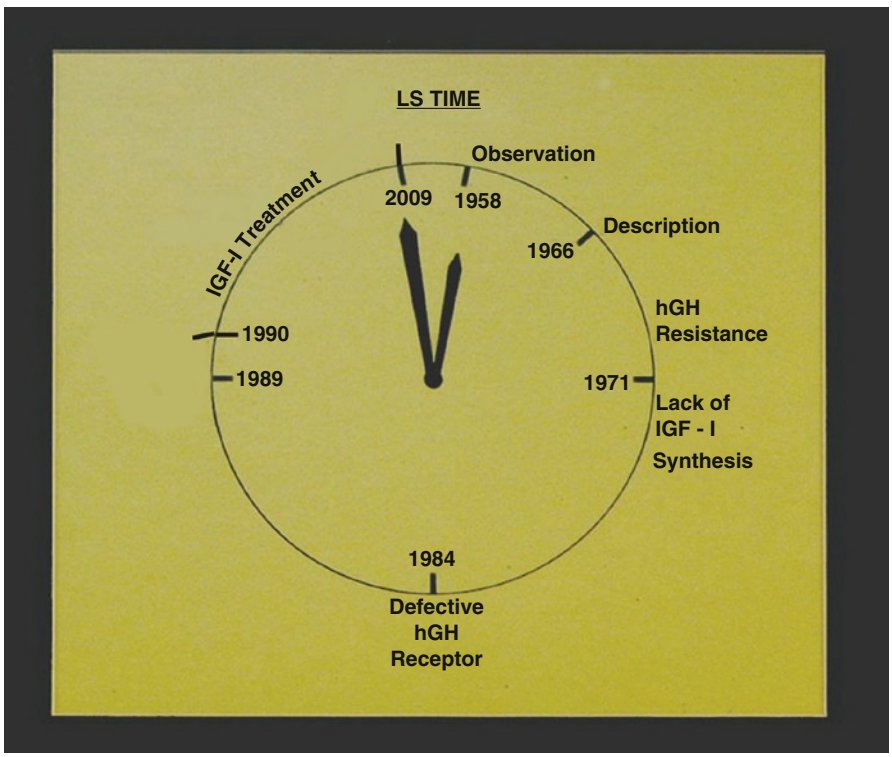

Fig. 48.1 The Laron syndrome clock

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Part II

The Growth Hormone Receptor "Null" Mouse or the Laron Mouse

The Laron Mouse

49

John J. Kopchick and John D. Blischak

Core Message

› Studies of GHR–/– mice have helped to reveal the role of GH in many physiological processes such as reproduction, growth, GH/IGF-1 feedback loops to the pituitary, insulin and glucose metabolism, longevity, and obesity. This mouse model has also been used to investigate *in vivo* intracellular signaling systems specific for the GH/GHR interaction. Additionally, these mice have been and continue to be used to study disease states in which GH has been implicated in the pathophysiology of the disease such as Type 1 diabetes and certain cancers. In the following sections, we will elaborate on these findings, and where possible, point out similarities/differences between results found in mice versus human LS individuals.

49.1 Introduction

The growth hormone receptor/growth hormone binding protein (GHR/BP) gene-disrupted or knockout mouse (GHR–/–) was generated over a decade ago (Zhou et al. 1997). This has facilitated the study of GH in a wide variety of subject areas resulting in more than 100 publications from research groups worldwide (Appendix 1). GHR–/– mice are unique because of their insensitivity (or resistance) to the action of GH. This defect results in growth retardation or dwarfism (Fig. 49.1), delayed sexual maturation, and severely depressed IGF-1 and elevated serum GH levels (List et al. 2001). As we allowed the original mice to age, we were surprised to learn that they became obese, somewhat hypoglycemic and hypoinsulinemic and had extended life spans (Coschigano et al. 2000, 2003; Berryman et al. 2004). This is counterintuitive to models of obesity-associated insulin resistance and Type 2 diabetes where obesity is correlated with insulin resistance, Type 2 diabetes and decreased life spans (Surwit et al. 1988; Qiu et al. 2005).

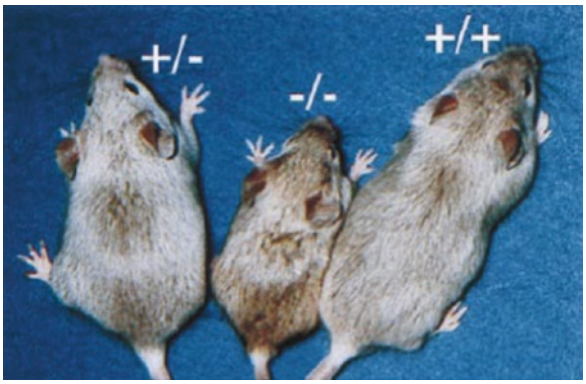

Fig. 49.1 Female mice at 4 weeks of age representing the three different genotypes. +/+ indicates wild type, +/– indicates heterozygous, and –/– indicates GHR–/– mice. From (Zhou et al. 1997)

The various phenotypes of these GHR–/– mice render them ideal models for the study of Laron syndrome (LS) in humans, a condition characterized by resistance or insensitivity to GH (Kopchick and Laron 1999). Physical and physiological characteristics of the GHR–/– mice (except for the hypoinsulinemia) are

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very similar to those observed in LS individuals and will be described throughout this section of the book.

49.2 GHR Disruption Produces a Dwarf Phenotype

As would be expected, the GHR−/− mice are smaller than their wild type counterparts (Fig. 49.1). In our original study, we observed no difference in body size or weight at birth between the three mouse genotypes (GHR+/+, GHR+/−, GHR−/−) (Zhou et al. 1997). However, GHR−/− mice were significantly smaller by 3 weeks of age. In contrast, there was no significant difference in the growth of heterozygotes vs. wild type controls. After 12 weeks of age, the female heterozygote mice were only slightly, yet significantly, smaller than wild type controls, but the difference in body weights of the male heterozygotes and controls did not reach significance. The same pattern was observed for measurements of body length (nose-anus) at 8 weeks of age. GHR−/− had a shorter body length than heterozygotes and wild type controls. Female heterozygotes were shorter than wild type mice, while male heterozygotes and controls showed no significant difference. In a second study (Coschigano et al. 2000) of growth of these animals, we found that by 4 weeks of age, the GHR−/− weighed ~45% less than wild type controls. Not only did GHR−/− mice not become as large as controls, but they also reached their maximum weight at an earlier age. Male GHR−/− mice attained maximum weight at ~14 weeks of age and female GHR−/− at ~34 weeks. Male GHR+/+ stopped growing at ~42 weeks of age, while females stopped at 46 weeks. Male +/− growth peaked at ~32 weeks of age, while that of females at ~44 weeks. However, there was no significant difference in the final body weights between +/− and +/+ mice.

Organ weights are also affected in the GHR−/− mice. The kidney, heart, spleen, and testis were proportionally smaller when compared to control littermates. However, the brain increased (~151% of +/+) (Sjögren et al. 2000) and the liver decreased in size relative to body weight (~84.4% of +/+) (Zhou et al. 1997; Sjögren et al. 2000). Analysis of bone growth and remodeling revealed that the mice had a decrease in bone size and bone mineral content, and that these defects could be rescued by treatment with IGF-I (Sims et al. 2000; Sjögren et al. 2000). GH's influence on the development of the teeth has also been characterized. For example, GHR−/− mice had little to no cellular cementum in their first molars, a GH-responsive mineralized tissue similar in composition to bone, (Smid et al. 2004).

49.3 GHR Disruption Produces Endocrine Disruption

Several groups have reported effects on reproduction and reproductive parameters using the GHR−/− mice. Male mice exhibited reduced fertility as a result of a decrease in gonadotropin-releasing hormone (GnRH)-induced stimulation of serum luteinizing hormone (LH), attenuation of LH-stimulated testosterone release, fewer LH receptors in the testes, and hyperprolactonemia (Chandrashekar et al. 1999, 2001). GHR−/− female mice showed a delay in vaginal opening and when pregnant had increased placental and decreased fetal weights. Furthermore, they gave birth to litters with smaller numbers and smaller individual pup weight than wild type littermates (Danilovich et al. 1999).

In the pituitary of the GHR−/− mouse, the long- and short-feedback loops have been altered due to low IGF-1 levels and the lack of GHR in the pituitary (Asa et al. 2000). This results in hyperplasia of the somatotrophs with increased levels of GH synthesis and secretion. In term of glucose metabolism, the GHR−/− mice are extremely insulin-sensitive with low-normal levels of glucose (Coschigano et al. 2003). This can be explained by removing the diabetogenic effect of GH, thus rendering the animals insulin-sensitive. Additionally, the observation that GHR−/− mice have an increased life span has led to a variety of studies attempting to dissect the action of GH as it relates to longevity (Coschigano et al. 2000; Masternak et al. 2005a, b, c).

Since GH secretion is negatively correlated with obesity, the effects of GH on adipose tissue have been studied in the GHR−/− mice. GH is believed to act in a depot-specific manner since the increased adiposity observed in GHR−/− mice was observed to be primarily due to an increase in the subcutaneous fat pads when normalized to body weight (Berryman et al. 2004).

Another interesting revelation was that serum levels of adiponectin increased in GHR−/− mice, contrary to previous studies where adiponectin was inversely correlated with obesity (Arita et al. 1999; Yamauchi et al. 2001). This finding could help explain the insulin hypersensitivity in GHR−/− mice (Berryman et al. 2004; Nilsson et al. 2005).

49.4 GHR Disruption Produces Other Phenotypes

In terms of GH-induced intracellular signaling, the function of different functional domains in the GHR has been studied by Waters and colleagues. Using a gene "knock-in" strategy, mutated GHR genes encoding truncations in the intracellular domain of the GHR were introduced into the GHR−/− mice (Rowland et al. 2005). Recall that the GHR is composed of ~620 amino acids including ~245 aa in the extracellular region, ~24 aa comprising the transmembrane area, and a ~350 aa intracellular region (Talamantes and Ortiz 2002). One mouse line expressed a truncated GHR at residue 569 and also possessed amino acid changes of tyrosines 539 and 545 to phenylalanines. Similarly, a second mouse line was generated in which the GHR was truncated at residue 391 (lysine). These mice showed growth retardation that was proportional to the truncation of the GHR. Surprisingly, it was found that GH-induced JAK2 and ERK1/2 intracellular signaling that initiates at residues 298–391 accounted for 11% of GH-dependent growth. Furthermore, 44% of the total GH-dependent growth was achieved between GHR residues 392–569 (excluding residues 539 and 545) which accounted for 30% of STAT5 activation. Finally, inclusion of the distal 80 residues of the GHR and restoration of the normal tyrosines at residues 539 and 545 restored the missing 70% of STAT5 signaling and resulted in 100% of GH-dependent growth. These findings support claims that STAT5 signaling plays a larger role in GH-dependent growth than ERK1/2 in vivo.

Finally, the GHR−/− mice also have been used as models of human diseases. Streptozotocin (STZ) can induce a Type 1 diabetic state. STZ-treated mice are extremely sensitive to nephropathy, in particular glomerulosclerosis. However, STZ-treated GHR−/− mice were protected from STZ-induced glomerulosclerosis suggesting that GH plays a crucial role in diabetic kidney damage (Bellush et al. 2000). In the cancer area, the GHR−/− mice have been used to monitor the progression of breast and prostate cancer. Crossing the C3(1)/T antigen (Tag) mouse, a model of prostate and mammary cancer due to expression of the SV-40 large T antigen, with the GHR−/− mouse, resulted in mice with an attenuation in the development of mammary (Zhang et al. 2007) and prostate cancers (Wang et al. 2005) compared to Tag/GHR+/+ mice.

In the following chapters, we describe some of these findings.

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Role of GH/IGF-I Deficiency in Aging

50

Edward O. List

Core Message

› Our laboratory and others have been able to use the GHR–/– mice to elucidate the impacts of GH on aging. While many questions persist, these mice have helped to establish that a lack of GH action can extend life span in a manner similar to caloric restriction. Moreover, insulin sensitivity appears to be the most prominent mechanism shared between these two interventions that result in life span extension. Further analysis of the contribution of each of the insulin-sensitive tissues as they relate to GH should help focus on these mechanisms. Thus, our laboratory is currently generating liver, muscle, and adipose tissue-specific GHR gene disrupted mice. By investigating the dynamic interactions between GH and insulin signaling within these tissues, how they alter the physiology of other tissues in compensation for the lack of GHR, and the effect on aging will further our understanding of the GH-related mechanisms that extend longevity.

50.1 Introduction

Aging studies in GHR–/– mice were initiated in our laboratory with the finding that GHR–/– mice have significantly extended life spans when compared to wild type control mice (Coschigano et al. 2000). Since this initial finding in 2000, the GHR–/– mice have been used in many studies to explore the possible mechanisms involved in extended longevity in the absence of GH action. The notion that inhibition of the GH/IGF-I axis can increase life span is not unique to this mouse model, but rather an ever-growing theme found across a diverse range of species from invertebrates to mammals. However, due to the specific absence of GH action, the GHR–/– mouse has provided a mammalian model to precisely explore the role of GH in aging. In this section we will discuss a broad range of topics related to GH and aging with special emphasis on the role of the GHR–/– mice. Specifically, we will examine the definition of aging, influence of the GH/IGF axis on life span, insulin's influence on life span, caloric restriction, caloric restriction in dwarf mice, and comparisons of two dwarf mouse models produced in our laboratory: transgenic GH antagonist (GHA) mice and GHR–/– mice.

50.2 Aging

Aging, in evolutionary terms, is likely related to a selection for genes that are beneficial in early life, but become deleterious after reproductive age. Hence, such genes would not possess selective force on the organism, thereby escaping natural selection (Kirkwood and Austad 2000; Kowald 2002). There are several theories of aging that deal with an organism's inherent susceptibility and how they are exploited to promote aging. For example, the oxidative-stress theory proposes that the age-related decline in physiological function is due to the progression and irreversible accumulation of oxidative damage to important biological molecules such as lipids, proteins, and nucleic acids (Sohal and

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Weindruch 1996; Finkel and Holbrook 2000). Furthermore, oxidative stress results in mutations to the mitochondrial genome (mtDNA), which may be of particular importance for the aging phenotype (Cortopassi et al. 1992; Tanhauser and Laipis 1995; Van Remmen and Richardson 2001). The replicative senescence theory is based on observations that inhibition of cyclin-dependent kinases and the shortening of telomeres limit the number of times a somatic cell can divide *in vitro* and presumably in living organisms (Campisi 1996; Blackburn 2000; Hasty et al. 2003). In humans, there are multiple autosomal recessive disorders (such as Werner's syndrome, Cockayne syndrome, and trichothiodystrophy) that can accelerate the onset of age-related diseases and shorten life span (Martin and Oshima 2000; Hasty et al. 2003). One common theme with these disorders is disruption of DNA repair mechanisms. Werner's syndrome, for example, results from a mutation of a specific DNA helicase (WRN) involved in DNA repair (Yu et al. 1996). The 3′ 5′ exonuclease activity of this helicase is stimulated by an interaction with the Ku86/70 complex, which is necessary to mend double-strand DNA breaks including those caused by reactive oxygen species or ionizing radiation (Cooper et al. 2000). Mice deficient in Ku86 display an early onset of many age-related disorders and die prematurely (Vogel et al. 1999).

50.3 Influence of the GH/IGF Axis on Longevity

The release of GH from the anterior pituitary stimulates the production of insulin-like growth factor-1 (IGF-I) in many tissues throughout the body. The decline in circulating GH and IGF-I with age has lead to the theory that the consequences of aging are related to decreased GH/IGF-I action (Bartke et al. 2000). Several animal models with diminished GH/IGF-I activity have extended longevity which directly contradicts this theory (Longo and Finch 2003). Ames dwarf mice and Snell dwarf mice carry homozygous recessive mutations at the Prop-1 and Pit-1 loci, respectively, which results in an impairment in the development of somatotroph, lactotroph, and thyrotroph residing in the anterior pituitary. Because of the impairment to these specific pituitary cell types, these mice have deficiencies in GH, prolactin, and thyroid-stimulating hormone.

Fig. 50.1 Photo of a 3-year-old GHR–/– mouse. While the average life span of *Mus musculus* is ~2–2.5 years, our laboratory has generated mice that live ~3 years. One GHR–/– mouse lived 1,819 days and is recognized as the longest lived laboratory mouse. The mouse in this picture is a 3-year-old male GHR–/– mouse in the C57BL/6J background

Both the Ames and Snell genotypes lead to mice with severe decreases in the GH/IGF-I axis signaling and increased longevity. While the decreased GH/IGF-I action is thought to be responsible for this extended longevity (Wolf et al. 1993; Bartke et al. 2001), the fact that multiple endocrine systems are affected limits conclusions one can make about GH's role in these GH and IGF-I-deficient mouse models. Furthermore, additional phenotypes including altered glucose metabolism and compensatory changes in the hypothalamic-pituitary-adrenal (HPA) axis (Carter et al. 2002) are confounding. Another approach to help understand GH's role in aging using mouse models is to selectively alter the activity of the GH/IGF-I axis by disrupting specific genes. To this end, our laboratory has disrupted the gene encoding the GHR and binding protein (GHR–/–) (Zhou et al. 1997a). Because the GHR is absent, GH is functionally inactivated. This section of the book is dedicated to these mice. Suffice it to say, GHR–/– mice are dwarf and long-lived (Fig. 50.1). They have increased GH and severely reduced IGF-I levels (Coschigano et al. 2003). On record, a single GHR–/– mouse lived 1,819 days and is considered the longest lived laboratory mouse. This mouse remains the holder of the "Methuselah Mouse Prize" for the world's oldest mouse. The Methuselah Mouse Prize is organized by the Methuselah foundation (http://www.methuselah-foundation.org/) founded by Aubrey de Grey of the University of Cambridge, UK.

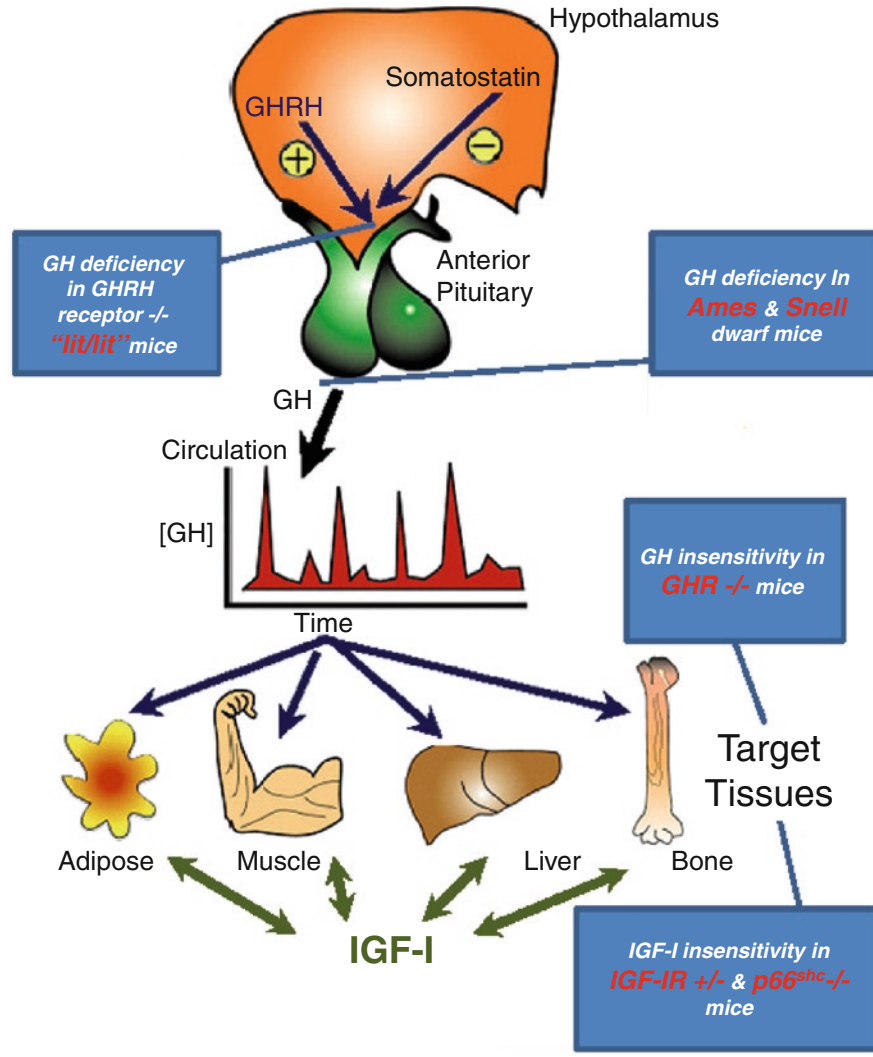

Fig. 50.2 Cartoon description of the GH/IGF-I axis. Six long-lived dwarf mouse lines with disruptions to the GH/IGF-I axis are indicated

Other mice lacking genes in the GH/IGF-I pathways have also been developed. Mice lacking both alleles that encode the IGF-I receptor (IGF-IR−/−) die at birth; however, heterozygous IGF-IR+/− mice live about 25% longer than their IGF-IR+/+ littermates (Holzenberger et al. 2003). Similarly, mice with disruptions to the IGF-I receptor substrate, p66shc (p66shc−/−), live 30% longer than their wild type littermates and are more tolerant to paraquat-induced oxidative stress (40% increased survival time) (Migliaccio et al. 1999). Fat-specific insulin receptor knockout (FIRKO) mice initially display a normal growth curve, but show a decreased body weight of approximately 15–25% and have 50–75% less fat mass at 3 months of age. These mice are resistant to the age-related decline in glucose tolerance that occurs in their wild type littermates and also have an increased median and maximal life span (Bluher et al. 2003). A summary of mice with a mutation or gene disruption directly resulting in a decreased signaling in GH/IGF-I axis is depicted in Fig. 50.2.

50.4 Insulin/IGF-I Influence on Lifespan: From Worms, Flies, and Yeast to Mammals

Insulin/IGF signal transduction has been shown to influence life span in many species. The basic machinery of the signaling cascades is highly conserved and has been

studied most extensively as it applies to the field of aging, in the roundworm *Caenorhabditis elegans* (Guarente and Kenyon 2000; Kenyon 2001; Tatar et al. 2003). Sensory neurons responding to signals, both internal and environmental, secrete peptides similar to insulin and IGF-I. These peptides bind DAF-2 which is a receptor tyrosine kinase. Binding of the DAF-2 receptor triggers a signaling cascade where AGE-1 (which is a homolog of the phosphinostide-3-OH kinase (PI3K)) activates AKT/PKB (a protein kinase), which then phosphorylates DAF-16 (a forkhead/winged-helix transcription factor) and prevents DAF-16 migration into the nucleus. Thus, DAF-16-mediated transcription is inhibited by DAF-2 stimulation. Mutations to the genes encoding DAF-2 (Kenyon et al. 1993; Kimura et al. 1997) or AGE-1 (Johnson et al. 1987; Dorman et al. 1995) resulting in partial loss of function increases *C. elegans* life span by more than double. Complete inactivation of DAF-16 abolishes the life span extension in DAF-2 mutants (Lin et al. 1997; Ogg et al. 1997). In *Drosophila melanogaster* (fruit flies) and *Saccharomyces cerevisiae* (yeast), mutations to genes encoding components of insulin/IGF-I signaling pathway also affect longevity. An example of this can be seen in female fruit flies that lack the insulin-like receptor (Tatar et al. 2001) or CHICO (the insulin receptor substrate) (Clancy et al. 2001) and have extended life span. Similarly, yeast lacking SCH9 (a protein kinase AKT/PKB homolog) (Fabrizio et al. 2001) also have extended longevity. In mice, disruption of the IGF-I gene, heterozygous disruption of the IGF-IR, and disruption of the IGF-I receptor substrate, p66shc–/–, result in life span extension (Migliaccio et al. 1999). Further, p66shc has been shown to be necessary for phosphorylation, cellular redistribution, and inactivation of FKHRL1, a mammalian homolog to DAF-16 (Nemoto and Finkel 2002).

50.5 Caloric Restriction

Caloric restriction is recognized as the most constant intervention that extends life span across all species that have been studied. While the precise mechanisms that allow caloric restriction to extend life span are not completely known, they likely involve the cumulative effects of multiple mechanisms related to glucose, insulin and IGF-I, reactive oxygen species, and oxidative damage (Koubova and Guarente 2003; Masoro 2003). The auto-oxidation of glucose as well as other reactions generates chemicals (such as glyoxyl, methylglyoxyl, 3-deoxyglucosone) that react with proteins and lipids to form advanced-glycation end-products (AGEs) (Singh et al. 2001). AGEs accumulate with age (and are accelerated with the glucose-rich environment of diabetes) and damage cellular components by interfering with protein function and through inappropriate interactions with receptors, leading to negative cellular responses (including the secretion of inflammatory cytokines, transcription of stress-related genes, the generation of reactive oxygen species and altered cell migration and adhesion). The receptor for advanced-glycation end-products (RAGE) is upregulated by high concentrations of ligand. This occurs with aging and is increased in diabetic patients (Nishikawa et al. 2000; Brownlee 2001; Evans et al. 2002). Caloric restriction has been shown to decrease AGEs in the vasculature of older rats (Teillet et al. 2000) and in skin samples taken from older monkeys (Sell et al. 2003).

50.6 Caloric Restriction in Dwarf Mice

Bartke et al. (2001) demonstrated that caloric restriction in long-lived GH-deficient Ames dwarf mice increased both mean and maximal life span (Bartke et al. 2001). Thus, caloric restriction was able to increase life span in an additive way to an already long-lived mouse model, suggesting that GH-deficient dwarfism and caloric restriction are separate and distinct mechanisms that result in increased life span. In stark contrast, when caloric restriction was applied to GHR–/– mice, no additional extension of life span was obtained (Bonkowski et al. 2006) (Fig. 50.3). Since Ames dwarf mice are deficient in several pituitary hormones, it is likely that the difference between these two models involves hormones other than GH. Thus, the specific removal of GH action in the GHR–/– mice appears to extend life span utilizing mechanisms similar to that of caloric restriction. The overlap observed by Miller et al. (2002) combined with the finding that caloric restriction does not increase life span in GHR–/– mice (Bonkowski et al. 2006) provides evidence that the mechanisms by which caloric restriction and lack of GH action increase longevity are similar. To further explore this overlap, Bonkowski et al. (2009) investigated insulin signaling events in GHR–/– mice and wild type mice subjected to mild caloric restriction (more specifically every other day feeding was used which results in a ~15% CR). In

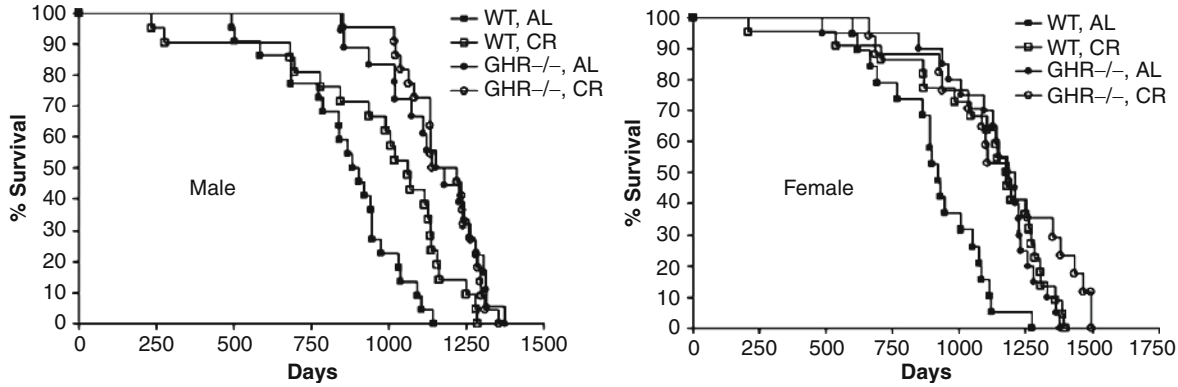

Fig. 50.3 Comparison of life span in male (*left chart*) and female (*right chart*) wild type mice fed ad libitum (WT, AL), wild type mice with caloric restriction (WT, CR), GHR–/– mice fed ad libitum (GHR–/–, AL), GHR–/– mice with caloric restriction (GHR–/–, CR). This figure is modified with permission (Bonkowski et al. 2009)

wild type mice, mild caloric restriction increased insulin-stimulated activation of insulin signaling molecules in both liver and muscle. However, GHR–/– mice responded differently with increased activation in only the early steps of insulin signaling in liver, while muscle had increased activation of only downstream signaling events. Moreover, chronic caloric restriction in GHR–/– mice did not result in further changes to insulin signaling compared to wild type mice, which may explain why caloric restriction does not increase insulin sensitivity and/or longevity in the GHR–/– mice. They also reported that muscle of GHR–/– mice exhibited severely reduced serine phosphorylation of IRS-1. Since serine phosphorylation of IRS-1 is inhibitory to insulin signaling and is reduced in the muscle of GHR–/– mice, it is possible that reduced serine phosphorylation of IRS-1 accounts for the heightened insulin sensitivity found in GHR–/– mice (Bonkowski et al. 2009).

50.7 Comparison of Two Dwarf Mouse Models Produced in Our Laboratory: Transgenic GHA Mice and GHR–/– Mice

While it is established that GH-deficient Ames and Snell dwarf mice live longer than wild type mice (Bartke 2000; Flurkey et al. 2001, 2002; Dozmorov et al. 2002), questions about the specific involvement of GH in longevity have persisted from these studies because GH-deficient Ames and Snell dwarf mice lack other pituitary hormones such as prolactin and thyroid-stimulating hormone (TSH). Therefore, to better assess GH's role in aging, our laboratory studied aging in two mouse lines generated in our laboratory that are not deficient in any other pituitary hormone, but are specifically deficient in GH signaling. The first engineered mouse was a GHA transgenic mouse. Since GHA competes with endogenous GH for receptor binding, GH signaling is inhibited (Chen et al. 1990, 1991a, b; Okada et al. 1992; Xu et al. 1995). The second mouse was the GHR–/– mouse (Zhou et al. 1997b). Although these two dwarf mouse models share many phenotypic similarities, a few differences have been observed. GHR–/– and GHA mice differ in size, in both body length and whole body mass. At 4 months of age, the body mass of the GHR–/– and GHA mice is 40% and 60% the mass of littermate controls, respectively. This difference in body mass has been shown to continue to increase with age, as the GHA mice approach the body mass of their littermate controls, while the body mass of GHR–/– mice remains significantly less than littermate controls (Coschigano et al. 2003). Further, circulating levels of IGF-I are significantly reduced in both dwarf lines when compared to littermate controls; however, IGF-I is more decreased in GHR–/– mice with 80% decrease in IGF-I compared to a 25% decrease in circulating IGF-I in GHA mice. Food consumption was also found to differ between the two dwarf lines as GHA mice consume a similar amount of food as their littermate controls, while the GHR–/– mice consume 52% less than littermate controls. When normalized to body weight, GHA and the GHR–/– mice consume more than their respective littermate controls at a young age. However, this effect

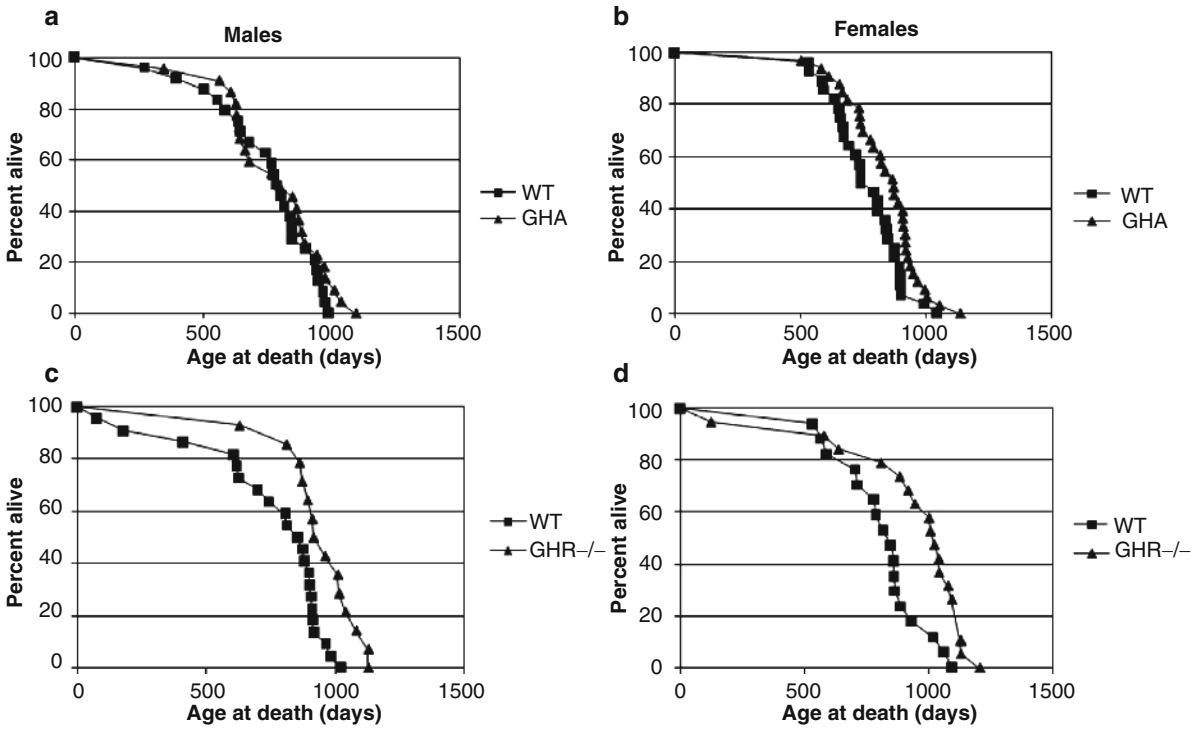

Fig. 50.4 Comparison of life span in "normal lived" male (**a**) and female (**b**) dwarf mice expressing the GH antagonist (GHA), to that of "long lived" male (**c**) and female (**d**) GHR–/– mice. This figure is modified with permission (Coschigano et al. 2003)

was only slightly significant and was not observed at later ages (Coschigano et al. 2003). Several insulin-like growth factor binding proteins (IGFBP) were also shown to differ between the two mouse models. While IGFBP-3 levels were reduced in both the models, this reduction was only observed at an older age (11 months). IGFBP-1, -2, and -4 levels did not change in GHA. In contrast, GHR–/– mice have reduced levels of IGFBP-1 and -4, and levels of IGFBP-2 are elevated as compared to littermate controls. Another important difference between these two mouse lines was insulin and insulin sensitivity. In the GHA mice, fasting blood glucose and insulin did not differ from littermate controls. However, GHR–/– mice were shown to have decreased fasting blood glucose and insulin (65–86% and 10–26% the level of controls, respectively). Finally, in terms of life span, the GHA mice are similar to their littermate controls, while GHR–/– mice have been shown to have extended mean and median lifespans (Coschigano et al. 2000, 2003) (Fig. 50.4). The precise reason why life span is extended in GHR–/– mice and not GHA mice is not completely known; however, the most plausible explanation would be the much lower levels of both IGF-I and insulin found in the GHR–/– mice. The insulin exposure theory postulates that declines in insulin exposure can result in a global decrease in other growth factors such as GH (Meites 1990; Quigley et al. 1990) and IGF-I (Breese et al. 1991), which ultimately results in increased longevity (Parr 1997, 1999). In agreement, both the long-lived Ames and Snell dwarf mice lines have reduced insulin levels (Dominici et al. 2002; Hsieh et al. 2002). Since Ames, Snell, and GHR–/– long-lived dwarf mouse models have decreased insulin levels, while GHA mice do not, there is a strong possibility that decreased insulin is indeed a key component for increased life span in these long-lived mouse models.

