Children and Young Adults Born Small for Gestational Age (SGA)

GH-IGF-IGFBP axis, insulin sensitivity, adipocytokines and body composition during and after growth hormone treatment

Marije van Dijk

Cover design: Adria Ceelen
Lay-out: Legatron Electronic Publishing, Rotterdam
Printer: PrintPartners IPSKAMP, Enschede

ISBN: 978-90-9021683-6

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Kinderen en jong-volwassenen Small for Gestational Age (SGA)

GH-IGF-IGFBP-as, insulinesensitiviteit,
adipocytokines and lichaamssamenstelling
tijdens en na groeihormoonbehandeling

Proefschrift

ter verkrijging van de graad van doctor aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op vrijdag 23 maart 2007 om 11.00 uur

door

Marije van Dijk

geboren te Eindhoven

Promotiecommissie:

Promotor: Prof.dr. A.C.S. Hokken-Koelega

Overige leden: Prof.dr. A.J. van der Lelij
Prof.dr. J.B. van Goudoever
Prof.dr. J.M. Wit

The printing of this thesis and the studies described were financially supported by Novo Nordisk Farma B.V., Alphen a/d Rijn, the Netherlands and Novo Nordisk A/S, Bagsværd, Denmark.

Aan Robert, Isa en mijn familie

Contents

Chapter 1

Introduction

Introduction

This doctoral thesis gives a detailed account of various studies, performed in short children born small for gestational age (SGA) participating in the third Dutch GH trial (IUGR-3 study), and in young SGA adults previously treated with GH in the first Dutch GH trial (SGA follow-up study). This chapter describes definitions of SGA, prevalence and etiology of SGA, and clinical and endocrinological aspects associated with SGA. Also the effects of GH treatment in short SGA subjects are discussed. Finally, the aims and outline of this thesis, inclusion and exclusion criteria and study designs of the IUGR-3 study (appendix A), IUGR-1 study (appendix B) and SGA follow-up study (appendix C) are presented.

1 Small for gestational age (SGA)

Definitions of SGA

Until recently, several definitions have been used for SGA, ranging from a birth weight and/or length below the 10th or 3rd percentile for gestational age. In 2001, the International SGA Advisory Board Panel reached consensus on the definition of SGA, by defining SGA as a birth weight and/or length below −2 standard deviations (SD) for gestational age using appropriate reference data.[1]

SGA refers to the size of the infant at birth, and not to intrauterine growth. The term intrauterine growth retardation (IUGR) is used to describe reduced growth velocity in the fetus. This can be observed as a deviation of the fetal growth chart, as documented by at least two intrauterine growth assessments. Although SGA is often related to IUGR, not all SGA infants have suffered from IUGR, and infants who are born after a short period of IUGR are not necessarily SGA. Figure 1 shows the fetal growth patterns of SGA and IUGR newborns.

Prevalence and etiology of SGA

When SGA is defined as a birth weight and/or length below −2 SD for gestational age, 2.3% of all live-born infants are born SGA. In 2005, 187,910 infants were born in the Netherlands (Central Bureau of Statistics, Voorburg, the Netherlands). According to the definition, 4322 of them were born SGA.

SGA can be caused by several factors, including fetal, maternal, placental and demographic factors.[2-4] The primary determinant of fetal growth is the ability of the uteropacental unit to deliver oxygen and nutrients to the fetus.[5] Identification of the cause of SGA is important, as underlying mechanisms may affect prognosis and treatment. However, the cause of impaired fetal growth remains unidentified in up to 40% of cases. Table 1 shows factors that are associated with SGA.

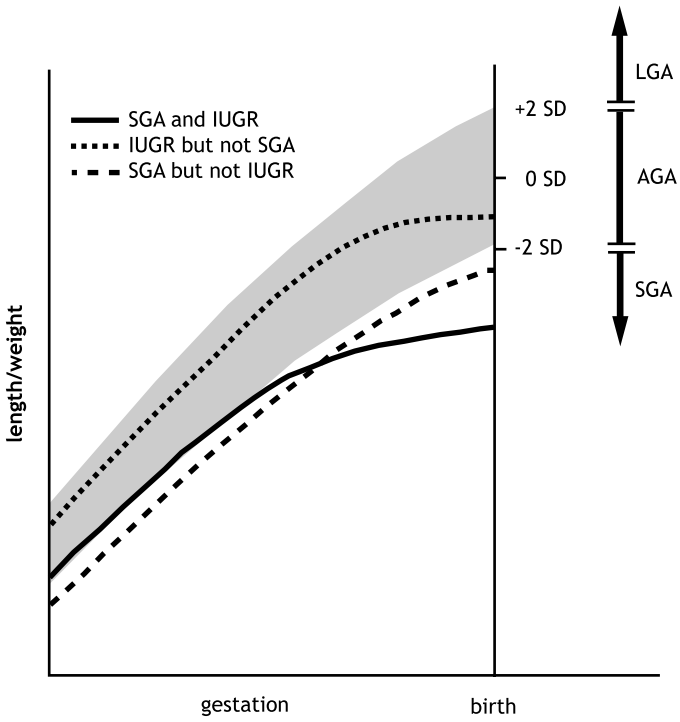

Figure 1. Fetal growth chart showing difference between SGA and IUGR newborns

Fetal factors include chromosomal abnormalities, genetic defects, metabolic problems and congenital anomalies.

Maternal factors can be divided into medical complications and environmental factors. Medical complications include: chronic vascular diseases (secondary to hypertension, diabetes mellitus, renal disease, collagen vascular disease), conditions associated with maternal hypoxemia, infections (particularly toxoplasmosis, rubella, cytomegalovirus and herpes virus) and malnutrition. Environmental factors include cigarette smoking, alcohol abuse, use of illicit drugs (heroin, cocaine) and therapeutic drugs (eg, anticonvulsants, anticoagulants).

Placental factors are associated with problems in placental perfusion resulting in reduced fetal oxygenation. These include structural abnormalities of the placenta, infarctions and suboptimal implantation site.

Demographic factors involve maternal age, parental race and height, obstetric history, and multiple gestation, particularly in case of shared fetal circulation.

Table 1. Factors associated with increased incidence of infants who are born SGA. Adapted from Bernstein and Divon,[2] Pollack and Divon,[3] Wollmann,[4] and Keller et al.[6]

Fetal factors	
Karyotypic abnormalities	Trisomy 21 (Down syndrome)
	Trisomy 18 (Edward syndrom)
	Monosomy X (Turner syndrome)
	Trisomy 13 (gonadal dysgenesis)
Other chromosomal abnormalities	Autosomal deletions
	Ring chromosomes
Genetic diseases	Achondroplasia
	Bloom syndrome
Congenital anomalies	Potter syndrome
	Cardiac abnormalities
Maternal factors	
Medical conditions	Hypertension
	Renal disease
	Diabetes (advanced stages) mellitus
	Collagen vascular diseases (eg, systemic lupus erythematosus)
	Maternal hypoxemia (cyanotic heart disease, chronic anemia, chronic pulmonary disease)
Infection	Toxoplasmosis
	Rubella
	Cytomegalovirus
	Herpesvirus
	Malaria
	Trypanosomiasis
	Human immunodeficiency virus
Nutritional status	Low prepregnancy weight
	Low pregnancy weight with poor weight gain during pregnancy
Substance use/abuse	Cigarette smoking
	Alcohol
	Illicit drugs
	Therapeutic drugs (eg, warfarin, anticonvulsants, antineoplastic agents, folic acid antagonists)

Uterine/placental factors	
Gross structural placental factors	Single umbilical artery
	Velamentous umbilical cord insertion
	Bilobate placenta
	Placental hemangiomas
	Infarcts, focal lesions
Insufficient uteroplacental perfusion Placenta previa Low-lying placenta Placental abruption	Suboptimal implantation site
Demographic factors	
Maternal age	Very young age
	Older age
Maternal height Maternal weight Maternal and paternal race	
Parity	Nulliparity
	Grand multiparity
Maternal history	Previous delivery of SGA infants
Multiple gestation	Particularly severe in syndromes associated with shared fetal circulation

2 Clinical and endocrinological aspects associated with SGA

Short stature

SGA is a common cause of short stature in childhood and adulthood, accounting for 20% of all cases.[7] Most children born SGA show spontaneous catch-up growth to a normal height above −2 SDS. However, approximately 10% of them do not and remain short throughout life. In a Swedish cohort of 111 infants born SGA, defined as a birth length below −2 SD, 13% still had a height below −2 SDS at the age of 2 years.[8] In another group of 724 infants born SGA, defined as a birth length below −1.88 SDS, Hokken-Koelega et al. found that 15% did not show catch-up growth to a normal height at the age of 2 years.[9] Catch-up growth is usually completed in the first 2 years of life, and is most pronounced during the first 6 months. However, in premature SGA infants, catch-up growth may take longer (Figure 2).[9]

Chaussain et al. reported that SGA children with a birth length < −2 SD who remained short during childhood reached a mean adult height of 161.9 (8.0) cm for boys and 147.6 (7.0) cm for girls.[10] If a normal height above −2 SDS has not been achieved

by 3 years of age, there is a 7-fold increased risk for short stature for those born with a low birth length and a 5-fold increased risk for those born with a low birth weight.[7] Therefore, a child born SGA who is still short at 3 years of age, should be referred to a pediatrician with expertise in endocrinology.[1]

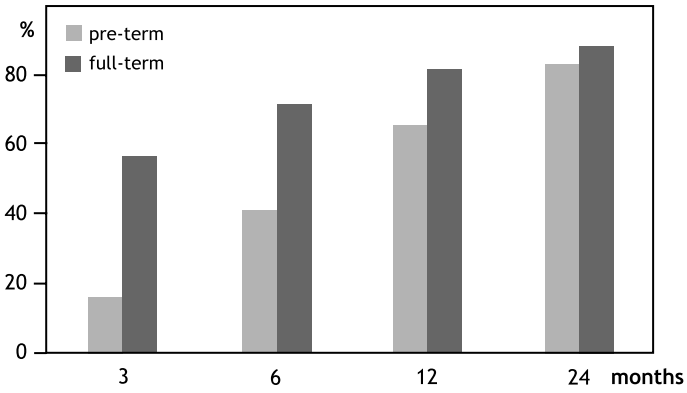

Figure 2. Percentage of pre-tem and full-term SGA infants with postnatal catch-up growth to a height > −1.88 SDS. Adapted from Hokken-Koelega et al..[9]

Growth hormone (GH), insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs)

The physiology of the GH-IGF-IGFBP axis is shown in Figure 3. GH is secreted by the pituitary gland under control of the hypothalamic hormones, GH-releasing hormone (GHRH) and somatostatin, as well as ghrelin, a hormone mainly produced in the stomach.[5] GHRH and ghrelin bind to their respective receptors in the pituitary and stimulate GH secretion. Somatosatin inhibits GH release. Most of the anabolic actions of GH are mediated by IGF-I, but GH has also many cellular effects that are independent of IGF-I.[11]

The IGF system consists of three primary ligands: IGF-I, IGF-II, insulin, and three closely related membrane-bound receptors. IGF-I is an important member of the IGF system and shows structural and functional similarities with insulin and has important anabolic and metabolic effects. IGF-I is present in two forms: circulating IGF-I which is primarily liver-derived and the extra-hepatic autocrine/paracrine form of IGF-I, which is produced by local tissues.[12]

The majority of circulating IGF-I is bound to IGFBPs, of which six classes have been identified. IGFBP-3 is the major carrier protein of IGF-I and binds 70−95% of IGF-I as a binary complex or a ternary complex together with the acid-labile subunit (ALS).[13] IGFBP-3 and ALS are both regulated by GH. IGFBP-1 binds only a small fraction of IGF-I, but has direct inhibitory effects on IGF-I mediated processes *in vivo*.[13]. The hepatic production of IGFBP-I is inversely regulated by the portal supply of insulin.[14]

Less than 1% of IGF-I is unbound and circulates in its free form. Free IGF-I is believed to be the biological active form and several studies have indicated the importance of free versus total IGF-I in short-term dynamic metabolic changes, but also in long-term steady-state changes, like linear growth.[15,16]

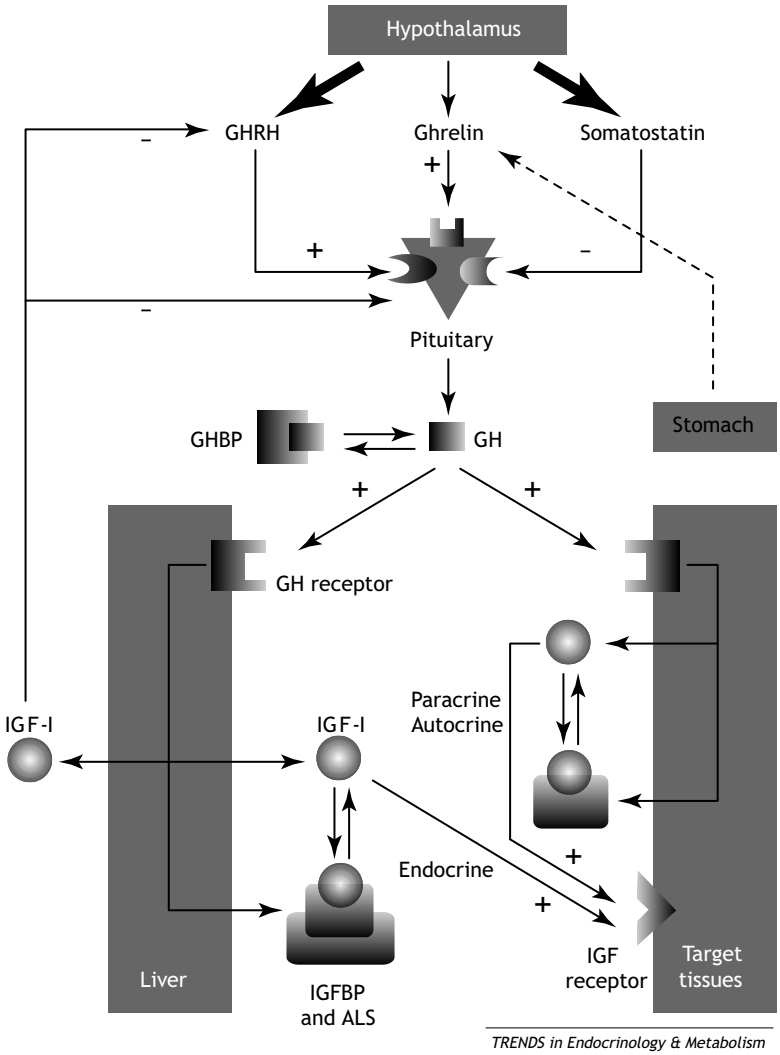

Figure 3. Physiology of the GH-IGF-IGFBP axis. Adapted from Holt.[5]

Fetal growth

The exact role of GH in fetal growth remains unclear. Although high levels of GH in the fetal circulation have been reported,[17,18] it appears that GH is not a major hormonal

regulator of fetal growth, since the number of GH receptors in prenatal life is low.[19] On the other hand, in neonates with congenital GH deficiency, birth length was on average 1 SD score lower compared with healthy neonates, suggesting that GH does have a small effect on linear growth *in utero*.[20] In SGA fetuses, GH levels were found to be comparable with AGA fetuses during the second half of gestation.[18] In SGA neonates, however, most studies have reported elevated GH concentrations in cord blood as compared with AGA subjects.[21-23] The latter suggests that SGA neonates have a state of GH resistance, which is characterized by increased GH levels.[20]

Fetal IGF-I and IGF-II are the most important endocrine determinants of fetal growth and their concentrations are mainly regulated by genetic factors, fetal nutrition and insulin levels. IGF-I and IGF-II are already detectable in cord blood from the first trimester and show a 2–3-fold increase during the last trimester.[24,25] In newborn SGA infants, IGF-I and IGF-II levels were significantly reduced compared with AGA infants, suggesting an important role for low IGF levels in fetal growth retardation.[18,24,25] Gene deletion studies in mice demonstrated that IGF-I and IGF-II knock-out mice had a birth weight which was 60% of normal.[26] The growth-promoting effect of IGF-II is also exerted through the IGF-I receptor. Hence inactivation of the IGF-I receptor even resulted in a more severe reduction in birth weight to 45% of normal, due to loss of both IGF-I and IGF-II action. Interestingly, inactivation of hepatic IGF-I production, as in the liver IGF-I deficient (LID)-mice, had only a minimal effect on fetal growth, despite strongly reduced levels of circulating IGF-I.[27] Serum levels of free IGF-I were comparable for LID- mice and wild-type controls, suggesting that normal growth in utero may result from normal circulating free IGF-I levels. It might also be that the extra-hepatic autocrine/paracrine production of IGF-I was sufficient to maintain normal growth. In SGA fetuses, IGFBP-3 levels were also lower than in AGA fetuses, whereas IGFBP-1 levels were elevated.[25,28] IGFBP-1 has been inversely related to insulin levels and it might be that low insulin levels in the growth-retarded fetus are responsible for the elevation of IGFBP-1.[5]

Initially, insulin was thought to be the major growth-promoting hormone in fetal life. More recently, it has been shown that insulin acts directly via stimulation of cellular nutrient (glucose) uptake and indirectly by stimulation of IGF-I production.[29] Glucose availability and the subsequent increase in fetal insulin are the major regulators of fetal IGF-I production. Fetal pancreatectomy in sheep resulted in low fetal IGF-I levels and caused severe intrauterine growth retardation.[30] In addition, intrafetal infusions with either glucose or insulin increased fetal IGF-I.

SGA subjects

The pathophysiology of persistent short stature in some children born SGA is not fully understood. Disturbances in the GH-IGF-IGFBP axis may contribute to poor postnatal catch-up growth as several studies have demonstrated that up to 60% of SGA children

with persistent short stature show a reduced spontaneous GH secretion during a 24-hour GH-profile and/or low GH peaks during GH provocation tests.[31-35] Serum IGF-I and IGFBP-3 levels were also reduced in short SGA children when compared with healthy controls with normal stature.[36-38] Some short SGA children have, however, normal or high GH levels together with low levels of IGF-I and IGFBP-3, suggesting a reduced functioning of the GH receptor.[31] High-to-normal levels of GH an IGF-I have also been reported in few SGA individuals, which suggests a reduced functioning of the IGF-I receptor.[39]

Abnormalities in the GH-IGF-IGFBP axis have also been observed in chronic adult diseases associated with low birth weight, including type 2 diabetes mellitus and cardiovascular disease.[40-42] Therefore, in SGA subjects, disturbances in the GH-IGF-IGFBP axis may be related to the future development of these diseases.

Insulin sensitivity and cardiovascular risk factors

In epidemiological studies, an inverse relation has been reported between birth weight and the risk of hypertension, cardiovascular disease and type 2 diabetes mellitus in adulthood.[43-45] Insulin resistance plays an important role in the pathogenesis of these diseases.[46,47] The exact mechanisms underlying these associations are, however, still unknown. Several hypotheses have been proposed.

Fetal origins hypothesis: Based on a number of epidemiological observations linking reduced birth weight and adult diseases, such as hypertension, hyperlipidemia, diabetes mellitus type 2, coronary artery disease and metabolic syndrome, Barker et al. suggested that fetal malnutrition could result in permanent metabolic alterations and changes in organ structures in the fetus.[43,44] This programming would be in favor of short-term survival, but deleterious on the long-term and result in diseases in adulthood, as shown in Figure 4.

Fetal insulin hypothesis: The fetal insulin hypothesis was formulated by Hattersley et al. and postulated that the association between low birth weight and adult insulin resistance is principally genetically mediated.[49] Genes involved in insulin resistance could result in low-insulin-mediated fetal growth in utero as well as insulin resistance in childhood and adulthood (Figure 5).

Growth acceleration hypothesis: Singhal et al. suggested that rapid postnatal growth rather than birth weight per se, is responsible for programming of cardiovascular diseases. Fetal growth restriction relative to genetic growth potential could result in deleterious growth acceleration postnatally; increased infant growth rate by a nutrient enriched diet, may have adverse long-term effects and result in adult diseases (Figure 6).[50]

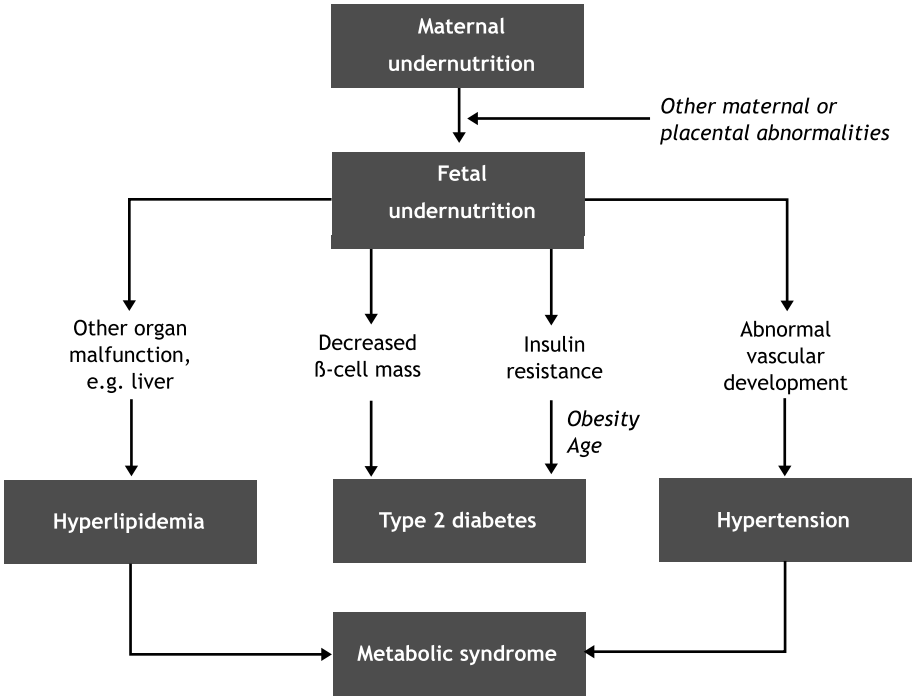

Figure 4. Representation of the fetal origins hypothesis. Adapted from Barker et al..[44,48]

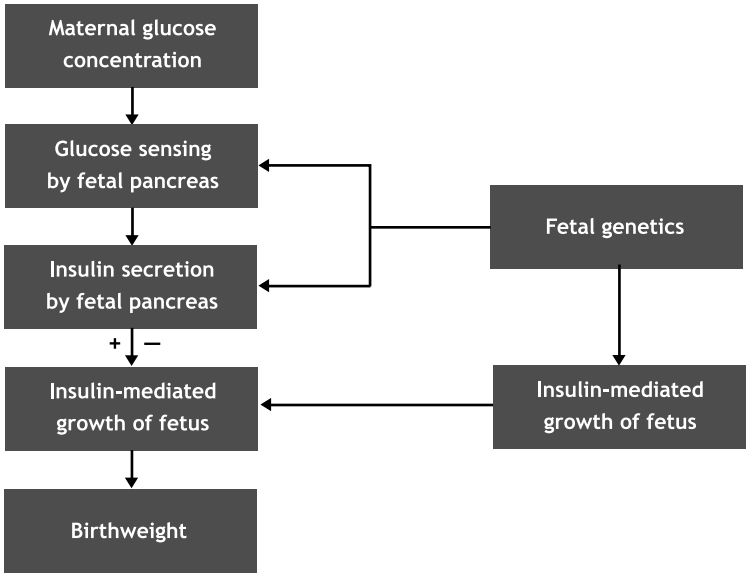

Figure 5. Simplified representation of the fetal insulin hypothesis. Adapted from Hattersley et al..[49]

Figure 6. Simplified representation of growth acceleration hypothesis. Adapted from Singhal et al.[50]

SGA subjects

In a group of prepubertal SGA children with a mean age of 8 years, we previously showed that 8% had an impaired oral glucose tolerance test.[51] Further studies indicated that short SGA children were more insulin resistant than children born AGA.[52,53] In addition to reduced insulin sensitivity, it was also found that SGA children and adolescents had a higher systolic blood pressure[54,55] and more often hypercholesterolemia.[56] Thus, also SGA children might have an increased risk of adult diseases later in life.

Adipocytokines

Adipocytokines are fat-cell derived hormones with important endocrine properties. Adiponectin was firstly described in 1995 as a new adipocytokine.[57] It is highly and specifically expressed in differentiated adipocytes and circulates at high levels in the bloodstream.[58] Expression of adiponectin is higher in subcutaneous than visceral adipose tissue.[59] Strong and consistent inverse associations between adiponectin and both insulin resistance and adiposity have been reported in humans[60-65] and animals.[66,67] In humans, serum adiponectin levels were low in obesity and type 2 diabetes and could predict subsequent changes in insulin resistance and risk of type 2 diabetes.[61-64,68] In obese children, weight loss resulted in an increase of serum adiponectin levels.[65] Adiponectin knockout mice had signs of the metabolic syndrome, such as insulin resistance, glucose intolerance, hyperlipidemia and hypertension.[66] Conversely, transgenic mice overexpressing adiponectin, were insulin sensitive.[67]

Resistin was initially identified in 2001 as an adipocyte-derived hormone in mice and has been suggested as a link between obesity and insulin resistance.[69] In mice, circulating resistin levels were decreased by the anti-diabetic drug rosiglitazone, and increased in diet-induced and genetic forms of obesity.[69] Human studies evaluating the associations between resistin, insulin resistance and obesity are controversial. Some studies did not report any association between resistin levels and fat mass,[70,71] whereas others found higher resistin levels with increasing fat mass.[72] In humans, the role of resistin in insulin resistance and obesity remains to be determined.[73]

SGA subjects

Adiponectin levels in cord blood of newborns were much higher than in adults and older children.[74-79] Cord blood adiponectin levels were positively related to birth weight

which is in contrast to adults, showing an inverse relation between adiponectin and body weight.[74-77,79] In newborns, adipose tissue is composed mainly of small newly differentiated adipocytes[80] and almost 90% of total body fat is in the subcutaneous compartment whereas only 4% in the visceral.[81] These factors may explain their high adiponectin levels and the positive associations with birth weight. Compatible with these associations is the finding that SGA neonates had lower adiponectin levels than AGA neonates.[76] In SGA children, data on adiponectin levels are inconsistent. Studies comparing adiponectin in SGA and AGA children reported similar adiponectin levels,[82] lower,[83] and higher levels of adiponectin in the SGA group.[84]

At start of our study, no data were available on resistin levels in SGA subjects. The contribution of circulating adiponectin and resistin levels to the risk of subsequent development of insulin resistance and type 2 diabetes mellitus in SGA subjects merits further studies.

Body composition and fat distribution

Short SGA children have a typical lean appearance which is characterized by a low BMI.[54] Increasing evidence suggests that birth weight is positively associated with lean body mass (LBM) and inversely with body fat mass.[85-94] Hediger et al. showed that SGA infants were smaller with regard to body size than infants born AGA through early childhood.[86] This discrepancy in weight was primarily attributable to a deficit in LBM, whereas fat mass was less affected.[86] In healthy normal children and adolescents, lower birth weight was also associated with lower LBM, but not with a lower fat mass.[94] These data suggest that in short SGA children, leanness is mainly the result of a lower LBM mass rather than fat mass, which may contribute to their predisposition to develop type 2 diabetes mellitus and cardiovascular disease in later life. In that case, assessment of body composition and fat distribution in SGA subjects might give clues which subjects are more prone to develop these adult diseases.

Body composition is greatly influenced by sex and height.[95] It is therefore important to match for these variables when comparing LBM and fat mass in SGA and AGA subjects. Since none of the previous studies have taken this into account,[86,88,89,91] it might be that some of the reported differences are related to differences in sex and height between the groups.

3 Growth hormone (GH) treatment in SGA children

Effects on linear growth

A significant number of short SGA children exhibit alterations in the GH-IGF-IGFBP axis.[31-38] These observations have led to the first GH studies in short SGA children. In 1991, the

first Dutch multi-center, randomized, double-blind, dose-response GH trial was started to investigate the efficacy of GH treatment on growth.[36,96] Children were treated with either 1 or 2 mg GH/m^2·day. In this study, 85% of the children reached a normal adult height above −2 SDS and 98% reached an adult height within their target height range.[96] Interestingly, adult height SDS was not significantly different between the two GH dosage groups. In 1996, the second Dutch GH trial was started with a randomized control group for 3 years. After 3 years, children treated with 1 mg GH/m^2·day showed a significant improvement in their height SDS, whereas those in the control group remained short.[97] In addition to the Dutch GH trials, other studies also demonstrated that GH treatment is an effective therapy for short SGA children. Table 2 summarizes the findings of long-term GH studies in short SGA children and data on adult height.

Table 2. Long-term GH trials in short SGA children

Definitions	GH dose	At start			Δ Ht SDS	Final Ht		Reference
SGA/short stature		n	Age (y)	Ht SDS	5y	n	Ht SDS	
<−1.88 SDS	Untreated	29	7.8	−2.6		15	−2.3	Sas et al.[36] and Van Pareren et al.[96]
	1 mg/m^2·day	41	7.3	−3.0	2.2	28	−1.1	
	2 mg/m^2·day	38	7.2	−3.1	2.6	26	−0.9	
< −2 SDS/< −2.5 SDS	Untreated	47	12.8	−3.2		33	−2.7	Carel et al.[98]
	2 mg/m^2·day	102	12.7	−3.2		91	−2.1	
< −2 SDS	Untreated	34	8.3	−2.2		34	−2.0	Dahlgren et al.[99]
	1 mg/m^2·day	36	8.9	−3.1		36	−1.2	
	1 mg/m^2·day	41	12.3	−2.5		41	−1.6	

Effects on the GH-IGF-IGFBP-axis

Previous reports have shown that GH treatment of short SGA children leads to increases in serum IGF-I and IGFBP-3 levels, which are positively related to the GH dose.[36,37,100] Boguszewski et al. reported a rise of 90% in IGF-I levels after one year and of 123% after 2 years of GH treatment with a dose of 1 mg/m^2·day.[37] Sas et al. reported a rise of IGF-I and IGFBP-3 levels up to 1.2 SDS and 0.2 SDS, respectively, during GH treatment with 1 mg GH/m^2·day for one year, whereas treatment with 2 mg GH/m^2·day resulted in IGF-I and IGFBP-3 levels of 1.9 SDS and 0.5 SDS, respectively. After 5 years of GH treatment, the IGF-I SDS and IGFBP-3 SDS were 1.7 and 1.0 in the 1-mg GH dose group and 2.0 and 1.2 in the 2-mg GH dose group, respectively.[36]

Although the effects of GH treatment on serum levels of IGF-I and IGFBP-3 in short SGA children have been well studied, no data were available on the GH-dose effects on serum GH levels in these children. It had been shown that administration of GH to healthy and GH-deficient adults resulted in a dose-dependent rise of serum GH levels.[14,101] Therefore, it is likely that short SGA children receiving high-dose GH treatment not only have higher levels of IGF-I, but also higher GH levels. It is also not known whether GH-induced increases in serum levels of GH, IGF-I and IGFBP-3 are reversible after discontinuation of GH treatment.

Effects on insulin sensitivity and cardiovascular risk factors

GH has well-documented insulin-antagonistic effects and its use has been associated with a reduction in insulin sensitivity and an increase in insulin levels.[102-104] Therefore, concern had been expressed regarding the possible late consequences of GH treatment on risk factors for type 2 diabetes and cardiovascular disease.

It had been previously shown that insulin sensitivity was reduced in short SGA children before start of GH treatment whereas GH resulted in a further decline.[51-53,105-107] Reassuringly, we showed a recovery of insulin sensitivity and insulin levels to reference values at 6 months after discontinuation of GH therapy.[107]

GH treatment also resulted in a significant reduction in systolic blood pressure as well as a reduction in serum lipids which remained so until 6 months after discontinuation.[107] However, long-term surveillance of insulin sensitivity and cardiovascular parameters is important to exclude any negative effects of GH.

Effects on adipocytokines

Data on the effects of GH treatment on adiponectin and resistin levels in SGA children are very limited. Ibáñez et al. recently reported a reduction in adiponectin levels in a group of SGA children after 6 months of high-dose GH treatment parallel to the reduction in insulin sensitivity.[108] At start of this study, reports describing the effects of GH treatment on resistin levels did not exist.

Effects on body composition and fat distribution

GH has well-documented anabolic effects on muscle mass and lipolytic effects on adipose tissue.[109,110] GH deficiency has been associated with increased fat mass and truncal obesity,[111,112] whereas GH excess, as in active acromegaly, has been related to reduced fat mass and increased LBM.[113] In short SGA children, GH treatment resulted in a normalization of BMI.[54] Leger et al. measured muscle and fat tissue mass of the thighs using magnetic resonance imaging (MRI) in 14 short SGA children during 3 years of GH therapy.[114] They reported a progressive increase in muscle tissue cross-sectional area and a transient decline in adipose tissue cross-sectional area. At the end of 3 years

the muscle tissue cross-sectional area change was significantly greater in GH-treated SGA children as compared with untreated AGA controls, whereas adipose tissue cross-sectional area change was similar. However, they did not measure total body muscle and body fat, nor fat distribution. It is also not known whether GH-induced changes in body composition and fat distribution are long-lasting.

