SMALL AT BIRTH

CARDIOVASCULAR AND METABOLIC HEALTH
OF SUBJECTS BORN SGA AND/OR PRETERM
AND EFFECTS OF GROWTH HORMONE TREATMENT

KLEIN BIJ DE GEBOORTE

CARDIOVASCULAIRE EN METABOLE STATUS
VAN TE KLEIN (SGA) EN/OF PREMATUUR
GEBOREN PERSONEN EN EFFECTEN
VAN GROEIHORMOONBEHANDELING

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Ruben Heinjan Willemsen

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PROMOTIECOMMISSIE:

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

OVERIGE LEDEN:
Prof.dr. A.J. van der Heijden
Prof.dr. W. Kiess
Prof.dr. J.B. van Goudoever

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SMALL AT BIRTH
cardiovascular and metabolic health of subjects born SGA and/or preterm and effects of growth hormone treatment

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CHAPTER 1

INTRODUCTION

Introduction

This doctoral thesis describes cardiovascular and metabolic risk factors in children and young adults with a small size at birth, either due to preterm or SGA birth. For those born SGA with persistent short stature, the effects of GH treatment on these risk factors were studied. This first chapter describes definitions, prevalence and etiologies of SGA, and clinical and endocrinological aspects associated with SGA. Finally, the aims of the study and outline of this thesis are described.

1. Small for gestational age (SGA)

1.1 Definitions of SGA

In the past years, several definitions have been used for SGA, ranging from a birth weight and/or length below the 10th to 3rd percentile or –2 SD 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 (1). This was confirmed in the consensus statement of 2007 (2).

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 prenatal ultrasound biometry. 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. Because the prenatal growth pattern is often unknown, we prefer to use the term SGA.

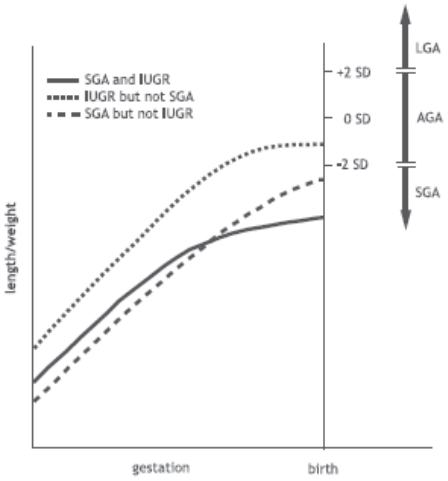

Figure 1. Fetal growth chart showing difference between SGA and IUGR newborns

1.2 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 (3-5). 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.

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

Maternal factors can be divided into medical conditions and environmental factors. Medical conditions 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 (e.g., anticonvulsants, anticoagulants).

Placental factors are associated with problems in placental perfusion resulting in reduced fetal oxygenation. These include structural abnormalities of the placenta, maternal or fetal thrombophilia, 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 (3), Pollack and Divon (5), Wollmann (4), and Keller et al. (6).

Fetal factors	
Karyotypic abnormalities	Trisomy 21 (Down syndrome)
	Trisomy 18 (Edward syndrome)
	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 mellitus (advanced stages)
	Collagen vascular diseases (e.g. systemic lupus erythematosus)
	Maternal hypoxemia (cyanotic heart disease, chronic anemia, chronic pulmonary disease)
	Toxoplasmosis
Infection	Rubella
	Cytomegalovirus
	Herpes virus
	Malaria
	Trypanosomiasis
	Human immunodeficiency virus
Nutritional status	Low pre-pregnancy weight
	Low pregnancy weight with poor weight gain during pregnancy
Substance use/abuse	Cigarette smoking
	Alcohol
	Illicit drugs
	Therapeutic drugs (e.g., 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
Maternal and/or fetal thrombophilia	
Insufficient uteroplacental perfusion	Suboptimal implantation site
Placenta praevia	
Low-lying placenta	
Placental abruption	

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

2.1 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 most pronounced during the first 6 months and is usually completed in the first 2 years of life. However, in premature SGA infants, catch-up growth may take longer (9, 10). This is most probably because prematures differ from term children with regard to several parameters, such as distance between height SDS at 2 years of age (height SDS_{2y}) and target height SDS, and difference between height SDS_{2y} and birth length SDS (9, 10). By the age of 8 years, 91% has reached a height > -2 SDS (10).

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 (~ -2.1 SDS) for boys and 147.6 (7.0) cm (~ -2.8 SDS) for girls (11, 12). These values were significantly lower than the target heights of these children ($p<0.001$) (11). If a normal height above – 2 SDS has not been achieved by 2 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).

2.2. Cardiovascular disease associated with SGA

2.2.1 *Historical data and hypotheses*

Based on epidemiological studies, an inverse relation has been reported between birth weight and the risk of hypertension, cardiovascular disease and diabetes mellitus type 2 in adulthood (13-15). Insulin resistance plays an important role in the pathogenesis of these diseases (16, 17), however, the exact mechanisms underlying these associations are 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 (13, 14). This re-programming would be in favor of short-term survival, but deleterious on the long-term as it would result in diseases in adulthood (Figure 2).

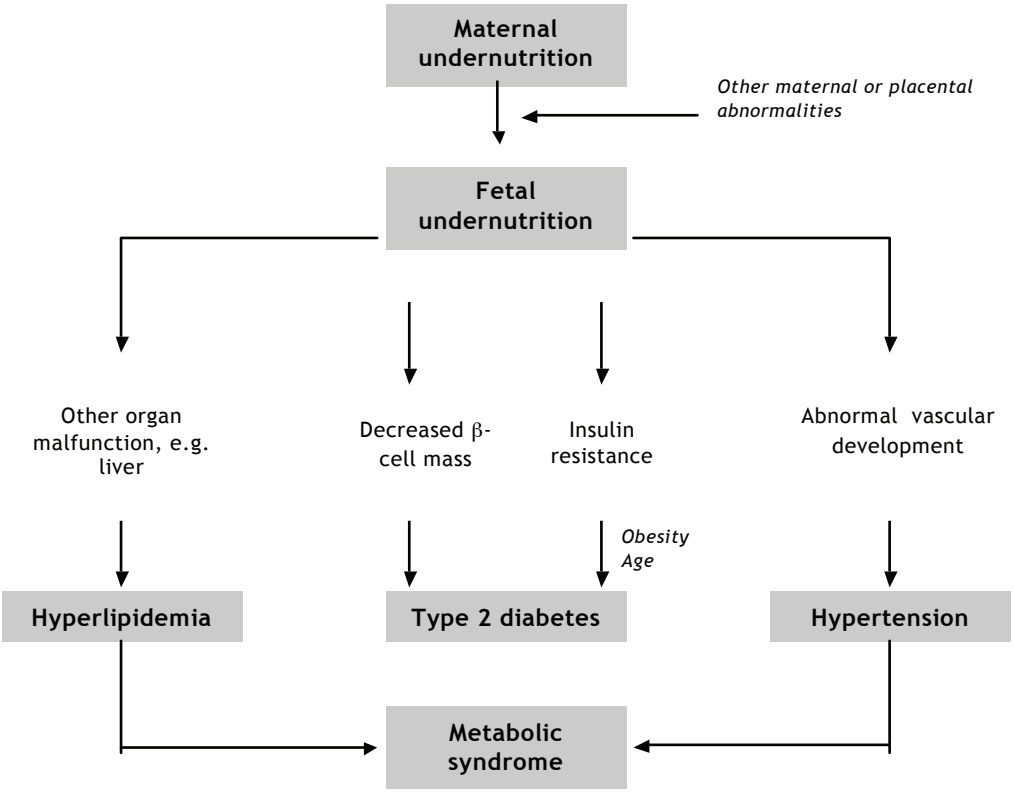

Figure 2. Representation of the fetal origins of the metabolic syndrome.
Adapted from Barker et al. (14, 18).

Fetal insulin hypothesis: The fetal insulin hypothesis was formulated by Hattersley et al. It was postulated that the association between low birth weight and adult insulin resistance is principally genetically mediated (19). 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 3).

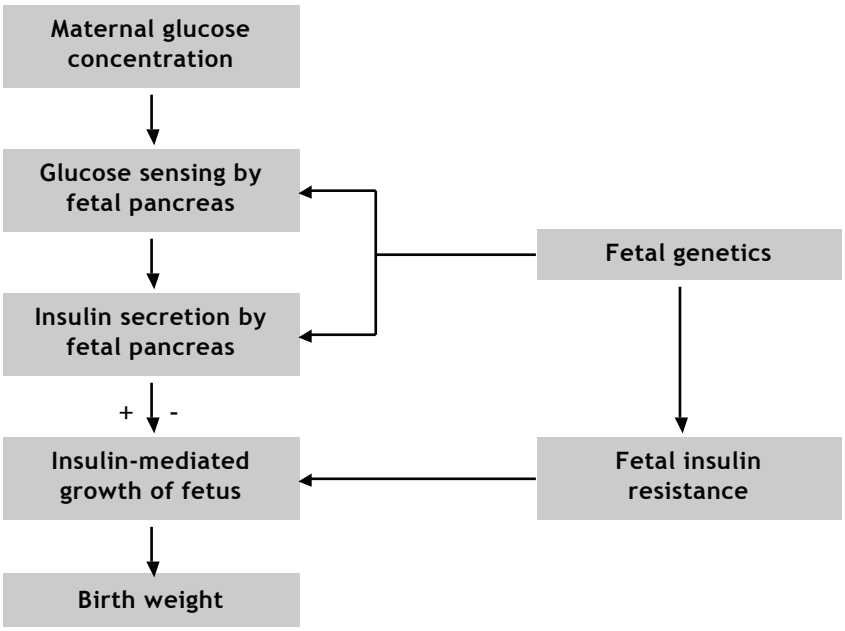

Figure 3. Simplified representation of fetal insulin hypothesis. Adapted from Hattersley et al. (19).

Growth acceleration hypothesis: Singhal and Lucas suggested that rapid postnatal growth rather than birth weight per se, could have adverse long-term effects and might result in adult diseases (Figure 4) (20).

Figure 4. Simplified representation of growth acceleration hypothesis.
Adapted from Singhal and Lucas (20).

Fat accumulation hypothesis: Based on detailed measurement of body composition by dual energy x-ray absorptiometry (DXA), our research group took the growth acceleration hypothesis by Singhal and Lucas a step further by specifying growth acceleration into fat accumulation (Figure 5) (21). According to this hypothesis, small size at birth followed by growth in height and weight as such is not a problem as long as a normal amount of fat is accumulated.

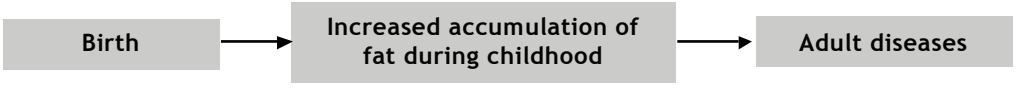

Figure 5. Simplified representation of fat accumulation hypothesis. Adapted from Leunissen et al. (21).

2.2.2 Assessment of cardiovascular risk

Novel cardiovascular risk markers

In the past, several new cardiovascular risk markers have been identified with additional predictive value with respect to the development of cardiovascular disease and diabetes mellitus type 2. A majority of these new markers are produced in adipose tissue (Figure 6). Some of them will be described below and the limited number of studies in SGA subjects will be summarized.

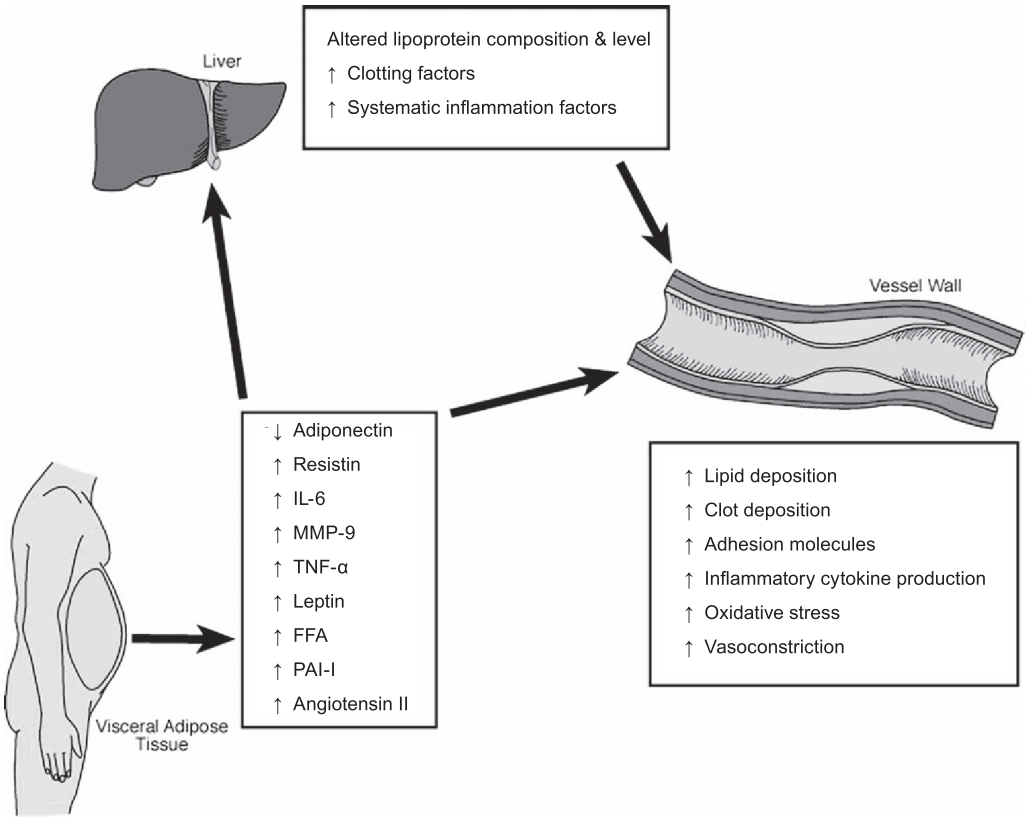

Figure 6. Adipose tissue produces several substances associated with the development of cardiovascular disease. Adapted from Fantuzzi et al. (22)

Adiponectin

Adiponectin was first described in 1995 as a novel adipocytokine, synthesized exclusively by adipocytes and secreted in relatively large amounts into plasma (23). Adiponectin has been inversely associated with insulin resistance and obesity parameters

in both animal models and human studies (24-31). Adiponectin knock-out mice developed insulin resistance, when fed a high fat/high sucrose diet (30), whereas in obese children, weight loss resulted in an increase of serum adiponectin levels (24). Though its mechanism of action is largely unknown, longitudinal studies have shown that low adiponectin is a risk factor for developing diabetes mellitus type 2 (31).

Resistin

Resistin was initially identified as an adipocytokine in mice that was downregulated by thiazolidinediones (TZDs, a class of Peroxisome Proliferator Activated Receptor gamma (PPARγ) agonists) (32). TZDs are used in the treatment of type 2 diabetes mellitus and are known to enhance insulin sensitivity in vivo. Increased resistin levels were found in genetically and diet-induced animal models of obesity (32). In animals, inverse associations were described between resistin levels and glucose tolerance and positive correlations with obesity (32). In humans, however, the associations with insulin resistance and obesity were less clear and could often not be reproduced (29, 33-36). Therefore, the role of resistin as a marker for insulin resistance in humans has been questioned (35, 37). Recent reports suggest that, instead of being a marker for insulin resistance, resistin might have proinflammatory properties as it is a member of the cytokine family (38, 39). Jung et al. showed that resistin is secreted from macrophages in atheromas and may contribute to atherogenesis because of its effects on vascular endothelial cells and smooth muscle cells (40). So, resistin might rather be considered as a parameter for inflammation and atherosclerosis than for insulin resistance. Because important crosstalks have been described between metabolic and inflammatory pathways (41), resistin might still be a marker of interest.

Interleukin-6 and C-reactive protein

There is increasing evidence that the innate immune system in combination with nutritional factors is involved in the pathogenesis of DM2 (42). Growing evidence indicates that the GH-IGF-I system and the immune system are correlated and several bidirectional interactions have been described (43). Besides, the receptors and signaling cascades activated by GH and IL-6 appear to be very similar (44). Hypopituitary adults have higher CRP and IL-6 levels (45). Interleukin-6 (IL-6) is an inflammatory cytokine and a primary determinant of the production of C-reactive protein (CRP). Both increased CRP and IL-6 levels have been associated with increased incidence of cardiovascular disease, obesity and diabetes mellitus type 2 in adults (46-52). In children, CRP levels were positively associated with BMI, systolic blood pressure and triglycerides and inversely with HDL-C levels (53).

MMP-9

Matrix metalloproteinases (MMPs) are zinc-dependent endoproteinases, which participate in remodelling the extracellular matrix (ECM) (54, 55). Since alterations in the elastic properties of the vasculature in atherosclerosis result from constant remodelling of the arterial wall, MMPs are thought to play a role in the development of athero-

sclerosis and cardiovascular disease (55, 56). One of the interesting MMPs with respect to atherosclerosis is MMP-9, which can degrade elastin. Elastin is the main elastic component of the arterial wall. In MMP-9 knock-out mice, MMP-9 deficiency resulted in less intima hyperplasia and less atherosclerosis (57). MMP-9 deficient smooth muscle cells had decreased migratory activity as well as a decreased capacity to contract collagen (57). Increased MMP-9 levels were found in hypertensive subjects (58, 59) and high plasma MMP-9 levels predict cardiovascular mortality in patients with coronary artery disease (60).

Novel cardiovascular risk factors in SGA

Contrasting data about adiponectin levels have been reported for SGA infants and children (61-64). Some found positive correlations with birth weight (61, 63, 65-67), but others did not (62, 68-70). Importantly, birth weight was not adjusted for gestational age in all of these studies, whereas adiponectin has been shown to increase impressively with gestational age (71). Compared with AGA children, previous studies reported lower (61, 63), similar (64) or higher (62) levels in SGA children. This might be explained by the fact that the studied SGA groups for which lower adiponectin levels were reported, all consisted of a mixture of short SGA children and SGA children with catch-up growth (61, 63).

Data on inflammatory markers in SGA children are very scarce. One study reported IL-6 levels in short SGA children (64), which were comparable with normal AGA children. Unfortunately, they did not provide any information on recent infections, surgery or trauma, which obviously can influence inflammatory markers.

Resistin, CRP and MMP-9 levels have not been described in SGA children before.

Glucose homeostasis and conventional cardiovascular risk factors

In a group of prepubertal short SGA children with a mean age of 8 years, we previously showed that 8% had an impaired oral glucose tolerance test (72). Further studies indicated that short SGA children were more insulin resistant than children born AGA (73, 74). Notably, there was a compensatory increase in insulin secretion in short SGA children (73, 74). The relationship between insulin sensitivity and insulin secretion is best described by a hyperbolic function (Figure 6). When insulin sensitivity varies, a proportionate and reciprocal alteration in insulin secretion is required to maintain a constant glucose tolerance (75). If insulin secretion does not change appropriately, impaired glucose tolerance and ultimately diabetes mellitus type 2 will develop (76). In addition to reduced insulin sensitivity, it was also reported that SGA children and adolescents had a higher systolic blood pressure (77, 78) and more often hypercholesterolemia (79).

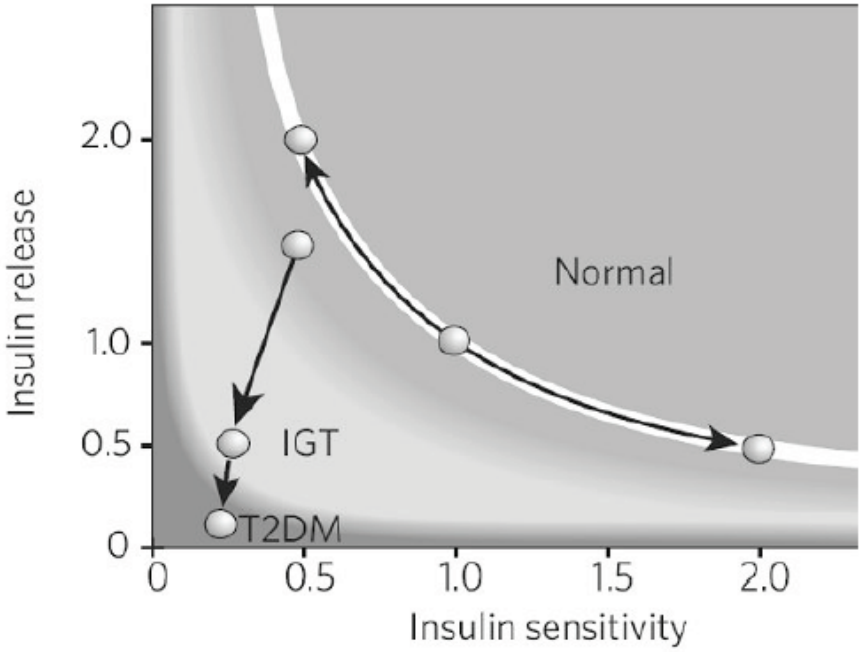

Figure 7. Hyperbolic association between insulin secretion and insulin sensitivity. Adapted from Kahn et al. (80). IGT: impaired glucose tolerance; T2DM: type 2 diabetes mellitus

Body composition

It is well known that obesity is an important risk factor for the development of cardiovascular disease and diabetes mellitus type 2 (22). In children born SGA with spontaneous catch-up in weight, early development of adiposity has been reported (81). However, short children born SGA have a different phenotype. They have a typical lean appearance, which is confirmed by a low body mass index (BMI) SDS and a low sum of skinfolds SDS (77). Short SGA children also have lower IGF-I levels (82-84), and because abnormalities in body composition in GH deficient children have previously been demonstrated (85), it might well be that body composition in short SGA children is also altered. However, data on detailed body composition in short SGA children are very scarce (86, 87).

3. Growth hormone (GH) treatment in SGA children

3.1 Effects on linear growth

A significant number of short SGA children exhibit alterations in the GH-IGF-IGFBP axis (82, 84, 88-92). These observations have led to the first GH studies in short SGA children. In 1991, the first Dutch multi-centre randomized double-blind dose-response GH trial was started to investigate the efficacy of GH treatment on growth (84, 93). Children were treated with either 1 or 2 mg GH/m2/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 (93). 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, GH treatment with a dose of 1mg GH/m2/day resulted in a normalization of height, whereas children in the control group remained short (94). In addition to the Dutch GH trials, several other studies have also demonstrated that GH treatment is an effective and safe 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 SGA/ short stature	GH dose	At start			Δ Height SDS	Adult Height		
		N	Age (yr)	Height SDS	5 y	N	Height SDS	
<–1.88 SDS	Untreated	29	7.8	–2.6		15	–2.3	Sas et al. (84) and van Pareren et al. (93)
	0.033 mg/kg/d	41	7.3	–3.0	2.2	28	–1.1	
	0.067 mg/kg/d	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. (95)
	0.067 mg/kg/d	102	12.7	–3.2		91	–2.1	
<–2 SDS	Untreated	34	8.3	-2.2		34	-2.0	Dahlgren et al. (96)
	0.033 mg/kg/d	36	8.9	-3.1		36	-1.2	
	0.033 mg/kg/d	41	12.3	-2.5		41	-1.6	

3.2 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 (84, 89, 97). 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/m²/day (89). Sas et al. reported a rise in IGF-I and IGFBP-3 levels up to 1.2 SDS and 0.2 SDS, respectively, during GH treatment with 1 mg GH/m²/day for one year, whereas treatment with 2 mg GH/m²/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 (84). Van Dijk et al. performed overnight GH profiles in short prepubertal SGA children (83). Mean and maximum GH levels during an overnight GH profile were 34.8 and 104 mU/l respectively during treatment with 1 mg GH/m²/day, and 64.4 and 161 mU/l during treatment with 2 mg GH/m²/day (83).

3.3 Effects on novel cardiovascular risk markers

Data regarding the effect on novel cardiovascular risk markers, such as adiponectin, resistin, IL-6, CRP and MMP-9 are very limited. Ibañez et al. reported a reduction in adiponectin and an increase in IL-6 levels in a small group of SGA children after 6 months of high-dose GH treatment (64). However, no information was given on recent infections, surgery or trauma. Given the important antidiabetic and anti-inflammatory properties of adiponectin, we hypothesized that adiponectin might be involved in the reduction in insulin sensitivity induced by GH treatment. At start of this study, reports describing the effects of GH treatment on resistin, CRP and MMP-9 levels did not exist.

3.4 Effects on insulin sensitivity and conventional 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 (98-100). Therefore, concern has been expressed regarding the long-term effects of GH-treatment on the insulin-glucose homeostasis in short SGA children. It has previously been shown that insulin sensitivity is reduced in short SGA children before start of GH treatment (74, 101). GH treatment resulted in a further decline of insulin sensitivity and a compensatory increase in insulin secretion (73, 102).

Previous studies reported conflicting results regarding the change in insulin sensitivity after GH was stopped. De Zegher et al. observed a decrease in insulin sensitivity in 9 short SGA children during GH treatment (using a very high dose of ffi3 mg GH/m2/day), which was reversible after stop of GH treatment (102). Cutfield et al. measured insulin sensitivity in 5 short SGA children and reported that insulin sensitivity did not

recover after stop of GH treatment (73). Both groups investigated insulin sensitivity after stop of GH treatment, but still before adult height had been achieved. Besides, in one study it remained unclear if some of the children had already entered puberty (102), which can reduce insulin sensitivity as well (103, 104). Furthermore, it is questionable if the number of subjects was sufficient to draw definitive conclusions. Up to date, it is not known how insulin sensitivity and secretion change longitudinally in SGA adolescents after adult height has been attained and GH treatment has been stopped.

With respect to other cardiovascular risk factors, GH treatment has been associated with a reduction in systolic blood pressure as well as a reduction in cholesterol levels which remained so until 6.5 years after discontinuation (105, 106). In the same study, at an age of 22 years, insulin sensitivity and secretion, body mass index and waist circumference in previously GH-treated SGA subjects were comparable with those of untreated SGA subjects.

Reassuringly, the results of the follow-up studies which have been performed until now in previously GH-treated SGA subjects do not indicate that GH treatment increases the risk for diabetes mellitus type 2 and metabolic syndrome (105, 106). However, long-term surveillance of insulin sensitivity and cardiovascular parameters in previously GH-treated SGA subjects is important to exclude any negative effects of GH. In the last SGA consensus meeting in 2007 long-term follow-up of previously GH-treated SGA subjects was considered essential (2).

3.5 Effects on body composition

GH has well-documented anabolic effects on muscle mass and lipolytic effects on adipose tissue (107, 108). GH deficiency has been associated with increased fat mass and truncal obesity (51, 109), whereas GH excess, as in active acromegaly, has been related to reduced fat mass and increased LBM (110).

Data regarding the effect of GH on body composition in short SGA children are scarce. Leger et al. measured muscle and fat tissue mass of the thighs by magnetic resonance imaging (MRI) in 14 short SGA children during GH therapy (86). They reported a progressive increase in muscle tissue cross-sectional area and a transient decline in adipose tissue cross-sectional area following 3 years of GH treatment (86). However, they did not measure total body fat and muscle mass. Besides, there are no data on the long-term consequences of GH treatment on body composition beyond the initial phase of catch-up growth during the first 2-3 years of GH treatment in SGA children.

From a methodological point of view, it is remarkable that most studies investigating body composition in children receiving GH therapy did not adjust for the GH-associated catch-up in height (85-87, 109, 111). Consequently, these studies might have reported an underestimation of lean and fat mass for height and an overestimation of the effect of GH treatment on these parameters.

3.6 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 (112). However, the authors concluded that major adverse events in relation to GH treatment were rare and 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 (72, 77, 105). Nevertheless, all SGA children receiving GH therapy should be monitored regularly for changes in glucose homeostasis, lipid profile, blood pressure and serum IGF-I levels to exclude any possible adverse effects of GH (2, 113).

4. Aims of the study

Novel cardiovascular risk markers

We compared adiponectin, resistin, IL-6 and CRP levels in short SGA children with those in healthy normal-statured children. Besides, we evaluated the short-term effect (2 years) of GH treatment on adiponectin and resistin levels compared with untreated SGA controls. Also, we assessed the long-term effects (7 years) of GH treatment in 2 different dosages (1 and 2 mg GH/m2/day) and the effect of discontinuation of GH treatment on adiponectin, resistin, IL-6 and CRP-levels.

We investigated whether GH treatment affects plasma MMP-9 levels and blood pressure in short SGA children compared with sex- and age-matched untreated SGA controls. Because MMP-9 levels are thought to play a role in remodelling the arterial wall we also investigated whether these levels are related to blood pressure.

Body composition and bone mineral density

We measured body composition and bone mineral density by Dual Energy X-ray Absorptiometry during 6 years in short SGA children participating in a GH study with a randomized controlled part of 3 years. Besides, we investigated in a larger group of short SGA children whether there were differences in body composition between premature and term short SGA children. At last, we investigated longitudinal changes in body composition after discontinuation of GH due to attainment of adult height.

Insulin sensitivity and secretion

We evaluated longitudinal changes in insulin sensitivity and secretion, as measured with a frequently sampled intravenous glucose tolerance test (FSIGT) with tolbutamide, in GH-treated SGA subjects at near adult height and 6 months after GH treat-

ment was stopped. Besides, we investigated whether insulin sensitivity and secretion were different between preterm and term short SGA children.

Prematurity

Since both SGA and preterm birth have been associated with increased incidence of adult cardiovascular disease and diabetes mellitus type 2, we investigated in short children born SGA if preterm birth had an independent effect on insulin sensitivity and secretion, body composition, blood pressure and lipid levels. In addition, we investigated the relative contributions of prematurity and SGA on insulin sensitivity and secretion in young adulthood as well as the effect of adult body composition and early postnatal growth on these parameters.

5. Outline of the thesis

Chapter 1 gives an introduction in the topics described in this thesis.
Chapter 2 describes adiponectin and resistin levels before and during 2 years of GH treatment in comparison with healthy AGA controls and untreated SGA controls, and reports the associations of these adipocytokines with cardiovascular risk factors.
Chapter 3 describes longitudinal changes in adiponectin, resistin, IL-6 and CRP levels during and after discontinuation of long-term GH treatment with either 1 or 2 mg GH/m2/day in comparison with healthy AGA controls.
Chapter 4 reports on MMP-9 levels and blood pressure in short SGA children during 3 years of GH treatment in comparison with untreated SGA controls.
Chapter 5 describes the long-term effects of GH treatment on body composition and bone mineral density during 6 years of GH treatment in short SGA children with a randomized controlled part of 3 years.
Chapter 6 reports on serial measurements of insulin sensitivity, insulin secretion and body composition in GH-treated SGA adolescents at discontinuation of GH due to attainment of adult height and 6 months after stop of GH.
Chapter 7 describes differences between preterm and term short prepubertal SGA children in insulin sensitivity, insulin secretion, body composition, blood pressure and lipid levels.
Chapter 8 reports on insulin sensitivity, insulin secretion and body composition in young adults that were born premature and/or SGA.
Chapter 9 discusses our data in relation to current literature and comments on the clinical implications and conclusions of our study results
Chapter 10 summarizes our findings in English
Chapter 11 presents a Dutch summary

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CHAPTER 2

Effect of growth hormone therapy on serum adiponectin and resistin levels in short SGA children and associations with cardiovascular risk parameters

Ruben H. Willemsen[1], Marije van Dijk[1],
Yolanda B. de Rijke[2,3], Albert W. van Toorenenbergen[3],
Paul G.H. Mulder[4], Anita C.S. Hokken-Koelega[1]

[1]*Dept. of Pediatrics, Division of Endocrinology, Erasmus MC Sophia,* [2]*Dept. of Internal Medicine, Diagnostic Laboratory Endocrinology, Erasmus MC,* [3]*Dept. of Clinical Chemistry, University Medical Centre Rotterdam, Erasmus MC,* [4]*Dept. of Epidemiology and Biostatistics, Erasmus MC, Rotterdam, The Netherlands*

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Abstract

Background: Adiponectin and resistin are fat-cell derived hormones, which are thought to be respectively protective and disadvantageous with regard to the development of cardiovascular disease (CVD) and diabetes mellitus type II (DM II). Low birth weight has been associated with increased risks for the development of these diseases. In short SGA children, GH therapy has several positive effects regarding cardiovascular risk factors. On the other hand concern has been expressed about the effects of GH therapy on insulin sensitivity.