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Body Composition, Adipose Tissue, and Energy Balance

51

Darlene E. Berryman

Core Message

› There is a dramatic alteration in body composition, adipose tissue, and energy balance in GHR–/– mice. Despite the fact that studies have utilized mice of differing ages, gender, and genetic backgrounds, the long-lived, insulin-sensitive GHR–/– mice are consistently found to be relatively obese with a striking depot-difference in adipose tissue accumulation. The increased adiposity is due to increases in both white adipose tissue, most notably the subcutaneous regions, and brown adipose tissue. In part, the increased adipose tissue mass is due to a positive energy balance marked by increased food consumption, even though this is somewhat offset by greater energy expenditure. Despite the many published studies related to adipose tissue and energy balance in these mice, there is still much to be learned. As we continue to appreciate that adipose tissue is a complex, interactive, modifiable endocrine tissue, the GHR–/– mice represent a counterintuitive state of obesity without the many metabolic complications that often accompany excess adiposity. Understanding the unique features of energy regulation and adiposity in these mice will help us better understand the metabolic dysfunction surrounding an obese state.

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51.1 Introduction

Adipose tissue is a well-established target for growth hormone (GH) with tissue mass being strongly and negatively correlated with GH levels. The ability of GH to alter adiposity appears to be due in part to increased lipolysis within the adipocyte and to GH's contribution to adipocyte differentiation and/or proliferation (Flint et al. 2003; Bluher et al. 2005). Likewise, GH is known to have an anabolic impact on muscle tissue, a feature that will be more thoroughly addressed in Chapter 53. Based on these well-accepted functions of GH, it is not surprising that an excess or deficiency in GH function leads to significant alterations of body composition in humans. That is, excess GH in humans, as occurs with acromegaly, results in increased muscle mass and decreased body fat mass (Katznelson 2008). At the other extreme, deficiency or absence of GH function, as with individuals with Laron syndrome, is associated with marked increases in fat mass and decreases in lean body mass (Laron et al. 2006). Fat tissue is altered in an analogous manner in many animal models with altered GH function. That is, bovine GH transgenic mice with excess GH signaling have relatively less adipose tissue in adulthood (Palmer et al. 2009), whereas the GHR–/– mouse as well as several different GH-deficient mouse models have been reported to have elevated levels of adipose tissue mass (Heiman et al. 2003; Berryman et al. 2004).

Interest in adipose tissue has been reinvigorated in recent years. Much of this renewed interest stems from a fairly recent appreciation that adipose tissue is much more complex than formerly realized and is not just a mere storage site for triglycerides. Specifically, adipose tissue is now recognized as a major intrinsic endocrine organ with a variety of physiologically important secretory products.

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Table 51.1 Comparison of % body fat data reported for GHR−/− mice

Genetic background	Gender	Body composition method	Age	% Body fat of control mice	% Body fat of GHR−/− mice	Reference
Mixed OlaBalbC	Male	NMR	6 Months	13	21	Berryman et al. (2004)
C57Bl/6J	Male	NMR	5 Months	12	27	Berryman et al. (2006)
Mixed	Male	DEXA	"Young" (6–7 weeks) "Adult" (7–10 months) "Aged" (28–32 months)	15 21 17	17 28 28	Bonkowski et al. (2006)
Mixed	Female	DEXA	"Young" (6–7 weeks) "Adult" (7–10 months) "Aged" (28–32 months)	15 20 21	16 21 32	Bonkowski et al. (2006)
Mixed OlaBalbc	Male	DEXA	3.5 Months	14	34	Egecioglu et al. (2006)
C57Bl/6J	Male	NMR	6 Weeks–2 years	5–22	12–42	Berryman et al. (2010)
C57Bl/6J	Female	NMR	6 Weeks–2 years	8–28	11–35	Berryman et al. (2010)

Further, the potential for macrophage infiltration (Weisberg et al. 2003; Xu et al. 2003; Clement et al. 2004), for remodeling (Charriere et al. 2003; Ukropec et al. 2008), for a more prominent role of brown adipocytes (Lefterova and Lazar 2009; Lehr et al. 2009), and for clear depot-specific differences in metabolism (Casteilla et al. 2008; Kloting et al. 2009; Macotela et al. 2009) has stimulated clinical and basic research in this tissue.

In this chapter, we will highlight what is known about body composition and adipose tissue mass and functional changes in GHR−/− mice. As the balance of energy intake and expenditure greatly influence these parameters, we will also review the available data on food intake and energy expenditure in GHR−/− mice. All data will be compared to that of individuals with Laron syndrome.

51.2 Body Composition

Based on the known lipolytic function of GH, it is expected that an absence of GH action will result in increases in whole-body adiposity among GHR−/− mice. Indeed, several reports in which whole-body composition analyses were performed consistently show that GHR−/− mice are relatively obese in comparison to littermate controls (Table 51.1). The only exception appears in one report in which very young mice (6–7 weeks) did not show significantly different increases in percent whole-body fat (Bonkowski et al. 2006). Further, some of these reports also indicate that despite the significantly reduced size of the GHR−/− mice, the absolute weight of their total fat mass is comparable to that of littermate controls (Berryman et al. 2004; Berryman et al. 2006; Berryman et al. 2010). Thus, whole adipose tissue mass appears to be one of the few tissues not reduced in these dwarf mice. Finally, in our laboratory, we have been tracking body composition using NMR technology of male and female GHR−/− mice over the course of their lifespan (Fig. 51.1). As shown, male GHR−/− have a greater increase in percent fat mass (normalized to body weight). In contrast, total fat mass for male GHR−/− mice is similar to control mice. Similar to male GHR−/− mice, female mice also have greater percent fat mass throughout their lives, yet the difference is not as profound. Overall, these results demonstrate an intriguing gender and age-dependent difference in body composition for GHR−/− mice. Individuals with Laron syndrome also

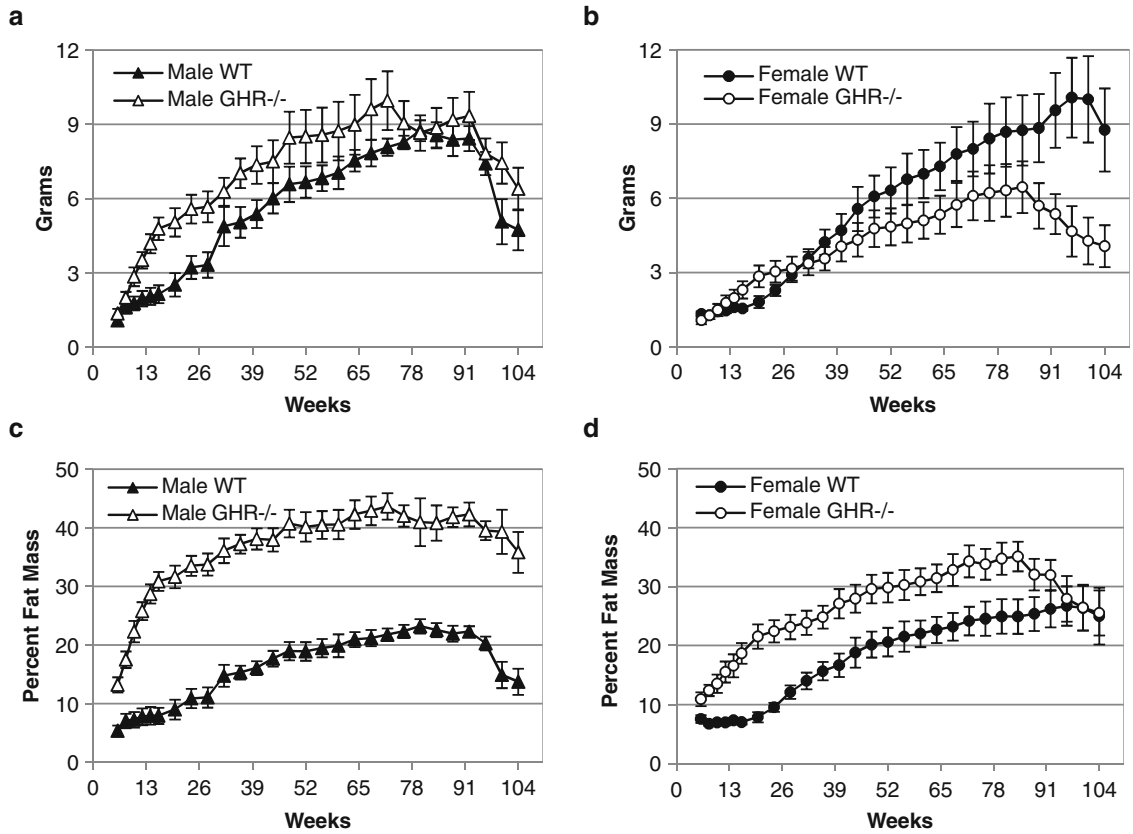

Fig. 51.1 Absolute fat mass and percent fat mass for male and female GHR−/− mice. Data are expressed as mean ± SEM, $n = 7$ male GHR−/−, 6 male NT, 8 female GHR−/−, and 8 female NT mice. Using the same cohort of mice, weight and body composition measurements were taken periodically up to 112 weeks of age in duplicate using the Bruker Minispec. Reproduced with permission from Berryman et al (2010)

have increased percent fat mass (Chap. 12), although females appear to have a greater percent fat mass than males. Further, the increase in percent body fat for Laron is initiated in early life as shown for the GHR−/− mice.

51.3 White Adipose Tissue

51.3.1 Depot-Specific Accumulation

Studies consistently show that GHR−/− mice have excess total body fat accumulation. However, the accumulation of fat mass is not uniform, with different depots being disproportionately enlarged. Specifically, GH has site/depot-specific effects on adipose tissue with multiple reports showing a profound increase preferentially in the mass of subcutaneous depots in male mice (Li et al. 2003; Berryman et al. 2004; Liu et al. 2004; Berryman et al. 2006; Berryman et al. 2010). Data from our lab (Berryman et al. 2010) and data from Flint et al. (2006) show a similar depot-specific trend in GHR−/− female mice. The increased subcutaneous fat mass can be easily visualized by MRI images captured for select male GHR−/− mice (Fig. 51.2). Two separate papers have also indicated that the fat pad next to the kidney (retroperitoneal) is increased in male GHR−/− mice (Berryman et al. 2004; Egecioglu et al. 2006), although these findings are not consistent and may depend on age or genetic background of the mice (Liu et al. 2004; Berryman et al. 2006). It is important to note that early reports (List et al. 2001; Coschigano et al. 2003) suggested that GHR−/− mice were not

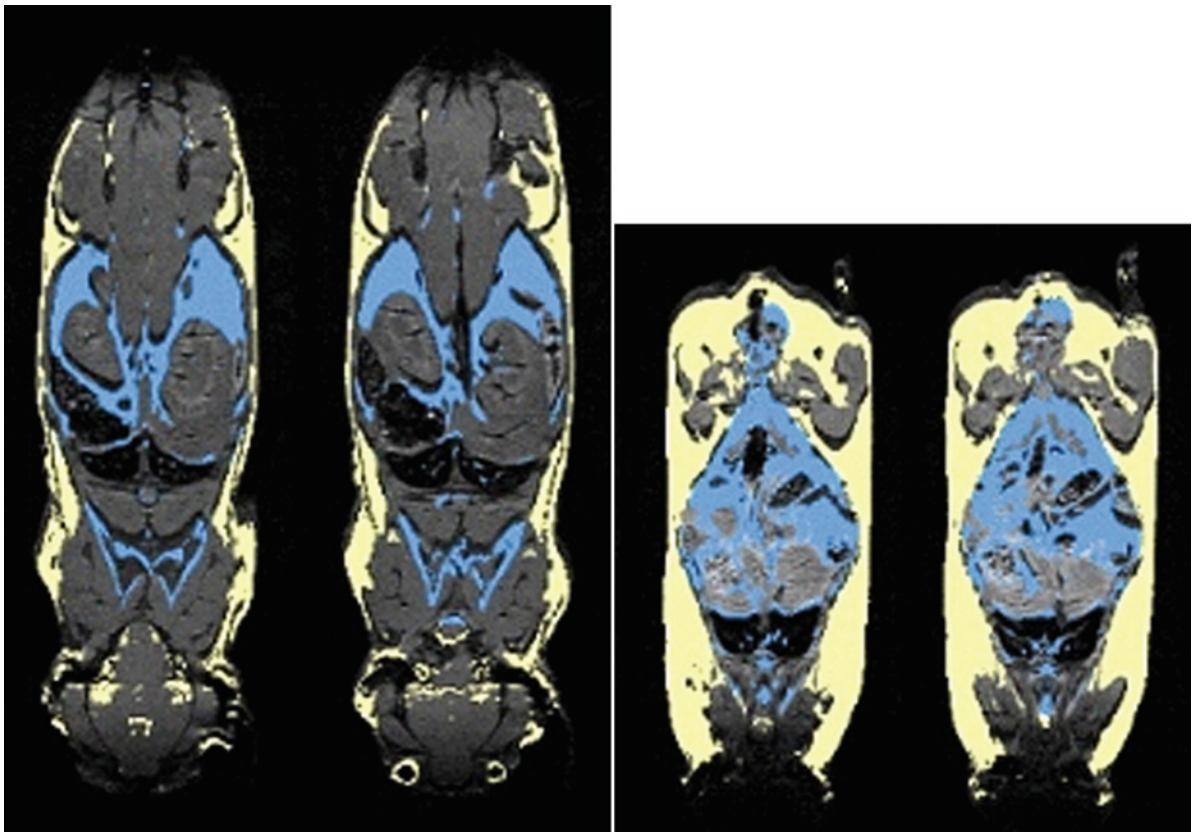

Fig. 51.2 Regional body fat distribution of male control mice (*left*) and male GHR−/− mice (*right*) using magnetic resonance imaging. Images were acquired using a Bruker 4T small animal MR scanner and a T1-weighted multiecho spin-echo acquisition (Matrix 128×512×128; FOV 2.5×10×2.5 cm; Resolution 0.1953×0.1953×0.1953 mm^3 T1-Weighted, 15-echos TE=15, 30, 4,.... 225 ms). The mouse is positioned with the anterior part at the bottom of the image. Image voxels were classified using a multichannel classification algorithm into three classes: (i) fat, (ii) lean muscle, and (iii) bone/air (Papademetris et al. 2005). An interactive segmentation method is used to identify the peritoneum and to label the fat into two groups (inter-abdominal and subcutaneous). Subcutaneous fat is noted by the color yellow and intra-abdominal by the color blue

obese as compared to control mice. This discrepancy can be easily explained due to the particular depot used to assess adiposity in these studies. That is, these studies utilized solely the epididymal fat pad, which is the depot most commonly studied due to its ease of dissection, to extrapolate whole-body adiposity levels. However, this particular depot is consistently proportional to body size in GHR−/− mice and not enlarged (Berryman et al. 2004; Berryman et al. 2006). Overall, there is no doubt that an adipose depot-dependent effect occurs in GHR−/− mice. It should be noted that marked increases in subcutaneous adipose tissue have been reported for individuals with Laron syndrome (Chap. 12) as well as GH-deficient humans (Tanner and Whitehouse 1967; Toogood et al. 1996), although other depots may also be enlarged (Laron et al. 2006).

51.4 Response to High-Fat (HF) Feeding

Providing HF diets to specific mouse strains results in diet-induced obesity and subsequent diabetes (Surwit et al. 1988; Surwit et al. 1995; Qiu et al. 2005; List et al. 2007). Two separate groups have reported the effect of HF feeding on GHR−/− mice (Berryman et al. 2006; Robertson et al. 2006). A comparison of these two studies is provided in Table 51.2. Overall, both studies report that GHR−/− mice appear more susceptible to diet-induced obesity with greater percent body weight gains than control mice and with most fat pads increased with calorically dense diets. Despite the increased obesity with HF feeding, GHR−/− mice remain relatively protected from the hyperglycemia that often accompanies increased adiposity.

Table 51.2 Comparison of low-fat (LF) and high-fat (HF) feeding studies with GHR–/– mice

	Berryman et al. (2006)	Robertson et al. (2006)
Mice	Male GHR–/– mice Backcrossed into C57Bl/6J	Male GHR–/– mice Mixed OlaBalbC/C57Bl/6J
Diet manipulation	12 Weeks of HF diet feeding Nutrient-matched LF diet Started at 2.5 months	17 Weeks of HF diet feeding Chow as LF diet Started at 3.5 months
% Weight change with HF feeding	32 For GHR–/– 17 For controls	31 For GHR–/– 21 For controls
Body composition	All weight gain due to fat mass gains	NA
Fat pad mass	All fat depots measured (inguinal subcutaneous, retroperitoneal, epididymal) were enlarged with HF feeding for both GHR–/– and control mice	Visceral, leg, scapula were enlarged with HF diet feeding for both GHR–/– and control mice; no change in renal fat pad mass for GHR–/– mice, but significant increase for controls with HF diet
Glucose and Insulin	No change in glucose Plasma insulin levels increase in GHR–/– mice with HF feeding, but still hypoinsulinemic relative to control mice	No change in glucose No increase in insulin resistance or serum insulin levels for either GHR–/– or control mice

Table 51.3 Adipocyte size in various fat pads from GHR–/– mice vs. littermate controls. Asterisk indicates that mean areas of adipocytes were significantly different than WT adipocytes of the same fat pad

	WT Mean area ± SE (μm^2)	GHR–/– Mean area ± SE (μm^2)	Change (%)
Epididymal	1990 ± 31	1875 ± 35	–5.8
Retroperitoneal	1933 ± 31	2138 ± 44*	10.6
Subcutaneous	1337 ± 18	1783 ± 33*	33.3

51.5 Depot-Specific Differences in Function and Expression

The striking differences in adipose depot accumulation in GHR–/– are likely accompanied by differences in function, composition, innervation, and protein/gene expression. Although a full understanding of why these depot differences are seen remain unanswered, several reports have illustrated some significant differences. Differences in adipocyte cell size and number in different depots in GHR–/– mice have been reported (Flint et al. 2006; Kelder et al. 2007). For cell size, cross-sectional area of adipocytes was determined from male mice using whole adipose tissue, which has an advantage of preserving the fragile, larger adipocytes (Hirsch and Gallian 1968; Chen and Farese 2002). As shown in Table 51.3 and Fig. 51.3, the mean cross-sectional area of adipocytes was greater for subcutaneous and retroperitoneal depots in GHR–/– mice, while there was no significant cell size increase in the epididymal fat pads. This contradicts findings available for a single individual with Laron syndrome in which fat cell size was smaller in subcutaneous and omental depots than control subjects (Chap. 13). Thus, an increase in adipocyte size is at least in part responsible for the enlarged subcutaneous depot in GHR–/– mice. The trend in female mice may be different as suggested by Flint et al. (2006). Flint reported that cell number may be the main factor influencing the increased mass of subcutaneous adipose tissue in female GHR–/– mice.

Intrinsic depot differences have been most clearly shown for differentiation and proliferation for isolated adipocytes from female mice. Flint et al. (2006) demonstrated that isolated preadipocytes from subcutaneous depots were able to proliferate, differentiate, and respond to hormones in an identical fashion as the preadipocytes isolated from control animals. In contrast, cells isolated from the parametrial fat pads were not able to proliferate and differentiate in vitro. The fundamental requirement for GH action in the

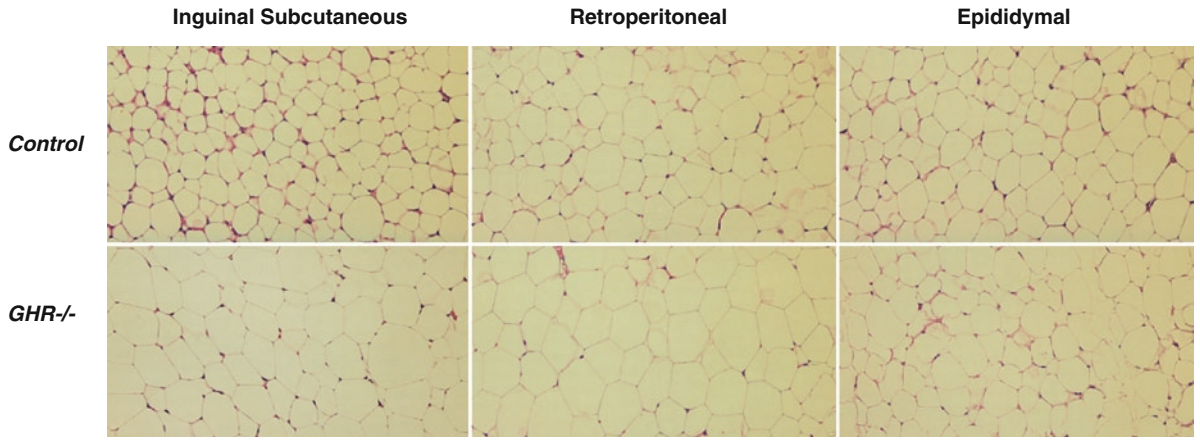

Fig. 51.3 Histology of fat pads from GHR−/− mice and matched littermate controls. Fat pads from inguinal, epididymal, and retroperitoneal regions were dissected from 6-month-old mice and fixed in 10% formalin. Tissue was embedded in paraffin, cut into 5 um sections, and stained with hematoxylin and eosin. Histological samples were viewed at 20x magnification and images obtained with a SPOT digital camera

parametrial depot and the independence of GH action in the subcutaneous depot for proliferation and differentiation provides another example of the inherent depot-specific effect in GHR−/− mice. Unfortunately, the underlying mechanism for this difference has not been resolved.

The long-lived GHR−/− mice represent an interesting model to study additional depot-specific differences. Our laboratory and others are actively exploring proteomic, genomic, and cell-type variation that explain the functional differences in these depots. We have preliminary data suggesting genotype and depot-specific expression differences in genes or proteins related to macrophage infiltration, brown adipocyte composition, and glucose/lipid metabolism (unpublished results). The use of the GHR−/− mouse model to study depot-specific differences may help unravel the underlying mechanism for how these differences are established. Moreover, the use of tissue-specific GHR gene disrupted mice will help uncover specific contributions of the GH/IGF-1 axis to these depot differences.

51.6 Brown Adipose Tissue

A second form of adipose tissue, brown adipose tissue or BAT, is altered in GHR−/− mice. Brown adipocytes within BAT are unique in that they contain densely packed mitochondria and have uncoupling protein 1 (UCP1) located in the inner mitochondrial membrane. UCP1 causes protons to leak across the innermitochondrial membrane, leading to a loss of the electrochemical gradient that mitochondria normally use to produce ATP. Thus, respiration is uncoupled from ATP formation, which results in heat production. BAT is located in the interscapular region in small mammals and in newborn humans. However, BAT has been shown to persist after birth in small animals, and although BAT is abundant at birth in humans, it is mostly replaced by WAT with age. Nonetheless, brown adipocytes do persist and are interspersed within WAT depots. Recent data suggest an important, although not fully understood, role in the proportion of brown adipocytes within WAT depots in humans (Lehr et al. 2009).

GHR−/− mice have been reported to have enlarged intrascapular brown adipose depots (Li et al. 2003; Egecioglu et al. 2006). The enlargement of BAT tissue is accompanied by an increase in UCP-1 expression in both younger (10 weeks) and older mice (52 weeks) (Li et al. 2003). These authors further suggest that UCP1 gene expression may be negatively regulated by GH as GH transgenic mice with excess GH have lowered UCP1 expression.

51.7 Endocrine Function

Adipocytes release a variety of proteins that act both locally and distally through autocrine, paracrine,

and endocrine mechanisms. Two of the most thoroughly studied are leptin and adiponectin. Both of these hormones have been assessed in GHR−/− mice. Specifically, leptin levels of GHR−/− mice are consistently elevated in male mice at younger (Berryman et al. 2004; Egecioglu et al. 2006) and older ages (Al-Regaiey et al. 2005). This trend is not surprising considering that this hormone has been shown to be consistently and positively correlated with adiposity and GHR−/− mice are relatively obese. Laron individuals also appear to have excess serum leptin levels (Laron et al. 1998). Adiponectin levels have also been shown to be consistently elevated in male (Berryman et al. 2004; Al-Regaiey et al. 2005; Nilsson et al. 2005) and female GHR−/− mice (Nilsson et al. 2005). While adiponectin is often negatively correlated with obesity, adiponectin is also implicated in the regulation of lipid and glucose metabolism in the liver, skeletal muscle, and adipose tissue and in the improvement of insulin sensitivity. Interestingly, GHR−/− mice represent a counterintuitive situation in which obesity is accompanied by an improvement in insulin sensitivity. Thus, the high adiponectin levels in the obese GHR−/− mice may relate more to adiponectin's role in glucose homeostasis than in obesity. Individuals with Laron syndrome also have elevated total and high molecular weight adiponectin, as described in Chap. 16.

51.8 Energy Balance

Accumulation of excess adipose tissue results from an imbalance of food intake and energy expenditure. Several groups have reported food intake data for GHR−/− mice. These data are summarized in Table 51.4. Almost all studies show that GHR−/− mice consume less total energy, which would be expected with their dwarf size. This finding is confirmed with individuals with Laron Syndrome (Chap. 13). However, several studies, but not all, show GHR−/− mice consume signficantly more energy when values are normalized to body weight. In terms of the other half of the energy balance equation, data on energy expenditure in GHR−/− mice are limited. Only two studies have been published that measure energy expenditure and both used male adult mice (Berryman et al. 2006; Westbrook et al. 2009). Surprisingly, GHR−/− mice were reported to have increased oxygen consumption in both studies. However, one study reported lower respiratory quotient (RQ) values (Westbrook et al. 2009), an indicator of metabolic fuel preference with a lower value being indicative of preference for fat oxidation, whereas the other study found no statistical difference in RQ albeit the trend was similar (Berryman et al. 2006). A similar trend has been observed for female GHR−/− mice (Longo et al. 2010). The different results may be due to the vastly different times used to monitor energy expenditure in these mice.

Table 51.4 Food Intake Results from GHR−/− mice

Mice	Age/Method	Total kcalorie Intake	Kcalorie Intake normalized to body size	Reference
Male GHR−/− mice; backcrossed into C57Bl/6J background	2 months of age monitored for 1 week; 8 or 9 months of age monitored for 3 weeks; average amount of food consumed/mouse calculated by dividing the amount of food consumed/week/number of mice in the cage	↓ At 2 months ↓ At 9 months	↑ At 2 months No statistical difference at 9 months	Coschigano et al. (2003)
Male 129 Ola-Balb/c Mixed	3–5 months of age; mice singly housed; food weighed weekly	↓	No statistical difference	Berryman et al. (2004)
Male SV129Ola-Balb/c	3 months of age; monitored for 3 h; food weight before and after monitoring period	Not reported	↑	Egecioglu et al. (2006)
Male GHR−/− mice; backcrossed into C57Bl/6J background	10–20 weeks; metabolic cages; singly housed	↓	↑	Berryman et al. (2006)

Individuals with Laron syndrome show a similar trend toward lower RQ values and normal to elevated resting energy expenditure when normalized to lean body mass (Chap. 13). Westbrook et al. (2009) provides several possible explanations for why these mice have increased energy expenditure including greater energy costs to maintain body temperature due to their decreased mass-to-surface ratio and their unique adipokine profile. Although the reason for the increased energy expenditure remains unanswered, it is clear that the altered body composition in GHR−/− mice is accompanied by greater consumption and expenditure of energy.

One variable related to energy balance that has been assessed in these mice is ghrelin. Ghrelin is an endogenous ligand for the growth hormone secretagogue receptor (Howard et al. 1996) and has been shown repeatedly to increase food intake and stimulate appetite (Chen et al. 2009). Ghrelin mRNA expression and serum concentrations have been reported for GHR−/− mice. Nass et al. (2004) reported no significant difference in stomach mRNA expression and in fed or fasted serum concentrations of ghrelin in 4-month-old male mice as compared to littermate controls. Likewise, a study by Egecioglu et al. (2006) showed no change in serum concentrations in 4–5-month-old male mice. However, this same study does show that GHR−/− mice have a blunted feeding response to ghrelin when centrally injected.

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Metabolism and Metabolic Regulation

52

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Core Messages

› The data available on GHR–/– mice consistently show improved insulin sensitivity at all ages. Glucose levels, which are very low in young animals, seem to normalize at older ages. Insulin levels remain significantly lower than normal throughout the lifespan of GHR–/– mice. Together, these data suggest that insulin sensitivity decreases only slightly as GHR–/– mice age. In addition, circulating lipid levels tend to be decreased, adding to the scenario of improved metabolic health in GHR–/– mice. However, the need for closer inspection of the age-dependent changes in metabolism in these mice is apparent. Given the age-dependent variation observed in blood glucose levels, it becomes critical to establish the insulin responsiveness of target organs in GHR–/– mice of different ages. Interestingly, data available on liver suggest an insulin-resistant state in older animals. This seems counterintuitive given that whole-body insulin sensitivity is enhanced in GHR–/– mice even at old age. Therefore, it would be interesting to evaluate the insulin responsiveness of the liver in young GHR–/– animals to determine the role of this organ in whole-body insulin sensitivity. Similarly, a thorough characterization of the age-specific degree of insulin sensitivity in skeletal muscle and heart is needed to complement the data that are already available. Furthermore, focus needs to be applied on adipose tissue, given that it is one of the main target organs of insulin and GH action and a key player in metabolic regulation (see Chap. 51).

› However, insulin sensitivity is the result of complex metabolic regulation. Other factors, such as PPAR isoforms, mainly PPARγ, can also affect insulin responsiveness. In the liver, contrary to the low activation status of insulin signaling intermediates, PPARγ expression is high. Increased activity of this insulin-sensitizing molecule might compensate the apparent inactivity of the insulin signaling cascade, leading to the overall inhibition of glucose production. High PPARα levels leading to increased fatty acid oxidation may contribute to the insulin responsiveness by decreasing lipid levels in the blood. However, the action of PPAR isoforms in tissues such as liver, muscle, and heart is still not clear, and further research is necessary to establish the influence that these molecules have on metabolic regulation.

› Interestingly, GHR–/– mice are not resistant to weight gain induced by a high-fat diet, but their glucose and insulin levels remain significantly lower than controls even after the weight gain. On the other hand, soy-derived diets affect lipid and glucose levels differently, with a genotype-specific increase in cholesterol and glucose tolerance only in the high isoflavone diet.

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› The beneficial effects on insulin sensitivity, in spite of obesity observed in GHR–/– mice, raise interesting questions about the relationship between GH, insulin signaling, and metabolism. These topics are of major concern in today's world, given the widespread prevalence of obesity, insulin resistance and diabetes. However, the current information is far from enough to answer all the questions. Further research is needed to shed light on the complex mechanisms of metabolic regulation.

52.1 Introduction

Metabolic regulation in GHR–/– (growth hormone receptor knockout) mice has been the target of numerous studies, given their increased insulin sensitivity and extended lifespan. The absence of GHR signaling results in very low levels of insulin-like growth factor 1 (IGF-1) and subsequent lack of negative feedback on pituitary somatotropes, which leads to increased serum growth hormone (GH) levels (Venken et al. 2007; Zhou et al. 1997). For these reasons as well as others discussed throughout this book, the GHR–/– mice serve as a suitable model for the study of the human Laron syndrome (LS) observed in individuals with impaired GHR signaling.

In addition to changes in the GH/IGF-1 axis, GHR–/– mice show decreased blood glucose at a young age that seems to normalize as the animals become older (Al-Regaiey et al. 2005; Coschigano et al. 2003). Insulin sensitivity is significantly higher than that in wild type mice, with insulin levels being greatly decreased throughout the lifespan of GHR–/– mice (Al-Regaiey et al. 2005; Coschigano et al. 2003). Given that GH is well-known to display diabetogenic properties, it is not surprising to find enhanced insulin sensitivity in GHR–/– mice. Interestingly, GHR–/– mice also display enhanced accumulation of adipose tissue, mainly in the subcutaneous depot (see Chap. 51). The relative obesity observed in GHR–/– mice is consistent with the lack of GH's lipolytic action and increased insulin activity leading to lipogenesis. This obese phenotype is also found in LS individuals (see Chap. 12). Blood glucose levels in humans with LS are lower than normal, but their insulin levels are generally high, even at young ages (Laron et al. 1995). Thus, LS individuals display insulin resistance, and at older ages, may develop type 2 diabetes (Laron 2004). The reason why mice and humans differ with respect to insulin sensitivity is still not clear.

In an effort to explain the molecular mechanisms behind the increased insulin sensitivity observed in GHR–/– mice, the levels and activation of the insulin receptor and downstream molecules in the insulin signaling cascade have been characterized in GHR–/– liver, skeletal muscle, and heart. Peroxisome proliferator-activated receptor (PPAR) isoforms, which are known to enhance insulin sensitivity and play a role in lipid metabolism, have also been evaluated in those tissues. The data obtained from these studies are reviewed in this chapter.