Safety aspects

The National Cooperative Growth Study (NGCS) monitored the safety of GH treatment from 1984 until 1995 in children with various diagnoses. Reported adverse events included idiopathic intracranial hypertension, edema and lymphedema, carpal tunnel syndrome, slipped capital femoral epiphysis, diabetes mellitus and glucose intolerance.[115] The authors concluded that major adverse events in relation to GH treatment were rare and that their frequency may be affected by preexisting medical conditions.

In SGA children, several studies have shown that GH treatment was well tolerated and that side effects were uncommon.[51,54,107] However, since long-term follow-up data after discontinuation of GH are yet unknown, all SGA children receiving GH therapy should be monitored regularly for changes in blood pressure, serum IGF-I levels, fasting glucose and insulin levels and fasting serum lipids to exclude any possible adverse effects of GH.[116]

4 Aims of the study

This doctoral thesis describes the results of various studies performed in (i) short SGA children being treated with GH in the third Dutch GH trial (IUGR-3 study) and (ii) young SGA adults previously treated with GH treatment in the first Dutch GH trial (SGA follow-up study). The IUGR-3 study aimed to investigate the effects of GH treatment on (i) GH-IGF-IGFBP axis, (ii) insulin sensitivity and cardiovascular risk factors, (iii) adipocytokines and (iv) body composition in prepubertal short SGA children. The SGA follow-up study assessed the late consequences of long-term GH treatment on these variables at 6.5 years after discontinuation of GH in young SGA adults in comparison with untreated SGA controls.

GH-IGF-IGFBP axis

We investigated serum GH levels during an overnight GH-profile and serum levels of free and total IGF-I and IGFBP-3 in short SGA children before and after 6 months of treatment with either 1 or 2 mg GH/m^2·day. To assess whether GH-induced changes in serum IGF-I and IGFBP-3 levels are reversible after GH stop, we also evaluated serum IGF-I and IGFBP-3 levels longitudinally from start of treatment until 6.5 years after

discontinuation in young SGA adults. At 6.5 years after GH stop, both were compared with those of untreated short SGA controls.

In addition, we investigated the associations between the GH-IGF-IGFBP axis and insulin sensitivity and insulin secretion in short SGA children before and during GH treatment. We were particularly interested in the relative roles of GH, IGF-I and IGFBP-3 in the regulation of glucose homeostasis, since it has been proposed that disturbances in the GH-IGF-IGFBP axis may serve as a link between reduced fetal growth and adult diseases.[39,117]

Insulin sensitivity and cardiovascular risk factors

We evaluated the changes in insulin sensitivity, acute insulin response and disposition index, as measured with an intravenous glucose tolerance test with Tolbutamide, in short SGA children during 24 months of GH treatment. To assess the possible long-term risks of GH treatment, we also investigated insulin sensitivity, acute insulin response and disposition index and cardiovascular parameters in young SGA adults at 6.5 years after discontinuation of long-term GH in comparison with untreated short SGA controls.

Adipocytokines

We evaluated changes in adiponectin and resistin levels in short SGA children during 24 months of GH treatment. Since serum adiponectin and resistin levels might be related with the relative insulin resistance induced by GH therapy, we also assessed their associations with insulin sensitivity and insulin secretion. Adiponectin levels were also measured in young SGA adults at 6.5 years after discontinuation of long-term GH treatment in comparison with untreated sex-and height-matched controls born either SGA or AGA.

Body composition and fat distribution

We measured body composition and fat distribution by Dual Energy X-ray Absorptiometry (DXA) in short SGA children during 24 months of GH treatment in comparison with untreated short SGA controls. We also assessed correlations with insulin sensitivity, cardiovascular risk factors and adiponectin levels. To assess the late consequences of earlier GH treatment in childhood on body composition, we also evaluated body composition in young SGA adults at 6.5 years after discontinuation of long-term GH treatment in comparison with untreated sex-and height-matched controls born either SGA or AGA.

5 Outline of the thesis

The studies presented in this thesis were part of the IUGR-3 study and the SGA follow-up study. The inclusion and exclusion criteria of the IUGR-3, IUGR-1 and SGA follow-up study are shown in Appendix A, B and C at the end of this chapter. Chapters 2–5 give a detailed description of various results during treatment in short SGA children (IUGR-3 study). Chapters 6 and 7 focus on the late effects of long-term GH treatment at 6.5 years after discontinuation of GH in young SGA adults (SGA follow-up study).

Chapter 1 gives an introduction in the SGA field.

Chapter 2 reports on the changes in overnight GH levels and serum levels of IGF-I and IGFBP-3 after 6 months of GH treatment with either 1 mg or 2 mg GH/m^2·day in short SGA children.

Chapter 3 describes the associations between serum levels of GH, free and total IGF-I, IGFBP-3 and IGFBP-1 and insulin sensitivity and insulin secretion in short SGA children, before and after 6 months of GH treatment.

Chapter 4 gives a detailed account of the associations between insulin sensitivity, insulin secretion, disposition index and serum levels of adiponectin and resistin before, during and after 24 months of GH treatment in short SGA children.

Chapter 5 describes the effects of GH therapy on body composition and fat distribution and their associations with insulin sensitivity, cardiovascular risk factors and adiponectin levels in short SGA children.

Chapter 6 evaluates risk factors for type 2 diabetes mellitus and cardiovascular disease and serum IGF-I and IGFBP-3 levels in young GH-treated SGA adults from start of GH treatment until 6.5 years after discontinuation of GH. Results were also compared with those of untreated short SGA controls.

Chapter 7 describes body composition, fat distribution and adiponectin levels in previously GH-treated young adults in comparison with untreated sex- and height-matched SGA and AGA controls.

Chapter 8 discusses our data in relation to current literature and the clinical implications and conclusions of our study results.

Chapter 9 summarizes our findings in English

Chapter 10 presents a Dutch summary.

Appendix A (IUGR-3 study)

Inclusion criteria IUGR-3 study:

1. Birth length and/or birth weight SDS below −2 for gestational age[118]
2. Uncomplicated neonatal period, without signs of severe asphyxia (Apgar score > 3 after 5 minutes) or long term complications of respiratory ventilation such as broncho-pulmonary dysplasia.
3. Short stature, defined as a height SDS score below −2.5[119]
4. Height velocity SD score below zero to exclude children with catch-up growth[119]
5. Age between 3−8 years at start of the study
6. Prepubertal stage, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys[120]

Exclusion criteria IUGR-3 study:

1. Chromosomal disorders and known syndromes, except for Silver-Russell syndrome
2. Disorders of major organs
3. Endocrine or metabolic disorders, such as diabetes mellitus, diabetes insipidus, hypothyroidism, or inborn errors of metabolism, except for GH-deficiency
4. Chondrodysplasia
5. Hydrocephalus
6. Active malignancy or increased risk on leukemia
7. Emotional deprivation
8. Previous anabolic sex steroid or GH therapy

Design IUGR-3 study

The IUGR-3 study started in 2002. The study design was an open-labeled, randomized, multicenter study. After stratification for gender, GH-status (maximum serum GH between 20−30 mU/L vs. serum GH > 30 mU/L during a GH stimulation test) and BMI (< −1 SD vs. > −1 SD), children were randomized into 2 different groups. During 6 months, children of group A received GH therapy with a dose of 1 mg GH/m^2·day and children of group B received a dose of 2 mg GH/ m^2·day. Subsequently, all children received the same dose of 1 mg GH/ m^2·day. Biosynthetic GH [Nordiptropin® SimpleXx™ (15 mg/1.5 ml), Novo Nordisk A/S, Bagsværd, Denmark] was administered subcutaneously once daily at bedtime using the Nordipen™ 15.

Appendix B (IUGR-1 study)

Inclusion criteria IUGR-1 study:

1. Birth length and/or birth weight SDS below −1.88 for gestational age[118]
2. Uncomplicated neonatal period, without signs of severe asphyxia (Apgar score > 3 after 5 minutes) or long term complications of respiratory ventilation such as broncho-pulmonary dysplasia.
3. Short stature, defined as a height SDS score below −1.88[119]
4. Height velocity SD score below zero to exclude children with catch-up growth[119]
5. Age between 3–11 years for boys and 3–9 years for girls at start of the study
6. Prepubertal stage, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys[120]

Exclusion criteria IUGR-1 study:

1. Chromosomal disorders and known syndromes, except for Silver-Russell syndrome
2. Disorders of major organs
3. Endocrine or metabolic disorders, such as diabetes mellitus, diabetes insipidus, hypothyroidism, or inborn errors of metabolism, except for GH-deficiency
4. Chondrodysplasia
5. Hydrocephalus
6. Active malignancy or increased risk on leukemia
7. Emotional deprivation
8. Previous anabolic sex steroid or GH therapy

Design IUGR-1 study

The IUGR-1 study started in 1991. The study design was a multi-center, randomized, double-blind, dose-response GH trial. After stratification for age and for spontaneous GH secretion, all children were randomly and blindly assigned to either one of two GH dosage groups: group A received GH therapy with a dose of 1 mg GH/m^2·day and children of group B received a dose of 2 mg GH/ m^2·day. Biosynthetic GH [Nordiptropin® SimpleXx™ (15 mg/1.5 ml), Novo Nordisk A/S, Bagsværd, Denmark] was administered subcutaneously once daily and GH treatment was stopped after reaching adult height.

Appendix C (SGA follow-up study)

Inclusion criteria SGA follow-up study:

1. Previous participation in the IUGR-1 study
2. Previously being treated with GH for more than 4 years
3. Period of at least 4 years after discontinuation of GH treatment

Design SGA follow-up study

The SGA follow-up study was designed to evaluate the possible long-term risks of GH treatment in short SGA subjects. The SGA follow-up study was performed in 2005 and involved young SGA adults previously participating in the IUGR-1 study. For the SGA follow-up group (n = 37), mean GH treatment period had been 7.3 (1.3) years and mean period after discontinuation 6.5 (1.4) years. Risk factors for type 2 diabetes mellitus and cardiovascular disease were measured longitudinally from start of GH treatment until 6.5 years after discontinuation of GH therapy. At 6.5 years after GH stop, outcome variable were also compared with those of untreated SGA controls.

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Chapter 2

High serum levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I) during high dose growth hormone treatment in short children born small for gestational age

[1]Marije van Dijk, [2]Paul Mulder, [3]Mieke Houdijk, [4]Jaap Mulder, [5]Kees Noordam, [6]Roelof J. Odink, [7]Ciska Rongen-Westerlaken, [8]Paul Voorhoeve, [9]Johan Waelkens, [10]Wilhelmina Stokvis-Brantsma, [1]Anita Hokken-Koelega

[1]Department of Pediatrics, Division of Endocrinology, Sophia Children's Hospital, Erasmus Medical Center, Rotterdam, [2]Department of Epidemiology & Biostatistics, Erasmus Medical Center, Rotterdam, [3]Juliana Children's Hospital, The Hague, [4]Rijnstate Hospital, Arnhem, [5]University Medical Center, St Radboud, Nijmegen, [6]Beatrix Children's Hospital, Groningen, [7]Canisius-Wilhelmina Hospital, Nijmegen, [8]Vrij Universiteit Medical Center, Amsterdam, [9]Catharina Hospital, Eindhoven, [10]Leiden University Medical Center, Leiden, The Netherlands.

J Clin Endocrinol Metab, 2006, 91:1390-1396

Abstract

Context: Epidemiological studies have indicated that high serum GH levels and levels of IGF-I in the upper tertile to quintile are associated with long term risks.

Objective: The objective of the study was to evaluate the changes in serum levels of GH during overnight profiles, IGF-I and IGF binding protein 3 (IGFBP-3) in short children born small for gestational age (SGA)during GH treatment with two doses.

Patients: Thirty-six prepubertal short SGA children were the subjects of this study

Intervention: Subjects received either 1 mg (group A) or 2 mg (group B) GH /m^2·day.

Main Outcome Measures: At baseline and after 6 months of GH treatment, overnight GH profiles were performed, and fasting levels of serum IGF-I and IGFBP-3 were measured.

Results: After 6 months, group B had significantly higher GH levels during the profile [mean, maximum and area under the curve above zero line (AUC_0)] than group A ($p < 0.009$). In group B maximum GH levels increased from in 43.9 to 161 mU/L ($p < 0.0002$) and in group A from 57.2 to 104 mU/L ($p = 0.002$). During the profile (*i.e.* 12 hours per day), children of group B had mean GH levels of 64.4 mU/L *vs.* 34.8 mU/L in group A ($p = 0.001$). The IGF-I SDS and IGF-I to IGFBP-3 ratio SDS increased significantly in both groups, but were significantly higher in group B than in group A [1.5 *vs.* 0.2 ($p = 0.002$) and 1.4 *vs.* 0.3 ($p = 0.007$), respectively]. In group B, 74% of the children had IGF-I levels in the highest quintile during GH treatment compared with 19 % in group A.

Conclusion: Our study shows that high-dose GH treatment in short SGA children results in high serum GH and IGF-I levels in most children. We recommend monitoring IGF-I levels during GH therapy to ensure these remain within the normal range.

Introduction

Most children born small for gestational age (SGA) show catch-up growth to a normal height during the first two years of life, but approximately 10–15% of them remain short with a height below –2 SD scores.[1,2] Disturbances in the GH-IGF axis may play a role in SGA children with persistent short stature.[3-10]

It has been demonstrated that GH treatment of short children born SGA results in a normalization of height during childhood as well as a normal adult height for most of them.[6,11] Recently, Pareren et al. showed that long-term treatment with a GH dose of 1 mg/m^2·day (~0.033mg/kg·day) was as effective as the higher dose of 2 mg/m^2·day (~0.067/kg·day) for most children with regard to adult height.[11]

Previous reports have shown that GH treatment of short SGA children leads to increases in serum IGF-I and IGFBP-3 levels, which are positively related to the GH dose.[4-6] Sas et al. reported a rise of the IGF-I and IGFBP-3 SD score up to 1.2 and 0.2, respectively, during GH treatment with 1 mg GH/m^2·day for 1 year, whereas treatment with 2 mg GH/m^2·day resulted in an IGF-I and IGFBP-3 SD score of 1.9 and 0.5, respectively. After 5 years of GH treatment, the IGF-I and IGFBP-3 SD scores were 1.7 and 1.0 in the 1-mg GH dose group and 2.0 and 1.2 in the 2-mg GH dose group, respectively.[6]

Although the effects of GH treatment on serum levels of IGF-I and IGFBP-3 in short SGA children have been well studied, no data are available on the effect of GH therapy with various doses on serum GH levels in these children. It has been shown that administration of GH to healthy and GH-deficient adults results in a dose-dependent rise of serum GH levels.[12,13] Therefore, it is expected that short SGA children receiving high-dose GH treatment, not only have higher levels of IGF-I, but also higher GH levels.

Concern has been expressed regarding the possible harmful effects of high serum GH and IGF-I levels for many years.[14,15] Recent epidemiological studies on risk of breast,[16] prostate[17] and colon[18] cancer have indicated that serum levels of IGF-I in the upper tertile to quintile are associated with an increased risk of cancer. For that reason, it is important to evaluate the serum levels of GH and IGF-I in GH-treated short SGA children.

Therefore, we studied GH levels during an overnight GH-profile and serum levels of IGF-I and IGFBP-3 in 36 short SGA children, both before and after 6 months of treatment with either 1 mg or 2 mg GH/m^2·day.

Patients and methods

Subjects

The study group comprised 36 prepubertal short children born SGA. Children were included according to the following criteria: 1) birth length and/or birth weight standard deviation score (SD score) below −2 for gestational age,[19] 2) current height SD score below −2.5,[20] 3) height velocity SD score below zero to exclude children with spontaneous catch-up growth,[20] 4) prepubertal stage, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys,[21] 5) age between 5−8 years at start of the study, and 6) an uncomplicated neonatal period, without signs of severe asphyxia (Apgar score > 3 after 5 minutes) or long term complications of respiratory ventilation such as bronchopulmonary dysplasia. Children with endocrine or metabolic disorders, chromosomal defects, syndromes and growth failure caused by other conditions (*e.g.* emotional deprivation, severe chronic illness, chondrodysplasia) were excluded, except for Silver-Russell syndrome. The study was approved by the medical ethics committees of the participating centres and written informed consent was obtained from the parents.

Study design

After stratification for gender, GH-status (maximum serum GH between 20−30 mU/L vs. serum GH > 30 mU/L during a GH stimulation test) and body mass index (BMI) (< −1 SD *vs.* > −1 SD) all 36 children were randomized into two different groups. During 6 months, children of group A (n = 16) received GH therapy with a dose of 1 mg GH/ m^2·day and children of group B (n = 20) received a dose of 2 mg GH/ m^2·day. GH [Nordiptropin® SimpleXx™ 15 mg/1.5 ml (biosynthetic human growth hormone, Novo Nordisk A/S, Bagsværd, Denmark] was administered subcutaneously once daily at bedtime using the Nordipen™ 15. Overnight GH profiles were performed in all subjects at baseline and after 6 months of GH treatment. Children were admitted to the hospital and blood for determination of serum GH levels was withdrawn from an indwelling venous catheter at 20 minute intervals between 19.00 and 7.00 h. Children followed their normal eating pattern until midnight. The next morning a fasting blood sample was taken for measurement of IGF-I and IGFBP-3 levels. During the second GH profile, at 6 months after start of GH therapy, the daily subcutaneous GH injection was given under observation at 20:00 h, one hour after onset of the GH profile.

Assays

Serum IGF-I and IGFBP-3 were measured in one laboratory using specific RIAs, as previously described.[22-24] The intra-assay coefficient of variation (CV) was 4% and the inter-assay CV was 6%. GH levels were measured by IMMULITE 2000 (Diagnostic Products Corporation, L.A. CA 90045-5597 USA), with a lower detection limit of 0.13 mU/L. The intra-assay and inter-assay CV were 3.7% and 5.7%, respectively.

Calculations

All overnight GH profiles were analyzed using the Pulsar program.[25] The area under the curve above zero line (AUC_0), mean and maximum GH levels were derived. The AUC_0 was divided by 3 to rescale time into units of 1 hour and was similar when calculated by the trapezoidal method. Serum levels of GH were expressed in milliunits per L (mU/L). The serum levels of IGF-I and IGFBP-3 were converted into SD scores to adjust for age and sex, using reference values for healthy children with normal stature determined in the same laboratory.[26]

Statistics

Analyses were carried out using the computer statistical package SPSS (version 10.1, SPSS Inc., Chicago, Il, USA) for Windows. Results are expressed as the median (interquartile range), unless indicated otherwise. The Mann-Whitney U test was used for differences between groups. Differences between points in time were tested by the Wilcoxon signed rank test. To test for linear relationships between continuous variables, partial correlations were estimated for group A and B together, with adjustment for GH dosage. Multiple linear regression analysis was used to assess multivariable relationships. Factors showing a significant partial correlation with the 6-months change in height SD score were entered into the model. Only results of the best fitting model (in terms of R-squared) are shown. Statistical significance was defined as $p < 0.05$.

Results

Clinical data

Table 1 lists the baseline clinical data of both GH dosage groups. Children of both groups (A and B) had comparable baseline characteristics. Two children of group B had genetically proven Silver-Russell Syndrome. Six children in group A and six children in group B were born preterm.

Table 1. Baseline clinical characteristics of the study groups

	Group A (n = 16) (1mg GH/m^2·day)	Group B (n = 20) (2mg GH/m^2·day)
Male/female	9/7	11/9
Gestational age	37.8 (34.1 to 39.2)	38.0 (33.3 to 39.3)
Birthweight SD score	−1.8 (−3.4 to −1.1)	−2.1 (−2.6 to −1.3)
Birth length SD score	−2.6 (−3.4 to −1.6)	−2.8 (−3.4 to −2.1)
Age at start GH treatment (yrs)	6.2 (5.8 to 7.4)	6.2 (5.4 to 7.7)
Height SD score at start GH treatment	−3.3 (−3.4 to −2.8)	−3.1 (−3.4 to −2.7)
BMI SD score at start GH treatment	−0.8 (−2.0 to −0.1)	−1.2 (−2.2 to −0.7)

Data expressed as median (interquartile range)

Growth response to GH therapy

In group A, the height SD score increased significantly from −3.3 (−3.4 to −2.8) at start to −2.8 (−2.9 to −2.3) after 6 months of GH therapy ($p = 0.0004$). Group B showed an increase in height SD score from −3.1 (−3.4 to −2.7) to −2.4 (−2.8 to −2.2) after 6 months ($p < 0.0001$). The change in height SD score was significantly higher in group B than in group A ($p = 0.001$).

Overnight GH profiles

Table 2 lists the characteristics of the overnight GH profiles for both GH dosage groups at baseline and after 6 months. At baseline, the AUC_0, mean and maximum GH levels were comparable for group A and B. After 6 months of GH treatment (when a subcutaneous GH injections was given at 20.00 h), the AUC_0, mean, and maximum GH levels increased significantly in both groups. All values were significantly higher in group B compared with group A. For example, in group B mean GH levels increased from 9.6 to 64.4 mU/L and maximum GH levels from 43.9 to 161 mU/L, whereas group A showed an increase of mean GH levels from 10.8 to 34.8 mU/L and of maximum GH levels from 57.2 to 104 mU/l.

Figure 1 depicts the mean serum GH levels of both groups at each time point during the overnight GH profiles at baseline and after 6 months. After subcutaneous GH injection at 20:00 h, GH levels remained above 40 mU/L for 7.3 hours in group B compared with 4.5 hours in group A ($p = 0.0008$) and above 20 mU/L for 9.3 hours in group B compared with 6.5 hours in group A ($p = 0.017$). Figure 2 shows the individual mean GH levels during the overnight GH profiles for each child with the children ranked per group. During GH treatment, a wide interindividual variation in mean GH levels was seen. Two subjects in group A and one in group B showed strikingly high mean serum GH levels compared with the other children of their group.

Table 2. Characteristics of overnight GH release, IGF-I and IGFBP-3 levels in group A and B at baseline and after 6 months of GH treatment

	Group A (1mg GH/m^2·day)			Group B (2mg GH/ m^2·day)			
	0 months	6 months	p value[a]	0 months	6 months	p value[a]	p value[b]
AUC_0 (mU/L*12 h)	130 (113 to 150)	428 (344 to 638)	0.0004	113 (97.4 to 138)	791 (668 to 946)	0.0001	0.0009
Mean GH (mU/L)	10.8 (9.2 to 12.5)	34.8 (28.2 to 52.0)	0.0004	9.6 (8.0 to 11.3)	64.4 (54.6 to 76.9)	0.0001	0.001
Max GH (mU/L)	57.2 (44.4 to 73.5)	104 (94.3 to 149)	0.002	43.9 (32.0 to 53.9)	161 (110 to 206)	0.0002	0.009
GH > 40 mU/L (hrs)	0.7 (0.1 to 1.0)	4.5 (2.9 to 6.4)	0.0007	0.0 (0.0 to 0.9)	7.3 (6.0 to 8.7)	0.0001	0.001
GH > 20 mU/L (hrs)	2.0 (1.7 to 2.6)	6.5 (5.7 to 9.2)	0.0004	1.7 (0.8 to 2.2)	9.3 (7.7 to 10.7)	0.0001	0.017
IGF-I SDS	−1.6 (−2.1 to −1.3)	0.2 (−0.5 to 0.7	0.0008	−1.6 (−2.2 to −1.2)	1.5 (0.8 to 2.1)	0.0001	0.002
IGFBP-3 SDS	−1.5 (−1.7 to −0.8)	−0.2 (−0.7 to 0.2)	0.002	−1.5 (−1.8 to −1.1)	0.5 (0.44 to 0.68)	0.0001	0.005
IGF-I / IGFBP-3 ratio SDS	−1.1 (−1.7 to −0.6)	0.3 (−0.3 to1.0)	0.002	−1.0 (−1.6 to −0.4)	1.4 (0.6 to 2.1)	0.0002	0.007

Data expressed as median (interquartile range).

[a] compared with baseline

[b] group B *vs.* A after 6 months of GH treatment

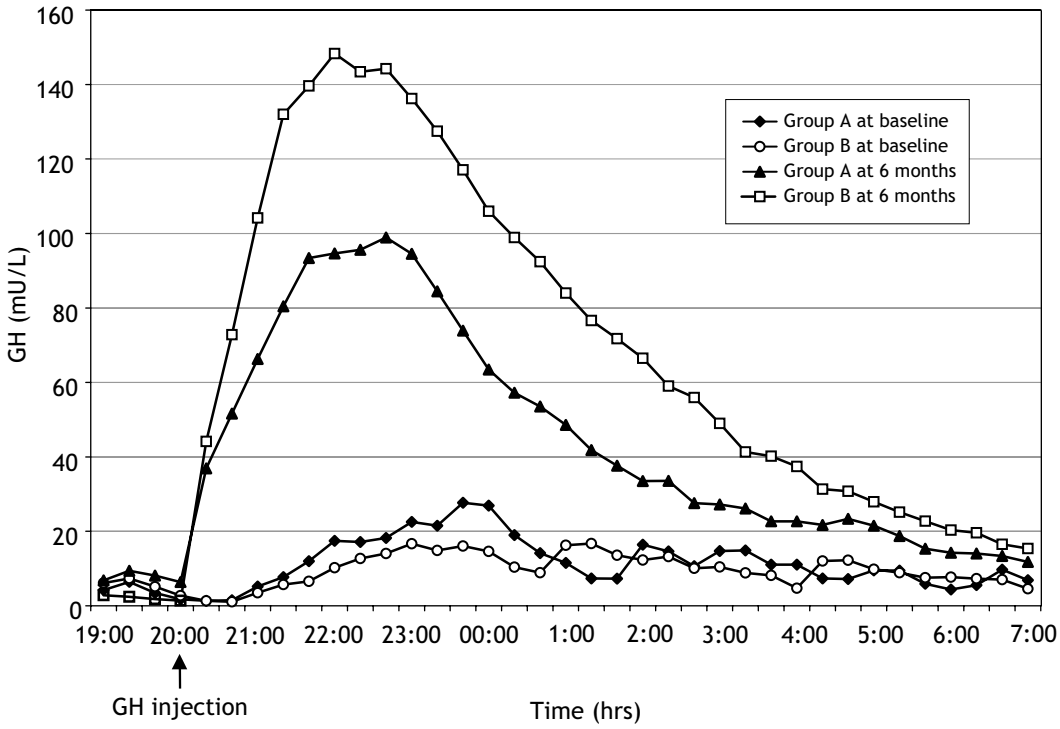

Figure 1. Mean GH levels for each time point during an overnight GH profile at baseline and after 6 months of GH treatment.

IGF-I and IGFBP-3 levels and IGF-I to IGFBP-3 ratio

Serum levels of IGF-I, IGFBP-3 and the IGF-I to IGFBP-3 ratio, expressed as SD scores, are shown in Table 2. Baseline IGF-I and IGFBP-3 SD scores were comparable for group A and B and significantly lower than zero ($p = 0.0001$ to 0.0009). After 6 months of GH treatment, the IGF-I and IGFBP-3 SD scores increased significantly in both GH dosage groups compared with baseline, but were significantly higher in group B than in group A. In group B, the IGF-I SD score increased from −1.6 to 1.5 and the IGFBP-3 SD score increased less markedly from −1.5 to 0.5. Both SD scores were significantly higher than zero ($p = 0.005$ and $p = 0.009$, respectively). In contrast, in group A, the IGF-I SD score increased from −1.6 to 0.2 and the IGFBP-3 SD score from −1.5 to −0.2, both being not statistically different from zero anymore. Seventy-four percent of the children of group B had serum IGF-I levels in the highest quintile (> 0.84 SD score) and 37% had levels above 2 SD score compared with only 19% ($p = 0.0014$) and 6% ($p = 0.034$) of the children of group A, respectively.

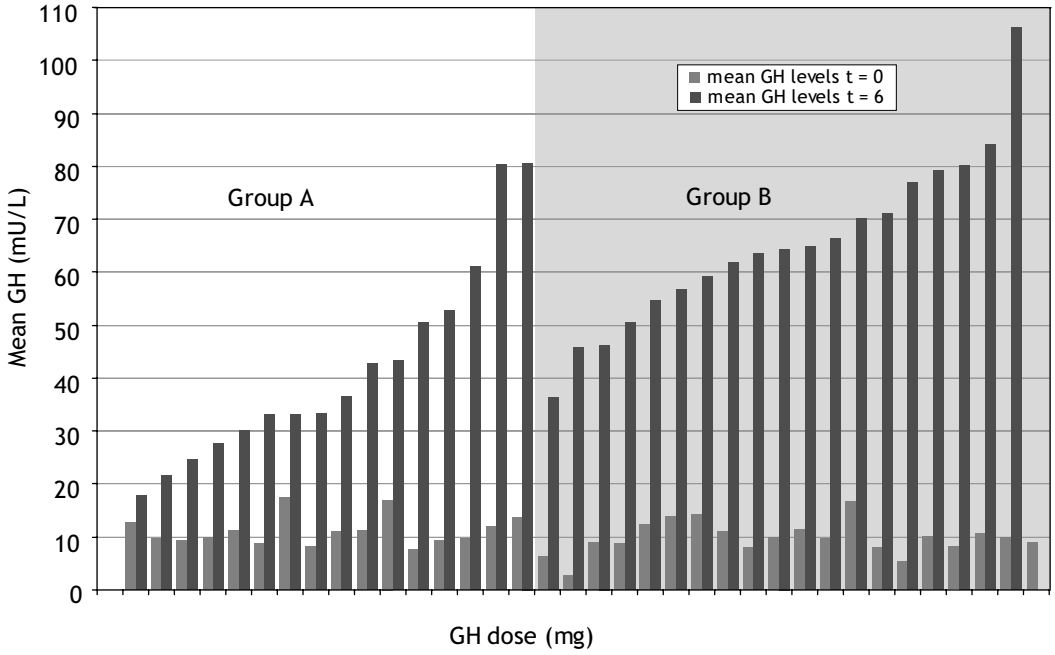

Figure 2. Mean GH levels for each individual during an overnight GH profile before and after 6 months of GH treatment

At baseline, the IGF-I to IGFBP-3 ratio SD score was significant lower than zero in both groups (p = 0.002 and p = 0.0001, respectively). After 6 months of GH treatment, the IGF-I to IGFBP-3 ratio SD score increased significantly in both groups compared with baseline, but was significantly higher in group B than in group A. In group B, there was an increase from −1.0 to 1.4, which was significantly higher than zero (p = 0.001). In contrast, in group A the IGF-I to IGFBP-3 ratio SD score increased from −1.1 to 0.3 and was no longer statistically different from zero. Sixty-three percent of children of group B had an IGF-I to IGFBP-3 ratio in the highest quintile (> 0.84 SD score) and 32% above 2 SD score, compared with 25% (*p* = 0.026) and 0% (p = 0.015) of the children in group A, respectively.

At baseline, the IGFBP-3 SD score correlated significantly with the AUC_0 (r = 0.48, p = 0.003), mean (r = 0.51, p = 0.002) and maximum GH levels (r = 0.57, p = 0.000), but no correlation was found after 6 months. In contrast, the IGF-I and IGF-I to IGFBP-3 ratio SD scores did not correlate with serum GH levels, neither at baseline, nor at 6 months.

Relationship between the growth response and other variables

Partial correlations were made for group A and B together, with adjustment for GH dose. The change in height SD score correlated significantly with the height SDS at start ($r = -0.34$, $p = 0.044$), age at start ($r = -0.50$, $p = 0.002$), AUC_0 at start ($r = -0.34$, $p = 0.044$), mean GH levels at start ($r = -0.36$, $p = 0.034$) and the baseline IGF-I ($r = -0.47$, $p = 0.004$) and IGFBP-3 SD scores ($r = -0.51$, $p = 0.002$). No significant partial correlation was found between gain in height SD score and the following parameters: birth weight and birth length SD score, target height SD score, maximum GH levels at start, baseline IGF-I to IGFBP-3 ratio SD score, AUC_0 at 6 months, mean and maximum GH levels at 6 months, IGF-I, IGFBP-3 and IGF-I to IGFBP-3 ratio SD scores at 6 months and the 6-month changes in GH levels, IGF-I, IGFBP-3 and IGF-I to IGFBP-3 ratio SD scores.

Using multiple regression, the following variables parameters were the best predictors of the 6-months increase in height SD score during GH treatment: GH dose (group B *vs.* group A) ($\beta = 0.51$, $p = 0.0002$), age (in years) at start of the study ($\beta = -0.370$, $p = 0.0043$) and IGF-I SD score at start ($\beta = -0.34$, $p = 0.0079$). These three variables explained 55% of the variation of the 6-months change in height SD score.

Discussion

Our study shows that short SGA children receiving high-dose GH treatment (2 mg/m^2·day) have very high mean serum GH levels of 64.4 mU/L during 12 hours per day. High-dose GH treatment also resulted in serum IGF-I levels and an IGF-I to IGFBP-3 ratio in the highest quintile (> 0.84 SD score) in 74 and 63% of the children, respectively.