Methods: We measured adiponectin and resistin levels in 136 short prepubertal children born SGA and their association with cardiovascular risk parameters and growth factors. Also, we compared the levels with normal-statured controls. The effect of GH treatment was evaluated in 50 short SGA children versus baseline and versus an untreated sex- and age-matched SGA control group.

Results: Short SGA children had similar adiponectin and lower resistin levels compared to normal-statured controls. In GH-treated SGA children, neither adiponectin nor resistin levels changed significantly during 2 years of GH treatment. Compared to untreated sex- and age-matched SGA controls, GH-treated SGA children had similar adiponectin and lower resistin levels. Adiponectin correlated inversely with age, but not with any cardiovascular risk parameter or growth factor. Higher IGF-1 levels in GH-treated children were associated with lower resistin levels.

Conclusions: Compared to normal-statured controls, short prepubertal SGA children had similar adiponectin and lower resistin levels. Two years of GH treatment had no effect on their adiponectin and resistin levels.

Introduction

The identification of so-called 'adipocytokines' has brought challenging opportunities in metabolic research. Adipocytokines are fat-cell derived hormones, which are thought to have an influence on metabolism and might predict susceptibility to cardiovascular disease.

Adiponectin was first described in 1995 as a novel adipocytokine, synthesized exclusively by adipocytes and secreted in relatively large amounts into plasma (1). Adiponectin has been inversely associated with insulin resistance and parameters of obesity in both animal models and human studies (2-9). Adiponectin knock-out mice developed insulin resistance, when fed a high fat/high sucrose diet (8), whereas in obese children, weight loss resulted in an increase of serum adiponectin levels (2). Though its mechanism of action is largely unknown, adiponectin is regarded as one of the factors related to the metabolic syndrome (9).

Resistin was recently discovered as an adipocytokine that was downregulated by thiazolidinediones (TZDs, a class of Peroxisome Proliferator Activated Receptor gamma (PPARγ) agonists) (10). TZDs are used in the treatment of type 2 diabetes mellitus and are known to enhance insulin sensitivity in vivo. Increased resistin levels were found in genetically and diet-induced animal models of obesity (10). In animals, inverse associations were described between resistin levels and glucose tolerance and positive correlations with obesity (10). In humans, however, the associations with insulin resistance and obesity are less clear and often cannot be reproduced (7, 11-14). Data on resistin levels in children and adolescents are very scarce (13, 14).

Low birth weight has been associated with increased risk for the development of adult cardiovascular disease and type 2 diabetes mellitus (15). Since growth hormone (GH) therapy has effects on insulin sensitivity, concern has been expressed about the long-term effects of GH therapy in short children born small for gestational age (SGA). GH therapy has various positive effects on cardiovascular risk factors, such as a reduction in blood pressure SD-score, the atherogenic index and a more favourable body composition with particularly more lean body mass (16). On the other hand, lower insulin sensitivity with higher insulin levels has been described during GH therapy (17, 18), which appeared to normalize after discontinuation of GH (19). Contrasting data about adiponectin levels have been reported for SGA infants and children (20-23). Some found positive correlations with birth weight (20, 22, 24-26), but others did not (21, 27-29). Importantly, birth weight was not adjusted for gestational age in all of these studies, whereas adiponectin has been shown to increase impressively with gestational age (30).

To the best of our knowledge resistin levels haven't been described in SGA children. Besides, adiponectin and resistin levels have not been described in relation to cardiovascular risk parameters in short SGA children.

The aims of the present study were (i) to evaluate adiponectin and resistin levels in prepubertal short SGA children before and after 24 months of GH-therapy, (ii) to compare the levels of the GH-treated short SGA children with a sex- and age-matched

untreated short SGA control group and (iii) to describe the relation between adipocytokine levels and parameters of obesity, blood lipids, glucose, insulin, blood pressure and growth factors.

Subjects and methods

Subjects

The study group comprised 136 prepubertal short children born SGA. All children fulfilled the same inclusion criteria: 1) birth length and/or birth weight standard deviation score (SDS) below –2 for gestational age (31), 2) height SDS below –2 according to Dutch standards (32), 3) height velocity SDS below zero to exclude children with spontaneous catch-up growth (32), 4) prepubertal stage, defined as Tanner breast stage 1 for girls and testicular volume less than 4 ml for boys (33), 5) an uncomplicated neonatal period without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis 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, with the exception of Silver-Russell syndrome. In 50 of the 136 children, adiponectin and resistin levels were tested before and after 24 months GH-therapy (while still receiving GH therapy). Baseline cardiovascular data and GH effects on height SDS, IGF-1 SDS and IGFBP-3 SDS of 28 of these 50 children have been published before(34, 35). In order to study the effect of GH, the GH-treated children were also compared with 27 untreated short prepubertal SGA-children. These 27 children were selected from the 86 children at baseline, which did not have a repeat measurement, to match in age and sex with the 50 GH-treated children. Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area (BSA). GH dose was 1 mg/m^2 BSA for all children, except for a subgroup of 19 children, who received 2 mg/m^2 BSA for the first 6 months of treatment followed by 1 mg/m^2 BSA thereafter. Since the 2-year change in adiponectin and resistin was similar for children receiving 1 or 2 mg/m^2 BSA in the first 6 months of GH treatment (data not shown), they were analysed together. The study was approved by the Ethics Committees. Written informed consent was obtained from the parents or custodians of each child.

Anthropometry

Standing height was measured using a Harpenden stadiometer and was expressed as SD-score for sex and chronological age using Dutch references (32). Body mass index (BMI) was calculated according to the formula weight/(height)^2 and was expressed as SD-score for sex and age (36). Biceps, triceps, subscapular and suprailiacal skinfold

thickness were measured using a Holtain skinfold calliper, the sum of four skinfolds was calculated and these were expressed as SDS using references for healthy Dutch children (37). Systolic and diastolic blood pressure (BP) was measured twice on the left arm. The mean of 2 measurements was used for analysis. BP was expressed as SDS adjusted for age and sex (38). Since height is an important determinant of BP in childhood and adolescence, BP was also expressed as SDS adjusted for height and sex(38).

Hormone and biochemical assays

All blood samples were taken after an overnight fast. Serum glucose and total cholesterol levels were measured as described previously (19). Insulin levels were all measured in one laboratory using the same method (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). Triglycerides (TG) were measured on the Chem-1 analyser according to the manufacturer's instructions (Technicon Instruments, Tarrytown, New York) and after 1998 on the Hitachi 917 analyser according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Both methods were comparable (y = x-0.030).

DHEAS levels were measured in 79 consecutive children recruited between 1991 and 2000 using a chemiluminescence-based competitive immunoassay (Immulite1, Diagnostic Products Corporation, Los Angeles, CA.) The interassay coefficient was 8 %. The limit of detection was 0.2 µmol/liter. Values below this limit of detection were considered to be 0.2 µmol/liter.

Insulin-like growth factor I (IGF-I) and insulin-like growth factor binding protein 3 (IGFBP-3) serum levels were measured as described previously (39). IGF-I and IGFBP-3 levels were adjusted for age and sex as SDS (40, 41).

Adiponectin and resistin levels were measured before and after 2 years of GH therapy, after an overnight fast. All samples were assayed in duplicate and the mean of these two measures used for analysis. Serum adiponectin levels were determined by ELISA (R&D Systems Inc., Minneapolis, MN; intra-assay coefficient of variation (CV) < 7 %; inter-assay CV < 7 %). Serum resistin levels were determined by ELISA (R&D Systems Inc., Minneapolis, MN; intra- and inter-assay coefficient of variation < 6% and <7 %, respectively). Adiponectin and resistin reference values were obtained from 40 healthy normal statured prepubertal children (23 boys and 17 girls), aged 5.0-10.1 years, attending the outpatient clinic for a minor surgical procedure. Children suffering from any systemic illness, syndrome or dysmorpic features were excluded. Normal stature was defined as a height SDS above –2 and below +2 according to Dutch standards (32).

Statistical analysis

All data are presented as mean ± SD, except for serum adiponectin and resistin levels, which are presented as median and interquartile range. The non-normally distributed

levels of adiponectin and resistin were logarithmically transformed. Differences between groups were tested using the independent Student's t-test. Differences in time within the same subjects (i.e. before and after 2 years of GH therapy) were calculated using a paired Student's t-test. Correlations were analysed using Spearman's correlation coefficient in the total group at baseline and in 55 children (50 GH-treated and 5 untreated) with a repeat measurement after 2 years. Backward multiple regression analyses with logarithmically transformed adiponectin and resistin as the dependent variable were used to assess multivariate relationships. Factors showing a linear correlation with adiponectin or resistin were entered in the model together with age and sex. Level of significance was determined at $p<0.05$. Statistics were performed using SPSS for Windows version 11.0.1.

Results

Baseline data

Table 1 shows the clinical and laboratory parameters of the total study population. Adiponectin did not significantly differ from the normal statured control group of the same age. Resistin levels were significantly lower in SGA children ($p<0.001$), when compared to normal statured controls. BMI SDS and sum of skinfolds SDS were significantly lower than zero SDS. Also, IGF-1 SDS and IGFBP-3 SDS were significantly lower than zero SDS. Whereas diastolic BP SDS was in the normal range, systolic BP SDS was significantly higher than zero SDS. Lipid, fasting glucose and insulin levels were in the normal range. Adiponectin and resistin levels at baseline were not different for girls and boys (data not shown). Table 2 shows anthropometric and laboratory parameters for the GH-treated SGA children versus the sex- and age-matched untreated SGA controls. Gestational age, birth weight SDS, birth length SDS, height SDS at baseline, age and sex were similar for the GH-treated SGA children and the untreated SGA controls.

Table 1 Baseline clinical and fasting laboratory parameters in 136 short SGA-children

		Reference values
N	136	40
Sex (m/f)	87/49	23/17
Gestational age (wks)	37.0 (3.6) [b]	39.0 (2.3)
Birth weight SDS	-2.3 (1.1) [ab]	0.1 (1.2)
Birth length SDS	-3.2 (1.4) [ab]	-0.3 (1.2)
Age (yr)	8.0 (2.3)	7.5 (1.5)
Height SDS	-3.0 (0.6) [a]	
BMI SDS	-1.3 (1.0) [a]	
Sum of 4 skinfolds SDS	-1.0 (1.2) [a]	
Systolic BP_{height} SDS	0.9 (1.0) [a]	
Diastolic BP_{height} SDS	0.1 (1.0)	
Adiponectin (μg/ml)	10.9 (6.8-15.0)	10.7 (8.3-13.0)
Resistin (ng/ml)	9.7 (7.5-11.9) [b]	14.7 (9.9-19.4)
Cholesterol (mmol/l)	4.2 (0.9)	2.8-5.4
Triglycerides (mmol/l)	0.8 (0.4)	0.3-1.4
Insulin (mU/l)	6.0 (2.8)	< 16
Glucose (mmol/l)	4.6 (0.8)	2.6-6.0
IGF-1 SDS	-1.0 (1.0) [a]	
IGFBP-3 SDS	-1.2 (0.9) [a]	

All values expressed as mean (SD), except for adiponectin and resistin, which are median (interquartile range).
BP_{height} SDS = blood pressure SD-scores adjusted for height and sex
[a] = significantly different from 0 SDS ($p<0.001$)
[b] = significantly different from reference group ($p<0.001$)

Correlations at baseline

At baseline, serum adiponectin levels in 136 short SGA children did not correlate with BMI SDS or sum of four skinfolds SDS. There was no correlation between adiponectin levels and systolic and diastolic blood pressure SDS (either corrected for age and sex or for height and sex). There was a weak, but significant negative correlation between age and adiponectin ($r = -0.229$, $p<0.01$). Adiponectin levels did not correlate with height SDS, fasting insulin, glucose, insulin/glucose-ratio, IGF-1 SDS, IGFBP-3 SDS, DHEAS, total cholesterol, triglyceride levels, birth weight SDS, birth length SDS and birth head circumference SDS. Resistin levels in all SGA children at baseline did not correlate with BMI SDS, sum of skinfolds SDS, systolic or diastolic blood pressure SDS, age, height SDS, fasting insulin and glucose, IGF-1 SDS, IGFBP-3 SDS, DHEAS, total cholesterol,

triglyceride levels, birth weight SDS, birth length SDS and birth head circumference SDS.

Adiponectin and resistin levels during 2 years of GH therapy

In the 50 GH-treated children, neither adiponectin nor resistin levels changed significantly during 2 years of GH treatment (Figure 1 and 2; Table 2). Height and BMI SDS increased significantly compared to baseline in the GH-treated children, whereas the sum of skinfolds SDS decreased significantly. IGF-1 SDS, IGFBP-3 SDS and fasting insulin increased significantly compared to baseline. 27 SGA children were selected from the remaining 86 untreated on the basis of matching sex and age with the treated group. Compared to these untreated sex- and age-matched SGA controls, the GH-treated SGA children had similar levels of adiponectin and lower resistin levels (Figure 2). Compared to the untreated SGA controls they had a significantly higher height SDS, systolic BP SDS, IGF-1 SDS and IGFBP-3 SDS, but lower cholesterol levels and sum of skinfolds SDS.

In the 5 untreated children with a repeat measurement after 2 years, adiponectin levels did also not change, but resistin levels tended to increase (8.6 (0.8) ng/ml at baseline to 13.0 (3.5) ng/ml after 2 years; p=0.08).

Correlations after 2 years

In the 55 children with a second measurement after 2 years (50 GH-treated and 5 untreated), adiponectin correlated weakly with fasting glucose ($r = -0.278$; $p<0.05$) but did not correlate with age, height SDS, IGF-1 SDS and IGFBP-3 SDS. There were also no correlations with BMI SDS, sum of skinfolds SDS, systolic and diastolic blood pressure SDS, fasting insulin, insulin/glucose-ratio, total cholesterol and triglyceride levels.

Resistin levels correlated inversely with IGF-1 SDS ($r = -0.291$; $p<0.05$), but not with age and IGFBP-3 SDS. Resistin levels did not correlate with height SDS, BMI SDS, sum of skinfolds SDS, blood pressure SDS, fasting glucose, insulin, insulin/glucose ratio, total cholesterol and triglyceride levels.

Multiple regression analysis

In multiple regression analyses on the data of 55 children, with a repeat measurement after 2 years (50 GH-treated and 5 untreated) the correlation between serum adiponectin levels and fasting glucose lost significance after adjustment for GH therapy (coded as yes/no), age and sex.

For serum resistin levels, a model containing IGF-1 SDS ($\beta = -0.123$; $p<0.01$) explained 14.7 % of the variance in resistin levels, after adjustment for GH therapy, age and sex.

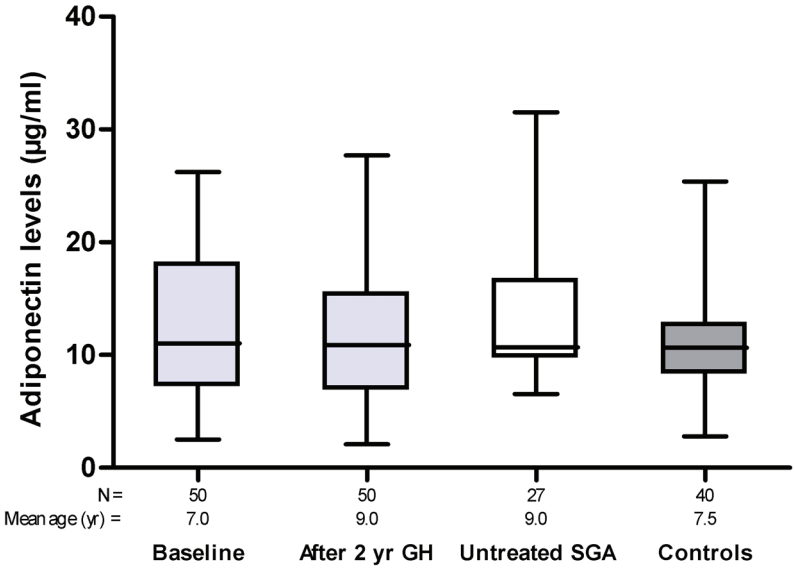

Figure 1. Adiponectin levels of GH-treated children *vs*. baseline, untreated SGA, and normal-statured controls.

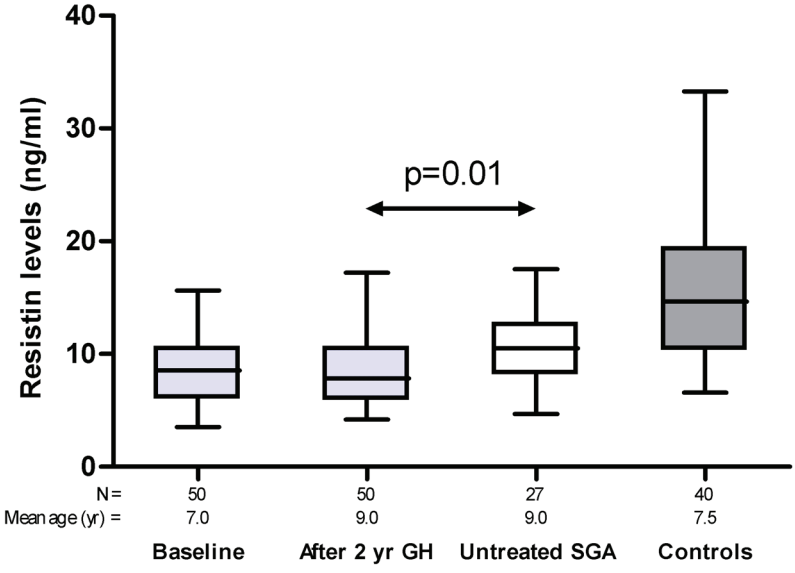

Figure 2. Resistin levels of GH-treated children *vs*. baseline, untreated SGA, and normal-statured controls.

Table 2. GH effects on anthropometric and laboratory parameters

	GH-treated SGA		P-value	Untreated SGA controls	P-value
	Baseline	After 2 yrs	2 yrs vs. baseline	[sex- and age-matched]	GH-treated vs. untreated
N	50	50		27	
Sex (m/f)	31/19	31/19		18/9	NS
Gestational age	37.2 (3.2)	37.2 (3.2)		36.6 (3.8)	NS
Birth weight SDS	-2.1 (1.1)[a]	-2.1 (1.1)[a]		-2.2 (1.1)[a]	NS
Birth length SDS	-2.9 (1.0)[a]	-2.9 (1.0)[a]		-2.7 (1.0)[a]	NS
Age (yr)	7.0 (1.7)	9.0 (1.7)	<0.001	9.0 (1.7)	NS
Height SDS	-3.0 (0.5)[a]	-1.8 (0.6)[a]	<0.001	-2.8 (0.6)[a]	<0.001
BMI SDS	-1.3 (1.0)[a]	-1.0 (1.0)[a]	<0.001	-1.1 (0.9)[a]	NS
Sum of 4 skinfolds SDS	-0.9 (0.9)[a]	-1.3 (0.8)[a]	<0.01	-1.0 (1.0)[a]	<0.05
Systolic BP_{height} SDS	1.2 (0.9)[a]	1.2 (0.9)[a]	NS	0.8 (1.0)[a]	<0.05
Diastolic BP_{height} SDS	0.3 (1.1)	0.3 (0.5)[b]	NS	0.2(1.1)	NS
Adiponectin (μg/ml)	11.0 (5.7-16.4)	10.9 (6.7-15.0)	NS	10.7 (9.8-16.7)	NS
Resistin (ng/ml)	8.6 (6.4-10.7)	7.9 (5.6-10.1)	NS	10.5 (8.2-12.7)	0.01
Cholesterol (mmol/l)	3.7 (0.7)	3.8 (0.6)	NS	4.3 (0.6)	<0.01
Triglycerides (mmol/l)	0.8 (0.4)	0.8 (0.3)	NS	0.8 (0.3)	NS
Insulin (mU/l)	6.4 (2.4)	7.6 (3.7)	<0.05	7.9 (6.5)	NS
Glucose (mmol/l)	4.7 (0.6)	4.7 (0.6)	NS	4.9 (1.0)	NS
IGF-1 SDS	-1.4 (1.0)[a]	0.8 (0.9)[a]	<0.001	-1.0 (0.9)[a]	<0.001
IGFBP3 SDS	-1.4 (0.7)[a]	0.4 (0.5)[a]	<0.001	-0.9 (0.8)[a]	<0.001

Data are expressed as mean (SD) except for adiponectin and resistin, which are median (interquartile range)

BP_{height} SDS = blood pressure SD-scores adjusted for height and sex

[a] = significantly different from 0 SDS (p<0.001) [b] = significantly different from 0 SDS (p<0.05)

Discussion

Our study reports serum adiponectin and resistin levels in 136 short prepubertal SGA children. The effect of 2 years of GH treatment was evaluated by testing fifty children before and after 24 months of GH treatment. In addition, we compared the GH-treated children with an untreated sex- and age-matched SGA control group and a normal statured reference group. At baseline, no strong relations were present between either adiponectin or resistin levels and known cardiovascular parameters, such as BMI and sum of four skinfolds SDS, blood pressure, lipids, fasting glucose and insulin. There was a weak inverse correlation between age and adiponectin levels. Compared to normal statured controls, SGA children had similar levels of adiponectin, but lower resistin levels. Compared to baseline neither serum adiponectin nor resistin levels changed significantly in 50 SGA children during 2 years of GH-treatment. Compared to untreated sex- and age- matched SGA controls, the GH-treated children had comparable levels of adiponectin and lower resistin levels. In multivariable analyses in the children which were also measured after 2 years, higher IGF-1 levels were associated with lower resistin levels after adjustment for age, sex and GH treatment.

At baseline, we did not find correlations between serum adiponectin levels and variables, such as BMI SDS, sum of skinfolds SDS, blood lipids, fasting glucose, insulin and blood pressure. Martin et al. investigated relationships between adiponectin, fasting insulin and lipids (42) and found that the association between adiponectin and fasting insulin could only be demonstrated in obese individuals, whereas the associations between adiponectin and lipid levels were very weak in lean children. Also, Knobler et al. found the association between adiponectin and measures of insulin sensitivity to be not present in non-obese coronary artery disease (CAD) patients when stratifying the total group by their BMI (43). Since short SGA children typically have a very lean appearance with a low muscle mass and fat mass (44), it is very plausible that associations between adiponectin and fasting insulin and blood lipids were not present in our study group.

We found adiponectin levels in short SGA children not to be different from those in normal statured controls. Some authors reported adiponectin levels in SGA children and found lower (20, 22), similar (23) or higher (21) levels when compared to AGA children. However, the studied SGA groups for which lower adiponectin levels were reported, all consisted of a mixture of short SGA children and SGA children with catch-up growth (20, 22). Ibanez et al. reported adiponectin levels in a group of exclusively short SGA children and also found normal levels (23). Lopez-Bermejo et al. found higher levels in lean SGA children and lower levels in overweight SGA children (21). Our SGA children were all short. More recent data indicate that SGA children with spontaneous catch-up growth, particularly those who become overweight, are probably more at risk than those who remain short (45). We did not find reduced adiponectin levels in our population of exclusively short SGA children, which is in agreement with this hypothesis.

Our study showed that 2 years of GH treatment in a group of short SGA children did not affect adiponectin levels. A previous study reported a decline in adiponectin levels during GH treatment of 16 short SGA children (46). However, numbers were smaller, the study period lasted 6 months instead of 2 years and the study population had a different ethnicity. Since the decline of adiponectin levels in the latter study was accompanied by an increase of DHEAS levels, as opposed to our population (47), we wanted to investigate whether DHEAS levels were associated with adiponectin levels. We could, however, not detect such a correlation. The longer duration of GH treatment in our study might also be important, due to possible time-dependent effects of GH on insulin sensitivity and growth factors, resulting in a different net outcome of adiponectin levels.

Data on the effects of GH and IGF-1 on adiponectin levels, as reported in the literature up to date, have been inconclusive. Acromegalic patients have been reported to have increased, similar as well as decreased levels when compared to healthy controls (48-51). In growth hormone deficient (GHD) patients substitution therapy led to either an increase in adiponectin levels or no change (52-56). In accordance with the reports in GHD patients, GH therapy in our SGA patients did not affect adiponectin levels.

The first reports on resistin levels in animals suggested that resistin could be a marker of insulin resistance (8, 10). In accordance with other reports in humans, we did not find positive correlations between resistin and fasting glucose and insulin levels (12, 13, 57, 58). Strikingly, short SGA children had reduced resistin levels. One of the explanations might be that short SGA children typically have a very lean appearance and low fat mass (44, 59). GH therapy, which has been reported to attenuate insulin sensitivity (17, 18), did not change resistin levels in the GH-treated group. The untreated SGA control group, which is expected to be less insulin resistant, showed even higher resistin levels. After 2 years, resistin levels correlated inversely with IGF-1 SDS. In multiple regression analysis with resistin levels as the dependent variable IGF-1 SDS had a negative association with resistin, independently of GH therapy, age and sex. This is in line with a report showing that in vitro IGF-1 downregulates resistin gene expression and protein secretion (60). The IGF-1/IGFBP-3 axis has been repeatedly linked with insulin sensitivity as well as with the pathogenesis of atherosclerosis (61, 62). Underlying associations between IGF-1 and resistin might have confounded the reported, yet inconclusive associations in the literature. On the other hand, we did not find significant changes in resistin levels during 2 years of GH treatment, suggesting that there are also other factors influencing resistin levels. Recent reports on resistin levels indicate that, instead of being a marker for insulin resistance, it might have proinflammatory properties, as it is a member of the cytokine family (63, 64). Our data do not support that resistin is a marker for insulin resistance.

A limitation of our study is the absence of a large untreated control group with a repeat measurement after 2 years. We had only 5 untreated children with a repeat measurement after 2 years. Therefore, we chose to compare the GH-treated children with a sex- and age-matched untreated SGA control group, with the same in- and exclusion criteria as the GH-treated children. We cannot exclude the possibility that this sex- and

age-matched group had already higher resistin levels 2 years prior to the measurement for this study. However, in our small group of untreated children a rise in resistin levels was observed, which is in agreement with the higher resistin levels in the sex- and age-matched control group when compared with the GH-treated group.

In conclusion, our study shows that short prepubertal SGA children have normal adiponectin and lower resistin levels, when compared to normal statured controls of the same age. Because of known associations between low birth weight and the development of cardiovascular disease and diabetes mellitus type II, concern has been expressed about the effects of GH treatment in short SGA children. Our data demonstrate that two years of GH treatment had no effect on their adiponectin and resistin levels. This is reassuring as it shows that GH treatment does not induce disadvantageous changes in these adipocytokines, which have emerged as one of the markers for the development of cardiovascular disease and diabetes mellitus type II.

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CHAPTER 3

Long-term growth hormone treatment is not associated with disadvantageous changes of inflammatory markers and adipocytokines in children born small-for-gestational-age

Ruben H. Willemsen[1], Paul G.H. Mulder[2],
Albert W. van Toorenenbergen[3],
Anita C.S. Hokken-Koelega[1]

[1]*Dept. of Pediatrics, Division of Endocrinology, Erasmus MC Sophia,* [2]*Dept. of Epidemiology and Biostatistics, Erasmus MC,* [3]*Dept. of Clinical Chemistry, University Medical Center Rotterdam, Erasmus MC, Rotterdam, The Netherlands*

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Abstract

Context: Low birth weight is associated with increased risks for adult cardiovascular disease (CVD) and diabetes mellitus type 2 (DM2). Adiponectin and resistin are hormones, considered respectively protective and disadvantageous regarding these risks. No data exist on the effect of long-term GH treatment on these hormones and inflammatory markers in children born small for gestational age (SGA).

Objective: To describe longitudinal changes in inflammatory markers and adipocytokines during and after a long-term dose-response GH study.

Design: Longitudinal dose-response study (group A: 1 mg/m2 body surface area (BSA) (~0.033 mg/kg·day) vs. group B: 2 mg/m2 BSA (~0.067 mg/kg·day)) and comparison with age-related controls

Patients: 103 SGA children

Measurements: We measured adiponectin, resistin, interleukin-6 (IL-6) and C-Reactive Protein (CRP) levels at baseline, after 1 and 7 years of GH treatment and 6 months after discontinuation of GH.

Results: Adiponectin levels decreased over time, but remained comparable with controls. Resistin levels increased and remained lower or comparable with controls. There were no significant differences between the GH dosage groups. After stop of GH, adiponectin decreased in group B and resistin increased in group A. GH therapy did not affect IL-6 and CRP levels at any time point. An increase in BMI SDS over time was associated with a decrease in adiponectin levels. None of the markers were associated with insulin sensitivity.

Conclusion: Long-term GH treatment is not associated with disadvantageous changes in adiponectin, resistin, IL-6 and CRP levels, neither during nor after GH treatment.

Introduction

Approximately 10 % of children born small for gestational age (SGA) fail to show sufficient catch-up growth (1, 2). Growth hormone (GH) therapy accelerates childhood growth and increases adult height (3-5). GH treatment for short children born SGA has recently been licensed and is increasingly applied as a growth promoting therapy in short SGA children.

However, low birth weight has been associated with increased risks for the development of adult cardiovascular disease (CVD) and type 2 diabetes mellitus (DM2)(6). Risk factors for the development of these diseases, such as a reduced insulin sensitivity and an increased systolic blood pressure, are present during childhood in short SGA children (7, 8). Since GH therapy has effects on insulin sensitivity, concern has been expressed about the long-term effects of GH treatment in short SGA-children. On one hand, GH therapy has various positive effects on cardiovascular risk factors, such as a decrease in blood pressure SD-score and the atherogenic index, and a more favourable body composition with particularly more lean body mass (8, 9). On the other hand, GH therapy decreases insulin sensitivity (10, 11).

Adiponectin was first described in 1995 as a novel adipocytokine, synthesized exclusively by adipocytes and secreted in relatively large amounts into serum (12). Decreased levels were described in obesity in both animal models and human studies and also in DM2 (13). Adiponectin knock-out mice developed insulin resistance, when fed a high fat/high sucrose diet (14). Weight loss in obese children resulted in an increase in adiponectin levels (15). Adiponectin has been inversely associated with insulin resistance and parameters of obesity (16-19). Though its mechanism of action is largely unknown, longitudinal studies have shown that low adiponectin is a risk factor for developing DM2 (20).

Resistin was discovered a few years later as an adipokine that was down regulated by thiazolidinediones (TZDs, a class of Peroxisome Proliferator Activated Receptor gamma (PPARγ) agonists) (21). TZDs are used in the treatment of DM2 and enhance insulin sensitivity in vivo. Increased resistin levels were found in genetically and diet-induced animal models of obesity (21). In animals, inverse associations were described between resistin levels and glucose tolerance and positive correlations with obesity (21). In humans, however, the associations with insulin resistance and obesity are less clear cut and often cannot be reproduced (13, 22-25). Data on resistin levels in children and adolescents are very scarce (24, 25).

There is increasing evidence that the innate immune system in combination with nutritional factors is involved in the pathogenesis of DM2 (26). IL-6 is an inflammatory cytokine and a primary determinant of the production of CRP. Both increased CRP and IL-6 levels have been associated with increased incidence of cardiovascular disease, obesity and diabetes mellitus type 2 in adults(27-33). In children, CRP levels were positively associated with BMI, systolic blood pressure and triglycerides and inversely with HDL-C levels (34). To the best of our knowledge, there are no data on CRP levels in short SGA children.