Circulating levels of other hormones involved in glucose homeostasis (glucagon, corticosterone, leptin, adiponectin) have been measured, along with the levels of various serum proteins and electrolytes as an indication of overall health. Also, studies of pancreas histology in GHR–/– mice have revealed structural and functional alterations in this organ and are discussed below. Regarding lipid profiles, triglycerides (TG), HDL, LDL, and total cholesterol levels in GHR–/– mice display normal to decreased levels. However, LS individuals show increased total and LDL-cholesterol (see Chap. 15). Finally, the effects of different diets (caloric restriction, high-fat diet, and soy-protein rich diet) on metabolic parameters of GHR–/– mice have been reported. Caloric restriction has mainly been studied related to aging, given the longer lifespan that GHR–/– mice display (see Chap. 50).

In this chapter, we summarize the current knowledge on these topics as they relate to metabolism and metabolic regulation in GHR–/– mice and how these compare to the metabolism in LS individuals.

52.2 Glucose Homeostasis

52.2.1 Glucose

A number of articles have reported glucose levels and hormones involved in glucose homeostasis in GHR–/– mice. At young ages (between 10 days and 9 months of age), male and female GHR–/– mice display fasting and nonfasting glucose levels that are significantly lower than those found in wild type mice (Bartke et al. 2004; Berryman et al. 2006; Coschigano et al. 2003; Dominici et al. 2000; Egecioglu et al. 2005; Hauck et al. 2001; Liu et al. 2004). These findings contrast with those found in

11-, 12-, and 21-month-old male mice, where no difference in glucose levels was found between GHR−/− mice and controls (Al-Regaiey et al. 2005; Bonkowski et al. 2009; Coschigano et al. 2003). Unfortunately, there are no reports on glucose levels at ages older than 5 months for female GHR−/− mice. LS individuals also display low glucose levels during infancy, which increase after a delayed puberty but remain below normal levels (Laron et al. 1995) at older ages; however, some cases of hyperglycemia, glucose intolerance, and diabetes were registered (Chap. 30). On the other hand, in wild type mice, a trend toward higher glucose levels in males compared to females was observed at 4–5 months. However, this variation between genders was not observed in GHR−/− mice (Hauck et al. 2001).

52.2.2 Insulin

Circulating insulin levels have also been examined in GHR −/− mice. Both fasting and nonfasting insulin levels were significantly lower when compared to controls, independent of gender or age (Al-Regaiey et al. 2005; Berryman et al. 2006; Bonkowski et al. 2009; Coschigano et al. 2003; Dominici et al. 2000; Egecioglu et al. 2005; Guo et al. 2005; Hauck et al. 2001; Liu et al. 2004). Consequently, insulin sensitivity is increased in GHR−/− mice (Bonkowski et al. 2009; Bonkowski et al. 2006; Guo et al. 2005; Liu et al. 2004; Masternak et al. 2005a). Also, insulin secretion in response to a glucose load seems to be impaired in GHR−/− mice (Guo et al. 2005). This is consistent with glucose intolerance found in young adult LS individuals (Laron et al. 1995). Unlike GHR−/− mice, LS individuals have higher insulin levels, indicating insulin resistance (Laron et al. 1995) (Chap. 30). However, their insulin levels are low when considering the degree of obesity these individuals display (Laron 2008). As LS individuals age, insulin resistance worsens and can lead to type 2 diabetes, as mentioned above, and the associated vascular complications (Laron 2004; Laron et al. 1995) (Chap. 30).

The increased insulin sensitivity observed in GHR−/− mice makes them an interesting model for the study of diabetes and associated organ end damage. When GHR−/− mice were treated with streptozotocin to induce type 1 diabetes, glucose levels increased to match those of control mice, but their kidneys were protected from glomerulosclerosis (Bellush et al. 2000). Thus, GH action is important in diabetes-induced nephropathy.

52.2.3 Glucagon

Circulating glucagon levels in the nonfasted state were lower in GHR−/− mice compared to controls at 2 months of age (Liu et al. 2004). Also, after a glucose load the decrease in glucagon secretion was more pronounced in GHR−/− than wild type mice at 2–4 months (Guo et al. 2005). Interestingly, glucagon levels were normal at 21 months of age in male GHR−/− mice (Al-Regaiey et al. 2005). Thus, glucagon levels seem to follow a trend similar to that of glucose in GHR−/− mice, with decreased levels in young animals and normalization at older ages. However, in young LS individuals the response of glucagon during an arginine infusion test was normal (Chap. 30).

52.2.4 Glucocorticoids

Regarding corticosterone levels, there seems to be an effect of gender in GHR−/− mice. At ~5 months of age, males had higher corticosterone levels than controls, whereas females showed no difference from wild type values (Egecioglu et al. 2005; Hauck et al. 2001). Males still showed increased corticosterone levels at 21 months of age (Al-Regaiey et al. 2005), but no information is available for older GHR−/− females. The elevated corticosterone levels observed in young and older males, in the presence of low circulating insulin levels, would be expected to promote hepatic glucose production, which is consistent with the activation of gluconeogenic enzymes measured in the liver of these mice (Al-Regaiey et al. 2005) (see below). Glucocorticoids can also promote fat accumulation, consistent with GHR−/− mice being obese (Egecioglu et al. 2005) (see Chap. 51).

52.2.5 Leptin and Adiponectin

Blood levels of other hormones related to glucose homeostasis have also been reported, such as leptin and adiponectin. These hormones are secreted by white adipose tissue and have known insulin-sensitizing actions. In GHR−/− mice, circulating leptin levels were increased in nonfasted ~5 and 21-month-old males (Al-Regaiey et al. 2005; Egecioglu et al. 2005). However, in 5-month-old GHR−/− mice, leptin levels were normal after a 4-h fast (Berryman et al. 2004). On

the other hand, circulating adiponectin levels were increased in ~6 and 21-month-old males and ~5-month-old female GHR–/– mice (Al-Regaiey et al. 2005; Berryman et al. 2004; Nilsson et al. 2005). LS individuals also display high adiponectin levels (Chap. 14). The relationships between adiponectin, leptin, fat mass, and food intake are covered in Chap. 51.

52.2.6 Pancreas Histology and Function

In the pancreas of GHR–/– mice several functional changes exist that likely contribute to the altered glucose homeostasis. Histological analyses of the pancreata of 2-month-old GHR–/– mice revealed smaller islet size that was not proportional to body size (Guo et al. 2005; Liu et al. 2004). The diminished islet size could be due to smaller islet cells, since lower β-cell mass was found in ~11-month-old GHR–/– mice (Liu et al. 2004). Despite this, α-cell mass was proportionally higher in GHR–/– mice when compared to controls (Liu et al. 2004). Consistent with a lower β-cell mass, insulin content and insulin mRNA were also lower in GHR–/– pancreata at 2–4 months of age when compared to controls (Guo et al. 2005; Liu et al. 2004). Apparently, the lower insulin demand along with the lack of GH action in GHR–/– mice results in smaller islet size when compared to wild type.

52.3 Insulin Signaling

GH has long been considered a diabetogenic hormone because of its ability to antagonize insulin action, and although several explanations have been offered, the mechanism(s) for this antagonism are not yet clear. At the cellular level, insulin signals through a complicated cascade of protein activation/inactivation that begins at the cell membrane where the insulin receptor (IR) is located (Appendix Fig. 3). Recently, some reports have suggested that the decreased response to insulin induced by GH is the result of the GH-dependent upregulation of the p85α subunit of phosphatidylinositol 3-kinase (PI3K) (Barbour et al. 2005; del Rincon et al. 2007). PI3K is a major player in the insulin signaling cascade downstream of the IR and the IR substrate 1 (IRS-1) and is present in the cell as a heterodimer, formed by a catalytic subunit (p110) and a regulatory subunit (p85α or β). Both p110 and p85 subunits are required for PI3K to be active. Upon tyrosine-phosphorylation of IRS-1, PI3K binds to it through the p85 subunit, promoting the activation of the p85-p110 heterodimer. However, when p85 levels are in excess of p110, free p85 monomers can compete with the heterodimeric form for IRS-1 binding. Indeed, insulin resistance has been linked to increased p85α levels in skeletal muscle and adipose tissue of mouse models of enhanced GH signaling (Barbour et al. 2005; del Rincon et al. 2007). The opposite also applies, where low p85α levels resulted in increased insulin sensitivity despite normal p85β expression (Barbour et al. 2005; del Rincon et al. 2007). Unfortunately, levels of monomeric p85α have not been evaluated in GHR–/– mice. However, lit/lit (GH releasing hormone receptor-deficient) mice, which are also dwarf and have GH deficiency with low circulating IGF-1 levels and enhanced insulin sensitivity at young age, displayed reduced p85α expression in white adipose tissue at 3 months of age (del Rincon et al. 2007). On the other hand, humans subjected to short-term (4-h) GH infusion who showed insulin resistance in muscle had no change in IRS-1 phosphorylation and IRS-1-associated PI3K activity (Jessen et al. 2005). Thus, more than one mechanism might exist for GH-induced insulin resistance: a short-term effect independent of PI3K and a long-term one through upregulation of p85α. However, the possibility still remains that antagonism of insulin by GH is different in humans and mice.

Many other proteins that play a role in insulin signaling (Appendix Fig. 3) have been studied in GHR–/– mice, establishing their level and activation status in the liver, skeletal muscle, and heart. These data are summarized in Table 52.1.

52.3.1 Liver

In liver homogenates, overall insulin signaling seemed to remain similar to controls in young mice. Although there are contradictory data for the first steps of insulin signaling in GHR–/– mice, normal levels of signaling downstream of IRS-1 seem to be a common finding. For example, total and tyrosine-phosphorylated (pY) levels of IR were increased in 3–5 and 14-month-old GHR–/– mice (Bonkowski et al. 2009; Dominici et al. 2000).

Table 52.1 Proteins involved in the insulin signaling cascade and their levels in GHR−/− mice as compared to controls

	Liver		Skeletal muscle		Heart	
	mRNA	Protein	mRNA	Protein	mRNA	Protein
IR	↑At 21 months (Masternak et al. 2005b)	↑ At 3–5 and 14 months (Dominici et al. 2000; Bonkowski et al. 2009) pY ↑ at 3–5 and 14 months (Dominici et al. 2000; Bonkowski et al. 2009) ↔ at 6 months (Robertson et al. 2006) pY/total ↓ at 3–5 months (Dominici et al. 2000)	↔ At 21 months (Masternak et al. 2005b)	↑ At 14 months (Bonkowski et al. 2009) pY ↔ at 14 months (Bonkowski et al. 2009) and ↓ (transiently delayed) at 6 months (Robertson et al. 2006)	↔ At 3 and 21 months (Masternak et al. 2006)	↑ At 14 months (Giani et al. 2008) pY ↑ at 14 months (Giani et al. 2008) pY/total ↔ at 14 months (Giani et al. 2008)
IRS-1	↑ At 21 months (Masternak et al. 2005b)	↔ At 3–5 months (Dominici et al. 2000) pY ↔ at 3–5 months (Dominici et al. 2000)	↔ At 21 months (Masternak et al. 2005b)	↔ At 14 months (Bonkowski et al. 2009) pY ↓ (transiently delayed) at 6 months (Robertson et al. 2006) pS ↓ at 14 months (Bonkowski et al. 2009)	↑ At 3 months, ↔ at 21 months (Masternak et al. 2006)	↑ At 14 months (Giani et al. 2008) pY ↑ at 14 months (Giani et al. 2008) pY/total ↔ at 14 months (Giani et al. 2008)
IRS-2	↑ At 21 months (Masternak et al. 2005b)		↔ At 21 months (Masternak et al. 2005b)		↑ At 3 months, ↔ at 21 months (Masternak et al. 2006)	
p85 Subunit of PI3K		↔ At 3–5 months (Dominici et al. 2000), ↓ at 14 months (Bonkowski et al. 2009) Associated to IRS-1 ↔ at 14 months (Bonkowski et al. 2009) Associated to IRS-1/total IRS-1 ↔ at 3–5 and 6 months (Dominici et al. 2000; Robertson et al. 2006)		Associated to IRS-1 ↑ at 14 months (Bonkowski et al. 2009) Associated to IRS-1/total IRS-1 ↔ at 6 months (Robertson et al. 2006)		↔ At 14 months (Giani et al. 2008) Associated to IRS-1/total IRS-1 ↑ at 14 months (Giani et al. 2008)
p55α, p50α (isoforms of p85)		↓ At 14 months (Bonkowski et al. 2009)				
PI3K activity		↔ At 3–5 months (Dominici et al. 2000)				

(*continued*)

Table 52.1 (continued)

	Liver		Skeletal muscle		Heart	
	mRNA	Protein	mRNA	Protein	mRNA	Protein
Akt/PKB	↑ At 21 months (Al-Regaiey et al. 2005)	↔ At 14 and 21 months (Bonkowski et al. 2009; Al-Regaiey et al. 2005) p ↔ at 14 months (Bonkowski et al. 2009), ↓ at 21 months (Al-Regaiey et al. 2005)	↔ Akt2 at 21 months (Al-Regaiey et al. 2007)	↑ Akt1 and Akt2 at 14 months (Bonkowski et al. 2009) ↑ Akt2 at 21 months (Al-Regaiey et al. 2007) p-Akt1 and Akt2 ↑ at 14 months (Bonkowski et al. 2009), ↔ total p-Akt at 21 months (Al-Regaiey et al. 2007)		↑ At 14 months (Giani et al. 2008) p ↑ At 14 months (Giani et al. 2008) p/total ↔ at 14 months (Giani et al. 2008)
Foxo1	↑ At 21 months (Al-Regaiey et al. 2005)	↑ At 21 months (Al-Regaiey et al. 2005)	↔ At 21 months (Al-Regaiey et al. 2007)	↓ At 21 months (Al-Regaiey et al. 2007)		
AMPK		↑ At 21 months (Al-Regaiey et al. 2005) p ↑ at 21 months (Al-Regaiey et al. 2005)		p ↔ at 21 months (Al-Regaiey et al. 2007)		
GLUT4			↔ At 21 months (Masternak et al. 2005b)	↑ At 14 months (Bonkowski et al. 2009)	↔ At 3 and 21 months (Masternak et al. 2006)	↓ At 14 and 21 months (Masternak et al. 2005b; Giani et al. 2008)

Arrows show the direction of change (increase ↑, decrease ↓, no change ↔) for the levels measured in GHR−/− mice as compared to wild type. The age of the mice involved in the study is also shown, with the reference in parentheses.
AMPK 5'-AMP-activated protein kinase; *Akt/PKB* protein kinase B; *Foxo1* forkhead box protein O1; *GLUT4* glucose transporter 4; *IR* insulin receptor; *IRS* insulin-receptor substrate; *PI3K* phosphatidylinositol 3-kinase; p phosphorylated; *pS* serine-phosphorylated; *pY* tyrosine-phosphorylated

However, in 6-month-old GHR−/− mice, pY-IR levels were normal (Robertson et al. 2006). Also, the velocity of tyrosine-phosphorylation of the IR and IRS-1 after an insulin stimulus was normal as well in GHR−/− mice at 6 months of age (Robertson et al. 2006). Interestingly, although pY-IR levels were elevated in 3–5-month-old GHR−/− mice, the next step in the signaling cascade (Appendix Fig. 3), tyrosine-phosphorylation of IRS-1, was unchanged from control levels (Dominici et al. 2000). Consistent with this, PI3K activity at 3–5 months and phosphorylated Akt (p-Akt) levels at 14 months were normal in GHR−/− mice (Bonkowski et al. 2009; Dominici et al. 2000). Phosphorylation of Akt (also known as PKB, protein kinase B) is promoted by PI3K upon recruitment and activation of PI3K by pY-IRS-1. Regarding PI3K activity, the levels of p85 and its isoforms p55α and p50α were decreased at 14 months of age (Bonkowski et al. 2009). However, for accurate evaluation of p85 levels regarding insulin sensitivity, it is critical to determine the levels of p85α separately from p85β and from p85α bound to p110. As explained above, this stems from the proposed role of free p85α (and not p85β) as a negative regulator of insulin signaling in skeletal muscle and adipose tissue (Barbour et al. 2005; del Rincon et al. 2007).

On the other hand, in older mice liver insulin signaling appeared to be downregulated (Al-Regaiey et al. 2005). When p-Akt levels were measured, they were lower in 21-month-old GHR−/− mice than controls (Al-Regaiey et al. 2005). One of the functions of p-Akt is

to inhibit the transcription factor Foxo1 (Forkhead box protein O1). Foxo1 can bind to PGC-1α (PPARγ coactivator 1α) and upregulate the expression of the gluconeogenic enzymes PEPCK (phosphoenolpyruvate carboxykinase) and G6Pase (glucose-6-phosphatase). Consistent with low p-Akt levels, Foxo1, PGC-1α, PEPCK, and G6Pase content were increased in 21-month-old GHR–/– mice when compared to controls (Al-Regaiey et al. 2005). Also, p-CREB levels were higher in 21-month-old GHR–/– mice than wild type (Al-Regaiey et al. 2005). Given that insulin inhibits CREB (cAMP response element-binding protein), increased levels of p-CREB are consistent with lower insulin signaling in older GHR–/– mice. High p-CREB levels are thought to promote gluconeogenesis and fatty acid oxidation through PGC-1α activity (Herzig et al. 2001). Increased fatty acid oxidation is also consistent with higher PPARα levels in GHR–/– livers than controls (see below). In addition, increased levels of AMPK (5'-AMP-activated protein kinase) and p-AMPK were found in 21-month-old GHR–/– mice (Al-Regaiey et al. 2005). Higher AMPK activity also results in gluconeogenesis and fatty acid oxidation, which could be the result of increased leptin and adiponectin signaling in GHR–/– mice (Al-Regaiey et al. 2005).

Overall, these results suggest that younger GHR–/– mice do not display major alterations in liver insulin signaling. Instead, at older ages, liver metabolism in GHR–/– mice is shifted toward glucose production and lipid oxidation, denoting low insulin sensitivity (Al-Regaiey et al. 2005). These results are surprising, given the enhanced whole-body insulin sensitivity observed in GHR–/– mice at all ages. Interestingly, the activation of gluconeogenesis at older age would serve to explain the normalized blood glucose levels observed in GHR–/– mice (see above). It would be interesting to measure lipid content in the liver of older GHR–/– mice, given that although TG levels were increased at 3 months of age (Bartke et al. 2004), enhanced lipid oxidation at older age may result in decreased TG accumulation in this organ.

52.3.2 Skeletal Muscle

In the case of skeletal muscle, initial reports in GHR–/– mice suggested that insulin signaling was decreased (delayed activation of IR and IRS-1 upon insulin-stimulation) in 6-month-old and unchanged (IR, IRS-1, and glucose transporter 4 (GLUT4) mRNA levels) in 21-month-old mice relative to controls (Masternak et al. 2005b; Robertson et al. 2006). However, recent data indicate that GHR–/– skeletal muscle has increased insulin sensitivity at 14 and 21 months of age (Al-Regaiey et al. 2007; Bonkowski et al. 2009). At 14 months of age, GHR–/– mice have normal pY-IR levels, but lower serine-phosphorylation of IRS-1 (pS-IRS-1) than wild type mice (Bonkowski et al. 2009). Given that IRS-1 is inhibited by serine-phosphorylation, low pS-IRS-1 levels indicate higher IRS-1 activity. In fact, lower levels of IRS-1 inhibition could account for increased glucose uptake and higher activation of downstream molecules even in the presence of unchanged pY-IR (Bonkowski et al. 2009). Consistent with this, p-Akt and GLUT4 levels were higher in GHR–/– mice than wild type littermates at 14 months (Bonkowski et al. 2009). Regarding PI3K, the levels of p85 associated to pY-IRS-1 were also increased in 14-month-old GHR–/– mice (Bonkowski et al. 2009). However, it was not determined if this increase was due to active p85-p110 heterodimers or inactive p85α monomers. Therefore, PI3K activity cannot be inferred from this measurement. At 21 months of age, molecules that induce the inactivation of IRS-1 through serine-phosphorylation, such as JNK1 (c-Jun N-terminal kinase 1) and PKCζ (protein kinase Cζ), were lower in GHR–/– mice than controls (Al-Regaiey et al. 2007). These data are similar to those obtained in 14-month-old GHR–/– mice and are consistent with increased insulin signaling. Interestingly, Foxo1 (and Foxo3) levels were decreased in 21-month-old GHR–/– mice, even though p-Akt and PGC-1α levels were normal (Al-Regaiey et al. 2007). This is opposite to the increase of Foxo1 found in the liver of 21-month-old mice. Although the function of Foxo transcription factors in muscle is not clear, a role for muscle atrophy and insulin resistance through the inhibition of glucose oxidation has been proposed. Therefore, decreased Foxo levels at old age would result in higher insulin sensitivity and delayed muscle wasting in GHR–/– mice (Al-Regaiey et al. 2007).

52.3.3 Heart

Regarding the heart, GHR–/– mice also appear to display increased insulin sensitivity. At 14 months of age, pY-IR, pY-IRS-1, and p-Akt were increased in

GHR−/− mice when compared to controls (Giani et al. 2008). Levels of p85 associated to IRS-1 were also increased in these mice (Giani et al. 2008), although no further characterization of p85 was performed to differentiate between p85-p110 dimers and p85α monomers. Interestingly, GLUT4 levels were decreased in 14-, and 21-month-old GHR−/− mice, while GLUT1 levels (measured only in 14-month-old mice) remained similar to controls (Giani et al. 2008; Masternak et al. 2006). Given the enhanced insulin sensitivity observed in the heart, the downregulation of GLUT4 could serve as a compensatory mechanism to avoid excess glucose uptake, allowing for normal function of this organ (Giani et al. 2008).

52.3.4 MAPK Pathway

A few molecules in the insulin-stimulated MAPK (mitogen-activated protein kinase) pathway (Appendix Fig. 3) have also been studied. In the liver of 3–5-month-old GHR−/− mice, Shc (Src homology 2 domain-containing-transforming protein C1) was found to be similar to controls (Dominici et al. 2000). Thus, similarly to the IRS-1 pathway, the MAPK cascade fails to display increased activation in the liver of young GHR−/− mice. On the other hand, in the heart of 14-month-old GHR−/− mice, levels of phosphorylated (active) ERK1 (extracellular signal-regulated kinase 1) and ERK2 were elevated (Giani et al. 2008). This increase has been suggested to compensate the lack of GH signaling and promote normal cardiac growth (Giani et al. 2008) (see Chap. 54). Therefore, insulin signaling in the heart appears to be enhanced not only through the IRS-1 pathway but via the MAPK pathway as well.

In conclusion, the enhanced insulin sensitivity observed in GHR−/− mice seems not to be reflected in the responsiveness of all individual organs. Instead, it might result from a combination of varying degrees of sensitivity in various organs. For instance, the liver appears to display normal insulin sensitivity in young GHR−/− mice and to shift toward insulin resistance at older ages. In contrast, skeletal and cardiac muscles seem to remain very insulin sensitive at all ages. However, the actions of insulin are carried out through numerous mechanisms, several of which have not been thoroughly studied. More studies are necessary to accurately assess the responsiveness to insulin in these and other target organs of insulin signaling, including adipose tissue. Furthermore, clarifying the action of other signaling molecules, such as PPAR isoforms (see below), may help to thoroughly characterize the degree of insulin sensitivity in each organ studied.

52.4 Lipid Profiles

Multiple studies have assessed the lipid profile of GHR−/− mice. Total cholesterol was significantly decreased in male GHR−/− mice at 3–5 and 21 months of age (Bartke et al. 2004; Egecioglu et al. 2005; Masternak et al. 2005a). Interestingly, when males and females were evaluated together at ~4 months of age, their cholesterol levels were found to be numerically but not statistically different from controls (Liu et al. 2004). Thus, the possibility exists that female total cholesterol levels remain normal. Unfortunately, no data on this are available since no studies have looked at female mice separately. Levels of HDL-cholesterol were decreased in males or showed a trend toward decrease in grouped males and females at 3–5 months (Egecioglu et al. 2005; Liu et al. 2004). LDL-cholesterol was also decreased in 3–5-month-old male GHR−/− mice (Egecioglu et al. 2005). Consistent with GHR−/− mice, LS individuals show low to normal total and LDL-cholesterol in early childhood (Laron 2004). However, these levels increase with age and obesity, commonly resulting in increased total and LDL-cholesterol (Laron 2004; Laron et al. 1995; Rosenbloom et al. 1999) (see Chap. 15).

Regarding circulating TG, conflicting data have been reported. Most studies have shown normal levels in GHR−/− mice: 3 and 21-month-old males and ~4-month-old grouped males and females (Bartke et al. 2004; Liu et al. 2004; Masternak et al. 2005a). However, decreased TG levels have also been reported for ~5-month-old male GHR−/− mice (Egecioglu et al. 2005). In adult LS individuals, TG levels were normal (Laron et al. 2008) (see Chap. 15). In addition, levels of apolipoprotein B (ApoB), an apolipoprotein affiliated with TG-rich lipoproteins, were lower in male GHR−/− mice than controls at ~5 months of age (Egecioglu et al. 2005). Regarding plasma free fatty acids (FFA), levels in 21-month-old male GHR−/− mice were similar to controls (Masternak et al. 2005a). In contrast, LS individuals have high FFA in circulation (Laron 2004) (see Chap. 15).

Table 52.2 PPAR and RXR isoforms in GHR−/− mice as compared to controls

	Liver (Masternak et al. 2005c)		Skeletal muscle (Masternak et al. 2005a)		Heart (Masternak et al. 2006)	
	mRNA	Protein	mRNA	Protein	mRNA	Protein
PPARγ	↑ At 21 months	↑ At 21 months	↔ At 21 months	↓ At 21 months	↔ At 3 and 21 months	↔ At 21 months
PPARα	↑ At 21 months	↑ At 21 months	↔ At 21 months	↓ At 21 months	↑ At 3 months and ↔ At 21 months	↓ At 21 months
PPARβ	↔ At 21 months	↓ At 21 months	↔ At 21 months	↓ At 21 months	↑ At 3 and 21 months	↓ At 21 months
RXRγ	↑ At 21 months		↔ At 21 months		↔ At 3 months and ↑ At 21 months	
RXRα	↑ At 21 months		↔ At 21 months		↑ At 3 and 21 months	
RXRβ	↑ At 21 months		↔ At 21 months		↑ At 3 and 21 months	

Arrows show the direction of change (increase ↑, decrease ↓, no change ↔) for the levels measured in GHR−/− mice as compared to wild type. The age of the mice involved in the study is also shown. References are included at the top of each column. Abbreviations: *PPAR* peroxisome proliferator-activated receptor; *RXR* retinoid X receptor

At the tissue level, livers of 3-month-old male GHR−/− had lower nonesterified cholesterol and higher esterified cholesterol than control mice (Bartke et al. 2004). Liver TG were also significantly increased in 3-month-old male GHR−/− mice (Bartke et al. 2004). This agrees with data from LS individuals, who tend to develop fatty livers (see Chap. 14). On the other hand, in skeletal muscle, there was no change in TG and FFA content between male 21-month-old GHR−/− mice and controls (Masternak et al. 2005a).

52.5 Peroxisome Proliferator-Activated Receptor (PPAR) Isoforms

PPAR isoforms are regulators of lipid and glucose metabolism. PPARγ is highly expressed in adipose tissue where it regulates adipocyte differentiation, lipid accumulation, and adipokine secretion, resulting in increased whole-body insulin sensitivity (Ferre 2004). PPARα, highly prevalent in the liver, activates fatty acid oxidation and decreases TG levels (Ferre 2004; Fruchart et al. 2001). In mice, PPARα decreases Apo A1 and A2 levels, thereby decreasing HDL production (Schoonjans et al. 1997). This effect is opposite in humans, resulting in increased ApoA1, ApoA2, and HDL-cholesterol (Fruchart et al. 2001; Schoonjans et al. 1997). PPARβ (also called PPARδ) in turn is involved in wound healing, but a role for it in lipid metabolism has also been proposed (Wang et al. 2003). Upon heterodimerization with retinoid X receptor (RXR) isoforms (γ, α, or β), PPAR isoforms bind to responsive DNA elements and activate the transcription of target genes. Levels of PPARγ, α and β, as well as RXR isoforms have been measured in liver, skeletal muscle, and heart of male GHR−/− mice (Table 52.2.).

PPARγ protein levels were increased in the liver, decreased in skeletal muscle, and unchanged in the heart of 21-month-old GHR−/− mice when compared to controls (Masternak et al. 2005a; Masternak et al. 2005c; Masternak et al. 2006). PPARγ target genes have been described mainly in adipose tissue, where this isoform's main functions are carried out. Therefore, the mechanisms of PPARγ action in liver, muscle, and heart are not well-established. For instance, muscle-specific PPARγ-null mice display whole-body insulin resistance, but the etiology of this condition remains unknown (reviewed by Kintscher and Law 2005). Therefore, the possibility exists that PPARγ has insulin-sensitizing effects that are tissue-specific. In certain tissues, these effects may be less important than those promoted by insulin signaling intermediates. Instead, in other tissues, insulin-sensitizing effects of PPARγ may override those of molecules in the insulin signaling cascade. Certainly,

other unknown factors also could be playing a role to modify insulin responsiveness in a tissue-specific manner. Future research will hopefully unveil these complex mechanisms of regulation.

In this hypothetical scenario, high PPARγ levels in the liver of 21-month-old GHR–/– mice could function to preserve high insulin responsiveness in this organ despite the low activation of insulin signaling (see above). And this would be consistent with high whole-body insulin sensitivity in these mice. Instead, low or unchanged PPARγ levels in skeletal muscle and heart suggest that in GHR–/– mice PPARγ action is less important for insulin sensitivity in these organs (Masternak et al. 2005a; Masternak et al. 2005c).

Protein levels of PPARα were increased in the liver and decreased in skeletal muscle and heart of 21-month-old male GHR–/– mice (Masternak et al. 2005a; Masternak et al. 2005c; Masternak et al. 2006). This suggests alterations in fatty acid oxidation in these tissues. In the liver, high levels of PPARα indicate increased fatty acid usage and may also lead to the low total cholesterol levels observed in GHR–/– mice (Masternak et al. 2005c) and prevent insulin resistance. Interestingly, given that PPARα increases HDL-cholesterol in humans, higher PPARα levels could be linked to the increased cholesterol levels measured in LS individuals (see above). Increased fatty acid oxidation is also consistent with high Foxo1, p-CREB, and p-AMPK levels in GHR–/– liver, as described above. On the other hand, decreased PPARα levels in skeletal muscle and heart of GHR–/– mice may indicate that fatty acid oxidation is low in these organs, which is consistent with high insulin sensitivity (Masternak et al. 2005a; Masternak et al. 2006).

PPARβ protein levels were decreased in the liver and skeletal muscle and increased in the heart of GHR–/– mice (Masternak et al. 2005a; Masternak et al. 2005c; Masternak et al. 2006). Although the role of PPARβ in the heart is not clear, in skeletal muscle and liver decreased levels of this molecule may reduce energy uncoupling and promote lipid accumulation (Masternak et al. 2005a). However, decreased levels of PPARβ in the liver may be counterbalanced by the increased PPARα levels in this organ (Masternak et al. 2005c).

The mRNA levels of RXRγ, α, and β were unchanged in skeletal muscle and increased in heart and liver (except RXRγ, which was unchanged in heart) of GHR–/– mice (Masternak et al. 2005a; Masternak et al. 2005c; Masternak et al. 2006). An increase in the expression of RXR isoforms suggests there is a higher probability of forming heterodimers with PPAR isoforms, which would lead to enhanced activity of PPARs in the liver and heart of GHR–/– mice (Masternak et al. 2005c; Masternak et al. 2006).

Finally, given that many PPARα-regulated genes are negatively controlled by GH through STAT5b (Sugiyama et al. 1994; Zhou and Waxman 1999a, 1999b), several PPARα-regulated genes were measured in the liver of different GH secretion or signaling deficient mice (Stauber et al. 2005). In ~4-month-old male GHR–/– mice and among genes involved in fatty acid catabolism, cytochrome P450 4a (Cyp4a) and thiolase displayed increased expression. Genes involved in stress resistance were mostly not altered (T-complex protein 1-α and 1-β, protein disulfide-isomerase A4 (ERp72), heat shock protein 25 (Hsp25), Hsp60, Hsp65, Hsp84) except for T-complex protein 1-ε, which was increased. Related to inflammation and atherosclerosis, expression of β-fibrinogen was decreased in the liver of GHR–/– mice, suggesting protection against cardiovascular disease (Stauber et al. 2005).

52.6 Effects of Diets on Glucose Homeostasis and Lipid Profiles

Several studies have evaluated the response of GHR–/– mice to caloric restriction, mainly in search of the mechanisms that give rise to the extended lifespan in these mice (see Chap. 50). Effects of caloric restriction have been characterized with respect to the expression of PPAR and RXR isoforms and to components of the insulin signaling cascade in the liver, skeletal muscle, and heart (Al-Regaiey et al. 2007; Bonkowski et al. 2009; Giani et al. 2008; Masternak et al. 2005a; Masternak et al. 2005b; Masternak et al. 2005c; Masternak et al. 2006).

The effects of high-fat feeding have been evaluated as well, and GHR–/– mice gained weight when subjected to the diet (see Chap. 51). The usually low fasting insulin levels observed in GHR–/– mice were increased in 5-month-old male mice after a high-fat diet, but remained significantly lower than those of low fat-fed wild type mice (Berryman et al. 2006). There were also no significant changes in fasting glucose levels between high fat-fed and low fat-fed GHR–/– mice (Berryman et al. 2006). No changes in insulin sensitivity or

nonfasted insulin levels were observed in a similar study of 3.5-month-old male mice (Robertson et al. 2006). In addition, an increase in β-cell mass in the pancreas was observed in GHR–/– mice, similar to that seen in wild type mice, seemingly to compensate the enhanced insulin need after high-fat feeding (Robertson et al. 2006). Thus, GHR–/– mice remain highly sensitive to insulin in spite of gaining weight when fed a high-fat diet.