This is the first report describing serum GH levels after GH administration in prepubertal short SGA children. We found great interindividual variations in mean serum GH levels among the short SGA children in both GH dosage groups. Comparable individual variations in GH levels after subcutaneous GH injection have previously been reported in GH deficient children and were attributed to different mechanisms of the degradation of GH at the site of injection or in the circulation.[27] Two children in group A and one child in group B had extremely high GH levels compared with the other children in the groups. Higher serum GH levels have been described when the GH injection was administered intramuscular instead of subcutaneous.[28] It is possible that the GH administration in these two children was not completely subcutaneous as in the other children, but partly intramuscular at time of the overnight profile. Their IGF-I levels were not different compared with the other children.

Previous studies concerning GH levels during GH treatment have mainly been performed in healthy adults,[29,30] GH-deficient patients[12,28,31] and in girls with Turner syndrome.[32] The short SGA children in our study showed remarkably higher mean and

maximum serum GH levels after subcutaneous GH injection. Vahl et al. found an inverse correlation between serum GH levels after a single GH dose and age as well as intra-abdominal fat mass.[33] This might partly explain the higher mean and maximum GH levels in our study group, which consisted of young prepubertal SGA children with a reported lower fat mass and a lower BMI SD score than their peers.[34]

In the high-dose group, mean serum GH levels were 64.4 mU/L during the 12 hours of the GH-profile, and remained above 20 mU/L for more than 9 hours, indicating that short SGA children treated with 2 mg GH/m^2·day have elevated GH levels for a great part of the day. At the end of the overnight GH-profiles, 11 hours after the subcutaneous GH injection, serum GH returned to near baseline levels in both groups. For comparison, overnight GH levels in normal prepubertal boys and girls are 10.5 and 10.8 mU/L, respectively, and increase during puberty reaching maximum values of 17.1 mU/L in boys and 20 mU/L in girls.[35]

In the 2-mg GH dose group, 74% of the children had IGF-I levels and 63% an IGF-I to IGFBP-3 ratio in the highest quintile (> 0.84 SD score) after GH-treatment and approximately 30% of them even had levels above 2 SD scores. In contrast, almost all children of the normal GH dose group had IGF-I levels and/or an IGF-I to IGFBP-3 ratio SD score within ± 1 SD score.

In another group of short SGA children receiving 1 and 2 mg GH /m^2·day, Sas et al. also showed an increase of the IGF-I SD score up to 1.2 and 1.9 in the first year and up to 1.7 and 2.0 after 5 years, respectively,[6] indicating that these levels remain at the same SD level when GH treatment is given for many years. De Zegher et al. reported a 3 to 6-fold increase of IGF-I levels after two years of high-dose GH treatment with 2 and 3 mg/m^2·day, respectively.[5] In all reports, there was also a significant increase of the IGF-I to IGFBP-3 ratio.[5,6]

We found a clear correlation between baseline GH levels and the baseline IGFBP-3 SD score, but not with the IGF-I SD score, which is in agreement with previous studies.[3,8,36] This might suggest that IGFBP-3 levels are a more valuable measure for the endogenous GH secretion in short SGA children.

The 6-months change in height SD score was inversely related to mean GH levels at start and the baseline IGF-I, IGFBP-3 and the IGF-I to IGFBP-3 ratio SD scores, indicating that children with lower levels of GH and IGF-I were more sensitive to GH treatment. No correlation was found between the growth response and the increases in GH, IGF-I, and IGFBP-3 levels. This may suggest a reduced GH and/or IGF-I receptor sensitivity, particularly in those children with higher GH and IGF-I levels in combination with a poorer growth response. Another explanation may be that IGF-I receptors in some short SGA children are already maximally stimulated, meaning that a further increase of GH and IGF-I levels has no extra effect. Previous reports concerning the relationship between growth response to GH therapy and the GH-IGF-I axis are contradictory. Some

studies suggest that the catch up growth during GH treatment is independent of the GH-IGF-I axis.[6,11,31] However, other studies have shown a clear correlation between the growth response and baseline IGF-I levels[4,36] or changes in IGF-I levels during GH treatment.[37]

Concern has been expressed regarding the possible detrimental effects of persistently high serum levels of GH and IGF-I.[14,15] Epidemiological studies have suggested that high serum levels of GH and IGF-I might increase cancer risk in human beings, especially when IGFBP-3 levels are low.[17,18] Serum IGF-I levels in the upper tertile to quintile have been associated with increased risk of breast, prostate and colon cancer.[16-18] These findings were supported by Renehan et al., who did a systematic review and meta-regression analysis of the association between concentrations of IGF-I and IGFBP-3 levels and cancer risks.[38] In contrast, low serum levels of GH and IGF-I, as found in individuals with a T1663A polymorphism in the human GH1 gene, are associated with a decreased risk of colorectal cancer.[39]

Most short SGA children receiving GH treatment with 2 mg /m^2·day have high GH levels for many hours per day and IGF-I levels in the upper quintile (> 0.84 SD score). As the majority of these children will be treated for 10−12 years until adult height is reached, serum GH and IGF-I levels will be elevated during their childhood and adolescence. Children treated with the higher GH dose for many years might therefore be at increased risk for complications in later life.

Recently, GH treatment for short children born SGA with persistent short stature has been approved by the European Agency for the Evaluation of Medicinal Products (EMEA). However, there is still debate about the optimal GH dose for these children. In the United States, the higher GH dose of 2 mg /m^2·day has been approved by the Food and Drug Administration (FDA), whereas we have recently shown that treatment with a GH dose of 1 mg/m^2·day was as effective as 2 mg/m^2·day with regard to reach a normal adult height.[11] In a recent epianalysis, it has been shown that height gain is less dose dependent over the long term than over the short term.[40] For that reason, there is no evidence to support long term treatment with the higher GH dose for all short SGA children with regard to adult height improvement.

In conclusion, our study shows that GH treatment with 2 mg GH /m^2·day in short SGA children results in high serum GH levels for 12 hours per day and IGF-I levels and an IGF-I to IGFBP-3 ratio in the highest quintile (> 0.84 SD score) in 74 and 63% of the children, respectively, whereas treatment with a dose of 1 mg GH /m^2·day completely normalizes IGF-I and IGFBP-3 levels (± 1 SD score) . The long term risks of high GH and IGF-I levels in short SGA children are still unknown. Therefore we recommend monitoring IGF-I levels during GH therapy to ensure these remain within the normal range. GH treatment could be started at a lower dose with individual adjustment based on growth response and IGF-I levels.

Acknowledgments

We would like to thank Mrs. Colette Wissink and Christel Bruinings, research nurses, for their technical assistance and Dr. W.H. Hackeng (PhD) for his GH assays.

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Chapter 3

Associations between insulin sensitivity, insulin secretion, overnight growth hormone (GH) levels, free and total IGF-I and IGF-binding proteins 3 and 1 in short children born small for gestational age (SGA) before and during GH treatment

[1]Marije van Dijk, [2]Mieke Houdijk, [3]Paul G Mulder, [4]Jaap C Mulder, [5]Kees Noordam, [6]Roelof J. Odink, [7]Ciska Rongen-Westerlaken, [8]Wilhelmina H Stokvis-Brantsma, [9]Paul G Voorhoeve, [10]Johan J Waelkens and [1]Anita C Hokken-Koelega

[1]Department of Pediatrics, Division of Endocrinology, Erasmus MC, Sophia Children's Hospital, Rotterdam, [2]Juliana Children's Hospital, The Hague, [3]Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, [4]Rijnstate Hospital, Arnhem, [5]University Medical Center, St Radboud, Nijmegen, [6]University Medical Center Groningen, Groningen, [7]Canisius-Wilhelmina Hospital, Nijmegen, [8]Leiden University Medical Center, Leiden, [9]Vrije Universiteit (VU) Medical Center, Amsterdam, [10]Catharina Hospital, Eindhoven, The Netherlands

Submitted for publication

Abstract

Context: Birth weight has been associated with an increased risk of diabetes mellitus type 2. A significant number of short children born small for gestational age (SGA) have disturbances in their GH-IGF-IGFBP axis, which may contribute to the future development of insulin resistance and chronic adult diseases.

Objective: We evaluated the relations between insulin sensitivity (Si), acute insulin response (AIR), disposition index (DI) and the GH-IGF-IGFBP axis in short SGA children before and during GH treatment.

Patients: Thirty-six prepubertal short SGA children participated in this study.

Intervention: Subjects received 1 or 2 mg GH/m^2·day.

Main outcome measure: At baseline and after 6 months, an iv glucose tolerance test (FSIGT) was performed and serum levels of GH, free and total IGF-I, IGFBP-3 and IGFBP-1 were measured.

Results: After 6 months, we observed a reduction in Si and a compensatory rise in AIR, by which DI remained constant. Overnight GH levels, free and total IGF-I and IGFBP-3 levels showed a dose-dependent increase, whereas IGFBP-1 decreased. During GH treatment, Si and AIR were closely related to total IGF-I, IGFBP-3 and IGFBP-1, but not to overnight GH levels or free IGF-I.

Conclusion: GH treatment of short SGA children results in a reduction of Si, apparently due to chronic elevation of GH and IGFBP-3 levels. The simultaneous increase in IGF-I levels may have attenuated the anti-insulin effects of GH and IGFBP-3. Our data underline the complexity of relations between glucose homeostasis and the GH-IGF-IGFBP-axis and further studies in this field are required.

Introduction

Birth weight has been inversely associated with the risk of cardiovascular disease and diabetes mellitus type 2 (DM-II) in adulthood.[1-3] In addition, children born small for gestational age (SGA) were more insulin resistant than those born appropriate for gestational age (AGA).[4,5] Being born SGA is also associated with short stature in childhood and adulthood in 10−15 % of cases.[6,7] A significant number of SGA subjects with persistent short stature have permanent abnormalities in their growth hormone (GH)-insulin-like growth factor (IGF)-IGF binding protein (IGFBP) axis, characterised by reduced spontaneous GH secretion and low IGF-I and IGFBP-3 levels.[8-15]

Increasing evidence indicates that the GH-IGF-IGFBP axis plays an important role in regulating glucose homeostasis and insulin sensitivity (16). GH has well-documented anti-insulin effects and its use has been associated with a reduction in insulin sensitivity and hyperinsulinemia.[17-19] In contrast, IGF-I exerts insulin-like effects which are opposite to those of GH. Several prospective studies have shown a positive association between low circulating IGF-I levels and the subsequent risk on impaired glucose tolerance, type 2 diabetes and ischemic heart disease.[20,21] Hence, in SGA subjects, alterations in the GH-IGF-IGFBP axis may contribute to the future development of insulin resistance and chronic adult diseases.

Most of circulating IGF-I is bound to IGFBPs. IGFBP-3 is the major carrier protein of IGF-I and binds 70−95% of IGF-I as a binary complex or a ternary complex together with the acid-labile subunit (ALS).[22] IGFBP-1 binds a small fraction of IGF-I, but has direct inhibitory effects on IGF-I mediated processes *in vivo*.[22] Only a minor fraction of the total IGF-I circulates in its free form, which is believed to be the biologically active form.[23] Nyomba et al. previously observed a significant correlation between free IGF-I and insulin sensitivity as estimated by an iv glucose tolerance test in 11 healthy subjects.[24] These results also suggest a possible role for free IGF-I in regulating glucose homeostasis.

In the present study we evaluated insulin sensitivity, insulin secretion and disposition index in relation to overnight GH levels, free and total IGF-I, IGFBP-3 and IGFBP-1 in 36 short SGA children, both before and after 6 months of treatment with either 1 mg or 2 mg GH/m^2·day. We also determined whether serum levels of GH, free and total IGF-I and IGFBP-3 and IGFBP-1 were valuable as markers of insulin resistance.

Patients and methods

Subjects

The study group comprised 36 prepubertal short children born SGA. Children were included according to the following criteria: 1) birth length and/or birth weight standard deviation score (SDS) below −2 for gestational age,[25] 2) current height SDS below −2.5,[26] 3) height velocity SDS below zero to exclude children with spontaneous catch-up growth,[26] 4) prepubertal stage, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys,[27] 5) age between 5−8 years at start of the study, and 6) an uncomplicated neonatal period, without signs of severe asphyxia (Apgar score > 3 after 5 minutes) or long term complications of respiratory ventilation such as bronchopulmonary dysplasia. Children with endocrine or metabolic disorders, chromosomal defects, syndromes and growth failure caused by other conditions (e.g. emotional deprivation, severe chronic illness, chondrodysplasia) were excluded, except for Silver-Russell syndrome. The study was approved by the medical ethics committees of the participating centres and written informed consent was obtained from the parents.

Study design

After stratification for gender, GH-status (maximum serum GH between 20−30 mU/L vs. serum GH > 30 mU/L during a GH stimulation test) and BMI (< −1 SD vs. > −1 SD), all 36 children were randomised into 2 different groups. During 6 months, children of group A (n = 16) received GH therapy with a dose of 1 mg GH/ m^2·day and children of group B (n = 20) received a dose of 2 mg GH/ m^2·day. GH [Nordiptropin® SimpleXx™ 15 mg/1.5 ml [biosynthetic human growth hormone, Novo Nordisk A/S, Bagsværd, Denmark] was administered subcutaneously once daily at bedtime using the Nordipen™ 15. At baseline and after 6 months of GH treatment, standing height and weight were measured and BMI was calculated. Height and BMI were expressed as SDS adjusting for sex and age according to Dutch reference data for children.[26,28] At the same time points, a frequently sampled iv glucose tolerance test (FSIGT) with Tolbutamide was performed, as previously described.[29] Glucose and insulin levels were measured in all samples. Before the FSIGT tests, overnight GH profiles were performed as previously described[15] and fasting blood samples were taken for measurement of free and total IGF-I, IGFBP-3 and IGFBP-1 levels. Data on the overnight GH profiles and serum levels of total IGF-I and IGFBP-3 have been previously published, however, not in combination with free IGF-I and IGFBP-1 levels, nor in relation to insulin sensitivity and insulin secretion.[15]

Assays

Serum glucose levels were determined on a VITROS analyser 750 (Orthoclinical Diagnostics, Johnson & Johnson Company, Beers, Belgium). Serum insulin levels were measured by an IRMA (Medgenix, Biosource Europe). The intra-assay coefficient of variation (CV) was 2 to 4.7% (19–405 pmol/l) and the inter-assay CV was 4.2 to 11.3% (32–375 pmol/l).

GH levels were measured by IMMULITE 2000 (Diagnostic Products Corporation, L.A. CA 90045-5597 USA), with a lower detection limit of 0.13 mU/L. The intra-assay and inter-assay CV were 3.7% and 5.7%, respectively. The free IGF-I assays were performed in the same laboratory, under standardized circumstances, i.e. the assays were performed at 5°C, serum samples were kept at 5°C, and incubated for exactly 2 hrs. Free IGF-I levels were measured in serum with a commercial two-site immunoradiometric assay (IRMA) using a commercial kit (Diagnostic System Laboratories, Inc., Webster, Texas, USA). This IRMA detects both the unbound IGF-I and the easily dissociable IGF-I.[29] The inter-assay CV was 9.7%. Serum levels of total IGF-I, IGFBP-3 and IGFBP-1 were measured in one laboratory using specific RIAs, as previously described.[30-32]

Calculations

Insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR) and disposition index (DI) were calculated using Bergman's MINMOD MILLENNIUM software.[33] Si quantifies the capacity of insulin to promote glucose disposal and Sg reflects the capacity of glucose to mediate its own disposal. AIR, an estimate of insulin secretory capacity, was measured as the area under the curve from zero to ten minutes corrected for baseline insulin levels. DI equals AIR*Si and indicates the degree of glucose homeostasis.

All overnight GH profiles were analysed using the Pulsar program.[34] Serum levels of GH were expressed in milliunits per L (mU/L). Free IGF-I levels were converted into SDS based on reference values of healthy children (116 girls and 211 boys, aged between 0 and 17 yrs) visiting the hospital for minor elective surgery. After centrifugation, blood samples were stored at −80°C until further analysis. Smoothed references for free IGF-I were constructed using the LMS method, designed for constructing normalized standards of nonparametric data, as previously described by Cole.[35] Serum levels of total IGF-I and IGFBP-3 were also expressed as SDS adjusting for age and sex, using reference values for healthy children with normal stature determined in the same laboratory.[36]

Statistics

Analyses were carried out using the computer statistical package SPSS for Windows (version 11.1, Chicago, Illinois, USA). Results are expressed as the median (interquartile range). The Mann-Whitney U test was used to test differences between groups. Differences in time within the same subjects (i.e. before and after 6 months of GH therapy) were tested by the Wilcoxon signed rank test. To test for linear relationships between continuous variables at baseline, Spearman's correlation coefficient was used. During GH treatment, partial correlations were estimated for group A and B together, after adjustment for GH dosage. Statistical significance was defined as $p < 0.05$.

Results

Clinical data and anthropometrics

Clinical data are shown in Table 1. Group A and B had comparable baseline characteristics. Table 2 shows height and BMI before and after 6 months of GH treatment. Baseline height SDS was −3.3 (−3.4 to −2.8) and −3.1 (−3.4 to −2.7) and BMI SDS −0.8 (−2.0 to −0.1) and −1.2 (−2.2 to −0.7) for group A and B, respectively. During GH treatment, height SDS increased significantly in both groups ($p < 0.001$), whereas BMI SDS remained constant. At 6 months, height and BMI SDS were not significantly different between the groups.

Table 1. Baseline clinical characteristics of the study groups

	Group A (n = 16) (1mg GH/m^2·day)	**Group B (n = 20) (2mg GH/m^2·day)**
Male/female	9/7	11/9
Gestational age	37.8 (34.1 to 39.2)	38.0 (33.3 to 39.3)
Birth weight SDS	−1.8 (−3.5 to −1.1)	−2.1 (−2.6 to -1.3)
Birth length SDS	−2.6 (−3.4 to -1.6)	−2.8 (−3.4 to -2.1)
Age at start GH treatment (yrs)	6.2 (5.8 to 7.4)	6.2 (5.4 to 7.7)
Height SDS at start GH treatment	−3.3 (−3.4 to -2.8)	−3.1 (−3.4 to -2.7)
BMI SDS at start GH treatment	−0.8 (−2.0 to -0.1)	−1.2 (−2.2 to -0.7)

Data expressed as median (interquartile range)

Insulin sensitivity and insulin secretion

FSIGT characteristics are shown in Table 2. At baseline, Si, Sg, AIR and DI were not significantly different for group A and B. After 6 months of GH treatment, Si decreased

significantly in group A and B ($p = 0.003$ and $p = 0.01$, respectively), whereas AIR increased significantly ($p < 0.001$). Sg, DI and fasting glucose levels remained unchanged in both groups. Fasting insulin levels did not change significantly in group A, but increased significantly in group B ($p = 0.004$). At 6 months, none of the variables were significantly different between group A and B.

Overnight GH levels, free and total IGF-I and IGFBP-3 and IGFBP-1

Results are shown in Table 2. At baseline, mean GH levels were comparable for both groups, whereas maximum GH levels were lower in group B ($p = 0.014$). In group A and B, GH treatment resulted in a significant increase in mean ($p < 0.001$) and maximum GH levels ($p = 0.002$ and $p < 0.001$, respectively). At 6 months, mean and maximum GH levels were significantly higher in group B compared with group A ($p = 0.001$ and $p = 0.009$, respectively).

At baseline, free and total IGF-I SDS and IGFBP-3 SDS were all significantly lower than zero in group A ($p < 0.001$ and $p < 0.001$ and $p = 0.01$, respectively) and B ($p < 0.001$), but not significantly different between the groups. In group A and B, GH treatment resulted in a significant increase in free IGF-I SDS (($p = 0.002$ and $p < 0.001$, respectively), total IGF-I SDS ($p < 0.001$) and IGFBP-3 SDS ($p = 0.002$ and $p < 0.001$, respectively). After 6 months, all values were significantly higher in group B than A ($p < 0.001$ and $p = 0.002$ and $p = 0.005$, respectively). Furthermore, all were comparable with zero in group A, whereas significantly higher than zero in group B ($p < 0.001$ and $p = 0.005$ and $p = 0.009$, respectively). Serum IGFBP-1 levels remained unchanged in group A, but decreased significantly in group B ($p = 0.019$).

Correlations

Baseline correlations are shown in Table 3. At baseline, Si was inversely related to AIR, but not to overnight GH levels and serum levels of free and total IGF-I levels, IGFBP-3 and −1. AIR was positively related to fasting insulin levels and total IGF-I SDS. Fasting insulin levels correlated positively with fasting glucose levels, total IGF-I and IGFBP-3 SDS.

Partial correlations adjusted for GH dose after 6 months of GH treatment are shown in Table 4. Si was inversely related to AIR, fasting glucose and insulin levels, total IGF-I and IGFBP-3 SDS, and positively to IGFBP-1 levels. In contrast, AIR correlated positively with fasting glucose and insulin levels, total IGF-I SDS and negatively with IGFBP-1 levels. Fasting insulin levels were also positively related to IGFBP-3 SDS.

The 6-months change in height SDS correlated significantly with height SDS at start ($r = -0.34$, $p = 0.044$), age at start ($r = -0.50$, $p = 0.002$), mean GH levels at start ($r = -0.36$, $p = 0.034$), total IGF-I SDS at start ($r = -0.47$, $p = 0.004$), IGFBP-3 SDS at start ($r = -0.51$, $p = 0.002$) and IGFBP-1 levels at start ($r = 0.34$, $p = 0.047$), but not with Si, AIR or fasting insulin levels.

Table 2. FSIGT results and serum levels of GH, free and total IGF-I, IGFBP-3 and -1 in group A and B at baseline and after 6 months of GH treatment. Parts of this table have previously been published.[15]

	Group A (1mg GH/m^2/day)			Group B (2mg GH/ m^2/day)				
	0 months	6 months	p value[a]	0 months	6 months	p value[a]	p value[b]	p value[c]
Height SDS	−3.3 (−3.4 to -2.8)	−2.8 (−2.9 to -2.3)	< 0.001	−3.1 (−3.4 to -2.7)	−2.4 (−2.8 to -2.2)	< 0.001	NS	NS
BMI SDS	−0.8 (−2.0 to -0.1)	−1.0 (−2.1 to -0.7)	NS	−1.2 (−2.2 to −0.7)	−1.0 (−1.8 to -0.6)	NS	NS	NS
Si*10^{-4}/min−1 (µU/ml)	13.7 (9.2 to 19.1)	7.5 (6.7 to 8.7)	0.003	13.4 (7.8 to 19.9)	6.5 (3.5 to 11.4)	0.01	NS	NS
Sg*10^{-2} (m/d)min−1	2.0 (1.5 to 2.6)	2.2 (1.6 to 2.9)	NS	2.1 (1.7 to 3.2)	2.3 (1.8 to 2.9)	NS	NS	NS
AIR (mU/L)	215 (162 to 346)	460 (376 to 651)	< 0.001	346 (2.5 to 480)	616 (384 to 1294)	< 0.001	NS	NS
DI (AIR * Si)	3024 (2253 to 5294)	3699 (3267 to 4250)	NS	4646 (3041 to 5777)	4003 (3276 to 4905)	NS	NS	NS
Glucose (mmol/L)	4.7 (4.5 to 5.2)	4.9 (4.5 to 5.0)	NS	4.8 (4.3 to 5.1)	5.0 (4.7 to 5.3)	NS	NS	NS
Insulin (mU/L)	6.0 (4.3 to 8.8)	7.0 (4.5 to 7.8)	NS	5.0 (4.0 to 6.8)	6.0 (4.0 to 11.5)	0.004	NS	NS
Mean GH (mU/L)	10.8 (9.2 to 12.5)	34.8 (28.2 to 52.0)	< 0.001	9.6 (8.0 to 11.3)	64.4 (55.6 to 76.9)	< 0.001	NS	0.001
Max GH (mU/L)	57.2 (44.4 to 73.5)	104 (94.3 to 149)	0.002	43.9 (32.0 to 53.8)	161 (110 to 206)	< 0.001	0.014	0.009
Free IGF−I SDS	−1.6 (−2.3 to -0.3)	0.4 (0.0 to 1.1)	0.002	−0.8 (−2.1 to -0.2)	1.7 (1.0 to 2.4)	< 0.001	NS	< 0.001
Total IGF−I SDS	−1.6 (−2.1 to -1.3)	0.2 (−0.5 to 0.7)	< 0.001	−1.6 (−2.2 to -1.2)	1.6 (0.8 to 2.1)	< 0.001	NS	0.002
IGFBP−3 SDS	−1.5 (−1.7 to -0.8)	−0.2 (−0.7 to 0.2)	0.002	−1.5 (−1.8 to -1.1)	−0.5 (0.4 to 0.7)	< 0.001	NS	0.005
IGFBP−1	163 (87 to 194)	146 (113 to 174)	NS	172 (134 to 195)	130 (99 to 163)	0.019	NS	NS

Data expressed as median (interquartile range)

[a] compared with baseline

[b] group B vs. A at baseline

[c] group B vs. A after 6 months of GH treatment

Table 3. Spearman's correlation coefficients at baseline for group A and B together

	Height SDS	BMI SDS	Si	Sg	AIR	DI	Glucose	Insulin	Mean GH	Max GH	Free IGF-I SDS	Total IGF-I SDS	IGFBP-3 SDS	IGFBP-1
Height SDS	–	–	–	−0.41[c]	–	–	–	–	–	–	–	0.34[a]	–	–
BMI SDS	–	–	–	−0.34[c]	–	–	–	–	–	–	–	–	–	–
Si	–	–	–	–	−0.53[a]	0.37[c]	–	–	–	–	–	–	–	–
Sg	−0.41[c]	−0.34[c]	–	–	–	–	–	–	–	–	–	−0.43[b]	–	–
AIR	–	–	−0.53[a]	–	–	0.53[a]	–	0.52[a]	–	–	–	0.36[c]	–	–
DI	–	–	0.37[c]	–	0.53[a]	–	–	–	−0.44[b]	–	–	–	–	–
Glucose	–	–	–	–	–	–	–	0.44[b]	–	–	–	–	–	–
Insulin	–	–	–	–	0.52[a]	–	0.44[b]	–	–	–	–	0.32[*]	0.34[c]	–
Mean GH	–	–	–	–	–	−0.44[b]	–	–	–	0.74[a]	–	–	0.37[c]	−0.35[c]
Max GH	–	–	–	–	–	–	–	–	0.74[a]	–	–	–	0.36[c]	–
Free IGF-I SDS	–	–	–	–	–	–	–	–	–	–	–	0.58[a]	–	–
Total IGF-I SDS	0.34[c]	–	–	−0.43[b]	0.36[c]	–	–	0.32[*]	–	–	0.58[a]	–	0.41[c]	–
IGFBP-3 SDS	–	–	–	–	–	–	–	0.34[c]	0.37[c]	0.36[c]	–	0.41[c]	–	–
IGFBP-1	–	–	–	–	–	–	–	–	−0.35[c]	–	–	–	–	–

Units: Si: insulin sensitivity*10^{-4}/min-1 (μU/ml); Sg : glucose effectiveness*10^{-2} (m/d)min-1; AIR : acute insulin response (mU/L); DI : disposition index (AIR * Si); Glucose (mmol/L); Insulin (mU/L); IGFBP-1; Mean GH (mU/L); Max GH (mU/L)

[a]$p < 0.001$; [b]$p < 0.01$; [c]$p < 0.05$; [*]$p = 0.058$

Table 4. Partial correlation coefficients adjusted for GH at 6 months for group A and B together

	Height SDS	BMI SDS	Si	Sg	AIR	DI	Glucose	Insulin	Mean GH	Max GH	Free IGF-I SDS	Total IGF-I SDS	IGFBP-3 SDS	IGFBP-1
Height SDS	–	–	–	–	–	–	–	–	–	–	0.38[c]	0.37[c]	0.34[c]	–
BMI SDS	–	–	–	–	–	–	0.35[*]	–	–	–	–	0.40[c]	0.36[c]	–
Si	–	–	–	–	−0.60[a]	–	−0.54[b]	−0.57[a]	–	–	–	−0.53[b]	−0.37[c]	0.41[c]
Sg	–		–	–	0.41[c]	0.42[c]	–	–	–	–	–	–	–	–
AIR	–	–	−0.60[a]	0.41[c]	–	0.63[a]	0.41[c]	0.63[a]	–	–	–	0.47[b]	–	−0.38[c]
DI	–	–	–	–	0.63[a]	–	–	–	–	–	0.36[c]	0.37[c]	–	–
Glucose	–	0.35[*]	−0.53[b]	–	0.41[c]	–	–	0.65[a]	–	–	–	0.48[b]	0.47[b]	−0.64[a]
Insulin	–	–	−0.57[a]	–	0.62[a]	–	0.65[a]	–	–	–	–	0.53[b]	0.35[c]	−0.54[b]
Mean GH	–	–	–	–	–	–	–	–	–	0.87[a]	–	–	–	–
Max GH	–	–	–	–	–	–	–	–	0.87[a]	–	–	–	–	–
Free IGF-I SDS	0.38[c]	–	–	–	–	0.37[c]	–	–	–	–	–	0.63[a]	0.52[b]	−0.38[c]
Total IGF-I SDS	0.37[c]	0.40[c]	−0.53[b]	–	0.47[b]	0.37[c]	0.48[b]	0.53[b]	–	–	0.63[a]	–	0.84[a]	−0.61[a]
IGFBP-3 SDS	0.34[c]	0.36[c]	−0.37[c]	–	–	–	0.47[b]	0.35[c]	–	–	0.52[b]	0.84[a]	–	−0.70[a]
IGFBP-1	–	–	0.41[c]	–	−0.38[c]	–	−0.65[a]	−0.54[b]	–	–	−0.38[c]	−0.61[a]	−0.70[a]	–

Units: Si: insulin sensitivity*10^{-4}/min−1 (μU/ml); Sg: glucose effectiveness*10^{-2} (m/d)min-1; AIR: acute insulin response (mU/L); DI: disposition index (AIR * Si); Glucose (mmol/L); Insulin (mU/L); IGFBP-1; Mean GH (mU/L); Max GH (mU/L)

[a]$p < 0.001$; [b]$p < 0.01$; [c]$p < 0.05$; [*]$p = 0.051$

Discussion

This study shows that 6 months of GH treatment in short SGA children results in a reduction in insulin sensitivity (Si) and a compensatory rise in acute insulin response (AIR), by which disposition index (DI) remained constant. Overnight GH levels, serum levels of free and total IGF-I and IGFBP-3 showed a dose-dependent increase, whereas IGFBP-1 levels decreased. During GH treatment, Si and AIR were closely related to serum levels of total IGF-I, IGFBP-3 and IGFBP-1, but not to serum GH and free IGF-I levels.

We hypothesized that in short SGA children, alterations in the GH-IGF-IGFBP axis may be related to the future development of insulin resistance and DM-II and cardiovascular disease. Therefore, we evaluated the relationships between Si, AIR and the GH-IGF-IGFBP axis in short SGA children before and during GH treatment.

In our study, baseline Si and AIR of the study subjects were comparable with those of another group of SGA children which we previously described.[5] Free and total IGF-I and IGFBP-3 SDS were all significantly lower than zero. During GH treatment, Si decreased in both GH dosage groups, whereas AIR increased. In addition, we measured DI, which reflects the capacity of the pancreatic beta-cell to upregulate insulin secretion in response to insulin resistance.[37] In our study, DI remained constant, indicating that glucose homeostasis was well maintained.

At the same time, GH treatment induced an increase in overnight GH levels and serum levels of free and total IGF-I and IGFBP-3 and a decline in IGFBP-1 levels. Given the well-established insulin-antagonistic effects of GH[17-19] and insulin-sensitising effects of IGF-I,[20,21,38] the reduction in Si is most likely due to elevated GH levels. Recently, the relative contributions of GH and IGF-I in inducing insulin resistance have been assessed, using different transgenic mouse models.[39,40] Liver-IGF-I-deficient (LID)-mice were crossed with GH antagonist (GHa) transgenic mice. This resulted in a further decrease in serum IGF-I levels and inactivation of GH action as well as an improvement of insulin sensitivity and a decrease in insulin levels. From these data, the authors concluded that insulin resistance was mainly the result of chronic elevation of GH levels rather than decreased IGF-I levels. IGF-I appears to have minor additional effects on insulin sensitivity.[40] Hence in conditions when both GH levels and IGF-I levels are high, like in our GH-treated SGA subjects, the anti-insulin effects of GH predominate, but may be attenuated by the insulin-like effects of high IGF-I levels.

An unexpected finding was that we did not find a significant correlation between Si and overnight GH levels, which is in contrast with a previous report.[41] It might be that GH receptors involved in the modulation of Si are already maximally stimulated at a certain serum GH level. In that case, Si decreases until a plateau has been reached after which a further increase in GH levels has no extra effect. The lack of a significant association between Si and GH levels may also be due to the opposite effects of IGF-I.