In this paper we present the effect of long-term GH treatment with an average duration of 7 years and the effect of GH discontinuation after reaching adult height (AH) on adiponectin, resistin, IL-6 and CRP levels for 103 short SGA individuals. For all markers we report the effect of two different doses of GH (1 vs. 2 mg/BSA·day) and compare the levels with those in age-related controls.

Subjects and methods

Subjects

The study group comprised 103 short children born SGA. Inclusion criteria have previously been described (3). In short, the children were prepubertal, had a birth length and height standard deviation score (SDS) below –1.88, did not show catch-up growth in height and had no growth failure caused by other disorders. The study was approved by the Medical Ethics Committees. Written informed consent was obtained from the parents or custodians of each child. 90 children participated in a double-blind dose-response trial and were randomly assigned to either 1 or 2 mg GH/m^2 body surface area (BSA)·day (~0.033 vs. 0.067 mg GH/kg·day) (4). 11 of these children were not randomized due to their advanced age at start of GH treatment; they received 2 mg GH/BSA·day. Thirteen children participated in another GH trial and received 1 mg GH/BSA·day (35). Due to ethical considerations the ethics committees did not allow for a control group until AH. Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S, Bagsværd, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated BSA. GH treatment was discontinued when height velocity dropped below 0.5 cm over the last 6 months and/or bone age was ≥ 15 years for girls and ≥ 16.5 years for boys.

40 healthy normal statured prepubertal children, attending the outpatient clinic for elective minor surgery were used as a control group for adiponectin and resistin levels at baseline and after 1 year of GH treatment. Children suffering from any systemic illness, syndrome or dysmorphic features were excluded. Since adiponectin levels change during puberty (36, 37) we compared adiponectin, resistin and IL-6 levels at near AH and 6 months after discontinuation of GH with a post-pubertal control group of 34 healthy normal statured adolescents. The collection and use of blood samples from the control groups was approved by the Medical Ethics Committee and written informed consent was obtained from all adolescents and the parents or custodians of each child. Normal stature was defined as a height SDS between –2 and +2 according to Dutch standards(38).

Anthropometry

Before start of treatment and subsequently every three months, height, weight and Tanner stage were recorded, as described previously (3). Height, body mass index (BMI)

and sum of four skinfolds were expressed as SD-score using references for healthy Dutch children, as described previously (3,8).

Parameters of insulin sensitivity

In a subgroup of 79 children a 2 hr oral glucose tolerance test (OGTT) was performed, as previously described (10). For these children, the whole body insulin sensitivity index (WBISI) was calculated (39) as an estimate for insulin sensitivity according to the formula (10,000/square root of [fasting glucose x fasting insulin] x [mean glucose x mean insulin during OGTT]). The WBISI has been shown to be highly correlated ($r=0.73$; $p<0.0001$) with the rate of whole-body glucose disposal during the euglycemic hyperinsulinemic clamp (39). For the total study group, the fasting glucose to insulin (G/I) ratio was calculated, which is another estimate of insulin sensitivity.

Hormone and biochemical assays

At baseline, after 1 year of GH treatment, at near AH (defined as 1.5 years or less before discontinuation of GH) and 6 months after discontinuation of GH treatment, a fasting blood sample was taken. After centrifugation all samples were frozen (-80 ˚C) until assayed. For the analyses of IL-6 and CRP levels, samples of children with reported infection, trauma or surgery in the month before blood sampling were excluded. Because values of CRP above 10 mg/l are suggestive of macro inflammation, these were also excluded from analysis. For the analyses of correlations between the markers, all available samples were used.

Serum glucose and insulin levels were measured as described previously (10). Serum adiponectin was determined by ELISA (R&D Systems Inc., Minneapolis, MN; intra-assay coefficient of variation (CV) < 7 %; inter-assay CV < 7 %). Serum resistin was determined by ELISA (R&D Systems Inc., Minneapolis, MN; intra- and inter-assay coefficient of variation < 6% and <7 %, respectively). Serum IL-6 levels were measured with a high sensitivity ELISA kit (R&D Systems Inc., Minneapolis, MN; intra- and inter-assay coefficient of variation < 12.3 % and 7.2 %, respectively). Serum CRP levels were determined with a sensitive method (high-sensitive CRP) on the Immage analyser (Beckman Coulter, Mijdrecht, the Netherlands) according to the manufacturer's instructions. The lower limit of detection was 0.2 mg/liter. Values below this limit were considered as 0.2 mg/liter. Since CRP values were very skewed, even after logarithmic transformation, the values were recoded as either <0.999 mg/l or ≥ 1 mg/l. Thus, the percentages of individuals with a CRP ≥ 1 mg/l in the 2 dosage groups at the different study times were calculated.

Statistical analysis

To test the time effect (0, 1 year of GH treatment, near AH and 6 months after discontinuation of GH treatment) and dose effect of 1 mg versus 2 mg GH/BSA·day on

adiponectin, resistin and IL-6, mixed model analyses of variance were performed. In these models, sex and pubertal status (coded as either prepubertal or pubertal) were entered as covariates. Besides, we tested whether there were interactions between sex and time as well as dose and time. Clinical data are presented as mean (SD), model estimates as mean (95% CI). Non-normally distributed levels of adiponectin, resistin, IL-6, WBISI and the fasting G/I ratio were logarithmically transformed before analysis. Differences between SGA individuals and controls were tested by one-way analysis of variance (ANOVA) with Dunnett's post-hoc test (several experimental groups versus one control group). Differences between groups at baseline were tested by one-way analysis of variance (ANOVA) with Bonferroni's post-hoc test. To test time and dose effects on the percentage of individuals having a CRP > 1 mg/ml, a logistic regression was performed using the GEE (Generalized Estimating Equations) method in order to account for repeated measurements. Correlations were analysed using Spearman's rank correlation coefficient. The significance level was set at 0.05. Statistics were performed using SPSS for Windows (version 11.0.1, Chicago, Illinois, USA) and SAS (SAS Institute Inc., Cary, NC, USA, release 8.2).

Results

Clinical characteristics at baseline

Gestational age, birth weight SDS, birth length SDS, height SDS, BMI SDS, sum of skinfolds SDS, age and sex were not significantly different for both GH dosage groups (Table 1). Sex distribution and pubertal status were comparable between the 2 GH-dosage groups at the various time points (data not shown). At baseline and after 1 year of GH treatment the SGA children were compared with a group of age-related children. For comparison at near AH and 6 months after discontinuation of GH, a group of postpubertal controls was used. As expected, the controls had a significantly higher birth weight SDS, birth length SDS and gestational age.

Table 1 Clinical characteristics at baseline

	Group A	Group B	Controls (children)	Controls (adolescents)
N	54	49	40	34
Sex (m/f)	35/19	28/21	23/17	17/17
Gestational age (wks)	37.3 (3.1) [a]	36.4 (4.1) [a]	39.0 (2.3)	39.3 (1.7)
Birth weight SDS	-2.6 (1.1) [b]	-2.7 (1.0) [b]	0.1 (1.2)	0.0 (1.3)
Birth length SDS	-3.5 (1.5) [b]	-3.8 (1.6) [b]	-0.3 (1.2)	0.1 (0.9)
Age (yr)	8.0 (2.4)	8.2 (2.8)	7.5 (1.5)	20.9 (1.5)
Height SDS	-3.0 (0.7)	-3.0 (0.8)		
BMI SDS	-1.2 (1.2)	-1.3 (1.0)		
Sum of skinfolds SDS	-0.7 (1.7)	-0.8 (1.3)		

Group A: 1 mg GH/BSA·day (~0.033 mg GH/kg·day); Group B: 2 mg GH/BSA·day (~0.067 mg GH/kg·day); SDS= standard deviation score
Data are presented as mean (SD)
[a] $p<0.05$ vs. controls; [b] $p<0.001$ vs. controls

Adiponectin

Adiponectin decreased in both dosage groups over time, but the decrease in group B was less marked than in group A (Table 2). The adiponectin level in group A decreased already significantly after 1 year of GH treatment, whereas in group B, adiponectin became only significantly lower than baseline at near AH. After discontinuation of GH treatment, adiponectin levels did not significantly change in group A, but decreased in group B ($p<0.01$). Compared with age-related controls, adiponectin levels remained normal during the entire course of GH treatment. Adiponectin levels did not significantly differ between the two GH-dosage groups at all time points, even after correction for sex. After adjustment for GH dosage, girls showed higher levels of adiponectin than boys, but this gender difference was only significant at near AH (baseline: $p=0.433$; 1 year GHRx: $p=0.190$; near AH: $p<0.01$; after discontinuation: $p=0.074$). In the mixed model, we assessed the influence of puberty on serum adiponectin levels. Puberty was associated with an 18.2 % decrease in adiponectin levels, though this did not reach statistical significance ($p=0.07$).

Resistin

After 1 year of GH treatment, resistin levels remained comparable to baseline (Table 2). At near AH after approximately 7 years of GH treatment, only group A had higher levels of resistin than at baseline ($p<0.01$). After discontinuation of GH, both dosage groups showed higher resistin levels than at baseline ($p<0.01$ for group A; $p<0.05$ for group B), but only group A had higher levels after discontinuation of GH treatment than at near AH ($p<0.05$). Resistin levels before, during and after stop of GH treatment were either lower or comparable to those of age-related controls. After adjustment for GH dosage, resistin levels were not significantly different for girls and boys at all time points. Resistin levels did not significantly differ between the two dosage groups at all time points, even after correction for sex. Puberty had no effect on resistin levels.

Interleukin-6

IL-6 levels remained similar before, during and after stop of GH treatment and were not significantly different between both GH-dosage groups (Table2). After adjustment for GH dosage, IL-6 levels were not significantly different for girls and boys at all time points. Compared with age-related controls, SGA individuals had similar IL-6 levels. This remained so after correction for sex. Puberty did not affect IL-6 levels.

High sensitivity CRP

The percentage of SGA individuals with CRP values above 1 mg/ml was comparable before, during and after stop of GH treatment and was not significantly different between both GH dosage groups (Table 2).

Table 2. Adipocytokines and parameters of insulin sensitivity before, during and after stop of GH treatment in SGA individuals

	Group	Pre-GH		After 1 yr of GH		Near-AH		Post-GH	
		Mean	95 % CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Age (yr)	A	7.7	(7.0-8.5)	8.7	(8.0-9.5)	15.5	(15.1-16.0)	16.7	(16.3-17.1)
	B	8.1	(7.4-8.9)	9.1	(8.4-9.9)	15.5	(15.0-15.9)	16.6	(16.1-17.0)
Adiponectin (μg/ml)	A	11.2	(9.8-12.9)	9.2[b]	(7.8-10.8)	7.1[a]	(6.0-8.3)	6.5[a]	(5.4-7.8)
	B	12.2	(10.6-14.0)	10.9	(9.3-12.9)	8.6[a]	(7.2-10.2)	6.6[ad]	(5.4-7.9)
	Controls	11.0	(9.7-12.3)	11.0	(9.7-12.3)	8.1	(6.9-9.4)	8.1	(6.9-9.4)
Resistin (ng/ml)	A	10.7[g]	(9.7-11.8)	11.2[i]	(10.1-12.4)	13.6[bi]	(12.1-15.4)	17.0[ae]	(14.6-19.7)
	B	11.2[h]	(10.1-12.4)	10.2[g]	(9.2-11.2)	11.7[h]	(10.2-13.4)	13.8[ci]	(11.8-16.0)
	Controls	15.5	(13.5-17.5)	15.5	(13.5-17.5)	17.4	(15.6-19.2)	17.4	(15.6-19.2)
IL-6 (pg/ml)	A	0.9	(0.7-1.2)	1.0	(0.8-1.2)	1.0	(0.7-1.3)	1.2	(0.9-1.6)
	B	1.0	(0.8-1.3)	1.0	(0.8-1.2)	1.0	(0.7-1.3)	1.0	(0.7-1.3)
	Controls					1.1	(0.9-1.3)	1.1	(0.9-1.3)
% hs-CRP > 1 mg/ml	A	18.6	(6.9-41.3)	15.2	(6.8-30.6)	23.6	(10.5-44.9)	20.3	(7.7-43.5)
	B	23.3	(10.7-43.4)	15.0	(7.3-28.5)	18.2	(5.3-4.7)	9.7	(2.3-33.0)
Fasting G/I ratio	A	0.89	(0.74-1.07)	0.78	(0.63-0.96)	0.41[a]	(0.32-0.52)	0.59[be]	(0.47-0.74)
	B	0.95	(0.77-1.17)	0.57[b#]	(0.46-0.70)	0.46[a]	(0.34-0.63)	0.56[b]	(0.43-0.73)
WBISI	A	11.9	(9.7-14.7)	7.1[a]	(6.0-8.5)	5.4[a]	(3.8-7.6)	8.2[cf]	(6.1-10.9)
	B	13.6	(10.9-16.9)	5.9[a]	(4.9-7.1)	5.7[a]	(4.1-8.0)	7.1[a]	(5.5-9.2)

Group A: 1 mg GH/BSA·day (~0.033 mg GH/kg·day); n=54; Group B: 2 mg GH/BSA·day (~0.067 mg GH/kg·day); n=49; AH= adult height; post-GH= 6 months after stop of GH; G/I = glucose/insulin; WBISI = whole body insulin sensitivity index

Data are presented as mean and 95% confidence intervals

[a] p<0.001 vs. baseline
[b] p<0.01 vs. baseline
[c] p<0.05 vs. baseline
[d] p<0.001 vs. near AH
[e] p<0.01 vs. near AH
[f] p<0.05 vs. near AH
[g] p<0.001 vs. controls
[h] p<0.01 vs. controls
[i] p<0.05 vs. controls
[#] p<0.05 vs. group A

Parameters of insulin sensitivity

The fasting glucose/insulin (G/I) ratio decreased significantly after 1 year of GH treatment in group B and in both groups at near AH (Table 2). After adjustment for sex, there were no significant differences between the two dosage groups, except after 1 year of GH treatment (group B lower than group A; $p<0.05$). In both groups, the fasting G/I ratio increased after discontinuation of GH treatment, though this did not reach statistical significance in group B. After adjustment for GH dose, girls had a lower fasting G/I ratio than boys, both at near AH ($p<0.02$) and at 6 months after GH discontinuation ($p<0.05$).

The whole body insulin sensitivity index (WBISI) decreased significantly in both groups during GH treatment (Table 2). After discontinuation of GH, the WBISI increased significantly in group A only. After adjustment for sex, there were no significant differences between the two dosage groups. After adjustment for GH dose, girls had a lower WBISI at near AH ($p<0.05$).

The G/I ratio correlated significantly with WBISI at all time points ($r=0.59$-0.91; $p<0.001$). Also, the changes in G/I ratio correlated significantly with the changes in WBISI at all time points ($r=0.47$-0.79; p-values <0.001-0.04).

Correlations between the adipocytokines

At all time points, IL-6 levels correlated with CRP levels ($r=0.342$; $p<0.05$; $r=0.467$; $p<0.001$; $r=0.470$; $p<0.01$; $r=0.499$; $p<0.001$, respectively). Adiponectin did not correlate with resistin, IL-6 and CRP levels. After 1 year of GH treatment and 6 months after discontinuation, resistin correlated with IL-6 and CRP levels ($r=0.225$; $p<0.05$ and $r=0.313$; $p<0.01$ after 1 year of GH; $r=0.431$; $p<0.01$ and $r=0.347$; $p<0.05$ 6 months after discontinuation of GH).

Correlations between changes in adipokines and inflammatory markers and changes in BMI SDS, height SDS, sum of skinfolds SDS and parameters of insulin sensitivity

The changes in adiponectin levels from baseline to 1 yr of GH and from baseline to near AH were inversely correlated with the corresponding changes in BMI SDS ($r=-0.399$; $p<0.01$ and $r=-0.478$; $p<0.05$, respectively). The change in adiponectin levels from baseline to post-GH was inversely associated with the change in sum of skinfolds SDS, but this association failed to reach statistical significance ($r=-0.350$; $p=0.08$). Changes in resistin and IL-6 levels did not correlate with changes in BMI SDS and sum of skinfolds SDS.

Changes in adiponectin, resistin and IL-6 levels from baseline to 1 yr GH, from baseline to near AH and from baseline to post-GH did not correlate with the corresponding changes in the WBISI, fasting G/I ratio and height SDS.

Discussion

In this paper we report the long-term effects of 7 years GH treatment in 2 different doses and the effect of discontinuation of GH in short SGA individuals on adiponectin, resistin, IL-6 and CRP levels. Also, we compared the levels with age-related controls. We observed a decrease in adiponectin levels and an increase in resistin levels over time, which was less marked in group B (2 mg GH/BSA·day) compared to group A (1 mg GH/BSA·day). GH therapy did not affect IL-6 and CRP levels at any time point. At all time points, adiponectin and IL-6 levels were comparable with controls, whereas resistin levels were either lower or comparable. Our results indicate that long-term GH therapy of SGA individuals is not associated with disadvantageous changes in adipocytokine levels over time.

During the course of GH treatment, adiponectin levels decreased, but were not different from age-related controls at any time point. Previous studies reported decreasing adiponectin levels with increasing age and pubertal status (36, 37). In our study population, we found puberty to be associated with a decrease in adiponectin levels of 18.2 %, though this did just not reach statistical significance. After adjustment for dosage, we also found girls to have higher adiponectin levels than boys, which became significant during GH therapy at near AH. Besides, adiponectin levels did not increase during the 6 months after discontinuation of GH in both GH-dosage groups. So, the observed decrease in adiponectin levels during the course of GH treatment as well as the emerging gender difference are most likely the effect of sex steroids in puberty. Ibañez et al. reported 6 months of GH treatment in short SGA children to be associated with a decrease of serum adiponectin levels (40). Our long-term longitudinal study shows, however, that 7 years of GH treatment of 103 SGA individuals is not associated with disadvantageous changes in adiponectin levels.

In our study, changes in adiponectin over time did not correlate with GH-induced changes in parameters of insulin sensitivity. Previously, no associations were found between adiponectin and fasting insulin or measures of insulin sensitivity in lean individuals as opposed to obese individuals (41, 42). Since the SGA individuals investigated in our study all were relatively lean (8), the lack of any association between adiponectin and parameters of insulin sensitivity is in line with these previous observations.

Notably, at most study times we found significantly lower resistin levels in SGA individuals than in controls. The lower resistin levels in SGA individuals might be explained by their typical body composition, with a reduced fat mass SDS (9). Resistin correlated with IL-6 and CRP levels, though this was not consistent at all time points. There were no correlations between changes in resistin levels and changes in any of the parameters of insulin sensitivity. The role of resistin as a marker for insulin resistance in humans has been questioned (24, 43). Recent reports suggest that, instead of being a marker for insulin resistance, resistin might have proinflammatory properties as it is a member of the cytokine family (44, 45). Jung et al. showed that resistin is secreted from macrophages in atheromas and may contribute to atherogenesis because of its effects on vascular endothelial cells and smooth muscle cells (46). So, resistin might

rather be considered as a parameter for inflammation and atherosclerosis than for insulin resistance. The correlations between resistin and IL-6 and CRP levels and the lack of any correlation between resistin and parameters of insulin sensitivity in our study support this.

Of note, the decrease in adiponectin levels and the increase in resistin levels over time in group B (2 mg/BSA·day) were less pronounced than in group A (1 mg/BSA·day). From this, one may argue that a GH dose of 2 mg/BSA·day might be favourable with regard to adipocytokine levels. However, the observed differences between both dosage groups were quite small and not significantly different at all time points. Besides, it has been shown that the 2 mg dose results in high GH and IGF-I levels in the majority of short SGA children and the long-term risks of GH and IGF-I levels in the higher quartile in SGA children are yet unknown (47).

Growing evidence indicates that the GH-IGF-I system and the immune system are correlated and several bidirectional interactions have been described (48). The receptors and signalling cascades activated by GH and IL-6 appear to be very similar (49). Hypopituitary adults have higher CRP and IL-6 levels (50). Conflicting results exist regarding the effect of GH therapy in GHD patients. Some authors found CRP levels to decrease during GH treatment in GHD adults (51), whereas others reported no change (52). In GHD children, increased IL-6 and TNF- levels were measured 6 hours after administration of GH, but not after 24 hours or 3 months (53). Another study reported increased levels of several cytokines (measured 12 hours after injection) in normal short statured children after 4 days of GH in a GH generation test protocol (54). Data on inflammatory markers in SGA children are very scarce. Ibañez et al. reported a significant increase of IL-6 levels in short SGA children after 6 months of GH treatment (40), but no information was given on recent infections, surgery or trauma. Our study shows that long-term GH treatment in 2 different doses does not affect IL-6 and CRP levels in SGA individuals who were free of recent infections, surgery and trauma.

Previously, we reported the results of our pilot study on short term effects of GH treatment on adiponectin and resistin levels in comparison with untreated SGA controls (55). In this other, smaller group of SGA children, the effect of 2 years of GH treatment in a single dose (1 mg GH/BSA·day) was studied. Our present paper is the only one describing the longitudinal effects of long-term continuous GH treatment during 7 years with 2 different GH doses and the effect of GH discontinuation on inflammatory markers and adipocytokines in a large cohort of SGA individuals.

We found that an increase in BMI SDS in SGA individuals during childhood and adolescence was associated with a decrease in serum adiponectin levels. The same trend was observed for the sum of skinfolds SDS, but this association failed to reach statistical significance. Our data suggest that gaining more fat mass during childhood and puberty is associated with a greater decrease in serum adiponectin levels. Recent data indicate that low birthweight children that exhibit rapid weight gain might be more at risk than those who remain lean (56). The GH-treated children in our SGA cohort remained relatively lean during treatment and puberty with an average sum of skinfolds of –1 SDS which remained significantly lower than 0 SDS during 6 years of GH treat-

ment (8). This could be one of the reasons why adiponectin levels remained comparable with the controls.

In conclusion, our data show that long-term GH treatment in SGA individuals does not induce unfavourable changes in serum adiponectin, resistin and IL-6 levels, because all levels remained normal compared with age-related controls. Adiponectin levels decreased over time, but this is most likely the effect of sex steroids in puberty. Resistin levels increased, but remained lower or comparable than the controls at all time points. Serum IL-6 en CRP levels remained the same during and after discontinuation of GH treatment. Thus, our results are reassuring, because they indicate that long-term GH treatment does not disadvantageously change these adipocytokines, which have been suggested as determinants of the development of diabetes mellitus type 2 and cardiovascular disease in later life.

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CHAPTER 4

Plasma matrixmetalloproteinase-9 levels and blood pressure in short children born small-for-gestational-age and effects of growth hormone treatment

Ruben H. Willemsen[1], Marije van Dijk[1],
Sandra W.K. de Kort[1], Albert W. van Toorenenbergen[2],
Anita C.S. Hokken-Koelega[1]

[1]*Dept. of Pediatrics, Division of Endocrinology, Erasmus MC Sophia,* [2]*Dept. of Epidemiology and Biostatistics, Erasmus MC,* [3]*Dept. of Clinical Chemistry, University Medical Center Rotterdam, Erasmus MC, Rotterdam, The Netherlands*

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Abstract

Context: Short SGA children have an increased systolic blood pressure, which decreases during long-term GH treatment. The underlying mechanism is still unknown. Matrix metalloproteinases (MMPs) are zinc-dependent endoproteinases, which are involved in the remodelling of the extracellular matrix and are thought to play a role in atherosclerosis. High MMP-9 levels are found in hypertensive patients and predict cardiovascular mortality.

Objectives: To investigate whether GH treatment affects plasma MMP-9 levels in short SGA children and whether these are related to blood pressure (BP)

Design: Case-control study

Intervention: GH treatment versus no treatment during 36 months

Patients: 38 short SGA children receiving GH treatment versus 17 sex- and age-matched untreated short SGA controls

Outcome measure: Plasma MMP-9 levels and BP were measured at baseline, and after 6, 12 and 36 months of study

Results: MMP-9 decreased significantly during 3 years of GH treatment, whereas it remained similar in untreated SGA controls. After 3 years of GH treatment, MMP-9 levels were significantly lower in the GH group than in untreated SGA controls. Systolic BP SDS significantly decreased in the GH group, but remained unaltered in untreated SGA controls. MMP-9 levels did not correlate with systolic or diastolic BP.

Conclusions: Plasma MMP-9 levels and systolic BP SDS decreased to almost 50% of baseline values in the GH group, whereas these remained unchanged in untreated SGA controls. Our data indicate that GH has a positive effect on both MMP-9 levels and systolic BP SDS.

Introduction

Matrix metalloproteinases (MMPs) are zinc-dependent endoproteinases, which participate in remodelling the extracellular matrix (ECM) (1, 2). Since alterations in the elastic properties of the vasculature in atherosclerosis result from constant remodelling of the arterial wall, MMPs are thought to play a role in the development of atherosclerosis and cardiovascular disease (2, 3). One of the interesting MMPs with respect to atherosclerosis is MMP-9, which can degrade elastin. Elastin is the main elastic component of the arterial wall. In MMP-9 knock-out mice, MMP-9 deficiency resulted in less intima hyperplasia and less atherosclerosis (4). MMP-9 deficient smooth muscle cells had decreased migratory activity as well as a decreased capacity to contract collagen (4). Increased MMP-9 levels were found in hypertensive subjects (5, 6) and high plasma MMP-9 levels predict cardiovascular mortality in patients with coronary artery disease (7).

Smaller size at birth has been associated with higher risks for the development of adult cardiovascular disease (8). Various studies reported that short children born small-for-gestational-age (SGA) had a high systolic blood pressure (BP) (9, 10), which declined during growth hormone (GH) therapy (9). The mechanism by which this reduction was achieved, is still unclear. Sixty percent of short SGA children also have abnormalities in the GH-insulin-like-growth factor-I (IGF-I)-axis with low normal plasma levels of insulin-like-growth-factor-I (IGF-I) and IGF-binding-protein-3 (IGFBP-3) (11). Low IGF-I levels in adulthood have been associated with increased risk of ischaemic heart disease (12). In GH-deficient adults, GH replacement led to a progressive decrease in MMP-9 and vascular endothelial growth factor (VEGF) levels and an initial decrease of MMP-2 reaching a plateau thereafter, in parallel to an increase in IGF-I levels (13).

In short SGA children, GH treatment increases IGF-I levels to physiological or supraphysiological levels, depending on the GH dose (14). However, little is known about the vascular growth factors in short SGA children and about the effect of GH therapy on these parameters. To the best of our knowledge, no data have been reported on MMPs in short SGA children. We hypothesized that GH treatment in short SGA children affects MMP-9 levels and that these are related to blood pressure.

To investigate if GH treatment affects MMP-9 levels, we measured plasma MMP-9 levels in short SGA children during 36 months of GH treatment and compared them with those in an untreated sex- and age-matched short SGA control group. Furthermore, we investigated whether MMP-9 levels were associated with blood pressure.

Subjects and methods

Subjects

The study group consisted of 55 prepubertal short children born SGA (38 GH-treated and 17 sex-and age-matched untreated). All children fulfilled the same inclusion criteria: 1) birth length and/or birth weight standard deviation score (SDS) below –2 for gestational age (15), 2) height SDS below –2 according to Dutch standards (16), 3) height velocity SDS below zero to exclude children with spontaneous catch-up growth (16), 4) prepubertal stage, defined as Tanner breast stage 1 for girls and testicular volume less than 4 ml for boys (17), 5) an uncomplicated neonatal period without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia. None of the children were growth hormone deficient. 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, with the exception of Silver-Russell syndrome. IGF-I levels (14, 18) and part of the blood pressure data (10, 19) have previously been published. 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

38 children were randomised to receive either 1 or 2 mg GH/m^2 body surface area (BSA)/day during the first 6 months. After the initial 6 months, all children continued with 1 mg/BSA/day. Biosynthetic GH (Norditropin, Novo Nordisk A/S, Bagsværd, Denmark) was administered subcutaneously once daily at bedtime. Every three months, the GH dose was adjusted to the calculated BSA.

Anthropometry

Standing height and weight were measured and body mass index (BMI) was calculated as previously described (18). Height and BMI were expressed as SDS adjusted for sex and age according to Dutch reference data for children (16, 20). Systolic and diastolic blood pressure (BP) was measured twice in the left arm. The mean of 2 measurements was used for analysis. Because height is an important determinant of BP in childhood, BP was expressed as SDS adjusted for height and sex (21). None of the children received anti-hypertensive therapy.

Assays

Insulin-like growth factor I (IGF-I) serum levels were measured in one laboratory as previously described (14). The intra-assay coefficient of variation (CV) was 4%

and the inter-assay CV was 6%. IGF-I levels were adjusted for age and sex as SDS (22, 23). Heparinized plasma MMP-9 levels were determined by ELISA (total MMP-9 Immunoassay, R&D Systems Inc., Minneapolis, MN); intra-assay coefficient of variation (CV) < 3 %; inter-assay CV < 8 %.

Statistics

All data are presented as mean ± SD, except for MMP-9 levels, which are presented as median and interquartile range. The non-normally distributed levels of MMP-9 were logarithmically transformed prior to analysis. Because the change in MMP-9 levels and blood pressure during the first 6 months was not significantly different between the 2 GH dosage groups, the groups were combined to one GH-group. 4 children of the GH group and 3 children of the untreated control group had started puberty at the 36 months visit. Exclusion of these subjects for the statistical analyses gave exactly the same results and therefore it was not considered to affect our results. SD-scores were compared with zero using Student's one sample t-test. Differences between groups were tested using the independent Student's t-test. Differences in time within the same subjects (i.e. before and during GH therapy) were calculated using a paired Student's t-test. Multiple linear regression was used to adjust differences in outcome between the groups for possible baseline differences. Correlations were analysed using Spearman's correlation coefficient. With respect to anthropometry at birth and in childhood and blood pressure, a value of 0 SDS corresponds with the mean value of the parameter in a child with the same age and sex (anthropometry) or with the same height and sex (blood pressure). Therefore, SD-scores were compared with 0. The significance level was set at 0.05. Statistics were performed using SPSS for Windows (version 12.0.1, SPSS Inc., Chicago, IL, USA).

Results

Clinical data

Age, sex, gestational age, anthropometric parameters at birth and at baseline, and baseline diastolic BP SDS were comparable for both groups (Table 1). Baseline systolic BP SDS was significantly higher than zero SDS in both groups, whereas birth weight SDS, birth length SDS, current height SDS and BMI SDS were all significantly lower than zero SDS.

Table 1. Characteristics at birth and at baseline

	GH-treated SGA	Untreated SGA controls
N	38	17
Sex (m/f)	1 8/15	1 3/7
Gestational age (wks)	36.5 (3.8)	35.1 (4.0)
Birth weight SDS	-2.2 (1.2)*	-2.8 (1.1)*
Birth length SDS	-3.2 (1.0)*	-3.1 (1.1)*
Age (yr)	6.4 (1.1)	6.2 (0.9)
Height SDS	-3.1 (0.5)*	-3.2 (0.5)*
BMI SDS	-1.2 (1.1)§	-1.2 (1.1)*
Systolic BP SDS	1.2 (0.9)*,a	0.6(1.0)#
Diastolic BP SDS	0.3 (1.2)	0.4 (0.8)#

*= p<0.001; §= p<0.01; #= p<0.05 versus zero SDS
a= p<0.05 vs. SGA controls
SDS = standard deviation score; BP = blood pressure

Changes during GH treatment

Plasma MMP-9 levels decreased significantly and progressively during 3 years of GH treatment, whereas they remained at the same level in the untreated SGA controls (Figure 1; Table 2). After 3 years of GH treatment, plasma MMP-9 levels were significantly lower in the GH group than in the untreated SGA controls (p=0.04), also after correction for baseline IGF-I SDS and systolic BP SDS (p=0.018). The change in plasma MMP-9 levels during 3 years of follow-up was also significantly greater in the GH-treated children than in the untreated SGA controls (p=0.006), and this remained so after correction

for baseline IGF-I SDS and systolic BP SDS (p=0.035). At all time points, MMP-9 levels were not different between boys and girls.