Finally, the effects of soy-derived diets were tested on 3-month-old male GHR–/– mice, to evaluate their beneficial role in overall health and longevity. A diet containing high isoflavone-soy protein content was shown to increase plasma cholesterol levels to normal in GHR–/– mice, while no change was observed in wild type animals. Also, glucose clearance was markedly enhanced by the diet in GHR–/– mice with no change in controls (Bartke et al. 2004). On the other hand, the high isoflavone diet had no effect on fasting glucose and TG levels in both GHR–/– and wild type mice. Interestingly, a similar diet containing low isoflavone-soy protein decreased plasma TG levels and fasting glucose in GHR–/– and wild type mice. However, the low isoflavone diet did not affect cholesterol levels or glucose tolerance in either of the genotypes (Bartke et al. 2004). Unfortunately, insulin levels were not measured in this study. In conclusion, the data show that glucose and TG levels behaved similarly in GHR–/– and wild type mice. They decreased in low isoflavone diet and showed no change in high isoflavone diets. Instead, cholesterol levels and glucose tolerance increased only in GHR–/– mice under a high isoflavone-soy protein diet. This suggests a genotype-specific impact on insulin sensitivity for the high isoflavone diet.

52.7 Additional Metabolic Parameters

Certain studies have measured the circulating levels of additional molecules in GHR–/– mice, and when compared to LS individuals, some inconsistencies exist. At ~4 months, GHR–/– mice displayed normal levels of calcium, total protein, bilirubin, uric acid, γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), and creatinine, (Liu et al. 2004). They also displayed increased levels of albumin, urea, alanine aminotransferase (ALT/GPT), creatine kinase (CPK), and chloride (Liu et al. 2004). Potassium levels were normal or increased, and sodium levels were normal or decreased when 4–5-month-old GHR–/– mice were studied (Egecioglu et al. 2007; Liu et al. 2004). Along with these results, lower plasma renin and unchanged aldosterone were reported (Egecioglu et al. 2007) (see Chap. 54). LS individuals also display normal sodium, potassium, ALP, creatinine, and GGT (mainly in females) (Chaps. 31 and 34). However, opposite of what was observed in GHR–/– mice, LS individuals have normal levels of chloride, ALT (only a few elevated cases), CPK, and generally elevated GGT in males (Chaps. 31 and 34). Low ALP and creatinine have also been reported in early childhood in LS (Laron 2004),

Also, the thyroid hormones 3',3,5-L-triiodothyronine (T_3) and thyroxine (T_4) were both decreased in ~5-month-old female GHR–/– mice, and the T_3/T_4 ratio was normal, suggesting mild hypothyroidism (Hauck et al. 2001). In LS individuals, however, normal T4 (free and total) were found (Chap. 29).

Expression of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), a glucocorticoid activating enzyme, was elevated in the liver of 3-month-old male GHR–/– mice (Itoh et al. 2004), consistent with circulating corticosterone levels being elevated in these mice (Egecioglu et al. 2005; Hauck et al. 2001) (see above). However, the observed increase did not seem to be sufficient to promote the expression of a glucocorticoid-inducible enzyme in skeletal muscle (Itoh et al. 2004) (see Chap. 53).

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Skeletal Muscle

53

Juan Ding and John J. Kopchick

Core Messages

- Consistent with LS individuals, GHR−/− mice have reduced muscle mass. The animal study on skeletal muscle greatly complements the human data, where studies are limited due to ethnic issues. For instance, GHR−/− mice had reduced muscle fiber size, but not the number of muscle fibers, resulting in reduced muscle mass. Contradictory data on fiber type proportion have been reported. Skeletal muscle IGF-1 mRNA was decreased in GHR−/− mice, but protein level remained the same compared to that in control mice, thus suggesting that autocrine actions of IGF-1 are not sufficient to rescue the sarcopenia phenotype of GHR−/− mice. GH/IGF-1 increased muscle mass independently of androgens and glucocorticords in WT mice. Muscle insulin sensitivity was increased in GHR−/− mice, but some studies found no change in key insulin signaling molecules or even delayed insulin response in GHR−/− muscle.
- Conflicting results may arise because of differences in age, gender, genetic background, muscle groups investigated, and technique differences. To address these issues and elucidate the gender- and age-specific effects of GH in skeletal muscle, it will be important to analyze animals of both genders across greater age spans. Also, it will be necessary to find out if GH exerts differential effects on different muscles. If so, then a representative skeletal muscle or group of muscles may be designated for use by all laboratories, so that legitimate comparisons may be made across studies. Ultimately, a clear mechanism of GH on muscle biology will be helpful in treating LS individuals who suffer from weakened muscle as well as other human diseases involving skeletal muscle such as muscle wasting and muscle atrophy.

53.1 Introduction

Growth hormone (GH) is known to have an anabolic effect on skeletal muscle directly or indirectly via insulin-like growth factor-1 (IGF-1), which is induced by GH in many tissues including skeletal muscle and acts through endocrine, paracrine, and/or autocrine actions. Accordingly, recombinant human GH (rhGH) has been used to increase lean mass in GH-deficient adult patients (Gotherstrom et al. 2009). Moreover, GH abuse among elite athletes has become problematic as they seek to increase muscle mass and enhance athletic performance (Saugy et al. 2006). This has lead to the World Anti-Doping Agency (WADA) listing rhGH as a prohibited substance (WADA 2009).

Laron syndrome (LS) individuals (see Chap. 17) have decreased muscle mass (Laron et al. 2006), reduced muscle force and endurance that significantly improved after IGF-1 treatment, although never

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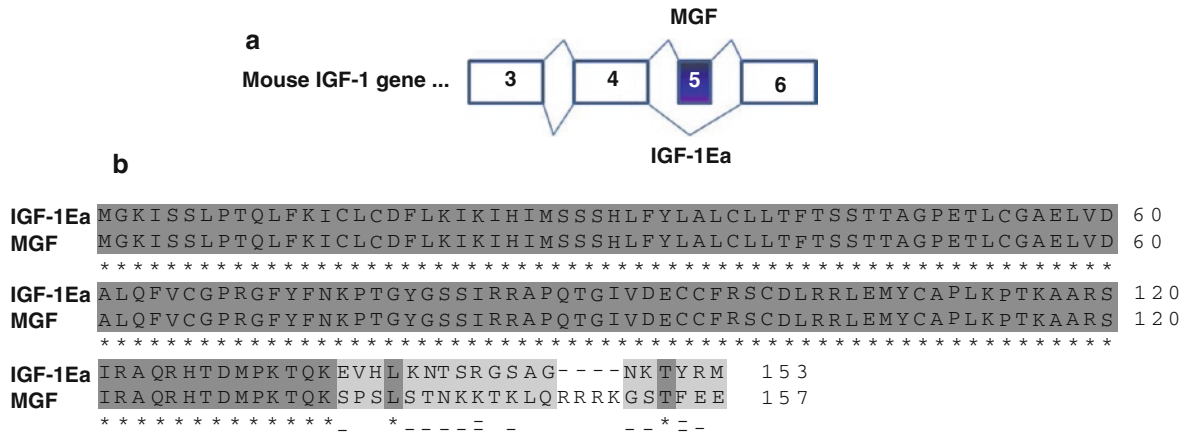

Fig. 53.1 Alternative splicing of IGF-1 precursor mRNA in the mouse. (**a**) Exons 3–6 of IGF-1 gene are shown. IGF-1Ea is transcribed by skipping exon 5. MGF is produced by alternative splicing that includes exon 5. This insert of exon 5 results in subsequent translational frameshift and a different carboxyl terminal sequence of MGF compared to IGF-1Ea (shown in (**b**)). (**b**) Sequence alignment of preproIGF-1Ea and preproMGF. Sequences in *dark gray* are identical between the two isoforms (designated by *asterisk*); sequences in *light gray* are similar amino acids (designated by *dash* or *double dashes*)

achieving as much as GH treatment on GH-deficient patients (Brat et al. 1997), and reduced exercise capacity partly due to peripheral muscle dysfunction (Ben-Dov et al. 2003). However, in order to reveal the underlying mechanism responsible for phenotypic differences in the muscle of LS individuals, animal studies are needed as humans have much greater genetic variations than in-bred mice and that human studies are restricted by ethics and usually only involve noninvasive procedures. Studies using hypophysectomized rodents have reported conflicting results, likely due to elimination of other pituitary hormones by hypophysectomy. Preservation of these hormones in GHR–/– mice allows examination of the specific effects of GH/IGF-1 on skeletal muscle. In this chapter, skeletal muscle research using GHR–/– mice will be discussed. The muscle phenotype of GHR–/– mice including fiber type, fiber size, and cell number will be summarized in this review. As GHR–/– mice are also insulin-sensitive and long-lived, insulin signaling has been studied in several target tissues including skeletal muscle. Therefore, this review also summarizes studies on insulin sensitivity in the muscle of GHR–/– mice.

53.2 IGF-1 Production in Skeletal Muscle of GHR–/– Mice

GH induces IGF-1 expression in many tissues, including skeletal muscle, with ~70% of circulating IGF-1 derived from the liver. Because of complete insensitivity to GH, GHR–/– mice have significantly reduced circulating IGF-1 levels. In mouse skeletal muscle, there are two IGF-1 mRNA isoforms (Fig. 53.1), IGF-1Ea – the same one expressed by liver – and mechano growth factor (MGF)– which is derived from alternative splicing of the IGF-1 gene. MGF was originally discovered to be markedly increased after mechanical stimulation (McKoy et al. 1999). MGF mRNA has a 52 nucleotide insert that leads to a translational frameshift and results in a different carboxyl terminal sequence compared to IGF-1Ea. MGF was more effective than IGF-1Ea in promoting motorneuron survival and activating muscle satellite cells when the cDNA of both isoforms was injected into the facial muscles of adult rats subjected to facial nerve damage (Aperghis et al. 2004). At the mRNA level, expression of both IGF-1 isoforms was found to be reduced in 3-month-old male GHR–/– mice (Iida et al. 2004). In addition, total IGF-1 mRNA was found to be decreased in 10-week-old male GHR–/– mice (Venken et al. 2007). This suggests that muscle IGF-1 expression at the mRNA level relies to a large extent on an intact GH signaling. However, it does not appear that protein expression levels of IGF-1 in skeletal muscle of 4-month-old female GHR–/– mice agree with mRNA data (Kraemer et al. 2009). Analysis of plantaris, soleus, and gastrocnemius muscles from GHR–/– mice and littermate controls showed similar protein levels of IGF-1. Unfortunately, no distinction between the two isoforms was made. While it is not known why

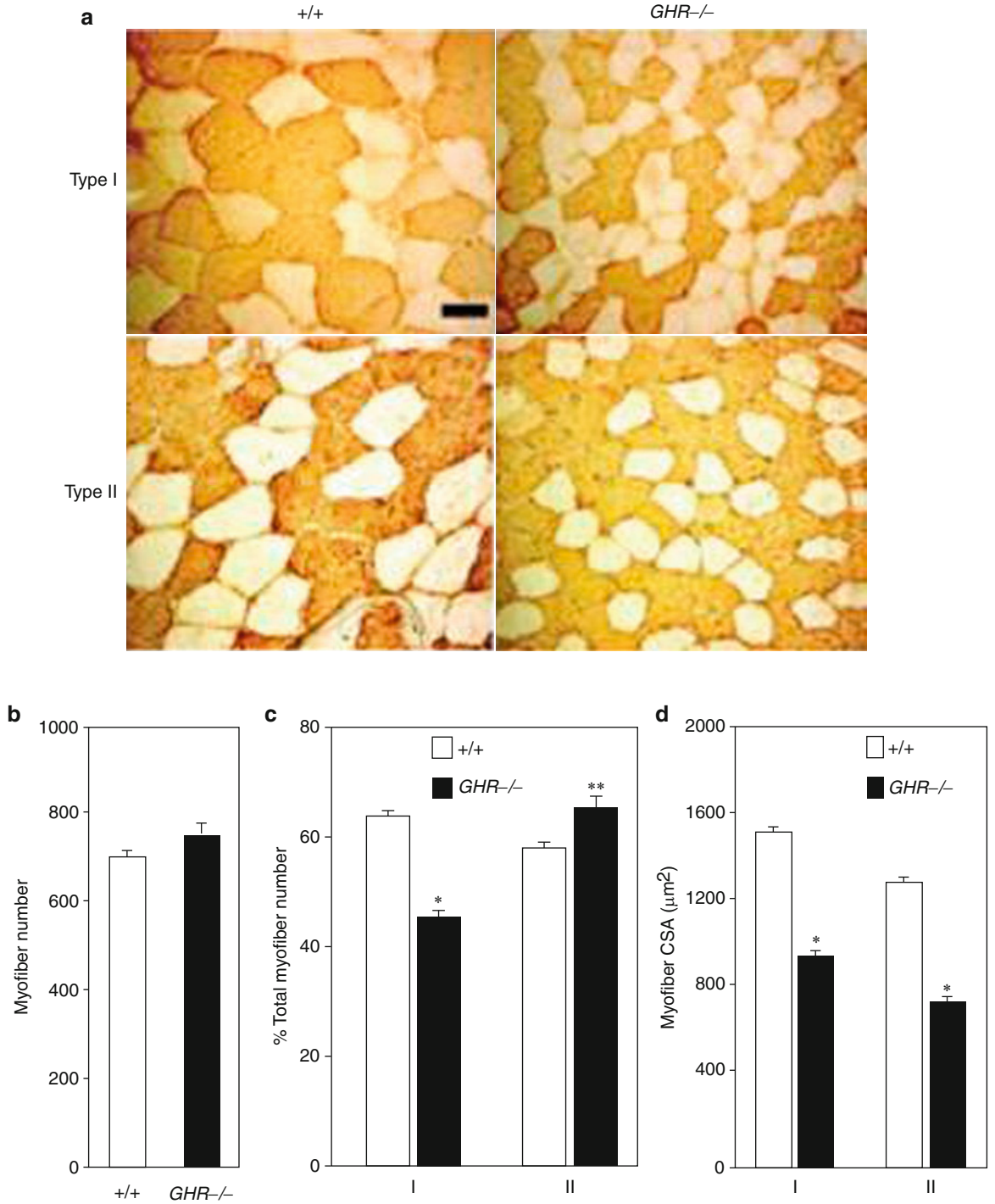

Fig. 53.2 Muscle phenotype of GHR−/− mice. (**a**) Representation of wild type (+/+) and GHR−/− soleus immunostained with antitype I and antitype II MyHC antibodies. (Scale bar: 50 μm in the *upper left*.) (**b**) No difference in myofiber number of +/+ and GHR−/− soleus. (**c**) Fiber type proportion in +/+ and GHR−/− soleus. GHR−/− had fiber type switched to more type II fibers (slow fibers). (**d**) The cross-section area (CSA) of myofibers in soleus muscle. Data are presented as means± standard error of the mean ($n=4$). *Single asterisk*, $p<0.005$; *double asterisk*, $p<0.05$ comparing to +/+. (Reproduced with permission from (Sotiropoulos et al. 2006)) (Copyright (2006) National Academy of Sciences, U.S.A)

GHR–/– female mice had similar level of IGF-1 protein as the controls, the similarity does suggest that IGF-1 in these muscle groups does not strongly rely on GH stimulation in female mice and that a basal level of IGF-1 production is present in both genotypes. More studies involving both genders and multiple ages are needed to study the gender- and age-specific effects of GH on IGF-1 in skeletal muscle. It is interesting that in healthy young male human subjects, 2-week administration of rhGH (0.075 IU/kg/day) did not change muscle IGF-1 mRNA expression, although circulating IGF-1 did increase threefold (Aperghis et al. 2009). These results also support those observed in female GHR–/– mice in that muscle IGF-1 production may be less dependent on GH. In humans, other factors such as nutritional state and/or basal production may be important in this tissue.

53.3 Skeletal Muscle Phenotype

Skeletal muscle is composed of multinucleated myofibers (muscle cells, also called myocytes), which undergo hypertrophy through expanding cell volume as well as fusing with mononucleated satellite cells, which are differentiated from myoblasts, the muscle stem cells. Satellite cells are located between the myofiber cell membrane and the basal lamina and are normally dormant. Once activated, satellite cells proliferate, differentiate, and fuse with existing multinucleated myofibers to increase myofiber size.

GHR–/– mice are known to have decreased absolute muscle mass (Zhou et al. 1997; Coschigano et al. 2003). What is the basis of this muscle reduction? It seems to be the result of reduced size of the muscle cell, rather than cell number. Sotiropoulos et al. (2006) showed that 2-month-old GHR–/– mice (both male and female) had the same number of myofibers, but that myofibers were smaller in GHR–/– mice (Fig. 53.2a, b, d). In vitro experiments revealed that in limb muscles cells of 4-week-old WT mice, GH exposure (or treatment) increased myofiber size independently of IGF-1 by promoting fusion of satellite-cell-type myoblasts to nascent myotubes (formed by initial fusion of myoblasts themselves), whereas no effect was observed in muscle cells derived from GHR–/– mice (Sotiropoulos et al. 2006). In addition, GH had no effect on the size, proliferation, or differentiation of myoblast precursor cells from WT mice, indicating that the effect of GH on muscle is at the level of cell fusion rather than hypertrophy or hyperplasia of myoblast precursor cells.

Skeletal muscle fibers are divided into two major types: I and II fibers. Type I or slow twitch fibers produce relatively lower force over a long time using energy generated primarily from aerobic respiration. In contrast, type II or fast twitch fibers produce relatively higher force over a short time duration using energy generated primarily via glycolysis.

Muscle fiber type was assessed in GHR –/– mice in two separate studies (Sotiropoulos et al. 2006; Schuenke et al. 2008). Data from these studies are summarized in Table 53.1. Sotiropoulos et al. found that 2-month-old GHR–/– mice (both male and female) had a fiber type switch from type I (fast) to type II (slow) (Fig. 53.2c). However, the other study by Schuenke et al. using 4-month-old female mice revealed that GHR–/– mice had smaller muscle fibers, but unchanged proportions of fiber types. The difference may be due to the difference in the strain, age, and gender of the animals used.

53.4 Interaction of Androgen and GH on Skeletal Muscle Hypertrophy

Androgens are essential for an increase of muscle mass during puberty and subsequent maintenance of muscle mass in male adulthood. It is therefore of interest to

Table 53.1 Comparison of muscle fiber size and type between GHR–/– mice and controls

Genetic background	Age	Gender	Muscle	Fiber size	Fiber type
Sv129Ola (Sotiropoulos et al. 2006)	8 weeks	Both	Soleus, tibialis anterior	↓	More slow fiber
C57BL/6J (Schuenke et al. 2008)	16 weeks	Females	Plantaris soleus gastrocnemius	↓	Unchanged fiber type proportion

evaluate whether there is an interaction of androgens with the GH/IGF-1 axis regarding muscle size. To address this question, dihydrotestosterone (DHT) or testosterone (T) was administered to orchidectomized (testicles removed) GHR−/− and wild type mice during late puberty. Both treatments stimulated muscle mass equally in GHR−/− and control mice, without affecting muscle IGF-1 mRNA expression (Venken et al. 2007). Since no difference in muscle mass stimulation occurred following testosterone treatment regardless of GH action, this study demonstrated that androgens and GH/IGF-1 stimulate muscle growth via independent mechanisms.

Table 53.2 Insulin signaling activity in the skeletal muscle of GHR−/− mice

Genetic background	Age	Gender	Muscle used	Insulin signaling molecules in the muscle	
				mRNA	Protein
C57BL/6J (Masternak et al. 2005b)	21 months	Male	Hind limb muscle	IR ↔ IRS1 ↔ IRS2 ↔ GLUT4 ↔	
C57BL/6J (Masternak et al. 2005a)	21 months	Male	Hind limb muscle	PPARγ ↔ RXRγ ↔	PPARγ ↓
C57BL/6J (Al-Regaiey et al. 2007)	21 months	Male	Hind limb muscle	AKT2 ↔ PKCζ ↔ PGC-1α ↔ Foxo1 ↔ Foxo3 ↔	AKT2 ↑ pAKT2 ↔ pAMPK ↔ pPKCγ/ζ ↓ pJNK1 ↓ pJNK2 ↔ PGC-1α ↔ Foxo1 ↓ Foxo3 ↓
129/Ola-Balb/c-C57BL/6 (Robertson et al. 2006)	6 months	Not specified	Soleus and gastrocnemius		p-IR ↔, delayed in response to insulin; p-IRS-1↔, delayed in response to insulin; p85 (associated with IRS-1) ↔
C57BL/6J (Bonkowski et al. 2009)	14 months	Male	Hind limb muscle		IR ↑ p-IR ↔ IRS1 ↔ pS IRS1 ↓ p85/pY99 (associated with IRS-1) ↑ AKT1 ↑ p-AKT1 ↑ AKT2 ↑ p-AKT2 ↑ GLUT4 ↑ mTOR ↓ p-mTOR ↓

↑ increased; ↓ decreased; ↔ unchanged

IR insulin receptor; *IRS* insulin receptor substrate; *GLUT4* glucose transporter-4; *PI3K* phosphatidylinositol 3-kinase (downstream of and activated by IRS-1; comprised of two subunits- p85 and p110. p85 is the regulatory subunit and p110 the catalytic subunit. Excess p85 causes insulin resistance); *AKT* (also called protein kinase B (PKB), a serine-threonine kinase that is activated by PI3K); *PGC* peroxisome proliferator-activated receptor-γ coactivator (transcription coactivator that promotes insulin sensitivity); *PPARγ* peroxisome proliferator-activated receptor γ (ligand-dependent transcription factor, increases insulin sensitivity); *RXRγ* retinoid X receptor γ (form heterodimers with PPAR γ for transcriptional activation of target genes); *AMPK* adenosine monophosphate-activated protein kinase (activated to favor ATP generation; increase glucose uptake and fatty acid oxidation in muscle); *PKC* protein kinase C (downstream of and activated by PI3K, required for insulin-stimulated glucose transport; also a negative regulator of insulin signaling cascade); *JNK* c-Jun N-terminal kinase (promote insulin resistance by serine-phosphorylating IRS-1); *mTOR* mammalian target of rampamycin (serine/threonine kinase, negative regulator of insulin signaling cascade); *Foxo* fork head box protein o (transcription factor, negative regulator of glucose uptake in muscle)

53.5 Interaction of Glucocorticoids and GH on Skeletal Muscle Atrophy

Glucocorticoids (GCs) are known to induce muscle loss in a process called steroid myopathy (Boumpas et al. 1993). In rats, this process has been shown to be prevented by GH and/or IGF-1 treatment (Matsushita et al. 1996). The enzyme that catalyzes the conversion of inert cortisone and 11-dehydrocorticosterone into the active GCs (cortisol and corticosterone), 11 beta-hydroxysteroid dehydrogenase 1 (11βHSD1), is decreased by GH replacement therapy in adult hypopituitary patients (Weaver et al. 1994). However, in skeletal muscle of 3-month-old male GHR−/− mice, the mRNA of neither 11βHSD1 nor glutamine synthetase (a GC-inducible enzyme of glutamine synthesis) was found to be significantly different from those of the wild type mice (Itoh et al. 2004). Therefore, it appears that muscle GCs do not contribute significantly to the muscle atrophy observed in GHR−/− mice.

53.6 Insulin Signaling in GHR−/− Skeletal Muscle

GHR−/− mice are more insulin sensitive than wild type mice. Given the insulin-antagonistic action of GH and that most of the insulin-sensitive tissues express GHR, it is not difficult to imagine that GH will dampen insulin signaling in insulin-responsive organs such as liver, fat, and muscle. There have been several studies on insulin signaling in the skeletal muscle of GHR−/− mice, as summarized in Table 53.2 (see Fig. 3 in appendix for insulin signaling pathway). In skeletal muscle of 21-month-old GHR−/− mice, mRNA levels of insulin receptor (IR), insulin receptor substrate-1 (IRS-1), IRS-2, and glucose transporter-4 (GLUT4) were the same as controls (Masternak et al. 2005b), but protein levels of phophorylated (p) AKT2 were increased, and negative regulators of insulin signaling such as phosphorylated protein kinase Cζ (PKC Cζ) and c-Jun N-terminal kinase 1 (JNK1), as well as Foxo 1 and 3, were decreased (Al-Regaiey et al. 2007), which indicated increased insulin sensitivity in the muscle of GHR−/− mice. Moreover, in 14-month-old GHR−/− mice, there were increased basal protein levels of IR, AKT1, AKT2, and GLUT4 compared to control mice. Three minutes after insulin stimulation, these mice also exhibited increased levels of phosphorylated AKT1, phosphorylated AKT2, as well as reduced levels of inhibitory molecules such as serine phosphorylated IRS-1, mammalian target of rampamycin (mTOR) protein, and activated form p-mTOR comparing to controls, supporting a model of increased insulin sensitivity in the muscle of GHR−/− mice (Bonkowski et al. 2009).

However, a report by Robertson et al. showed that 6-month-old GHR−/− mice had delayed insulin response in the muscle (Robertson et al. 2006). Phosphorylation of IR and IRS-1 in the muscle was lower than controls 5 min after insulin stimulation and only reached the control level after 15 min (Robertson et al. 2006). The authors argued that IGF-1 might be more important in insulin signaling in the muscle and that the diminished level of IGF-1 in the GHR−/− mice was responsible for the delay in response to insulin. One note is that they used 6-month-old 129/Ola-Balb/c-C57BL/6 mice instead of 14-month-old C57BL/6 mice as used by Bonkowski et al. This discrepancy in mouse age and strain may be responsible for the inconsistency regarding insulin sensitivity in GHR−/− skeletal muscle.

Another contradictory piece of data was reduced peroxisome proliferator-activated receptor γ (PPARγ), an insulin sensitizer, in GHR−/− muscle (Masternak et al. 2005a). The authors argued that whole-body insulin sensitivity in the GHR−/− mice might be regulated independently of muscle PPARγ. Indeed, the whole-body insulin sensitivity (as defined by serum insulin and glucose levels) observed in GHR−/− mice may not depend solely on several known components of insulin signaling cascade, instead may be controlled by other factors involved in glucose homeostasis at much more complicated levels yet unknown to humans.

Regarding fat metabolism in skeletal muscle of GHR−/− mice compared to controls, 21-month-old males had equal levels of free fatty acids and triglycerides, as well as equal levels of mRNA expressions of PPAR α and β/δ, and retinoid X receptor α and β/δ (Masternak et al. 2005a). However, the protein levels of PPAR α and β/δ were decreased in GHR−/− mice, suggesting reduced fatty acid metabolism in the muscle of these mice (Masternak et al. 2005a) (also see Chap. 52).

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Cardiac Function in GHR–/– Mice

54

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Core Message

› The studies discussed in this chapter highlighted the role of GH signaling in heart function, cardiovascular disease, and glucose homeostasis. Interestingly, GHR–/– mice do not present major cardiovascular abnormalities, and their overall cardiovascular risk seems to be diminished, independently of their age and/or gender. Thus, the extended longevity of GHR–/– mice may be due in part to their improved cardiovascular health and increased insulin sensitivity. Although important aspects of GH signaling and the cardiovascular system have been revealed, the underlying mechanisms linking GH insensitivity and cardiovascular health/disease need to be investigated further. In a recent study, the effects of GH in coagulation or thrombosis were evaluated in a model of pulmonary embolism. Expression of coagulation inhibitors was strongly modulated by sex-specific GH secretion patterns (Wong et al. 2008).This study highlights the significance of GH on thrombosis in vivo and raises interesting questions. What are the specific effects of reduced IGF-1 vs. increased GH on thrombotic events? Thus, the thrombotic evaluation of GHR–/– mice will aid in redefining the role of GH/IGF-1 in thrombosis-related diseases.

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54.1 Introduction

Growth hormone (GH) is primarily known as a regulator of growth and development; however, its physiological role extends beyond growth regulation. GH plays an important role in determining body composition and affects muscle strength, bone mineral density, reproductive parameters, and metabolic functions (de Boer et al. 1995; Drake et al. 2001; Zofkova 2003; Maison et al. 2004) (for details see Chaps. 51, 52, 53, 55, 58).

GH has also been implicated in the development, structure, and function of the cardiovascular system (Colao et al. 2005; Colao et al. 2006).The existence of a relationship between acromegaly and the cardiovascular system was described at the end of the nineteenth century by H. Huchard in the "Anatomie pathologique, lesions et trouble cardiovasculaires de l'acromegalie" (Huchard 1895). Huchard postulated for the first time that enlargement of the heart and increased atherosclerotic plaque accumulation occurred as a consequence of acromegaly (Acierno 1994). Acromegalic cardiomyopathy is characterized by ventricular hypertrophy, interstitial fibrosis, and extracellular collagen deposition associated with myofibrillar derangement, lympho-mononuclear cell infiltration, and areas of monocyte necrosis. Left ventricle hypertrophy is the most widely observed abnormality in acromegaly; however, the right ventricle can be equally involved (Colao et al. 2001). In addition, acromegalic patients present a more atherogenic lipoprotein profile, characterized by augmented triglyceride levels in plasma (Boero et al. 2009). Increased prevalence of cardiovascular disease is also associated with long-standing GH deficiency (GHD). Thus, cardiac abnormalities are associated with both GH excess and deficiency. Altered lipid profiles, hypercoagulability, atherosclerosis,

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decreased muscle performance, endothelial dysfunction, and reduced cardiac dimension are negative factors associated with GHD (Brickman and Silverman 2000; Colao et al. 2001; McCallum et al. 2002). Decreased thickness of the left ventricle posterior wall and the interventricular septum is among the most common morphological abnormalities observed in patients with childhood-onset GHD (Colao et al. 2006). Also, endothelial cell dysfunction associated with decreased production of systemic nitric oxide (NO) is a hallmark feature in GHD (McCallum et al. 2002). Decreased NO activity leads to impaired blood flow, vasoconstriction, thrombosis, and inflammation (Ueno et al. 2000). Therefore, the morphological cardiac abnormalities, along with the systemic vascular impairments observed in GHD, compromise cardiac function and lead to increased risk of cardiovascular disease.

Although the relationship between GH excess/deficiency and the cardiovascular system has been described using genetic mouse models of acromegaly (Bollano et al. 2000; Bogazzi et al. 2008) and GHD (Ren and Brown-Borg 2002), the role of an intact GH/IGF-1 axis in the development of normal cardiovascular structure and function is still not well-established. Interestingly, LS individuals appear to have normal cardiac function, despite the occurrence of reduced cardiac dimensions, with reductions in left ventricular volume and mass, and diminished stroke volume and cardiac output (Feinberg et al. 2000). The GHR−/− mouse is a suitable model for human LS, since homozygous GHR−/− mice accurately resemble the phenotype typical of individuals with Laron syndrome: severe growth retardation, proportionate dwarfism, absence of the growth hormone receptor and GH binding protein, decreased serum IGF-1, and elevated serum GH concentrations (Zhou et al. 1997; Coschigano et al. 2000).Therefore, GHR−/− mice provide an excellent model to study the impact of a disrupted GH/IGF-1 axis in cardiac structure, development, and maintenance.

This chapter will review some of the most recent experimental evidence of the role of GH on cardiac morphology and function, cardiovascular disease, and future work to define the mechanisms of action of GH on the vascular system in the context of the GHR−/− mouse.

54.2 Heart Structure and Physiology

Indications of possible cardiac dysfunction in GHR−/− mice were noted by their reduced cardiac weigh and volume (Coschigano et al. 2003; Egecioglu et al. 2007; Izzard et al. 2009), which correlated with clinical observations on heart morphology in LS individuals (Feinberg et al. 2000). Relative left ventricle (LV) weight was found to be lower in GHR−/− mice compared to wild type (WT) controls; however, no difference existed in the relative weight of the right ventricle (RV) between genotypes. Interestingly, echocardiography measurements revealed that GHR−/− mice were able to produce a cardiac output (CO) to meet their oxygen supply needs (Table 54.1). Heart rates (HR) were not significantly different between groups (Table 54.1) (Egecioglu et al. 2007). In contrast, LS individuals present a significantly lower cardiac output than control subjects (Feinberg et al. 2000), which are consistent with the reduced cardiac dimensions.

Table 54.1 Body weight and ECG measurements (Egecioglu et al. 2007)

	WT	GHR−/−
Background	Sv129Ola-Balb/c	Sv129Ola-Balb/c
Sex	Females	Females
BW g	36 ± 3	17 ± 1*
HR beats/min	404 ± 55	332 ± 31
Left ventricular (LVDd) mm/10 g BW	0.98 ± 0.04	1.47 ± 0.11*
LVDs mm/10 g BW	0.38 ± 0.02	0.67 ± 0.05*
PWth mm/10 g BW	0.28 ± 0.01	0.44 ± 0.01*
RWth	0.57 ± 0.02	0.62 ± 0.05
VCf circ/s	10.03 ± 0.97	7.63 ± 0.57*
FS %	61 ± 2	55 ± 2*
E Wave cm/s	0.60 ± 0.05	0.51 ± 0.03
A Wave cm/s	0.39 ± 0.09	0.30 ± 0.04
E/A	1.75 ± 0.26	1.80 ± 0.14

BW body weigh; *HR* heart rate; *LVDd* LV diameter at diastole; *LVDs* LV diameter at systole; *PWth* posterior wall thickness; *RWth* relative wall thickness; *Vcf* velocity of circumferential shortening; *FS* fractional shortenings; *E/A* diastole/late atrial contraction ratio. This table was modified from Egecioglu et al. (2007)
*P value < 0.05

On the other hand, the LV posterior wall thickness (PWth) of GHR−/− mice was decreased by 25% compared to WT mice, which correlated with a significant reduction in LV diameter at diastole (LVDd) (Table 54.1); however, the relative wall thickness (RWth), calculated as the ratio of 2× posterior wall thickness/end-diastolic diameter, was similar in GHR−/− mice and WT controls, indicating no relevant difference between genotypes (Table 54.1). In addition, the average early diastole/late atrial contraction (E/A) ratio that characterizes LV filling was not significantly altered, indicating no difference in diastolic function between groups (Table 54.1) (Egecioglu et al. 2007). In contrast, echocardiographic data of LS individuals showed that LV septum, posterior wall, and end-diastolic diameter are significantly reduced compared with the control group (Feinberg et al. 2000). However, in agreement with data from the GHR−/− mouse, it was also reported that there was no major difference in E/A ratio between LS individuals and control subjects (Feinberg et al. 2000).