During GH treatment, Si correlated negatively with total IGF-I SDS, whereas AIR and fasting insulin levels correlated positively with total IGF-I SDS. These correlations appear contradictory, but are consistent with previous findings in normal boys and girls.[42,43] It has been shown that insulin has direct and indirect positive effects on serum IGF-I levels. Studies in rats demonstrated that insulin stimulates IGF-I mRNA production in hepatocytes[44] and in vitro studies in human hepatoma cells showed that insulin promotes binding of GH to GH receptors in the liver.[45] Insulin is also able to promote IGF-I bioactivity indirectly by suppression of IGFBP-1 levels, which inhibits IGF-I mediated processes *in vivo*.[46] Free IGF-I showed no significant correlation with Si or fasting insulin. Its contribution to glucose homeostasis requires further studies.

We also found an inverse relation between Si and serum IGFBP-3 levels during GH treatment and a positive relation between fasting insulin levels and IGFBP-3. It has been demonstrated that IGFBP-3 is able to induce insulin-antagonistic effects. Transgenic IGFBP-3 mice overexpressing IGFBP-3 had impaired glucose homeostasis and were insulin resistant[47] and it was recently also reported that IGFBP-3 inhibits insulin-stimulated glucose uptake in cultured adipocytes.[48]

GH treatment resulted in a significant decrease in fasting IGFBP-1 levels in the high-dose group. At 6 months, Si was positively related to IGFBP-1 levels, which is in agreement with previous reports.[49,50] It has been suggested that measurement of fasting IGFBP-1 levels may be useful in identifying those individuals with insulin resistance. AIR and fasting insulin levels correlated negatively with fasting IGFBP-1 levels. It has been clearly demonstrated that hepatic IGFBP-1 production is inversely regulated by portal insulin.[51]

In summary, we hypothesized that GH treatment induces an increase in serum levels of GH, free and total IGF-I and IGFBP-3. Elevation of GH and IGFBP-3 levels results in a worsening of insulin resistance, which in turn leads to a compensatory rise in insulin levels. Increased insulin levels further increase IGF-I levels directly and indirectly by suppressing IGFBP-1 levels. Increased IGF-I levels may prevent further reduction in insulin sensitivity.

The 6-months change in height SDS was inversely related to height SDS at start, age at start, mean GH levels at start, total IGF-I and IGFBP-3 SDS at start and positively with IGFBP-1 levels at start, but not with Si, AIR or fasting insulin levels. De Zegher et al. previously reported in a group of 9 SGA children that those with higher insulin levels had the poorest growth response to GH therapy.[19] The authors suggested that SGA children who are more insulin resistant at baseline, may have the highest risk of glucose intolerance and their lack of growth response to GH treatment should be considered as a warning against continuation of treatment.[38] However, we could not confirm these results and further research in this field is warranted.

In conclusion, GH treatment of short SGA children resulted in a reduction in insulin sensitivity apparently due to chronic elevation of GH and IGFBP-3 levels. The simultaneous increase in IGF-I levels may have attenuated the insulin-antagonistic effects of GH and IGFBP-3. Determination of free IGF-I levels in addition to total IGF-I and IGFBP-3 had no significant advantage in the evaluation of insulin sensitivity in short SGA children, whereas IGFBP-1 levels had. Our data underline the complexity of relations between glucose homeostasis and the GH-IGF-IGFBP axis in SGA children.

Acknowledgments

We greatly acknowledge Mrs. Christel Bruinings and Mrs. Colette Wissink, research nurses, for their technical assistance, Dr. W.H. Hackeng (PhD) for his GH, glucose and insulin assays, and Dr. J. van Doorn (PhD) for determination of free and total IGF-I, IGFBP-3 and IGFBP-1 levels.

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Chapter 4

Changes in insulin sensitivity are associated with changes in adiponectin levels during 24 months of growth hormone (GH) treatment in short children born small for gestational age (SGA)

[1]M. van Dijk, [1]R.H. Willemsen, [2]P.G.H. Mulder, [3]A.W. van Toorenenbergen, [4]Y. B. de Rijke and [1]A.C.S. Hokken-Koelega

[1]Department of Pediatrics, Division of Endocrinology, Sophia Children's Hospital, [2]Department of Epidemiology & Biostatistics, [3]Department of Clinical Chemistry, [4]Department Internal Medicine, Diagnostic Laboratory Endocrinology, Erasmus MC, Rotterdam, The Netherlands.

Submitted for publication

Abstract

Context: Low birth weight has been associated with insulin resistance. Adiponectin and resistin are adipocytokines with opposite properties. Adiponectin has been inversely related to obesity and insulin resistance, whereas resistin positively.

Objective: We evaluated changes in insulin sensitivity (Si) in relation to changes in adiponectin and resistin levels during 24 months of GH treatment. During the first 6 months, also GH-dose effects were assessed.

Patients: Thirty-eight prepubertal short SGA children.

Intervention: During the first 6 months, group A (n = 18) received 1 mg GH/m^2·day and group B (n = 20) 2 mg GH/m^2·day. Subsequently, between 6 and 24 months, both groups received 1 mg GH/m^2·day.

Main Outcome Measures: At baseline and after 6 and 24 months of GH treatment, Si and disposition index (DI) were calculated from an iv glucose tolerance test with Tolbutamide and fasting levels of adiponectin and resistin were determined.

Results: GH treatment resulted in parallel changes in Si and serum adiponectin levels: a reduction between 0–6 months, followed by an improvement thereafter. At 24 months, Si and adiponectin were closer to baseline than at 6 months. The reduction in Si was well compensated by increased insulin secretion, by which DI remained constant. Resistin increased between 0–6 months, but completely returned to baseline levels thereafter.

Conclusion: Our data in short SGA children show that glucose homeostasis was well maintained during GH treatment. The effects of GH on adiponectin and resistin appeared to be time-dependent and most marked in the early phase of GH treatment. Adiponectin levels might play a role in GH-induced insulin resistance.

Introduction

Birth weight has been inversely associated with the incidence of cardiovascular disease and type 2 diabetes mellitus (DM-II) in adulthood.[1-3] Insulin resistance plays an important role in the pathogenesis of DM-II and is often present before clinical symptoms become apparent.[4,5] It was previously shown that prepubertal short children born small for gestational age (SGA) had lower insulin sensitivity compared with short controls with normal birth weight.[6,7]

Adiponectin and resistin are both adipocytokines, though, with opposite properties. Adiponectin is an anti-inflammatory adipocytokine and has been inversely related to adiposity and insulin resistance.[8-11] Resistin has pro-inflammatory properties and has been positively related to obesity.[12,13] Cianfarani et al. recently reported reduced adiponectin levels in a group of SGA children with a mean height SDS of −1.4 (1.3).[14] SGA children who do not show catch-up growth and remain short (height SDS < −2.5) have a typical lean appearance, characterized by a low LBM.[15] Given the fact that adiponectin correlates negatively with fat mass, levels might be different in SGA children without catch-up growth compared with those with catch-up growth.

Growth hormone (GH) treatment has recently been approved for short SGA children. Its use has been associated with a reduction in insulin sensitivity which appears to be reversible after withdrawal of GH treatment.[16,17] It was recently shown that adiponectin levels decreased during 6 months of GH treatment of short SGA children, however, no data are available on the longer term.[18] Also, the effects of GH on adiponectin and resistin in relation to insulin sensitivity in short SGA children have not yet been evaluated in detail.

In the present study we aimed to evaluate the changes in insulin sensitivity in relation to changes in adiponectin and resistin levels during 24 months of GH treatment in short SGA children. During the first 6 months, children were treated with either 1 or 2 mg GH/ m^2·day in order to investigate GH-dose effects.

Patients and methods

Subjects

The study group consisted of 38 prepubertal short children born SGA. Children were included according to the following criteria: 1) birth length and/or birth weight standard deviation score (SDS) below −2 for gestational age,[19] 2) current height SDS score below −2.5,[20] 3) height velocity SD score below zero to exclude children with catch-up growth (20), 4) prepubertal stage, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys,[21] 5) age between 5−8 years at start of the study and 6) an uncomplicated neonatal period, without signs of severe asphyxia

(Apgar score > 3 after 5 minutes) or long term complications of respiratory ventilation such as bronchopulmonary dysplasia. Children with endocrine or metabolic disorders, chromosomal defects, syndromes and growth failure caused by other conditions (e.g. emotional deprivation, severe chronic illness, chondrodysplasia) were excluded, except for Silver-Russell syndrome. The study was approved by the Medical Ethics Committees of the participating centers and written informed consent was obtained from the parents.

GH treatment protocol

After stratification for gender, GH-status (maximum serum GH levels < 30 mU/L *vs*. > 30 mU/L during a GH stimulation test) and BMI (< −1 SDS *vs*. > −1 SDS), all 38 children were randomised into two groups. During the first 6 months, children of group A (n = 18) received 1 mg GH/ m^2·day and children of group B (n = 20) 2 mg GH/ m^2·day. Subsequently, between 6 and 24 months, all children received the same dose of 1 mg GH/ m^2·day. Biosynthetic GH [Nordiptropin ® SimpleXx™ 15 mg/1.5 ml, Novo Nordisk A/S, Bagsværd, Denmark] was administered sc once daily at bedtime using the Nordipen™ 15.

Study design

At baseline and after 6 and 24 months of GH treatment, standing height and weight were measured and BMI was calculated. Height and BMI were expressed as SD scores adjusting for sex and age according to Dutch reference data for children.[20,22]

At the same time points, a modified frequently sampled iv glucose tolerance test (FSIGT) with Tolbutamide was performed, as previously described.[23] Glucose and insulin levels were measured in all samples and insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR) and disposition index (DI) were calculated using Bergman's MINMOD MILLENNIUM software.[24] Insulin sensitivity quantifies the capacity of insulin to promote glucose disposal and glucose effectiveness reflects the capacity of glucose to mediate its own disposal. The acute insulin response, an estimate of insulin secretory capacity, was measured as the area under the curve from zero to ten minutes corrected for baseline insulin levels. Disposition index equals AIR*Si and indicates the degree of glucose homeostasis.

Before the test, additional fasting blood samples were taken for determination of adiponectin and resistin levels. Adiponectin and resistin levels were compared with controls of the same age and sex, recruited among children visiting the outpatient clinic for minor elective surgery (reference group).

Assays

Serum glucose levels were determined on a VITROS analyser 750 (Orthoclinical Diagnostics, Johnson & Johnson Company, Beers, Belgium). Serum insulin levels were

measured by an IRMA (Medgenix, Biosource Europe, Nivelles, Belgium). The intra-assay coefficient of variation (CV) was 2 to 4.7% (19–405 pmol/l) and the inter-assay CV was 4.2 to 11.3% (32–375 pmol/l). Serum adiponectin and resistin levels were assessed by an ELISA (R&D Systems Inc. Minneapolis, USA) The intra-assay CV was < 7% and < 6%, respectively, and the inter-assay CV was < 7% for both.

Statistics

Analyses were carried out using the computer statistical package SPSS for Windows (version 11.1, Chicago, Illinois, USA). Results are expressed as the mean (SD), unless indicated otherwise. Insulin sensitivity (Si), glucose effectiveness (Sg) and acute insulin response (AIR) were logarithmically transformed before analyses and these variables are expressed as the median (interquartile range). Repeated measures of variance (mixed model ANOVA) was used to analyse changes over time and differences between the GH dosage groups. Differences between the GH dosage groups and controls were tested by Independent-Samples t test. To test for linear relationships between continuous variables at baseline, Pearson's correlation coefficient was used, whereas during GH treatment, partial correlations were estimated for group A and B together after adjustment for GH dosage. Statistical significance was defined as $p < 0.05$.

Results

Clinical characteristics

Table 1 lists the clinical data of both GH dosage groups. Group A and B had comparable clinical characteristics. At start of GH treatment, mean age was 6.4 (1.1) years and mean height SD score was −3.1 (0.5) in both groups.

Table 1. Clinical characteristics of the study groups

	Group A (n = 18)	Group B (n = 20)
Male/female	11/7	11/9
Gestational age	36.3 (3.3)	36.6 (4.3)
Birth weight SD score	−2.2 (1.5)	−2.1 (0.9)
Birth length SD score	−3.2 (1.1)	−3.2 (0.9)
Age at start of study (yrs)	6.4 (1.1)	6.4 (1.1)
Height SD score at start of study	−3.1 (0.5)	−3.1 (0.5)
BMI SD score at start of study	−1.2 (1.2)	−1.3 (0.9)

Data expressed as mean (SD)

FSIGT results

FSIGT results are shown in Table 2. At baseline, S), AIR, DI, and Sg, and fasting levels of glucose and insulin were not significantly different between groups A and B.

During the first 6 months of GH treatment, when group A and B had received 1 and 2 mg GH/m^2·day, respectively, Si decreased significantly in both GH dosage groups ($p < 0.001$) whereas AIR increased significantly ($p < 0.001$). DI remained constant. Sg and fasting glucose levels did not change during the first 6 months, whereas fasting insulin levels only increased in group B ($p = 0.001$). After 6 months of GH treatment, none of the variables were significantly different between the GH dosage groups.

Between 6 and 24 months, when group A and B received the same dose of 1 mg GH/m^2·day, Si improved significantly in both groups ($p = 0.043$ and $p = 0.002$, respectively) whereas AIR decreased ($p = 0.015$ and $p = 0.032$, respectively). As a result, DI remained unchanged. At 24 months, Si and AIR were still different from baseline in group A ($p = 0.013$ and $p = 0.007$, respectively) and B ($p = 0.002$ and $p < 0.001$, respectively), but less significantly than at 6 months. Fasting glucose levels decreased significantly between 6 and 24 months in group A and B ($p = 0.004$ and $p = 0.004$, respectively), resulting in levels lower than baseline for group A only ($p = 0.005$), whereas insulin levels did not change between 6 and 24 months.

Adiponectin and resistin levels

Adiponectin and resistin levels of group A and B and the reference group are shown in Table 2. At baseline, resistin levels were lower in group B compared with group A ($p = 0.011$).

During the first 6 months, when group A received 1 mg GH and group B received 2 mg GH/m^2·day, adiponectin decreased significantly in both groups ($p < 0.001$ and $p = 0.007$, respectively). Resistin levels increased in both groups, however, this only reached statistical significance in group B ($p = 0.007$). After 6 months, serum adiponectin and resistin levels were not significantly different between the GH-dosage groups.

Between 6 and 24 months, when all children received 1 mg GH/m^2·day, adiponectin levels increased significantly in group A and B ($p = 0.024$ and $p = 0.047$, respectively), whereas resistin levels decreased, although only significantly in group B ($p = 0.035$). At 24 months, adiponectin and resistin levels were not significantly different from baseline, except for adiponectin levels in group A, which were lower ($p = 0.021$).

Adiponectin levels of the SGA subjects were comparable with those of the age- and sex-matched reference group, whereas resistin levels were significantly lower in SGA children, both at baseline and at 24 months ($p < 0.001$).

Table 2. FSIGT results and adipocytokine levels in group A and B and reference group

		0 months	6 months	24 months
Group A	Si*10^{-4}/min−1 (μU/ml)†	13.7 (9.0–20.6)	7.5 (6.4–8.9)[a]	11.2 (8.1–14.2)[c]
0–24 months: 1 mg GH	Sg*10^{-2} (m/d)min−1†	2.0 (1.5–2.8)	2.2 (1.7–2.9)	1.8 (1.5–2.0)
	AIR (mU/L) †	249 (179–339)	442 (350–632)[a]	328 (209–388)[c]
	DI (AIR * Si)	3717 (1863)	3659 (1202)	3279 (790)
	Glucose (mmol/L)	4.8 (0.6)	4.8 (0.4)	4.4 (0.5)[b]
	Insulin (mU/L)	6.1 (2.4)	6.4 (2.6)	5.7 (1.6)
	Adiponectin (μg/ml)	14.1 (6.8)	8.8 (4.9)[a]	11.7 (5.5)[c]
	Resistin (ng/ml)	10.4 (2.9)[e]	12.5 (4.3)	9.8 (3.1)[e]
Group B	Si*10^{-4}/min−1 (μU/ml)†	13.4 (7.8–19.9)	8.0 (3.5–11.4)[a]	9.5 (6.0–13.3)[c]
0–6 months: 2 mg GH	Sg*10^{-2} (m/d)min−1†	2.1 (1.7–3.2)	2.3 (1.7–2.9)	2.0 (1.7–2.9)[d]
6–24 months: 1 mg GH	AIR (mU/L) †	346 (205–480)	616 (384–1294)[a]	456 (302–639)[a,d]
	DI (AIR * Si)	4591 (1960)	4439 (2081)	5108 (3324)[d]
	Glucose (mmol/L)	4.8 (0.6)	4.9 (0.5)	4.5 (0.3)
	Insulin (mU/L)	5.5 (2.6)	8.5 (5.0)[a,d]	6.5 (3.3)
	Adiponectin (μg/ml)	12.1 (6.7)	9.1 (5.6)[c]	11.4 (6.3)
	Resistin (ng/ml)	7.9 (2.7)[d,e]	11.4 (6.8)[c]	8.2 (3.2)[e]
Reference group	Adiponectin (μg/ml)	11.0 (3.8)	–	10.8 (4.5)
	Resistin (ng/ml)	16.0 (6.4)	–	14.6 (6.1)

Data expressed as mean (SD) † Median (interquartile range)

Reference group: 0 months, age 6.5 (1.0), n=22 (13 males); 24 months, age 8.3 (1.1), n = 26 (15 males).

Significantly different from baseline: [a]$p < 0.001$, [b]$p < 0.005$ [c]$p < 0.05$

Group B significantly different from group A: [d]$p < 0.05$

Group A and B significantly different from reference group at 0 and 24 months: [e]$p < 0.001$

Correlations

At baseline, Si and adiponectin levels were not related to each other, whereas during the first 6 months of GH treatment, the reduction in Si correlated positively with the reduction in adiponectin levels ($r = 0.42$, $p = 0.016$). We did not observe an association between changes in insulin sensitivity and changes in resistin levels. Disposition index was inversely related to resistin levels ($r = -0.39$, $p = 0.017$).

Discussion

Our study demonstrates that GH treatment of short SGA children results in parallel changes in Si and serum adiponectin levels: a reduction during the first 6 months, followed by a subsequent improvement between 6 and 24 months. At 24 months, Si and adiponectin levels were closer to baseline than at 6 months. The reduction in Si was well compensated by an increase in insulin secretion, by which disposition index remained constant. Resistin levels showed an increase during the first 6 months of GH treatment and a subsequent decrease between 6 and 24 months, resulting in levels comparable with baseline.

GH has well-documented insulin-antagonistic effects.[25-27] During the first 6 months of GH therapy, Si decreased significantly. Interestingly, between 6 and 24 months, Si increased, resulting in values closer to baseline values. Our results are different from those of de Zegher et al., who reported a progressive reduction in Si during the first and second year of GH treatment of short SGA children. In that study, however, the short SGA children were treated with a higher GH dose (3 mg GH/m^2·day) compared with ours. We recently reported that GH treatment with 2 mg GH/m^2·day resulted in very high serum GH levels during an overnight GH profile.[28] Given the anti-insulin effects of GH, the children in the study of de Zegher et al. might have been more insulin resistant due to higher serum GH levels, preventing improvement of Si over time. Two other explanations for the discrepancy in results may be differences in sample size and methodology. In the study of de Zegher et al., 9 children were treated with GH compared with 38 in ours, and secondly, in the mentioned study no Tolbutamide was used during the iv glucose tolerance test. Longer-term studies are needed to evaluate whether insulin sensitivity remains constant or further improves after 2 years.

After 6 months of GH treatment, Si did not differ between the GH-dosage groups, suggesting that changes in Si were not dose-dependent within the GH-dose range we used.

In addition to Si, we evaluated DI, which reflects the ability of the pancreatic beta-cells to upregulate insulin secretion in response to insulin resistance in order to maintain normoglycemia.[29] It has been reported that disposition index is a strong predictor for

the subsequent development of DM-II.[30] In our study, DI remained unchanged during GH treatment in both GH dosage groups, indicating that glucose homeostasis was well maintained.

During GH treatment, changes in serum adiponectin levels paralleled the changes in Si whereas resistin showed opposite changes. At 24 months, adiponectin levels were comparable with baseline levels in group B, whereas still lower in group A, although less markedly than at 6 months. Resistin levels, however, were comparable with baseline values in both groups.

One other study in short SGA children also reported a decline in adiponectin levels after 6 months of GH treatment.[18] In addition, we previously did not find any significant differences in both adiponection and resistin levels after 24 months of GH treatment compared with baseline values, in another group of short SGA children.[31] These results suggest that the effects of GH on adiponectin and resistin levels are time-dependent and most significant in the initial phase of treatment.

Adiponectin levels were not significantly different between SGA children and children of the reference group. Reports on adiponectin levels in SGA children are inconsistent. Two previous studies also found no differences between adiponectin levels in SGA children and AGA controls,[18,32] whereas two other publications reported reduced[14] or higher levels[33] of adiponectin.

Resistin levels were lower in SGA children compared with the reference group. In literature, resistin has been positively related to obesity.[12,13] The fact that our SGA children were lean, might explain their reduced resistin levels.

At baseline, Si and serum adiponectin levels were not related to each other. It has previously been shown that in Pima Indian children, fasting adiponectin levels correlated negatively with fasting insulin levels. However, this association was not present after adjustment for percentage body fat.[34] This suggests an important role for adipose tissue in the relationship between Si and adiponectin levels. This was confirmed by a study of Martin et al., showing a stronger relationship between adiponectin and insulin levels with increasing adiposity and a lack of correlation in lean individuals.[35] It was speculated that the association between adiponectin and Si is stronger in obese subjects because of the pro-inflammatory, insulin resistant environment associated with adiposity. In such an environment, the anti-inflammatory properties of adiponectin might play an important role in influencing Si.[35] However, in lean individuals, such as short SGA children, the impact of adiponectin levels on Si remains unclear.

Interestingly, during GH treatment, Si and adiponectin did correlate. As previously suggested by Martin et al., it might be that the associations between Si and adiponectin levels increase in insulin resistant conditions, due to either obesity or GH administration.[35] We did not find any relationship between Si an resistin levels, which is in agreement with previous studies in Pima Indians[13] and adults.[36]

In summary, our study demonstrates that GH treatment of short SGA children results in parallel changes in Si and serum adiponectin levels, whereas resistin showed opposite changes. At 24 months, Si and adiponectin levels were closer to baseline than at 6 months, whereas resistin levels were comparable. These results suggest that the effects of GH on adiponectin and resistin are time-dependent and most marked in the early phase of GH treatment. Disposition index remained unchanged, indicating that glucose homeostasis was well maintained. During GH treatment, adiponectin levels were positively related to Si, suggesting a possible role for adiponectin in GH-induced insulin-resistance. Longer term studies are necessary to evaluate whether GH treatment has lasting effects on Si and adiponectin levels in short SGA children.

Acknowledgments

We thank Mrs. J.C. Bruinings-Vroombout and C. Wissink, research nurses, for their technical assistance during the study, G. Klein Heerenbrink and H. van Toor for adiponectin and resistin determinations and W.H. Hackeng for analyzing the FSIGT tests.

We greatly acknowledge the physicians of the participating centers: E.C.A.M. Houdijk, Juliana Children's Hospital, The Hague; J.C. Mulder, Rijnstate Hospital, Arnhem; C. Noordam, St Radboud University Medical Center Nijmegen, R.J.H. Odink, University Medical Center Groningen; C. Rongen-Westerlaken, Canisius Wilhelmina Hospital, Nijmegen; W.H. Stokvis, Leiden University Medical Center; P.G. Voorhoeve, VU University Medical Center, Amsterdam; J.J.J. Waelkens, Catharina Hospital, Eindhoven, The Netherlands.

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Chapter 5

The effects of GH treatment on body composition and fat distribution and relations with cardiovascular risk factors in short children born small for gestational age (SGA)

[1]Marije van Dijk, [1]Ruben Willemsen, [2]Johan Waelkens, [3]Jaap Mulder, [4]Mieke Houdijk, [5] Roelof J. Odink, [6]Wilhelmina H. Stokvis-Brantsma, [7]Paul Voorhoeve, [8]Ciska Rongen-Westerlaken, [9]Kees Noordam, [10]Paul Mulder, [1]Anita Hokken-Koelega

[1]Department of Pediatrics, Division of Endocrinology, Sophia Children's Hospital, Erasmus MC, Rotterdam, [2]Catharina Hospital, Eindhoven, [3]Rijnstate Hospital, Arnhem, [4]Juliana Children's Hospital, The Hague, [5]Beatrix Children's Hospital, Groningen, [6]Leiden University Medical Center, Leiden, [7]Vrije Universiteit Medical Center, Amsterdam, [8]Canisius-Wilhelmina Hospital, Nijmegen, [9]University Medical Center, St Radboud, Nijmegen, [10]Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands.

Submitted for publication

Abstract

Context: Many studies have demonstrated an inverse relationship between birth weight and the risk of type 2 diabetes mellitus and cardiovascular disease. In subjects born small for gestational age (SGA), body composition and fat distribution might affect their predisposition of these disorders.

Objective: We evaluated body composition and fat distribution and associations with cardiovascular risk factors before and during 24 months of GH treatment. During the first 6 months, also GH-dose effects were assessed

Patients: Thirty-eight prepubertal short SGA children and 10 untreated short SGA controls.

Intervention. During the first 6 months, group A (n=18) received 1 mg GH/m^2·day and group B (n=20) 2 mg GH/m^2·day. Subsequently, between 6 and 24 months, both groups received 1 mg GH/m^2·day.

Main Outcome Measures. At baseline and after 6 and 24 months, body composition and fat distribution were measured by Dual Energy X-ray Absorptiometry (DXA). Also associations between fat mass, fat distribution with cardiovascular risk factors were investigated

Results: At baseline, lean body mass (LBM) SDS, fat mass SDS and fat percentage SDS were significantly lower than zero. GH treatment resulted in a progressive increase in LBM SDS parallel to the increment in height, and a temporary reduction in fat mass and fat percentage SDS during the first 6 months. This reduction was due to a decline in peripheral rather than central fat. Total fat mass and trunk fat correlated negatively with insulin sensitivity.

Conclusion: GH treatment of short SGA children results in an increase in LBM and a transient decline in fat mass. Fat mass was inversely associated with insulin sensitivity, which underscores the importance of preventing overweight in SGA subjects.

Introduction

Many studies have demonstrated an inverse relationship between birth weight and the risk of adult diseases, including type 2 diabetes mellitus (DM-II), cardiovascular disease, hypertension and metabolic syndrome.[1-3] A well-known risk factor for the development of these disorders is obesity, which may already be present in childhood.[4-6]

There is evidence that birth weight is positively associated with subsequent lean body mass (LBM)[7-15] and negatively with fat mass.[7,9,11,12,16] This suggests that in children born small for gestational age (SGA), LBM may be reduced, whereas fat mass relatively increased. This might affect their risk of the later development of DM-II and cardiovascular disease. In that case, assessment of body composition may be helpful to identify those individuals who are more prone to develop these adult diseases.

GH has well-documented anabolic effects on muscle mass and lipolytic effects on adipose tissue.[17,18] GH deficiency has been associated with increased fat mass and truncal obesity,[19,20] whereas GH excess, as in active acromegaly, has been related to reduced fat mass and an increase in LBM.[21] Leger et al. measured muscle and fat tissue mass of the thighs using magnetic resonance imaging (MRI) in 14 short SGA children during 3 years of GH therapy and one year off therapy.[22] They reported a progressive increase in muscle tissue cross-sectional area and a transient decline in adipose tissue cross-sectional area. At the end of 3 years, the muscle tissue cross-sectional area change was significantly greater in the GH-treated SGA children as compared with untreated controls born appropriate for gestational age (AGA), whereas adipose tissue cross-sectional area change was similar. In this study, however, total body muscle and body fat were not measured, nor was fat distribution. The associations between body composition and insulin sensitivity and other cardiovascular risk factors have also not yet been evaluated in detail in short SGA children.

In the present study, we therefore evaluated body composition and fat distribution, as measured by Dual Energy X-ray Absorptiometry (DXA), in 38 short children born SGA during 24 month of GH treatment in comparison with 10 untreated short SGA controls. We also assessed associations with insulin sensitivity, adiponectin levels and other cardiovascular risk factors. During the first 6 months, children were treated with either 1 or 2 mg GH/ m^2·day in order to investigate possible GH-dose effects.

Patients and methods

Subjects

The study group consisted of 38 prepubertal short children born SGA. Children were included according to the following criteria: 1) birth length and/or birth weight standard deviation score (SDS) below −2 for gestational age,[23], 2) current height SDS score below −2.5,[24] 3) height velocity SD score below zero to exclude children with catch-up growth,[24] 4) prepubertal stage, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys,[25] 5) age between 5−8 years at start of the study and 6) an uncomplicated neonatal period, without signs of severe asphyxia (Apgar score > 3 after 5 minutes) or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia. Children with endocrine or metabolic disorders, chromosomal defects, syndromes and growth failure caused by other conditions (e.g. emotional deprivation, severe chronic illness, chondrodysplasia) were excluded, except for Silver-Russell syndrome. The study was approved by the Medical Ethics Committees of the participating centers and written informed consent was obtained from the parents.

GH treatment protocol

After stratification for gender, GH-status (maximum serum GH levels < 30 mU/L *vs.* > 30 mU/L during a GH stimulation test) and BMI (< −1 SDS *vs.* > −1 SDS), all 38 children were randomized into two groups. During the first 6 months, children of group A (n=18) received 1 mg GH/ m^2·day and children of group B (n = 20) received 2 mg GH/ m^2·day. Subsequently, between 6 and 24 months, all children received the same dose of 1 mg GH/ m^2·day. Biosynthetic GH [Nordiptropin ® SimpleXx™ 15 mg/1.5 ml, Novo Nordisk A/S, Bagsværd, Denmark] was administered sc once daily at bedtime using the Nordipen™ 15.

Study design

At baseline and after 6 and 24 months of GH treatment, standing height and weight were measured and BMI was calculated. Height and BMI were expressed as SD scores adjusting for sex and age according to Dutch reference data for children.[24,26]

At the same time points, a Dual-Energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare) was performed in all children. LBM, total fat mass, fat percentage, trunk and limb fat were measured. For this type of DXA, the intra-assay coefficient of variation (CV) for lean tissue and fat tissue has been reported to be 1.57−4.49% and 0.41−0.88%, respectively.[27] Trunk fat, a measure of central adiposity, is the total amount of fat in the chest, abdomen, and pelvis. Limb fat, a measure of peripheral fat is the total amount of fat in both arms and legs. We also calculated the LBM to height ratio, expressed in grams/cm (g/cm) to adjust for the increase in height during GH treatment.

LBM, total fat mass and fat percentage were transformed into SDS adjusting for sex and age, using Dutch reference values for children with normal stature.[28,29] At baseline and after 24 months, we also measured body composition and fat distribution in 10 untreated short SGA children of the same age as our study subjects, in order to evaluate the natural changes over time.

At baseline and at 24 months, we investigated correlations between fat mass, fat distribution and risk factors for DM-II and cardiovascular disease: insulin sensitivity and acute insulin response as measured with an iv glucose tolerance test (FSIGT),[30] fasting levels of glucose, insulin, adiponectin, resistin, total cholesterol (TC), LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c), serum triglycerides (TG) and systolic and diastolic blood pressure. Systolic and diastolic blood pressure were measured by a Dinamap Critikon (Southern Medical Corp., Baton Rouge, LA) and expressed in SDS, using sex- and age-matched reference values.[31]

Assays

Serum glucose levels were determined on a VITROS analyser 750 (Orthoclinical Diagnostics, Johnson & Johnson Company, Beers, Belgium). Serum insulin levels were measured by an IRMA (Medgenix, Biosource Europe, Nivelles, Belgium). The intra-assay coefficient of variation (CV) was 2 to 4.7% (19–405 pmol/l) and the inter-assay CV was 4.2 to 11.3% (32–375 pmol/l). Serum levels of TC, LDL-c, HDL-c and TG were measured using a enzymatic colorimetric test on the Hitachi 917 analyser (Roche Diagnostics, Mannheim, Germany). Serum adiponectin and resistin levels were assessed by an ELISA (R&D Systems Inc. Minneapolis. USA) The intra-assay CV was < 7% and < 6%, respectively, and the inter-assay CV was < 7% for both.

Statistics

Analyses were carried out using the computer statistical package SPSS for Windows (version 11.1, Chicago, Illinois, USA). Results are expressed as mean (SD), except for total fat mass, fat percentage, trunk and limb fat, which were log-transformed before analysis and expressed as median (interquartile range). In group A and B, repeated measures of variance (mixed model ANOVA) was used to analyse the changes over time at baseline, 6 and 24 months, and to test the differences between the groups. In the untreated short SGA controls, paired Student's t test was used to test differences between baseline and 24 months. To test for linear relationships between continuous variables at baseline, Pearson's correlation coefficient was used for group A and B together, whereas at 24 months partial correlations with adjustment for GH dose were used. Statistical significance was defined as $p < 0.05$.