Systolic BP SDS tended to increase during the first 6 months of GH treatment. However, after 3 years of follow-up, systolic BP SDS had significantly decreased in the GH group, but had remained similar in the untreated SGA controls. Diastolic BP SDS decreased in both the GH group and the untreated SGA controls.

IGF-I SDS significantly increased in the GH group, whereas it remained unchanged in the untreated SGA controls.

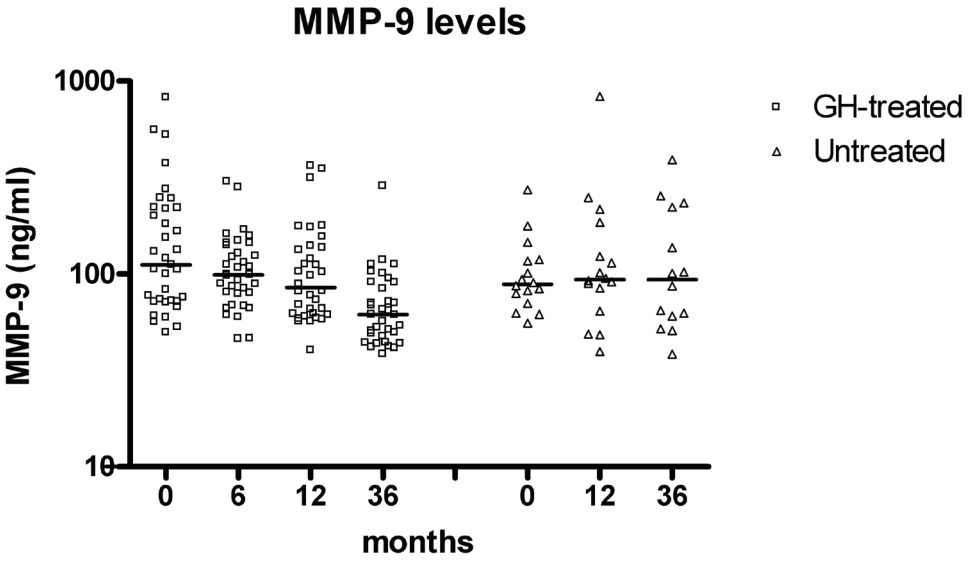

Figure 1. MMP-9 levels in GH-treated SGA children *vs.* untreated sex- and age-matched SGA controls. Horizontal lines represent medians.

Table 2. Changes in blood pressure, MMP-9 and IGF-I SDS during GH treatment

		GH Group	P-value vs. baseline	Untreated SGA controls	P-value vs. baseline
Age (yr)	Baseline	6.4 (1.1)		6.2 (0.9)	
	6 mo	6.9 (1.1)		6.5 (1.2)	
	12 mo	7.5 (1.1)		7.2 (1.0)	
	36 mo	9.4 (1.1)		9.2 (1.1)	
MMP-9 (ug/l)	Baseline	111 (72-220)		88 (73-118)	
	6 mo	99 (79-141)	**34**	-	
	12 mo	85 (62-137)	53	93 (69-169)	52
	36 mo	61 (47-90) [§,1]	**<0.001**	94 (60-222)	88
SBP SDS[a]	Baseline	1.2 (0.9) [§]		0.6 (1.1)	
	6 mo	1.6 (1.1) [#]	**30**	0.7 (0.7)	79
	12 mo	1.1 (0.9)	57	0.8 (1.0)	68
	36 mo	0.5 (1.0)	**3**	0.5 (1.0)	56
DBP SDS[a]	Baseline	0.3 (1.2)		0.4 (0.9)	
	6 mo	0.5 (0.9)	59	0.2 (0.9)	14
	12 mo	0.0 (0.9)	32	0.2 (1.1)	66
	36 mo	0.0 (0.8)	**23**	-0.4 (0.8)	5
IGF-I SDS	Baseline	-1.6 (0.9) [&]		-0.6 (0.9)	
	6 mo	0.8 (1.3)	**<0.001**	-	
	12 mo	0.7 (1.0) [&,2]	**<0.001**	-0.7 (1.4)	795
	36 mo	0.9 (1.1) [&,2]	**<0.001**	-1.2 (1.3)	14

GH Group: n=38; Untreated SGA: n=17
SBP= systolic blood pressure; DBP= diastolic blood pressure; SDS= standard deviation score
[a] = corrected for height and sex
[&]= p<0.001; [#]=p<0.01; [§]= p<0.05 vs. untreated SGAcontrols
[1]= p<0.01 change in MMP-9 levels in GH-group vs. that in untreated SGA controls
[2]= p<0.001 change in IGF-I SDS in GH-group vs. that in untreated SGA controls
Data are presented as mean (SD), except for MMP-9, which is median (interquartile range)

Correlations

MMP-9 levels were not associated with IGF-I SDS and systolic or diastolic blood pressure SDS at the various time points. Changes in MMP-9 levels over time were also not correlated with changes in IGF-I SDS and systolic or diastolic blood pressure SDS. Changes in IGF-I SDS over time did not correlate with changes in systolic or diastolic blood pressure SDS. At the various time points, IGF-I SDS did not correlate with systolic BP SDS as well. There was a weak positive correlation between IGF-I SDS and diastolic BP SDS at baseline and at 36 months, but the correlation coefficient was <0.3 and therefore considered not to be clinically relevant.

Discussion

In this study, we investigated whether GH treatment affected MMP-9 levels in short SGA children and whether changes in MMP-9 levels were related with changes in blood pressure during GH treatment. Plasma MMP-9 levels decreased significantly and progressively during 3 years of GH treatment to almost 50% of the level at baseline, whereas they remained unchanged in sex- and age-matched untreated SGA controls. After 3 years of GH treatment, plasma MMP-9 levels were significantly lower in the GH group than in the untreated SGA controls. After 3 years of GH treatment, systolic BP SDS had significantly decreased, whereas it had remained similar in the untreated SGA controls. MMP-9 levels did however not correlate with systolic or diastolic BP SDS at the various time points; neither did the changes in MMP-9 levels correlate with the changes in systolic and diastolic BP SDS.

In our study, MMP-9 levels decreased during GH treatment. Likewise, in a study by Randeva et al. (13), GH-replacement in GH-deficient adults was accompanied by a progressive decrease in MMP-9 levels. The fact that 2 independent studies observed a reduction of MMP-9 during GH treatment suggests that GH treatment affects this parameter. This finding is of interest, since SGA and GH-deficiency have both been linked with an increased risk for the development of cardiovascular disease. Recently, our group showed that systolic blood pressure measured 6.5 years after cessation of GH treatment in young adults born SGA had remained lower than at start of GH treatment in childhood (24). The systolic BP was also significantly lower compared with untreated short SGA adults. The mechanism by which this reduction was achieved is still unclear. In this paper we show that both MMP-9 levels and systolic BP decreased during GH treatment. We could however not detect a relation between MMP-9 and blood pressure. This does not necessarily imply that the MMP system is not involved in blood pressure. There might be several explanations for the absence of this correlation.

Firstly, blood pressure might not be high enough in our population of healthy SGA children to detect a correlation. Yasmin et al. (5) investigated the association between MMP-9 levels and blood pressure in 2 study groups and found the association to be weaker in a group of healthy individuals with a normal mean blood pressure than in a case-control study of hypertensive subjects. Secondly, besides MMP-9, other MMPs might play a role in remodelling the vascular tree. MMP-1 is another metalloproteinase, which degrades collagen type I (2). Collagen type I has high tensile strength and rigidity (25). Hypertensive patients had lower MMP-1 levels (26), and thus, more collagen type I in their hearts and vascular tree, than other types of collagen. Thirdly, the activity of the various MMPs can be affected by tissue inhibitors of metalloproteinases (TIMPs) (2). Lastly, atherosclerosis has a complex multifactorial pathogenesis in which inflammation and lipid levels also play a prominent role and this should be taken into account when interpreting our data.

In another study, short SGA children also had a significantly higher systolic BP SDS than healthy controls (9). It has been questioned whether the reduction in systolic BP SDS during 6 years of GH treatment in this study was due to a reduction of anxiety af-

ter repeated hospital visits rather than a true effect of GH treatment (9). In the present study, we confirm the previous finding that systolic BP SDS decreases on the longer term. In addition, we show that systolic BP SDS remained similar in untreated SGA controls, although the initial value was lower than in the GH-group.

An unexpected finding was that systolic BP SDS significantly increased after 6 months of GH treatment. Since this increase was small, we are not sure if this should be regarded as a true increase. Alternatively, a transient increase in systolic BP SDS might be the consequence of a temporary increase in extracellular and/or plasma volume after GH treatment as described in GH-deficient adults (27-29), though this observation was not accompanied by an increase in systolic BP in all studies (27, 28). Besides, the increase in systolic BP is most probably only an acute effect of GH treatment (28, 29). Data regarding longitudinal changes in BP in GH-deficient children are very scarce (30).

In conclusion, our data show that MMP-9 levels decreased by approximately 50 % during 3 years of GH treatment. Systolic BP SDS was significantly higher than zero at baseline, but decreased significantly on the longer term in the GH-group. In contrast, both MMP-9 levels and systolic BP SDS remained unchanged in the untreated sex- and age-matched SGA controls. Future research should be directed at the effects of GH treatment on other MMPs, such as MMP-1, and TIMPs, which can inhibit the activity of MMPs.

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CHAPTER 5

Long-term effects of growth hormone (GH) treatment on body composition and bone mineral density in short children born small for gestational age: six-year follow-up of a randomized controlled GH trial

Ruben H. Willemsen[1], Nicolette J.T. Arends[1], Willie M. Bakker-van Waarde[2], Maarten Jansen[3], Edgar G.A.H. van Mil[4], Jaap Mulder[5], Roelof J. Odink[6], Maarten Reeser[7], Ciska Westerlaken[8], Wilhelmina H. Stokvis-Brantsma[9], Johan J.J. Waelkens[6], Anita C.S. Hokken-Koelega[1]

[1]*Dept. of Pediatrics, Division of Endocrinology, Erasmus MC Sophia, Rotterdam,* [2]*Beatrix Children's Hospital, Groningen,* [3]*Wilhelmina Children's Hospital, Utrecht,* [4]*Free University Medical Center, Amsterdam,* [5]*Rijnstate Hospital, Arnhem,* [6]*Catharina Hospital, Eindhoven,* [7]*Juliana Children's Hospital, The Hague,* [8]*Canisius Wilhelmina Hospital, Nijmegen,* [9]*Leiden University Medical Center, Leiden, the Netherlands*

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Abstract

Context: Alterations in the GH-IGF-I axis in short SGA children might be associated with abnormalities in bone mineral density (BMD) and body composition. In addition, birth weight has been inversely associated with diabetes and cardiovascular disease in adult life. Data on detailed body composition in short SGA children and long-term effects of GH treatment are very scarce.

Objective: To investigate effects of long-term GH treatment on body composition and BMD by Dual Energy X-Ray Absorptiometry (DXA) in short SGA children

Design: Longitudinal 6-year GH study with a randomized controlled part for 3 years

Results: At baseline, fat percentage standard deviation score (SDS) and lumbar spine BMD SDS corrected for height ($BMAD_{LS}$ SDS) were significantly lower than zero. Lean body mass (LBM) SDS adjusted for age was also reduced, but LBM adjusted for height (LBM SDS_{height}) was not decreased. GH treatment induced a decrease in fat percentage SDS and an increase in $BMAD_{LS}$ SDS. LBM SDS_{height} remained similar in GH-treated children, but deteriorated in untreated controls. When these untreated controls subsequently started GH treatment, their LBM SDS_{height} rapidly normalized to values comparable with zero.

Conclusion: During long-term GH treatment in short SGA children, fat percentage SDS decreased and $BMAD_{LS}$ SDS increased. These effects of GH treatment were most prominent in children who started treatment at a younger age and in those with greater height gain during GH treatment. LBM SDS_{height} remained around 0 SDS in GH-treated children, but declined to low normal values in untreated controls.

Introduction

Approximately 10 % of children born small-for-gestational-age (SGA) fail to show sufficient catch-up growth (1, 2). Growth hormone (GH) therapy in short children born SGA improves childhood growth and increases adult height (3-5). GH treatment for short children born SGA has recently been licensed (Food and Drug Administation (FDA), 2001 and European Agency for the Evaluation of Medicinal Products (EMEA), 2003) and will thus be increasingly applied as a growth promoting therapy in short SGA children.

Birth weight has been inversely associated with risk of diabetes mellitus type 2 and cardiovascular disease in adult life (6). It is well known that obesity is an important risk factor for the development of these diseases (7). Therefore, it is relevant to monitor body composition in this specific population. In children born SGA with spontaneous catch-up in weight, early development of adiposity has been reported (8). However, short children born SGA have a different phenotype. They have a lean appearance, which is confirmed by a low body mass index (BMI) SDS and a low sum of skinfolds SDS (9). On the other hand, short SGA children have lower IGF-I levels (4, 10, 11), and because abnormalities in body composition in growth hormone deficient children have previously been demonstrated (12), it might well be that body composition in short SGA children is also altered. However, data on detailed body composition in short SGA children as well as the effects of GH treatment in this population are very scarce (13, 14). Besides, there are no data on the long-term consequences of GH treatment on body composition beyond the initial phase of catch-up growth during the first 2-3 years of GH treatment in SGA children. From a methodological point of view, it is remarkable that most studies investigating body composition in children receiving GH therapy did not adjust for the GH-associated catch-up in height (12-16). Consequently, these studies might have reported an underestimation of lean and fat mass for height and an overestimation of the effect of GH treatment on these parameters.

There is increasing evidence that size at birth and in childhood may affect bone mineral density (BMD) and the risk of osteoporotic fracture in adulthood (17). In various animal models, the GH-IGF-I axis appeared to be an important factor regarding the regulation of bone growth and BMD (14, 18-20). Since short SGA children have lower IGF-I levels (4, 10, 11), it is interesting to investigate their BMD, and the effects of long-term GH treatment on this parameter.

In this paper, we report the 6-year longitudinal data of short SGA children participating in a randomized controlled GH study investigating body composition and BMD by Dual Energy X-Ray Absorptiometry (DXA). After the first 3 years, the untreated control group started GH treatment as well. In our study we expressed lean and fat mass as SD-scores adjusted for age and sex, and also as SD-scores adjusted for height and sex.

Subjects and methods

Subjects

The study group comprised 25 short prepubertal children born SGA. Inclusion criteria have previously been described (21). In short, the children were prepubertal, had a birth length and current height standard deviation score (SDS) below –2, did not show catch-up growth in height and had no growth failure caused by other disorders. None of the children were growth hormone deficient, which was defined as a GH peak <10 µg/l during two GH stimulation tests. During the first 3 years of the study, all children remained prepubertal. Puberty was defined as Tanner breast stage > 2 for girls and a testicular volume > 4 ml for boys (22). The study was approved by the Medical Ethics Committees. Written informed consent was obtained from the parents or custodians of each child.

Study design

This study was an open-labelled study with a randomized control group for 3 years. After 3 years, the initially untreated SGA controls started GH treatment as well. Before randomisation, the children were stratified according to chronological age of the children (3.00-5.50 years versus 5.50-7.99 years) and height of the parents (height of both parents above –2.00 SDS versus height of at least one parent below –2.00 SDS). Subsequently, the patients were randomly assigned to either the GH-group (2/3 of children) or the control group (1/3 of children). The GH-group started immediately with GH treatment at a dose of 1 mg/m^2 body surface area (BSA)·day. The control group remained untreated for 3 years followed by GH treatment with the same dose as the GH-group. Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S, Bagsværd, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated BSA.

DXA measurements

During 6 years, a Dual-Energy X-ray Absorptiometry scan (DXA, type Lunar DPX-L, GE Healthcare) was yearly performed in a group of 30 SGA children living near Rotterdam (20 of the GH-group and 10 of the control group). Lean body mass (LBM), total fat mass (FM), fat percentage (% fat) and lumbar spine bone mineral density (BMD_{LS}) were measured. To correct for bone size, we calculated bone mineral apparent density (BMAD) of lumbar spine (gram/cm^3) with the model $BMAD_{LS} = BMD_{LS} \times [4/(\pi \times \text{width})]$ (23). All values were transformed into SD-scores for sex and chronological age using Dutch reference values for children (24, 25).

Since body composition, particularly LBM, is strongly related to height, LBM and FM expressed as SD-score for age and sex might result in an underestimation in short stature. Besides, we were interested to know whether GH had an additional effect on LBM

and FM beyond the effects due to catch-up in height. For these reasons, LBM and FM were also expressed as SD-score for height and sex. These height-adjusted SD-scores were calculated as follows. Heights of the children of the reference group (24) were plotted against the corresponding DXA parameters (LBM, FM). The SD_{height}-scores, that were computed using these reference values, compared the DXA parameters of the SGA children with those of children with the same height and sex. Reference values were obtained using the same instrumentation and software (24, 25).

This paper presents 6-year data of this randomized controlled study. The preliminary findings on 1-year changes in body composition and 3-year changes in BMD were previously published (14, 21).

Statistics

Five children were excluded from analysis. These children received gonadotropin releasing hormone agonist (GnRHa) treatment because of relative early puberty and an adult height prognosis < -2 SDS (4 of the GH-group and 1 of the control group). During the total duration of the study there were only 3 missing values (1 at 6 years in the GH-group; 1 at 3 years and 1 at 5 years in the control group). Because the group for which DXA scans were available was a part of the original cohort, we tested whether there was any selection bias. Gestational age, birth weight SDS, birth length SDS, age, sex, height SDS, BMI SDS, IGF-I SDS and IGFBP-3 SDS were comparable for children who had DXA scans compared with children who had not (data not shown). Data are expressed as mean (SD). SD-scores were compared with zero using Student's one sample t-test. Differences between groups at the various time points, differences in changes during the first 3 years of the study (0-3 yr for both groups) and differences in changes during the first 3 years of GH treatment (0-3 yr for GH-group vs. 3-6 yr for control group) were tested using independent Student's t-test. Differences between points in time were tested by paired Student's t-tests. Correlations were analysed using Spearman's correlation coefficient. Backward multiple regression analysis was used to assess predictors for the changes in various parameters during 3 years of GH treatment. Level of significance was determined at $p<0.05$. Statistics were performed using the computer statistical package SPSS (version 11.0.1; SPSS Inc. Chicago, IL) for Windows.

Results

Baseline characteristics

Table 1 shows the baseline characteristics of the study groups. Gestational age, anthropometric parameters at birth and before entering the study, age, sex, IGF-I and IGFBP-3 levels were not significantly different between groups.

Table 1. Clinical characteristics at baseline

	SGA GH group	SGA Control group
N	16	9
Sex (m/f)	7/9	2
Gestational age (wks)	35.6 (3.1)	35.9 (2.5)
Birth weight SDS	-2.2 (1.4)	-2.9 (0.9)
Birth length SDS	-3.3 (1.3)	-3.3 (1.3)
Age (yr)	6.1 (1.5)	5.9 (1.7)
Height SDS	-3.0 (0.7)	-3.2 (0.5)
BMI SDS	-1.0 (1.0)	-1.2 (0.9)
IGF-1 SDS	-0.4 (1.1)	-0.1 (1.2)
IGFBP-3 SDS	-1.2 (1.1)	-0.9 (1.4)

SDS= standard deviation score
BMI= body mass index
IGF-1= insulin-like growth factor 1
IGFBP-3= insulin-like growth factor binding protein 3

Body composition

At baseline, both groups had a significantly reduced mean LBM SDS adjusted for age and sex (LBM SDS_{age}; Table 2). LBM SDS_{age} increased rapidly after start of GH treatment but remained significantly lower than zero up to 6 years after start of GH treatment. The untreated randomized control group showed a further decline in LBM SDS_{age} ($p<0.01$), whereas height SDS did not decrease. After start of GH treatment, the initially untreated control group showed a catch-up in LBM SDS_{age}, which was comparable in magnitude with the catch-up in LBM SDS_{age} during the first 3 years of treatment in the GH-group ($p=0.209$).

Table 2. Six year changes in LBM SDS_{age}, body fat % SDS_{age} and height SDS of the GH- and control group

	SGA GH group	SGA Control group	
		Untreated 0-3 yr	GH 3-6 yr
LBM SDS_{age}			
Baseline	-2.0 (0.3) [3]	-2.2 (0.4) [3]	
1 year	-1.4 (0.4) [3, ¶, c]	-2.3 (0.3) [3]	
2 year	-1.1 (0.5) [3, ¶, c]	-2.6 (0.4) [3, #]	
3 year	-1.1 (0.5) [3, ¶, c]	-2.8 (0.4) [3, #] ⇒	-2.8 (0.4) [3, #]
4 year	-1.1 (0.5) [3, ¶, c]		-2.3 (0.5) [3]
5 year	-1.0 (0.6) [3, ¶, b]		-2.0 (0.5) [3]
6 year	-0.9 (0.7) [3, ¶, a]		-1.6 (0.7) [3, #]
% Body fat SDS			
Baseline	-1.0 (0.8) [3]	-0.9 (1.0) [1]	
1 year	-1.7 (0.8) [3, #, a]	-0.8 (0.8) [1]	
2 year	-1.4 (0.8) [3, *, a]	-0.5 (1.1)	
3 year	-1.5 (0.7) [3, #]	-0.7 (1.3) ⇒	-0.7 (1.3)
4 year	-1.5 (0.6) [3, *]		-1.1 (0.9) [2]
5 year	-1.6 (0.7) [3, #]		-1.2 (1.2) [1]
6 year	-1.6 (0.7) [3, *]		-1.1 (1.1) [1]
Height SDS			
Baseline	-3.0 (0.7) [3]	-3.2 (0.5) [3]	
1 year	-2.0 (0.8) [3, ¶, b]	-3.2 (0.4) [3]	
2 year	-1.5 (0.9) [3, ¶, c]	-3.1 (0.4) [3]	
3 year	-1.3 (0.9) [3, ¶, c]	-3.1 (0.4) [3] ⇒	-3.1 (0.4) [3]
4 year	-1.2 (0.9) [3, ¶, c]		-2.6 (0.5) [3, #]
5 year	-1.1 (1.0) [3, ¶, a]		-2.2 (0.7) [3, #]
6 year	-1.0 (1.1) [2, ¶, a]		-1.9 (0.7) [3, ¶]

All values expressed as mean (SD)
[1] = p<0.05 compared to zero
[2] = p<0.01 compared to zero
[3] = p<0.001 compared to zero
[*] = p<0.05 versus baseline
[#] = p<0.01 versus baseline
[¶] = p<0.001 versus baseline
[a] = p<0.05 compared to control group
[b] = p<0.01 compared to control group
[c] = p<0.001 compared to control group

Because either of the groups SGA children were short for age at baseline (Table 2), LBM expressed as SD-score for age and sex might result in an underestimation. We therefore expressed LBM also as SD-score adjusted for height and sex (LBM SDS_{height}; Table 3). Importantly, LBM SDS_{height} at baseline was not significantly reduced for all groups.

Whereas LBM SDS_{height} did not significantly change for the GH-treated children up to 6 years, it tended to decrease in the untreated ones and became significantly lower than zero after 2 years. As a result, LBM SDS_{height} of the GH-group was significantly higher than that of the untreated control group after 2 and 3 years of GH treatment ($p<0.05$ and $p<0.01$, respectively). When the initially untreated SGA controls started GH treatment, they showed a rapid increase in their LBM SDS_{height}, resulting in a similar LBM SDS_{height} as the GH-group.

Table 3. Six year changes in LBM SDS_{height} and fat mass SDS_{height} of the GH- and control group

	SGA GH group	SGA Control group	
		Untreated 0-3 yr	GH 3-6 yr
LBM SDS_{height}			
Baseline	0.5 (2.1)	-1.1 (2.5)	
1 year	0.2 (1.3)	-1.1 (2.0)	
2 year	-0.1 (0.9) [a]	-1.9 (1.7) [1]	
3 year	-0.3 (0.9) [a]	-2.0 (1.4) [2]	⟹ -2.0 (1.4) [2]
4 year	-0.5 (0.9) [1]		-1.0 (0.8) [2]
5 year	-0.4 (0.9)		-0.5 (0.8)
6 year	-0.3 (0.9)		-0.4 (0.8)
Fat mass SDS_{height}			
Baseline	-1.3 (1.3) [2]	-1.5 (1.6) [1]	
1 year	-2.1 (1.1) [3, #]	-1.4 (1.4) [1]	
2 year	-1.6 (1.0) [3]	-1.1 (1.4)	
3 year	-1.7 (1.0) [3]	-1.2 (1.7)	⟹ -1.2 (1.7)
4 year	-1.7 (0.8) [3]		-1.5 (1.2) [2]
5 year	-1.7 (0.9) [3]		-1.4 (1.4) [1]
6 year	-1.8 (0.6) [3]		-1.3 (1.3) [1]

All values expressed as mean (SD)
[1] = $p<0.05$ compared to zero
[2] = $p<0.01$ compared to zero
[3] = $p<0.001$ compared to zero
[#] = $p<0.01$ versus baseline
[a] = $p<0.01$ compared to control group

Before GH treatment, all children had a body fat percentage SDS significantly below zero (Table 2). During the first 3 years of GH treatment, the GH-group showed a significant decrease in % body fat SDS, which remained at the same level up to 6 years of GH treatment. When GH treatment was started in the initially untreated control

children, these children also showed a reduction in their % body fat SDS, which was comparable with the reduction during the first 3 years of the GH-group.

At baseline, fat mass adjusted for height and sex (fat mass SDS_{height}) was significantly lower than zero in all children (Table 3). Fat mass SDS_{height} decreased significantly during the first year of GH treatment in the GH-group. After 6 years of GH treatment, fat mass SDS_{height} was still significantly lower than zero.

Bone mineral density

To correct for short stature, we calculated lumbar spine bone mineral apparent density SDS ($BMAD_{LS}$ SDS). At baseline, $BMAD_{LS}$ SDS was significantly lower than zero (Table 4). Compared to BMD_{LS} SDS, $BMAD_{LS}$ SDS was only moderately reduced. The GH-group showed a significant increase in their $BMAD_{LS}$ SDS after 2 years of GH treatment, which was maintained up to 6 years after start of GH treatment. The initially untreated control group showed no increase in their $BMAD_{LS}$ SDS after start of GH treatment.

Table 4. Six year changes in lumbar spine bone mineral density (BMD_{LS}) and bone mineral apparent density ($BMAD_{LS}$) SDS of the GH- and control group

	SGA GH group	SGA Control group		
		Untreated 0-3 yr		GH 3-6 yr
BMD_{LS} SDS				
Baseline	-1.5 (1.0) [3]	-1.4 (0.8) [2]		
1 year	-0.8 (0.8) [2, ¶]	-1.3 (0.8) [2]		
2 year	-0.4 (0.8) [¶, a]	-1.3 (0.8) [2]		
3 year	-0.1 (0.6) [¶, b]	-1.0 (0.7) [2, *]	⟹	-1.0 (0.7) [2, *]
4 year	-0.2 (0.7) [¶, a]			-0.9 (0.6) [2, *]
5 year	-0.3 (0.6) [¶]			-0.8 (0.4) [2, *]
6 year	-0.3 (0.7) [¶, a]			-0.9 (0.5) [2, #]
$BMAD_{LS}$ SDS				
Baseline	-0.7 (1.3) [1]	-0.6 (0.7) [1]		
1 year	-0.4 (1.0)	-0.6 (0.8)		
2 year	-0.3 (1.0) [#]	-0.3 (1.0)		
3 year	0.1 (0.9) [¶]	-0.3 (0.8)	⟹	-0.3 (0.8)
4 year	0.0 (1.1) [¶]			-0.5 (0.6) [1]
5 year	-0.2 (0.9)			-0.1 (0.7)
6 year	-0.2 (1.0) [*]			-0.5 (0.8)

All values expressed as mean (SD)
[1] = $p<0.05$ compared to zero
[2] = $p<0.01$ compared to zero
[3] = $p<0.001$ compared to zero
[*] = $p<0.05$ versus baseline
[#] = $p<0.01$ versus baseline
[¶] = $p<0.001$ versus baseline
[a] = $p<0.05$ compared to control group
[b] = $p<0.01$ compared to control group

Changes during the first 3 years of the study

We also compared the changes in the DXA parameters during the first 3 years of the study between the 2 study groups. GH-treated children had a significantly higher gain in LBM SDS_{age} ($p<0.001$) and BMD_{LS} SDS ($p<0.001$), and a larger reduction in fat % SDS ($p<0.01$) and fat mass SDS_{height} ($p<0.05$) than untreated controls. The changes in LBM SDS_{height} during the first 3 years of the study were not significantly different between both groups. The increase in BMAD SDS during the first 3 years of the study tended to be higher in the GH-group than the control group ($p=0.084$).

Correlations

During 3 years of GH treatment, the gain in BMD_{LS} SDS ($r=0.71$; $p<0.001$), the gain in $BMAD_{LS}$ SDS ($r=0.63$; $p<0.01$), the reduction in body fat percentage SDS ($r=-0.41$; $p<0.05$) and the gain in LBM SDS_{age} ($r=0.42$; $p<0.05$) were all strongly correlated with the gain in height SDS. As expected, the gain in LBM corrected for height and sex (LBM SDS_{height}) was not correlated with the gain in height SDS. The association between the reduction in fat mass SDS_{height} and the gain in height SDS was comparable with the association between the change in body fat percentage SDS and the gain in height SDS, but did not reach statistical significance ($r= -0.35$; $p=0.098$).

Multiple regression analysis

The gain in height SDS during 3 years of GH treatment was associated with age at start of GH treatment ($\beta= -0.10$; $p=0.012$), height at start of GH treatment ($\beta=0.22$; $p=0.083$) and sex ($\beta= -0.27$; $p=0.078$), regardless of puberty. This model explained 41% of the variance of the gain in height SDS during 3 years of GH treatment.

The gain in LBM SDS_{height} during 3 years of GH treatment was associated with age at start of GH treatment ($\beta= 0.26$; $p<0.01$) and LBM SDS_{height} at start of GH treatment ($\beta= -0.72$; $p<0.001$), regardless of sex and puberty. This model explained 91% of the variance of the gain in LBM SDS_{height} during 3 years of GH treatment.

The reduction in fat mass SDS_{height} during 3 years of GH treatment was associated with age at start of GH treatment ($\beta= -0.24$; $p<0.01$), fat mass SDS_{height} at start of GH treatment ($\beta= 0.50$; $p<0.001$), regardless of sex and puberty. The explained variance of the reduction in fat mass SDS_{height} of this model was 57%. A regression model with body fat percentage SDS instead of fat mass SDS_{height} showed comparable results.

The gain in $BMAD_{LS}$ SDS during 3 years of GH treatment was associated with age at start of GH treatment ($\beta= -0.16$; $p<0.05$), $BMAD_{LS}$ SDS at start of GH treatment ($\beta= -0.30$; $p<0.05$), regardless of sex and puberty. This model explained 52% of the variance of the gain in BMADLS SDS during 3 years of GH treatment.

Discussion

This paper shows 6-year longitudinal results of GH treatment on body composition and BMD measured by DXA in short SGA children during a study with a randomized controlled part for 3 years. Our longitudinal cohort is the only group of short SGA children in which these parameters have been studied by DXA over the long term. We show that short SGA children have a significantly reduced body fat % SDS and BMD at baseline. Lean mass adjusted for age and sex was also reduced, but when it was adjusted for height and sex (LBM SDS_{height}), it was not. GH treatment induced a decrease in body fat % SDS and an increase in $BMAD_{LS}$ SDS during the first years of treatment, which was maintained at the same level up to 6 years after start of GH treatment. LBM SDS_{height} remained at the same level in GH-treated children, but deteriorated in the untreated control group. When the initially untreated control group started GH treatment, their LBM SDS_{height} rapidly normalized to values comparable with zero. Children, who grew better during 3 years of GH treatment, also had higher gains in their $BMAD_{LS}$ SDS and larger reductions in body fat % SDS. Finally, a younger age at start of GH treatment was associated with a larger decline in body fat % SDS and a higher gain in height SDS and $BMAD_{LS}$ SDS during GH treatment.