Echocardiography measurements also showed that GHR−/− mice present significant reductions in LV diameter at systole (Table 54.1), which correlated with decreases in fractional shortening (FS) and velocity of circumferential shortening (Vcf) (Table 54.1), indicating decreased systolic cardiac function. These findings correlated with the reduced systolic blood pressure observed in female GHR−/− mice at 4 months (Egecioglu et al. 2007) and in 9-month-old male GHR−/− mice (Izzard et al. 2009). Blood pressure measurements indicated that the systolic blood pressure was decreased by 25% in female GHR−/− mice (4 months) compared to WT controls (Egecioglu et al. 2007). In contrast, the systolic and diastolic functions in LS individuals are intact, and their cardiac response to stress is normal (Feinberg et al. 2000). The decreased systolic blood pressure observed in this mouse model may be explained by the lower levels of renin and increased levels of K^+ in plasma of GHR−/− mice; however, the aldosterone plasma levels were unaltered in 4-month-old female GHR−/− mice compared to WT controls (Egecioglu et al. 2007). Thus, the specific mechanisms underlying reduced systolic blood pressure are unclear. It is hypothesized that the upregulation in endothelial NO synthase (eNOs) expression by large vessels may contribute to this effect (Egecioglu et al. 2007).

Studies have shown that eNOs expression contributes to maintain vasodilator tone pressure by inducing the release of NO in response to stress (Rees et al. 1996). Thus, augmentation in NO production may lead to increased vasodilation. Interestingly, increased systemic NO production has been observed in GH-deficient patients, suggesting impaired endothelial cell function and hypotension (Boger et al. 1996; McCallum et al. 2002). Real time PCR (RT-PCR) studies revealed that female GHR−/− mice showed significant increase in aortic eNOs mRNA compared to WT mice (Egecioglu et al. 2007). Upregulation of eNOS expression may be associated with increased systemic production of NO. This may explain the reduced systolic blood pressure observed in GHR−/− mice; however, further experiments are needed for confirmation. It is worthwhile to point out that despite the increase in aortic eNOs expression, endothelial cell function is not compromised in GHR−/− mice. Functional studies employing norepinephrine and acetylcholine revealed that GHR−/− mice present an intact endothelial function in blood vessels with a normal contractile response independently of their gender and age (Egecioglu et al. 2007; Izzard et al. 2009).

Overall, these findings suggest that although GHR−/− mice present morphological changes in heart and vasculature, these abnormalities do not seem to compromise the animals' life span suggesting that compensatory mechanisms may be activated to overcome these negative physiological effects on the cardiovascular system.

54.3 The Impact of GH Resistant in Cardiovascular Health in GHR−/− Mice

54.3.1 Cardiovascular Risk Factors in GHR−/−

Impaired cardiovascular function is associated with both GH excess and deficiency. In acromegaly, cardiovascular and metabolic impairments contribute to significantly enhanced mortality. The high cardiovascular risk associated with excess of GH, and IGF-1 is attributed to the increased incidence of diabetes, hypertension, and lipid disorders in acromegalic patients (Colao et al. 2004). A recent study showed

that acromegalic patients present a more atherogenic lipid and lipoprotein profile, alteration in the carbohydrate metabolism such as insulin resistance, high levels of endothelin-1 (the most potent vasoconstrictor of human vessels), and increased numbers of CD49d positive lymphocytes, which are the inflammatory cells typically associated with atherosclerotic processes observed in acromegaly (Boero et al. 2009). On the other hand, cardiovascular alterations have also been reported in adult patients with GHD: lipid profile abnormalities, visceral adiposity, glucose intolerance, insulin resistance, hypertension, and cardiac abnormalities (Colao et al. 2006).

Obesity is an important risk factor for cardiovascular disease. One important phenotypical characteristic of LS individuals is truncal obesity characterized by increased subcutaneous and visceral fat. Related to obesity, LS individuals present altered lipid profiles (hyperlipidemia) and insulin resistance (Laron et al. 2008). GHR−/− mice present similar phenotypical characteristics to those observed in LS individuals: reduced body size accompanied by an elevated percentage of adiposity, mainly subcutaneous (Berryman et al. 2004); high serum levels of GH; and very low levels of IGF-1 (Zhou et al. 1997; Coschigano et al. 2000). One important difference is that GHR−/− mice showed increased insulin sensitivity. Interestingly, GHR−/− mice show decreased levels of glucose between 10 days and 5 months old of age, independently of gender (Dominici et al. 2000; Liu et al. 2004; Berryman et al. 2006; Egecioglu et al. 2006); however, no differences in glucose levels were observed at older ages (10–21 months) in male mice (Coschigano et al. 2003; Al-Regaiey et al. 2005; Bonkowski et al. 2009). In contrast, insulin levels remained lower than controls independently of gender (Dominici et al. 2000; Coschigano et al. 2003; Liu et al. 2004; Al-Regaiey et al. 2005; Berryman et al. 2006; Egecioglu et al. 2006; Bonkowski et al. 2009), although a slight increase in insulin levels was observed as these mice aged (Coschigano et al. 2003).Thus, these findings indicate that insulin sensitivity is decreased as a function of time in GHR−/− mice, while still remaining significantly higher than that of WT animals (see Chap. 52). Blood chemistry studies showed that total cholesterol levels, HDL-cholesterol, LDL-cholesterol, triglycerides, and Apolipoprotein B serum levels were decreased in male mice of various ages (Bartke et al. 2004; Egecioglu et al. 2005; Masternak et al. 2005), or tended to be decreased in a group of ~4-month-old males and

Table 54.2 Insulin signaling cascade in the heart

	mRNA	Protein
IR	↔ At 3 and 21 months ♂ (Masternak et al. 2006)	↑ At 14 months pY ↑ at 14 months pY/Total ↔ at 14 months (Giani et al. 2008)
IRS-1	↑ At 3 months, and ↔ at 21 months ♂ (Masternak et al. 2006)	↑ At 14 months pY ↑ at 14 months pY/Total ↔ at 14 months (Giani et al. 2008)
IRS-2	↑ At 3 months, and ↔ at 21 months ♂ (Masternak et al. 2006)	
p85 subunit of PI3K		↔ At 14 months ↑ Associated to IRS-1/Total IRS-1 at 14 months (Giani et al. 2008)
Akt/PKB		↑ At 14 months p ↑ At 14 months p/Total ↔ at 14 months (Giani et al. 2008)
GLUT-1		↔ At 14 months (Giani et al. 2008)
GLUT-4	↔ At 3 and 21 months ♂ (Masternak et al. 2006)	↓ At 14 months (Giani et al. 2008) and 21 months (♂) (Masternak et al. 2006)

IR insulin receptor; *IR* insulin-receptor substrate; *PI3K* phosphatidylinositol-3 kinase; *Akt/PKB* protein kinase B; *GLUT-1* glucose transport 1; *GLUT-4* glucose transport 4; *p* phosphorylation; *pY* tyrosine phosphorylation; *p/Total* ratio of phosphorylation to total protein content; *pY/Total* ratio of tyrosine phosphorylation to total protein content
Increase ↑; no change ↔; Gender, male ♂

females (Liu et al. 2004) in GHR−/− mice as compared to WT; however, free fatty acid levels in plasma were similar between 21-month-old male GHR−/− and WT (Masternak et al. 2005) (see Chap. 52). The improved lipid profile observed in GHR−/− mice correlates with their reduced glucose levels, increased insulin sensitivity, delayed aging, and extended life span.

54.3.2 Insulin Signaling in the Heart of GHR−/− Mice

One important aspect to explore is the impact of insulin sensitivity on cardiovascular health. A recent study showed that heart tissue of 14-month-GHR−/− mice presented elevated protein levels of IR and IRS-1(Table 54.2) (Giani et al. 2008); however, while both IRS-1 and IRS-2 mRNA expression were elevated in hearts of 3-month-old male GHR−/− mice, no differences in IR mRNA levels were found at the same age (Table 54.2) (Masternak et al. 2006). In addition as compared to WT controls, GHR−/− mice also exhibited increased tyrosine phosphorylation of IR and IRS-1 (Table 54.2) (Giani et al. 2008). These findings correlate with the state of insulin sensitivity exhibited by GHR−/− mice. Upregulation of IR and IRS-1 protein levels may be a consequence of the reduced insulin levels exhibited by GHR−/− mice. In order to compensate the otherwise reduced response to insulin, over-expressed IR and IRS-1 may lead to increased activation of downstream molecules. Although GHR−/− mice presented nonsignificant changes in heart p85 protein levels in response to insulin, a significant increase in the association of IRS-1 with p85 was observed (Table 54.2) (Giani et al. 2008). In addition, GHR−/− mice exhibited an increased expression and increased phosphorylation of Akt/PKB as compared to WT controls (Table 54.2) (Giani et al. 2008). These findings suggest increased translocation of GLUT-4 to the cell membrane. However, male GHR−/− mice (at 3 and 21 months) presented no significant changes in GLUT-4 mRNA levels in heart (Table 54.2) (Masternak et al. 2006). Moreover, GLUT-4 protein levels were significantly decreased in heart tissue of 14- and 21-month-old GHR−/− mice as compared to WT controls (Table 54.2) (Masternak et al. 2006; Giani et al. 2008). In contrast, GLUT-1 protein levels were unchanged in heart tissue of 14-month-old GHR−/− mice (Table 54.2) (Giani et al. 2008). Studies have shown that heart muscle expresses decreased amount of GLUT-4 as compared to GLUT-1 (Brownsey et al. 1997); however, glucose uptake by cardiac myocytes in response to insulin is mainly mediated by GLUT-4 (Slot et al. 1991). Furthermore, recent evidence obtained by the generation of mice with cardiac-selective GLUT-4 deletion indicated that GLUT-1 mediates glucose uptake in basal conditions in fed mice, but the major mechanism by which the heart uptakes glucose in response to ischemia is GLUT-4-mediated (Tian and Abel 2001). Since cardiac GLUT-4 expression is downregulated in diabetic rat hearts (Slieker et al. 1992), these results suggest that the upregulation of GLUT-4 may play a cardioprotective role against carbohydrate metabolism imbalances. Moreover, two studies have shown that GLUT-4 overexpression leads to a positive increase in heart glucose uptake in diabetic mice (db/db), preventing diabetes-induced cardiopathy (Belke et al. 2000; Belke et al. 2001). Thus, the decreased expression of GLUT-4 in the heart of GHR−/− is contradictory, since these mice are hypersensitive to insulin. A possible explanation is that lower levels of GLUT-4 correspond to a compensatory effect to the otherwise exacerbated response to insulin due to elevated expression, production, and activation of IR, IRS-1, and Akt (for details on insulin signaling cascade see Appendix Fig. 3). In the context of GHR−/− mice, lower levels of GLUT-4 may favor normal heart function, preventing excessive glucose uptake which may lead to glycogen storage disorders (Wolf et al. 2008).

The activation of the MAPK signaling cascade is critical for cardiac myocyte differentiation, growth, and response to stress. Deletion of cardiac ERK1/2 leads to heart failure in response to pressure overload induced by transverse aortic constriction and exercise (Purcell et al. 2007). Decreased MAPK downstream signaling is also associated with hyperglycemia and insulin resistance (Zhang et al. 2002). Hearts of 14-month-old male GHR−/− mice display a large increase in ERK1/2 phosphorylation compared to WT controls, whereas protein levels remain similar (Table 54.2). This finding suggests that increased ERK1/2 activity may reflect a cardioprotective mechanism against increased insulin signaling by stimulating cardiomyocyte growth and remodeling rather than glucose uptake (Giani et al. 2008).

In conclusion, the evaluation of echocardiography measurements and vascular function showed not major abnormalities in the cardiac function of GHR−/− mice, despite their reduced heart dimensions and decreased systolic blood pressure (Egecioglu et al. 2007). Another

interesting aspect is that despite their obesity, GHR−/− mice do not suffer from some abnormalities associated with increased cardiovascular risk. GHR−/− mice are insulin sensitive and display better lipid profiles than WT (Bartke et al. 2004; Liu et al. 2004; Egecioglu et al. 2005; Masternak et al. 2005). Analysis of heart tissues of GHR−/− mice showed increased phosphorylation and protein levels of IR and IRS-1, enhanced association of IRS-1 with p85 and upregulation of Akt/PKB signaling. These findings correlate with GHR−/− mice hypersensitivity to insulin. In contrast, GLUT-4 levels were significantly decreased in GHR−/− heart tissues, as a compensatory effect to favor heart health by preventing excessive glucose uptake (Giani et al. 2008).

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Bone

55

Shigeru Okada and Jacob Wright-Piekarski

Core Message

› The effects of GH and IGF-1 on somatic growth have been closely linked since the somatomedin hypothesis was proposed over 50 years ago (Salmon and Daughaday 1957). However, it has also been established that GH exerts its direct effects independent of IGF-1 mediation in the regulation of growth. Furthermore, roles of estrogen and androgen on bone growth have been elucidated using GHR-/- mice. Androgens stimulate bone formation independent of systemic or local IGF-1 production. Estradiol, on the other hand, upregulates systemic IGF-1 levels by stimulating hepatic IGF-1 synthesis independent of GH. Thus, estrogen or androgen action might be clinically useful on bone modeling for patients with age-dependent GH-IGF-1 deficiency and/or GH-resistant conditions. Since the contributions of GH and IGF-1 as well as other hormones are complex, the generation of temporal or bone-specific GHR knockout mice will be valuable to further characterize the contribution of GH in bone growth.

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55.1 Introduction

Longitudinal bone growth has been studied as a model system for the growth-promoting activity of GH because of its clear dose-dependency (Cheek and Hill 1974). Determination of the width of the tibial growth plate in hypophysectomized rats was found to be a sensitive and specific bioassay for GH action (Greenspan et al. 1949) and is still used as one of the reference methods for determining GH activity in vivo. Growth of long bones is dependent on the proliferation of chondrocytes in the epiphyseal growth plate, where cells are located between two areas of calcified tissue (Kember and Sissons 1976). In such areas, fibroblasts reside in a band of rarely-dividing stem cells, the germinal layer (Green et al. 1985). During bone growth, germinal cells differentiate into chondrocytes, which enter an actively proliferating layer where they undergo limited clonal expansion. As cells divide, they are displaced away from the germinal layer, enter the hypertrophic cell layer, and become ossified. When sexual maturity stimulates epiphyseal closure, longitudinal bone growth ceases, thereby ending normal growth (Isaksson et al. 1987).

The presence of a mediating serum factor of GH action secreted from the liver, termed the sulfaction-factor, somatomedin C, or IGF-1, was postulated based on the observation that cultured cartilage fragments did not respond to exogenous GH, but actively divide when sera from animals or men with normal pituitary function were added (Salmon and Daughaday 1957). Sera from hypophysectomized animals were less potent than those from normal animals, and GH replacement therapy in hypophysectomized animals regained the activity of the serum factor, indicating that such factor was regulated by GH (Daughaday and Reeder 1966). Later, purified IGF-1 confirmed that IGF-1 induced tibial growth and thymidine incorporation in vivo (Schoenle et al. 1986).

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Thus, the somatomedin hypothesis was formulated, describing the relationship between GH and IGF-1, with GH acting mainly on the liver to generate IGF-1, which in turn stimulates proportional body growth (Daughaday et al. 1981; Daughaday and Rotwein 1989). However, the ability of purified IGF-1 to induce body weight gain in dwarf mice or hypophysectomized rats was considerably lower than that of GH (van Buul-Offers and Van den Brande 1979; Holder et al. 1981; Skottner et al. 1986). In addition, a number of nonhepatic tissues have been shown to produce IGF-1, including smooth muscle, skin, lung, bone, and cartilage, as well as a number of fetal mouse tissues (Atkison et al. 1980; D'Ercole et al. 1980; Stracke et al. 1984; Clemmons and Van Wyk 1985). Furthermore, GH and IGF-1 infusion caused disproportional growth of kidneys, adrenal glands, and spleen, indicating that distinct organs respond differently to systemic IGF-1 and GH (Skottner et al. 1989). Together, these observations supported a possibility that not only hepatic IGF-1 but also IGF-1 secreted from many different tissues by GH-stimulation might act locally by autocrine or paracrine mechanisms (D'Ercole et al. 1984; Isaksson et al. 1987; Isgaard et al. 1988) This modified form of the somatomedin hypothesis is known as the dual effector theory (Green et al. 1985). In the case of bone growth, the hypothesis states that GH directly stimulates the differentiation of prechondrocytes and the young differentiating cells acquire responsiveness to IGF-1. Mitogenic activity of IGF-1 selectively stimulates these chondrocytes by an autocrine or paracrine mechanism. As a result, the differentiating chondrocytes enter clonal expansion (Isaksson et al. 1987).

Thus, the clear evidence demonstrating both GH and IGF-1 stimulate growth makes it difficult to assign relative importance to their actions and establish the nature of growth regulation when both factors exist. Interestingly, when rats were maximally stimulated with IGF-1, GH could stimulate growth further (Fielder et al. 1996). Thus, systemic injection of GH and IGF-1 produces additive or synergistic effects (Ohlsson et al. 1998). Two independent studies using IGF-1–/– mice clearly demonstrated the crucial role of IGF-1 in intrauterine growth as well as perinatal survival; however, due to high rate of fatality after birth and severe developmental defects, it was difficult to study its relationship to GH effects (Liu et al. 1993; Powell-Braxton et al. 1993). Later, introduction of conditional knockout mice using the Cre/loxP system improved the postnatal survival rate and successfully demonstrated that IGF-1 is essential for GH-stimulated postnatal growth in mice (Liu et al. 1998; Liu and LeRoith 1999). On the other hand, generation of liver-specific IGF-1–/– mice demonstrated that liver-derived IGF-1 is not required for normal growth (Yakar et al. 1999). In the complete absence of IGF-1 secretion from the liver and the subsequent reduction of serum IGF-1 levels by 75%, both male and female mice exhibited normal bone length and overall body length (Yakar et al. 1999). Together with the fact that GH transgenic mice reach nearly twice the size of their nontransgenic littermates, while IGF-1 transgenic mice do not grow beyond the size of controls (Ohlsson et al. 1998), the need for revision of the original somatomedin hypothesis has become apparent.

55.2 GH and Longitudinal Bone Growth in the GHR–/– Mouse

With the availability of GHR–/– mice, it became possible to directly compare the effect of GH vs. IGF-1 signaling on animal growth. An X-ray image of GHR +/+, +/–, and –/– mice is shown in Fig. 55.1. Note the remarkable similarity in the overall bone structure. However, differences certainly exist, not the least of which is the dwarfism seen in the GHR–/– animals. Other changes include reduction in epiphyseal plate width in GHR–/– mice by 20 (20 days old) to 30% (10 weeks old) compared to wild type littermates (Wang et al. 2004; Davies et al. 2007), while the reduction in IGF-1–/– was 13% (20 days old) (Wang et al. 2004). Tibial linear growth rate was reduced by approximately 35 and 65% in IGF-1–/– and GHR–/– mice, respectively, between postnatal days 20 and 40 (Wang et al. 2004). The growth plate of IGF-1–/– mice showed significant enlargement of the germinal zone with normal proliferation and amount of chondrocytes, and reduced overall hypertrophy. In contrast, the germinal zone in GHR–/– mice was hypoplastic with reduced proliferation, number, and hypertrophy of chondrocytes (Wang et al. 2004). These observations are consistent with the observed phenotype in GHR–/– mice (Fig. 55.1). The rate of longitudinal bone growth is determined by the rate of new chondrocyte formation and the size of hypertrophic chondrocytes at the growth plate (Hunziker 1988). Thus, the physiological defects in GHR–/– mice explain the greater linear growth deficiency compared to IGF-1–/– mouse (Wang et al.

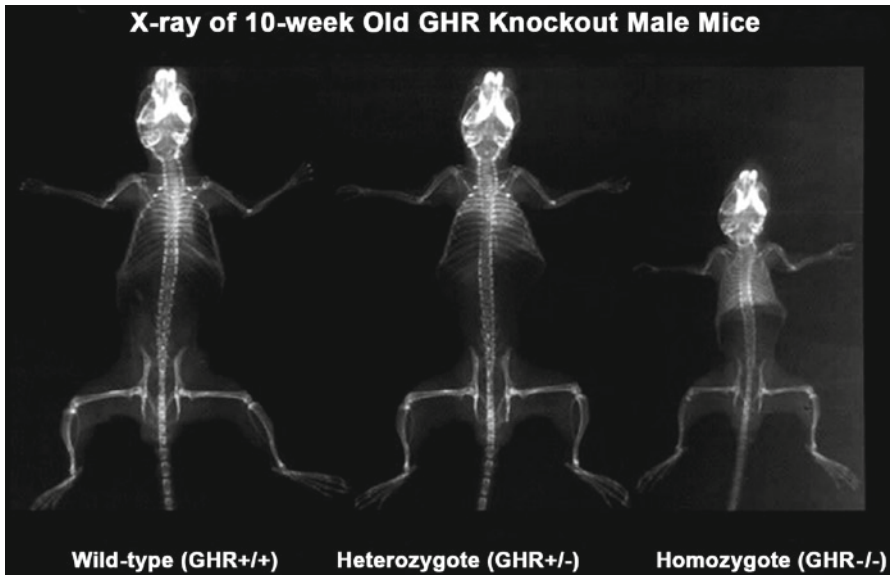

Fig. 55.1 X-rays of wild type (+/+), heterozygous (GHR+/−), and homozygous (GHR−/−) male mice at 10 weeks of age (Image kindly provided by Professor Paul Kelly)

2004). Lupu and coworkers also reported expansion of the germinal zone in IGF-1−/− mice; however, they ascribed it to delayed ossification rather than to GH excess (Lupu et al. 2001). On the other hand, the same group reported that GHR−/− mice did not have delayed ossification (Lupu et al. 2001). Also, another report observed delayed ossification in GHR−/− mice (Sjögren et al. 2000). These conflicting observations may be due to the difficulty of the alignment of tissue sections obtained from dwarf mice to those from much larger wild type mice (Wang et al. 2004). There are also conflicting data reporting reduction in number and proliferation of chondrocytes in IGF-1−/− mice (Lupu et al. 2001). Since locally injected GH increased thymidine incorporation in the prechondrocyte layer of the growth plate (while IGF-1 injected mice showed no change) and GH reduces chondrocyte cell cycle time to half of that induced by IGF-1, the notion that some of the GH-promoting effects involve direct stimulation of prechondrocytes is consistent with the observation (Ohlsson et al. 1998; Wang et al. 2004).

55.3 Bone Morphology in GHR−/− Mice

Typical images of femurs and the corresponding cross-sectional area from 2-year-old wild type and GHR−/− are shown in Fig. 55.2 (Berryman et al. 2009). The

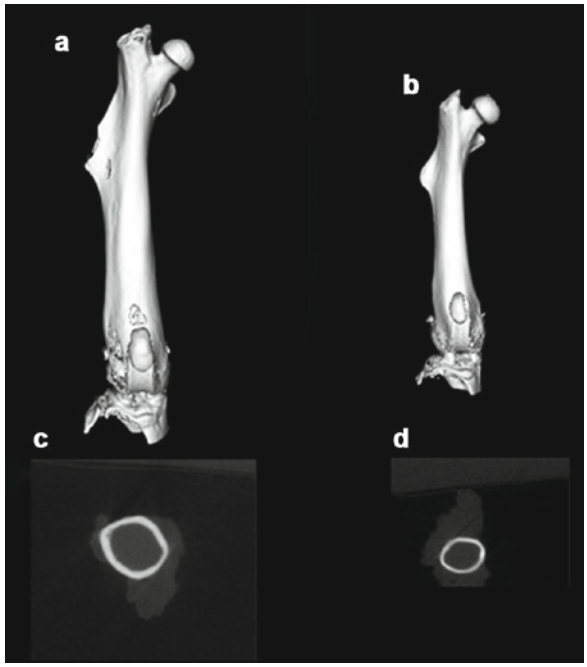

Fig. 55.2 Scanned images of femurs from wild type (**a**, **c**) and GHR−/− mice (**b**, **d**). A 3D reconstitution (**a**, **b**) and cross-sectional area (**c**, **d**) of the femur is shown. Right-hind legs of 2-year-old males were dissected at the hip, and soft tissue was removed. Legs were then mounted onto small foam blocks and scanned using a GE eXplore Locus Small Animal MicroCT Scanner at a voxel size of 20 μm, 80 kV, 450 μA, and 2,000 ms of exposure time. Calibration was performed using an acrylic phantom, containing samples of equal densities to air, water, and bone. Images were obtained using the GE eXplore Microview software

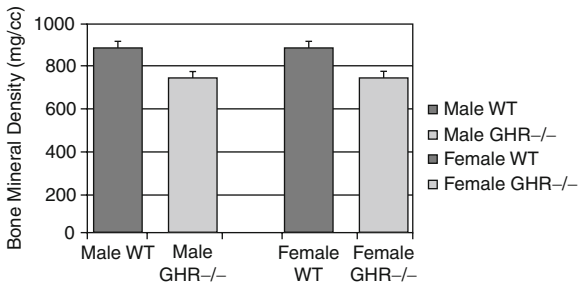

Fig. 55.3 Bone mineral density of mid-diaphyseal femoral region in male and female GHR –/– and wild type mice. Bone mineral density was determined using a GE eXplore Locus Small Animal MicroCT Scanner. Data are expressed as mean ± SEM, $n=10$ (male GHR–/– and WT), $n=6$ (female WT), $n=9$ (GHR –/– female). Right-hind legs of 2 years old mice were dissected at the hip and soft tissue was removed. Legs were then mounted onto small foam blocks and scanned using the GE eXplore Locus Small Animal MicroCT Scanner at a voxel size of 20 μm, 80 kV, 450 μA, and 2,000 ms of exposure time. Calibration was performed using an acrylic phantom, containing samples of equal densities to air, water, and bone. A mid-diaphyseal region of the femur was selected using a threshold of 800 HU to separate bone tissue from the background image. Bone mineral density of the selected tissue was determined using the GE eXplore Microview software (Berryman et al. 2009)

ratio of total bone mineral content to crown-rump length and that of total bone area to crown-rump length were decreased by 45 and 23%, respectively, in 3.5-month-old GHR–/– mice compared to wild type (Egecioglu et al. 2006). Bone mineral density was also decreased in total body, spine, femur, tibia, and cranium by 11, 20, 32, 29, and 29%, respectively, at 3 months of age (Sjogren et al. 2000). Also, bone mineral density measurements performed in our laboratory at 2 years of age is shown in Fig. 55.3. Cortical bone mineral content of the mid-diaphyseal section of the femur and tibia was decreased by approximately 50% in GHR–/– mice (Sjogren et al. 2000), while serum levels of osteocalcin were unchanged. In addition to the general decrease in bone size, GHR–/– mice displayed disproportional skeletal growth (Sjogren et al. 2000).

55.4 Sex Steroids and Bone

In addition to the well-established role of GH and IGF-1 in the regulation of bone growth, steroid hormones play essential roles in pubertal growth spurt and bone mineral accrual. Estrogens stimulate longitudinal growth during early puberty and induce closure of the epiphyseal growth plate during late puberty, which limits the longitudinal growth and determines the ultimate bone size. Estrogen deficiency or resistance results in low bone mineral density (BMD) and failure to establish peak bone mass. Estrogen plays a major role in bone growth regulation not only in females but also in males. Estrogen-deficient males do not have pubertal growth spurt and display sustained linear growth without closure of epiphyses (Juul 2001). The stimulation of longitudinal growth by estrogen involves the GH-IGF-1 axis, and estrogens can stimulate GH secretion and induce hepatic synthesis of IGF-1 (Venken et al. 2005). However, whether or to what extent estrogens may be able to regulate pubertal growth spurt and bone mineral acquisition independently of GH/IGF-1 is still unclear. GHR–/– mice provide a unique opportunity to address these questions. When GHR–/– male mice were orchidectomized and treated with 17β-estradiol, longitudinal growth of bone as well as periosteal growth rate was restored (Venken et al. 2005). Also, 17β-estradiol treatment fully rescued the reduced cross-sectional area, cortical thickness, and periosteal perimeter in bone of GHR–/– mice by a net increase in osteoblast number. 17β-estradiol upregulated hepatic IGF-1 gene expression in GHR–/– mice and restored serum IGF-1 levels to approximately 25% of normal levels. These results demonstrated a direct effect of IGF-1 on chondrocyte proliferation and growth plate thickness independent of GH action. Given that skeletal IGF-1 is not affected in GHR–/– mice (Venken et al. 2007), these data indicate that circulating IGF-1 is the major determinant of radial cortical bone growth.

On the other hand, the markedly low serum IGF-1 levels in GHR–/– mice did not affect trabecular bone modeling as measured by trabecular BMD, bone volume, number, width, and bone turnover (Venken et al. 2007). Orchidectomy decreased trabecular bone volume significantly in wild type and GHR–/– mice to a similar extent, and this was prevented fully by dihydrotestosterone and testosterone administration. Treatment with androgens also stimulated periosteal bone growth and increased cortical thickness without affecting serum or skeletal IGF-1 levels. These observations strongly suggest that androgens stimulate trabecular and cortical bone modeling independently of local or systemic IGF-1 production (Venken et al. 2007). Since the stimulatory effects of androgens on cortical bone mass during puberty parallel the effects of androgens on muscle, these effects may be, at least in part, indirectly mediated through an increase in muscle mass, resulting in increased mechanical loading (Venken et al. 2007). In conclusion, the study with GHR–/– mice demonstrated

that the development and maintenance of trabecular bone do not require GHR signaling or circulating IGF-1. Local IGF-1 expression may regulate trabecular bone modeling independent of GH. In contrast, GHR activation and circulating IGF-1 are major determinants of periosteal bone growth and cortical bone mass. Also, GHR signaling, systemic IGF-1, and androgen receptor signaling seem to stimulate periosteal growth and cortical bone mass independently.

55.5 Effect of GH on Craniofacial Complex

Modulation of craniofacial bone growth has been well-documented both in humans and animals (Pirinen et al. 1994; Bravenboer et al. 1996; Forsberg et al. 2002; Ramirez-Yanez et al. 2005). GH regulates cartilage formation, thus GH treatment accelerates craniofacial growth in children. On the contrary, facial bone growth is particularly retarded and fontanelle closure is delayed in Laron individuals as described in Chap. 9. Although the overall growth of the skull is relatively normal, a disproportionate cephalofacial relation with frontal bossing, saddle nose, shallow orbits, small chin, and "sunset" look is observed with this condition (Laron 2002). GH influences the height of face, in particular the posterior height (Van Erum et al. 1997), which is determined by the length of the mandibular ramus (Ramirez-Yanez et al. 2005). Ramirez-Yanez et al. compared craniofacial length of 45 days old mice with bovine (b) GH transgenic (Kopchick et al. 1999), GH antagonist (A) transgenic (Chen et al. 1991), and GHR–/– (Kopchick et al. 1999). As described earlier for humans, effect of GH was different in different regions of scull. bGH mice resulted in greater dimensions of craniofacial length and upper face height. In contrast, GHR–/– and GHA mice showed significant reduction in craniofacial length, upper height, and the length of mandibular corpus (Ramirez-Yanez et al. 2005). In humans, a lack of GH affects posterior face height, which is determined by the length of mandibular ramus (Pirinen et al. 1994). In mice, not only was the length of mandibular ramus, thus the posterior face height, significantly reduced in GHA and GHR–/– mice, but also the upper face height (Ramirez-Yanez et al. 2005). It was speculated that the reduced GH activity resulted in the reduced chondral growth in the mandibular condyles and in the nasal bone (Ramirez-Yanez et al. 2004).

55.6 Effect of GH on the Development of Tooth

In Laron individuals, tooth development is delayed, and teeth are often defective and also crowded due to the small mandible (Laron 2002). Delayed tooth eruption and hypodontia were also reported in Laron individuals (Sarnat et al. 1988). On the other hand, patients with gigantism and acromegaly show normal size of dentitions (Levin 1965). GH influences the cementum, a thin layer of mineralized tissue that attaches periodontal ligament to the root surface of tooth (Smid et al. 2004). Cross-sectional area of this tissue is reduced by tenfold in GH–/– and threefold in GHA mice, whereas increased by twofold in bGH transgenic mice (Smid et al. 2004). bGH transgenic mice showed increase in the length of incisors, while GHR–/– mice showed opposite effect (Ramirez-Yanez et al. 2005). Presence of GH and GHR in enamel organs at the embryonic cap and bell-shaped stage during tooth bud formation was demonstrated (Zhang et al. 1997). Also, odontoblasts and ameloblasts express functional GHR, and GH increases cell proliferation of the inner dental epithelium, dental papilla, and Hertwig's epithelial sheath before the differentiation of odontoblasts (Young et al. 1995). GH has shown to have effects not only on size but also on shape of the molar dentin in mice (Smid et al. 2004). Interestingly, total crown area and mesio-distal width at the cemento-enamel junction axis were unchanged in the first lower molars of bGH mice (Smid et al. 2004) as observed in humans with GH excess (Levin 1965). In contrast, crown dimensions were significantly reduced in GHR–/– and GHA mice (Smid et al. 2004), again as observed in individuals in Laron syndrome (Laron 2002). In conclusion, GH influences dentin size and shape not only in dentin appositional growth but also during crown and root morphogenesis prior to dentinogenesis (Smid et al. 2004).

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GHR Knockout and the CNS

56

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Core Message

› The GHR –/– mouse has proven to be a useful and unique model to study the role of GH on growth, development, and function of the CNS. GHR–/– mice show a relative increase in the size of the brain and pituitary with concomitant changes in the morphology of these tissues. The disruption of GH signaling alters the feedback mechanisms that control GH expression. This results in changes in expression of several hypothalamic proteins that influence GH expression and also results in structural changes in the pituitary. Several measures of motorneuron development were unaltered in the GHR–/– mouse, indicating this is a GH-independent process. An examination of the brain of GHR–/– animals shows increased neuron cell density and hypoplasia of glial cells compared to controls. The single investigation of cognition in these animals has shown protection from age-related decline in memory in the GHR–/– mice. There is a great opportunity to use these mice to facilitate further research into the role GH plays in neural development and function.