Results

Clinical characteristics and anthropometrics

Table 1 lists the baseline clinical data of both GH dosage groups and the untreated short SGA controls. Group A and B had comparable clinical characteristics and did not differ from the untreated SGA controls. Mean age at start of the study was 6.4 (1.1), years for the children in group A and B and 6.0 (1.2) years for the short SGA controls. Table 2 shows height and BMI SDS at all time points. In both GH-treated groups, height SDS increased significantly between baseline and 24 months ($p < 0.001$), whereas it remained unchanged in the untreated short SGA controls. Between baseline and 24 months, BMI SDS did not change in group A, increased in group B ($p = 0.003$) and decreased in the untreated SGA controls ($p = 0.020$).

Table 1. Clinical characteristics of the study groups

	Group A (n = 18)	Group B (n = 20)	Untreated SGA controls (n = 10)
Male/female	11/7	11/9	6/4
Gestational age	36.3 (3.3)	36.6 (4.3)	36.3 (2.7)
Birth weight SD score	−2.2 (1.5)	−2.1 (0.9)	−2.8 (0.9)
Birth length SD score	−3.2 (1.1)	−3.2 (0.9)	−3.3 (1.3)
Age at start of study (yrs)	6.4 (1.1)	6.4 (1.1)	6.0 (1.7)
Height SD score at start of study	−3.1 (0.5)	−3.1 (0.5)	−3.2 (0.4)
BMI SD score at start of study	−1.2 (1.2)	−1.3 (0.9)	−1.1 (0.9)

Data expressed as mean (SD)

Lean body mass (LBM)

Table 2 shows LBM in all groups over time. At baseline, LBM SDS was significantly lower than zero in all groups ($p < 0.001$). After 6 and 24 months of GH treatment, LBM SDS had progressively increased in both GH dosage groups ($p < 0.001$). In the untreated SGA controls, however, LBM SDS had significantly decreased after 24 months ($p < 0.001$). The LBM to height ratio had significantly increased after 24 months in group A ($p < 0.001$), group B ($p < 0.001$), and in the SGA control group ($p = 0.004$). However, the increases were significantly greater in both GH-dosage groups than in the untreated SGA controls ($p < 0.001$). After 6 months, when group A had been treated with 1 mg GH/ m^2·day and group B with 2 mg GH/ m^2·day of GH treatment, no differences between the two GH dosage groups were observed.

Fat mass and fat percentage

Total fat mass and fat percentage are shown in Table 2. At baseline, total fat mass SDS and fat percentage SDS were significantly lower than zero in group A ($p = 0.002$ and $p = 0.001$, respectively) and B ($p < 0.001$ and $p = 0.002$, respectively). In the untreated short SGA controls, fat mass SDS was also lower than zero ($p = 0.009$), whereas fat percentage SDS was comparable with zero. During the first 6 months, when group A received 1 mg GH/ m^2·day and group B 2 mg GH/ m^2·day, total fat mass SDS and fat percentage SDS decreased significantly in group A ($p = 0.008$ and $p < 0.001$, respectively) and B ($p < 0.001$). At 6 months, total fat mass SDS and fat percentage SDS were not significantly different between both GH dosage groups. Between 6 and 24 months, when both GH dosage groups received the same dose of 1 mg GH/ m^2·day, total fat mass SDS and fat percentage SDS increased in group A ($p = 0.002$ and $p < 0.001$, respectively) and B ($p < 0.001$). At 24 months, total fat mass SDS was comparable with baseline values in group A, whereas slightly higher than baseline in group B ($p = 0.049$). Fat percentage SDS was lower compared with baseline in group A ($p = 0.006$), but similar in group B. In untreated SGA controls, total fat mass SDS and fat percentage SDS did not change significantly over 24 months.

Fat distribution

Trunk and limb fat are also listed in Table 2. During the first 6 months of GH treatment, the decrease in total fat mass was mainly due to a reduction in limb fat ($p < 0.001$ for group A and B) rather than in trunk fat ($p = 0.040$ and $p = 0.014$, respectively). Between 6 and 24 months, both trunk and limb fat increased significantly in both GH dosage groups ($p < 0.001$). The relative increments in trunk and limb fat between 6 and 24 months were nearly comparable. At 24 months, trunk fat was significantly higher compared with baseline in both GH dosage groups ($p < 0.001$), and limb fat was only significantly higher than baseline in group B ($p = 0.016$). In the untreated SGA controls, trunk fat was significantly higher than baseline values after 24 months ($p = 0.022$), whereas limb fat was not significantly different.

Cardiovascular risk factors

At baseline and at 24 months, several risk factors for DM-II and cardiovascular disease were measured which are shown in Table 3.

Table 2. Body fatness in group A and B and untreated SGA controls

		0 months	6 months	24 months
Group A	Height SDS	−3.1 (0.45)	−2.6 (0.39)[a]	−1.8 (0.4)[a]
0–24 mo: 1 mg GH	BMI SDS	−1.2 (1.2)	−1.3 (1.0)	−1.0 (1.1)
	LBM (g)	13670 (1964)	15492 (2012)[a]	19367 (2141)[a]
	LBM SDS	−2.2 (0.6)	−1.9 (0.5)[a]	−1.5 (0.5)[a]
	LBM to height ratio (g/cm)	127.6 (13.5)	138.0 (13.1)[a]	156.0 (13.0)[a]
	Total fat mass (g) †	1735 (1326–2715)	1268 (962–1994)[a]	2078 (1283–3208)[c]
	Total fat mass SDS	−1.9 (1.4)	−3.5 (2.2)[c]	−2.0 (1.4)
	Fat percentage (%) †	10.6 (8.5–15.9)	7.3 (5.6–10.9)	8.9 (6.7–13.2)[d]
	Fat percentage SDS	−0.9 (1.0)	−2.1 (1.1)[a]	−1.6 (1.1)[c]
	Trunk fat (g) †	635 (379–855)	507 (372–726)[d]	844 (494–1204)[a]
	Limb fat (g) †	871 (595–1469)	541 (381–990)[a]	961 (549–1517)
Group B	Height SDS	−3.1 (0.5)	−2.5 (0.5)[a]	−1.7 (0.6)[a]
0–6 mo: 2 mg GH	BMI SDS	−1.3 (0.9)	−1.1 (0.8)	−0.89 (0.8)[b]
6–24 mo: 1 mg GH	LBM (g)	13147 (1913)	15628 (2336)[a]	19552 (2681)[a]
	LBM SDS	−2.4 (0.4)	−1.8 (0.5)[a]	−1.5 (0.7)[a]
	LBM to height ratio (g/cm)	123.3 (11.5)	138.9 (14.7)[a]	155.8 (16.2)[a]
	Total fat mass (g) †	1864 (1506–2487)	1381 (1016–1617)[a]	2697 (1718–3970)[a]
	Total fat mass SDS	−2.3 (1.5)	−4.3 (1.7)[a]	−1.7 (1.3)[d]
	Fat percentage (%) †	12.3 (9.5–14.8)	7.0 (6.3–8.8)[a]	10.3 (6.9–16.4)
	Fat percentage SDS	−0.9 (1.1)	−2.2 (0.8)[a]	−1.3 (1.1)
	Trunk fat (g) †	681 (453–939)	583 (401–744)[d]	1205 (703–1651)[a]
	Limb fat (g) †	920 (700–1158)	504 (411–659)[a]	1180 (778–1872)[d]

Untreated SGA	Height SDS	−3.2 (0.4)	–	−3.1 (0.3)
Controls	BMI SDS	−1.1 (0.9)	–	−1.4 (1.1)[d]
	LBM (g)	13156 (2706)	–	15240 (2521)[a]
	LBM SDS	−2.1 (0.3)	–	−2.6 (0.3)[a]
	LBM to height ratio (g/cm)	126.1 (14.2)	–	132.6 (12.9)[b]
	Total fat mass (g) †	2593 (1581–3261)	–	2273 (2023–5738)[d]
	Total fat mass SDS	−1.3 (0.9)	–	−1.5 (1.3)
	Fat percentage (%) †	15.5 (12.2–17.9)	–	15.3 (11.6–23.6)
	Fat percentage SDS	−0.2 (0.6)	–	−0.1 (0.7)
	Trunk fat (g) †	673 (594–902)	–	764 (674–1714)[d]
	Limb fat (g) †	1423 (843–1916)	–	1221 (1028–3104)

Data expressed as mean (SD) † Median (interquartile range)
Significantly different from baseline: [a]$p < 0.001$, [b]$p < 0.005$, [c]$p < 0.01$, [d]$p < 0.05$

Table 3. Risk factors for DM-II and cardiovascular disease at baseline and at 24 months in group A and B

	Group A		Group B	
	0 months	24 months	0 months	24 months
Si*10^{-4}/min−1 (μU/ml)†	13.7 (9.0–20.6)	11.2 (8.1–14.2)	13.4 (7.8–19.9)	9.5 (6.0–13.3)
Sg*10^{-2} (m/d)min−1†	2.0 (1.5–2.8)	1.8 (1.5–2.0)	2.1 (1.7–3.2)	2.0 (1.7–2.9)
AIR (mU/L) †	249 (179–339)	328 (209–388)	346 (205–480)	456 (302–639)
DI (AIR * Si)	3717 (1863)	3279 (790)	4591 (1960)	5108 (3324)
Glucose (mmol/L)	4.8 (0.6)	4.4 (0.5)	4.8 (0.6)	4.5 (0.3)
Insulin (mU/L)	6.1 (2.4)	5.7 (1.6)	5.5 (2.6)	6.5 (3.3)
Adiponectin (μg/ml)	14.1 (6.8)	11.7 (5.5)	12.1 (6.7)	11.4 (6.3)
Resistin (ng/ml)	10.4 (2.9)	9.8 (3.1)	7.9 (2.7)	8.2 (3.2)
Total cholesterol (mmol/L)	3.8 (0.6)	4.0 (0.8)	3.9 (0.7)	3.9 (0.4)
LDL–cholesterol (mmol/L)	2.0 (0.7)	2.3 (0.7)	2.2 (0.6)	2.2 (0.4)
HDL–cholesterol (mmol/L)	1.5 (0.3)	1.4 (0.3)	1.5 (0.4)	1.6 (0.3)
Triglycerids (mmol/L)	0.8 (0.4)	0.9 (0.4)	0.7 (0.3)	0.9 (0.4)
Systolic BP SDS	1.1 (0.6)	0.9 (1.2)	1.1 (1.0)	0.7 (1.0)
Diastolic BP SDS	0.0 (1.0)	−0.1 (1.0)	0.1 (0.6)	0.1 (0.5)

Data expressed as mean (SD) † median (interquartile range)

Correlations

At baseline, total fat mass and trunk fat correlated negatively with insulin sensitivity (r = −0.34, p = 0.045 and r = −0.46, p = 0.005, respectively), but not with adiponectin, serum lipids or blood pressure. After 24 months of GH treatment, total fat mass and trunk fat were still inversely related to insulin sensitivity (r = −0.55, p = 0.001 and r = −0.60, p < 0.001, respectively). In addition, trunk fat showed a positive correlation with acute insulin response (r = 0.35, p = 0.041) and fasting insulin levels (r = 0.34, p = 0.048).

Discussion

Before GH treatment, LBM SDS, fat mass SDS and fat percentage SDS of short SGA children were significantly lower than zero. GH treatment resulted in a progressive increase in LBM SDS, parallel to the increment in height SDS. We found a temporary reduction in fat mass and fat percentage SDS, due to a decline in peripheral rather than central fat. Total fat mass and trunk fat were inversely associated with insulin sensitivity.

In short SGA children assessment of body composition and fat distribution is important as it might give clues which children are most at risk for developing DM-II and cardiovascular disease in later life. In the present study, we measured LBM, fat mass and fat percentage, and body fat distribution by DXA. DXA is an accurate method for measuring fat distribution by quantification of fat mass in anatomically defined regions of interest, i.e. trunk and limb region.[32,33]

Several studies have demonstrated a positive association between birth weight and subsequent LBM,[7-15] indicating that low-birth-weight subjects have less muscle mass. In our short SGA children, LBM SDS was significantly reduced at baseline. However, our results were compared with reference values obtained from healthy children with normal height. As LBM was strongly correlated with height SDS (r = 0.86, p < 0.001), at least part of the reduction of LBM SDS can be attributed to the short stature of our study subjects.

Total fat mass SDS and fat percentage SDS were also significantly lower than zero at baseline. Both did not correlate with height SDS. Literature relating birth size to later adiposity is rather inconsistent. Of three other studies with DXA measurements in children, one found no association,[14] one found an inverse relation[16] and one found a positive association.[15] Our study groups consisted of SGA children who were short prior to GH treatment. It might be that fetal programming of body composition is different in short SGA children compared with those who showed spontaneous catch-up growth. Previous studies showed that SGA children with catch-up growth were heavier, taller, and had a higher BMI than those who remained short.[34] In addition, they gained more abdominal fat and body adiposity than AGA children between age 2 and 4 years.[35]

GH treatment resulted in a sustained increase of LBM SDS in both GH dosage groups, whereas in untreated short SGA controls, LBM SDS decreased. Although part of the increment in LBM SDS is due to the parallel increment in height, our data on the LBM to height ratio suggest that GH has also some direct effects on LBM. The increase in LBM to height ratio after 24 months of GH treatment was significantly greater than in the untreated short SGA controls. This implies that for every cm gain in height, the increase in LBM in grams is higher in GH-treated SGA subjects. This is in agreement with the well-documented anabolic effects of GH.[17,18]

Our study showed that GH treatment induced a short-term reduction in fat mass SDS and fat percentage SDS during the first 6 months, which was followed by an increase for both between 6 and 24 months. At 24 months, fat mass SDS and fat percentage SDS were similar to baseline or only slightly different. In untreated SGA controls, fat mass SDS and fat percentage SDS at 24 months were also comparable with baseline. This transient lipolytic effect of GH has been repeatedly found in other studies in SGA children as well as GH-deficient children.[20,36,37]

During the first 6 months of GH treatment, the decrease in total fat mass was mainly due to a decrease in limb fat rather than trunk fat. It appears that in the initial phase of GH treatment, there is a preferential reduction in peripheral fat rather than central fat in short SGA children, suggesting that GH affects adipose tissue differently in the various fat depots. Between 6 and 24 months, the increases in trunk and limb fat were nearly comparable. In another study in GH-deficient children, the reduction in fat percentage of trunk and limbs was largely comparable during 6 months of GH treatment.[20] However, GH-deficient children are known with marked truncal obesity,[38,39] whereas short SGA children have a lean appearance.[40]

During the first 6 months, children of group A and B received 1 and 2 mg GH/m^2·day, respectively, in order to investigate possible GH-dose effects on body composition and fat distribution. Within the GH-dose range we used, we did not observe any significant differences in LBM, total fat mass, trunk, and limb fat, suggesting that the effects of GH on body composition and fat distribution are not dose-dependent.

At baseline and after 24 months of GH treatment, total fat mass and trunk fat correlated negatively with insulin sensitivity. This is consistent with previous reports in chidren.[4,41] These data underline the importance of preventing overweight and obesity in children, especially in those who might already be at increased risk for adult diseases, such as SGA children.

In conclusion, our study shows that GH treatment results in an increase in LBM and a temporary reduction in total fat mass and fat percentage, which appears to be due to decrease in peripheral rather than central fat. These data may suggest that the anabolic effects of GH are stronger than its lipolytic effects in childhood. Total fat mass and trunk fat were inversely associated with insulin sensitivity, which highlights the need

for preventing overweight in SGA subjects. Long-term follow-up studies are required to investigate whether the effects of GH on body composition and fat distribution are persistent.

Acknowledgments

We greatly acknowledge Mrs. J.C. Bruinings-Vroombout and Mrs. M. Huibregtse-Schouten, research nurses, for their assistance during the study, Prof. E.P Krenning, PhD, head of the Nuclear Medicine Department and Mrs. J.P. Sluimer for performing and analysing the DXA measurements.

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Chapter 6

Risk factors for diabetes mellitus type 2 and metabolic syndrome are comparable for previously growth hormone (GH)-treated young adults born small for gestational age (SGA) and untreated short SGA controls

[1]Marije van Dijk, [1]Ellen M.N. Bannink, [1]Yvonne K. van Pareren, [2]Paul G.H. Mulder, and [1]Anita C.S. Hokken-Koelega

[1]Department of Pediatrics, Division of Endocrinology, Sophia Children's Hospital, Erasmus MC, Rotterdam, [2]Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands.

J Clin Endocrinol Metab, 2007, 92: 160-165

Abstract

Context: Low birth weight might increase risk of diabetes mellitus type 2 (DM-II) and metabolic syndrome (MS). Growth hormone (GH) has insulin-antagonistic properties. Therefore, long-term follow-up of GH-treated children born small for gestational age (SGA) is important.

Objective and patients: The objective of the study was to evaluate insulin sensitivity (Si) and disposition index (DI), all components of the MS and IGF-I and IGFBP-3 levels in 37 previously GH-treated young SGA adults in comparison with 25 untreated short SGA controls.

Results: GH-treated subjects were 22.3 (1.7) years. Mean duration of GH treatment had been 7.3 (1.3) years. Mean period after discontinuation was 6.5 (1.4) years. Si and DI were comparable for GH-treated and untreated SGA subjects. Fasting glucose and insulin levels increased during GH treatment, but recovered after discontinuation. BMI, waist circumference, HDL-c levels and triglycerids were equivalent. Systolic and diastolic blood pressure and cholesterol were significantly lower in GH-treated subjects. Thirty-two percent of untreated controls had an increased blood pressure versus none of the GH-treated subjects. GH-induced rises in IGF-I and IGFBP-3 levels had completely recovered after GH stop.

Conclusion: At 6.5 years after discontinuation of long-term GH treatment, Si, DI, fasting levels of glucose and insulin, BMI, waist circumference, IGF-I and IGFBP-3 levels were equivalent for GH-treated and untreated young SGA adults. Systolic and diastolic blood pressure and serum cholesterol were even lower in GH-treated subjects. These data are reassuring because they suggest that long-term GH-treatment does not increase the risk for DM-II and MS in young adults.

Introduction

In epidemiological studies, an inverse association has been reported between birth weight and the risk of diabetes mellitus type 2 (DM-II) and metabolic syndrome in adulthood.[1-3] Approximately 10% of children born small for gestational age (SGA) fail catch-up growth and remain short with a height below −2 standard deviation scores (SDS).[4,5] Growth hormone (GH) treatment has recently been approved for short SGA children, and nowadays, they comprise a large group of GH-treated children, accounting for 30% of new cases (Dutch Growth Foundation, Rotterdam, The Netherlands).

Because GH has been associated with increased insulin levels and insulin resistance,[6-9] concern has been expressed regarding the late consequences of GH treatment on risk factors for DM-II and associated comorbidities, especially in possibly predisposed subjects, such as SGA children. As GH use in this population will sharply increase in the coming years, long-term follow-up is important.

It was previously shown that short SGA children had reduced insulin sensitivity before receiving GH, which further declined during GH therapy.[10-12] Most studies have reported a recovery of insulin sensitivity and insulin levels to pre-treatment levels within 3 to 6 months after withdrawal of GH treatment.[13,14] It was also reported that SGA children had a higher systolic blood pressure and more often hypercholesterolemia.[15,16] GH treatment resulted in a significant reduction in systolic blood pressure, as well as a reduction in cholesterol levels which remained so until 6 months after discontinuation of GH therapy.[15]

However, there are no long-term follow-up data on risk factors for DM-II and metabolic syndrome after discontinuation of GH treatment in subjects born SGA. In the present study, we evaluated fasting insulin and glucose levels, blood pressure, body mass index (BMI), fasting serum lipids, and serum levels of IGF-I and IGF-binding protein (IGFBP)-3 in young adults born SGA from start of GH treatment until 6.5 years after discontinuation of GH. At 6.5 years after discontinuation, all these outcome variables were compared with those of untreated short SGA controls and a frequently sampled iv glucose tolerance test (FSIGT) with Tolbutamide was performed .

Patients and methods

Subjects

Previously GH-treated SGA subjects. The study group comprised 37 subjects born SGA who had previously been participating in a multi-center, double-blind, randomised, dose-response GH trial which originally involved 79 children.[17,18] The dose-response GH trial started in 1991 and evaluated the effects of 2 doses of GH,

1 and 2 mg GH/ m^2·day, on long-term growth and adult height. Inclusion criteria for the GH trial have previously been described.[17] In short, the children were included when prepubertal, with a birth length and height standard deviation score (SDS) below −1.88, without signs of any catch-up growth in height and without growth failure caused by other disorders. All children were randomly and blindly assigned to either group A or B: group A received 1 mg GH/ m^2·day and group B received 2 mg GH/ m^2·day. Biosynthetic GH was administered subcutaneous once daily and GH treatment was stopped after reaching adult height.

The present follow-up study was performed in 2005. Inclusion criteria were a period of at least 4 years after discontinuation of GH treatment and being treated with GH for more than 4 years. Forty-two of the original 79 participants were not included for the following reasons: for 20 subjects, the period after discontinuation of GH treatment was less than 4 years, 4 children dropped out during the original GH trial due to either lack of motivation (n = 2), precocious puberty (n = 1), or GH insensitivity (n = 1), 2 subjects were lost to follow-up, 2 emigrated, 1 subject died due to a road accident, 5 persons did not respond to the invitation letter, and 8 subjects did not want to participate due to either lack of interest (n = 4) or fear for venous punctures (n = 4). Initial characteristics of the eligible 37 GH-treated SGA subjects were comparable with those of the 42 subjects who were excluded, except for age at inclusion (8.5 *vs.* 6.3 years, respectively; $p < 0.001$) and duration of GH treatment (7.4 *vs.* 9.4 years, respectively; $p < 0.001$).

Untreated short SGA controls: All outcome variables at 6.5 years after GH stop were compared with those of 25 short young adults born SGA who had never received GH treatment. These subjects were part of a large cohort of young adults participating in a follow-up study evaluating risk factors for DM-II and cardiovascular disease. They were selected on their birth length and current height which were both below −1.88 SDS.

The GH trial was approved by the Medical Ethics Committees (MEC) of the participating centers, and the follow-up study by the MEC of Erasmus University Medical Center, Rotterdam, The Netherlands. Written informed consent was obtained from all participants or their parents.

Study design

The previously GH-treated SGA subjects were monitored longitudinally. At start, after 6 years of GH treatment and 6 months and 6.5 years after discontinuation of GH, height and weight were measured and BMI was calculated. Height and BMI were expressed in SDS adjusting for sex and age according to Dutch reference data.[19,20] Systolic and diastolic blood pressure (BP) were measured by a Dinamap Critikon (Southern Medical Corp., Baton Rouge, LA, USA) and expressed in SDS, using sex- and height-matched reference values.[20,21] At the same time points, fasting blood samples were taken for

determination of glucose, insulin, fasting glucose to insulin ratio, hemoglobin A1c (HbA1c), serum cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), and IGF-I and IGFBP-3 levels. Serum IGF-I and IGFBP-3 levels were converted into SDS to adjust for sex and age, using reference values for healthy children with normal stature determined in the same laboratory.[22] After centrifugation all samples were frozen (−80 °C) until assayed.

At 6.5 years after discontinuation of GH, we also performed a frequently sampled iv glucose tolerance test (FSIGT) with Tolbutamide.[23] Glucose and insulin levels were measured in all samples and used for calculation of insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR) and disposition index (DI) using Bergman's MINMOD MILLENNIUM software.[24] Si quantifies the capacity of insulin to promote glucose disposal and Sg reflects the capacity of glucose to mediate its own disposal. The AIR, an estimate of insulin secretory capacity, was measured as the area under the curve from zero to ten minutes corrected for baseline insulin levels. DI index equals AIR*Si and indicates the degree of glucose homeostasis. In addition, family history of DM-II was recorded and waist circumference was measured at the level of the umbilicus using a non-extendable measuring tape.

Metabolic syndrome components

At 6.5 years after discontinuation of GH treatment, the various components of the metabolic syndrome were assessed in both the previously GH-treated and untreated SGA subjects. According to criteria formulated by Adult Treatment Panel III (ATP III), metabolic syndrome is diagnosed if 3 or more of the following symptoms are present: central obesity (waist circumference ≥ 102 (males) or 88 cm (females), raised TG levels (TG ≥ 1.7 mmol/L), reduced HDL-c levels (HDL-c < 1.0 (males) or 1.3 (females) mmol/L), high blood pressure (systolic ≥ 130 and/or diastolic BP ≥ 85 mm Hg) and increased fasting glucose levels (glucose ≥ 6.1 mmol/L).[25]

Assays

Glucose levels were determined on a VITROS analyser 750 (Orthoclinical Diagnostics, Johnson & Johnson, Beers, Belgium). Serum insulin levels were measured by an IRMA (Medgenix, Biosource Europe, Nivelles, Belgium). The intra-assay coefficient of variation (CV) was 2 to 4.7% (19–405 pmol/l) and the inter-assay CV was 4.2 to 11.3% (32–375 pmol/l). HbA1c levels were measured using an automatic HPLC analyser (DIAMAT; Bio-Rad Laboratories, Inc., Edgemont, CA, USA). The upper-normal assay limit was 6.6%. Serum levels of cholesterol, LDL-c, HDL-c and TG were measured using an enzymatic colorimetric test on the Hitachi 917 analyser (Roche Diagnostics, Mannheim, Germany). Serum IGF-I and IGFBP-3 levels were determined in one laboratory by a specific RIA as previously described.[26,27]

Statistics

Analyses were carried out using the computer statistical package SPSS for Windows (version 11.1, Chicago, Illinois, USA). Statistical analyses in the GH-treated SGA subjects were performed for group A and B separately and for the groups together. Because outcome variables were not different between the GH dosage groups, data are shown for both groups together, unless indicated otherwise. Results are expressed as mean (SD), except for Si, AIR, Sg and DI which were log-transformed before analysis and expressed as median (interquartile range). Changes over time were analysed with repeated measures of variance (mixed models ANOVA). Firstly, an F test was performed to test whether time had a significant effect. To correct for multiple testing, a p value < 0.005 ($\alpha = 0.05/10$) was considered statistical significant. Then, only when $p < 0.005$, repeated measures of variance (mixed models ANOVA) was used to test differences between baseline and the different time points. Differences between GH-treated SGA subjects and untreated short SGA controls were evaluated using an Independent-Samples t-test and Fisher's Exact test for proportions. For linear relationships between continuous variables, Pearson's correlation coefficients were used. Before the study, a power calculation with a significance levels (α) of 0.05 and a chosen power of 80% estimated that there should be at least 17 subjects in each group to identify a difference of 20% in insulin sensitivity. A difference of 20% in insulin sensitivity was considered as clinically relevant.

Results

Clinical characteristics and family history of DM-II

Clinical characteristics of the previously GH-treated SGA subjects (n = 37) and untreated SGA controls (n = 25) are shown in Table 1. Within the GH-treated SGA group, only gestational age was different between groups A (n = 19) and B (n = 18) [37.8 (3.2) *vs.* 35.2 (4.3) weeks, respectively; $p = 0.042$]. Compared with untreated SGA controls, gestational age, birth length and birth weight SDS were lower in GH-treated SGA subjects, whereas current height SDS was significantly higher ($p < 0.001$). At 6.5 years after GH stop, GH-treated SGA subjects were 1.3 years older. Mean duration of GH treatment had been 7.3 (1.3) years and period after discontinuation of GH was 6.5 (1.4) years.

Of the GH-treated SGA subjects, 10/25 (40.0%) had a positive family history for DM-II compared with 10/23 (43.5%) of the untreated SGA controls.

Table 1. Clinical characteristics of previously GH-treated SGA subjects and untreated SGA controls

	GH–treated SGA group (n = 37)	Untreated short SGA controls (n = 25)
Male/female	22/15	11/14
Gestational age	36.6 (3.9)[a]	39.6 (1.3)
Birth length SDS	−3.6 (1.5)[b]	−2.9 (0.7)
Birth weight SDS	−2.6 (1.0)[a]	−1.8 (0.8)
Height SDS at start GHRx	−2.9 (0.7)	–
Height SDS at present study	−1.4 (1.0)[a]	−2.6 (0.6)
Age at start GH treatment (yrs)	8.5 (1.7)	–
Age at stop GH treatment (yrs)	15.8 (1.4)	–
Age at follow–up study (yrs)	22.3 (1.7)[b]	21.0 (1.7)
GH duration	7.3 (1.3)	–
Period after GH stop	6.5 (1.4)	–

Data expressed as mean (SD)
GH-treated SGA subjects *vs.* untreated SGA controls: [a]$p < 0.001$; [b]$p < 0.05$

Insulin sensitivity and glucose homeostasis

FSIGT results are listed in Table 2. At 6.5 years after GH stop, Si, Sg, AIR and DI were not significantly different between the previously GH-treated and untreated SGA subjects. Interestingly, DI tended to be higher in GH-treated SGA subjects, although not significantly ($p = 0.077$).