At baseline, both lean mass and body fat % were significantly lower compared with normal children of the same age and sex. The reduced LBM at baseline appeared to be caused by short stature, since lean mass adjusted for height and sex (LBM_{height} SDS) was not reduced. On the other hand, short SGA children did have a reduced fat mass for their short stature, which was indicated by their reduced body fat % SDS and the reduced fat mass adjusted for height and sex (fat mass SDS_{height}). Therefore, it seems unlikely that development of childhood obesity in short SGA children is responsible for the association between low birth weight and adult cardiovascular disease and diabetes. It might be that SGA children with spontaneous catch-up in weight are the ones at risk, rather than SGA children that remain short (26). On the other hand, we cannot rule out that other mechanisms are responsible for the negative association between birth weight and CVD and diabetes in adult life, such as insulin resistance or low IGF-I levels levels (27, 28). Although the short SGA children in our study were not growth hormone deficient, they did have relatively low IGF-I levels (21). Another important aspect is the distribution of fat mass in the body, which we did not analyze in this study. It has been demonstrated that central obesity, as opposed to peripheral obesity, confers the highest risk for cardiovascular disease (7). Future studies should be directed at this issue.

During GH treatment, both body fat % SDS and fat mass SDS_{height} declined. The greatest reduction was seen during the first year of GH treatment causing a significant difference compared with the untreated controls after 1 year. Children, who were younger and had a higher fat mass SDS_{height} at start of GH treatment, showed a greater reduction in their fat mass SDS_{height} during 3 years of GH treatment. The same associations were seen when we used body fat % SDS as the dependent variable. Thus, it might be more favourable to start GH treatment at a younger age to obtain a larger reduction in body

fat %, although most SGA children already had a low body fat % SDS at baseline.

Most studies investigating the effects of GH treatment on body composition in children did not adjust for the increased height which accompanies GH therapy and thus, were unable to study the effects of GH treatment beyond the increases of muscle and fat mass as a consequence of catching-up in height (12-16). In contrast to lean mass adjusted for age and sex (LBM SDS_{age}), lean mass adjusted for height and sex (LBM SDS_{height}) did not increase during GH treatment. Thus, GH did not result in an increase of lean mass beyond the normal increase as consequence of a gain in height.

Notably, the untreated children had a further decline of their LBM SDS_{age} to almost –3 SDS, without significant changes in their height SDS. Also, lean mass adjusted for height and sex (LBM SDS_{height}) tended to decrease over time in the untreated controls resulting in a significantly lower LBM SDS_{height} after 3 years compared with GH-treated children. Thus, lean mass in the untreated SGA children declined, whereas it was preserved in the GH-treated children. A possible explanation for this decline might be that short SGA children have a reduced food intake, which improves during GH treatment (14). Singhal et al. reported an inverse association between birth weight and lean mass, which was stronger in older children (29). Our data are in line with this report as they show that older SGA children have indeed a lower lean mass SDS compared with younger SGA children. The reduced muscle mass might also have played a role in the reduced insulin sensitivity which has been found in a comparable group of short SGA children with the same age (27, 30).

When lean mass was adjusted for age and sex only, the initially untreated children tended to have a lower lean mass after 3 years of GH treatment compared to the GH-group after 3 years of GH treatment. However, when lean mass was adjusted for height and sex (LBM SDS_{height}), it appeared that the initially untreated children showed a rapid catch–up in their LBM SDS_{height} during 3 years of GH treatment, resulting in levels comparable with the GH-group after 3 as well as 6 years of GH treatment. The difference between LBM corrected for age and LBM corrected for height is explained by the fact that the control children also gained less height, because they were older at start of GH treatment. LBM SDS_{height} increased in the initially untreated children during GH treatment, whereas no change was observed during the first 3 years in the GH-group. This might be explained by the fact that the increase in LBM SDS_{height} during GH treatment is higher when the baseline level was lower, as was shown in the multiple regression analysis.

Bone mineral density SDS at baseline was significantly lower than zero. However, DXA measures an areal density (g/cm^2), which is obtained by dividing bone mineral content (BMC, gram) by the projected bone image (area, cm^2). Therefore, DXA may underestimate BMD in children with short stature. A widely used and validated model to correct for bone size is BMAD (23). This is a better parameter for assessing bone mineralization in short children. In contrast to BMD_{LS} SDS, $BMAD_{LS}$ SDS was only moderately reduced at baseline. In our study, GH treatment resulted in an increase in $BMAD_{LS}$ SDS after 3 years of GH treatment. The change in $BMAD_{LS}$ SDS during 3 years of GH treatment correlated strongly with the increase in height SDS. Thus, the chil-

dren with the best growth response also had the highest gain in their bone mineral density SDS. Multiple regression analysis showed that children who were older at start of GH treatment gained less $BMAD_{LS}$ SDS than children who started GH therapy at an earlier age. Our data suggest that starting GH at an earlier age is more favourable, because it is accompanied by a higher gain in $BMAD_{LS}$ SDS. Since lean mass adjusted for height (LBM SDS_{height}) did not increase during GH treatment, it seems that the increase in BMAD cannot be regarded as a consequence of gaining more muscle mass. IGF-I, ALS (Acid Labile Subunit) and IGFBP-3 play a prominent role in the regulation of bone growth and density(18-20). In short SGA children, IGF-I and IGFBP-3 levels are low and increase during GH treatment (4, 11, 21). In addition, a better nutritional intake and increased physical activity during GH treatment, as was reported by parents, might have played a role in the increase in BMD (14).

In conclusion, short SGA children have a low body fat percentage and BMD. Long-term GH treatment in short children born SGA causes a decrease in body fat percentage and body fat adjusted for height as well as an increase in BMD. Lean mass increased along with height in GH-treated children but GH treatment did not result in an increment of lean mass beyond the normal growth-related increase. Lean mass adjusted for height and sex remained around 0 SDS in GH-treated children, but declined to low normal values in untreated controls. The effects of GH treatment on body fat and BMD were most prominent in children who started GH treatment at a younger age with a higher gain in height SDS during 3 years of GH treatment. Our study shows that it is necessary to correct DXA parameters for height in order to obtain a good interpretation of body composition and bone mineral density in short SGA children at baseline as well as during GH treatment.

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CHAPTER 6

Longitudinal changes in insulin sensitivity and body composition of small for gestational age adolescents after stop of growth hormone treatment

Ruben H. Willemsen[1], Sten P. Willemsen[2], Anita C.S. Hokken-Koelega[1]

[1]*Dept. of Pediatrics, Division of Endocrinology, Erasmus MC Sophia,* [2]*Dept. of Epidemiology and Biostatistics, Erasmus MC, Rotterdam, The Netherlandss*

Submitted

Abstract

Context: Growth hormone (GH) treatment reduces insulin sensitivity (Si). For small-for-gestational-age (SGA) subjects, who might have an increased risk to develop cardiovascular disease and type 2 diabetes, it is still uncertain how Si, ß-cell function and body composition change over time after stop of GH treatment.

Objective: To investigate longitudinal changes in Si, ß-cell function and body composition after stop of long-term GH treatment

Design: Longitudinal study

Patients: 48 SGA adolescents were studied at adult height, while still on GH, and 6 months after GH stop, and compared with 38 AGA controls

Outcome measure: Paired measurements of Si and ß-cell function, assessed by frequently sampled intravenous glucose tolerance tests (FSIGT), and body composition, measured by dual energy x-ray absorptiometry (DXA).

Results: After stop of GH, Si (p=0.006), glucose effectiveness (Sg; p=0.009) and ß-cell function (disposition index= DI; p=0.024) increased, whereas insulin secretion (AIR; NS) decreased. Fat percentage increased (p<0.0005), and lean body mass (LBM) decreased (p<0.0005), but fat distribution remained unaltered and body composition remained within the normal range. Compared with AGA controls, Si was lower during GH and became similar after GH stop, AIR was higher at both time points, and Sg and DI became higher.

Conclusions: The GH-induced lower insulin sensitivity in SGA adolescents increases after stop of long-term GH treatment and becomes similar to that of AGA controls. Discontinuation of GH treatment is, however, also associated with an increase in percent body fat and a decrease in LBM, without changes in fat distribution.

Introduction

It is well known that growth hormone (GH) treatment reduces insulin sensitivity. Since short children born small-for-gestational-age (SGA) have a lower insulin sensitivity than normal children prior to start of GH treatment, concern has been expressed regarding the long-term effects of GH-treatment on the insulin-glucose homeostasis of SGA children (1-3).

Previously, we showed that the insulin response during an oral glucose tolerance test (OGTT) normalized after stop of GH treatment (4). An OGTT is, however, dependent on the uptake of glucose in the digestive system and does not provide any information regarding changes in insulin sensitivity and ß-cell function.

Two studies reported conflicting results regarding the change of insulin sensitivity after discontinuation of GH treatment (5, 6). In the first study in 9 short SGA children, the observed decrease in insulin sensitivity during GH treatment was reversible (5). In the second study in 12 children, insulin sensitivity did not recover after stop of GH treatment (6). Both studies investigated insulin sensitivity after stop of GH treatment, but before adult height was achieved. Besides, in one study it was unclear whether some children had already entered puberty (5), which can reduce insulin sensitivity as well (7, 8). Furthermore, it is questionable if the number of subjects was sufficient to draw definite conclusions. Thus, up to date, it is not known how insulin sensitivity and secretion change longitudinally in SGA adolescents after adult height has been attained and GH treatment has been stopped. Regarding body composition, there are no data on changes after stop of GH treatment.

We hypothesized that the GH-induced reduction in insulin sensitivity recovers after stop of GH treatment and that the changes in body composition, if any, would be limited. We performed paired measurements of insulin sensitivity and ß-cell function, using the frequently sampled intravenous glucose tolerance test (FSIGT) in 48 GH-treated SGA adolescents, at near adult height (AH) and 6 months after cessation of GH. Besides, we measured body composition by dual energy x-ray absorptiometry (DXA) at the same time points. In addition, we compared their data with those of 38 AGA controls.

Subjects and methods

Subjects

The study group comprised 48 adolescents born SGA, who participated in a GH-trial, of which the inclusion criteria have previously been described (9). In short, the children were prepubertal, had a birth length and height standard deviation score (SDS) below –2.0, did not show catch-up growth in height and had no growth failure caused by other disorders. Once daily at bedtime, 1 mg biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S, Bagsværd, Denmark) per square meter body surface area (BSA) was given subcutaneously. Every three months, the GH dose was adjusted to the calculated

BSA. GH treatment was discontinued when height velocity dropped below 0.5 cm over the last 6 months and/or bone age was ≥ 15 years for girls and ≥ 16.5 years for boys. SGA subjects were compared with 38 healthy normal-statured AGA (defined as birth length and height >-2 SDS (10, 11)) controls, which were matched for gestational age and sex. The GH trial and the current follow-up study were approved by the Medical Ethics Committees. Written informed consent was obtained from all controls and subjects and, if they were younger than 18 years, also from the parents or custodians of each adolescent.

Study design

Subjects were studied twice after an overnight fast: (i) at near adult height, while still on GH, and (ii) 6 months thereafter, without GH. Standing height and weight were measured and body mass index (BMI) was calculated. Height and BMI were expressed as SDS adjusting for sex and age according to Dutch reference data (10, 12). A modified frequently sampled iv glucose tolerance test (FSIGT) with tolbutamide was performed, as previously described (13, 14). Insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR) and disposition index (DI) were calculated using Bergman's MINMOD MILLENNIUM software (15). 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.

Body composition was measured with dual-energy x-ray absorptiometry scans on one machine (DXA, type Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Lean body mass (LBM), fat mass (FM) and fat percentage (% fat) were determined. Fat percentage was transformed into SD-scores for sex and age using Dutch reference values (16, 17). Since body composition is strongly related to height, LBM and FM expressed as SD-score for age and sex might result in an underestimation in case of short stature. Therefore, LBM and FM were expressed as SD-score for height and sex. Height-adjusted SD-scores were calculated as previously described (18).

Assays

All serum glucose levels were determined on a VITROS analyser 750 (Orthoclinical Diagnostics, Johnson&Johnson Company, Beerse, Belgium). All serum insulin levels were measured by 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). All assays were performed in one central laboratory.

Statistics

To normalize the distribution, all FSIGT parameters were logarithmically transformed prior to analyses. With respect to body composition data, a value of –2 to 2 SDS corresponds with a normal body composition corrected for age and sex (SDS_{age}) or height and sex (SDS_{height}). To test the time effect of discontinuation of GH treatment on FSIGT and body composition parameters, mixed model analyses of variance were performed. There were no interactions between sex and time and oral contraceptive use and time. Therefore, these data were not entered in the model. Differences between SGA subjects and controls were tested by one-way analysis of variance (ANOVA) with an LSD post-hoc test. Clinical data are presented as mean (SD), model estimates as geometric mean (95% confidence interval) for FSIGT parameters, and mean (95% confidence interval) for body composition data. Level of significance was determined at $p<0.05$. Statistics were performed using the computer statistical package SPSS (11.0.1; SPSS Inc., Chicago, IL).

Results

Clinical characteristics

Table 1 shows the clinical characteristics of the SGA subjects and AGA controls at various time points. SGA subjects had a mean age of 8.6 years at start of GH treatment and 16.1 years at stop of GH treatment. After a mean duration of 7.5 years of GH treatment, height had increased from –2.8 SDS at start of GH treatment to –1.3 SDS at adult height (AH).

Table 1. Characteristics at birth, at start and at stop of GH treatment

	SGA	AGA controls	P-value SGA vs AGA
N	48	38	
Sex (m/f)	18/30	14/24	0.950
Gestational age (wks)	36.6 (3.3)	35.5 (3.5)	0.132
Birth weight SDS	-2.4 (1.0)*	-0.1(1.2)	<0.0005
Birth length SDS	-3.0 (1.5)*	-0.3 (1.0)	<0.0005
Age at start of GH treatment (yrs)	8.6 (2.4)	N/A	
Height SDS at start of GH treatment	-2.8 (0.7)*	N/A	
Age at current study (yrs)	16.1 (1.3)	19.2 (0.9)	<0.0005
Adult height SDS	-1.3 (0.8)*	-0.3 (1.1)	<0.0005
GH duration (yrs)	7.5 (1.7)	N/A	

SDS = standard deviation score
*= p<0.0005 versus zero SDS
N/A = not applicable

Longitudinal changes in insulin sensitivity and ß-cell function

Figure 1 shows the data of the serial FSIGT tests at stop of GH treatment (SGA at stop GH) and 6 months thereafter (SGA after stop GH) in comparison with AGA controls. Si increased significantly after stop of GH treatment (Fig. 1A: 3.8 (3.3-4.4) to 5.1 (4.2-6.3) x 10^{-4}/min^{-1} (mU/l); p=0.006). Si was significantly lower in SGA subjects at stop of GH than in AGA controls (p=0.001), but became comparable after GH treatment was stopped. Also, Sg improved significantly after stop of GH treatment (Fig. 1C: 1.5 (1.3-1.8) to 1.9 (1.8-2.1) x 10^{-2}/min^{-1} (mg/d); p=0.009), and became significantly higher than Sg in AGA controls (p=0.024). After stop of GH treatment, insulin secretion decreased (Fig. 1B: 699 (577-848) to 623 (514-754) mU/l; p=0.130) and disposition index increased significantly

(Fig. 1D: 2561 (2063-3178) to 3258 (2768-3835); p=0.024). Compared with AGA controls, SGA subjects had a significantly higher insulin secretion at and 6 months after stop of GH (p<0.0005), and a significantly higher DI 6 months after stop of GH (p=0.004).

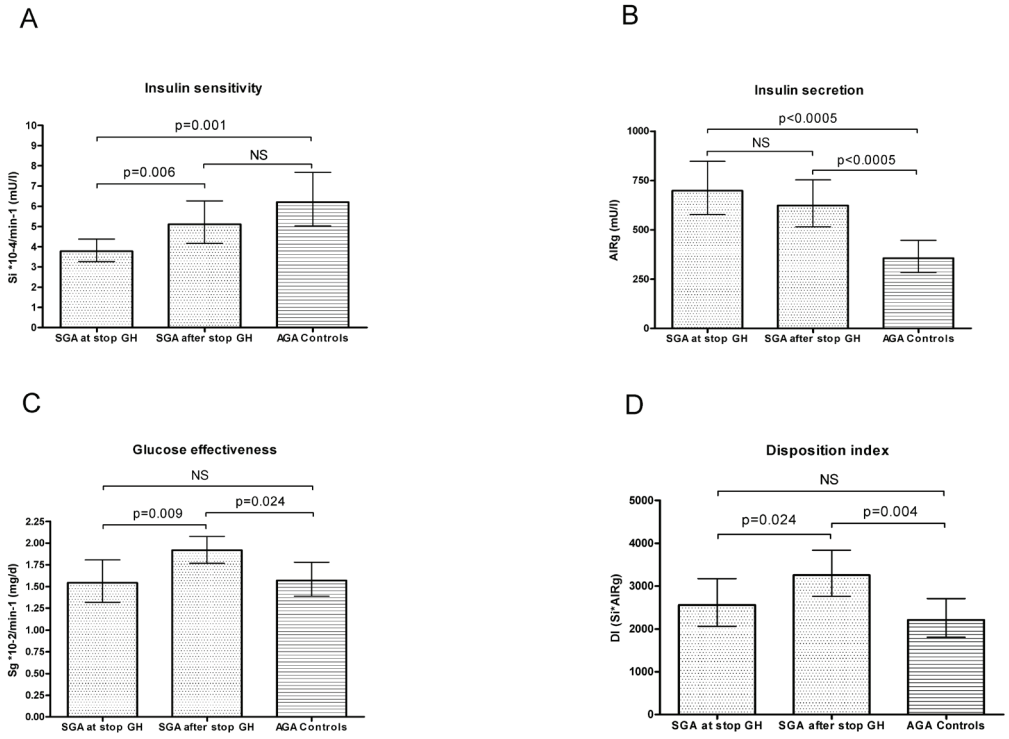

Figure 1. Longitudinal changes in FSIGT parameters of SGA adolescents from stop of GH treatment to 6 months thereafter in comparison with AGA controls. A: Insulin sensitivity; B: Insulin secretion; C: Glucose effectiveness; D: Disposition index.
Si= insulin sensitivity. AIRg= acute insulin response to glucose. Sg= glucose effectiveness. DI= disposition index. NS= not significant

Longitudinal changes in body composition

Table 2 shows the changes in body composition from stop (SGA at stop GH) to 6 months after stop of GH treatment (SGA after stop GH). There was a significant increase in fat percentage SDS and fat mass corrected for height and sex ($p<0.0005$), and a significant decrease in lean body mass corrected for height and sex ($p<0.0005$). Trunk to total fat mass ratio did not significantly change and remained within the normal range at both time points. Body composition was comparable with the AGA controls, with the exception of a higher trunk to total fat mass ratio in SGA subjects at stop of GH. Body mass index (BMI) SDS did not change after discontinuation of GH.

Table 2. Longitudinal changes in body composition after stop of GH treatment

	SGA at stop GH	SGA after stop GH	P-value at vs. after stop GH	AGA
Fat percentage SDS	-0.20 (-0.53 – 0.12)	0.14 (-0.16 – 0.44)	**<0.0005**	0.26 (-0.06 – 0.58)
Fat mass SDS_{height}	-0.04 (-0.32 – 0.23)	0.25 (-0.01 – 0.49)	**<0.0005**	0.30 (0.01 – 0.60)
Lean body mass SDS_{height}	0.06 (-0.27 – 0.39)	-0.21 (-0.53 – 0.12)	**<0.0005**	-0.41 (-0.80 – -0.03)
Trunk fat/total fat ratio	0.50# (0.48 - 0.51)	0.49 (0.48 - 0.50)	0.158	0.47 (0.45 – 0.49)
BMI SDS	0.05 (-0.20 – 0.29)	0.03 (-0.22 – 0.27)	0.648	-0.01 (-0.40 – 0.38)

Values are represented as mean (95 percent confidence interval)
# = $p<0.01$ vs. AGA
SDS= standard deviation score adjusted for age and sex
SDS_{height}= standard deviation score adjusted for height and sex

Discussion

In this paper we report longitudinal changes in insulin sensitivity, ß-cell function and body composition in SGA adolescents from stop to 6 months after stop of GH treatment in comparison with AGA controls. Insulin sensitivity was significantly reduced in SGA subjects at stop of GH, but increased significantly after stop of GH treatment to a similar value as in AGA controls. Insulin secretion decreased compensatory after stop of GH, but remained significantly higher than in AGA controls. Glucose effectiveness increased significantly after stop of GH treatment and became higher than that of controls. Disposition index, which indicates how well the ß-cells compensate for

lower values of insulin sensitivity, improved after stop of GH treatment, reaching a higher value than controls. Regarding body composition, fat percentage SDS and fat mass adjusted for height and sex increased significantly after stop of GH, whereas lean body mass adjusted for height and sex decreased significantly, but the values remained within the normal range.

This is the first study describing serial measurements of insulin sensitivity and secretion in SGA subjects at stop and 6 months after stop of GH treatment at attainment of adult height. Our data are in contrast to those of Cutfield et al. (6), who reported that the decrease of insulin sensitivity during GH treatment was irreversible after its discontinuation. However, they only studied 5 children after stop of GH treatment. De Zegher et al. (5) reported a reversible decrease of insulin sensitivity after stop of GH treatment in 9 children, but in this study it was unclear if the children had become pubertal. Finally, both studies investigated insulin sensitivity before adult height had been attained.

Previously, we investigated glucose tolerance with OGTT in another group of GH-treated SGA subjects and showed that the glucose and insulin response during OGTT normalized after stop of GH treatment (4). Unfortunately, OGTT do not provide information on insulin sensitivity and ß-cell function. Our current data show that the decrease in insulin sensitivity during GH treatment is reversible after discontinuation of GH. Van Dijk et al. (19) investigated insulin sensitivity in previously GH-treated young adults who had discontinued GH treatment for 6.5 years, and found that their insulin sensitivity and ß-cell function were similar to those of untreated SGA controls. However, insulin sensitivity and ß-cell function were not measured in these subjects during GH treatment. Therefore, that study was unable to study longitudinal changes in insulin sensitivity and ß-cell function.

To the best of our knowledge, this is the first study investigating changes in body composition in SGA after stop of GH. Interestingly, we found significant changes in body composition after stop of GH treatment, which could not be detected with BMI SDS. Fat percentage SDS and fat mass SDS increased, whereas lean body mass SDS decreased. These changes are opposite to those which occur when GH treatment is started in SGA children (18). At this moment, the clinical relevance of the observed changes is unclear. It remains to be elucidated how body composition changes on the longer term after GH has been discontinued.

Insulin sensitivity is known to have a strong correlation with fat percentage. Despite the increase in fat percentage SDS after stop of GH, insulin sensitivity increased. This indicates that discontinuation of GH treatment has a beneficial effect on insulin sensitivity, which is greater than the opposite effect on insulin sensitivity due to gaining more fat mass.

An unexpected finding was the increase in Sg after stop of GH treatment. Sg is a measure for insulin-independent glucose disposal. In previous studies, Sg was not significantly different, albeit lower in short SGA than in normal AGA children (1). During GH treatment, Sg did not change on the short-term (6), but the long-term effects are not known. In first-degree relatives of type 2 diabetic individuals, who were followed

longitudinally, Sg was lower at the first assessment in those subjects, who progressed from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) (20). In another follow-up study on first degree relatives of type 2 diabetics, Sg was found to be an independent predictor for the development of type 2 diabetes (DM2) (21). The combination of a low Si and a low Sg was associated with the highest cumulative risk of DM2 (21). Importantly, in our study Sg was similar for GH-treated SGA subjects and AGA controls and improved significantly when GH treatment was stopped, indicating that Sg in SGA subjects, both on and off GH, is not worse than that in AGA controls.

Interestingly, insulin secretion remained significantly higher in SGA subjects, also after GH was discontinued. One could argue that the ß-cells need to secrete relatively large amounts of insulin to maintain glucose homeostasis, leading to a possible exhaustion of the ß-cells and thus DM2 on the longer term. However, there is no support for this view in the available literature on the development of DM2 in persons at risk, because published data indicate that low rather than high first-phase insulin secretion is associated with progression from NGT to IGT as well as progression from IGT to DM2 (22, 23). Moreover, the decline in glucose tolerance over time in relatives of type 2 diabetic individuals was strongly related to the loss of ß-cell function, measured by DI (20). In our study DI was even higher in SGA subjects than in controls. Nevertheless, our data cannot guarantee that glucose homeostasis remains unaffected on the longer term. Therefore, long-term follow-up of previously GH-treated SGA subjects remains important.

In conclusion, the GH-induced reduction in Si in SGA adolescents is reversible after stop of long-term GH-treatment and Si becomes similar to that of AGA controls, which is reassuring. Discontinuation of GH treatment was, however, also associated with a significant increase in percent body fat and a decrease in lean body mass, without changes in fat distribution. It remains to be elucidated how body composition changes on the longer term after GH has been discontinued. Our data underscore the importance of follow-up studies after discontinuation of GH treatment.

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CHAPTER 7

Independent effects of prematurity on metabolic and cardiovascular risk factors in short small-for-gestational-age children

Ruben H. Willemsen[1],
Sandra W.K. de Kort[1],
Danielle C.M. van der Kaay[2],
Anita C.S. Hokken-Koelega[1,2]

[1]*Dept. of Pediatrics, Division of Endocrinology, Erasmus MC Sophia,* [2]*Dutch Growth Foundation, Rotterdam, the Netherlands*

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Abstract

Context: Both small-for-gestational-age (SGA) and preterm birth have been associated with increased incidence of adult cardiovascular disease and diabetes mellitus type 2. It is however unclear if preterm birth has an additional effect on cardiovascular risk factors in short children born SGA.

Objective: To investigate if prematurity has an independent influence on several cardiovascular risk factors within a population of short SGA children.

Design: Cross-sectional observational study

Patients: 479 short SGA children (mean age 6.8 years), divided in preterm (< 36 weeks) and term (≥ 36 weeks) children.

Outcome measure: Insulin sensitivity, beta cell function, body composition and lipid levels were studied in subgroups; blood pressure, anthropometry at birth and during childhood in the total group

Results: Preterm SGA children were significantly lighter and shorter at birth after correction for gestational age than term SGA children ($p<0.001$), but had a comparable head circumference. In preterm SGA children, we found a significantly higher systolic ($p=0.003$) and diastolic blood pressure SDS ($p=0.026$), lower body fat % SDS ($p=0.011$), and higher insulin secretion ($p=0.033$) and disposition index ($p=0.021$), independently of the degree of SGA. Insulin sensitivity, serum lipid levels, muscle mass and body fat distribution were comparable for preterm and term SGA children.

Conclusions: Within a population of short SGA children, preterm birth has divergent effects on several cardiovascular risk factors. Whereas preterm SGA children had a higher systolic and diastolic blood pressure, they also had a lower body fat % and a higher insulin secretion and disposition index than term SGA children.

Introduction

A small size at birth has been associated with adult diseases, such as cardiovascular disease and diabetes mellitus type 2 (1-3). However, size at birth is determined by 2 factors: intrauterine growth and duration of pregnancy.

We and others have previously shown that short small-for-gestational-age (SGA) children have a lower insulin sensitivity (4, 5) and a higher systolic blood pressure (4, 6). Recent reports indicate that also prematurity itself might have adverse consequences with regard to cardiovascular risk factors. Compared with controls born at term, premature born young adults had higher blood pressure and fasting glucose levels, irrespective of being SGA (7). In a cohort of young adult Swedish men, prematurity was recognized as a risk factor for high blood pressure, independent of birth weight standard deviation score (SDS) (8).

In contrast, others reported that among children born preterm, only those who were born SGA had an increased systolic blood pressure and pulse wave velocity, which is a measure for arterial stiffness (9). Singhal et al. found comparable levels of fasting glucose, insulin and lipids between preterm SGA and AGA adolescents (10).

Most aforementioned studies investigated the influence of SGA within a population of preterm born or 'low birth weight' subjects. However, because only a minority of all preterm children is born SGA (~2-3 %), these studies may not have been suitable to investigate whether a combination of SGA and preterm birth is worse with regard to cardiovascular risk parameters than being born SGA at term.

The aim of our study was to investigate if prematurity has an independent influence on insulin sensitivity, beta cell function and other cardiovascular risk factors within a large population of short SGA children. We hypothesized that being born both preterm and SGA is associated with a worse cardiovascular risk profile than being born SGA at term. To test our hypothesis, we compared preterm short SGA children with term short SGA children.

Subjects and methods

Subjects

The study group comprised 479 prepubertal short children born SGA. All children fulfilled the same inclusion criteria: 1) birth length and/or birth weight standard deviation score (SDS) below –2 for gestational age(11), 2) height SDS below –2 according to Dutch standards(12), 3) height velocity SDS below zero to exclude children with spontaneous catch-up growth(12), 4) prepubertal stage, defined as Tanner breast stage 1 for girls and testicular volume less than 4 ml for boys(13), 5) an uncomplicated neonatal period without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis 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, with the exception of Silver-Russell syndrome. When we performed the analyses after exclusion of the Silver-Russell subjects, the results were the same. The following data have been published before: insulin sensitivity and secretion data from 28 of 77 children (4), body composition from 30 of 149 children (14, 15), blood pressure, total cholesterol, HDL-c, LDL-c and triglyceride levels of 159 children (4, 6, 16) and FFA levels of 28 children (4). However, these papers had another objective and did not focus on the influence of prematurity. The study was approved by the Medical Ethics Committees. Written informed consent was obtained from the parents or custodians of each child.

Study design

Children were divided into 2 groups on the basis of their gestational age: (i) preterm (gestational age <36 wks) and (ii) at term (gestational age ≥36 wks). The gestational age of the subjects was determined by ultrasound in the first trimester, if available, and otherwise calculated from the date of the last menstruation.

Anthropometry

Standing height was measured using a Harpenden stadiometer and was expressed as SD-score for sex and chronological age using Dutch references (12). Body mass index (BMI) was calculated according to the formula weight/(height)^2 and was expressed as SD-score for sex and age (17). Systolic and diastolic blood pressure (BP) was measured twice on the left arm with an automated device and appropriate cuff size (Dinamap Critikon, Southern Medical Corp., Baton Rouge, LA, USA). The mean of 2 measurements was used for analysis. Since height is an important determinant of BP in childhood and adolescence, BP was expressed as SDS adjusted for height and sex (18).

Body composition

Dual-Energy X-ray Absorptiometry scans (DXA, type Lunar DPX-L, GE Healthcare, Madison, Wisconsin, USA) were performed in a subgroup of 149 children. Lean body mass (LBM) and fat percentage (% fat) were determined. All values were transformed into SD-scores for sex and chronological age using Dutch reference values for children, which were obtained using the same instrumentation and software (19, 20). Since body composition, particularly LBM, is strongly related to height, LBM expressed as SD-score for age and sex might result in an underestimation in short stature. Therefore, LBM was also expressed as SD-score for height and sex. Height-adjusted SD-scores were calculated as previously described (15).

Insulin sensitivity and secretion

In a subgroup of 77 children, a modified frequently sampled intravenous glucose tolerance test (FSIGT) with tolbutamide was performed, as previously described (21, 22). 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 (23). 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. Sex and ethnic distribution, gestational age, birth weight SDS, birth length SDS, birth head circumference SDS, height SDS and BMI SDS were not significantly different between children who underwent an FSIGT and those who did not. Only age was slightly higher in the FSIGT group (7.3 vs. 6.7 years).