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56.1 Introduction

The role of GH in the development and maintenance of a healthy central nervous system (CNS) has been a topic of study and speculation for many years. While a number of research groups have reported expression of GHR and GH in various neural cell types at different stages of development (Harvey et al. 2001; Turnley et al. 2002; Donahue et al. 2006), the exact role of GH signaling in neural tissues has been elusive due to sparse and disparate data. In vivo and in vitro studies have suggested a direct effect of GH on neuron survival and differentiation (Pelton et al. 1977; Noguchi et al. 1988; Scheepens et al. 2001; Otteson et al. 2002; Ajo et al. 2003). Additionally, data from LS patients indicate structural abnormalities and IQ deficiencies in some patients, while others appear normal (see Chap. 38). However, animal models of impaired GH action do not show significant abnormalities in brain structure or functional deficits. Some of these animal models have significant limitations, such as the absence of other pituitary hormones or intact capacity for autocrine/paracrine GH signaling, making the specific role of GH difficult to elicit. The invention of the GHR–/– mouse has provided researchers with a unique animal model that has a complete lack of GHR signaling. Several researchers have examined various effects of the GHR gene disruption on the CNS. These studies have reported impacts of the gene disruption on gross morphology of the CNS, hypothalamus and pituitary function, memory, neural differentiation, and CNS development.

56.2 Effects of GH on Brain Growth and Development

To gain some insight into a possible role of GH on brain development, Sjogren et al. (2000) examined the effect of the GHR disruption on brain size. They collected and

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weighed organs from 3-month-old GHR–/– animals and controls. Organ weights were reported relative to total body weight. They found that the brain of GHR–/– mice was significantly increased at 151% of controls, relative to total body weight. The authors speculate that early brain development occurs during a period of predominantly GH-independent growth, although GHR has been reported to show high expression in prenatal rat brain with a decline after birth (Lobie et al. 1993). A later report (Ransome et al. 2004) confirmed the relative increase in brain size of the GHR–/– animals. While the GHR–/– body weight was 45% of controls, the brain weight was 80% of controls (see Fig. 56.1) (Asa et al. 2000; Ransome et al. 2004).

Similar to the brain, pituitaries had increased weight relative to body weight in the GHR–/– animals. Asa et al. (2000) found that while the GHR–/– animals weighed 45% of control animals, the weight of their pituitaries was 58% of controls. Chandrashekar et al. (1999 and Peng et al. 2001b) both confirmed the finding of increased pituitary weight in GHR–/– animals relative to body weight. There also appear to be differences in pituitary cell structure in the GHR–/– animals. Kineman et al. (2001) reported that somatotrophs in GHR–/– mice were packed together more densely and showed decreased cell size while exhibiting signs of hyperplasia. Overall, the GHR–/– pituitary showed a 27% increase in the density of nuclei (Kineman et al. 2001).

56.3 The Pituitary and Hypothalamus

The pituitary and hypothalamus function in concert to exert precise control of the production and release of several hormones, including thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), adrenocorticotropic hormone (ACTH), leutinizing hormone (LH), gonadotropin, PRL, and GH itself. Production and release of GH by the pituitary are influenced by multiple levels of regulation, as shown in Fig. 56.2 and reviewed by Wong et al. (2006). Neurons in the hypothalamus signal to the pituitary via GH-releasing hormone (GHRH) and somatostatin (SRIF). A long-loop feedback system exists whereby circulating IGF-1 acts directly on somatotrophs or via the hypothalamus to inhibit GH production. GH can also directly influence its own production through a short-loop feedback system, in which circulating GH signals reduce GH production via the hypothalamus. Additionally, an ultra-short feedback loop has been described whereby GH acts directly on somatotrophs to inhibit GH production.

The long-loop, short-loop, and ultra-short feedback mechanisms all rely on intact GHR signaling. Disruption of GHR is expected to alter this regulatory system, which would ultimately affect hormone production, and possibly the structure and function of the pituitary. GHR–/–mice show an increase in circulating GH levels, indicating alterations in these regulatory feedback loops. Asa et al. (2000) examined the effect of the GHR disruption on pituitary structure and function. The disruption of GHR signaling in the mouse leads to several changes in gross morphology and cell populations of the pituitary. In the GHR–/– pituitary, there was a marked disruption of the reticulin fiber network accompanied by acini expansion, indicative of pituitary cell hyperplasia. An expansion in the proportion of GH-immunoreactive cells in the

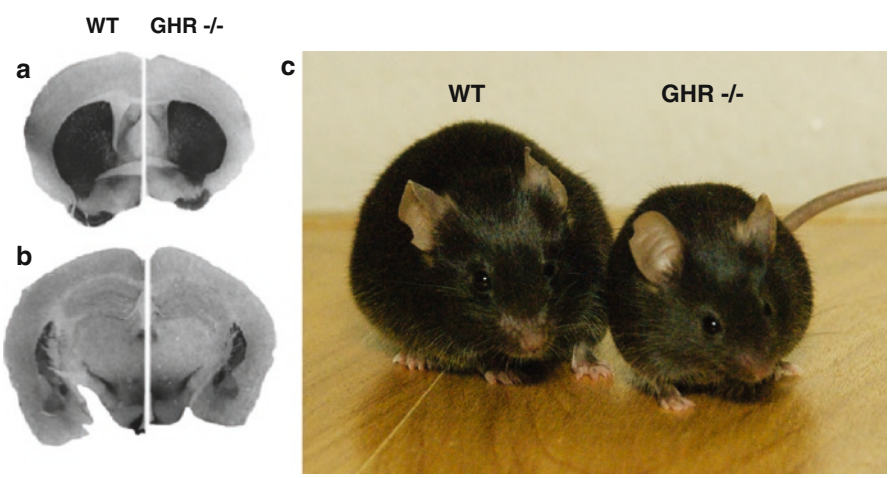

Fig. 56.1 Comparison of brain and body size of WT and GHR–/– male mice. TH immunohistochemistry of paired brain sections of WT and GHR–/– mice of (**a**) striatal sections and (**b**) hippocampal sections. A photograph of a WT and a GHR–/– mouse is shown (**c**). GHR–/– brain weight is 20% reduced, while overall body weight is drastically reduced compared to WT controls. (**a**) and (**b**) reproduced with permission from Ransome et al. (2004)

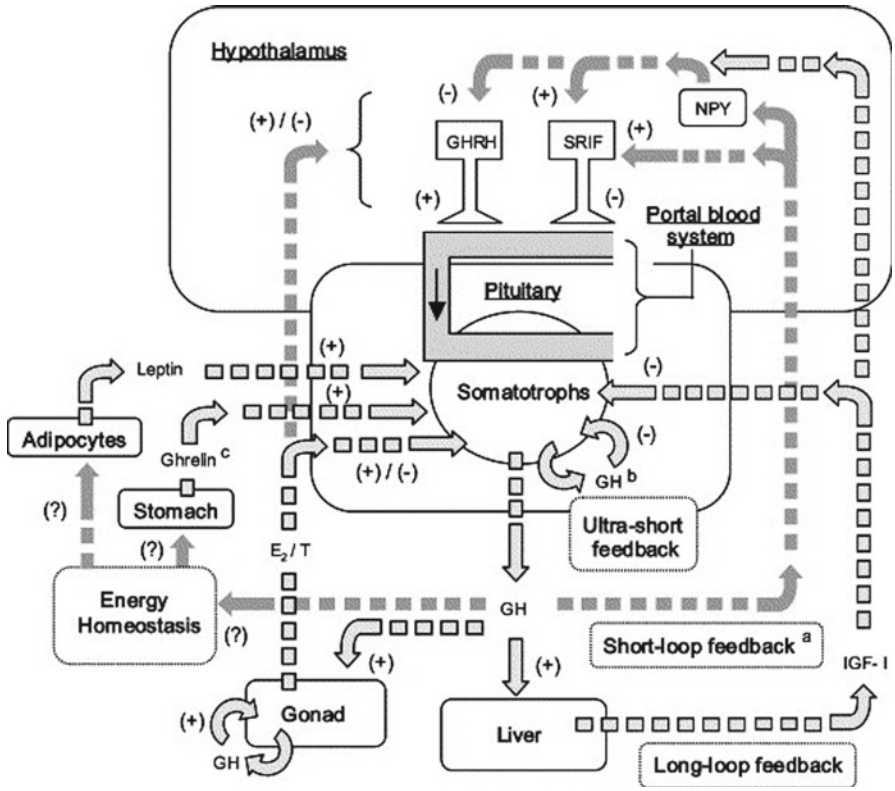

Fig. 56.2 Feedback regulation of pituitary GH release. GH release is under dual control by GH-releasing hormone (GHRH) and somatostatin (SRIF), which are delivered from neurons in the hypothalamus to the anterior pituitary. Three levels of GH feedback on somatotrophs have been described. A long-loop feedback negatively regulates GH production via systemic IGF-1 produced by the liver and other GH-responsive organs in response to GH. A second level of regulation is the short-loop feedback system, whereby GH travels to the hypothalamus and increases expression of SRIF while decreasing expression of GHRH. The third level or regulation is an ultra-short feedback system whereby autocrine/paracrine GH action within the pituitary negatively regulates GH production. Reproduced with permission from Wong et al. (2006)

GHR−/− pituitary was first observed by Asa et al. 2000 and confirmed by subsequent groups (Kineman et al. 2001; Peng et al. 2001a). Electron microscopy of the GHR−/− pituitary revealed hyperplasia of an abnormal population of somatotrophs characterized by sparsely granulated cells with expanded mitochondria, Golgi complexes, and endoplasmic reticulum membranes. These somatotrophs showed reduced cytoplasmic, but increased juxtanuclear Golgi staining for GH (Asa et al. 2000). The proportion of TSH and ACTH staining-pituitary cells was not altered in the GHR−/− mouse (Kineman et al. 2001).

The proportion of pituitary cells positive for PRL immunostaining was diminished in the GHR−/− mice, an interesting finding given the data that circulating levels of PRL are not lower than controls and may in fact be elevated (Chandrashekar et al. 1999; Kineman et al. 2001). In close agreement with data from Laron individuals, GHR−/− mice showed elevated levels of plasma PRL (Silbergeld et al. 1992; Chandrashekar et al. 1999), a somewhat paradoxical outcome considering that bGH transgenic mice also display hyperprolactinemia (Chandrashekar and Bartke 1996).

Peng et al. (2001a) show increased GHRH and decreased NPY mRNA expression in the hypothalamus of GHR−/− mice, both of which could increase pituitary GH production. The same study also showed a doubling of GHRH-R and GHS-R mRNA levels in the pituitary. The increased receptor levels in the pituitary may partially be explained by an increase in the proportion of somatotrophs in GHR−/− mice.

In summary, the GHR−/− mouse shows severe deregulation of the regulatory feedback systems that influence pituitary GH production. This results in

drastic changes in pituitary structure and alterations in pituitary function.

56.4 Memory

GHRs are known to be expressed in a variety of brain tissues, including regions of the brain that are essential for cognitive function (Lai et al. 1991). It is also known that GH can cross the blood-brain barrier (Johansson et al. 1995). Biologically, it is feasible that GH signaling may play a role in memory and learning either directly via GHR or through an increase in circulating IGF-1. Indeed, a small study of male patients with GH deficiency revealed deficits in memory performance (Deijen et al. 1996). Examination of Laron syndrome individuals has suggested that GHR gene mutations may cause mild to severe mental deficits, depending on the nature of the mutation (Shevah et al. 2005).

To help elucidate a possible role of GH in memory, Kinney et al. (2001) examined memory retention in young (2–4 months of age) and aged (17–30 months of age) GHR−/− animals and controls. In one of the experiments, mice were placed in an inhibitory avoidance apparatus that gave equivalent shocks to GHR−/− and control animals if they entered a specific compartment. Avoidance time of that compartment in subsequent trials was recorded at 24 h, 7 days, and 28 days posttraining. At the 24 h and 7 day time points, all animals showed strong avoidance behavior, with no evidence of genotype or age differences. By 28 days posttraining, the aged GHR−/− animals had the same avoidance retention as young GHR−/− animals and as the young wild type controls. Importantly, the aged wild type controls showed a decline in avoidance behavior compared to younger mice and to aged GHR−/− mice (Kinney et al. 2001). Thus, in this assay of avoidance learning and memory, GHR−/− mice did not show a decline with age that is characteristic of wild type control animals. There is mixed and sometimes contradictory data from studies of patients with GH deficiency, some showing certain cognitive deficits and others showing little or no effect (reviewed by van Nieuwpoort and Drent (2008)). Further research with GHR mice could help elucidate the impact of GH signaling on memory and other cognitive functions.

56.5 Prenatal Motorneuron Development

Several researchers have reported extensive expression of both GHR and GH throughout the developing CNS, indicating a likely role of GH signaling in neuron growth and development (Garcia-Aragon et al. 1992; Lobie et al. 1993; Harvey et al. 2001). Parsons et al. examined various motorneuron characteristics in prenatal and perinatal GHR−/− mice. Unlike adult GHR−/− animals, the body mass of GHR−/− animals at E18 through P2 was not different from controls (Parsons et al. 2003). Spinal cord volume and gross morphology at E13.5, E18.5, and P2 did not show a difference from controls, indicating that spinal cord growth is not dependent on GHR signaling (Parsons et al. 2003). Similarly, there was no change in the morphology or number of cranial motor nuclei motorneurons in GHR−/− animals at E18.5. There was, however, a slight but statistically significant decrease in the size of the nuclei of brachial LMC motorneurons at E13.5, E18.5, and P2, possibly indicating a reduction in motorneuron size (Parsons et al. 2003). The lack of significant changes in spinal cord and motorneuron development in GHR−/− animals was unexpected. This research indicates that either prenatal motorneuron development is not dependent on GH, or that GH is exerting an effect independent of GHR. One possible explanation could be that an intact PRL signaling pathway, which shares signaling components with the GH pathway, is able to compensate for the GHR gene disruption. Interestingly, a study of one cohort of LS patients suggests impairments in the visuomotor recall Bender test (see Chap. 38).

56.6 Neuron Populations of the CNS

Raonsome et al. analyzed various cell populations in the GHR−/− brain. The GHR−/− mice were found to have a similar number of total cells in the somatosensory cortex, but with an increase in neuron cell density of 23–26% compared to controls (Ransome et al. 2004). To further explore this increase in neuron density, markers of specific cell populations were used. The GHR−/− mice showed an increase in calretinin positive cells as well as calbindin positive interneurons compared to controls (Ransome et al. 2004). Remarkably, even though the GHR−/− mice have smaller brains,

they showed the same number of cholinergic neurons as controls, indicating an increased density of these cells in the GHR−/− striatum. While most neuron development occurs prenatally, gliogenesis is largely postnatal. Staining for glial cells indicated a decrease in both the number and size of these cells in adult GHR−/− mice, indicating a possible deficiency in this cell type. The GHR−/− mice also showed sparse branching of dendritic trees compared to controls (Ransome et al. 2004). The impact of glial cell hypoplasia and changes to dendritic tree branching on cognitive function in GHR−/− animals will require further study.

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Cancer

57

Brian D. Bower and John J. Kopchick

Core Message

› Despite discrepancies both within and between data from human patient populations and mouse models of altered GH induced signaling, abrogation of this signaling via mutation of the GHR appears to protect both mice and humans from cancer development and progression. While research on this phenomenon has been hindered by the low prevalence of Laron syndrome in the human population, research in the GHR−/− mouse is more extensive. The GHR−/− mouse exhibits statistically significant reductions in the overall incidence of malignancy (Ikeno et al. 2009), particular breast cancer (Zhang et al. 2007), lung cancer (Ikeno et al. 2009), lymphoma (Ikeno et al. 2009), and pituitary adenoma (Kineman et al. 2001) either under normal conditions or after carcinogen challenge as compared to control mice. While many of these phenotypes have not been confirmed in LS individuals, ongoing work has demonstrated that Laron patients exhibit resistance to malignancies (Shevah and Laron 2007). Continuing clinical and experimental work in both human patients and using the GHR−/− mouse shall further elucidate the intricacies and, hopefully, the mechanisms behind these phenotypes.

57.1 Introduction

Growth hormone (GH) induced signaling modulates the proliferation, differentiation, and apoptosis of several cell types and tissues (McLenachan et al. 2008; Pandey et al. 2008; Dardenne et al. 2009). As such, altered GH induced signaling could be expected to confer a variety of cancer related phenotypes. In general, changes in GH induced signaling in humans have not been found to produce profound changes in cancer incidence or cancer mortality as compared to either the general population or to specific control populations after adjusting for variables such as previous radiotherapy and family cancer histories. The changes that do occur are modest and likely of marginal clinical significance (Jenkins et al. 2006; Rutter and Rose 2007). In contrast, increases and decreases in GH induced signaling in mice have been found to increase and decrease the development of cancers respectively. The discrepancy between human clinical data and mouse experimental data may stem from the comparable heterogeneity of human patients, difficulties in comparing patients with altered GH induced signaling to appropriate control populations, and difficulties in controlling a number of variables other than GH induced signaling that may affect cancer susceptibility in human patients (Bogarin and Steinbok 2009). Additionally, the discrepancy may

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be due to differences in the etiologies of altered GH induced signaling between human patient populations and mouse models. As the clinical aspects of the relationship between GH induced signaling and cancer have been reviewed in previous chapters and by other authors (Cohen et al. 2000; Renehan et al. 2000; Melmed 2001; Jenkins et al. 2006; Ayuk and Sheppard 2008; Loeper and Ezzat 2008; Renehan and Brennan 2008; Bogarin and Steinbok 2009), this chapter shall examine cancer related data gathered from a variety of mouse lines with altered GH induced signaling, with a particular focus on experimental data from the GHR−/− mouse.

57.2 Oncogenic Mechanisms of the GH/IGF-1 Axis

GH induces several intertwined, intracellular pathways, which have been previously described (Lanning and Carter-Su 2006). GH releasing hormone (GHRH) is secreted from the arcuate nucleus into the pituitary portal system, where it binds to GHRH receptors on somatotrophs in the anterior pituitary, which in turn secrete GH into systemic circulation. Circulating GH binds to growth hormone receptors (GHR) in numerous cell populations and initiates a cascade of events which include autophosphorylation of the GHR (Stred et al. 1992), phosphorylation of Janus Kinase 2 (JAK2) (Argetsinger et al. 1993), and JAK2 activation of STAT5b (signal transducers and activators of transcription) and IRS1 (insulin receptor substrate 1) (Argetsinger et al. 1995).

STAT signaling has been implicated in development and progression of several cancers (Wagner and Rui 2008). Additionally, numerous signal transduction pathways promoting cell survival and proliferation including PI3K, P38 and ERK1/2 lie downstream of IRS1, and the activity of these pathways have been found to be increased by exogenous GH expression and decreased by GHR antagonist (GHA) treatment in mice (Bogazzi et al. 2008; Miquet et al. 2008). Furthermore, proteins not clearly connected to GH induced signaling have been found to be similarly increased and decreased by exogenous GH and GHA treatment, including the P53 tumor suppressor and proteins associated with the mitochondrial apoptosis pathway (Bogazzi et al. 2008). In each case, up and down regulation of GH induced signaling produced changes that encouraged or discouraged cell survival and proliferation respectively.

In many cells, GH binding to the GHR promotes the expression and secretion of insulin-like growth factor 1 (IGF-1) (Woelfle and Rotwein 2004) with the liver being the primary source of circulating IGF-1 (Mathews et al. 1986). IGF-1 acts as a potent growth factor in autocrine, paracrine and endocrine manners (Stratikopoulos et al. 2008). Concordantly, IGF-1 signaling has been implicated in the development and progression of several cancers, including mammary cancer (Creighton et al. 2008; Jones and Moorehead 2008; Law et al. 2008) skin cancer (Keehn et al. 2004; Moore et al. 2008) and colon cancer (Ealey et al. 2008; Fuchs et al. 2008). However, both GH and IGF-1 stimulate production of several IGF-1 associated proteins including IGF-1 binding proteins 1 (IGFBP1) and 3 (IGFBP3), and the IGF-1 acid labile subunit (ALS). IGFBP1 promotes IGF-1's activity by extending IGF-1's half life, while IGFBP3 and the ALS inhibits IGF-1's actions by sequestering IGF-1 in a ternary complex (Gourmelen et al. 1991; Jorgensen et al. 1991). IGFBPs have also been found to regulate tissue growth through IGF-1 independent methods (Yamada and Lee 2009). By signaling through these downstream pathways, GH induced signaling has both growth promoting and growth inhibiting actions (Cohen et al. 2000).

In addition to IGF-1 autocrine/paracrine activity, expression of GH and GH autocrine/paracrine activity have been identified as contributors to the development, growth and migration of human mammary epithelia cells (Zhu et al. 2005) and to promote the growth of endometrial carcinoma (Pandey et al. 2008). While GH stimulates the secretion of IGF-1, both GH and IGF-1 reduce GH secretion from the pituitary and GHRH secretion from the arcuate nucleus via negative feedback (Asa et al. 2000). This interplay has complicated attempts at elucidating the independent effects of GH and IGF-1 in vivo, as alterations of one component of this regulatory system affects the activity of many other components.

Mutation of the GHR has a variety of affects on the regulatory systems described above. Abrogation of GHR signaling disrupts GH negative feedback at the anterior pituitary and arcuate nucleus, and as such results in increased levels of circulating GH (Peng et al. 2001). However, GH insensitivity at the liver and peripheral tissues results in reduced circulating IGF-1 (Peng et al. 2001). This combination of changes has made the GHR−/− mouse model particularly useful for investigating the influence of GH induced signaling on the development and progression of cancer.

57.3 GH Induced Signaling and Cancer Incidence

Several human disease states have permitted examination of the influence of altered GH induced signaling on cancer incidence and mortality in humans. These states include excess GH in individuals with acromegaly, exogenous GH in GH deficient patients, and GHR mutations in individuals with Laron syndrome (LS). However, many of the data gathered through the study of human patients are complicated by lack of appropriate controls, cancer detection biases, differences in disease morbidity, and other variables (Jenkins et al. 2006). The difficulties inherent in studying human subjects has led to the examination of mice with altered GH signaling, including transgenic mice expressing exogenous GH (simulating acromegaly), mice treated with a GHs from a variety organisms (simulating GH therapy), and mice exhibiting disruption of the GHR (simulating LS). In contrast to human data, many mouse data suggest that increases and decreases in GH induced signaling respectively induce increases and decreases cancer incidences or mortality (Chen and Sonenberg 1977; Tornell et al. 1991; Murphy et al. 1992; Tornell et al. 1992; Yang et al. 1996; Kineman et al. 2001; McCutcheon et al. 2001; Pollak et al. 2001; Snibson et al. 2001; Dagnaes-Hansen et al. 2004; Wang et al. 2005; Divisova et al. 2006; Liang et al. 2006; Liu et al. 2006; Zhang et al. 2007; Ikeno et al. 2009). However, some data show no effect of altered GH induced signaling on mouse cancer incidence or mortality (Bechensteen et al. 1993; Chen et al. 2004; Farris et al. 2007; Bogazzi et al. 2008).

Clinical data regarding cancer development and progression in individuals with LS is relatively scarce, owing to the low prevalence (~approximately 300–400 individuals) of LS in humans (Rosenfeld et al. 1994). Data gathered from approximately half of the LS population (169) showed no incidence of malignancy, as compared to normal levels of malignancy in first degree relatives (Shevah and Laron 2007). These data are in good agreement with experimental data gathered from the GHR−/− mouse, which exhibits reduced overall incidence of malignancy (Ikeno et al. 2009) and reduced incidence of specific cancers including lymphoma (Ikeno et al. 2009), and cancers of the lung (Ikeno et al. 2009), pituitary (Kineman et al. 2001), breast (Zhang et al. 2007), and prostate (Wang et al. 2005) (Table 57.1).

57.4 GH Induced Signaling and Pituitary Hypertrophy and Neoplasia

Alterations of GH induced signaling in humans do not appear to produce distinct phenotypes regarding the development of pituitary adenomas. GH therapy has been shown to produce either no change (Arslanian et al. 1985; Ogilvy-Stuart et al. 1992; Ogilvy-Stuart and Shalet 1992) or a reduction (Moshang et al. 1996) in the recurrence of pituitary adenomas as compared to selected control populations. However, data from GHR−/− mice suggest a different phenotype (Kineman et al. 2001). GHR−/− mice have been found to exhibit reduced absolute but increased relative pituitary mass and remarkable resistance to adenoma formation as compared to wild-type (WT) control mice (Kineman et al. 2001). Additionally, pituitaries from GHR−/− mice showed a decreased proportion of lactotrophs, and increased proportion of somatotrophs that were of smaller size and that possessed greater resistance to GHRH-induced hypertrophy than those of WT controls (Kineman et al. 2001) (Fig. 57.1). Concordantly, GHR−/− mice exhibited early resistance to exogenous GHRH induced pituitary hypertrophy, which in contrast produced a significant increase in the pituitary mass of WT controls at all ages. However, at 12 months of age both WT controls and GHR−/− mice expressing exogenous GHRH exhibited remarkable pituitary hypertrophy and the formation of somatotroph derived pituitary adenomas demonstrating that the GHR was

Table 57.1 Change in cancer incidence due to GHR mutation

Organism	Overall	Breast	Lung	Lymphoma	Pituitary	Prostate
Humans	↓ (Shevah and Laron 2007)					
Mice	↓ (Ikeno et al. 2009)	↓ (Zhang et al. 2007)	↓ (Ikeno et al. 2009)	↓ (Ikeno et al. 2009)	↓ (Kineman et al. 2001)	↓ (Wang et al. 2005)

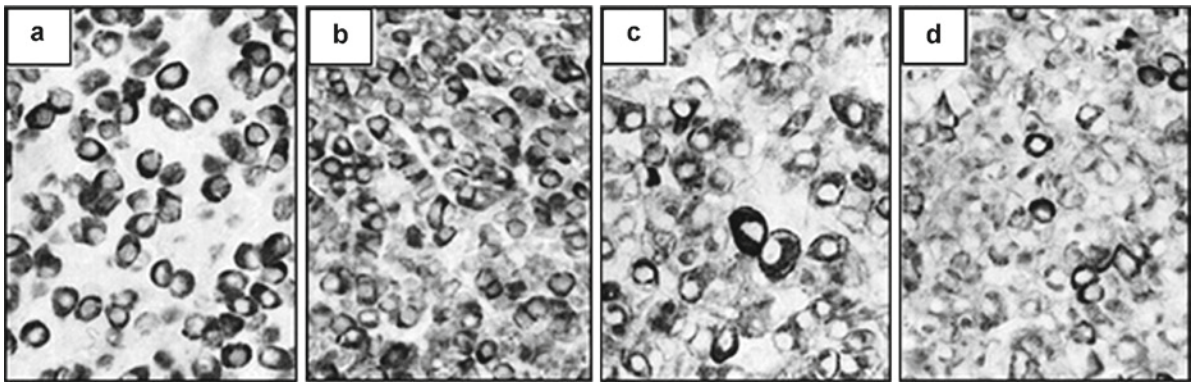

Fig. 57.1 Pituitary somatotrophs (*stained dark*) were smaller in GHR−/− mice (**b**) than in WT mice (**a**). Exogenous hGHRH expression caused marked hypertrophy of somatotrophs in WT mice (**c**), while having minimal effect on GHR−/− mice (**d**). Reproduced with permission from Kineman et al. (2001)

unnecessary for GHRH induced pituitary adenoma formation (Kineman et al. 2001).

Interestingly, several phenotypes in GHR−/− mice observed at 12 months of age showed gender or genotype specificity. Susceptibility to GHRH induced pituitary hypertrophy was gender dependent in WT controls, but not in GHR−/− mice, with WT males showing less susceptibility to GHRH induced hypertrophy than females (Kineman et al. 2001). Similarly, exogenous GHRH significantly elevated prolactin only in WT females – a difference which remained even after normalization for pituitary mass. Kineman et al. (2001) suggested that this gender related susceptibility of females to pituitary hypertrophy and hyperprolactinemia was due to cooperation of estrogens with GHRH elevated IGF-1 to promote lactotroph growth and prolactin secretion as previously described in rats (Spady et al. 1999). In contrast to gender specificity, resistance to GHRH induced pituitary hypertrophy appeared genotype specific in males, but not in females, with WT mice being relatively protected against GHRH induced pituitary hypertrophy (Kineman et al. 2001).

Taken together these data suggest that GHR−/− mice display remarkable resistance, but not complete immunity to the development of pituitary adenomas. The unexpected gender specific effects were only significant during exceptional circumstances (i.e., with exogenous GHRH). No significant decrease in the incidence of pituitary cancer has been described in LS individuals.

57.5 GH Induced Signaling and Breast Cancer

There is considerable disagreement in and between clinical and experimental data from human patients and mouse models regarding whether GH induced signaling promotes the development of breast cancer. While *in vitro* data has suggested that GH induced signaling promotes the growth of mammary tumors (Perry et al. 2008), studies have shown no increase in the breast cancer incidence of acromegalic individuals (Orme et al. 1998; Baris et al. 2002). Some studies have suggested that GH treatment after childhood cancer may increase the incidence of secondary malignant neoplasms (Ergun-Longmire et al. 2006; Rutter and Rose 2007) but the degree and clinical significance of this increase remains debatable (Perry et al. 2008). Mouse data from earlier studies showed increased cancer incidences in GH transgenic mice (Tornell et al. 1991; Tornell et al. 1992), but a recent large scale, long term (2 year) study has shown that even high dose GH treatment does not elicit an increase in breast cancer incidence in mice or rats (Farris et al. 2007). However, a well designed investigation of tumor development and growth using GH deficient dwarf rats has shown that carcinogen mediated mammary cancer development and progression are GH dependent (Shen et al. 2007). Further complicating the data, breast cancer xenograft growth has been shown to be inhibited in mice treated with a GHA, pegvisomant (Divisova et al. 2006). Data concerning this topic obtained from the GHR−/− mouse

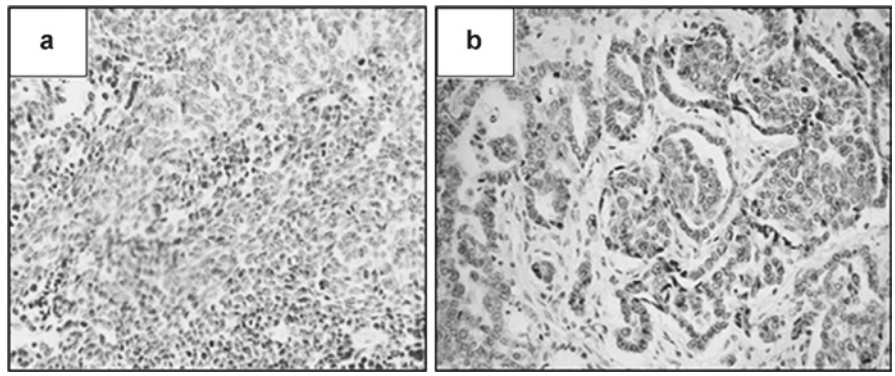

Fig. 57.2 TAg/WT mice (**a**) exhibited less differentiated and more aggressive morphology than TAg/GHR−/− mice (**b**). Reproduced with permission from Zhang et al. (2007)

model has suggested that GH induced signaling does promote breast cancer development (Zhang et al. 2007).

To explore the topic of GHR mediated breast cancer development, Zhang et al. (2007) crossed the GHR−/− mouse with a mouse line expressing a potent oncogene, the simian virus 40 (SV40) large T antigen (TAg), which has been shown to promote the development of estrogen independent mammary tumors in mice (Maroulakou et al. 1994). As compared with TAg mice with intact GHR signaling (Tag/WT), TAg/GHR−/− mice showed reduced frequency of mammary hyperplasia, and decreased tumor multiplicity and volume in mice that did developed mammary tumors. Additionally, TAg/GHR−/− mice exhibited increased cancer latency compared to TAg/WT controls.

Despite similar follicular and papillary organization in TAg/WT and TAg/GHR−/− mice, tumors in TAg/GHR−/− mice had markedly different morphologies than those in TAg/WT mice. TAg/GHR−/− tumors were smaller, more differentiated and less necrotic than those of TAg/WT controls. This morphology was suggestive of a less aggressive cancer and resembled mammary ductal carcinoma (Zhang et al. 2007) (Fig. 57.2).

The mechanism by which GH induced signaling contributed to mammary cancer development is not yet fully understood, but likely included autocrine and paracrine IGF-1 signaling – processes that were abrogated by GH resistance. Additionally, recent finding have implicated GH autocrine signaling in the proliferation and migration of mammary epithelia (Zhu et al. 2005), another process that would have been abrogated by GHR disruption. Again, no reduction in the incidence of mammary cancer has been described in LS individuals.

57.6 GH Induced Signaling and Colorectal Cancer

The impact of GH induced signaling on the development and progression of colorectal cancer in humans remains controversial. While many studies have shown increased incidence of precancerous colon polyps and colon cancers in acromegalic patients (Klein et al. 1982; Ituarte et al. 1984; Brunner et al. 1990; Ron et al. 1991; Terzolo et al. 1994; Delhougne et al. 1995; Colao et al. 1997; Jenkins et al. 1997; Orme et al. 1998; Baris et al. 2002; Colao et al. 2004; Matano et al. 2005; Terzolo et al. 2005; Matyja et al. 2006; Kurimoto et al. 2008; Rokkas et al. 2008) other studies have found no significant difference (Barzilay et al. 1991; Ladas et al. 1994; Ortego et al. 1994; Renehan et al. 2000; Bhansali et al. 2004; Biermasz et al. 2004; Martino et al. 2004) in the incidence of such maladies as compared with either the general population or selected control populations. There is some evidence that mortality from colorectal cancer is increased after GH therapy (Swerdlow et al. 2002). However, while it is generally recognized that acromegalic patients experience higher incidences of colorectal cancers, the magnitude of this increase and its clinical relevance remain debatable (Jenkins et al. 2006; Loeper and Ezzat 2008).

Mouse data regarding the impact of altered GH induced signaling on the development of colorectal

cancers is similarly unconvincing. GH transgenic mice have not been found suffer from significantly elevated colon cancer incidences (Bogazzi et al. 2009), but colorectal cancer xenograft growth in mice has been found to be inhibited by treatment with a GHA (Dagnaes-Hansen et al. 2004). While no colorectal cancer phenotype has been described in GHR−/− mice, experiments in liver specific IGF-1 deficient (LID) mice have suggested that the GH/IGF-1 axis contributes to the development and progression of precancerous colon polyps (Ealey et al. 2008).