Table 3 shows fasting levels of glucose, insulin and HbA1c and fasting glucose to insulin ratio. Fasting glucose and insulin levels increased significantly during GH treatment ($p = 0.002$ and $p < 0.001$), but were not significantly different from baseline anymore at 6 months after discontinuation. At 6.5 years after discontinuation, glucose and insulin levels were higher than at baseline ($p = 0.003$ and $p < 0.001$, respectively), but comparable with untreated SGA controls. Fasting glucose to insulin ratio did not change significantly over time and was comparable for GH-treated and untreated SGA subjects at 6.5 years after discontinuation. HbA1c decreased during GH treatment ($p < 0.001$), but returned to baseline values at 6.5 years after discontinuation of GH. At that time, HbA1c was lower in GH-treated than untreated SGA subjects ($p = 0.007$). None of the GH-treated or untreated SGA subjects had elevated fasting glucose levels according to ATP III criteria.[25]

Table 2. FSIGT results in previously GH-treated SGA subjects and untreated SGA controls

	GH–treated SGA group	Untreated short SGA controls
Si*10^{-4}/min–1 (μU/ml)	5.8 (4.6–8.6)	5.7 (3.3–8.5)
Sg*10^{-2} (m/d)min–1	1.85 (1.63–2.18)	1.95 (1.76–2.25)
AIR (mU/L)	466 (305–800)	446 (259–720)
DI (AIR * Si)	3516 (1846–4638)	2289 (1529–3534)

Data expressed as median (interquartile range)
Si, insulin sensitivity; Sg, glucose effectiveness; AIR, acute insulin response;
DI, disposition index

Blood pressure (BP)

Systolic and diastolic BP are shown in Table 3. In the GH-treated SGA subjects, baseline systolic BP SDS was significantly higher than zero ($p < 0.001$), whereas diastolic BP SDS was similar to zero. During GH treatment, both systolic and diastolic BP SDS decreased significantly ($p < 0.001$ and $p = 0.004$, respectively). At 6.5 years after discontinuation of GH treatment, systolic BP SDS was significantly lower than at baseline ($p < 0.001$), whereas diastolic BP SDS was equivalent to baseline values. Both were not different from zero SDS. The previously GH-treated SGA subjects had a significantly lower systolic and diastolic BP than untreated SGA controls ($p < 0.001$). According to ATP III criteria, none of the GH-treated SGA subjects had an increased systolic or diastolic BP, compared with 8/25 (32.0%) of the untreated SGA controls ($p < 0.001$).[25]

Body mass index and waist circumference

BMI SDS and waist circumference are shown in Table 3. In the GH-treated SGA subjects, baseline BMI SDS was significantly lower than zero ($p < 0.001$). During GH treatment, BMI SDS increased significantly ($p < 0.001$) to values similar to zero. At 6.5 years after discontinuation of GH, BMI SDS of the previously GH-treated SGA subjects was not different from the untreated SGA controls. Waist circumference was similar for GH-treated and untreated SGA subjects, also after adjustment for sex and height. None of the GH-treated SGA subjects had an increased waist circumference compared with 1/25 (4.0%) of the untreated SGA controls according to ATP III criteria.[25]

Table 3. Glucose and insuling levels, blood pressure, BMI, serum lipids and IGF-I and IGFBP-3 levels in previously GH-treated SGA subjects and untreated SGA controls

	GH–treated SGA group				Untreated short SGA controls
	Baseline	6 yrs of GH	6 mo post GH	6.5 yrs post GH	
Glucose (mmol/L)	4.2 (1.0)	5.0 (0.6)[b]	4.7 (0.5)	4.9 (0.5)[b,f]	5.0 (0.4)
Insulin (mU/L)	6.2 (3.5)	16.0 (8.0)[a]	8.8 (7.4)	9.3 (3.9)[a]	10.7 (5.4)
Fasting G/I	1.1 (1.4)	0.4 (0.2)	0.7 (0.4)	0.6 (0.2)	0.6 (0.3)
HbA1c (%)	5.0 (0.3)	4.8 (0.4) [a]	4.7 (0.4)[a]	5.2 (0.3)[d,i]	5.4 (0.3)
Systolic BP (mm Hg)	106.7 (11.1)	111.4 (12.7)[c]	115.3 (12.6)[a]	111.9 (8.9)	121.5 (11.6)
Diastolic BP (mm Hg)	57.9 (9.5)	55.7 (7.2)	59.4 (9.2)	63.8 (6.3)[b,f,g]	75.7 (6.3)
Systolic BP SDS	1.1 (0.9) [j]	0.3 (1.2)[a]	0.3 (1.2)[a]	0.0 (1.7)[a,g]	1.3 (0.9)
Diastolic BP SDS	0.0 (1.1)	−0.5 (0.6)[b,j]	0.3 (0.8)	0.1 (0.6)[f,h,g]	1.0 (0.5) [j]
BMI (kg/m^2)	14.7 (1.8)	19.1 (2.8)[a]	20.6 (2.8)[a]	22.4 (2.2)[a,d]	22.6 (3.3)
BMI SDS	−0.9 (1.3)[a]	0.1 (1.0)[a]	0.2 (1.0)[a]	−0.1 (0.7)[a]	0.2 (1.2)
Waist circumference (cm)	–	–	–	74.9 (6.7)	74.9 (9.2)
Cholesterol (mmol/L)	4.6 (0.8)	3.6 (0.6)[a]	4.1 (0.6)[a]	4.3 (0.8)[c,f,i]	4.8 (1.1)
LDL–c (mmol/L)	2.8 (0.8)	2.2 (0.6)[a]	2.5 (0.6)[a]	2.6 (0.7)[a]	–
HDL–c (mmol/L)	1.4 (0.3)	1.1 (0.2)[a]	1.1 (0.2)[a]	1.3 (0.3)[d]	1.2 (0.3)
TG (mmol/L)	1.0 (0.6)	1.4 (0.8)	1.2 (0.6)	1.4 (1.3)	1.0 (0.6)
IGF–I SDS	−0.9 (1.1) [j]	1.9 (0.7)[a,j]	–	−0.4 (0.7)[c,k]	−0.6 (1.0)[k]
IGFBP–3 SDS	−0.9 (0.9) [j]	1.2 (1.0)[a,j]	–	−1.6 (0.6)[a,i,j]	−1.2 (0.7)[j]

Data expressed as mean (SD)
G/I, glucose to insulin ratio
Compared with baseline values: [a]p < 0.001; [b]p < 0.005; [c]p < 0.05
Compared with 6 months post GH: [d]p < 0.001; [e]p < 0.005; [f]p < 0.05
GH-treated SGA subjects *vs.* untreated short SGA controls: [g]p < 0.001; [h]p < 0.005; [i]p < 0.05
Compared with zero: [j]p < 0.001; [k]p < 0.005; [l]p < 0.005

Serum lipid levels

Fasting serum lipid levels are listed in Table 3. During GH treatment, serum cholesterol, LDL-c and HDL-c levels decreased significantly (p < 0.001). At 6.5 years after stop, cholesterol and LDL-c levels were still lower than baseline values (p = 0.016), whereas HDL-c levels were equivalent. TG levels did not change during GH treatment. At 6.5 years after GH stop, serum cholesterol levels were significantly lower in GH-treated SGA subjects than untreated SGA controls, whereas HDL-c and TG levels were comparable. According to ATP III criteria, 6/37 (16.2%) of the GH-treated SGA subjects had high TG

levels and 6/37 (16.2%) had low HDL-c levels compared with 4/24 (16.7%) and 10/23 (43.5%) (p = 0.034) of the untreated SGA controls, respectively.[25]

Metabolic syndrome

Table 4 shows the different components of the metabolic syndrome. According to ATP III criteria, none of the GH-treated SGA subjects had metabolic syndrome compared with 2/25 (8.0%) of the untreated short SGA controls.[25]

Table 4. Metabolic syndrome components in previously GH-treated SGA subjects and untreated SGA controls, according to ATP III criteria.[25]

Symptoms	GH-treated SGA group	Untreated short SGA controls
Central obesity	None	1/25 (4.0%)
High triglycerides	6/37 (16.2%)	4/24 (16.7%)
Low HDL-c levels	6/37 (16.2%)[a]	10/23 (43.5%)
High blood pressure	None[b]	8/25 (32.0%)
High fasting glucose	None	None
> 3 symptoms	None	2/25 (8.0%)

Compared with untreated short SGA controls: [a]p = 0.034; [b]p < 0.001

Serum IGF-I and IGFBP-3 levels

Table 3 shows serum IGF-I and IGFBP-3 levels. In GH-treated SGA subjects, baseline IGF-I and IGFBP-3 SDS were significantly lower than zero. During GH treatment, IGF-I and IGFBP-3 SDS increased significantly (p < 0.001), resulting in values higher than zero (p < 0.001). At 6.5 years after discontinuation of GH, IGF-I and IGFBP-3 SDS had decreased and were significantly lower than zero again (p = 0.003 and p < 0.001, respectively). IGF-I SDS was comparable for GH-treated and untreated SGA subjects, whereas IGFBP-3 SDS was slightly lower in the GH-treated group (p = 0.046). None of the SGA subjects had IGF-I levels > 2 SDS.

Correlations

Si did not correlate with blood pressure, waist circumference, serum lipids or IGF-I and IGFBP-3 SDS in the GH-treated SGA subjects, whereas in untreated short SGA controls, Si was inversely related to cholesterol levels (r = -0.45, p =0.031) and IGF-I (r = −0.53, p = 0.008) and IGFBP-3 SDS (r = −0.51, p = 0.011). DI did not correlate with any of the outcome variables.

Discussion

Our longitudinal follow-up study shows that at 6.5 years after discontinuation of long-term GH treatment, insulin sensitivity (Si), acute insulin response (AIR), disposition index (DI), fasting glucose and insulin levels, BMI, waist circumference and IGF-I levels were comparable for previously GH-treated and untreated SGA subjects. Systolic and diastolic blood pressure (BP) and serum cholesterol were significantly lower in previously GH-treated SGA subjects.

Small size at birth has been associated with a higher risk of DM-II and metabolic syndrome in adulthood.[1-3] In the present study, risk factors for DM-II and metabolic syndrome were longitudinally measured in previously GH-treated SGA subjects and compared with untreated short SGA controls.

At 6.5 years after discontinuation of GH, Si, AIR, and DI were equivalent in GH-treated SGA subjects and untreated SGA controls. In addition, the GH-induced rise in glucose and insulin levels recovered after GH was stopped. At 6.5 years after discontinuation, none of the GH-treated subjects either had increased fasting glucose levels or developed DM-II. GH has well-known insulin-antagonistic effects and its use has been associated with a reduction in insulin sensitivity and hyperinsulinemia.[13,14,23,28] We show that these changes are reversible after discontinuation of GH treatment and remain so until at least 6.5 years after discontinuation. Because insulin sensitivity and insulin secretory capacity are both strong predictors of the subsequent development of DM-II,[29] our data are reassuring and suggest that long-term GH treatment of short SGA children does not have permanent effects on glucose homeostasis or increase the risk of DM-II.

Young GH-treated SGA adults had a normal systolic and diastolic BP SDS at 6.5 years after discontinuation of GH treatment. In contrast, both systolic and diastolic blood pressure SDS were significantly higher than zero in untreated SGA controls. Low birth weight has been associated with hypertension in later life and several studies have reported an increased systolic BP in SGA adolescents.[30,31] Before start of treatment, we also found an elevated systolic blood pressure in our SGA subjects which decreased during GH treatment.[15] Taken these data together, GH treatment might have long-lasting beneficial effects on blood pressure in short SGA subjects.

Before start of GH treatment, our short SGA children had a low BMI, which normalized during GH treatment.[15] Both BMI SDS and waist circumference were comparable for GH-treated and untreated SGA subjects. It has been demonstrated that the GH-induced increase in BMI is due to a rise in muscle mass rather than fat mass.[32,33] Given the fact that waist circumference is positively related to height[34] and that the GH-treated SGA subjects were taller than the untreated SGA controls, it might be that the latter have relatively more fat mass. Further studies comparing body composition

and fat distribution in GH-treated and untreated SGA subjects are necessary to confirm this.

In the present study, serum cholesterol was lower in GH-treated SGA subjects than in the untreated SGA controls, whereas HDL-c and TG were equivalent for both groups. During GH treatment, serum levels of cholesterol, LDL-c and HDL-c, fell during the first year and remained stable thereafter.[15] After discontinuation, cholesterol and LDL-c levels were lower than baseline values. Tenhola et al. previously reported a higher incidence of hypercholesterolemia among SGA children.[16] and it has also recently been shown that young SGA adults had significantly higher TG and lower HDL-c levels compared with controls born appropriate for gestational age (AGA).[35] Hence our data imply that GH treatment might have positive effects on lipid metabolism, which still persist after discontinuation of GH.

IGF-I and IGFBP-3 levels were significantly lower than zero SDS at baseline. During GH treatment, both increased significantly, resulting in values higher than zero. Previous studies have shown that GH treatment of short SGA subjects induces dose-dependent rises in GH, IGF-I and IGFBP-3 levels.[17,36,37] Concern has been expressed that persistently high GH and IGF-I levels could increase cancer risk in later life.[38] Reassuringly, at 6.5 years after discontinuation, serum IGF-I and IGFBP-3 levels had decreased and were comparable with those of untreated short SGA controls, indicating that the GH-induced rise in IGF-I and IGFBP-3 levels is completely reversible after discontinuation of GH.

In conclusion, our follow-up study shows that at 6.5 years after discontinuation of long-term GH treatment, Si, DI, fasting levels of glucose and insulin, BMI, waist circumference, IGF-I and IGFBP-3 levels were comparable for GH-treated and untreated young SGA adults. In addition, it turned out that systolic and diastolic BP and serum cholesterol were even lower in GH-treated subjects. These data are reassuring because they suggest that long-term GH-treatment does not increase the risk for DM-II and metabolic syndrome in young adults.

Acknowledgments

We thank Mrs. J. Dunk, J. van Nieuwkasteele and J.C. Bruinings, research nurses, for their technical assistance and help with data collection. We greatly acknowledge the physicians who participated in the original GH trial: E.C.A.M. Houdijk, MD, PhD, VU University Medical Centre, Amsterdam; M. Jansen, MD, PhD, Wilhelmina Children's Hospital, Utrecht; H.M. Reeser, MD, PhD, Juliana Children's Hospital, The Hague, The Netherlands.

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Chapter 7

Body composition and adiponectin levels in previously growth hormone (GH)-treated young adults born small for gestational age (SGA): comparisons with sex- and height-matched untreated SGA and AGA subjects

[1]M. van Dijk, [1]R.H. Willemsen, [1]E.M.N. Bannink, [2]Y.B. de Rijke, [3]A.W. van Toorenenbergen, [4]P.G.H. Mulder and [1]A.C.S. Hokken-Koelega

[1]Department of Pediatrics, Division of Endocrinology, Sophia Children's Hospital, [2]Department Internal Medicine, Diagnostic Laboratory Endocrinology, [3]Department of Clinical Chemistry, [4]Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands.

Submitted for publications

Abstract

Context: Birth weight is associated with subsequent body composition. GH treatment of short children born small for gestational age (SGA) results in a decline in fat mass and an increase in lean body mass (LBM). It is unknown whether these changes persist on the long term.

Objective: We investigated the impact of earlier GH treatment in short SGA children on body composition and fat distribution in young adulthood.

Patients: Thirty-seven previously GH-treated young SGA adults and 37 untreated sex- and height- matched SGA and AGA controls participated in the study.

Main outcome measures: In all groups, body composition and fat distribution were evaluated by Dual Energy X-ray Absorptiometry (DXA), and adiponectin levels were measured.

Results: GH-treated subjects were 21.9 (1.3) years. Mean duration of GH treatment and period after discontinuation were 7.3 (1.3) and 6.5 (1.4) years, respectively. Waist circumference was significantly lower in GH-treated than untreated SGA subjects ($p = 0.008$). Fat mass and percentage, trunk and limb fat tended to be higher in untreated SGA controls, but did not reach statistical significance. LBM and adiponectin levels were comparable for all groups.

Conclusion: At 6.5 years after discontinuation of long-term GH treatment, body composition and adiponectin levels were not significantly different in previously GH-treated SGA subjects and untreated sex- and height-matched SGA and AGA controls, except for waist circumference. These data are reassuring as they suggest that long-term GH of short SGA children treatment does not have unfavourable effects on body composition in adulthood.

Introduction

Epidemiological studies have shown an inverse association between birth size and the risk of adult diseases, including coronary artery disease, hypertension, diabetes mellitus type 2 (DM-II) and metabolic syndrome. Obesity is an important risk factor for these diseases and several studies have investigated the relationship between birth weight and subsequent adiposity and fat distribution.[1-10] Most of these studies showed a positive association between birth weight and lean body mass (LBM)[2-10] and an inverse relation with body fat mass,[1,2,4,5,7] indicating that low-birth weight subjects have less muscle mass and are more likely to become obese as adults than those with normal birth weight.

Most children born small for gestational age (SGA) show spontaneous catch-up growth in height, whereas approximately 10% of them remain short with a height below −2 SD scores.[11,12] SGA children who remain short have a typical lean appearance with a low BMI.[13] We previously showed by Dual Energy X-ray Absorptiometry that their leanness is characterised by a reduction in fat mass as well as LBM.[14] GH treatment of these children resulted in a transient decline in fat mass and an increase in LBM, which is consistent with the lipolytic and anabolic properties of GH.[14,15] The long-term effects of GH treatment on body composition in SGA subjects are, however, unknown.

Body composition is greatly influenced by age, sex and height.[16] It is therefore important to match for these parameters when comparing LBM and fat mass in low- and normal-birth weight subjects. Given the facts that SGA subjects remain on average shorter and that none of the previous studies have matched for height, some of the reported differences in body composition might be related to height differences between the study groups.

Adipose tissue secretes a number of hormones and cytokines that have been implicated in the development of obesity-related disorders. In contrast to other adipocytokines that are markedly increased in obesity, adiponectin is decreased in obese subjects and inversely associated with insulin resistance.[17-19] In SGA subjects, no data are available on the long-term effects of GH on adiponectin levels and its relation with body composition.

In the present study, we measured body composition and fat distribution by Dual Energy X-ray Absorptiometry (DXA), and adiponectin levels in 37 previously GH-treated young SGA adults at 6.5 years after discontinuation of GH. Results were compared with those of 37 untreated sex- and height-matched SGA and 37 AGA controls.

Patients and methods

Subjects

GH-treated SGA subjects. The study group comprised 37 subjects born SGA who had previously been participating in a multi-center, double-blind, randomised, dose-response GH trial which originally involved 79 children.[20,21] The dose-response GH trial started in 1991 and evaluated the effects of 2 doses of GH, 1 and 2 mg GH/ m^2·day, on long-term growth and adult height. Inclusion criteria for the GH trial have previously been described.[20] In short, the children were included when prepubertal, with a birth length and height standard deviation score (SDS) below −1.88, without signs of any catch-up growth in height and without growth failure caused by other disorders. All children were randomly and blindly assigned to either group A or B: group A received 1 mg GH/ m^2·day and group B received 2 mg GH/ m^2·day. Biosynthetic GH was administered subcutaneously once daily and GH treatment was stopped after reaching adult height.

The present follow-up study was performed in 2005. Inclusion criteria were a period of at least 4 years after discontinuation of GH treatment and being treated with GH for more than 4 years. Forty-two of the original 79 participants were not included for the following reasons: for 20 subjects, the period after discontinuation of GH treatment was less than 4 years, 4 children dropped out during the original GH trial due to either lack of motivation (n = 2), precocious puberty (n = 1), or GH insensitivity (n = 1), 2 subjects were lost to follow-up, 2 emigrated, 1 subject died due to a road accident, 5 persons did not respond to the invitation letter, and 8 subjects did not want to participate due to either lack of interest (n = 4) or fear for venous punctures (n = 4). Initial characteristics of the eligible 37 GH-treated SGA subjects were comparable with those of the 42 subjects who were excluded, except for age at inclusion (8.5 *vs*. 6.3 years, respectively; $p < 0.001$) and duration of GH treatment (7.4 *vs*. 9.4 years, respectively; $p < 0.001$).

Untreated sex- and height-matched SGA and AGA controls: Previously GH-treated SGA subjects were compared with untreated sex- and height-matched SGA and AGA subjects who had never been exposed to GH treatment. These subjects were part of a cohort of young adults participating in a national study evaluating risk factors for DM-II and cardiovascular disease. Each subject of the GH-treated group was individually matched for sex and height SDS with an untreated SGA and AGA subject.

The GH trial was approved by the Medical Ethics Committees (MEC) of the participating centers, and the follow-up study by the MEC of Erasmus University Medical Center, Rotterdam, The Netherlands. Written informed consent was obtained from all participants or their parents.

Study design

Standing height and weight were measured and body mass index (BMI) was calculated. Waist circumference was measured at the level of the umbilicus using a non-extendable measuring tape. All measurements were performed according to standardised methods, repeated three times after which the mean was calculated. Height and BMI were expressed as SD scores adjusting for sex and age according to Dutch reference data for children.[22,23] Dual-Energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK) was performed in all participants. Lean body mass (LBM), total fat mass, fat percentage, trunk and limb fat were measured. For this type of DXA, the intra-assay coefficient of variation (CV) for lean tissue and fat tissue has been reported to be 1.57–4.49% and 0.41–0.88%, respectively.[24] Trunk fat and limb fat were considered as estimates for central and peripheral fat, respectively.

Assays

Serum adiponectin levels were assessed by an ELISA according to the manufacturer's instructions (R&D Systems Inc. Minneapolis, USA). As reported by the manufacturer, the intra-assay CV was 2.7% and 4.7% at serum adiponectin levels of 19.8 and 143 ng/ml, respectively, and the inter-assay CV was 6.8 and 6.9% at serum adiponectin levels of 20.5 and 157 ng/ml, respectively.

Statistics

Analyses were carried out using the computer statistical package SPSS for Windows (version 11.5, Chicago, Illinois). Within the GH-treated SGA group, statistical analyses were performed for group A and B separately and for the groups together. Since outcome variables were not different between the GH dosage groups, data are shown for both groups together, unless indicated otherwise. Data are shown as mean (SD). Differences between GH-treated SGA subjects and untreated SGA and AGA controls were evaluated using One-Way ANOVA. The results of the ANOVA test are presented in the right column of the Tables. If the overall F-ratio was significant, an LSD post hoc test was performed to determine which means were different. P values of the LSD post hoc test are mentioned in the Table footnote and in the text. Differences between males and females were tested with regression analysis with adjustment for age and height. Before the study, a power analysis with a significance levels (α) of 0.05 and a chosen power of 80% estimated that there should be at least 22 subjects in each group to enable detection of relevant differences in body composition (LBM). A difference of 10% was considered clinically relevant.

Results

Clinical characteristics

Clinical characteristics of the three groups are shown in Table 1. Mean duration of GH treatment had been 7.3 (1.3) years and period after discontinuation of GH was 6.5 (1.4) years. Height SDS and age at start of GH treatment were −2.9 (0.7) and 8.5 (1.7) years, respectively.

Gestational age and birth length were significantly lower in GH-treated SGA subjects than in untreated controls born SGA ($p < 0.001$) and AGA ($p < 0.001$). Birth weight SDS was comparable in GH-treated and untreated SGA subjects. As expected, both birth length and birth weight SDS were lower in SGA than AGA subjects ($p < 0.001$). At time of the present study, previously GH-treated SGA subjects were 1.0 and 1.1 years older than untreated SGA ($p = 0.006$) and AGA controls ($p = 0.003$).

Table 1. Clinical characteristics of previously GH-treated SGA subjects and sex- and height-matched untreated SGA and AGA controls

	GH-treated SGA subjects	Untreated SGA controls	Untreated AGA controls	p value*
N (male/female)	22/15	22/15	22/15	
Gestational age	36.6 (4.0)[a,c]	39.1 (1.3)	39.0 (1.6)	< 0.001
Birth weight SDS	−2.6 (1.0)[c]	−2.3 (0.6)[e]	−0.8 (0.9)	< 0.001
Birth length SDS	−3.6 (1.5)[a,c]	−2.8 (0.7)[e]	−0.6 (0.6)	< 0.001
Age present study	21.9 (1.3)[b,d]	20.9 (1.7)	20.8 (1.5)	0.004

SDS: standard deviation score
Data expressed as mean (SD)
*Differences between the groups tested with One-Way ANOVA
LSD post hoc test: GH-treated SGA vs. untreated SGA: [a]$p < 0.001$; [b]$p < 0.01$
LSD post hoc test: GH-treated SGA vs. untreated AGA: [c]$p < 0.001$; [d]$p < 0.01$
LSD post hoc test: untreated SGA vs. untreated AGA: [e]$p < 0.001$

Height

As groups were sex- and height-matched, height SDS was similar for all groups. Each group comprised subjects with normal stature (defined as height SDS > −2) as well as short stature (defined as height SDS < −2). Twenty-seven subjects in the GH-treated SGA group had a normal stature, 26 in the untreated SGA control group and 27 in the AGA control group. Thus, the untreated SGA control group comprised subjects with and without spontaneous catch-up growth. Birth characteristics were not significantly different between subjects with normal stature and short stature (data not shown).

Body composition

Body composition of the three groups is shown in Table 2. BMI SDS was comparable for all groups. Waist circumference, however, was significantly lower in GH-treated SGA subjects than untreated SGA controls (p = 0.008), and not significantly different from AGA controls.

LBM was equivalent in the three groups. Total fat mass, fat percentage, trunk and limb fat were not statistically different between the groups, but tended to be higher in the untreated SGA controls.

Table 2. Body composition and adiponectin levels in previously GH-treated SGA subjects and sex- and height-matched untreated SGA and AGA controls

	GH−treated SGA subjects	Untreated SGA controls	Untreated AGA controls	p value*
Height (cm)	170.0 (7.7)	166.9 (8.0)	166.9 (8.0)	1.0
Weight (kg)	62.5 (8.9)	65.5 (12.8)	61.4 (12.8)	0.31
BMI (kg/m^2)	22.4 (2.2)	23.4 (3.5)	22.0 (3.9)	0.15
Height SDS	−1.4 (1.0)	−1.5 (1.0)	−1.5 (1.0)	1.0
BMI SDS	−0.1 (0.7)	0.4 (1.1)	−0.1 (1.2)	0.84
Waist circumference (cm)	74.9 (6.7)[a]	80.5 (10.5)	78.7 (9.4)	0.027
LBM (g)	46342 (10074)	45389 (13683)	48247 (9124)	0.56
Total fat mass (g)	14301 (6478)	17245 (8873)	15161 (7058)	0.25
Fat percentage (%)	23.5 (10.4)	25.9 (11.0)	23.6 (9.3)	0.53
Trunk fat (g)	7482 (3487)	8711(4775)	7498 (4153)	0.38
Limb fat (g)	6249 (3060)	7894 (4147)	7064 (3074)	0.14
Adiponectin (µg/ml)	8.0 (3.1)	7.3 (3.8)	7.8 (3.6)	0.67

BMI: body mass index; LBM: lean body mass; SDS: standard deviation score
Data expressed as mean (SD)
* Differences between the three groups tested with One-Way ANOVA
[a] LSD Post hoc test: GH-treated SGA *vs.* untreated SGA controls: p = 0.008

Adiponectin levels

GH-treated SGA subjects had mean adiponectin levels of 8.0 (3.1) µg/ml compared with 7.3 (3.8) µg/ml and 7.8 (3.6) µg/ml in untreated SGA and AGA subjects, respectively. Adiponectin levels were not statistically different between the groups.

Table 3. Body composition and adiponectin levels in males and females in each group

	GH-treated SGA subjects		Untreated SGA controls		Untreated AGA controls	
	males	females	males	females	males	females
Waist circumference (cm)	76.8 (6.3)	72.1 (6.6)	82.0 (10.6)	78.4 (10.4)	80.3 (8.6)	76.2 (10.2)
LBM (g)	52507 (8346)[b]	37711 (4172)	49886 (15929)	38645 (4202)	53679 (6669)[a]	39471 (4443)
Total fat mass (g)	12036 (6256)[b]	17473 (5525)	13772 (8043)[a]	22205 (7761)	13195 (7102)	18335 (5937)
Fat percentage (%)	18.3 (8.8)[a]	30.7 (8.0)	19.8 (8.4)[a]	35.1 (7.6)	19.2 (8.2)[c]	30.6 (6.2)
Trunk fat (g)	6810 (3716)[d]	8423 (3009)	7305 (4705)[a]	10719 4258)	6949 (4655)	8385 (3252)
Limb fat (g)	4710 (2468)[a]	8402 (2489)	5905 (3269)[a]	10735 (3640)	5996 (2401)[b]	9273 (2798)
Adiponectin (µg/ml)	7.3 (3.4)[d]	8.9 (2.4)	6.0 (2.7)[d]	9.2 (4.5)	6.6 (3.2)[c]	9.6 (3.5)

LBM: lean body mass

Data expressed as mean (SD)

Males versus females after adjustment for sex and height: [a]$p < 0.001$; [b]$p < 0.005$; [c]$p < 0.01$; [d]$p < 0.05$

Males versus females

Body composition for males and females separately is shown in Table 3. Differences were tested after adjustment for age and height. In most groups, males had a significantly higher LBM, and a lower amount of total fat mass, fat percentage, trunk and limb fat. Males had a more central fat distribution than females with relatively greater fat in the trunk than limbs. Adiponectin levels were lower in males than females in all groups ($p = 0.44$, $p = 0.048$ and $p = 0.008$, respectively)

Discussion

Our study shows that at 6.5 years after discontinuation of GH, waist circumference was significantly lower in previously GH-treated SGA subjects than in sex- and height-matched untreated SGA controls, whereas not different from AGA controls. LBM was comparable for all groups. Fat mass, fat percentage, trunk and limb fat were also not significantly different between the groups, but tended to be higher in untreated SGA controls compared with the other two groups.

Our study investigated the impact of earlier GH treatment on body composition and fat distribution in young adulthood. Body composition and fat distribution were measured by Dual Energy X-ray Absorptiometry (DXA). Since body composition is greatly influenced by age, sex and height,[16] results were compared with untreated sex- and height-matched young adults born either SGA or AGA. Our study design excluded any confounding influence from differences in sex- and height between groups.

Previous studies in SGA children have shown an increase in muscle mass and a decrease in fat mass during GH treatment.[14,15] At 6.5 years after discontinuation of GH, body composition was not significantly different between previously GH-treated SGA subjects and untreated SGA and AGA controls, except for waist circumference. In previously GH-treated SGA subjects, however, birth size was significantly smaller than in untreated SGA controls. Given the well-established positive association between birth size and subsequent LBM[2,6,7,9,10] and the inverse association between birth weight and fat mass,[1,4,5,7] one would expect a lower LBM and higher fat mass in those subjects with the smallest birth size. Our study shows that this was not the case, which might suggest that GH did have some long-lasting effects. It might also be that the anabolic and lipolytic effects of GH did not persist until 6.5 years after discontinuation.

Body fat indices tended to be higher in untreated SGA subjects. The untreated SGA control group comprised subjects with and without spontaneous catch-up growth, whereas none of the GH-treated SGA subjects had shown spontaneous catch-up growth prior to GH treatment. Ibáñez et al. recently reported that SGA subjects with spontaneous catch-up growth gained more body adiposity and abdominal fat when compared with

AGA infants.[25] It appears that GH-induced catch-up growth is not associated with an increase in fat mass.

Interestingly, LBM was comparable for untreated SGA and AGA subjects, which is in contrast with a number of studies.[2,6,7,9,10] One study in young men with the same age as our study subjects also reported comparable LBM in low- and normal birth weight subjects.[26] Some of the previously reported differences in LBM might be related to differences in height. However, other factors, such as lifestyle and genetic background might also be involved.

Adiponectin levels were comparable for GH-treated SGA subjects and untreated sex- and height-matched SGA and AGA controls. We recently showed that adiponectin levels were comparable with baseline values after 2 years of GH treatment in short prepubertal SGA children.[27] Our present study supports these findings and indicates that GH has no permanent effects on adiponectin levels. Jacquet et al. recently reported reduced adiponectin levels in young SGA adults compared with AGA controls.[28] However, differences between both groups were very small [12.7 (6.8) *vs.* 13.2 (6.4) µg/ml].

In all groups, adiponectin levels were lower in males than females, which is in agreement with previous publications.[29,30] It has been demonstrated that androgens decrease plasma adiponectin concentrations *in vivo*.[30] In addition, males had a more central fat distribution, which has shown to be an independent negative predictor of adiponectin levels.[31]

Due to the lack of a randomized untreated SGA control group, we cannot answer the question to which extent GH has been responsible for the reported findings. Some of the reported differences might already have been pre-existent in childhood. The Medical Ethics Committee, however, did not allow for an untreated SGA control group until adult height and such a control group will not become available, because short statute after SGA is now an indication for GH treatment.

The subjects in the control groups were sex- and height-matched. As a consequence, 70% (26/37) of the untreated SGA subjects had a normal stature and 30% (11/37) a short stature. Normally, approximately 90% of SGA children shows spontaneous catch-up to a normal height. Therefore, our data are not directly transferable to all SGA subjects.

In conclusion, our study shows that at 6.5 years after discontinuation of long-term GH treatment, waist circumference was significantly lower in previously GH-treated SGA subjects than untreated sex-and height-matched SGA controls, whereas comparable with AGA controls. Indices of fat mass tended to be higher in untreated SGA subjects, whereas LBM was comparable for the three groups. Adiponectin levels were similar for the three groups. Our data are reassuring as they might suggest that previous long-term GH treatment of SGA children does not have unfavorable effects on body composition and adiponectin levels in young adulthood.

Acknowledgments

We greatly acknowledge Mrs. J. Dunk, Mrs. J. van Nieuwkasteele and Mrs. J.C. Bruinings, research nurses, for their technical assistance and help with data collection; Prof. E.P Krenning, PhD, head of the Nuclear Medicine Department, and Mrs. J.P. Sluimer for performing and analyzing the DXA measurements; G. Klein Heerenbrink and H. van Toor, research analysts, for their laboratory analysis. We also greatly acknowledge the physicians who participated in the original GH trial: E.C.A.M. Houdijk, MD, PhD, VU Medical Center, Amsterdam; M. Jansen, MD, PhD, Wilhelmina Children's Hospital, Utrecht; H.M. Reeser, MD, PhD, Juliana Children's Hospital, The Hague, and Y.K. van Pareren, MD, PhD, Erasmus MC/Sophia Children's Hospital, Rotterdam, The Netherlands.

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Chapter 8

General Discussion

General Discussion

The studies presented in this thesis were part of the IUGR-3 study and the SGA follow-up study. The IUGR-3 study evaluated various endocrinological outcome variables in prepubertal short SGA children during GH treatment: (i) GH-IGF-IGFBP axis, (ii) insulin sensitivity and cardiovascular risk factors, (iii) adipocytokines and (iv) body composition. The SGA follow-up study assessed the late consequences of long-term GH treatment on these parameters at 6.5 years after discontinuation of GH in young SGA adults in comparison with untreated SGA controls.

In this chapter, results of the IUGR-3 and SGA follow up study are compared and discussed in view of current literature. Subsequently, clinical implications and conclusions are presented and directions for future research are given.

GH-IGF-IGFBP-axis

Overnight GH levels during GH treatment

We evaluated serum GH levels during an overnight GH profile in short children born SGA, prior to and after 6 months of GH treatment with either 1 mg or 2 mg GH/m^2·day. GH treatment resulted in a significant increase in mean and maximum serum GH levels in both GH dosage groups, but all values were significantly higher in the 2-mg GH group. In this group, mean GH levels were 64.4 mU/L during the 12 h of the GH profile, and remained above 20 mU/L for more than 9 hours, indicating that short SGA children receiving 2 mg GH/m^2·day have elevated GH levels for a great part of the day.

This is the first study describing serum GH levels after subcutaneous GH administration in short SGA children. Previous studies regarding GH levels during GH treatment have mainly been performed in healthy adults,[1,2] GH-deficient patients,[3-5] and in girls with Turner syndrome.[6] Interestingly, our SGA children showed remarkably high mean and maximum GH levels after subcutaneous GH injection compared with these studies. Vahl et al. found an inverse relation between serum GH levels and age and fat mass.[7] This might partially explain the higher GH levels in our group, which consisted of young prepubertal children with a reported lower fat mass and a low BMI.[8] It might also be that degradation of GH at the site of injection or in the circulation is different in short SGA children.

Serum levels of IGF-I and IGFBP-3 during GH treatment

We also evaluated the effects of GH therapy with either 1 mg or 2 mg GH/m^2·day on free and total IGF-I and IGFBP-3 levels and the IGF-I to IGFBP-3 ratio. Prior to start of GH treatment, all values were significantly lower than zero SDS. GH treatment resulted in

a dose-dependent rise in free and total IGF-I and IGFBP-3 levels, which is in agreement with previous studies (9-11). IGFBP-3 levels increased less markedly than IGF-I, resulting in an increase in the IGF-I to IGFBP-3 ratio SDS. In the high-dose group (2 mg GH/m^2·day), 63% of the children had total IGF-I levels and 74% an IGF-I to IGFBP-3 ratio in the highest quintile (> 0.84 SD score) during GH-treatment and approximately 30% of them even had levels above 2 SDS. In contrast, almost all children of the 1-mg GH group had IGF-I levels and/or an IGF-I to IGFBP-3 ratio SD score within ± 1 SD score.