Hormone and biochemical assays

All blood samples were taken after an overnight fast. Serum glucose and total cholesterol levels were measured as previously described (24). Insulin levels were all measured in one laboratory using the same method (IRMA; Medgenix, Biosource Europe, Nivelles, Belgium). The intra-assay coefficient of variation (CV) was 2 % to 4.7 % and the inter-assay CV was 4.2 % to 11.3%. Triglycerides (TG) were measured on the Chem-1 analyser according to the manufacturer's instructions (Technicon Instruments, Tarrytown, New York) and after 1998 on the Hitachi 917 analyser according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Both methods were comparable (y = x-0.030).

Non-esterified fatty acids (FFA) were measured in serum using an enzymatic colorimetric method (WAKO Chemicals, Germany). Apolipoprotein A-1, Apolipoprotein B and lipoprotein (a) were determined by rate nephelometry on the Immage Immunochemistry system, according to the manufacturers' instructions (Beckman Coulter, Mijdrecht, Netherlands). Between-run coefficients of variation were 4.2%, 2.8% and 6.9% for these lipoproteins at levels of 0.94, 0.53 and 0.35 g/l respectively.

Statistical analysis

FSIGT parameters, triglycerides, free fatty acids, limb fat and trunk fat were logarithmically transformed prior to analysis because of a skewed distribution. Because Lp(a) levels were very skewed, also after logarithmic transformation, we tested whether the percentage of individuals having a value $\geq$ 0.3 g/l was different between the groups. All data are presented as mean $\pm$ SD, except for the skewed parameters mentioned above, which are presented as median and interquartile range. Differences

between groups were tested using Student's t test for continuous variables and χ^2 test for categorical variables. Multiple linear regression (for continuous variables) and binary logistic regression analyses (for dichotomous variables) were used to adjust differences in outcome between the groups for possible baseline differences. Correlations were analysed using Spearman's correlation coefficient. With respect to body composition and blood pressure data, a value of –2 to 2 SDS corresponds with a normal body composition corrected for age and sex and a normal blood pressure corrected for height and sex. Level of significance was determined at p<0.05. Statistics were performed using the computer statistical package SPSS (12.0; SPSS Inc., Chicago, IL).

Results

Clinical characteristics

Table 1 lists the clinical data of the total study group and the preterm and term subgroups. Birth weight SDS, birth length SDS, current height SDS and BMI SDS were significantly lower than zero for both groups. Compared with term SGA children, preterm SGA children were significantly lighter and shorter at birth after correction for gestational age. Age and BMI SDS were significantly lower in preterm than term SGA children. Current height SDS was slightly higher in preterm SGA children than in term SGA children. Sex distribution and birth head circumference SDS were comparable.

Table 1. Clinical characteristics at birth and at baseline

	All	**SGA born Preterm < 36 wks**	**SGA born Term ≥ 36 wks**	**P-value**
N	479	164	315	
Age (yr)	6.8 (2.4)	6.3 (2.2)	7.0 (2.5)	<0.01
Sex (m/f)	259/220	93/71	166/149	NS
Ethnicity (% caucasian)	87.5	93.3	84.4	<0.01
Gestational age (wks)	36.4 (3.7)	32.1 (2.5)	38.7 (1.6)	<0.001
Birth weight SDS	-2.3 (1.1)[a]	-2.8 (1.3)[a]	-2.1 (1.0)[a]	<0.001
Birth length SDS	-3.2 (1.6)[a]	-3.9 (2.0)[a]	-2.9 (1.2)[a]	<0.001
Birth head circumference SDS	-2.0 (1.3)[a]	-2.2 (1.4)[a]	-1.8 (1.2)[a]	NS
Height SDS	-3.1 (0.6)[a]	-3.0 (0.6)[a]	-3.1 (0.7)[a]	<0.05
BMI SDS	-1.3 (1.0)[a]	-1.6 (1.0)[a]	-1.2 (1.0)	<0.0005

Data expressed as mean (SD)
SDS: standard deviation score
[a] p<0.001 compared with zero

Body composition

Because preterm SGA children had a significantly lower BMI SDS, we were interested whether this was due to a lower fat or a lower muscle mass. Preterm SGA children had a significantly lower body fat % SDS than term SGA children, also after adjustment for possible confounding factors (age, sex, ethnicity, birth weight SDS and birth length SDS) (Table 2). Both trunk fat and limb fat were significantly lower in preterm SGA children, though the difference for limb fat did not reach significance anymore after adjustment for possible confounding factors (age, sex, ethnicity, birth weight SDS, birth length SDS and total body weight). Fat distribution, defined as trunk fat/total fat-ratio, was comparable between preterm and term SGA children. Lean mass SDS was comparable, also when height-adjusted instead of age-adjusted SD-scores were used.

	All		SGA born Preterm < 36 wks		SGA born Term ≥ 36 wks		Unadjusted P-value	Model 1* P-value	Model 2* P-value
	N		N		N				
Body fat % SDS_{age}	144	-0.8 (0.9) [§]	51	-1.2 (0.8) [§]	93	-0.6 (0.9) [§]	<0.0005	0.011	N/A
Lean mass SDS_{age}	144	-2.5 (0.6) [§]	51	-2.4 (0.5) [§]	93	-2.6 (0.6) [§]	0.107	0.932	N/A
Lean mass SDS_{height}	148	-1.0 (1.9) [§]	54	-0.9 (2.1) [†]	94	-1.0 (1.7) [§]	0.973	0.776	N/A
Limbs fat (g)	146	728 (362-1248)	53	453 (260-826)	93	1088 (493-1520)	<0.0005	0.008	0.053
Trunk fat (g)	146	441 (226-799)	53	273 (166-451)	93	609 (338-936)	<0.0005	0.006	0.043
Trunk fat/total fat	146	0.33 (0.05)	53	0.33 (0.05)	93	0.34 (0.05)	0.302	0.594	0.795

Data expressed as mean (SD), except limbs and trunk fat which are median (interquartile range)
[§] = p<0.0005 versus zero; [†] = p<0.005 versus zero
[*] Model 1: adjusted for age, sex, ethnicity, birth weight SDS and birth length SDS (n=107 for body fat % SDS_{age} & lean mass SDS_{age}; n=110 for lean mass SDS_{height}; n=109 for fat distribution). Model 2: adjusted for age, sex, ethnicity, birth weight SDS, birth length SDS and weight (n=109)
N/A = not applicable

Insulin sensitivity and secretion

Table 3 shows the results of the FSIGT tests. Insulin sensitivity (Si) was comparable for preterm and term SGA children. Insulin secretion (AIR) tended to be higher and glucose effectiveness (Sg) tended to be lower in preterm than term SGA children, but the difference was not statistically significant. Disposition index (DI) was significantly higher in preterm than in term SGA children. After adjustment for possible confounding factors (age, sex, ethnicity, birth weight SDS and birth length SDS), Si, Sg and AIR were comparable for both groups, but DI was still higher for preterm SGA children, though this did not reach significance (p=0.066). After additional adjustment for body fat % SDS and height SDS, both AIR and DI were significantly higher in preterm than in term SGA children.

Fasting glucose and insulin levels and HOMA insulin resistance index (HOMA-IR) were comparable for preterm and term SGA children. This remained so after adjustment for potential confounding factors (age, sex, ethnicity, birth weight SDS, birth length SDS, fat % SDS and height SDS).

	All			SGA born Preterm < 36 wks			SGA born Term ≥ 36 wks			Unadjusted P-value	Model 1* P-value	Model 2* P-value
	N			N			N					
Si*10^{-4}/min^{-1} (μU/ml)	77	14.0	(10.5-19.3)	31	13.2	(10.7-19.9)	46	14.6	(10.4-19.0)	0.876	0.947	0.715
Sg*10^{-2}/min^{-1}	77	2.0	(1.6-2.7)	31	1.9	(1.5-2.5)	46	2.1	(1.7-2.9)	0.072	0.289	0.177
AIR (mU/L)	77	267	(176-350)	31	282	(200-397)	46	220	(162-336)	0.061	0.159	0.033
DI (AIR * Si)	77	3312	(2602-4750)	31	3729	(2886-5503)	46	3010	(2514-4654)	0.046	0.066	0.021
F Glucose (mmol/L)	217	4.6	(0.7)	68	4.5	(0.7)	149	4.7	(0.7)	0.183	0.885	0.599
F Insulin (mU/L)	156	6.0	(2.8)	55	6.0	(2.5)	101	6.0	(2.9)	0.900	0.371	0.913
HOMA-IR	155	0.7	(0.4)	55	0.8	(0.3)	100	0.7	(0.4)	0.962	0.355	0.988

Data are expressed as mean (SD), except FSIGT parameters, which are median (interquartile range); F=fasting

* Model 1: adjusted for age, sex, ethnicity, birth weight SDS and birth length SDS (n=57 for FSIGT; n=185 for F glucose; n=136 for F insulin; n=135 for HOMA-IR)
Model 2: adjusted for age, sex, ethnicity, birth weight SDS, birth length SDS, fat % SDS and height SDS (n=43 for FSIGT; n=82 for F glucose; n=33 for F insulin and HOMA-IR)

Blood pressure

Preterm SGA children had a significantly higher systolic (p=0.010) and diastolic (p<0.0005) blood pressure SDS than term SGA children (Table 4). This remained so after adjustment for possible confounding factors (age, sex, ethnicity, birth weight SDS, birth length SDS, BMI SDS and height SDS). Also, the percentage of children with a high systolic blood pressure was higher in preterm SGA children (26.3 %) compared with term SGA children (16.8 %).

Lipids

Preterm SGA children had significantly lower total cholesterol levels than term SGA children, but the difference was small and disappeared after adjustment for possible confounding factors (age, sex, ethnicity, birth weight SDS, birth length SDS, BMI SDS and height SDS) (Table 4). Serum levels of HDL-c, LDL-c, triglyceride, FFA, apo-A1, apo-B, the apo-B/apo-A1 ratio and the percentage children with a Lp(a) level ≥ 0.3 g/l were similar for both groups, also after adjustment for possible confounding factors (age, sex, ethnicity, birth weight SDS, birth length SDS, BMI SDS and height SDS).

	All		SGA born Preterm < 36 wks		SGA born Term ≥ 36 wks		Unadjusted P-value	Model 1* P-value	Model 2* P-value
	N		N		N				
Systolic BP SDS#	454	0.8 (1.0) §	156	1.0 (1.1) §	298	0.7 (1.0) §	0.010	0.003	0.003
Diastolic BP SDS#	454	0.4 (1.0) §	156	0.6 (1.0) §	298	0.2 (1.0) §	<0.0005	0.058	0.026
High Systolic BP (% >P95)	454	20.0	156	26.3	298	16.8	0.016	0.030	0.022
High Diastolic BP (% >P95)	454	11.0	156	14.7	298	9.1	0.066	0.964	0.713
TC (mmol/l) [3.0-5.5]	235	4.3 (0.7)	71	4.1 (0.7)	164	4.4 (0.8)	0.019	NS	NS
HDL-c (mmol/l) [0.9-1.9]	233	1.4 (0.3)	70	1.4 (0.3)	164	1.4 (0.3)	NS	NS	NS
LDL-c (mmol/l) [1.3-3.4]	232	2.5 (0.7)	71	2.4 (0.7)	161	2.5 (0.7)	NS	NS	NS
TG (mmol/l) [0.4-1.6]	196	0.7 (0.6-0.9)	60	0.6 (0.5-0.8)	136	0.6 (0.5-0.8)	NS	NS	NS
FFA (mmol/l) [0.2-1.2]	132	0.7 (0.4-0.9)	39	0.8 (0.5-1.0)	93	0.7 (0.4-0.9)	NS	NS	NS
Apo-A1 (g/l) [0.9-1.6)	106	1.4 (0.2)	33	1.4 (0.2)	73	1.4 (0.2)	NS	NS	NS
Apo-B (g/l) [0.5-1.3]	106	0.7 (0.2)	33	0.7 (0.1)	73	0.7 (0.2)	NS	NS	NS
Apo-B/Apo-A1 ratio	106	0.53 (0.14)	33	0.52 (0.13)	73	0.53 (0.14)	NS	NS	NS
Lp(a) ≥ 0.3 g/l (%)	105	17.1	33	12.1	72	19.4	NS	NS	NS

Data expressed as mean (SD), except TG and FFA, which are median (interquartile range)
TC: total cholesterol; TG: triglycerides; HDL-c= high density lipoprotein cholesterol; LDL-c= low density lipoprotein cholesterol; FFA= free fatty acids; Apo-A1= apolipoprotein A1; Apo-B= apolipoprotein B; Lp(a)= lipoprotein A; SDS: standard deviation score ; BP= blood pressure
= corrected for height and sex
The values in brackets after the variables in column 1 represent reference ranges for children of the same age.
§ = p<0.001 versus zero
* Model 1: adjusted for age, sex, ethnicity, birth weight SDS & birth length SDS (n=331 for RR; 197 for TC; 195 for HDL-c; 194 for LDL-c; 158 for triglycerides; 103 for FFA; 82 for apo-A1&B∶ 81 for Lp(a))
Model 2: adjusted for age, sex, ethnicity, birth weight SDS, birth length SDS, BMI SDS & height SDS (n=331 for RR; 197 for TC; 195 for HDL-c; 194 for LDL-c; 158 for triglycerides; 103 for FFA; 82 for apoA1&B&ratio)

Multiple regression analyses

Because we were also interested in the relative contribution of several parameters, such as anthropometry at birth and gestational age, to the various cardiovascular risk parameters, we performed backward multiple regression analyses on the total study group (Table 5). The multiple regression analyses indicated that gestational age was indeed a significant contributor to the variance in body fat % SDS, insulin secretion, disposition index, and systolic and diastolic blood pressure SDS. Gestational age was not a significant determinant of insulin sensitivity.

	Insulin sensitivity (Si)*		Insulin secretion (AIR)*		Disposition index (DI)*		Body fat % SDS		Systolic BP SDS		Diastolic BP SDS	
Variables	ß	P-value	ß	P-value	ß	P-value	ß	P-value	ß	P-value	ß	P-value
Age (yr)	-0.398	<0.0005	0.342	0.002		NS		NS	0.074	0.001	-0.076	0.001
Sex #		NS		NS		NS	-0.339	0.045	0.220	0.053		NS
Birth weight SDS		NS		NS		NS		NS		NS		NS
Birth length SDS		NS		NS	0.093	0.027	0.139	0.012		NS		NS
Gestational age (wks)		NS	-0.039	0.094	-0.044	0.011	0.041	0.079	-0.052	0.001	-0.036	0.023
Body fat % SDS	-0.156	0.097	0.297	0.009		NS		N/A		N/A		N/A
BMI SDS		N/A		N/A		N/A		N/A	0.171	0.002		NS
Overall		0.001		0.005		0.020		0.002		<0.0005		<0.0005
R^2		0.37		0.36		0.23		0.14		0.08		0.05
Adjusted R^2		0.33		0.29		0.18		0.11		0.07		0.05

* Dependent variable was log-transformed prior to analysis
# Coded as male=1 and female=2
SDS = standard deviation score
BMI = body mass index
NS = not significant
N/A = not applicable

Discussion

In this study we investigated whether preterm birth had an independent influence on several cardiovascular risk factors in short children born SGA. Preterm SGA children were significantly lighter and shorter at birth after correction for gestational age than term SGA children, but had a comparable head circumference. In preterm SGA children, we found a significantly higher systolic and diastolic blood pressure, lower body fat % SDS and higher disposition index, also after adjustment for the degree of SGA. Insulin sensitivity, serum lipid levels, muscle mass and body fat distribution were comparable for preterm and term SGA children.

Our study shows that premature SGA children had a more severe degree of growth retardation. Previously, we showed that SGA children with a more severe phenotype were more likely to be delivered by an elective caesarean section (25). It is likely that the preterm elective delivery of these SGA children was prompted by the severe growth retardation. Preterm SGA children had a lower body fat % SDS than term SGA children. Because preterm SGA children also had a more severe degree of SGA, as indicated by their lower birth weight SDS and birth length SDS, we adjusted for these possible confounding factors. After adjustment for anthropometry at birth, premature SGA children still had a lower body fat % SDS than term SGA children. As the postnatal period of children born preterm is often characterized by nutritional problems (26), this might be held responsible for the reduced percentage of body fat in preterm SGA children.

Insulin sensitivity was comparable between preterm and term SGA children, but body fat % SDS, which is known to strongly correlate with insulin sensitivity, was lower in preterm SGA children. Also, insulin secretion tended to be higher in preterm SGA children. Both findings might indicate a relative insulin resistance in preterm SGA children. However, after adjustment for body fat % SDS, insulin sensitivity remained comparable between preterm and term SGA children. Therefore, we cannot conclude that preterm SGA children have a relative insulin resistance.

An unexpected finding was that the disposition index was higher in preterm than in term SGA children. The disposition index reflects the capacity of pancreatic islets to compensate for a lower insulin sensitivity (27). Our data are in line with those of the Dutch famine studies. Ravelli et al. (29) showed that glucose tolerance was worse in those that were exposed to famine during mid or late gestation. Hofman et al. (5, 30) also reported a higher insulin secretion and disposition index in preterm SGA children than in term SGA children, but unfortunately they did not provide statistical comparisons. The difference in disposition index between preterm and term SGA children might be explained by the timing of prenatal growth retardation. All children in our study were born SGA. Therefore, the children that were born prematurely must have had retarded growth before the third trimester. Of the children born at term, it is not known when they had their growth retardation, but it seems likely that if growth was impaired early in pregnancy, these children would not have been born at term. The higher disposition index in preterm SGA children indicates that their insulin secretion is higher than would be expected from their level of insulin sensitivity. This could be

due to either a greater insulin secretion or a lower hepatic insulin extraction (28). It has been suggested that a lower liver mass might disadvantageously affect hepatic insulin extraction and it is not unthinkable that this is the case in preterm SGA subjects. Due to the wide range and the modest difference in disposition index between preterm and term SGA children, the clinical relevance is questionable. Nevertheless, our data show that short preterm SGA children had a glucose homeostasis that was not worse than that of term SGA children.

Preterm SGA children had a significantly higher systolic and diastolic blood pressure. Notably, more than 25% of all premature SGA children had high systolic BP according to the modified ATP (Adult Treatment Panel) III criteria for children(31). An elevated blood pressure in childhood is known to be associated with an increased risk for the development of hypertension in adulthood (32). In previous studies, prematurity itself has been associated with increased blood pressure (7, 8), irrespective of the degree of SGA. However, other reports suggested that among preterms only those born SGA had abnormalities in their blood pressure and vascular function (9, 10). The etiology of the relationship between small size at birth and an elevated blood pressure is still unclear, but there are several hypotheses. According to Brenner et al. (33) intra-uterine growth retardation leads to a reduced number of nephrons. This may lead to a reduced filtration surface area, renal sodium retention and ultimately hypertension. Rodríguez et al. (34) demonstrated in renal autopsy tissue that glomerulogenesis continues after preterm birth, but stops after 40 days. Preterm infants had less glomeruli than infants born at term (34). So, both intra-uterine growth retardation and prematurity may lead to a reduced number of nephrons. In our study, we demonstrate that both preterm and term SGA children have an increased systolic and diastolic blood pressure, but that preterm SGA children are more affected than term SGA children.

The multiple regression analyses indicated that gestational age was indeed a significant contributor to the variance in body fat % SDS, insulin secretion, disposition index, and systolic and diastolic BP SDS. The regression models for insulin secretion and disposition index resulted in an explained variance of approximately 30%. Since the models for systolic and diastolic BP SDS explained not more than 7.0% of the total variation, we must, however, conclude that gestational age is not a major factor in determining childhood blood pressure levels. A limitation is that we measured blood pressure by two measurements and this might not reflect the 24-hour blood pressure. Other parameters were measured in a subgroup, which means that the results might not be applicable to the total study population. However, subjects of the subgroups were recruited randomly and consecutively and there was no evidence that these were different from the others.

In conclusion, within a population of short SGA children, preterm birth has divergent effects on several cardiovascular risk factors. Whereas preterm SGA children had a higher systolic and diastolic blood pressure, they had a lower body fat % and a higher insulin secretion and disposition index than term SGA children.

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CHAPTER 8

Insulin sensitivity in young adults born premature

Ruben H. Willemsen[1], Ralph W.J. Leunissen[1], Theo Stijnen[2], Anita C.S. Hokken-Koelega[1]

[1]*Dept. of Pediatrics, Division of Endocrinology, Erasmus MC Sophia, Rotterdam,* [2]*Dept. of Epidemiology and Biostatistics, Leiden University Medical Center, Leiden, the Netherlands*

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Abstract

Background: In 2005, 12.7% of all babies was born preterm, and the incidence is rising. Nowadays, due to improved survival, an increasing number of children born premature reach young adulthood. A recent report suggested a lower insulin sensitivity in children born premature, which may put them at risk for the development of type 2 diabetes. It is, however, still unknown whether this reduced insulin sensitivity persists into adulthood.

Methods: We determined insulin sensitivity and ß-cell function with frequently sampled intravenous glucose tolerance tests, in 305 young adults (aged 18-24; 169 preterm and 136 term). We investigated the effect of gestational age, size at birth and adult body composition on insulin sensitivity. In a more detailed analysis, we studied the same parameters in clinically relevant subgroups: small-for-gestational-age (SGA) without catch-up growth, SGA with catch-up growth and appropriate-for-gestational-age (AGA) subjects, either born preterm or term.

Results: In contrast to previous reports, we found no evidence that preterm birth has deleterious effects on insulin sensitivity in young adulthood. Trunk fat was the most important determinant of insulin sensitivity, independently of size at birth and duration of pregnancy. Subgroup comparison showed that only term SGA subjects with spontaneous catch-up growth to a normal height had significantly lower insulin sensitivity than term AGA controls. However, taking insulin secretion into account, glucose homeostasis in all clinically relevant subgroups was comparable with term AGA controls.

Conclusion: Contrary to our hypothesis, preterm birth was not associated with reduced insulin sensitivity in young adulthood.

Introduction

Small size at birth has been associated with an increased incidence of adult cardiovascular disease and diabetes mellitus type 2 (1-4). However, size at birth is determined by 2 factors: intrauterine growth and duration of pregnancy. Most epidemiologic studies focused on subjects who were born with a lower weight. Unfortunately, size at birth was often not adjusted for gestational age. Therefore, it has been difficult to interpret whether the negative effect of a small size at birth on cardiovascular risk factors was due to a small size for gestational age (SGA) or due to prematurity.

Because of advances in neonatal intensive care, survival of preterm infants has improved and most children are now reaching young adulthood. In 2005, the preterm birth rate in the United States was 12.7%, which corresponds with approximately 500,000 preterm births per year (5). Moreover, this percentage is 20% higher than the preterm birth rate in 1990 (5). There has been quite some attention to the late effects of prematurity on intellectual capacities and psychomotor development, but data regarding risk factors for cardiovascular disease and diabetes are scarce.

A previous study in prepubertal children indicated that preterm children, either born SGA or appropriate-for-gestational-age (AGA), had lower insulin sensitivity than those born at term (6). It is, however, still unknown whether this difference persists into adulthood. In a group of young adults with low birth weight, glucose homeostasis was studied with oral glucose tolerance tests (OGTT) (7). This study showed higher indexes of glucose intolerance in those born premature (7). Unfortunately, OGTT are dependent upon the uptake of glucose in the digestive system and are therefore less accurate than intravenous tests for the assessment of glucose homeostasis. Besides, no information regarding insulin sensitivity and secretion is obtained.

The aim of our study was to determine insulin sensitivity in young adults with very low birth weight and to examine the influence of prematurity and SGA on insulin sensitivity. As a secondary aim, we investigated the effect of adult body composition and early postnatal growth on the same outcome parameters. We hypothesized that preterm birth is associated with a lower insulin sensitivity in young adulthood, independently of size at birth.

Subjects and methods

Subjects

The study population consisted of 305 healthy subjects with an age between 18 and 24 years. Premature subjects (n=169) were born before 36 weeks of gestation and had been admitted to the neonatal intensive care unit shortly after birth. Term subjects (n=136) were born after at least 36 weeks of gestation and randomly selected from hospitals in the Netherlands, where they had been registered because of 1) being small at birth (SGA with a birth length <-2 SD) (8), 2) showing short stature in childhood,

either after being born SGA or AGA (defined as a birth length >-2 SD) or 3) having a minor accidental health problem, but otherwise having a normal size at birth and in adulthood. All subjects fulfilled the same inclusion criteria: 1) age 18-24 years; 2) Caucasian; 3) singleton; 4) a neonatal period without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes) or long-term complications of respiratory ventilation, such as broncho-pulmonary dysplasia; 5) maximum duration of respiratory ventilation and/or oxygen supply in the neonatal period of 2 weeks. The following subjects were excluded: those with a serious complication (including necrotizing enterocolitis, intraventricular haemorrhage with a degree of 3 or more, spastic hemiplegia or quadriplegia), endocrine or metabolic disorders, chromosomal defects, syndromes, dysmorphic symptoms suggestive for a yet unknown syndrome or a condition known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, growth hormone treatment, treatment with glucocorticosteroids, radiotherapy). Birth data were taken from records of hospitals, community health services and general practitioners. The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam, the Netherlands, approved this study. Written informed consent was obtained from all the participants.

Based on SD-scores of birth length (8) and adult height (9), the study population was also subdivided into six groups: short SGA (birth length and adult height <-2 SDS), SGA with catch-up growth (birth length <-2 SDS and adult height >-1 SDS) and AGA (birth length and adult height > -1 SDS), either born premature (<36 weeks) or at term (≥36 weeks). In order to increase the statistical power for subgroup comparison, the cut-off values for these subgroups were set at -2 SDS and -1 SDS (+/- 0.1 SDS). Of the 305 subjects, 182 could be included in one of the subgroups.

Anthropometry

Height was measured using a Harpenden stadiometer and expressed as SD-score for sex and age using Dutch references (9). Body mass index (BMI) was calculated according to the formula weight/(height)^2 and expressed as SD-score for sex and age (10).

Body composition

Body composition was assessed by Dual-Energy X-ray Absorptiometry (DXA, type Lunar-Prodigy, GE Healthcare, Chalfont St Giles, UK). All scans were made on the same machine and quality assurance was performed daily.

Insulin sensitivity and secretion

After an overnight fast, a modified frequently sampled intravenous glucose tolerance test (FSIGT) with Tolbutamide was performed, as previously described (11, 12). 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 (13). 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.

Assays

All blood samples were taken after an overnight fast. Serum glucose levels were determined on a VITROS analyser 750 (Orthoclinical Diagnostics, Johnson&Johnson Company, Beerse, Belgium). Insulin levels were measured using the same method (IRMA; Medgenix, Biosource Europe, Nivelles, Belgium). The intra-assay coefficient of variation (CV) was 2.0-4.7% and the inter-assay CV was 4.2-11.3%. All assays were performed in one central laboratory.

Statistical analysis

To normalize the distribution, total fat mass, Si, Sg and AIR were logarithmically transformed and for DI the square root was taken prior to analyses. Differences between groups were tested by one-way analysis of variance (ANOVA) with Bonferroni's post-hoc test. Differences in categorical variables were tested with Chi square test. Multiple regression analyses with logarithmically transformed Si and the square root of DI as the dependent variable were used to assess multivariate relationships. The interaction term birth length SDS x adult height SDS was added to all MR models because the study group had been selected on birth length and adult height, in order to ensure that the effect of these variables was modeled correctly. Level of significance was set at $p<0.05$. Statistics were performed using the computer statistical package SPSS (version 12.0; SPSS Inc., Chicago, IL).

Results

Table 1 shows the baseline characteristics. The total group of 305 subjects had a mean age of 20.9 years. Figure 1 depicts insulin sensitivity on the x-axis and insulin secretion on the y-axis, in the total study population. Markers indicate the preterm and term subjects. The hyperbolic relation between insulin sensitivity (Si) and insulin secretion (AIR) is evident as well as the overlapping pattern of measurements in preterm and term subjec

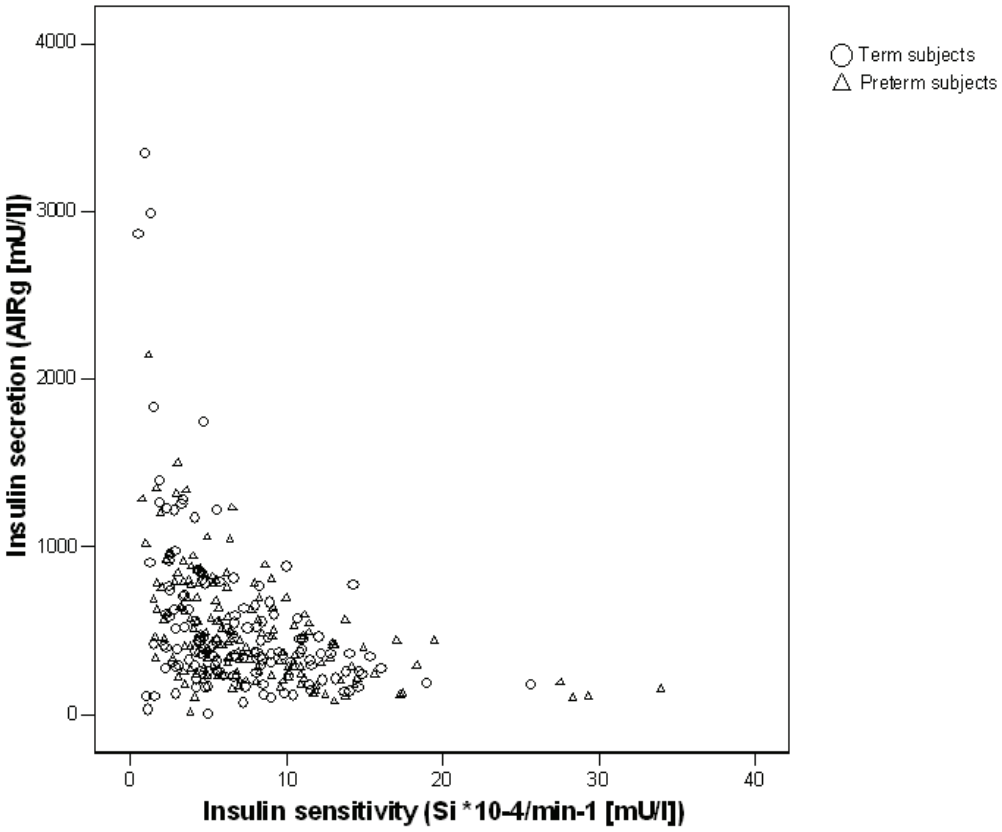

Figure 1. Insulin sensitivity and insulin secretion of preterm and term subjects

Table 1 Baseline characteristics of the total group and the subgroups

	Total group	Preterm SGA-S	Term SGA-S	Preterm SGA-CU	Term SGA-CU	Preterm AGA	Term AGA
N	305	9	25	34	23	65	26
Gender (M/F)	154/151	5/4	11/14	15/19	13/10	39/26	14/12
Gestational age (wks)	35.2 (4.0)	32.3 (1.5) [1]	39.4 (1.3)	32.3 (2.0) [1]	38.1 (1.3)	32.2 (2.4) [1]	39.1 (1.5)
Birth length SDS	-1.4 (1.7)	-3.6 (1.0) [2]	-2.7 (0.6) [2]	-3.1 (0.7) [2]	-2.7 (0.7) [2]	0.4 (0.8)	0.1 (0.7)
Birth weight SDS	-0.8 (1.7)	-2.5 (0.9) [2]	-2.0 (0.7) [2]	-2.2 (1.0) [2]	-2.3 (0.6) [2]	0.8 (1.1) [3]	0.0 (1.1)
Age (yrs)	20.9 (1.6)	21.7 (1.8)	20.9 (1.6)	20.5 (1.8)	21.2 (1.4)	21.1 (1.6)	20.9 (1.6)
Height SDS	-0.9 (1.2)	-2.3 (0.3) [4]	-2.6 (0.6) [4]	-0.1 (0.6)	-0.4 (0.6) [5]	0.1 (0.6)	0.1 (0.9)
BMI SDS	0.0 (1.2)	0.4 (1.3)	0.0 (1.4)	-0.3 (1.2)	0.5 (1.3)	0.2 (1.0)	0.0 (1.3)
Adult Fat %	24.4 (10.6)	25.4 (10.1)	24.9 (8.6)	23.8 (10.5)	25.9 (10.9)	24.7 (11.1)	22.8 (9.9)
Adult Fat (kg)	14.7 (9.3-21.4)	16.6 (8.6-21.3)	13.6 (9.0-19.0)	14.9 (8.7-20.2)	18.1 (10.5-23.1)	17.0 (10.2-24.5)	14.2 (9.8-21.0)
Adult LBM (kg)	46.7 (10.0)	42.9 (8.1)	40.0 (7.7) [2]	47.4 (9.9)	51.0 (9.7)	51.9 (9.3)	51.8 (10.8)
Trunk fat/total fat ratio	0.49 (0.06)	0.52 (0.06)	0.48 (0.06)	0.48 (0.06)	0.51 (0.07)	0.50 (0.05)	0.47 (0.05)

All values presented as mean (SD), except for adult fat, which is median (interquartile range)
SDS= standard deviation score; LBM= lean body mass
[1]: p<0.0005 compared with the term subgroups; [2]: p<0.0005 compared with AGA; [3]: p<0.01 compared with term AGA; [4]: p<0.0005 compared with SGA-CU & AGA;
[5]: p<0.05 compared with preterm AGA

To investigate the most important determinants for insulin sensitivity, we made several regression models (Table 2). The initial multiple regression model had insulin sensitivity as dependent variable, and age, sex, oral contraceptive use (OC-use), gestational age, adult height SDS, birth length SDS, the interaction term adult height SDS x birth length SDS and birth weight SDS as independent variables. In Model A, birth weight SDS and OC-use were significant determinants of insulin sensitivity (adjusted $R2 = 0.10$). Addition of adult weight SDS to the model resulted in a model with OC-use, adult height SDS and adult weight SDS as significant determinants of insulin sensitivity (Model B). Specifying adult weight SDS into adult fat mass and lean mass, measured by DXA, showed that adult fat mass was the most important determinant of insulin sensitivity ($p<0.0005$; adjusted $R2 = 0.33$; Model C). In our final model D, we specified adult fat mass into trunk and limbs fat mass, and it appeared that trunk fat had a negative effect on insulin sensitivity, whereas limbs fat was not a significant determinant.