Unlike GHR−/− mice, LID mice are GH sensitive. However, disruption of the IGF-1 gene in the liver has been found to produce profound reductions of circulating IGF-1 and increases in circulating GH (Yakar et al. 2004a; Yakar et al. 2004b), similar to those observed in GHR−/− mice. Concordantly, LID mice have been found to exhibit decreased incidences of breast and colon cancer, and decreased incidences of metastases (Yakar et al. 2004a; Yakar et al. 2004b). Ealey et al. (2008) investigated the development of colon carcinogenesis in LID mice treated with a potent carcinogen, azoxymethane (AOM), as compared to similarly treated control mice. Interestingly, while female AOM treated LID mice had significantly fewer aberrant crypt foci (ACF) than WT controls, male AOM treated LID mice showed no significant difference. The number of ACF in females were correlated to circulating IGF-1 levels, while in males there was no such correlation. Failure of the LID mutation to constrain tumorigenesis in males may have been due to sexually dimorphic insulin sensitivity in the LID mice. Ealey et al. (2008) observed that male LID mice became hyperinsulinemic as compared to WT mice, while insulin levels in females were not significantly different from controls. Insulin, which signals through IRS-1 (Sun et al. 1991), has similar growth promoting effects as IGF-1 and is a potent mitogen (Pollak 2008). The growth promoting action of hyperinsulinemia appeared to overcome the growth inhibiting action of reduced circulating IGF-1 in the males. However, no correlation between insulin levels and cancer incidence or growth was observed in the male LID mice (Ealey et al. 2008).

Despite dissimilarities with the GHR−/− mouse, the LID mouse appears to support the notion that decreased signaling in the GH/IGF-1 axis decreases the incidence and progression of cancers in mice. And while no colon cancer phenotype has been described in LS individuals, resistance to the development of ACF in LID mice appears to partially corroborate the reduced overall cancer incidence observed in LS individuals (Shevah and Laron 2007).

57.7 GH Induced Signaling and Prostate Cancer

While benign prostate hyperplasia and calcification is common in acromegalic males (Colao et al. 1998), there does not appear to be a clinically significant increase in the incidence of prostate cancer in the acromegalic population (Kurimoto et al. 2008; Loeper and Ezzat 2008). However, two mouse studies suggest that GH induced signaling may affect the development and progression of prostate cancer in mice (Torosian and Donoway 1991; Wang et al. 2005). Interestingly, GH treatment of prostate cancer xenografts has been shown to have no effect on tumor growth, but to inhibit metastases (Torosian and Donoway 1991). Additionally, disruption of the GHR has been shown to protect mice from prostate neoplasia (Wang et al. 2005).

To investigate the impact of abrogated GH induced signaling in mice, (Wang et al. 2005) crossed GHR−/− mice with mice expressing the SV40 large T-antigen (TAg) under the control of the prostate specific C1(3) promoter. The C1(3)TAg mouse model developed prostatic neoplasia at an increased rate compared to WT controls, in a manner similar to that observed in human patients (Shibata et al. 1998). While both TAg/GHR−/− and TAg/WT mice showed similar expression levels of protein markers of prostate epithelial differentiation, TAg/GHR−/− showed increased apoptosis via terminal dideoxynucleotidyl transferase (TUNEL) assay and decreased proliferation as measured by proliferating cell nuclear antigen (PCNA) staining. Concordantly, TAg/GHR−/− mice developed neoplasia at significantly lower rates and with longer latency than observed in TAg/WT mice (Wang et al. 2005).

Histological examination demonstrated that TAg/GHR−/− mice developed normal albeit hypotrophic prostate structures (Fig. 57.3). Additionally, immunohistochemical staining demonstrated that along with comparable expression of the TAg, both TAg/GHR−/− and TAg/WT mice expressed androgen receptors (AR), testosterone, and cell differentiation markers equally. These similar results suggest that TAg induced mouse

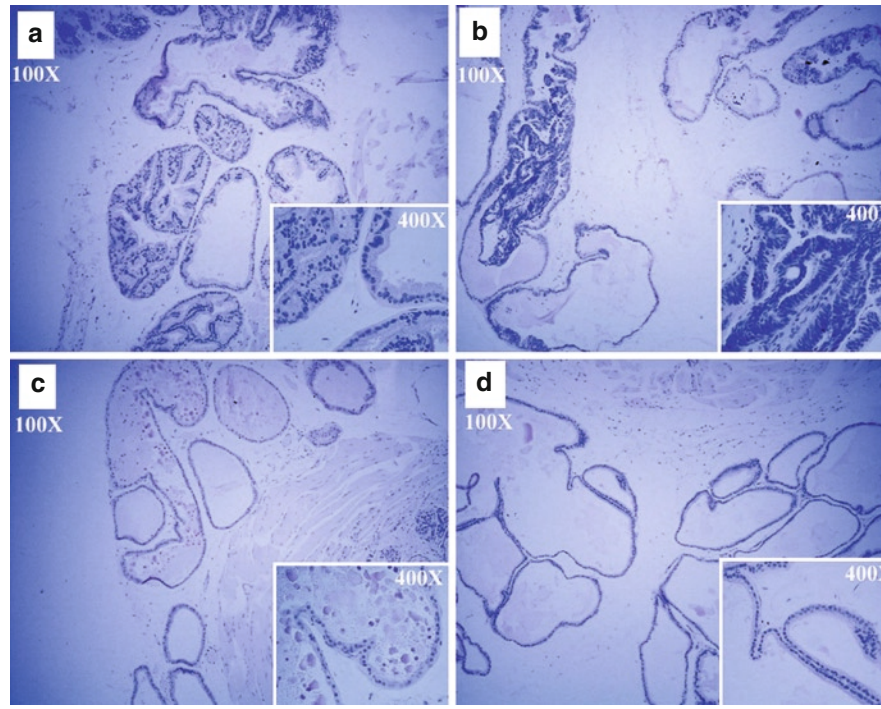

Fig 57.3 WT mice expressing TAg develop low to high grade lesions in both the dorsolateral (**a**) and ventral prostate (**b**), while GHR−/− mice expressing TAg predominately show normal histology in both the dorsolateral (**c**) and ventral (**d**) lobes of the prostate. Reproduced with permission from Wang et al. (2005)

prostate carcinogenesis is androgen independent. In contrast, differential apoptosis and proliferation observed via TUNEL and PCNA staining suggest that increased proliferation and decreased apoptosis were responsible for the increased abundance of precancerous cells (Wang et al. 2005). These data suggest that blockade of GH induced signaling may be an effective method of controlling both androgen dependent and independent prostate tumor progression (Wang et al. 2005). While no prostate cancer phenotype has been described in LS individuals, resistance to the development of prostate lesions in GHR−/− mice appears in agreement with the reduced overall cancer incidence observed in LS individuals (Shevah and Laron 2007).

57.8 GH Induced Signaling and Hematological Neoplasms

Data concerning the effects of alterations of GH induced signaling on the incidence of hematological neoplasms including lymphoma and leukemia are incomplete. Acromegalic patients seem to exhibit increased incidence of leukemia (Au et al. 2000) and other hematological neoplasms including non-Hodgkin's lymphoma (Alves et al. 1998). However, the studies that identified these trends investigated only a small number of subjects, and their results have not been corroborated by subsequent large scale studies (Cohen et al. 2000; Jenkins et al. 2006; Loeper and Ezzat 2008). GH therapy produces less clear effects on leukemia incidences. While one study has shown that GH therapy increased *de novo* development of leukemia (Fradkin et al. 1993), other studies have shown no significant difference as compared to either the general population or control populations (Allen et al. 1997; Nishi et al. 1999). Additionally, GH therapy has not been associated with relapse of leukemia (Clayton et al. 1987; Ogilvy-Stuart et al. 1992; Ogilvy-Stuart and Shalet 1992). However, GH therapy is associated with increased mortality from Hodgkin's lymphoma (Swerdlow et al. 2002). Again, many of the above described results are based on small numbers of subjects, or are refuted by other studies (Cohen et al. 2000; Jenkins et al. 2006; Loeper and Ezzat 2008). As such the true degree of increased cancer incidence and its clinical significance in acromegalic patients or patients undergoing GH therapy remains unknown (Cohen et al. 2000; Jenkins et al. 2006; Loeper and Ezzat 2008).

Data from mouse models are similarly controversial. GH treatment has been shown to improve the engraftment and oncogenesis of human peripheral blood lymphocytes in severe combined immunodeficiency (SCID) mice (Murphy et al. 1992). In contradiction, one study has shown that GH treatment does not increase the incidence of leukemia in a mouse model of leukemia (Bechensteen et al. 1993). This data is partially corroborated by a more recent, larger scale and longer term investigation that has shown that GH treatment does not increase cancer incidences in rats or mice (Farris et al. 2007).

Data gathered from the GHR−/− mouse obtained by Ikeno et al. (2009) suggests that GH induced signaling may promote the development of lymphoma. Histological examination of GHR−/− mice necropsied during a longevity study showed that a lower proportion of GHR−/− mice died from lymphoma as compared to WT controls (Ikeno et al. 2009). No significant reduction in the incidence of lymphoma has not been described in LS individuals (2007).

57.9 GH Induced Signaling and Lung Cancer

The impact of alteration of GH induced signaling on the development and progression of lung neoplasia is not fully understood. Studies of lung cancer patients have shown that single nucleotide polymorphisms (SNPs) of many genes in the GH/IGF-1 axis, including the GHR and IGFBP3, are more common in lung cancer patients than the general population (Moon et al. 2006; Cao et al. 2008). Additionally, elevated circulating IGF-1 has been associated with the development of numerous cancers, including lung cancer (Toniolo et al. 2000). Finally, *in vitro* knockdown or inhibition of GHRH has been shown to inhibit the growth of several lung cancers (Barabutis and Schally 2008; Sacewicz et al. 2008; Schally et al. 2008).

However, despite these apparent trends in epidemiological and *in vitro* data, there is little evidence from patient populations with altered GH induced signaling or mouse models to suggest that gross alterations in GH induced signaling alter the incidence of lung cancer. Data gathered from the GHR−/− mouse via histopathological examination during longevity monitoring has shown that GHR−/− mice exhibit fewer cases of fatal adenocarcinoma of the lung than WT control mice (Ikeno et al. 2009). However, no lung cancer phenotype has been described in LS individuals.

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Reproduction

58

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Core Message

› The creation of the GHR−/− mouse (or Laron mouse) has allowed for the elucidation of GH-specific and GH-dependent IGF1 effects on reproduction. Results obtained from experiments using these mice have confirmed earlier observations in individuals with Laron Syndrome, such as the delayed onset of puberty and morphological differences. The underlying message regarding GH insensitivity with respect to reproduction is that while the lack of GH signaling has effects upon sexual maturation and fertility, eventually the individual attains sexual maturity and is capable of reproduction. This is in stark contrast to the lack of IGF-1 signaling where both male and females are sterile. This difference is most likely due to the effects of local GH-independent IGF-1 production on the respective reproductive organs.

58.1 Introduction

While the growth promoting and metabolic effects of growth hormone have long been known, information regarding the role of GH in reproduction has only been more recently forthcoming. Observation of delayed sexual maturation in men with Laron syndrome provided early evidence of a role for GH in reproduction (Laron 1993). In mice, data suggesting a role for GH in reproduction was gleaned from mouse models of GH deficiency such as the lit/lit and Ames dwarf mice. However, the specific contributions of GH were impossible to determine since these mouse models are also deficient in prolactin (PRL) or PRL and thyroid-stimulating hormone, respectively.

More recent data have come from the creation and characterization of the growth hormone receptor/binding protein (GHR−/−) gene disrupted mouse model (Zhou et al. 1997). Evidence suggesting GH effects on reproduction arose from the observation that the breeding of GHR−/− male to GHR−/− female mice produced significantly smaller litter sizes with increased perinatal and postnatal pup mortality compared to wild-type or heterozygous KO mice (GHR+/−). Subsequent analysis has led to observations that include a delayed onset of puberty in both males and females, and decreased female development and reproductive function. This chapter summarizes the evidence that GH directly and indirectly via IGF-1 plays a physiological role in controlling male and female reproductive development and function.

58.2 Early Observations

The observation that men who suffer from GH resistance due to mutations in the GH receptor (Laron syndrome) experience delayed sexual maturation provided an early indication that GH plays a role in reproduction (Laron 1993; Rosenfeld et al. 1994; Strobl and Thomas 1994). Following the generation of the GHR−/− mouse model, conclusive evidence that GH affects reproduction was obtained (Zhou et al. 1997). Attempts to expand the GHR−/− population via GHR−/− inbreeding yielded

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surprisingly few offspring. While GHR+/+ inbreeding or GHR+/– inbreeding produced, on average, 6–7 pups per litter, GHR–/– inbreeding produced only 2–3 pups per litter. In addition to litter size, the GHR–/– pups experience an increase in perinatal mortality. Pups born from GHR–/– inbreeding have a 26% perinatal mortality rate vs. approximately 5% for GHR+/+ or GHR+/– inbreeding. While the reason for this increased perinatal mortality has not been conclusively demonstrated, observation of the stomachs of GHR–/– neonates suggests that a decrease in milk consumption may play a role in the increased mortality (Zhou et al. 1997).

58.3 Effects of GH on Male Reproductive Development

While early observations indicated that most men affected by Laron syndrome are fertile, their sexual maturation is delayed and they exhibit reduced sexual behavior (Laron 1993; Rosenfeld et al. 1994; Strobl and Thomas 1994). Delayed puberty in males has also been observed in Snell dwarf mice, GHR–/– mice, and rats that were deprived of GH by passive immunization against rat growth hormone releasing factor (GRF) (Hochereau-de Reviers et al. 1987; Arsenijevic et al. 1989; Zhou et al. 1997). Therefore, while the actions of GH are not absolutely required for fertility, GH plays an important role in normal sexual development. This is in contrast to GH's downstream effector, IGF-1, where both male and female mice that carry the IGF1 gene null mutation are infertile (Baker et al. 1996). One example of an effect of GH on male sexual development involves the age of balanopreputial separation. Balano-preputial separation refers to the separation of the prepuce from the glans penis, which is androgen dependent and is used as an external index for the onset of puberty in rodents. The average age at which the prepuce can be retracted in WT is approximately 28 days while retraction in GHR–/– mice is delayed to approximately 32 days (Keene et al. 2002). In humans, GH deficiency is associated with micropenis in men despite normal androgen levels (Laron and Sarel 1970). Humans suffering from GH deficiency also suffer from a loss of sexual desire and erection (Fujita et al. 1997). A similar reduction in copulatory behavior has been observed in male GHR–/– mice (Bartke 2000).

A lack of GH signaling has been shown to have effects on the secretion of hormones necessary for reproductive development. Luteinizing hormone (LH) is produced by the gonadotroph cells of the anterior pituitary gland and is essential for both male and female reproduction. The release of LH from gonadotrophs is controlled by pulsatile releases of gonadotropin-releasing hormone (GnRH) from the hypothalamus. In males, LH acts upon the Leydig cells to produce testosterone, an androgen that then acts upon the Sertoli cells of the testes to stimulate spermatogenesis. Ames dwarf mice have lower basal LH levels than WT mice, while basal LH secretion in GHR–/– mice is similar to that of WT mice (Chandrashekar and Bartke 1993; Chandrashekar et al. 2001). However, the increase in plasma LH levels in response to GnRH treatment is attenuated in GHR–/– mice (Chandrashekar et al. 1999). Since the GHR–/– mice have elevated levels of circulating GH, this result indicates that the attenuated GnRH response is due to a lack of circulating IGF-1. In addition, while plasma testosterone levels are normal in GHR–/– mice, LH-stimulated testosterone release is attenuated (Chandrashekar et al. 2001). GHR–/– mice also have decreased levels of circulating follicle-stimulating hormone (FSH). This hormone is also secreted from gonadotrophs of the anterior pituitary in response to hypothalamic release of GnRH. FSH acts upon testicular Sertoli cells and enhances the production of androgen binding protein which is critical for spermatogenesis (Chandrashekar et al. 2001).

The GH/IGF1 axis has also been shown to affect the development of several structures within the reproductive tract. Keene et al. (2002) have shown that GHR–/– mice have reduced testicular weights compared to WT mice. While the GHR–/– testicular weights were reduced, significant increases in testicular weights occurred in both GHR–/– and WT mice between 35 and 40 days of age. In addition, at 25–30 days of age, there is no significant difference in empty seminal vesicle weights between GHR–/– and WT mice. However, at 35 days of age and thereafter, the seminal vesicle weight of GHR–/– mice is significantly reduced compared to WT mice. WT mice experience an increase in seminal vesicle weight between 30 and 35 days of age. In contrast, not only does this increase occur later in the GHR–/– mice (35–40 days), but the increase is less dramatic (Keene et al. 2002). The GHR–/– mice also have reduced epididymal weights, and the age-related increase in epididymal weights is delayed in GHR–/– mice compared to WT mice. These data indicate that IGF-1 is an important mediator of normal sexual development.

The delay in sexual maturation in GHR–/– mice is also evidenced in the level of spermatogenesis within the testes. At 35 days of age, WT mice exhibit an elongation of spermatids. In contrast, elongated spermatids can be detected in fewer than 50% of GHR–/– mice (Keene et al. 2002). One explanation for the delayed formation of elongated spermatids would be reduced levels of testosterone within the testes. Testosterone and FSH have been shown to act synergistically on spermatogenesis (Kerr et al. 1992; McLachlan 2000). FSH supports the conversion of spermatocytes to spermatids (meiosis) and prevents the premature death of spermatogonial cells and spermatocytes (Sun et al. 1990; Kerr et al. 1992; Russell et al. 1993). The principle action of testosterone is to facilitate the conversion of round to elongated spermatids (Sun et al. 1990; O'Donnell et al. 1994). Indeed, GHR–/– mice have reduced intratesticular testosterone levels, both in absolute amount and amount per mg of testis at 60 days of age (Keene et al. 2002). This implies a lack of GH action on Leydig cells. Along this line, Lobie et al. 1990 have demonstrated that the GHR is expressed in Leydig and Sertoli cells of the testes, and Kanzaki and Morris 1999 have shown that GH stimulates testosterone secretion from isolated Leydig cells. The absence of GH receptors in the GHR–/– mice would therefore preclude normal GH stimulation in these cells. Chandrashekar et al. have also shown that GHR–/– mice have a reduced volume of Leydig cells per testis (Kanzaki and Morris 1999; Chandrashekar et al. 2001). Another potential contributing factor to the delay in male sexual maturation of GHR–/– mice is the observation that these mice have significantly reduced numbers of testicular LH and PRL receptors compared to WT mice (Childs 2000; Chandrashekar et al. 2001). PRL has been shown to increase LH receptor numbers and increase LH action on the testes (Bartke et al. 1978; Bartke et al. 1986). Therefore, even though the GHR–/– mice are hyperprolactinemic, this increase cannot fully restore LH receptor numbers due to the reduced PRL receptor number. In summary, the lack of GH receptors, plasma IGF-1 and the reduced FSH and testosterone secretions in the GHR–/– mice, causes a delay in sexual maturation but does not inhibit final maturation (Fig. 58.1).

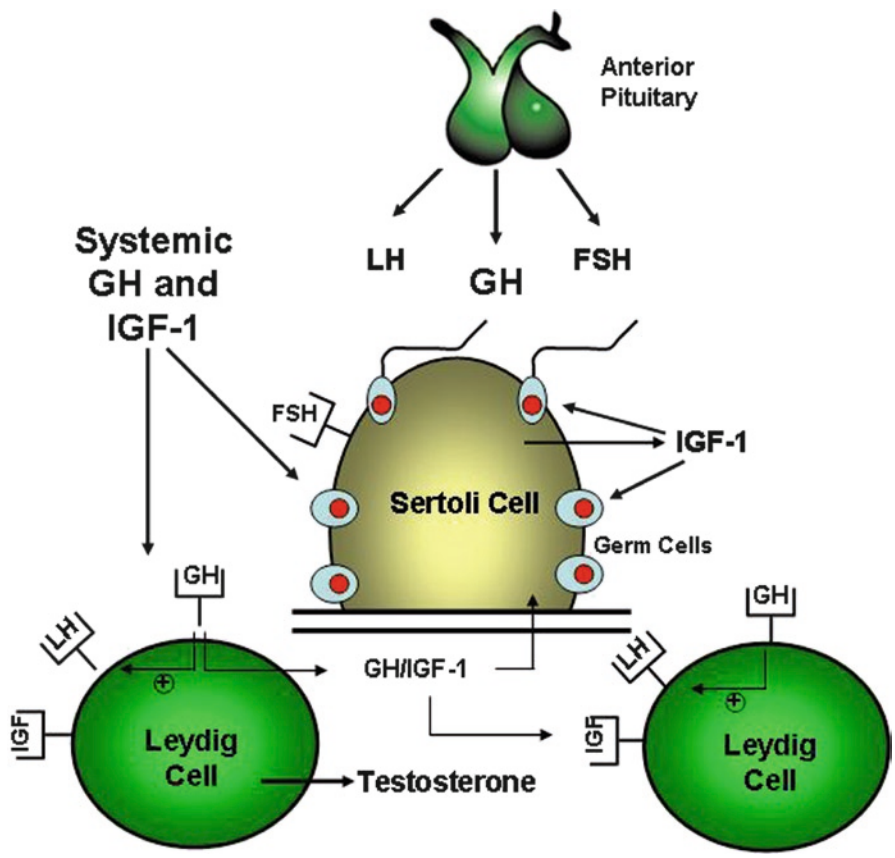

Fig. 58.1 Actions of GH on Leydig, Sertoli, and germ cells (Adapted from Bartke, 2000)

58.4 Effects of GH on Female Reproductive Development

This section will review the current evidence that GH and IGF-1 play a role in female reproductive development and function. Similar to what is seen in males, most females suffering from Laron syndrome are fertile. However, they exhibit a delay in sexual maturation which suggests a role for GH/IGF-1 in reproduction (Laron et al. 1980). Again, the generation of the GHR−/− mouse model has been essential in characterizing GH effects on reproduction (Zhou et al. 1997). In addition to decreased litter size and increased pup mortality, GHR−/− females are delayed in the time of their first pregnancy suggesting delayed puberty. GHR−/− females reach sexual maturity at 10 weeks of age compared to 6 weeks of age for GHR+/+ and GHR−/+ mice (Zhou et al. 1997).

An example of an effect of GH on female sexual development involves the age of vaginal introitus, or opening. Similar to the use of Balano-preputial separation as an external index for the onset of puberty in male rodents, the age of vaginal opening, can be used as an external index for female sexual maturation. The average age of vaginal opening in GHR−/− mice is delayed approximately 7 days compared to WT mice (Danilovich et al. 1999). In addition, the administration of recombinant human IGF-1 to female GHR−/− mice accelerated the vaginal opening by approximately 3 days. GHR−/− females treated with IGF-1 also demonstrated significantly greater uterine weight at the time of first vaginal estrus compared to untreated GHR−/− females. The GH/IGF-1 axis has also been shown to affect mammary gland development. Gallego et al. have demonstrated that GH preferentially activates STAT5 in the stromal compartment of mammary tissue. The lack of GH signaling in the GHR−/− females results in a greatly retarded ductal outgrowth and branching during the development of the mammary gland (Fig. 58.2a, b) (Gallego et al. 2001). However, the reduction in ductal growth is overcome during pregnancy, resulting in the ability of GHR−/− females to nurse their pups.

As mentioned previously, GHR−/− females produce fewer pups per litter than do GHR+/+ or GHR+/− females, with an increase in perinatal mortality (Zhou et al. 1997). Danilovich et al. demonstrated that GHR−/− females carry significantly fewer live fetuses during their pregnancy than do normal females. The reduced number of fetuses was speculated to be due to a reduced ovulatory rate since no increase in fetal mortality was observed. Also, fetal weight and crown-rump length are reduced in GHR−/− mice indicating effects of IGF1 on fetal growth and possible viability (Danilovich et al. 1999). This is similar to that seen in humans where congenital IGF-1 deficiency affects fetal length but does not have a significant effect on fetal weight. In addition, the average length of pregnancy for GHR−/− females is increased compared to WT females as is the placental weight. Considering that the average weight of GHR−/− pups is significantly lower than those born from WT females, it is possible that the increase in gestation and placental weight may represent a means of compensation for the reduced fetal growth due to the lack of IGF-1 or the hypoglycemic state of the mother.

To further characterize the potential of reduced ovulation rates in GHR−/− mice, subsequent investigation focused on the role of the GH/IGF-1 axis on follicular growth and development. Ovulation is dependent upon the growth and development of follicles within the ovary. While the total number of primordial follicles within the ovary becomes fixed early in life, they are continually recruited to the pool of growing follicles until their depletion signals reproductive senescence (McGee and Hsueh 2000). Primordial follicles develop

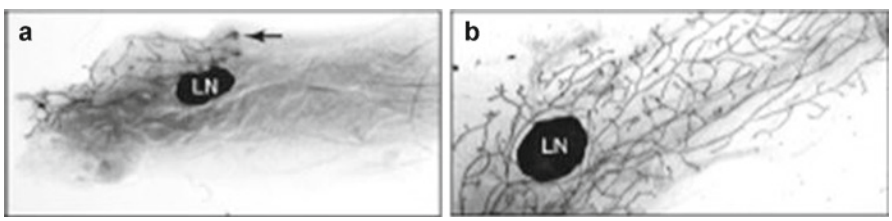

Fig. 58.2 Mammary development and function in GHR−/− mice. Whole-mount analysis of ductal outgrowth and branching in GHR−/− mammary glands (**a**) and corresponding WT mice (**b**). The *arrow* in (**a**) shows how far the epithelial tree has grown. Magnification is 10×. *LN* lymph node

into primary and secondary follicles prior to the formation of the antrum, a cavity that forms within the follicle. This preantral follicular development occurs prior to the onset of puberty, in a gonadotropin-independent manner, and can be referred to as the "initial" follicular recruitment (Hirshfield 1991; McGee and Hsueh 2000). The onset of puberty and reproductive cycling result in an increase in circulating FSH. The increased FSH serves to "recruit" a number of antral follicles for further maturation. During this maturation process, few follicles reach the ovulatory stage. The majority of recruited follicles undergo follicular atresia, an apoptotic process that results in their degeneration and subsequent resorption (Hsueh et al. 1994). In response to the release of LH from the anterior pituitary during ovulation, mature follicles on the surface of the ovary rupture and release the oocyte for fertilization. The portion of the follicle that remains in the ovary is transformed into the corpus luteum, a glandular structure that secretes progesterone to maintain pregnancy.

GH and IGF-1 receptors, as well as IGF-1 are widely expressed within the ovary (Levy et al. 1992; Adashi et al. 1997; Monget and Bondy 2000; Slot et al. 2006). Baker et al. (1996) demonstrated that IGF-1 is required for reproduction since IGF-1 gene-disrupted female mice are sterile and fail to ovulate. The analysis of ovulation in GHR−/− female mice has similarly shed light on the effects of GH on reproduction. The number of preimplanted eggs observed soon after fertilization and vaginal plug formation, as well as the uterine implantation rate are decreased in GHR−/− females compared to WT females (Bachelot et al. 2002; Zaczek et al. 2002). As expected, the number of corpora lutea observed in ovarian sections was also decreased in these GHR−/− females. In addition, superovulation of GHR−/− females with exogenous gonadotropin increased the number of eggs produced, but the GHR−/− females still produced fewer eggs than WT females. This indicates that the reduced ovulation is due to an ovarian defect rather than a lack of ovarian responsiveness to gonadotropin (Bachelot et al. 2002). Also, the fertility or ovarian response to exogenous gonadotropin of GHR−/− females was not restored by IGF1 treatment indicating that these effects of a lack of GH are independent of IGF-1.

The decreased ovulation rate in the GHR−/− mice can be explained by the impaired growth and development of the ovarian follicles. Examination of ovaries isolated from GHR−/− females indicated that while all categories of follicles are present within the ovary, there is a significant decrease in the numbers of healthy follicles in the antral and preovulatory stages (Bachelot et al. 2002; Zaczek et al. 2002). Slot et al. (2006) subsequently demonstrated that ovaries from GHR−/− females contain a higher number of primordial follicles compared to WT ovaries. A significantly greater percentage of follicular atresia was also observed in the GHR−/− ovaries. Interestingly, treatment of GHR−/− females with IGF-1 resulted in a decrease in the number of primordial follicles and a concomitant increase in the number of healthy antral follicles. This result suggests that GH-dependent IGF-1 plays a role in the recruitment and growth of primordial follicles possibly by decreasing the rates of follicular atresia and thereby increasing follicle survival. Along this line, ovaries from GH transgenic females have an increased number of healthy preovulatory follicles with a corresponding decreased level of follicular apoptosis (Danilovich et al. 2000).

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Conclusions and Future Studies

59

John J. Kopchick, Hiroyoshi Tanda, Darlene E. Berryman, and Zvi Laron

Core Message

› Individuals with LS and GHR–/– mice allow for an interesting comparison between a human genetic condition and genetically manipulated mice. Clearly, much caution has to be used in direct comparison not only because of species differences but also as various factors including age, gender, and other environmental influences (such as pharmaceutical management and diet) can alter the findings. Importantly, the similarities and differences can provide insight into important biological processes in a growth hormone-resistant state. For example, the increased longevity in GHR–/– mice, but not as yet determined in Laron individuals, could provide useful information about aging. Further, the more thorough understanding of the similarities and differences between LS and GHR–/– may aid in the treatment and in the understanding of the physiological and molecular basis of LS individuals. Finally, a compilation of the data in this book from a selected set of LS patients in combination with data from the GHR–/– mouse provides a valuable reference resource.

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59.1 Introduction

Laron syndrome and the GHR–/– mouse are unique models to study the effects of IGF-I deficiency in the presence of blocked GH activity. Additionally, the effects of IGF-I replacement therapy in these mice and patients will provide important physiological data. In this regard, information on the physiology and pharmacological effects of IGF-I have been gathered during short and long-term IGF-I treatment of the LS patients. In chapters 1–48, we documented various physiological, genetic, and clinical issues related to the cohort of Israeli LS patients. While some of the results have been published, a significant portion of the accounts were derived from compilations of patient charts, notes, and communications. A major objective of this book was to document this collection of human data. In Chaps. 49–59, we described results related to GHR–/– mice. Most of the mouse data have been published.

As we know, it is much easier to obtain controlled experimental results from mice vs. man. Coupled with the relative scarcity of LS patients, controlled experiments in LS patients are often not possible. Thus, the second objective of this book was to compare and contrast results between LS patients and GHR–/– mice in the hope of gleaning information that may serve as a guide for future use in LS patients.

We have generated a table depicting various parameters comparing LS patients to GHR–/– mice (Table 59.1). Significant similarities along with many

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Table 59.1 Comparison of various parameters between Laron patients and GHR−/− mice

Characteristic	Laron patients	GHR−/− mice	Additional notes
Sexual development and reproduction			While sexual development in both are delayed, maturity is eventually attained and both are reproductively competent
Age of puberty onset	↑	↑	
Genital size	↓ (male)	↓ (male)	GHR−/− female mice demonstrate a reduced ovulation rate due to impaired growth and development of ovarian follicles
Gonad size	↓ (male)	↓ (male)	
Fetal length	↓	↓	
Fetal weight	↔	↓	
Gestation period	↔	↑	
Lactation	↔	↔	
Perinatal mortality	↔	↑	
Energy balance and obesity			Total energy intake is decreased, but energy intake normalized to body size is either not different from controls or elevated
Energy expenditure	↔	↓	
Energy intake	↓	↓	
Incidence of obesity	↑	↑	Depot-specific differences in fat accumulation have not been well evaluated in LS individuals
Percent body fat	↑	↑	
Subcutaneous fat	↑	↑	
Adipocyte cell size	↓	↑	
Hormone levels and glucose metabolism			
Glucose	↓ (young)	↓ (young)	In LS there is no data for glucagon levels. Glucagon secretion in response to arginine infusion is normal. Normal TSH, total and free T4 in LS. Low T3 and T4 in GHR−/− female mice
	↔ (adult)	↔ (adult)	
Glucose tolerance	↔ (↓ young)	↓	
Insulin	↑	↓	Insulin levels in LS are low compared to the degree of obesity
Insulin sensitivity	↓	↑	
Aldosterone	N/A	↔	
Glucagon	↔	↓ (young)	
		↔ (adult)	
Glucocorticoids	N/A	↑ (males)	
		↔ (females)	
Renin	N/A	↓	
Adiponectin	↑	↑	
Ghrelin	↓	↔	
Leptin	↑	↑	
Prolactin	↑	↑	
Thyroid hormones	↔	↓ (females)	
Lipids			LS individuals tend to develop NAFLD
Apolipoprotein B	N/A	↓	

Table 59.1 (continued)

Characteristic	Laron patients	GHR−/− mice	Additional notes
Free fatty acids	↑	↔	
Liver triglycerides	↑/↔	↑	
Lp (a)	↔	N/A	
Total cholesterol	↓ (young)	↓	
	↑ (adult)		
HDL-cholesterol	↔/↓ (young)	↓/↔	
LDL-cholesterol	↑ (adult)	↓	
Triglycerides	↔ (adult)	↔	
Clinical chemistry			
Alanine aminotransferase (ALT/GPT)	↔	↑	
Aspartate aminotransferase (AST/GOT)	↔/↑	N/A	
Albumin	N/A	↑	
Alkaline phosphatase	↔/↓ (young)	↔	
Bilirubin	N/A	↔	
Creatine kinase (CPK)	↔	↑	
Creatinine	↔/↓ (young)	↔	
Calcium	↔	↔	
Chloride	↔	↑	
Gamma-glutamyltransferase	↑ (males)	N/A	
Lactate dehydrogenase	↔ (females)	↔/↑	
Phosphate	↔	↔/↓	
Potassium	↔/↓	↔	
Sodium	↔	N/A	
Total protein	↔	↔	
Urea	N/A	↑	
Uric acid	N/A	↔	
Muscle			Muscle fiber type is switched to slow fiber in GHR−/− mice in one study, but unchanged in another study
Muscle mass	↓	↓	
Muscle capacity	↓	N/A	
Muscle force	↓	N/A	
Muscle endurance	↓	N/A	
Muscle fiber size	N/A	↓	
Muscle fiber type	N/A	Inconclusive	
Muscle IGF-1 production	N/A	↓ (mRNA)	
		↔ (protein)	
Muscle insulin sensitivity	↓	↑	

(*continued*)

Table 59.1 (continued)

Characteristic	Laron patients	GHR−/− mice	Additional notes
Cardiovascular function			Systolic pressure is reduced by 25% in female GHR−/− mice at 4 months The risk for cardiovascular disease in LS individuals is inconclusive, with a few cases reported of congenital heart disease, coronary heart disease, and myocardial infections
Blood pressure	↔	↓	
Cardiac function	↔	↔	
Cardiac size and dimensions	↓	↓	
Blood vessel endothelial function	↔	↔	
Risk of cardiovascular disease	Inconclusive		
Neurology			Hearing loss is not detected in young LS patients treated with IGF-1 Several patients with LS are mentally retarded, and others show low normal IQ while still normal in others. Major brain structural defects are present in some patients, while normal in others
Brain size	N/A	↑	
Intelligence	↓/↔	N/A	
Hearing loss	↑	N/A	
Neurological abnormalities	↑	↔	
Ocular abnormalities	↑	N/A	
Longevity	↔	↑	LS does not seem to influence lifespan, especially when metabolic and cardiovascular complications remain untreated. However, the protection from cancer may positively influence lifespan
Cancer			
Breast	↓	↓	
Lung	↓	↓	
Lymphoma	N/A	↓	
Overall cancer incidence	↓	↓	
Prostate	↓	↓	
Pituitary	N/A	↓	
Bone			Osteopenia is found in both LS individuals and GHR−/− mice BMD is reduced in LS individuals and GHR−/− mice
Bone mineral density		↓	
Cranial base size	↓	↓	
Tooth development and size	↓	↓	

differences exist between the human and mouse data. We have summarized some of these points below.