Serum levels of IGF-I and IGFBP-3 after discontinuation of GH treatment

We wanted to know whether GH-induced elevations in serum IGF-I and IGFBP-3 levels declined after discontinuation of GH therapy. Therefore, serum levels of IGF-I and IGFBP-3 were measured longitudinally from start of GH treatment until 6.5 years after GH stop. We showed that in previously GH-treated young SGA adults, serum IGF-I and IGFBP-3 levels had decreased and were comparable with those of untreated short SGA controls. In addition, all previously GH-treated SGA subjects had serum IGF-I and IGFBP-3 levels within the normal range (±1 SD). These data indicate that GH-induced increases are reversible after stop of treatment.

Clinical implications and conclusions

Concern has been expressed regarding the possible detrimental effects of persistently high serum levels of GH and IGF-I.[12,13] Epidemiological studies have suggested that high serum levels of GH and IGF-I might increase cancer risk in human beings, especially when IGFBP-3 levels are low.[14,15] Serum IGF-I levels in the upper tertile to quintile have been associated with increased risk of breast, prostate and colon cancer.[14-16] A meta-regression analysis regarding associations between concentrations of IGF-I and IGFBP-3 levels and cancer risks supported these findings.[17]

From our data, it is clear that most short SGA children receiving long-term GH treatment with 2 mg /m^2·day have high GH levels for many hours per day as well as IGF-I levels in the upper quintile (> 0.84 SDS). As the majority of these children will be treated for 10−12 years until adult height is reached, serum GH and IGF-I levels may be elevated during almost their entire childhood and adolescence. Reassuringly, our follow-up study showed that IGF-I and IGFBP-3 levels were normal in young adults born SGA at 6.5 years after discontinuation of long-term GH treatment, indicating that GH-induced increases are reversible on the long term.

We have previously shown that GH treatment with a dose of 1 mg /m^2·day was as effective as 2 mg /m^2·day for most short SGA children with regard to adult height.[18] In addition, a recent meta-analysis indicated that GH treatment with 1 mg /m^2·day was adequate for the majority of SGA children.[19] However, SGA children who are very short (for example a height < −3 SDS) or relatively old at start of GH treatment, might profit

from a different treatment strategy. These children could be treated with a higher dose for 2–3 years until adequate short-term catch-up growth is achieved, followed by treatment with a lower dose to ensure a normal height on the long term.[19]

However, it is important to monitor IGF-I levels during GH therapy to ensure that these remain within the normal range, since the long-term risks of high GH and IGF-I levels in childhood are still unknown.

Associations between the GH-IGF-IGFBP-axis and insulin sensitivity

Increasing evidence suggests that the GH-IGF-IGFBP axis plays an important role in regulating glucose homeostasis and insulin sensitivity.[20,21] Our study showed that 6 months of GH therapy resulted in a reduction in insulin sensitivity and IGFBP-1 levels and a rise in acute insulin response, parallel to the dose-dependent rises in serum levels of GH, free and total IGF-I and IGFBP-3. GH has well-documented insulin-antagonistic properties and its use has been associated with a decrease in insulin sensitivity.[22-24] IGF-I, however, exerts insulin-sensitising effects, which are opposite to those of GH.[25-27] Given these data, the reduction in insulin sensitivity in our SGA children is most likely due to elevated GH levels. Recently, the relative contributions of GH and IGF-I in inducing insulin resistance have been assessed, using different transgenic mouse models.[28-30] Liver-IGF-I-deficient (LID)-mice show a marked reduction in circulating IGF-I levels and had elevated GH levels and signs of insulin resistance. Crossing of LID-mice with GH antagonist (GHa) transgenic mice resulted in a further decrease in serum IGF-I levels and an inactivation of GH action. This was associated with an improvement in insulin sensitivity and a decline in insulin levels. Given the fact that insulin sensitivity could be improved by inactivating GH action despite low IGF-levels, the authors suggested that chronic elevation of GH levels plays a major role in insulin resistance, whereas IGF-I may have a direct modulatory role. Hence in conditions when both GH levels and IGF-I levels are high, like in our GH-treated SGA subjects, the anti-insulin effects of GH predominate, but may be attenuated by the insulin-like effects of high IGF-I levels.

An unexpected finding, however, was that we did not find a significant correlation between insulin sensitivity and GH levels, which is in contrast to a previous report.[31] It might be that GH receptors involved in the regulation of insulin sensitivity are already maximally stimulated at a certain GH level. In that case, increasing GH levels would decrease insulin sensitivity until a plateau has been reached, after which a further increase in GH levels has no additional effect. The simultaneous actions of high IGF-I may also have weakened associations between GH and insulin sensitivity.

During GH therapy, total IGF-I SDS correlated negatively with insulin sensitivity and positively with acute insulin response and insulin levels. These results appear contradictory, but are consistent with previous findings in normal prepubertal boys and girls.[32,33] In addition, several studies have shown that insulin has direct and indirect

positive effects on serum IGF-I levels. Studies in rats demonstrated that insulin stimulates IGF-I mRNA production in hepatocytes[34] and *in vitro* studies in human hepatoma cells showed that insulin promotes binding of GH to GH receptors in the liver.[35] Insulin is also able to promote IGF-I bioactivity indirectly by suppression of IGFBP-1 levels, which inhibit IGF-I mediated processes *in vivo*.[36] Free IGF-I showed no significant relationship with insulin sensitivity or insulin levels. The contribution of free IGF-I levels to glucose homeostasis in SGA subjects requires further studies.

IGFBP-3 levels were also inversely related to insulin sensitivity during GH treatment. It has been demonstrated that IGFBP-3 is able to induce insulin antagonistic effects. Transgenic IGFBP-3 mice overexpressing IGFBP-3 had impaired glucose homeostasis and were insulin resistant[37] and it was recently also reported that IGFBP-3 inhibits insulin-stimulated glucose uptake in cultured adipocytes.[38]

GH treatment resulted in a significant decrease in fasting IGFBP-1 levels in the high-dose group. During GH therapy, IGFBP-1 levels were positively related to insulin sensitivity, which is in agreement with previous reports.[39,40] It has been suggested that measurement of fasting IGFBP-1 levels may be useful in identifying those individuals with insulin resistance.[39,40] IGFBP-1 levels showed an inverse relation with fasting insulin levels. It has been clearly demonstrated that hepatic IGFBP-1 production is inversely regulated by portal insulin.[41]

In summary, we hypothesized that GH treatment induces an increase in serum levels of GH, free and total IGF-I and IGFBP-3. Elevation of GH and IGFBP-3 levels results in a worsening of insulin resistance, which in turn leads to a compensatory rise in insulin levels. Increased insulin levels further increase IGF-I levels directly and indirectly by suppressing IGFBP-1 levels. Increased IGF-I levels may prevent further reduction in insulin sensitivity. A schematic representation is shown in Figure 1.

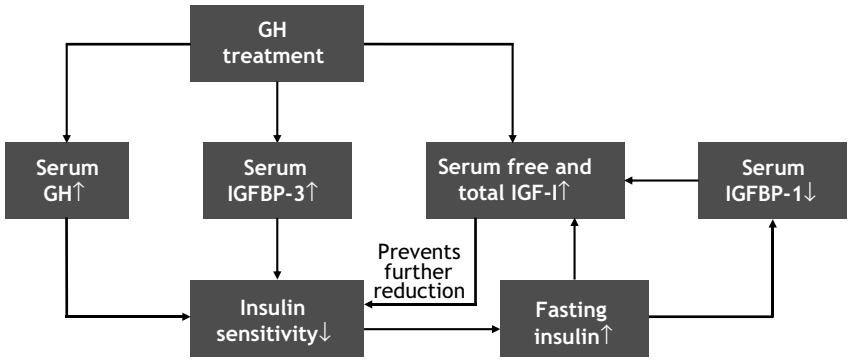

Figure 1. Schematic representation of possible interactions between the GH-IGF-IGFBP axis and insulin sensitivity during GH treatment

Clinical implications and conclusions

In conclusion, GH treatment of short SGA children resulted in a reduction in insulin sensitivity, apparently due to chronic elevation of GH and IGFBP-3 levels. The simultaneous increase in IGF-I levels may have attenuated the insulin-antagonistic effects of GH and IGFBP-3. Determination of free IGF-I levels, in addition to total IGF-I and IGFBP-3 had no significant advantage in the evaluation of insulin sensitivity in short SGA children, whereas IGFBP-1 levels had. Measurement of IGFBP-1 may therefore be valuable in identifying those individuals with insulin resistance. Our data underline the complexity of relations between the GH-IGF-IGFBP axis and glucose homeostasis in SGA children, indicating that further research in this field is warranted.

Insulin sensitivity and cardiovascular parameters

Insulin sensitivity, insulin secretion and disposition index during GH treatment

We evaluated insulin sensitivity, insulin secretion and disposition index with an intravenous glucose tolerance test with Tolbutamide in a group of prepubertal short SGA children prior to and during GH therapy. This test and the hyperinsulinaemic-euglycaemic clamp are considered to be “gold standards”, since they have been validated in children and are able to detect small differences in insulin sensitivity.[42]

During the first 6 months, when children were treated with either 1 mg or 2 mg GH/m^2·day, insulin sensitivity decreased significantly in both GH-dosage groups whereas acute insulin response increased. At 6 months, both were not significantly different between both GH dosage groups, suggesting that the effects of GH treatment were not dose-dependent within the GH-dose range we used. Between 6 and 24 months, when all children received 1 mg GH/m^2·day, we observed a rise in insulin sensitivity and a decline in acute insulin response, resulting in values closer to baseline. De Zegher et al. recently reported a progressive reduction in insulin sensitivity during the first and second year of GH treatment in a small group of 9 short SGA children.[23] However, those children were treated with a very high GH dose of 3 mg GH/m^2·day.

In addition to insulin sensitivity and acute insulin response, we evaluated disposition index. In normal conditions, the pancreatic beta-cell is able to upregulate insulin secretion in response to insulin resistance in order to maintain normoglycemia. Disposition index reflects the capacity of the beta-cell to do this.[43] In our study, disposition index remained unchanged during GH treatment, suggesting that glucose homeostasis was well maintained.

Insulin sensitivity and cardiovascular risk factors after discontinuation of GH treatment

Our study showed that at 6.5 years after discontinuation of long-term GH treatment, risk factors for type 2 diabetes and metabolic syndrome were comparable for previously GH-treated and untreated young SGA adults. Systolic and diastolic blood pressure and serum cholesterol were significantly lower in previously GH-treated SGA subjects.

During GH treatment glucose and insulin levels increased, but normalised after stop of GH. At 6.5 years after discontinuation, none of the GH-treated subjects either had high fasting glucose levels or developed type 2 diabetes mellitus. Also, insulin sensitivity, acute insulin response and disposition index were comparable with untreated young SGA adults. These data are reassuring, because they suggest that long-term GH treatment does not have permanent effects on glucose homeostasis or risk factors for type 2 diabetes.

Low birth weight has been associated with hypertension in later life and several studies have reported an increased systolic blood pressure in SGA children and adolescents.[8,44,45] Our study showed a decline in systolic blood pressure during the first years of GH treatment, which remained so until 6.5 years after discontinuation of GH.[24,46] At 6.5 years after discontinuation, both systolic and diastolic blood pressure SDS were within the normal range in previously GH-treated SGA adults and significantly lower than in untreated SGA controls. Taken these data together, GH treatment might have long-lasting beneficial effects on blood pressure in short SGA subjects.

Prior to start of GH treatment, our short SGA children had a low BMI, which normalized during GH treatment.[8] This remained so after discontinuation of GH treatment. Waist circumference was also comparable for GH-treated and untreated SGA subjects at 6.5 years after GH stop. Given the fact that waist circumference is positively related to height,[47] and that the GH-treated subjects were taller than the untreated controls, it might be that the latter have relatively more fat mass.

It has previously been demonstrated that hypercholesterolemia occurs more frequently among SGA children than AGA children.[48] In addition, young SGA adults had significantly higher serum triglyceride and HDL-cholesterol levels compared with AGA controls.[49] In our short SGA group, serum levels of total cholesterol, LDL-cholesterol and HDL-cholesterol fell during the first year of GH treatment and remained stable thereafter until GH stop.[24,46] At 6.5 years after discontinuation, serum total cholesterol levels were significantly lower in GH-treated SGA subjects than in the untreated SGA controls, whereas other serum lipids were equivalent for both groups. Our data suggest that GH treatment has positive effects on lipid metabolism, which might persist after discontinuation of GH.

Clinical implications and conclusions

Epidemiological studies have reported an inverse association between birth weight and the risk of type 2 diabetes mellitus and cardiovascular disease.[50-52] Because GH has insulin-antagonistic properties, concern had been raised regarding the late consequences of GH treatment on insulin resistance and risk factors for cardiovascular disease in SGA individuals. Although insulin sensitivity declined during GH therapy, the beta-cells showed an adequate increase in insulin secretion, by which disposition index remained constant. After discontinuation of GH, insulin sensitivity, acute insulin response and disposition index were comparable for GH-treated and untreated SGA subjects. These results are reassuring, because disposition index is a strong predictor for the subsequent development of type 2 diabetes.[53] In addition, BMI and waist circumference were also comparable for GH-treated and untreated young SGA adults, whereas systolic and diastolic BP and serum cholesterol were even lower in GH-treated subjects. Taken together, our data suggest that long-term GH-treatment does not increase the risk of DM-II and cardiovascular disease for short SGA children.

Adipocytokines

Serum adiponectin and resistin levels during GH treatment

We investigated the effects of GH treatment on serum adiponectin and resistin levels in short SGA children. During the first 6 months, when children received either 1 mg or 2 mg GH/m^2·day, we observed a reduction in adiponectin and an increase in resistin levels in both GH dosage groups. No differences between the groups were reported. Between 6 and 24 months, when all children received 1 mg GH/m^2·day, adiponectin and resistin levels increased and decreased, respectively. At 24 months, adiponectin levels were comparable with baseline values in the 2-mg group, whereas still lower than at baseline in the 1-mg group, although less markedly than at 6 months. Resistin levels were comparable with baseline values in both groups. Ibáñez et al. recently reported a reduction in adiponectin levels during 6 months of GH treatment in 16 short SGA children. In addition, Willemsen et al. showed no significant changes in both adiponectin and resistin levels after 24 months of GH treatment in 50 short SGA children.[54] These and our results suggest that the effects of GH on adiponectin and resistin levels are time-dependent and most significant in the early phase of treatment.

Adiponectin levels after discontinuation GH treatment

To assess whether the GH-induced changes in adiponectin levels would persist on the long term, we evaluated adiponectin levels in young SGA adults at 6.5 years after discontinuation of long-term GH treatment in comparison with untreated sex- and height

matched controls born either SGA or AGA. Our study showed that adiponectin levels were equivalent in all three groups, indicating that the effect of GH on adiponectin levels is only temporary.

Associations between adiponectin, resistin levels and insulin sensitivity

During GH treatment, changes in adiponectin levels paralleled the changes in insulin sensitivity, whereas resistin levels showed opposite changes. These findings suggest an interaction between adiponectin, resistin and GH-induced insulin resistance. Therefore, we also investigated associations between adiponectin, resistin and insulin sensitivity before, during, and after GH therapy.

At baseline and 6.5 years after discontinuation, serum adiponectin levels did not correlate with insulin sensitivity, which is in agreement with a previous study in SGA infants.[55] In Pima Indian children, who are known with marked obesity, adiponectin levels did correlate with insulin sensitivity.[56] However, this correlation disappeared after adjustment for percentage body fat, indicating that adipose tissue plays a major role in the associations between adiponectin and insulin sensitivity. Martin et al. recently investigated the relationships between adiponectin and insulin levels in a large group of 14-year olds. Adiponectin levels were significantly related to insulin levels in the obese group, but not in the lean group.[57] It appeared that associations between adiponectin and insulin sensitivity strengthened with increasing adiposity. Martin et al. suggested that the anti-inflammatory properties of adiponectin are particularly important in obesity, since it may reduce the negative effects of adipose tissue on insulin sensitivity. In that case, heavy, insulin resistant individuals might have a greater benefit from actions of adiponectin than their lean, insulin sensitive peers. In our study, most SGA children had a low BMI and a lean appearance, which might explain the lack of correlation between baseline adiponectin levels and insulin sensitivity.

Interestingly, during GH treatment, the reduction in adiponectin levels did correlate with the reduction in insulin sensitivity. This is in line with previous studies showing low adiponectin levels in insulin resistant conditions due to elevated GH levels. In acromegalic patients, serum adiponectin levels were significantly reduced,[58] and adiponectin levels were also low in insulin resistant mice overexpressing bovine GH.[59] This might suggest that correlations between insulin sensitivity and adiponectin are stronger with decreasing insulin sensitivity, due to either obesity[57] or GH administration.

We did not find a relation between resistin levels and insulin sensitivity, neither at baseline, nor during GH treatment. These data are in agreement with previous studies in Pima Indians[60] and adults[61] and suggest that resistin plays a minor role in the development of insulin resistance in SGA subjects before and during GH treatment.

Clinical implications and conclusions

We showed that GH treatment resulted in parallel changes in adiponectin levels and insulin sensitivity, whereas resistin showed opposite changes. After 24 months, adiponectin levels were closer to baseline values than at 6 months, whereas resistin levels were comparable. At 6.5 years after discontinuation of long-term GH therapy, adiponectin levels were equivalent in previously GH-treated and untreated young SGA adults. These results suggest that the effects of GH on adiponectin and resistin are time-dependent and not long-lasting.

Interestingly, adiponectin levels were associated with insulin sensitivity during GH-treatment, but not before start and after discontinuation of GH therapy. These data suggest that interactions between insulin sensitivity and adiponectin levels are stronger as insulin sensitivity declines. Our data do not provide evidence that resistin levels play a major role in the development of insulin resistance in SGA subjects

Body composition and fat distribution

Body composition and fat distribution during GH treatment

In short SGA children, assessment of body composition and fat distribution may help to identify which individuals are most at risk to develop insulin resistance and associated diseases in later life. Therefore, we measured lean body mass, fat mass and body fat distribution using Dual Energy X-ray absorptiometry (DXA) in relation to insulin sensitivity and cardiovascular risk factors before and during GH treatment.

Lean body mass, fat mass and fat percentage SDS adjusted for sex and age were significantly lower than zero before start of GH treatment, when compared with reference values of healthy children with normal height. Since lean body mass is positively related to height SDS, at least part of the reduction in lean body mass is due to the short stature of our SGA children. Height SDS did not correlate significantly with fat mass fat percentage.

Previous reports have shown a positive relationship between birth weight and subsequent lean body mass.[62-70] It has been suggested that poor fetal growth results in lower lean body mass later in life.[62] Several hypotheses have been proposed. One explanation might be that genetically determined insulin resistance results in impaired insulin-mediated growth of fetal muscle.[71] It might also be that growth of fetal muscle is reduced in favor of brain development.[72] Another possible explanation for the reduction in lean body mass might be low IGF-I levels due to poor fetal nutrition.[73]

Reports relating birth size to subsequent adiposity are less consistent. Of the 3 studies in children with DXA measurements of body composition, one found no association,[62] one found a negative association[74] and one found a positive association.[66]

In addition, two studies with DXA measurements in older men and women reported either an inverse relation[63] or no significant correlation.[64]

GH treatment resulted in a progressive increase in lean body mass which was comparable in both GH dosage groups. As mentioned above, due to the positive relation between height and lean body mass, this increment can be partially attributed to the increase in height SDS. However, our data on the lean body mass to height ratio, expressed in g/cm, suggested that GH also has some direct positive effects on lean body mass. The increase in the lean body mass to height ratio after 24 months was significantly greater in GH-treated SGA subjects than in untreated SGA controls. This indicates that for every cm gain in height, the increment in grams lean body mass is higher in GH-treated SGA subjects than untreated SGA controls. Other studies investigating body composition during GH treatment in children also reported an increase in muscle mass,[75,76] which is in agreement with the well-documented anabolic properties of GH.[77,78]

In the untreated SGA children, we observed a reduction in lean body mass SDS after 24 months compared with baseline, whereas height SDS remained unchanged. This suggests that without GH treatment, lean body mass decreases further with increasing age, which is consistent with studies in animals showing that the inverse relationship between birth weight and lean body mass amplifies with age.[79]

In addition, our study showed that GH treatment resulted in a short-term decline in fat mass and fat percentage SDS, followed by an increase between 6 and 24 months. At 24 months, both were comparable or only slightly different from baseline. This transient effect is a common phenomenon, which is also found in other GH studies.[75,76,80]

We also determined trunk and limb fat. Trunk fat was considered as a measure of central fat and limb fat as a measure of peripheral fat. During the first 6 months of GH treatment, both trunk and limb fat decreased, but the reduction in limb fat was greater. It appears that in the initial phase of GH treatment, there is a preferential reduction in peripheral fat rather than central fat. It might be that GH affects adipose tissue differently in the various fat depots. Between 6 and 24 months, the relative increments in trunk and limb fat were nearly comparable.

During the first 6 months of GH therapy, children received either 1 or 2 mg GH/m^2·day in order to investigate possible GH-dose effects on body composition and fat distribution. We did not observe any differences, suggesting that the effects of GH on body composition are not dose dependent within the GH-dose range we used.

Body composition and fat distribution after discontinuation of GH treatment

Body composition is greatly influenced by age, sex, and height.[47] Preferably, all these variables should be taken into account when comparing body composition in different patient groups, to exclude any confounding influences from differences in age, sex and height. In the SGA follow-up study, we investigated BMI, waist circumference, lean body

mass, fat mass and fat distribution in previously GH-treated young SGA adults at 6.5 years after discontinuation of long-term GH treatment in comparison with those of untreated sex-and height matched young adults born either SGA or AGA.

Our study showed that waist circumference in previously GH-treated SGA subjects was significantly lower than in sex- and height-matched untreated SGA controls, and comparable with AGA controls. Lean body mass was comparable for the three groups, whereas total fat mass, trunk and limb fat tended to be higher in untreated SGA subjects.

Previously GH-treated young SGA adults did not have greater lean body mass than untreated sex- and height-matched SGA controls, whereas our data in SGA children showed an increase in lean body mass during GH therapy. In GH-treated young SGA adults, birth size was, however, significantly smaller than in the untreated ones. Given the positive relation between birth size and later lean body mass, it is not unlikely that lean body mass would have been more reduced compared with untreated SGA controls in case no GH treatment had been given. It might also be that the GH-induced effect on lean body mass was not noticeable anymore at 6.5 years after discontinuation.

Interestingly, lean body mass and lean body mass percentage were also equivalent for untreated SGA and AGA controls, which is in contrast with several other studies demonstrating a positive relationship between birth weight and lean body mass.[62-66] One other study in young men with the same age as our study subjects, also reported similar lean body mass in low- and normal-birth weight subjects.[81] It might be that in some studies, the reported differences in lean body mass were related to differences in height rather than birth weight. Other factors, however, such as differences in life style or genetic background, might also be involved.

Waist circumference was significantly lower in previously GH-treated young SGA adults than in untreated SGA controls, and comparable with AGA controls. Total fat, trunk, and limb fat mass tended to be higher in untreated SGA subjects compared with the other two groups. Our study in prepubertal short SGA children showed only temporary effects of GH on fat mass. It might well be that the lipolytic effects of GH on adipose tissue are stronger during puberty and consequently, still evident in young adulthood, even after discontinuation of GH. Longitudinal evaluation of body composition during and after discontinuation of GH treatment will help to better understand the GH actions on lean body mass and fat mass in SGA subjects.

Associations between fat mass, insulin sensitivity and cardiovascular risk factors

In short SGA children, total fat mass and trunk fat correlated negatively with insulin sensitivity. This is consistent with previous reports in children and adults showing positive associations between the degree of obesity and the risk of type 2 diabetes mellitus and associated diseases.[82,83]

Clinical implications and conclusions

Our results in short SGA children showed that GH treatment results in a sustained increase in lean body mass and a temporary reduction in total fat mass and fat percentage, suggesting that in childhood, the anabolic effects of GH are stronger than its lipolytic effects. In our SGA follow-up study, we matched for sex and height when comparing previously GH-treated SGA subjects with untreated SGA and AGA controls. This allowed valid comparisons and excluded any confounding influences due to differences in sex and height. Despite smaller birth size in the previously GH-treated young SGA adults, lean body mass was equivalent to untreated SGA and AGA controls, whereas fat mass tended to be lower compared with untreated SGA subjects. During GH treatment, total fat mass and trunk fat were inversely associated with insulin sensitivity. This highlights the need for maintaining body weight within the normal range to prevent higher risks for type 2 diabetes mellitus. In conclusion, it appears that long-term GH treatment has no unfavorable effects on body composition in short children and young adults born SGA.

Considerations and general conclusions

GH treatment for short children born SGA with a birth weight and/or birth length below −2 SDS and an actual height below −2.5 SDS was approved by the US Food and Drug Administration (FDA) in 2001, and by the European Agency for the Evaluation of Medicinal Products (EMEA) in 2003. Nowadays, short SGA children comprise a large group of GH-treated children, and this number will increase in the coming years.

Our studies in short SGA children demonstrated that GH treatment resulted in a dose-dependent increase in GH, IGF-I and IGFBP-3 levels. Our SGA follow-up study in previously GH-treated young SGA adults showed that IGF-I and IGFBP-3 had decreased and were comparable with those of untreated short SGA controls, indicating that the rise in IGF-I and IGFBP-3 levels is reversible after GH stop.

We also investigated the associations between the GH-IGF-IGFBP axis and insulin sensitivity before and during GH treatment. We hypothesized that insulin resistance due to GH administration might be attributable to chronic elevation of GH and IGFBP-3 levels, whereas the simultaneous rise in IGF-I prevented a further decline in insulin sensitivity.

During GH treatment, the reduction in insulin sensitivity was associated with an increase in acute insulin response, by which disposition index remained constant. This indicates that glucose homeostasis was well maintained. At 6.5 years after discontinuation of GH, insulin sensitivity, acute insulin response and disposition index were similar for GH-treated and untreated SGA subjects, whereas systolic and diastolic blood pressure and serum cholesterol were even lower. These results are reassuring, because they

indicate that long-term GH treatment does not increase the risk for type 2 diabetes mellitus and cardiovascular disease in young adulthood.

We showed that adiponectin paralleled the changes in insulin sensitivity during GH treatment, whereas resistin showed opposite changes, which is in line with normal physiology. In addition, at 6.5 years after discontinuation, adiponectin levels were effects of GH are time-dependent and not long-lasting.

Finally, we demonstrated that GH induced a sustained increase in lean body mass and a temporary reduction in fat mass in prepubertal short SGA children. In previously GH-treated young adults, lean body mass was equivalent to untreated sex-and height matched controls born either SGA or AGA. Fat mass was lower than in untreated SGA controls, but similar to AGA controls. Fat mass showed strong correlations with insulin sensitivity and other risk factors for cardiovascular disease, which underscores the importance of maintaining body weight within the normal range. Our results imply that long-term GH treatment in SGA subjects is not unfavorable with regard to body composition.

In summary, our results do not provide evidence that long-term GH treatment has permanent adverse effects on the GH-IGF-IGFBP axis, risk factors for type 2 diabetes mellitus and adipocytokines. In fact, our data suggest that GH might even have beneficial effects on blood pressure, serum lipids and body composition, also after discontinuation.

Directions for future research

Since GH treatment with 1 mg/m^2·day has proven to be as effective as 2 mg/m^2·day for most children with regard to adult height, current data support treatment with 1 mg GH/m^2·day. However, higher doses during the first few years until appropriate catch-up growth is achieved, may give better results in SGA children who are very short or relatively old at start of GH treatment. The development of advanced growth prediction models is required, since such models may support individualization of GH treatment for short SGA children. Although side effects are very rare, all children should be monitored for changes blood pressure, IGF-I levels, fasting glucose and insulin levels and fasting serum lipids. At this moment, data at 6.5 years after discontinuation are reassuring. It remains, however, important to perform follow-up studies at regular intervals (every 5 years) in SGA cohorts after discontinuation of GH. Future studies should include genetic investigations in order to associate genotypes with clinical parameters. Such studies might improve our onderstanding of fetal and postnatal growth patterns, persistent short stature, as well as risk factors for type 2 diabetes mellitus and cardiovascular disease in SGA subjects.

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Chapter 9

Summary

This doctoral thesis describes the results of various studies performed in (i) short SGA children being treated with GH in the third Dutch GH trial (IUGR-3 study) and (ii) young SGA adults previously treated with GH treatment in the first Dutch GH trial (SGA follow-up study). The IUGR-3 study investigated the effects of GH treatment on (i) GH-IGF-IGFBP axis, (ii) insulin sensitivity and cardiovascular risk factors, (iii) adipocytokines and (iv) body composition, in prepubertal short SGA children (Chapter 2–5). The SGA follow-up study assessed the late consequences of long-term GH treatment on these variables at 6.5 years after discontinuation of GH in young SGA adults, in comparison with untreated SGA controls (Chapter 6 and 7).

Chapter 1

This chapter gives an overview of the definitions, prevalence and etiology of SGA. In addition, several clinical and endocrinological aspects associated with SGA are described, including (i) short stature, (ii) the GH-IGF-IGFBP axis and its role in fetal and postnatal growth, (iii) insulin sensitivity and cardiovascular risk factors, (iv) adipocytokines, and (v) body composition and fat distribution. It also gives a summary of previously reported data on the effects of GH treatment on these parameters in short SGA children. At the end of this chapter, the aims and outline of this thesis are presented as well as the study designs and in- and exclusion criteria of the IUGR-3 study, IUGR-1 study, and SGA follow-up study.

Chapter 2

Concern has been expressed regarding the possible harmful effects of high serum levels of GH and IGF-I. Epidemiological studies have suggested that high GH and IGF-I levels might increase cancer risk, especially when IGFBP-3 levels are low. We investigated the changes in overnight GH levels, serum levels of IGF-I and IGFBP-3, and IGF-I to IGFBP-3 ratio in 36 prepubertal short SGA children after 6 months of GH treatment with either 1 mg GH (group A) or 2 mg GH/m^2·day (group B). After 6 months of GH treatment, overnight GH levels increased significantly in both GH dosage groups. Group B had significantly higher GH levels during the profile (mean, maximum and AUC_0) than group A. In group B, maximum GH levels increased from 43.9 to 161 mU/L and in group A from 57.2 to 104 mU/L. During the profile (i.e. 12 hours per day), children of group B had a mean GH level of 64.4 mU/L versus 34.8 mU/L in group A. At baseline, IGF-I SDS, IGFBP-3 SDS and the IGF-I to IGFBP-3 ratio SDS were significantly lower than zero. During GH treatment, IGF-I and IGF-I to IGFBP-3 ratio SDS increased significantly in both groups, but were significantly higher in group B than in group A In group B, 74% of the children had IGF-I levels in the highest quintile during GH treatment compared with 19% in group A. In conclusion, our study shows that most short SGA children receiving GH treatment with 2 mg GH/m^2·day have high serum GH levels for many hours per day and IGF-I levels in the

upper quintile. Since the long-term risks of high GH and IGF-I levels during childhood are still unknown, it is important to monitor IGF-I levels during GH therapy to ensure these remain within the normal range.

Chapter 3

Increasing evidence indicates that the GH-IGF-IGFBP axis plays an important role in the regulation of glucose homeostasis and insulin sensitivity. A significant number of short SGA subjects have permanent abnormalities in their GH-IGF-IGFBP axis, which may contribute to the future development of insulin resistance and chronic adult diseases. We evaluated the relations between insulin sensitivity (Si), acute insulin response (AIR), disposition index (DI) and the GH-IGF-IGFBP axis in 36 prepubertal short SGA children during 6 months of GH treatment with either 1 mg GH (group A) or 2 mg GH/m^2·day (group B). After 6 months, we observed a reduction in Si and a compensatory rise in AIR, by which DI remained constant. Overnight GH levels, free and total IGF-I and IGFBP-3 showed a dose-dependent increase, whereas IGFBP-1 decreased. Given the well-documented insulin-antagonistic effects of GH, and the insulin sensitizing effects of IGF-I, the reduction in Si was most likely due to elevated GH levels. We did, however, not find a significant correlation between Si and GH levels. During GH treatment, Si was inversely related to total IGF-I and IGFBP-3 and positively to IGFBP-1 levels, whereas insulin levels correlated positively with total IGF-I and IGFBP-3 and negatively with IGFBP-1 levels. We hypothesized that GH treatment induced an increase in serum levels of GH, free and total IGF-I and IGFBP-3. Elevation of GH and IGFBP-3 levels resulted in a worsening of insulin resistance, which in turn might lead to a compensatory rise in insulin levels. Increased insulin levels further increased IGF-I levels directly and indirectly by suppressing IGFBP-1 levels. Increased IGF-I levels may have prevented further reduction in Si. Our data underline the complexity of relations between glucose homeostasis and the GH-IGF-IGFBP-axis in short SGA children and further research in this field is warranted.