In none of the models, gestational age was a significant determinant of insulin sensitivity. There were no significant interactions between birth weight SDS, birth length SDS or gestational age and adult fat mass, indicating that the effect of adult fat mass on insulin sensitivity was not modified by size at birth or gestational age.

Table 2. Multiple regression analyses for insulin sensitivity in the total group

	Natural logarithm of Si							
	Model A		Model B		Model C		Model D	
Variables	ß	p-value	ß	p-value	ß	p-value	ß	p-value
Gestational age (wks)	0.004	0.953	0.004	0.945	-0.001	0.987	-0.009	0.878
Birth length SDS	-0.019	0.855	0.040	0.679	0.057	0.533	0.050	0.583
Birth weight SDS	0.234	**0.018**	0.168	0.065	0.115	0.180	0.112	0.191
Age (yrs)	0.019	0.740	0.028	0.595	0.071	0.159	0.079	0.116
Gender*	0.061	0.489	0.089	0.278	-0.066	0.613	0.031	0.823
Oral contraceptive use#	-0.213	**0.017**	-0.180	**0.030**	-0.174	**0.025**	-0.174	**0.024**
Height SDS	0.126	0.119	0.367	**<0.0005**	0.203	**0.022**	0.201	**0.022**
BL*AH (SDS)	0.176	0.052	0.177	**0.035**	0.148	0.059	0.150	0.055
Weight SDS			-0.438	**<0.0005**				
Fat mass (kg)					-0.523	**<0.0005**		
Trunk fat mass (kg)							-0.571	**<0.0005**
Limbs fat mass (kg)							0.069	0.660
Lean body mass (kg)					-0.030	0.807	-0.037	0.763
Overall		<0.0005		<0.0005		<0.0005		<0.0005
R^2		0.12		0.26		0.35		0.36
R^2 adjusted		0.10		0.23		0.33		0.34

Beta's represent standardized regression coefficients
SDS= standard deviation score
*= coded as 1=male; 0= female
#= coded as 1= OC-use; 0= no OC-use

Subgroup analysis

For a more detailed analysis, we also investigated differences in insulin sensitivity and ß-cell function between 6 clinically relevant subgroups: SGA subjects with persistent short stature (SGA-S), SGA subjects with spontaneous catch-up growth (SGA-CU) and normal AGA subjects, divided in term and preterm subgroups. Sex distribution, age, BMI SDS, fat percentage, total fat mass and trunk fat/total fat ratio were comparable in all the subgroups (Table 1). Next to differences due to the selection criteria, lean body mass in term SGA-S subjects was significantly lower than AGA controls, in line with their reduced height SDS.

Table 3 shows the unadjusted results of the FSIGT-tests in the various subgroups. Insulin sensitivity, glucose effectiveness, insulin secretion and disposition index were comparable in all the subgroups. Insulin sensitivity was lowest in term SGA-CU subjects. The difference with the other subgroups reached significance in preterm AGA subjects only ($p<0.05$).

Table 3. FSIGT results*

	Total group	Preterm SGA-S	Term SGA-S	Preterm SGA-CU	Term SGA-CU	Preterm AGA	Term AGA
N	294	8	25	32	23	62	25
Si $*10^{-4}/min^{-1}$ (mU/l)	6.2 (3.8-9.9)	8.4 (5.9-10.6)	5.5 (2.8-9.3)	5.4 (4.0-11.7)	4.1 (2.8-6.7)[1]	7.0 (4.3-10.3)	8.0 (4.9-10.8)
Sg $*10^{-2}/min^{-1}$ (mg/d)	1.8 (1.5-2.2)	1.8 (1.6-2.1)	1.9 (1.6-1.9)	1.8 (1.5-2.2)	1.9 (1.3-2.1)	1.7 (1.4-2.1)	1.8 (1.4-2.0)
AIR (mU/l)	417 (262-659)	338 (205-707)	441 (255-592)	567 (299-774)	551 (378-921)	339 (236-560)	362 (266-584)
DI (Si*AIR)	2523 (1585-3903)	2991 (1465-3702)	2466 (1611-3786)	2820 (1976-4279)	2182 (1388-4015)	2539 (1534-3661)	2877 (1599-4155)

* Unadjusted data
[1] $p<0.05$ vs. preterm AGA

Table 4 shows differences in insulin sensitivity in the clinically relevant subgroups compared with term AGA controls, after adjustment for several confounding factors. Using term AGA subjects as the reference group, only term SGA-CU subjects had a significantly reduced insulin sensitivity (Model 1; Table 4). This remained so after adjustment for age, sex, and parameters known to influence insulin sensitivity (Models 2-4; Table 4). Disposition index, which indicates how well the ß-cells compensate for lower values of insulin sensitivity, was not different between the clinically relevant subgroups and term AGA controls in all models (data not shown). Thus, taking insulin secretion into account, glucose homeostasis was comparable with term AGA controls in all subgroups.

Table 4. Differences in insulin sensitivity in clinically relevant subgroups compared with term AGA controls

	Natural logarithm of Si							
	Model 1 Unadjusted		Model 2 Adjusted for age, gender and OC-use		Model 3 Adjusted for age, gender, OC-use, total fat and total lean mass		Model 4 Adjusted for age, gender, OC-use, trunk & limbs fat mass and total lean mass	
Subgroups	ß	P-value	ß	P-value	ß	P-value	ß	P-value
Preterm SGA-S*	0.125	0.649	0.029	0.913	0.105	0.658	0.166	0.480
Term SGA-S*	-0.327	0.086	-0.281	0.131	-0.249	0.161	-0.234	0.182
Preterm SGA-CU*	-0.153	0.393	-0.111	0.522	-0.105	0.467	-0.077	0.587
Term SGA-CU*	-0.555	**0.005**	-0.558	**0.004**	-0.403	**0.011**	-0.349	**0.026**
Preterm AGA*	-0.040	0.803	-0.060	0.696	0.034	0.790	0.040	0.751
Overall		0.017		0.001		<0.0005		<0.0005
R^2		0.08		0.14		0.44		0.46
R^2 adjusted		0.05		0.10		0.40		0.42

* Term AGA group is the reference group
OC-use= oral contraceptive use
SGA-S= small-for-gestational-age with persistent short stature
SGA-CU= small-for-gestational-age with spontaneous catch-up growth
AGA= appropriate-for-gestational-age

Effect of early postnatal growth on adult insulin sensitivity, insulin secretion and ß-cell function

For subjects born premature, we studied the association between early postnatal growth and insulin sensitivity, insulin secretion and ß-cell function. There was a weak association between postnatal weight gain from birth until term age and insulin secretion (r=0.22; p=0.006), and between postnatal weight gain from birth until 3 months and insulin secretion (r=0.20; p<0.05), but significance was lost after adjustment for the parameters used in model D (gestational age, birth weight and length SDS, age, gender, OC-use, height SDS, BL*AH, trunk and limbs fat mass and lean body mass). In contrast, insulin sensitivity and ß-cell function (determined as DI) were not associated with postnatal growth in length or weight from birth until term age or from birth until 3 months.

Discussion

In this study we investigated the relative contribution of prematurity and small size at birth (SGA), either with or without catch-up growth, on insulin sensitivity and secretion in young adulthood. Trunk fat and the use of oral contraceptives were the most important determinants of insulin sensitivity in young adulthood, independently of gestational age and size at birth. Contrary to our hypothesis, gestational age was not associated with reduced insulin sensitivity. From all clinically relevant subgroups, only term SGA subjects with spontaneous catch-up growth to a normal height had a significantly lower insulin sensitivity compared with normal term AGA controls. However, taking insulin secretion into account, glucose homeostasis in all clinically relevant subgroups was comparable with term AGA controls.

Our data are in line with a recent study in prepubertal children, comparing preterm and term children born either AGA or SGA (with catch-up growth) (14). Only term SGA children had increased fasting insulin levels and Homeostasis Model Assessment Insulin Resistance (HOMA-IR), whereas preterm children born AGA or SGA were not worse than term AGA controls. Likewise, we previously investigated insulin sensitivity in short SGA children, aged 6 years, and found no differences between preterm and term children (15). The study by Hofman et al. suggested that both AGA and SGA preterm children had reduced insulin sensitivity (6). We can, however, not reproduce their findings in our large study of 305 young adults. Our data clearly show that gestational age has no effect on insulin sensitivity in young adulthood.

Adult trunk fat mass was the most important determinant of insulin sensitivity in young adulthood, regardless of size at birth and gestational age. Finken et al. investigated surrogate measures of insulin resistance, i.e. HOMA-IR, C-peptide and fasting insulin, in a group of 19-year olds born before 32 weeks of gestation (16). They reported that adult obesity measures, such as BMI, waist circumference and fat mass, which was calculated from skinfolds measurements, were the strongest predictors of higher

HOMA-IR, insulin and C-peptide levels.

Hovi et al. performed OGTT in a cohort of preterm born young adults with very low birth weight and term AGA controls and demonstrated differences in glucose regulation between preterms and controls (7). We found no evidence for a worse glucose regulation, as measured by intravenous glucose tolerance tests, in preterm born young adults compared to term controls. Possible explanations for the differences between their and our study are the following. Firstly, Hovi et al. used OGTT, which are dependent upon the uptake of glucose in the digestive system. This test is therefore less accurate than an intravenous test for the assessment of insulin sensitivity and ß-cell function. Secondly, there were some differences in the selection of subjects. We did not include subjects with serious complications from preterm birth, such as cerebral palsy and necrotizing enterocolitis or subjects with prolonged respiratory ventilation or oxygen supply. Thirdly, all subjects in our cohort were Caucasian and singleton, whereas this was not clear in the study of Hovi et al.

We found a negative effect of the use of oral contraceptives in women. Also when we analyzed women only, this adverse effect remained significant. The deleterious effect of oral contraceptives on insulin sensitivity is well documented and most likely due to estrogens (17). In all previous studies on the effect of birth weight on parameters of glucose homeostasis, this factor was not taken into account. We feel that it is certainly a factor that should be included in such analyses.

We found no evidence that preterm birth has long-lasting deleterious effects on insulin sensitivity in this cohort of young adults. The fact that only term SGA subjects with spontaneous catch-up growth had a significantly lower insulin sensitivity than term AGA controls suggests that intrauterine events in the third trimester followed by spontaneous catch-up growth might be associated with lower insulin sensitivity. Notably, this subgroup had the highest fat percentage and total fat mass, which is associated with a lower insulin sensitivity (18). Our data clearly show that a large trunk fat mass, and not size at birth or the duration of pregnancy, is the main determinant of insulin resistance in young adulthood.

Our data indicate that insulin sensitivity and ß-cell function are not adversely affected in survivors of preterm birth at the age of 18-24 years. This is reassuring, but does not guarantee that glucose homeostasis remains normal on the long term, later in life. Therefore, long-term follow-up is still warranted.

In conclusion, in a cohort of 305 young adults, adult trunk fat mass was the most important predictor of insulin sensitivity, and birth weight, birth length and gestational age were no significant determinants. Compared with term AGA controls, only term SGA subjects with spontaneous catch-up growth had a significantly lower insulin sensitivity, whereas preterm subjects, regardless of being born AGA or SGA, with or without catch-up growth, had comparable insulin sensitivity. Taking insulin secretion into account, glucose homeostasis in all clinically relevant subgroups was comparable with term AGA controls, indicating that glucose homeostasis is not adversely affected at this age in any of the clinically relevant subgroups.

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CHAPTER 9

General discussion

General Discussion

The present thesis describes cardiovascular and metabolic risk factors in two conditions associated with a small size at birth: SGA and preterm birth. The first part consists of several studies in SGA children with persistent short stature. The following parameters for the assessment of cardiovascular risk were studied: (i) novel cardiovascular risk markers, (ii) body composition and (iii) insulin sensitivity and β-cell function. For all outcome parameters also the effect of GH treatment was studied. The second part focuses on long-term consequences of preterm birth on cardiovascular and metabolic risk parameters. We investigated the effect of prematurity on glucose homeostasis, blood pressure and lipid levels within a population of short SGA children. Besides, insulin sensitivity and β-cell function were studied in young adults born premature.

Part 1: Short SGA children

Novel cardiovascular risk markers

Serum adiponectin and resistin levels before and during 2 years of GH treatment

In a group of short SGA children we studied serum adiponectin and resistin levels before and during 2 years of GH in comparison with untreated sex- and age-matched short SGA children and a normal-statured control group. Before treatment, serum adiponectin levels of short SGA children were comparable with normal-statured controls and resistin levels were lower. Neither adiponectin nor resistin levels changed significantly during 2 years of GH treatment. Compared with untreated age- and sex-matched short SGA children, GH-treated SGA children had similar adiponectin levels and lower resistin levels. This suggests an increase in resistin levels over time in untreated short SGA children, which could be confirmed in paired samples of untreated SGA children, though this group was small and the p-value did just not reach significance. Adiponectin correlated inversely with age, but not with systolic or diastolic blood pressure, fasting insulin, glucose and lipid levels or any growth factor.

The absence of associations with cardiovascular and metabolic risk factors in these lean SGA subjects is in line with data from Martin et al. (3), who reported that the association between adiponectin and fasting insulin and lipids was dependent on the degree of adiposity, such that the association was absent or weaker in leaner subjects. Short SGA children have a low fat mass, as described in chapter 5 of this thesis, which makes it very plausible that these associations were not present.

Previous studies on adiponectin levels in SGA reported lower (4, 5), similar (6) or higher (7) levels compared with AGA children. Notably, the SGA groups for which lower adiponectin levels were reported all consisted of a mixture of short SGA children and SGA children with spontaneous catch-up growth. More recent data indicate that SGA children with spontaneous catch-up growth, particularly those who become overweight, are more at risk for cardiovascular disease than those who remain short (8).

Previously, Ibañez et al. (6) reported a decrease of adiponectin levels in 16 short SGA children during 6 months of GH treatment. Our data demonstrate that 2 years of GH treatment had no effect on adiponectin and resistin levels in 50 short SGA children. Van Dijk et al. (9) also found a decrease in adiponectin levels during the first 6 months of GH treatment, but this was followed by an improvement thereafter. Taking these data together, we can conclude that the reducing effect of GH on adiponectin is only temporary. This is reassuring because it shows that GH treatment is not associated with disadvantageous changes on the longer term in these adipocytokines, which have emerged as possible new markers for the development of cardiovascular disease and diabetes mellitus type 2.

Long-term dose effects of GH treatment and GH discontinuation on serum adiponectin, resistin, IL-6 and CRP levels

In another group of 103 short SGA children, we studied the effect of 7 years of GH treatment in 2 different doses and the effect of discontinuation of GH on serum adiponectin and resistin levels, as well as the inflammatory markers IL-6 and CRP.

Regarding adiponectin, the GH-treated SGA children had a similar decrease over time as healthy controls. The reduction in adiponectin levels in SGA as well as AGA controls over time is most probably a consequence of puberty (10, 11). Six months after GH was stopped, the levels remained comparable with controls. Resistin levels increased over time, but remained lower than or comparable with controls at all time points. The inflammatory markers IL-6 and CRP did not change during the entire duration of GH treatment and after its discontinuation.

An increase of BMI SDS during childhood and adolescence was associated with a decrease in adiponectin levels. Our data suggest that gaining more fat mass during childhood and adolescence is associated with a worsening of adiponectin levels. The fact that most GH-treated SGA children remained relatively lean during treatment, might thus explain why adiponectin levels remained comparable with controls. Thus, our data underscore that it is important to prevent the development of adiposity, as holds true for every individual.

IL-6 levels correlated with CRP levels at all time points. Also, several correlations between resistin and IL-6 and CRP were demonstrated. Initially, resistin was described as a marker of insulin resistance in mice (12). Since then, several authors have highlighted the proinflammatory properties of resistin (13-15). The associations between resistin and IL-6 and CRP, and the lack of any correlation between resistin and markers of insulin sensitivity in our study, support this view. More and more, subclinical inflammation is considered an independent risk factor for the development of atherosclerosis (16-23). Therefore, resistin is a marker that deserves attention, although the initial ideas about its function as a marker for insulin resistance have changed.

Clinical implications and conclusions

In 2 different studies in short SGA children, we have shown that GH treatment is not associated with disadvantageous changes in adiponectin, resistin, IL-6 and CRP levels. Also 6 months after discontinuation of GH treatment, these markers were comparable with healthy controls. In addition, van Dijk et al. (24) showed that at 6.5 years after stop of long-term GH treatment, adiponectin levels in previously GH-treated young SGA adults were similar to those of untreated young SGA adults as well as those of normal AGA young adults. This is in contrast to a previously reported reduction of adiponectin and an increase of IL-6 levels in 16 short SGA children during 6 months of GH treatment (6). Taking together our data and those of van Dijk et al. (9, 24), we can conclude that GH treatment might coincide with a temporary reduction of adiponectin during the first 6 months only. There are, however, no deleterious effects

of GH on adiponectin levels on the longer term. GH treatment does also not have any disadvantageous effect on IL-6, resistin and CRP levels, which is reassuring, because subclinical inflammation is increasingly mentioned as an independent risk factor for the development of cardiovascular disease and diabetes mellitus type 2.

Plasma matrixmetalloprotein-9 (MMP-9) levels and blood pressure before and during GH treatment

Systolic blood pressure is increased in short SGA children (25, 26) and decreases during GH treatment (1, 26). The underlying mechanism is, however, still unknown. The MMP-system has previously been associated with blood pressure and the incidence of cardiovascular disease (27-31). Randeva et al. (32) demonstrated a decrease in MMP-9 levels during GH replacement therapy in adult growth hormone deficient (GHD) patients, but they did not report data on blood pressure. We hypothesized that MMP-9 levels would be associated with blood pressure in short SGA children. We investigated MMP-9 levels and blood pressure in a group of short SGA children before and during GH treatment in comparison with untreated sex- and age-matched SGA controls. Both MMP-9 levels and systolic blood pressure decreased by approximately 50% in GH-treated SGA children, and remained unchanged in the untreated SGA controls. We did, however, not detect a correlation between MMP-9 and blood pressure. This does not necessarily imply that the MMP system is not involved in the regulation of blood pressure. There might be several explanations for the absence of this correlation. Yasmin et al. (31) showed that the strength of the association between MMP-9 and blood pressure was dependent on the level of blood pressure. Blood pressure might not have been high enough in our group of healthy SGA children to detect a correlation. Moreover, the MMP-system is a complex network in which various agents, such as other MMP types and tissue inhibitors of metalloproteinases (TIMPs) work together in the remodelling of the arterial wall. Thus, future research might be directed at the effects of GH treatment on other MMPs, for example MMP-1, and TIMPs, which can inhibit the activity of MMPs. Also, investigating endothelial function by ultrasonographic assessment of intima media thickness (IMT), flow mediated vasodilation (FMD) and pulse wave velocity (PWV) in combination with parameters of the MMP-system could be valuable (33-37).

Clinical implications and conclusions

Matrixmetalloproteins (MMPs) levels play a role in remodelling of the arterial wall, which may lead to atherosclerosis. High MMP-9 levels have been found in hypertensive patients and predict cardiovascular mortality (38). We have shown that both MMP-9 levels and systolic blood pressure decreased during 3 years of GH treatment in short SGA children, whereas no changes were observed in an untreated sex- and age-matched SGA control group. Our data indicate that GH treatment has a beneficial effect on these cardiovascular risk factors.

Body composition

Long-term effects of GH treatment on body composition and bone mineral density in short SGA children

We investigated the effect of GH treatment on body composition and bone mineral density (BMD) in short SGA children in a longitudinal GH study with a randomized controlled part for 3 years. At baseline, short SGA children had a reduced fat percentage SDS and a low lumbar spine bone mineral density SDS corrected for height ($BMAD_{LS}$). During 6 years of GH treatment, fat percentage SDS decreased and $BMAD_{LS}$ SDS increased. These effects were most prominent in children who started GH at a younger age and in those with a greater height gain during GH treatment. Lean body mass SDS adjusted for height was not reduced at baseline, remained around 0 SDS in GH-treated children, but deteriorated in untreated children.

Data on detailed measurements of body composition is short SGA children are very scarce (39, 40). From a methodological point of view, it is remarkable that most studies investigating body composition in short children did not adjust for height. Therefore, these studies might have reported an underestimation of muscle and fat mass for short children, and an overestimation of the changes during GH treatment. Léger et al. (40) investigated fat and muscle tissue of short SGA children before, during and after GH treatment in a cross-sectional (cs) area by magnetic resonance imaging (MRI) of the thigh. During the first year of GH treatment, the data were compared with AGA controls, who were followed longitudinally as well. The authors reported an increase in muscle tissue cs area and a transient decrease in fat tissue cs area during GH treatment. Even in this important study the parameters were not adjusted for height or, in this case, the total cs area of the thigh, which obviously increases more in GH-treated children than in controls due to increased height velocity. Therefore, it remains unclear if muscle mass had a greater increase in response to GH treatment than expected from the increment in height. Another limitation of that study was the fact that only the thigh was studied.

Surprisingly, in our study the untreated SGA children had a further decline of their muscle mass over time, whereas it was preserved in GH-treated children. A possible explanation might be the reduced food intake in untreated short SGA children, which improved in GH-treated children (39). Our data are in line with those of Singhal et al. (41), who investigated body composition by skinfold thickness measurements. They showed that the association between birth weight z-score and lean mass is stronger in older children than in younger children, after adjustment for age, gender, socioeconomic status, physical activity and height.

Clinical implications and conclusions

Our data show that GH treatment coincides with a reduction in fat % SDS, which is favourable regarding cardiovascular risk, although fat % SDS was already low at baseline. Furthermore, a modest increase in height-adjusted bone mineral density of the lumbar spine was observed. In order to adequately interpret body composition and bone mineral density in short children, at baseline as well as during GH treatment, it is necessary to correct DXA parameters for height.

Insulin sensitivity

Longitudinal changes in insulin sensitivity, ß-cell function and body composition from stop of GH treatment to 6 months thereafter

It is well known that GH treatment reduces insulin sensitivity. A lower birth weight has been associated with an increased risk for the development of cardiovascular disease and diabetes mellitus type 2 (42-44). Because short SGA children appear to have a lower insulin sensitivity than normal children prior to start of GH treatment (25, 45), concern has been expressed regarding the long-term effects of GH treatment on the insulin-glucose homeostasis in this specific population. There have been two previous studies on the effects of discontinuation of GH treatment on insulin sensitivity in short SGA subjects (46, 47), but numbers were small, the children were studied before adult height was attained and the various pubertal stages might have confounded the results.

We measured insulin sensitivity and β-cell function with a frequently sampled intravenous glucose tolerance test (FSIGT), and body composition by dual energy x-ray absorptiometry (DXA) in 48 SGA adolescents and compared their data with 38 AGA controls. In order to study the effect of discontinuation of GH treatment, the subjects were measured longitudinally: at adult height, while still on GH, and 6 months after GH had been discontinued.

Insulin sensitivity was significantly reduced in SGA subjects on GH, but increased significantly after stop of GH treatment when it became similar to insulin sensitivity of AGA controls. There was a compensatory decrease of insulin secretion after stop of GH, not reaching significance. Insulin secretion was significantly higher in SGA subjects, both on and off GH, than in AGA controls. Glucose effectiveness increased significantly after stop of GH treatment and became higher than in controls. Disposition index, which indicates how well the ß-cells can compensate for lower values of insulin sensitivity, improved significantly after stop of GH treatment, reaching a higher value than in controls. Fat percentage SDS and fat mass adjusted for height and sex increased significantly after stop of GH, whereas lean body mass adjusted for height and sex decreased significantly. However, values remained within the normal range.

Our data indicate that the GH-induced reduction in insulin sensitivity is reversible after stop of GH treatment and that insulin sensitivity becomes similar to that in AGA controls. In contrast, discontinuation of GH treatment was associated with a significant increase in percent body fat and a decrease in muscle mass, though the values remained within the normal range. Thus, while insulin sensitivity improves after discontinuation of GH treatment, body composition deteriorates. Because the values of body composition remained similar to those of AGA controls, the clinical relevance of these changes is still unclear. It remains to be elucidated how body composition and insulin sensitivity change on the longer term after GH has been discontinued.

Part 2: Prematurity

Effects of preterm birth on cardiovascular and metabolic risk factors in short SGA children

Both prematurity and SGA birth have been associated with increased cardiovascular and metabolic risk factors (25, 26, 45, 48-50). It is, however, unknown if the combination of SGA and preterm birth results in an additional increase of these risk factors. Several studies investigated the influence of birth weight SDS and gestational age on cardiovascular and metabolic risk factors within the general population (49, 51) or within a population of preterm born or "low birth weight" subjects (52-58). However, since only a minority of all children is born SGA (ffi2–3%), these studies may not have been suitable to investigate whether a combination of SGA and preterm birth is worse with regard to cardiovascular risk parameters than being born SGA at term.

To answer this question, we investigated within a large population of short SGA children, if preterm birth was associated with an independent adverse risk profile. We measured insulin sensitivity and β-cell function, body composition, blood pressure and lipid levels, and compared these values between preterm and term short SGA children, after adjustment for confounding factors.

Premature SGA children had a more severe degree of growth retardation at birth, as shown by their lower birth weight and length SDS. Previously, we showed that short SGA children with a more severe degree of growth retardation were more likely to be delivered by an elective caesarean section (59). Most likely, the elective preterm birth was prompted by the severity of the fetal growth retardation. Independently of the degree of SGA, preterm children had a lower body fat % SDS, a higher systolic and diastolic blood pressure, a higher insulin secretion and a higher disposition index at a mean age of 6.8 years. Insulin sensitivity and muscle mass were comparable for preterm and term children.

The neonatal period of preterm children is often complicated by nutritional problems, resulting in malnutrition (60). It might well be that this has long-lasting effects on body composition. There have been several hypotheses about the cause of increased

blood pressure associated with SGA as well as preterm birth. Regarding SGA, Brenner et al. (61) showed that intra-uterine growth retardation leads to a reduced number of nephrons. This may lead to a reduced filtration surface area, renal sodium retention and ultimately hypertension. With respect to preterm birth, Rodríguez et al. (62) demonstrated in renal autopsy tissue that glomerulogenesis continues after preterm birth, but stops after 40 days. Preterm infants had less glomeruli than infants born at term (62). Thus, both SGA and preterm birth may lead to a reduced number of nephrons and ultimately hypertension. Also, the use of nephrotoxic agents in the neonatal period, such as antibiotics, might have caused permanent damage to the kidneys resulting in later hypertension (63, 64). In our study, we have demonstrated that the combination of SGA and preterm birth leads to a higher systolic blood pressure than being born SGA at term. The proportion of high systolic blood pressure according to the ATP III criteria for children (65) was 26.3% for preterm SGA children and 16.8% for term SGA children. During GH treatment, systolic blood pressure decreases (26). It would be interesting to investigate if preterm and term short SGA children benefit equally from the reduction in blood pressure during GH treatment with regard to their cardiovascular health later in life.

Unexpectedly, insulin sensitivity was similar for preterm and term SGA children. Apparently, preterm birth is not associated with worse insulin sensitivity in short SGA children. However, insulin secretion and disposition index were higher in those born preterm. Comparing our data with the available literature, we see some similarities. Using the data of the Dutch famine studies, Ravelli et al. (66) reported that glucose tolerance was better in those that were exposed to famine early in their pregnancy as compared to those that had exposure during mid or late gestation. It seems reasonable to assume that the preterm SGA children in our study also had their growth retardation early in pregnancy. Also Hofman et al. (45, 50) showed a higher insulin secretion and disposition index in premature SGA children than in term SGA children, but unfortunately they did not provide statistical comparisons.

Clinical implications and conclusions

We have shown that prematurity has independent and divergent effects on metabolic and cardiovascular risk factors in short SGA children. Preterm SGA children had a lower body fat percentage, a higher systolic and diastolic blood pressure and a higher insulin secretion than term SGA children, whereas insulin sensitivity and lipid levels were comparable. Thus, glucose homeostasis was not worse in preterm SGA children, but there was a relatively high prevalence of systolic hypertension (ffi26%) in preterm SGA children according to the ATP III criteria for children (65). Since GH treatment is accompanied by a decrease in systolic blood pressure (26), it would be interesting to study if preterm and term children benefit equally from this side effect during treatment with regard to their cardiovascular health later in life.

Insulin sensitivity in young adults born premature

In a group of 305 young adults (169 preterm and 136 term; PROGRAM study), we investigated insulin sensitivity and β-cell function with the frequently sampled intravenous glucose tolerance (FSIGT) test. In contrast to a previous report in children (50), we found no evidence that preterm birth has deleterious effects on insulin sensitivity in young adulthood. Adult trunk fat mass was the most important predictor of insulin sensitivity, independently of size at birth and duration of pregnancy. In a more detailed analysis, we studied the same parameters in clinically relevant subgroups: SGA without catch-up growth, SGA with catch-up growth and AGA subjects, either born preterm or term. This subgroup comparison showed that only term SGA subjects with spontaneous catch-up growth to a normal height had significantly lower insulin sensitivity than term AGA controls. Notably, this subgroup also had the highest fat percentage and total fat mass, which is associated with a lower insulin sensitivity (67). However, taking insulin secretion into account, glucose homeostasis in all clinically relevant subgroups was comparable with term AGA controls. This indicates that the lower insulin sensitivity in term SGA subjects with spontaneous catch-up growth is well compensated by an increased insulin secretion in young adulthood. Lastly, oral contraceptive use in women was found to significantly decrease insulin sensitivity. The deleterious effect of oral contraceptives on insulin sensitivity is well documented and most likely due to estrogens (68). In all previous studies evaluating the effect of birth weight on parameters of glucose homeostasis, this factor was not taken into account. We feel that it is certainly a factor that should be included in such analyses.