59.2 Sexual Development and Reproduction

Characteristics of sexual development and reproduction in LS patients and GHR−/− mice have many similarities. The average age of puberty in LS patients and GHR−/− mice is older and genital and gonad size in both human and mouse males are reduced, although sexual maturity is reached. While sexual development is similar between LS and GHR−/− mice, there are differences in reproductive characteristics. Reproduction in LS patients is similar to normal individuals with the exception of fetal length, but GHR−/− mice differ from control mice. In the GHR−/− mouse, fetal length and fetal weight are reduced, while the gestation period and perinatal mortality are increased. Future research may determine whether additional traits that have been demonstrated in the GHR−/− mouse model are seen in LS patients. For example, GHR−/− female mice have a decreased ovulation rate due to an impaired growth and development of the ovarian follicles.

59.3 Energy Balance and Obesity

Individuals with LS syndrome and GHR–/– mice are obese despite their dwarf size. This increased obesity does not appear to be due to decreased energy expenditure or increased energy intake in either group as would be expected, suggesting a unique physiological state or a significant alteration in nutrient partitioning. It is hypothesized that one major cause may be the impairment of GH regulation of adiponectin. Additional studies including the impact of macronutrient manipulation in GHR–/– mice, assessment of absorptive capacity in either group, or a more thorough assessment of energy intake or energy expenditure in LS individuals could offer additional insight. Interestingly, data from GHR–/– mice suggest that not all adipose depots respond to the lack of GH action in a similar manner with the subcutaneous depot specifically and notably enlarged. LS individuals have notable increases in subcutaneous fat as well although a systematic comparison of the accumulation of fat mass in various depots has not been done. This unique distribution of adipose tissue should be further explored. For LS individuals, there is a need to use additional techniques, such as MRI, to evaluate the adipose tissue distribution. In GHR–/– mice, the differential inflammatory properties, glucose/lipid uptake, insulin response, and the endocrine properties of the individual depots could be more fully explored. Of note, the only reported difference between LS and GHR–/– is in adipocyte cell size of the subcutaneous depot, which are reduced in LS but it is increased in GHR–/– mice.

59.4 Hormone Levels, Glucose Metabolism, and Clinical Chemistry

Glucose levels and glucose tolerance are reduced in both groups. However, differences between the groups exist for insulin levels and insulin sensitivity. LS patients have relatively high insulin levels compared to the glucose levels and are insulin insensitive, while GHR–/– mice have lower insulin levels and are highly insulin sensitive. As far as other hormone levels are concerned, LS patients and GHR–/– mice share several similarities, but also have differences. Specifically, LS patients and GHR–/– mice have differing concentrations of glucagon, serum ghrelin, and thyroid hormones compared to controls. Serum ghrelin levels are lower in LS patients, while glucagon secretion and thyroid hormones in GHR–/– mice are lower. Of note, adiponectin and leptin are the only two adipose-derived endocrine products that have been assessed in both LS individuals and GHR–/– mice. They show a similar trend in both; that is, both are extremely elevated. While the high leptin levels are anticipated, the high adiponectin levels are somewhat novel, as typically this hormone is associated with increased insulin sensitivity and decreased adiposity. Thus, exploring the unique regulation of adiponectin could be valuable in understanding this seemingly beneficial hormone.

Lipid characteristics widely vary between LS patients and GHR–/– mice. Free fatty acids, liver triglycerides, and cholesterol differ in some degree, while only total triglyceride levels are similar. Free fatty acid levels are elevated in LS, while they are normal in the GHR–/– mouse. Liver triglycerides are elevated in some LS patients and normal in others, and they are elevated in GHR–/– mice. Total and LDL cholesterol is lower in LS patients during childhood, but increase above normal levels in adulthood, contrasting with reduced levels in GHR–/– mice.

Overall, the reduced insulin insensitivity in LS patients is consistent with high levels of FFA, total and LDL cholesterol, high liver triglyceride content, and high percentage of fat mass. On the other hand, the mechanisms leading to high insulin sensitivity, normal FFA, and low cholesterol in GHR–/– mice in spite of increased fat accumulation and fatty liver are not clear. Many studies have looked at the activation of insulin signaling cascades in organs such as liver, muscle, and heart in GHR–/– mice but the available data is inconclusive. Given the age-dependent changes observed in some metabolic parameters, especially glucose levels, a thorough analysis of insulin responsiveness in different organs and developmental stages might provide insight. In addition, the study of adipose tissue may provide critical information as well, as it is one of the major sites of glucose uptake and it plays a key role in metabolic regulation. Interestingly, some adult LS patients develop glucose intolerance and type 2 diabetes mellitus with all its vascular complications, while we have not seen this in GHR–/– mice.

59.5 Muscle

Similar to LS individuals, GHR–/– mice have reduced muscle mass, which is most likely due to smaller muscle fiber size. Although not reported, it is reasonable to

speculate that GHR−/− mice have decreased muscular function including muscle capacity, force, and endurance, which are the characteristics of human LS. GHR−/− mice show better insulin sensitivity in the muscle, which may be one contributor of increased insulin sensitivity and lifespan of this mouse model. Study on the muscular system of GHR−/− mice provides a unique opportunity to unravel the mechanism of GH action as well as its interaction with other known effectors of muscle. For example, GH is found to promote the fusion of myotubules, thereby increasing muscle fiber size, and because of lack of GHR, GHR−/− mice have markedly smaller muscle fibers. Future studies using GHR−/− mice may lead to therapeutic strategies to treat patients with muscle dystrophy.

59.6 Cardiovascular Function

Cardiovascular characteristics in LS patients and GHR−/− mice have several similarities and differences. Evaluation of echocardiography measurements showed no major abnormalities in the cardiac function of LS patients and GHR−/− mice, despite their reduced heart dimensions. In addition, endothelial function is normal in both LS patients and GHR−/− mice. Blood pressure measurements indicate that systolic blood pressure is reduced by 25% in 4-month-old female GHR−/− mice; in contrast, the systolic and diastolic functions are intact in LS patients. Although the specific mechanisms underlying the reduced systolic blood pressure observed in GHR−/− mice are still unclear, preliminary results suggest that upregulation in endothelial NO synthase (eNOs) expression by large vessels of GHR−/− mice may contribute to this effect. Future studies are needed to clarify this issue. Another interesting aspect is that the risk of cardiovascular disease seems to be reduced in GHR−/− mice, despite their obesity, while studies are currently inconclusive in LS patients. Thus, it will be worthwhile to investigate the mechanisms linking GH insensitivity and cardiovascular health/disease. Recently, studies highlighted the role of GH in modulating the expression of coagulation inhibitors in a model of pulmonary embolism. Thus, the thrombotic evaluation of GHR−/− mice will aid in redefining the role of GH/IGF-I in thrombosis-related diseases and will lead to the discovery of novel therapeutic strategies to treat cardiovascular disease. It is of note that untreated Laron syndrome patients have an increased number of platelets, which correlates with the thrombopoietin levels.

59.7 Cancer

Overall, cancer incidence is reduced in LS patients and GHR−/− mice. Abrogation of the GH/IGF-I signaling appears to protect both mice and humans from cancer development and progression. GHR−/− mice exhibit statistically significant reductions in breast and prostate cancer. In addition, reduced incidence of lymphoma and pituitary adenomas has been reported in GHR−/− mice. Although these phenotypes have not been totally confirmed in LS patients, ongoing work by Dr. Zvi Laron and colleagues has demonstrated that LS patients do exhibit resistance to malignancies. Future clinical and experimental studies in both humans and mice are needed to fully clarify these findings.

59.8 Neurology

Research on LS patients indicates both ocular and auditory abnormalities. Investigations in GHR−/− mice should be undertaken, as neither of these topics has been researched in those animals. Useful studies would analyze ocular growth and development in GHR−/− mice. Research to assess vision and hearing in GHR−/− mice would also be helpful. There are cases of LS patients with neurological defects and disease that include epilepsy, enlarged ventricles, diabetic neuropathy, one patient with a mental illness and one patient with a severe mental deficiency. Relevant research from GHR−/− mice is limited on this topic, but shows enlarged brain size (relative to body weight), structural abnormalities of the pituitary, increased cholinergic neuron density, and a resistance to age-related decline in memory. Intelligence studies of LS patients show a wide spectrum of IQs. Several patients with LS are mentally retarded, some show low normal IQ, while others test in the normal range. Further, major structural defects of the brain are present in some patients, while others appear normal. The unpredictable nature of brain

structure and intelligence in LS patients is difficult to explain based solely on GHR mutations. Data from GHR−/− mice do not show gross structural abnormalities of the brain, and in fact, GHR−/− mice are resistant to age-related decline in memory seen in wild type control animals. It would be of interest to further evaluate brain structure and function in GHR−/− animals.

59.9 Longevity

Present knowledge shows that lifelong deficiency of IGF-I in untreated patients with LS does not seem to markedly influence lifespan unless metabolic and cardiovascular complications, such as in diabetes mellitus and the hyperlipidemia are neglected. This is in contrast to the greatly increased lifespan observed in GHR−/− mice. Of note, overall cancer incidence is reduced in both LS patients and GHR−/− mice. Since metabolic and cardiovascular complications are increased in LS patients and may contribute to mortality, protection from cancer observed in LS patients may balance out the negative aspects of obesity-related morbidities. One of the likely mechanisms for extended lifespan in GHR−/− mice involves increased insulin sensitivity and decreased levels of insulin. Since humans with LS have decreased insulin sensitivity and relatively increased levels of insulin, it is likely that the difference in lifespan (extended in mice but not yet ascertained in humans with LS) is due in large part to insulin sensitivity. In this regard, IGF-I treatment of Laron syndrome patients improves insulin sensitivity and is approved for use in LS individuals. Based on the GHR−/− mice (and many other animal models in which GH, IGF-1, and insulin are decreased), it may also hold true that greatly extended lifespan may be possible in LS patients if insulin levels can be reduced and maintained at low levels.

Since GHR−/− mice are obese but remain insulin sensitive, it would be of interest to determine if decreasing adiposity further extends an already extended lifespan. However, GHR−/− mice that are placed on caloric restriction do not exhibit a further extension in lifespan. Since caloric restriction is a fairly extreme dieting technique, placing GHR−/− mice on a less stringent diet (i.e., just enough reduction in calories to decrease obesity) may be more beneficial than standard caloric restriction diet regimes at extending lifespan in these mice. Furthermore, since insulin sensitivity is thought to be a primary mechanism responsible for extending lifespan in GHR−/− mice, it would be of interest to remove the GHR tissue specifically in key insulin sensitive organs (such as muscle, liver, and fat). Evaluating the crosstalk between the GH, IGF-I, and insulin signaling in the various organs of these mice, and determining the individual contributions of each organ to overall insulin sensitivity and lifespan would help elucidate how the absence of GHR leads to extreme lifespan in the GHR−/− mice. Furthermore, an understanding of organ-specific contributions to insulin sensitivity and the role of GHR may prove to be vital in future development of novel therapies that selectively and tissue specifically resolve insulin resistance in individuals with LS as well as the ever growing non-LS population of individuals with insulin resistance.

59.10 Bone

LS individuals and GHR−/− mice demonstrate clear effects of GH on long bone growth. Both chondrocyte generation and hypertrophy are affected in GHR−/− mice, whereas only chondrocyte hypertrophy is the major defect in IGF-1−/− mice, suggesting differential roles of GH and IGF-1 regulation in bone growth. Craniofacial bone growth is also similarly affected in LS individuals and in GHR−/− mice. Hypodontia, microdontia, and delayed tooth eruption are observed in LS individuals. Crown width, root length, and dentin thickness are similarly affected in GHR−/− mice. Bone mineral density and bone mineral content has been reported to be significantly reduced both in LS individuals and in GHR−/− mice. However, estimated volumetric bone mineral density indicated that there is no difference in bone mineral density in LS individuals. Since the very similar effects of GH on bone development has been demonstrated both in LS individuals and GHR−/− mice, results from detailed study using mouse model may be directly applicable to the assessment of LS individuals.

59.11 Effects of IGF-I treatment

The human data provides important information gathered from short and long-term IGF-I administration. IGF-I strongly stimulates longitudinal growth in the absence of hGH action; however, when compared to

the hGH effect in children with isolated GH deficiency, the growth stimulating effect is weaker. The greatest and fastest catch-up growth is seen in the head circumference (brain growth) denoting the important role IGF-I plays in the CNS development. IGF-I also increases the organomicria and muscle force.

Despite its insulin lowering effect, increase in insulin insensitivity and improved glucose metabolism, IGF-I treatment has an adipogenic effect, leading to increased obesity of the treated Laron syndrome patients. The progressing obesity is indeed a major adverse effect of IGF-I treatment. Data on IGF-I treated GHR−/− mice are still lacking.

Appendix

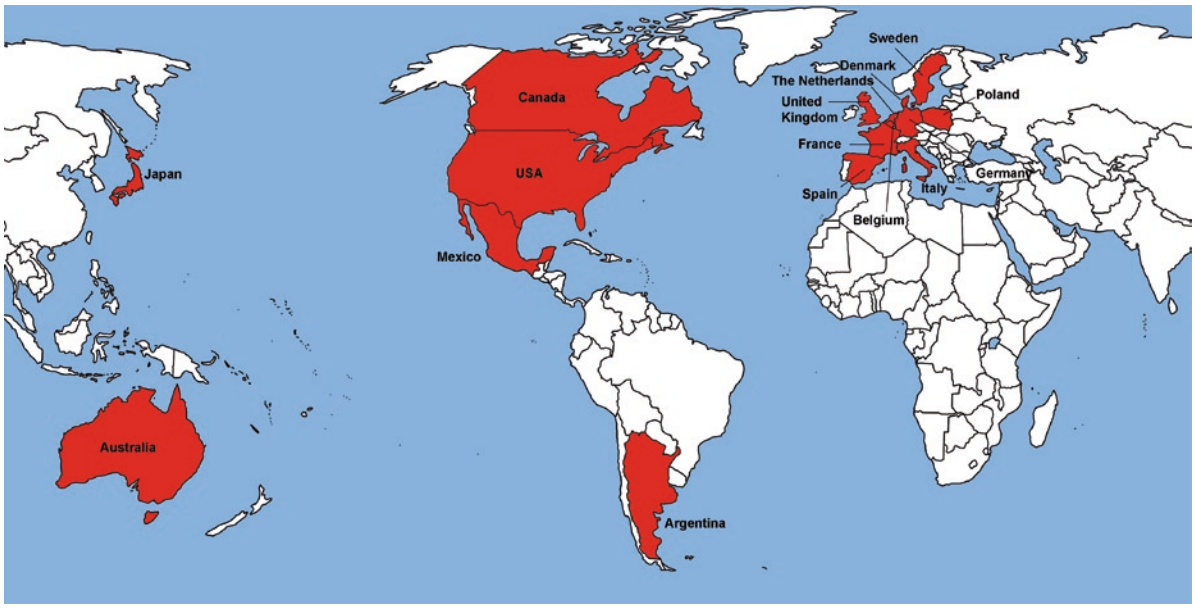

Fig. 1 World map denoting (in red) countries where the GHR–/– is used in a variety of research projects

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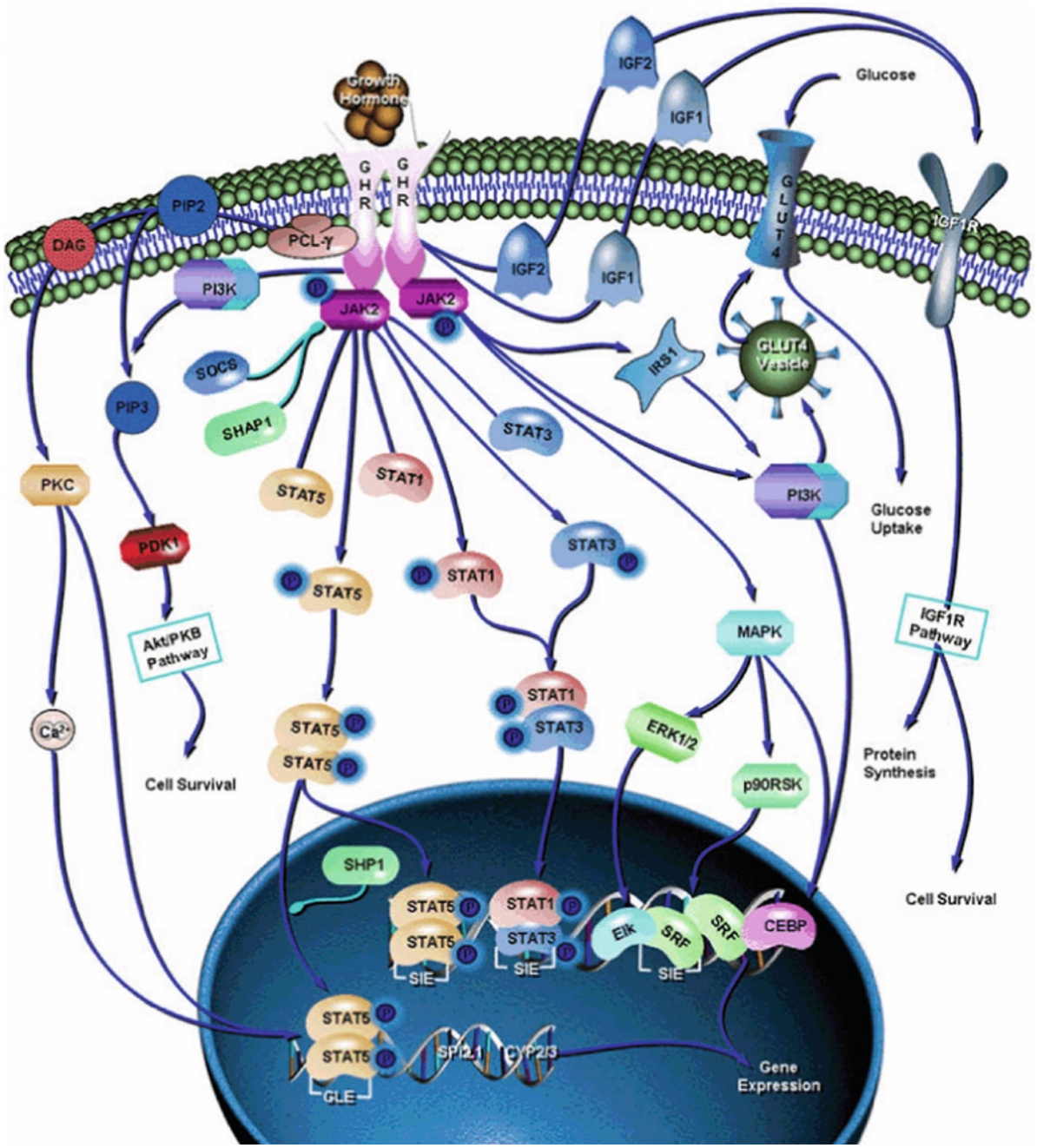

Fig. 2 **Growth Hormone Signaling Pathway**. Upon GH binding, the GHR homodimer becomes activated, resulting in phosphorylation and activation of JAK2. JAK2 then goes on to phosphorylate and activate a number of other signaling pathways including those of STAT, PI3K, and MAPK, which ultimately result in GH's physiological effects. *GH* growth hormone; *GHR* growth hormone receptor; *JAK2* Jjanus Kinase 2; *PLCγ* phospholipase C γ; *PIP2* phosphatidylinositol bisphosphate; *DAG* diacylglycerol; *PIP3* phosphatidylinositol (3, 4, 5)tri-phosphate; *PKC* protein kinase C; *CRAC* calcium release-activated channel; *SHP-1* SH2 domain-containing protein tyrosine phosphatase 1; *SOCS* suppressor of cytokine signaling; *IRS-1* insulin receptor substrate 1; *STAT5* signal transducer and activator of transcription-5 Adapted from SA BioSciences (www.SABiosciences.com)

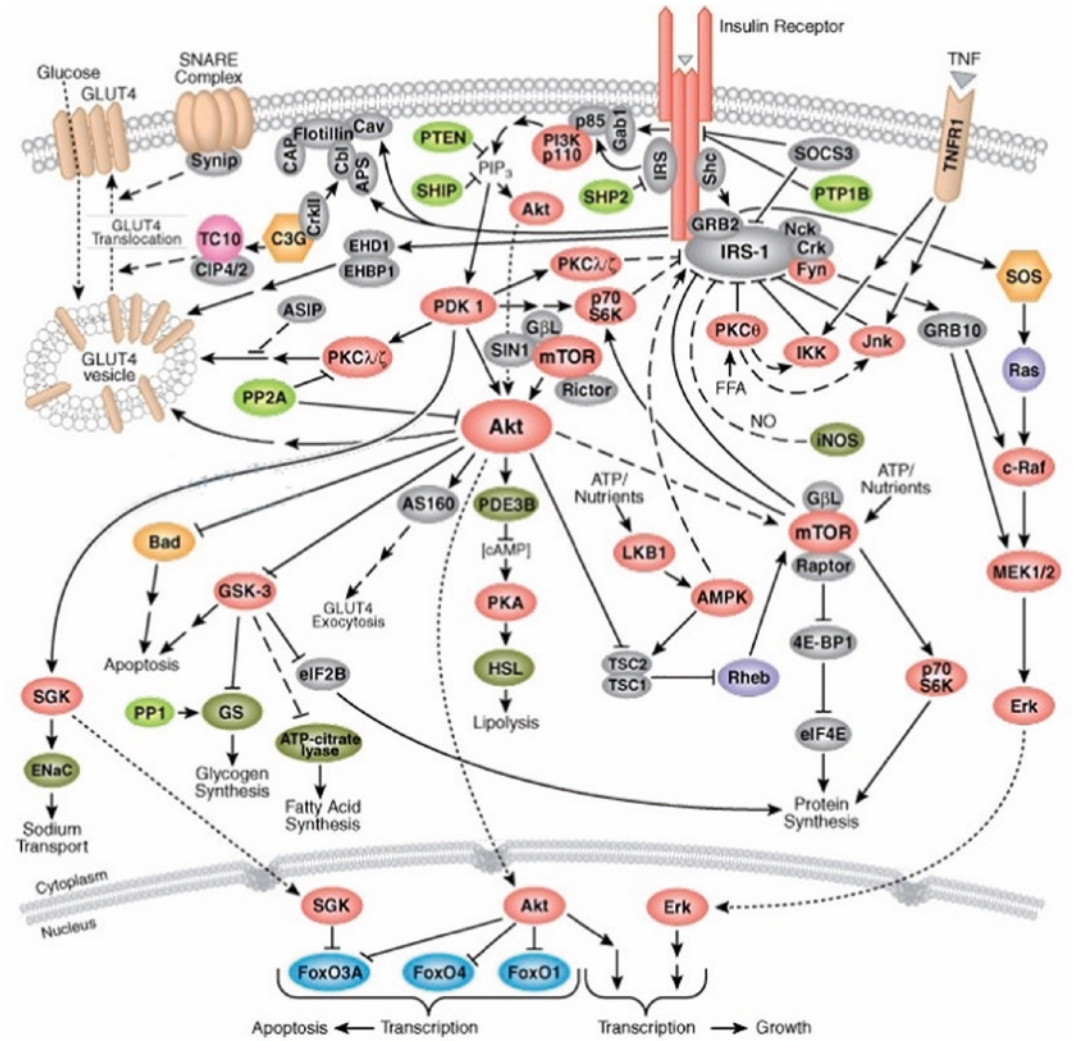

Fig. 3 **Insulin Signaling Pathway**. Insulin is the major hormone controlling critical energy functions including glucose and lipid metabolism. Upon binding insulin, the insulin receptor becomes activated and tyrosine phosphorylates and recruits different substrate adaptors including the IRS family of proteins. Tyrosine phosphorylated IRS then displays binding sites for numerous signaling partners. Among them, PI3K has a major role in insulin function mainly via the activation of the Akt/PKB and the PKCz cascades. Activated Akt induces glycogen synthesis through inhibition of GSK-3, protein synthesis via mTOR and downstream elements, and cell survival through inhibition of several pro-apoptotic agents (Bad, Forkhead family transcription factors, GSK-3). Insulin stimulates glucose uptake in muscle and adipocytes via translocation of GLUT4 vesicles to the plasma membrane. GLUT4 translocation involves the PI3K/Akt pathway and IR mediated phosphorylation of CAP and formation of the CAP:Cbl:CrkII complex. Insulin signaling also has growth and mitogenic effects that are mostly mediated by the Akt cascade as well as by activation of the Ras/MAPK pathway. A negative feedback signal emanating from Akt/PKB, PKCz, p70 S6K and the MAPK pathways results in serine phosphorylation and inactivation of IRS signaling. {{110 Cell Signaling Technology 2008}}. *Akt* AKT8 virus oncogene cellular homolog; *APS* Adapter protein with a PH and SH2 domain; *ASIP*, Atypical PKC isotype-specific interacting protein; *CAP* c-Cbl-associated protein; *Cav* Caveolin; *CBL* Cellular homologue of Cas NS-1 oncogene; *CRK* CT10 sarcoma oncogene cellular homolog; *EHD1* EH domain-containing 1; *Erk* Extracellular signal-regulated kinase; *FFA* Free Fatty Acids; *FoxO1,3,4* Forkhead box O1, O3A, O4; *Fyn*, A Src family tyrosine-protein kinase; *Gab1* GRB2-associated binder-1; *GLUT4* Glucose transporter type 4; *GRB* Growth factor receptor-bound protein; *GS/GSK* Glycogen synthase kinase; *HSL* Hormone Sensitive Lipase; *IKK* IκB kinase; *iNOS* Nitric oxide synthase, inducible; *IRS* Insulin receptor substrate; *JNK* c-Jun N-terminal kinase; *MAPK* Mitogen-activated protein kinase; *MEK* MAPK/Erk kinase; *mTOR* Mammalian target of rapamycin; *Nck* Nck adaptor protein; *PDE3B* cGMP-inhibited 3',5'-cyclic phosphodiesterase B; *PGC1α* peroxisome proliferator-activated receptor gamma co-activator; *PI3K* Phosphoinositide 3-kinase; *PIP2* Phosphatidylinositol 3,4-bisphosphate; *PIP3* Phosphatidylinositol 3,4,5-trisphosphate; *PKA* Protein kinase A; *PKC* Protein kinase C; *PLC* Phospholipase C; *PTEN* Phosphatase and tensin homologue; *PTP1B* Protein phosphotyrosylphosphatase 1B; *Shc* SH2-containing collagen-related proteins; *SHIP*, SH2-containing inositol phosphatase; *SHP1* SH2-containing phosphatase 1; *SHP2* SH2-containing phosphatase 2; *SOCS* Suppressor of cytokine signaling; *SOS* Son of sevenless guanine nucleotide exchange factor. Pathway provided courtesy of Cell Signaling Technology, Inc. www.cellsignal.com

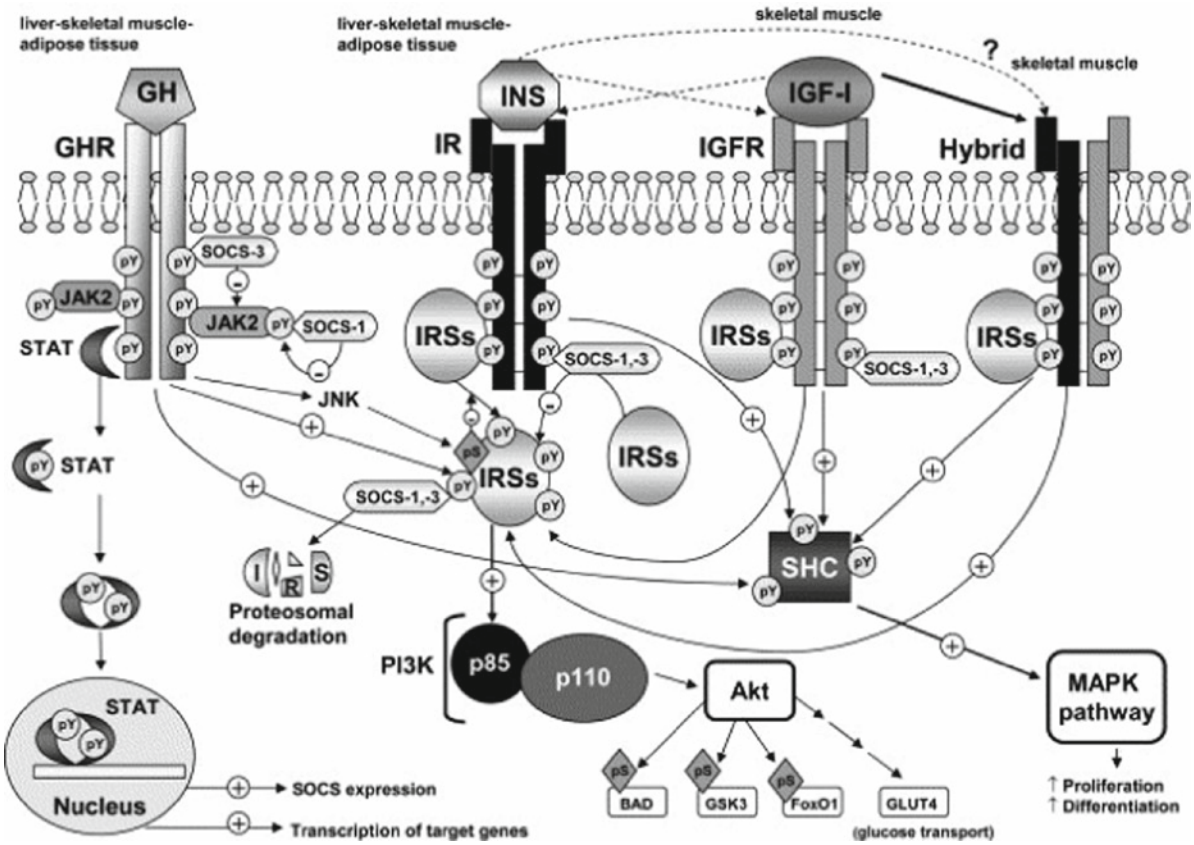

Fig. 4 Relationships between GH, IGF-I, and insulin signaling. Insulin and IGF-I binding to their cognate receptors result in tyrosine autophosphorylation in the receptor β subunits, which activates the tyrosine kinase and recruits the IRS proteins for tyrosine phosphorylation. Recruitment is regulated by Ser phosphorylation, which inhibits the interaction between IRSs and the phosphorylated receptor. Tyrosine phosphorylation of IRS-1 and IRS-2 recruits and activates various SH2 domain-containing proteins including the p85 regulatory subunit of PI3K, which activates the Akt cascade. Engagement of SHC is expected to activate the downstream MAPK cascade. The relative contribution of SHC and IRS proteins to this cascade appears to be cell and tissue specific. GH-induced dimerization of the GHR leads to activation of JAK2 and phosphorylation of several cytosolic proteins, including the IRS proteins and SHC. The regulation of gene transcription by GH involves two major signalling pathways: one mediated by the MAPK cascade and the other by the STAT proteins (mainly STAT5b) which after being phosphorylated by JAK2, dimerize and translocate to the nucleus, inducing the transcription of several genes including those encoding for IGF-1, ALS, and IGFB-3 (that comprise the major circulating IGF-I complex) and those of SOCS. SOCS-1 and -3 modulate the signalling potential of IRS via at least three potential mechanisms of inhibition. In the context of signalling via the IR/IGFR, SOCS introduce competition for receptor binding sites, inhibit JAK2 activity and induces proteosomal degradation of IRS. Insulin and IGF-I are capable of binding to the IR and the IGFR but with low affinity (indicate in dotted lines). The insulin sensitizing effect of IGF-I appears to be mediated mainly by hybrid receptors (Hybrid) that are present in large amounts in skeletal muscle (Dominici 2005). *Akt* v-Akt Murine Thymoma Viral Oncogene homolog; *BAD* BCL2-associated agonist of cell death; *FOXO1* forkhead box O1; *GH* growth hormone; *GHR* growth hormone receptor; *GLUT4* glucose transporter type 4; *GSK-3* glycogen synthase kinase-3; *IGF-1* insulin-like growth factor I; *IGFR* insulin-like growth factor receptor; *INS* insulin; *IR* insulin receptor; *IRS* insulin receptor substrate; *JAK2* janus kinase 2; *JNK* c-jun N-terminal kinase; *MAPK* mitogen-activated protein kinase; *p110* the p110 catalytic subunit of PI3K; *p85* the p85 regulatory subunit of PI3K; *PI3K* phosphoinositide 3-kinase; *pS* phosphoserine; *pY* phosphotyrosine; *SHC* Src homology 2 (SH2) and collagen domain protein; *SOCS-1,-3* suppressor of cytokine signaling-1,-3; *STAT* signal transducer and activator of transcription. This figure is taken from (Dominici 2005)

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