Chapter 4

Low birth weight has been associated with insulin resistance and an increased risk of type 2 diabetes mellitus (DM-II) and cardiovascular disease in adulthood. Adiponectin and resistin are adipocytokines, with opposite properties. Adiponectin has been inversely related to obesity and insulin resistance, whereas resistin positively. We evaluated the changes in insulin sensitivity (Si), acute insulin response (AIR), disposition index (DI) in relation to changes in adiponectin and resistin levels during 24 months of GH treatment in 38 prepubertal short SGA children. During the first 6 months, group A (n = 18) received 1 mg GH/ m^2·day and group B (n = 20) 2 mg GH/m^2·day. Subsequently, between 6 and 24 months, both groups received 1 mg GH/ m^2·day. During GH treatment, Si showed

a reduction during the first 6 months, followed by an improvement thereafter. At 24 months, Si was still lower than baseline, but less significantly than at 6 months. The reduction in Si was well compensated by an increase in AIR, by which DI remained constant. Changes in adiponectin levels paralleled the changes in Si, whereas resistin levels showed opposite changes. At 24 months, adiponectin levels were slightly lower in group A compared with baseline, but comparable with baseline in group B. Resistin levels had completely returned to baseline levels after 24 months in both groups. During GH treatment, adiponectin levels were positively related to Si. In conclusion, our data in short SGA children suggest that glucose homeostasis was well maintained during GH treatment. The effects of GH on adiponectin and resistin appeared to be time-dependent and most marked in the early phase of GH treatment. Adiponectin levels might play a role in GH-induced insulin resistance.

Chapter 5

Many studies have demonstrated an inverse relationship between birth weight and the risk of type 2 diabetes mellitus and cardiovascular disease. In subjects born SGA, body composition and fat distribution might affect their predisposition of these disorders. We evaluated body composition and fat distribution with Dual Energy X-ray Absorptiometry (DXA) in 38 prepubertal short SGA children before and during 24 months of GH treatment in comparison with 10 untreated short SGA controls. We also assessed associations with several cardiovascular risk factors. During the first 6 months, group A (n = 18) received 1 mg GH/m^2·day and group B (n = 20) 2 mg GH/m^2·day. Subsequently, between 6 and 24 months, both groups received 1 mg GH/m^2·day. At baseline, lean body mass (LBM) SDS, fat mass SDS and fat percentage SDS were significantly lower than zero. GH treatment resulted in a progressive increase in LBM SDS parallel to the increment in height, whereas in untreated short SGA controls, LBM had significantly decreased after 24 months. The LBM to height ratio increased significantly in all groups, but the increases were significantly greater in the GH-treated SGA subjects than in the untreated short SGA controls. Fat mass and fat percentage SDS decreased during the first 6 months of GH treatment, but increased thereafter. At 24 months, both were comparable or only slightly different from baseline. During the first 6 months of GH treatment, the decrease in total fat mass was mainly due to a decrease in limb fat rather than trunk fat. Between 6 and 24 months, the increases in trunk and limb fat were nearly comparable. Total fat mass and trunk fat were inversely related to Si. In conclusion, GH treatment results in an increase in LBM and a temporary reduction in total fat mass and fat percentage, which appears to be due to decrease in peripheral rather than central fat. The inverse relation between total fat mass, trunk fat and insulin sensitivity, highlights the need for preventing overweight in SGA subjects.

Chapter 6

Low birth weight might increase risk of diabetes mellitus type 2 (DM-II) and metabolic syndrome (MS). Since GH has insulin-antagonistic properties, long-term follow-up of GH-treated children born SGA is important. We evaluated insulin sensitivity (Si) and disposition index (DI), all components of the metabolic syndrome and IGF-I and IGFBP-3 levels in 37 previously GH-treated young SGA adults in comparison with 25 untreated short SGA controls. The previously GH-treated SGA subjects were 22.3 (1.7) years. Mean duration of GH treatment had been 7.3 (1.3) years and mean period after discontinuation was 6.5 (1.4) years. Si and DI were comparable for GH-treated and untreated SGA subjects. Fasting glucose and insulin levels increased significantly during GH treatment, but recovered after discontinuation. Systolic and diastolic blood pressures decreased significantly during GH treatment, and were significantly lower in previously GH-treated SGA subjects than untreated SGA controls at 6.5 years after discontinuation. BMI and waist circumference were equivalent in GH-treated and untreated SGA subjects. During GH-treatment, serum levels of cholesterol, LDL-c and HDL-c decreased significantly. At 6.5 years after stop, cholesterol levels were lower in previously GH-treated SGA subjects, whereas HDL-c and triglycerides were comparable with untreated controls. Thirty-two percent of untreated controls had an increased blood pressure versus none of the GH-treated subjects. In addition, none of the GH-treated SGA subjects had metabolic syndrome versus 2 untreated SGA controls. GH-induced rises in IGF-I and IGFBP-3 levels had completely recovered after GH stop. In conclusion, at 6.5 years after discontinuation of long-term GH treatment, Si, DI, fasting levels of glucose and insulin, BMI, waist circumference, IGF-I and IGFBP-3 levels were equivalent for GH-treated and untreated young SGA adults. Systolic and diastolic blood pressure and serum cholesterol were even lower in GH-treated subjects. These data are reassuring as they suggest that previous long-term GH-treatment does not increase the risk for DM-II and metabolic syndrome in young SGA adults.

Chapter 7

Birth weight has been associated with subsequent body composition and adiposity in childhood and adulthood. In short SGA children, GH treatment results in an increase in lean body mass (LBM) and a short-term reduction in fat mass. It is unknown whether these changes persist on the long term. We investigated the impact of earlier GH treatment in short SGA children on body composition and fat distribution in young adulthood. In 37 previously GH-treated young SGA adults, body composition and fat distribution were evaluated by Dual Energy X-ray Absorptiometry (DXA) and adiponectin levels were measured. Results were compared with 37 untreated sex- and height- matched SGA and AGA controls. GH-treated subjects were 21.9 (1.3) years. Mean duration of GH treatment

and period after discontinuation were 7.3 (1.3) and 6.5 (1.4) years, respectively. Waist circumference was significantly lower in GH-treated than untreated SGA subjects. Fat mass and fat percentage, trunk and limb fat tended to be higher in untreated SGA controls compared with the other two groups, but did not reach statistical significance. LBM and adiponectin levels were comparable for all groups. Our study design excluded any confounding influences due to differences in sex and height between the study groups. In conclusion, at 6.5 years after discontinuation of long-term GH treatment, body composition and adiponectin levels were not significantly different in previously GH-treated SGA subjects and untreated sex- and height-matched SGA and AGA controls, except for waist circumference. These data are reassuring as they suggest that long-term GH of short SGA children treatment does not have unfavourable effects on body composition in adulthood.

Chapter 8

In the general discussion, we discuss our findings in relation to the current literature. This chapter ends with general conclusions and suggestions for future research.

Chapter 10

Samenvatting

Dit proefschrift beschrijft de resultaten van een aantal studies in (i) kleine SGA kinderen voor en tijdens GH-behandeling, die deelnamen aan de 3e Nederlandse GH-studie (IUGR-3 studie) en (ii) SGA jong-volwassenen die in het verleden met GH zijn behandeld in de 1e Nederlandse GH-studie (SGA follow-up studie). De IUGR-3 studie heeft o.a. de effecten van GH-behandeling bestudeerd op (i) GH-IGF-IGFBP-as, (ii) insulinegevoeligheid en cardiovasculaire risicofactoren, (iii) adipocytokines, en (iv) lichaamssamenstelling en vetdistributie, in kleine prepubertaire SGA kinderen (Hoofdstuk 2-5). De SGA follow-up studie heeft de gevolgen onderzocht van voormalige GH-behandeling op deze factoren in een groep SGA jong-volwassenen, 6.5 jaar na afronding van de GH-behandeling. Resultaten werden vergeleken met die van een andere groep onbehandelde jong-volwassenen (6 en 7).

Hoofdstuk 1

Dit hoofdstuk beschrijft de definities, prevalentie en oorzaak van SGA. Vervolgens wordt een overzicht gegeven van een aantal klinische en endocrinologische aspecten die samenhangen met SGA, waaronder (i) kleine lengte, (ii) GH-IGF-IGFBP-as, en de rol van deze as in foetale en postnatale groei, (iii) insulinegevoeligheid en cardiovasculaire risicofactoren, (iv) adipocytokines en (v) lichaamssamenstelling en vetdistributie. Ook worden de effecten van GH- behandeling op deze factoren beschreven. Tenslotte wordt een overzicht gegeven van de doelstellingen van de studies, de in- en exclusiecriteria en de opzet van de studies.

Hoofdstuk 2

Er zijn aanwijzingen dat hoge GH- en IGF-I-spiegels schadelijke gevolgen kunnen hebben voor de gezondheid. Hoge GH- en IGF-I- spiegels zouden het risico op kanker kunnen verhogen, met name in combinatie met lage IGFBP-3-spiegels. Wij onderzochten de veranderingen in nachtelijke GH-spiegels, IGF-I- en IGFBP-3- spiegels, en de IGF-I/IGFBP-3-ratio in 36 prepubertaire SGA kinderen met een te kleine lengte. Zij werden gedurende 6 maanden behandeld met 1 mg GH (groep A) of 2 mg GH/m^2·dag (groep B). Nachtelijke GH-spiegels namen significant toe na 6 maanden GH-behandeling in beide groepen, maar alle waarden waren significant hoger in groep B (gemiddelde, maximum en AUC_0) dan in groep A. Maximum GH- spiegels stegen van 43,9–161 mU/L in groep B en van 57,2–104 mU/L in groep A. Tijdens het GH-profiel (gedurende 12 uur) hadden kinderen in groep B een gemiddelde GH-waarde van 64,4 mU/L en kinderen in groep A van 34,8 mU/L. Voor aanvang van GH-behandeling waren IGF-I SDS, IGFBP-3 SDS en IGF-I/IGFBP-3-ratio SDS significant lager dan nul. Tijdens GH-behandeling werd een significante toename gezien in IGF-I SDS en IGF-I/IGFBP-3-ratio SDS in beide groepen, maar de toename was groter in groep B dan in groep A. In groep B had 74% van de kinderen IGF-I spiegels in het hoogste quintiel (hoogste 20%) ten opzichte van 19% in groep A. Onze data laten

zien dat GH-behandeling met 2 mg GH/m^2·dag resulteert in hoge GH-spiegels voor een groot gedeelte van de dag en IGF-I-spiegels in het hoogste quintiel. Aangezien de risico's op de lange termijn nog onvoldoende bekend zijn, is het belangrijk om IGF-I spiegels te vervolgen tijdens GH-behandeling, opdat ze binnen de normale range blijven.

Hoofdstuk 3

Er zijn aanwijzingen dat de GH-IGF-IGFBP-as een belangrijke rol speelt in de regulatie van glucosehomeostase en insulinegevoeligheid. Een aanzienlijk deel van alle SGA personen heeft permanente stoornissen in deze as, wat kan bijdragen aan de latere ontwikkeling van bepaalde chronische aandoeningen, zoals type 2 diabetes mellitus en cardiovasculaire ziekten. Wij onderzochten de relaties tussen insulinegevoeligheid, insulinesecretie en dispositie-index en de GH-IGF-IGFBP-as voor en na 6 maanden GH-behandeling in 36 kleine prepubertaire SGA kinderen. Zij werden behandeld met 1 mg GH (groep A) of 2 mg GH/m^2·dag (groep B). Na 6 maanden daalde de insulinegevoeligheid en nam de insulinesecretie toe. Hierdoor bleef de dispositie-index onveranderd. Nachtelijke GH-spiegels en vrij en totaal IGF-I en IGFBP-3 lieten een dosis-afhankelijke stijging zien, terwijl IGFBP-1 spiegels daalden. Gezien de welbekende anti-insuline eigenschappen van GH en pro-insuline werking van IGF-I, is de daling van de insulinegevoeligheid waarschijnlijk het gevolg van de toename in GH-spiegels. We vonden echter geen relatie tussen insulinegevoeligheid en GH-spiegels. Tijdens GH-behandeling correleerde insulinegevoeligheid negatief met totaal IGF-I en IGFBP-3 en positief met IGFBP-1 spiegels. Insulinespiegels daarentegen, waren positief geassocieerd met totaal IGF-I en IGFBP-3 en negatief met IGFBP-1. Wij veronderstelden dat GH-behandeling resulteert in een toename van GH-spiegels, vrij en totaal IGF-I en IGFBP-3. De toegenomen GH- en IGFBP-3-spiegels veroorzaken een afname in insulinegevoeligheid. Dit heeft een toename van insulinespiegels tot gevolg, wat vervolgens zorgt voor een verdere toename in IGF-I, o.a. door remming van IGFBP-1. De verdere stijging van IGF-I spiegels heeft een beschermende invloed op de insulinegevoeligheid en voorkomt een verdere daling. Onze resultaten onderschrijven de complexiteit van de relaties tussen glucose homeostase en de GH-IGF-IGFBP-as in kleine SGA kinderen. Verdere studies op dit gebied zijn aan te bevelen.

Hoofdstuk 4

Een laag geboortegewicht is geassocieerd met een hoger risico op insulineresistentie, type 2 diabetes mellitus en cardiovasculaire aandoeningen op latere leeftijd. Adiponectine en resistine zijn hormonen die door vetcellen worden afgescheiden. Adiponectinespiegels zijn negatief gecorreleerd met obesitas en insulineresistentie en resistine juist positief. Wij bestudeerden de veranderingen in insulinegevoeligheid, insulinesecretie en dispositie-index in relatie tot veranderingen in adiponectine en resistine in 38 kleine,

prepubertaire SGA kinderen tijdens 24 maanden GH-behandeling. Tijdens de eerste 6 maanden werd groep A (n = 18) met 1 mg GH/m^2·dag behandeld en groep B met 2 mg GH/m^2·dag (n = 20). Daarna, tussen 6 en 24 maanden, ontvingen beide groepen 1 mg GH/m^2·dag. Tijdens GH-behandeling nam de insulinegevoeligheid af in de eerste 6 maanden, gevolgd door een toename tussen 6 en 24 maanden. Na 24 maanden was de insulinegevoeligheid nog steeds lager dan bij aanvang, echter in mindere mate dan na 6 maanden GH-behandeling. De afname in insulinegevoeligheid werd gecompenseerd door een toename in insulinesecretie, zodat de dispositie-index constant bleef. De veranderingen in adiponectine liepen parallel aan die van insulinegevoeligheid, terwijl veranderingen in resistinespiegels juist tegengesteld waren. Na 24 maanden waren adiponectinespiegels gelijk aan uitgangswaarden in groep B, en nog iets lager in groep A. Resistinespiegels waren gelijk aan de uitgangswaarden in beide groepen. Tijdens GH-behandeling correleerden adiponectinespiegels positief met insulinegevoeligheid. Onze resultaten in kleine SGA kinderen laten zien dat tijdens GH-behandeling glucosehomeostase gehandhaafd bleef. De effecten van GH op adiponectine en resistine lijken tijdsafhankelijk en het belangrijkst in de vroege fase van GH-behandeling. Adiponectine speelt mogelijk een rol in GH-geïnduceerde insulineresistentie.

Hoofdstuk 5

Een laag geboortegewicht is geassocieerd met een verhoogd risico op het ontstaan van type 2 diabetes mellitus en cardiovasculaire ziekten. Het is mogelijk dat lichaamssamenstelling en vetdistributie de aanleg voor deze aandoeningen in SGA personen beïnvloeden. Wij onderzochten de lichaamssamenstelling en vetdistributie met behulp van een DEXA scan (Dual Energy X-ray Absorptiometry) in 38 kleine prepubertaire SGA kinderen tijdens 24 maanden GH-behandeling in vergelijking met een onbehandelde SGA controlegroep. Tijdens de eerste 6 maanden werd groep A (n = 18) met 1 mg GH/m^2·dag behandeld en groep B (n = 20) met 2 mg GH/m^2·dag. Daarna, tussen 6 en 24 maanden, ontvingen beide groepen 1 mg GH/m^2·dag. We hebben ook de relaties met cardiovasculaire risicofactoren onderzocht. Bij aanvang waren de vetvrije massa SDS, vetmassa SDS en vetpercentage SDS significant lager dan gemiddeld. GH-behandeling resulteerde in een toename van de vetvrije massa SDS die parallel liep aan de toename in lengte SDS. In de onbehandelde SGA controlegroep, nam de vetvrije massa SDS echter af. In alle groepen nam de totale vetvrije massa ten opzichte van de lengte significant toe, maar deze toename was het grootst in groep A en B. In de eerste 6 maanden GH-behandeling namen vetmassa en vetpercentage significant af, maar deze namen weer toe tussen 6 en 24 maanden. Na 24 maanden waren beide nagenoeg gelijk aan de uitgangswaarden. De afname in vetmassa in de eerste 6 maanden was voornamelijk het gevolg van een afname in perifeer vet en in mindere mate in centraal vet. Daarna waren de veranderingen in centraal en perifeer vet vrijwel gelijk. De totale vetmassa en het centrale vet correleerden

negatief met insulinegevoeligheid. Onze bevindingen laten zien dat GH-behandeling in kleine SGA kinderen resulteert in een toename in de vetvrije massa en een tijdelijke afname in vetmassa, die hoofdzakelijk het gevolg is van een afname in perifeer vet. De negatieve associaties tussen vetmassa en insulinegevoeligheid bevestigen het belang om overgewicht te voorkomen in SGA personen.

Hoofdstuk 6

Epidemiologische studies hebben laten zien dat type 2 diabetes mellitus en het metabool syndroom vaker optreden bij personen met een laag geboortegewicht. Aangezien gebruik van GH is geassocieerd met insulineresistentie, is het belangrijk om SGA kinderen die met GH worden behandeld te vervolgen. Wij bestudeerden insulinegevoeligheid en dispositie-index, alle onderdelen van het metabool syndroom and IGF-I en IGFBP-3 spiegels in 37 SGA jong-volwassenen die in het verleden met GH werden behandeld. Uitkomsten werden vergeleken met die van 25 onbehandelde SGA jong-volwassenen met een kleine lengte. De GH-behandelde SGA personen waren 22,3 (1,3) jaar. De gemiddelde behandelingsperiode was 7,3 (1,3) jaar geweest en de periode tussen afronding van de behandeling en de huidige studie was 6,5 (1,4) jaar. Insulinegevoeligheid en dispositie-index waren niet verschillend tussen GH-behandelde en onbehandelde SGA jong-volwassenen. Tijdens GH-behandeling waren glucose- en insulinespiegels significant toegenomen, maar beide hadden zich weer hersteld na staken van de behandeling. Systolische en diastolische bloeddruk namen beide af tijdens GH-behandeling en waren beide significant lager in de GH-behandelde SGA personen dan de onbehandelde. BMI en tailleomvang waren gelijk in beide groepen. Tijdens GH-behandeling namen cholesterol-spiegels, HDL-c- en LDL-c-spiegels significant af. Cholesterol-spiegels waren nu significant lager in de GH-behandelde SGA personen dan in de onbehandelde, terwijl HDL-c en LDL-c spiegels gelijk waren in beide groepen. In de GH-behandelde groep had niemand een te hoge bloeddruk of het metabool syndroom. In de onbehandelde groep had echter 32% een te hoge bloedruk en voldeden 2 personen aan de voorwaarden van het metabool syndroom. IGF-I- en IGFBP-3-spiegels namen significant toe tijdens GH-behandeling, maar beide herstelden zich weer na staken van de behandeling. Onze resultaten laten zien dat insulinegevoeligheid, dispositie-index, nuchtere glucose- en insulinewaarden, BMI, taille-omvang, IGF-I- en IGFBP-3-spiegels gelijk waren in SGA jong-volwassenen die in het verleden met GH zijn behandeld en onbehandelde SGA jong-volwassenen. Systolische en diastolische bloeddruk en serum cholesterolspiegels waren zelfs lager in de GH-behandelde groep. Deze bevindingen zijn gunstig, omdat zij suggereren dat langdurige GH-behandeling in het verleden, niet het risico op type 2 diabetes mellitus en het metabool syndroom verhoogt in SGA jong-volwassenen.

Hoofdstuk 7

Verschillende studies hebben laten zien dat er een relatie bestaat tussen geboortegewicht en latere lichaamssamenstelling en vetdistributie. GH-behandeling resulteert in een toename van de vetvrije massa en een tijdelijke afname van de vetmassa in SGA kinderen met een persisterend kleine lengte. Het is niet bekend of deze veranderingen ook op de lange termijn blijven bestaan. Wij bestudeerden de effecten van vroegere GH-behandeling in kleine SGA kinderen op de lichaamssamenstelling en vetdistributie op volwassen leeftijd, alsmede de effecten op adiponectinespiegels. In 37 SGA jong-volwassenen die voorheen waren behandeld met GH, werden lichaamssamenstelling en vetdistributie bepaald met behulp van een DEXA scan en adiponectinespiegels gemeten. Uitslagen werden vergeleken met die van 37 onbehandelde SGA en AGA jong-volwassenen, met gelijke lengte en geslacht. De GH-behandelde SGA personen waren 22,3 (1,3) jaar. De gemiddelde behandelingsperiode was 7,3 (1,3) jaar geweest en de periode tussen afronding van de behandeling en de huidige studie was 6,5 (1,4) jaar. Tailleomvang was significant lager in de GH-behandelde SGA groep dan in de onbehandelde SGA controle-groep. De totale vetmassa en het vetpercentage, centraal en perifeer vet waren iets hoger in de onbehandelde SGA controlegroep dan in de andere 2 groepen, maar niet significant. De vetvrije massa en adiponectinespiegels waren gelijk in de drie groepen. De mogelijkheid dat bepaalde resultaten en verschillen tussen de groepen het gevolg waren van verschillen in lengte en geslacht, werd voorkomen door onze studieopzet. Onze resultaten laten zien dat lichaamssamenstelling en vetdistributie niet verschillend zijn in SGA jong-volwassenen die voorheen zijn behandeld met GH en onbehandelde SGA en AGA jong-volwassenen. Dit suggereert dat GH-behandeling geen nadelige effecten heeft op de lichaamssamenstelling en vetdistributie op de langere termijn in SGA jong-volwassenen.

Hoofdstuk 8

In de algemene discussie worden de resultaten van de verschillende studies besproken en vergeleken met de huidige literatuur. Aan het einde van dit hoofdstuk wordt een overzicht gegeven van onze algemene conclusies en worden er suggesties gedaan voor toekomstig onderzoek.

Dankwoord

Eindelijk is het zover. Het proefschrift is echt af! De afgelopen jaren zijn heel wat mensen direct en indirect betrokken geweest bij de totstandkoming ervan en daarvoor wil ik iedereen hartelijk bedanken. Een aantal personen echter in het bijzonder.

Alle kinderen en hun ouders die hebben meegewerkt aan de IUGR-3 studie en de deelnemers van de SGA follow-up studie ben ik zeer dankbaar. Zonder hen waren de onderzoeken die beschreven staan in dit proefschrift niet mogelijk geweest.

Mijn promotor, Prof. dr. A.C.S. Hokken-Koelega. Beste Anita, eindelijk is het dan zover. Het boekje is af. Heel erg bedankt voor de mogelijkheid die je me hebt gegeven om dit onderzoek te doen. Ik waardeer je hulp, je enthousiasme, je begeleiding en betrokkenheid heel erg.

Prof. dr. J.M. Wit , Prof. dr. J.B. van Goudoever en Prof. dr. A.J. van der Lelij wil ik hartelijk bedanken voor hun snelle beoordeling van mijn manuscript en hun bereidheid plaats te nemen in de kleine commissie.

Prof. dr. S.L.S. Drop wil ik hartelijk bedanken voor de prettige samenwerking binnen de subafdeling Endocrinologie. Daarnaast ook voor zijn bereidheid plaats te nemen in de grote commissie.

I would like to thank Prof. dr. W. Kiess for his willingness to participate in the Committee.

Prof. dr. A.J. van der Heijden wil ik hartelijk bedanken voor zijn plaatsneming in de grote commissie.

Dr. E.C.A.M. Houdijk ben ik ook zeer dankbaar voor haar plaatsneming in de grote commissie en daarnaast voor haar medewerking aan de IUGR-3 studie.

Ook de andere kinderarts-endocrinologen van alle centra die hebben meegewerkt aan dit onderzoek ben ik zeer dankbaar. Drs. J.C. Mulder, Dr. C. Noordam, Drs. R.J.H. Odink, Dr. C. Rongen-Westerlaken, Dr. W.H. Stokvis-Brantsma, Drs. P.G. Voorhoeve en Dr. J.J.J. Waelkens.

Dr. P.G.H. Mulder wil ik hartelijk bedanken voor alle hulp bij de statistische analyses.

Dr. W.H. Hackeng wil ik hartelijk bedanken voor het bepalen van de GH-profielen en de FSIGT testen.

Dr. A.W. van Toorenenbergen en Y.B. de Rijke wil ik hartelijk bedanken voor de mogelijkheid adiponectine en resistine te laten bepalen. Dhr. G. Klein Heerenbrink en dhr. H. van Toor wil ik bedanken voor het verrichten van die bepalingen.

Prof. dr. E.P. Krenning wil ik bedanken voor de mogelijkheid die wij hebben gekregen om DEXA-metingen te verrichten op de afdeling Nucleaire geneeskunde. Jopie Sluimer ben ik zeer dankbaar voor alle DEXA metingen en de analyses daarvan.

Novo Nordisk Farma BV Nederland en Novo Nordisk Farma A/S Denemarken wil ik hartelijk bedanken voor de plezierige samenwerking en de financiële ondersteuning van de studies en dit proefschrift.

Alle (ex-)arts-onderzoekers wil ik bedanken voor de gezelligheid tijdens de lunches, kerstdiners en weekenden.

Mijn collega's van de afdeling Endocrinologie.
Christel Bruinings. Beste Christel, heel erg bedankt voor al je hulp bij de IUGR-3 studie. Je was een steunpilaar voor de studie. Het was superleuk om met je samen te werken.
Colette Wissink. Beste Colette, heel erg bedankt voor je hulp bij het opzetten van de IUGR-3 studie en de samenwerking in de beginfase.
Joke Dunk. Beste Joke, bedankt voor al je hulp bij de SGA follow-up studie.
Ingrid van Slobbe, Janneke van Nieuwkasteele, Marianne Huibregtse en Esther Lems. Jullie wil ik ook bedanken voor al jullie hulp, het bijspringen waar nodig en de gezelligheid.
Venje, Robert, Floor, Nicolette, Ruben, Daniëlle, Sandra, Laura, Wietske, Roderick en Ralph, mijn collega endo-onderzoekers. Hartelijk dank voor alle samenwerking, discussies en gezelligheid.

Ellen, Marieke, en Dederieke. Collega's en vriendinnen. Ik heb een enorm leuke tijd met jullie gehad tijdens het promotietraject en daarbuiten. Bedankt voor al jullie betrokkenheid. Ellen en Marieke, jullie zijn mij al voorgegaan. Ik hoop dat het bij mij net zo goed gaat als bij jullie. Dederieke, jij moet nog, maar kan bij mij alvast een beetje oefenen.

Mijn (studie-)vriendinnen uit Amsterdam (en inmiddels ook elders). Lieve Anemone, Anneke, Foka, Ingenet, Karina, Melissa en Suzanne. Superbedankt voor jullie vriendschap en alle leuke jaren tijdens en na onze studie. Dat er nog veel weekjes Toscane mogen komen.

Ook alle andere vrienden en bekenden wil ik bedanken voor alle steun, gezelligheid en interesse gedurende de afgelopen jaren.

Mijn lieve familie, Jan, Caroline en kleine Bartje. Jan, hopelijk breng ik het er net zo goed af als jij. Bij jou hoefde ik alleen maar stil te zitten en lief te lachen. Succes met je nieuwe baan. Caroline, nog even en dan breken voor jullie ook rustiger tijden aan.

Mijn schoonfamilie. Frans en Donna, Leida en Gerard, Laura en Annemiek, Milou en Loek. Bedankt voor al jullie steun en interesse. En ook voor alle dagjes met Isa als ik weer eens een deadline moest halen.

Petra, jou wil ik in het bijzonder bedanken. Supergezellig dat je nu zo dicht bij ons woont. Ik waardeer je betrokkenheid heel erg en je interesse voor mijn onderzoek, voor Isa en nog veel meer.

Mijn lieve ouders, Adria en Bart. Altijd betrokken, altijd klaar gestaan met hulp in welke vorm dan ook en nog veel meer. Dank jullie wel voor alles. Ad, ik wil jou nog wel speciaal laten weten hoe bijzonder ik het vind dat je elke week weer naar Gouda komt voor Isa.

Lieve Robert en Isa, door jullie weet ik wat echt belangrijk is in het leven. Robert, heel erg bedankt voor je interesse, steun, betrokkenheid, humor, relativeringsvermogen en nog veel meer. Ik hou van je.

Curriculum vitae of Marije van Dijk

Marije van Dijk was born on 23rd November, 1973 in Eindhoven. After finishing high school (VWO) in 1992, Marije was able to start her medical training in 1993 at the Vrije Universiteit (Free University) in Amsterdam. In 1997, she spent 3 months at the The Raymond Purves Bone and Joint Research Laboratories in Sydney, Australia, where she investigated the effects of a new drug on inflammation and cartilage destruction in osteo-arthritis (supervisor Prof. dr. P. Ghosh in Sydney and Dr. A.P.A. Prins in Amsterdam). After this research project, she traveled through Australia for another 3 months. In 1998, she started with her internships. In 1999, Marije participated in another research project at the VU medical center investigating the use of antibiotics in neonatal intensive care units in the Netherlands (supervisors Dr. R.M. van Elburg, Dr. A.M. van Furth, and Prof. dr. W.P.F. Fetter). At the end of her internships, Marije spent 2 months in the Deborah Retief Memorial Hospital in Mochudi, Botswana, where she worked on the pediatric department. After obtaining her medical degree in October 2000, Marije started to work as a resident at the Department of Pediatrics in the Albert Schweitzer Hospital in Dordrecht. In November 2001, she started as a research fellow at the Department of Pediatrics, Subdivision of Endocrinology, Erasmus MC-Sophia Children's Hospital in Rotterdam (supervisor Prof. dr. A.C.S. Hokken-Koelega), which has resulted in the present thesis.

List of Publications

van Dijk M, Mulder P, Houdijk M, Mulder J, Noordam K, Odink RJ, Rongen-Westerlaken C, Voorhoeve P, Waelkens J, Stokvis-Brantsma J, Hokken-Koelega A 2006 High serum levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I) during high-dose GH treatment in short children born small for gestational age. *J Clin Endocrinol Metab* 91:1390-1396

van Dijk M, Bannink EM, van Pareren YK, Mulder PG, Hokken-Koelega AC 2007 Risk factors for diabetes mellitus type 2 and metabolic syndrome are comparable for previously growth hormone-treated young adults born small for gestational age (sga) and untreated short SGA controls. *J Clin Endocrinol Metab* 92:160-165

Willemsen RH, van Dijk M, de Rijke YB, van Toorenenbergen AW, Mulder PG, Hokken-Koelega AC 2007 Effect of growth hormone therapy on serum adiponectin and resistin levels in short, small-for-gestational-age children and associations with cardiovascular risk parameters. *J Clin Endocrinol Metab* 92:117-123

den Brinker M, Joosten KF, Liem O, de Jong FH, Hop WC, Hazelzet JA, van Dijk M, Hokken-Koelega AC 2005 Adrenal insufficiency in meningococcal sepsis: bioavailable cortisol levels and impact of interleukin-6 levels and intubation with etomidate on adrenal function and mortality. *J Clin Endocrinol Metab* 90:5110-5117

van Dijk M, Houdijk M, Mulder PG, Mulder JC, Noordam K, Odink RJ, Rongen-Westerlaken C, Stokvis-Brantsma WH, Voorhoeve PG, Waelkens JJ, Hokken-Koelega AC Associations between insulin sensitivity, insulin secretion, overnight growth hormone (GH) levels, free and total IGF-I and IGF-binding proteins 3 and 1 in short children born small for gestational age (SGA) before and during GH treatment. *Submitted*

van Dijk M, Willemsen RH, Mulder PG, van Toorenenbergen AW, de Rijke YB, Hokken-Koelega AC Changes in insulin sensitivity are associated with changes in adiponectin levels during 24 months of growth hormone (GH) treatment in short children born small for gestational age (SGA). *Submitted*

van Dijk M, Willemsen RH, Waelkens JJ, Mulder JC, Houdijk M, Odink RJ, Stokvis-Brantsma WH, Voorhoeve PG, Rongen-Westerlaken C, Noordam K, Mulder PG, Hokken-Koelega AC The effects of growth hormone (GH) treatment on body composition and fat distribution and relations with cardiovascular risk factors in short children born small for gestational age (SGA). *Submitted*

van Dijk M, Willemsen RH, Bannink EM, de Rijke YB, van Toorenenbergen AW, Mulder PG, Hokken-Koelega AC Body composition and adiponectin levels in previously growth hormone (GH)-treated young adults born small for gestational age (SGA): comparisons with sex- and height-matched untreated SGA and AGA subjects. *Submitted*