Clinical implications and conclusions

Our data clearly show that preterm birth was not associated with a reduction in insulin sensitivity in young adulthood. These data are reassuring and provide important information for the ongoing discussion on the long-term sequelae of preterm birth and increased survival rate due to advances in neonatal intensive care. Nevertheless, we feel that long-term follow-up is still warranted, because our data do not guarantee that glucose homeostasis will remain normal on the longer term.

General conclusions

Since the original observations by Barker et al. (42-44), a considerable number of reports has been published investigating the association between birth weight and cardiovascular disease. Short SGA children have an extreme phenotype. Only 2-3 % of all newborns are born SGA, of which approximately 10% shows insufficient catch-up growth and has persistent short stature (69-72). GH treatment for short SGA children was approved by the US Food and Drug Administration (FDA) in 2001, and by the European Agency of Medicinal Products (EMEA) in 2003. Therefore, the number of GH-treated short SGA children is expected to rise in the coming years. Because of the associations between low birth size and cardiovascular disease and type 2 diabetes and the metabolic effects of GH treatment, it is important to know cardiovascular and metabolic risk factors in short SGA children. In the first part of this thesis, we investigated several of these risk factors in short SGA children, before, during and after long-term GH treatment. Preterm birth is a condition with low birth weight as well, and in the past years it was suggested that the increased cardiovascular and metabolic risk might also be applicable to subjects born preterm. In the second part of this thesis, we describe the contribution of preterm birth to cardiovascular and metabolic risk factors within a large group of short SGA children and also in a cohort of young adults.

We investigated the novel cardiovascular risk markers adiponectin, resistin, IL-6 and high sensitivity CRP in short SGA subjects before, during and after GH treatment. We showed that neither short-term (ffi2 years) nor long-term GH treatment (ffi7 years) was associated with disadvantageous changes in these markers.

Our studies show that GH treatment has a positive effect on MMP-9 levels and systolic blood pressure. Both MMP-9 levels and systolic blood pressure SDS decreased by approximately 50% during 3 years of GH treatment, whereas these remained unchanged in a sex- and age-matched untreated SGA control group.

We also investigated long-term changes in body composition. During GH treatment percent body fat decreased and size-adjusted bone mineral density of the lumbar spine increased. After stop of GH treatment, percent body fat increased and lean body mass decreased significantly, but values remained well within the normal range.

Furthermore, we showed that the GH-induced reduction of insulin sensitivity is reversible after stop of long-term GH treatment and the values become comparable with those in AGA controls.

Within a group of short SGA children, we showed that preterm birth has independent effects on cardiovascular and metabolic risk factors. Preterm SGA children had a higher systolic and diastolic blood pressure, a lower percent body fat and a higher insulin secretion and disposition index than term SGA children. Insulin sensitivity was comparable between preterm and term SGA children. Finally, we showed that preterm birth was not associated with reduced insulin sensitivity in a group of young adults. Trunk fat was the most important determinant of insulin sensitivity, independently of size at birth and duration of pregnancy.

Considerations and directions for future research

SGA children form a heterogeneous group with different etiologies of SGA. Although it is clear that preeclampsia and gestational hypertension are more common in mothers of SGA children, there are still a high percentage of SGA births with unknown etiology. Genetic research, preferably with a genome-wide approach, might give more insight in the etiology of persistent short stature after SGA birth as well as the association between low birth weight and cardiovascular disease.

Preeclampsia and intrauterine growth restriction are thought to be vascular-related pregnancy complications. The presence of these pregnancy complications has been linked with future cardiovascular disease (73). Short SGA children have an increased systolic blood pressure, which decreases during GH treatment (26). Approximately 6.5 years after discontinuation of GH treatment, blood pressure had remained lower than in untreated SGA subjects (2). It is unknown if this GH-induced decrease in blood pressure has a beneficial effect on the odds of SGA women to develop preeclampsia or gestational hypertension. Therefore, it would be highly interesting to study the pregnancies and offspring of GH-treated SGA mothers and compare the outcomes with untreated SGA mothers. Another related aspect that might link a small size at birth with adult cardiovascular disease and has not received much attention yet, is the possibility of disturbances in coagulation and/or fibrinolysis. These disturbances could be the cause of placental infarction and thus SGA. Future research might also be directed at identifying these disturbances, such as maternal and/or fetal thrombophilia as a cause for SGA birth.

Growth hormone treatment has well-known anti-insulinemic effects, such as a reduction in insulin sensitivity. Our data demonstrate that long-term GH treatment was not associated with disadvantageous changes in several novel cardiovascular risk markers and that the GH-induced reduction in insulin sensitivity was reversible after GH was discontinuated. Thus, the available data regarding the safety of GH treatment in short SGA subjects with respect to cardiovascular and metabolic health are reassuring. However, it remains important to perform follow-up studies in previously GH-treated SGA subjects at regular intervals because the development of cardiovascular disease and type 2 diabetes is age-dependent and might occur later in life. Figure 1 presents an overview of the follow-up studies that have been performed by our research group in previously GH-treated SGA subjects.

In the past decades, the survival rate of preterm children has dramatically increased due to improvements in neonatal intensive care. Several studies suggested that preterm subjects, like SGA subjects, might have an increased risk for the development of cardiovascular disease and diabetes type 2 (50, 51, 74-76). In a group of young adults born premature, we showed that gestational age had no effect on insulin sensitivity. It is, however, important to realize that we included subjects without major morbidities in the neonatal period, such as necrotizing enterocolitis, long-term ventilation or oxygen supply and bronchopulmonary dysplasia, and without major disabilities in young adulthood, such as hemiplegia and quadriplegia. Besides, it is important to

Figure 1. Follow-up studies in SGA subjects after discontinuation of GH treatment

study other cardiovascular risk factors as well, because preterm birth has divergent effects on cardiovascular risk factors in short SGA children, as described in chapter 7 of this thesis.

Nowadays, due to improved neonatal care for preterms, an increased number of preterms survive, whereas the quality of life of short SGA children has improved due to the availability of GH treatment. The studies described in this thesis aimed to investigate the cardiovascular and metabolic health of these 2 patient groups. These and future studies can improve our knowledge regarding cardiovascular and metabolic risk factors in preterm and SGA subjects and help to provide them with better treatment and counselling.

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CHAPTER10

SUMMARY

Summary

Chapter 1

This chapter provides an introduction in the definitions, prevalence and etiology of SGA birth. Furthermore, clinical and endocrinological aspects associated with SGA are described, such as short stature and the association with cardiovascular disease. Several hypotheses regarding the association between a low birth weight and increased prevalence of cardiovascular disease and type 2 diabetes are discussed. The assessment of cardiovascular risk by several parameters, e.g. novel cardiovascular risk markers, such as adiponectin, resistin, IL-6, high sensitivity CRP and MMP-9, glucose homeostasis and body composition is highlighted and the previously reported data regarding these parameters in short SGA children are summarized. Besides, the effects of GH treatment on these markers as well as the effects on height and the GH-IGF-I-IGFBP-axis are described. At the end of this chapter the aims and outline of this thesis are presented.

Chapter 2

Adiponectin and resistin are fat-cell derived hormones, which are thought to be respectively protective and disadvantageous with regard to the development of cardiovascular disease and diabetes mellitus type II. Low birth weight has been associated with increased risks for the development of these diseases. In short SGA children, GH therapy has several positive effects regarding cardiovascular risk factors, such as a decrease in systolic blood pressure and lipid levels. On the other hand concern has been expressed about the effects of GH therapy on insulin sensitivity. We measured adiponectin and resistin levels in 136 short prepubertal children born SGA and their association with cardiovascular risk parameters and growth factors. Also, we compared the levels with those in normal-statured controls. The effect of GH treatment was evaluated in 50 short SGA children versus baseline and versus an untreated sex- and age-matched SGA control group. Compared to normal-statured controls, short SGA children had similar adiponectin (10.9 vs. 10.7 μg/ml) and lower resistin levels (9.7 vs. 14.7 ng/ml). In GH-treated SGA children, neither adiponectin nor resistin levels changed significantly during 2 years of GH treatment. Compared to untreated sex- and age-matched SGA controls, GH-treated SGA children had similar adiponectin (10.9 vs. 10.7 μg/l) and lower resistin levels (7.9 vs. 10.5 ng/ml). Adiponectin correlated inversely with age, but not with any cardiovascular risk parameter or growth factor. Higher IGF-1 levels in GH-treated children were associated with lower resistin levels. In conclusion, short prepubertal SGA children had similar adiponectin and lower resistin levels as normal-statured controls. Two years of GH treatment had no effect on their adiponectin and resistin levels.

Chapter 3

Low birth weight is associated with increased risks for adult cardiovascular disease and diabetes mellitus type 2. Adiponectin and resistin are hormones, considered respectively protective and disadvantageous regarding these risks. No data exist on the effect of long-term GH treatment on these hormones and inflammatory markers in children born SGA.

We investigated longitudinal changes in inflammatory markers and adipocytokines during and after a long-term dose-response GH study. 103 short prepubertal SGA children received either 1 mg GH/m^2 body surface area (BSA)/day (group A) or 2 mg GH/m^2 BSA/day (group B). Adiponectin, resistin, IL-6 and CRP levels were measured at baseline, after 1 and 7 years of GH treatment and 6 months after discontinuation of GH. The levels in the 2 GH dosage groups were compared with age-related controls. We found that adiponectin levels decreased over time, but remained comparable with controls. Resistin levels increased over time and remained lower or comparable with controls. There were no significant differences between the GH dosage groups. After stop of GH, adiponectin decreased in group B and resistin increased in group A. GH treatment did not affect IL-6 and CRP levels at any time point. An increase in BMI SDS over time was associated with a decrease in adiponectin levels. None of the markers were associated with insulin sensitivity, which was derived from OGTT-tests by calculating the whole body insulin sensitivity index (WBISI). In conclusion, long-term GH treatment is not associated with disadvantageous changes in adiponectin, resistin, IL-6 and CRP levels, neither during nor after GH treatment.

Chapter 4

Short SGA children have an increased systolic blood pressure, which decreases during long-term GH treatment. The underlying mechanism is still unknown. Matrix metalloproteinases (MMPs) are zinc-dependent endoproteinases, which are involved in the remodelling of the extracellular matrix and are thought to play a role in atherosclerosis. High MMP-9 levels have been found in hypertensive patients and predict cardiovascular mortality. We investigated whether 3 years of GH treatment affects plasma MMP-9 levels in short SGA children and whether MMP-9 levels are related to blood pressure. MMP-9 levels and blood pressure were measured at baseline, and after 6, 12 and 36 months in a case-control study. The data of 38 short SGA children receiving GH treatment were compared with those of 17 sex- and age-matched untreated short SGA controls. We show that MMP-9 levels decreased significantly during 3 years of GH treatment, whereas they remained similar in untreated SGA controls. After 3 years of GH treatment, MMP-9 levels were significantly lower in the GH group than in untreated SGA controls. Systolic BP SDS significantly decreased in the GH group, but remained unaltered in untreated SGA controls. MMP-9 levels did not correlate with systolic or diastolic BP. In conclusion, plasma MMP-9 levels and systolic BP SDS

decreased to almost 50% of baseline values in the GH group, whereas these remained unchanged in untreated SGA controls. Our data indicate that GH treatment has a positive effect on both MMP-9 levels and systolic BP SDS.

Chapter 5

Alterations in the GH-IGF-I axis in short SGA children might be associated with abnormalities in bone mineral density (BMD) and body composition. In addition, birth weight has been inversely associated with diabetes and cardiovascular disease in adult life. As body composition is an important determinant of these diseases, it is important to be informed about body composition in short SGA children, as well as the effects of GH treatment. However, data on detailed body composition in short SGA children and long-term effects of GH treatment are very scarce. We investigated the effects of long-term GH treatment on body composition and BMD by Dual Energy X-Ray Absorptiometry (DXA) in short SGA children in a longitudinal 6-year GH study with a randomized controlled part of 3 years. We found that fat percentage SDS and lumbar spine BMD SDS corrected for height ($BMAD_{LS}$ SDS) at baseline were significantly lower than in normal children. Lean body mass (LBM) SDS adjusted for age was also lower than in normal children, but LBM adjusted for height (LBM SDS_{height}) was comparable. GH treatment induced a decrease in fat percentage SDS and an increase in $BMAD_{LS}$ SDS. LBM SDS_{height} remained similar in GH-treated children, but deteriorated in untreated controls. When these untreated controls subsequently started GH treatment, their LBM SDS_{height} rapidly normalized to values comparable with normal children. In conclusion, during long-term GH treatment in short SGA children, fat percentage SDS decreased and $BMAD_{LS}$ SDS increased. These effects of GH treatment were most prominent in children who started treatment at a younger age and in those with greater height gain during GH treatment. LBM SDS_{height} remained around 0 SDS in GH-treated children, but declined to low normal values in untreated controls. In addition, our data demonstrate that it is important to adjust body composition parameters for height in short children to prevent an underestimation at baseline and an overestimation of the effect of GH treatment.

Chapter 6

It is well known that GH treatment reduces insulin sensitivity (Si). For SGA subjects, who might have an increased risk to develop cardiovascular disease and type 2 diabetes, it is still uncertain how Si, ß-cell function and body composition change over time after stop of GH treatment. Therefore, we investigated longitudinal changes in Si, ß-cell function and body composition from stop to 6 months after stop of GH treatment. 48 SGA adolescents were studied at adult height, while still on GH, and 6 months after GH was discontinued, and compared with 38 AGA controls. We assessed Si and ß-cell function by frequently sampled intravenous glucose tolerance tests (FSIGT), and measured body composition by dual energy x-ray absorptiometry (DXA). We demonstrate that after stop of GH, Si ($p=0.006$), glucose effectiveness (Sg; $p=0.009$) and disposition index

(DI; p=0.024) increased, whereas insulin secretion (AIR; NS) decreased. Fat percentage increased (p<0.0005), and LBM decreased (p<0.0005), but fat distribution remained unaltered. Compared with AGA controls, Si was lower during GH and became similar after GH stop, AIR was higher at both time points, and Sg and DI became higher after stop of GH treatment. Body composition of SGA subjects was comparable with AGA controls at both time points. In conclusion, the GH-induced lower insulin sensitivity in SGA adolescents increases after stop of long-term GH treatment and becomes similar to that of AGA controls. Cessation of GH treatment is associated with a small, but significant increase in percent body fat and a decrease in LBM, without changes in fat distribution.

Chapter 7

Both small-for-gestational-age (SGA) and preterm birth have been associated with increased incidence of adult cardiovascular disease and diabetes mellitus type 2. It is however unclear if preterm birth has an additional effect on cardiovascular risk factors in short children born SGA. We investigated if prematurity has an independent influence on several cardiovascular risk factors within a population of short SGA children. 479 short prepubertal SGA children (mean age 6.8 years) were divided in preterm (< 36 weeks) and term (≥ 36 weeks) children. The following parameters were compared between preterm and term short SGA children: insulin sensitivity and ß-cell function (assessed with FSIGT), body composition (measured by DXA), lipid levels, systolic and diastolic blood pressure, and anthropometry at birth and during childhood. We demonstrate that preterm SGA children were significantly lighter and shorter at birth after correction for gestational age than term SGA children (p<0.001), but had a comparable head circumference. In preterm SGA children, we found a significantly higher systolic (p=0.003) and diastolic blood pressure SDS (p=0.026), lower body fat % SDS (p=0.011), and higher insulin secretion (p=0.033) and disposition index (p=0.021), independently of the degree of SGA. Insulin sensitivity, serum lipid levels, muscle mass and body fat distribution were comparable for preterm and term SGA children. In conclusion, within a population of short SGA children, preterm birth has divergent effects on several cardiovascular risk factors. Whereas preterm SGA children had a higher systolic and diastolic blood pressure, they also had a lower body fat % and a higher insulin secretion and disposition index than term SGA children.

Chapter 8

In 2005, 12.7% of all babies were born preterm, and the incidence is rising. Nowadays, due to improved survival, an increasing number of children that were born premature reach young adulthood. A recent report suggested a lower insulin sensitivity in children born premature, which may put them at risk for the development of type 2 diabetes. It is, however, still unknown whether this reduced insulin sensitivity persists into adulthood. We determined insulin sensitivity and ß-cell function with frequently

sampled intravenous glucose tolerance tests, in 305 young adults (aged 18-24; 169 preterm and 136 term). We investigated the effect of gestational age, size at birth and adult body composition on insulin sensitivity. In a more detailed analysis, we studied the same parameters in clinically relevant subgroups: small-for-gestational-age (SGA) without catch-up growth, SGA with catch-up growth and appropriate-for-gestational-age (AGA) subjects, either born preterm or term. In contrast to previous reports, we found no evidence that preterm birth has deleterious effects on insulin sensitivity in young adulthood. Trunk fat was the most important determinant of insulin sensitivity, independently of size at birth and duration of pregnancy. Subgroup comparison showed that only term SGA subjects with spontaneous catch-up growth to a normal height had significantly lower insulin sensitivity than term AGA controls. However, taking insulin secretion into account, glucose homeostasis in all clinically relevant subgroups was comparable with term AGA controls. Contrary to our hypothesis, preterm birth was not associated with reduced insulin sensitivity in young adulthood.

Chapter 9

In the general discussion, we discuss our findings in relation to the current literature and conclude with general considerations and direction for future research.

CHAPTER11

SAMENVATTING
DANKWOORD
CURRICULUM VITAE
LIST OF PUBLICATIONS

Samenvatting

Hoofdstuk 1

Dit hoofdstuk beschrijft de definities, prevalentie en oorzaken van SGA. Vervolgens wordt een overzicht gegeven van klinische en endocrinologische aspecten van SGA, zoals een kleine lichaamslengte en het verband met hart- en vaatziekten. De verschillende hypotheses over het verband tussen een laag geboortegewicht en het vaker voorkomen van hart- en vaatziekten en diabetes mellitus type 2 worden genoemd. Het vaststellen van het risico op hart- en vaatziekten door middel van verschillende parameters, namelijk nieuwe cardiovasculaire risicomarkers, zoals adiponectine, resistine, IL-6, CRP and MMP-9 spiegels, de suikerstofwisseling en lichaamssamenstelling wordt beschreven en de eerder gepubliceerde gegevens over deze parameters in te kleine SGA kinderen worden samengevat. Daarnaast worden de beperkte hoeveelheid gepubliceerde gegevens over de effecten van GH behandeling op deze markers alsmede de effecten op lengte en de GH/IGF-I/IGFBP-as beschreven. Aan het eind van dit hoofdstuk worden de doelstellingen en de indeling van dit proefschrift gepresenteerd.

Hoofdstuk 2

Adiponectine en resistine zijn hormonen geproduceerd in het vetweefsel, waarvan gedacht wordt dat ze respectievelijk een beschermende en nadelige rol spelen met betrekking tot de ontwikkeling van hart- en vaatziekten en diabetes mellitus type 2. Een laag geboortegewicht is geassocieerd met toegenomen risico's op het ontwikkelen van deze ziektes. GH behandeling heeft verschillende gunstige effecten in kleine SGA kinderen, zoals een daling van de systolische bloeddruk en de vetspiegels. Aan de andere kant is er ook bezorgdheid uitgesproken over de effecten van GH behandeling op de insulinegevoeligheid. In 136 kleine prepubertaire SGA kinderen werden adiponectine en resistine spiegels gemeten en het verband met cardiovasculaire risicofactoren en groeifactoren bepaald. Ook werden de adiponectine en resistine spiegels vergeleken met die in controle kinderen met een normale lichaamslengte. Het effect van GH behandeling werd geëvalueerd in 50 kleine SGA kinderen ten opzichte van de uitgangswaarden bij het starten van GH behandeling en ten opzichte van onbehandelde SGA kinderen van vergelijkbare leeftijd en geslacht. Vergeleken met controles met een normale lichaamslengte, hadden kleine SGA kinderen vergelijkbare adiponectine (10.9 vs. 10.7 µg/ml) en lagere resistine spiegels (9.7 vs. 14.7 ng/ml). Adiponectine en resistine spiegels veranderden niet tijdens 2 jaar GH behandeling. GH-behandelde SGA kinderen hadden gelijke adiponectine (10.9 vs. 10.7 µg/l) en lagere resistine spiegels (7.9 vs. 10.5 ng/ml) vergeleken met onbehandelde SGA kinderen van identieke leeftijd en geslacht. Adiponectine correleerde negatief met de leeftijd, maar niet met enige andere cardiovasculaire risicofactor of groeifactor. Hogere IGF-I spiegels in GH-behandelde kinderen waren geassocieerd met lagere resistine spiegels. Concluderend, te kleine prepubertaire SGA kinderen hadden vergelijkbare adiponectine en lagere resistine

spiegels ten opzichte van gezonde controles met een normale lengte. Twee jaar GH behandeling had geen effect op hun adiponectine en resistine spiegels.

Hoofdstuk 3

Er zijn geen gegevens bekend over de lange termijn effecten van GH behandeling op adiponectine, resistine en ontstekingsmarkers. Wij onderzochten longitudinale veranderingen in onstekingsmarkers en adipocytokines tijdens en na een lange termijn GH dosis-respons studie. 103 te kleine prepubertaire SGA kinderen kregen ofwel 1 (groep A) ofwel 2 mg GH/m^2 lichaamsoppervlakte/dag (groep B). Adiponectine, resistine, IL-6 and CRP spiegels werden gemeten bij aanvang, na 1 en 7 jaar GH behandeling en 6 maanden na het stoppen van GH. De spiegels in de 2 GH dosis groepen werden vergeleken met leeftijdsgerelateerde controles. We vonden dat adiponectine spiegels afnamen in de tijd, maar ze bleven vergelijkbaar met de spiegels in controles. Resistine spiegels stegen in de tijd en bleven lager of vergelijkbaar met de spiegels in controles. Er waren geen significante verschillen tussen de GH dosis groepen. Na het stoppen van GH behandeling, daalde adiponectine in groep B en steeg resistine in groep A. GH behandeling had geen effect op IL-6 en CRP spiegels. Een stijging in de BMI SDS was geassocieerd met een daling van de adiponectine spiegels. Geen enkele marker was geassocieerd met insulinegevoeligheid, berekend als "whole body insulinegevoeligheid index" uit de data van OGTT testen. Samenvattend, lange termijn GH behandeling is niet geassocieerd met nadelige veranderingen in adiponectine, resistine, IL-6 en CRP spiegels, noch tijdens noch na GH behandeling.

Hoofdstuk 4

Te kleine SGA kinderen hebben een hoge systolische bloeddruk, welke daalt tijdens lange termijn GH behandeling. Het onderliggende mechanisme is nog onbekend. Matrix metalloproteinases (MMPs) zijn zink-afhankelijke endoproteinases, welke betrokken zijn bij het remodelleren van de extracellulaire matrix, waardoor ze waarschijnlijk een rol spelen bij atherosclerose. Hoge MMP-9 spiegels werden gevonden in hypertensieve patienten en voorspellen sterfte aan hart-en vaatziekten. Wij onderzochten of 3 jaar GH behandeling effect had op MMP-9 plasma spiegels in te kleine SGA kinderen en of MMP-9 spiegels gerelateerd waren aan bloeddruk. MMP-9 spiegels en bloeddruk werden bij aanvang, na 6, 12 en 36 maanden GH-behandeling gemeten in een case-controle studie. De gegevens van 38 te kleine SGA kinderen tijdens GH behandeling werden vergeleken met die van 17 onbehandelde te kleine SGA controles met een identieke leeftijd en geslacht. MMP-9 spiegels namen significant af tijdens 3 jaar GH behandeling, terwijl ze gelijk bleven in onbehandelde SGA controles. Na 3 jaar GH behandeling waren MMP-9 spiegels significant lager in de GH groep dan in de onbehandelde SGA controles. Systolische bloeddruk SDS daalde significant in de GH groep, maar veranderde niet in onbehandelde SGA controles. MMP-9 spiegels correleerden niet met systolische of diastolische bloeddruk. Samenvattend, plasma

MMP-9 spiegels en systolische bloeddruk SDS daalden tot bijna 50% van de waarden bij aanvang in de GH groep, terwijl deze niet veranderden in onbehandelde SGA controls. Onze resultaten laten zien dat GH behandeling een gunstig effect heeft op zowel MMP-9 spiegels als systolische bloeddruk SDS.

Hoofdstuk 5

Veranderingen in de GH-IGF-I as in te kleine SGA kinderen zijn mogelijk geassocieerd met afwijkingen in de botdichtheid en lichaamssamenstelling. Daarnaast is een laag geboortegewicht geassocieerd met het vaker voorkomen van diabetes en hart- en vaatziekten op volwassen leeftijd. Aangezien lichaamssamenstelling een belangrijke determinant is van deze ziektes, is het belangrijk om geïnformeerd te zijn over de lichaamssamenstelling van te kleine SGA kinderen, en de effecten van GH behandeling. Echter, er zijn zeer weinig gegevens bekend van gedetailleerde lichaamssamenstelling in kleine SGA kinderen en de lange termijn effecten van GH behandeling. Wij onderzochten de lange termijn effecten van GH behandeling op lichaamssamenstelling en botdichtheid, gemeten met Dual Energy X-Ray Absorptiometry (DXA) scans in te kleine SGA kinderen tijdens een 6 jaar durende longitudinale GH studie met een gerandomiseerd gecontroleerd deel van 3 jaar. We vonden dat het vetpercentage SDS en de botdichtheid SDS van de lumbale wervekolom gecorrigeerd voor lengte ($BMAD_{LS}$ SDS) bij aanvang significant lager waren dan bij normale kinderen. Spiermassa SDS (gecorrigeerd voor leeftijd en geslacht) was ook lager dan bij normale kinderen, maar spiermassa gecorrigeerd voor lengte (LBM SDS_{height}) was vergelijkbaar. GH behandeling induceerde een afname van het vetpercentage SDS en een toename van de $BMAD_{LS}$ SDS. LBM SDS_{height} bleef gelijk in GH-behandelde kinderen, maar verslechterde in onbehandelde controles. Waneer deze onbehandelde controles vervolgens startten met GH behandeling, normaliseerde hun LBM SDS_{height} snel naar waardes vergelijkbaar met die in normale kinderen. Concluderend, tijdens lange termijn GH behandeling van te kleine SGA kinderen, nam het vetpercentage SDS af en de $BMAD_{LS}$ SDS toe. Deze effecten van GH behandeling waren het sterkst aanwezig in kinderen die op jongere leeftijd met GH behandeling startten en in de kinderen met een grotere lengtewinst tijdens GH behandeling. LBM SDS_{height} bleef rond de 0 SDS in GH-behandelde kinderen, maar daalde tot laag normale waardes in onbehandelde controles. Tenslotte laten onze resultaten zien dat het belangrijk is om de lichaamssamenstelling van te kleine kinderen te corrigeren voor lengte teneinde een onderschatting van de waardes bij aanvang en een overschatting van de veranderingen tijdens GH behandeling te voorkomen.

Hoofdstuk 6

Het is algemeen bekend dat GH behandeling gepaard gaat met een daling van de insulinegevoeligheid. Mensen die SGA geboren werden hebben mogelijk een verhoogd risico op het ontwikkelen van hart- en vaatziekten en type 2 diabetes. Het is echter nog onbekend hoe de insulinegevoeligheid, ß-cel functie en lichaamssamenstelling

veranderen in de tijd na het stoppen van GH behandeling. Daarom onderzochten wij longitudinale veranderingen in insulinegevoeligheid, ß-cel functie en lichaamssamenstelling vanaf het moment van stoppen van GH behandeling tot 6 maanden erna. 48 SGA adolescenten werden onderzocht op het moment dat ze hun eindlengte hadden bereikt, terwijl ze nog steeds GH gebruikten, en 6 maanden nadat de GH behandeling was gestopt. Ze werden vergeleken met 38 gezonde controles, die zowel bij de geboorte als op latere leeftijd een normale lengte hadden (AGA controles). Insulinegevoeligheid en ß-cel functie werden gemeten met zogenaamde frequently sampled intravenous glucose tolerance tests (FSIGT), en lichaamssamenstelling werd gemeten met dual energy x-ray absorptiometry (DXA) scans. Na het stoppen van GH namen de insulinegevoeligheid ($p=0.006$), de insuline-onafhankelijke glucose opname ($p=0.009$) en de dispositie index ($p=0.024$) toe, terwijl de insulinesecretie (NS) afnam. Het vetpercentage steeg ($p<0.0005$), en de spiermassa nam af ($p<0.0005$), maar de vetverdeling bleef gelijk. Vergeleken met AGA controles, was de insulinegevoeligheid in SGA adolescenten lager tijdens GH behandeling, maar werd die na het stoppen van GH vergelijkbaar. De insulinesecretie was hoger op beide tijdstippen, en de insuline-onafhankelijke glucose opname en dispositie index werden hoger na het stoppen van de GH behandeling. Lichaamssamenstelling van SGA personen was op beide tijdstippen vergelijkbaar met die van AGA controles. Samengevat, de GH-geïnduceerde lagere insulinegevoeligheid in SGA adolescenten neemt weer toe na het stoppen van GH behandeling en wordt vergelijkbaar met die van AGA controles. Stoppen van GH behandeling is geassocieerd met een kleine, maar significante toename van het vetpercentage en een daling van de spiermassa, zonder veranderingen van de vetverdeling.

Hoofdstuk 7

Zowel SGA als prematuriteit zijn geassocieerd met hogere cardiovasculaire en metabole risicofactoren. Het was echter nog onduidelijk of prematuriteit een additioneel effect heeft op deze risicofactoren in te kleine SGA kinderen. Wij onderzochten of prematuriteit een onafhankelijke invloed heeft op verschillende cardiovasculaire risicofactoren in een groep te kleine SGA kinderen. 479 te kleine prepubertaire SGA kinderen (gemiddelde leeftijd 6.8 jaar) werden verdeeld in premature (< 36 weken) en aterme (≥ 36 weeks) kinderen. De volgende parameters werden vergeleken tussen premature en aterme kleine SGA kinderen: insulinegevoeligheid en ß-cel functie (bepaald met FSIGT), lichaamssamenstelling (gemeten met DXA), lipiden spiegels, systolische en diastolische bloeddruk, en anthropometrie bij geboorte en op de kinderleeftijd. We laten zien dat premature SGA kinderen significant lichter en korter waren bij hun geboorte, na correctie voor de zwangerschapsduur, dan aterme SGA kinderen ($p<0.001$). De geboorteschedelomtrek was vergelijkbaar. Premature SGA kinderen hadden een significant hogere systolische ($p=0.003$) en diastolische bloeddruk SDS ($p=0.026$), een lager vetpercentage SDS ($p=0.011$), en een hogere insulinesecretie ($p=0.033$) en dispositie index ($p=0.021$), onafhankelijk van de ernst van SGA. Insulinegevoeligheid, serum lipiden spiegels, spiermassa en vetverdeling waren vergelijkbaar voor premature

en aterme SGA kinderen. Samengevat, prematuriteit heeft invloed op verschillende cardiovasculaire en metabole risicofactoren in een groep te kleine SGA kinderen. Premature SGA kinderen hadden een hogere systolische en diastolische bloeddruk, maar ook een lager vetpercentage en een hogere insulinesecretie en dispositie index dan aterme SGA kinderen.

Hoofdstuk 8

In 2005 werd 12.7% van alle babies prematuur geboren, en de incidentie is stijgende. Dankzij een verbeterde overleving, bereikt tegenwoordig een toenemend aantal prematuur geboren kinderen de volwassenheid. Een recent artikel suggereerde een lagere insuliegevoeligheid in prematuur geboren kinderen, hetgeen zou kunnen leiden tot de ontwikkeling van diabetes type 2 op latere leeftijd. Het was echter nog onduidelijk of deze lagere insulinegevoeligheid tot in de volwassenheid blijft bestaan. Wij bepaalden insulinegevoeligheid en ß-cel functie met behulp van zogenaamde “frequently sampled intravenous glucose tolerance” testen in 305 jong volwassenen (leeftijd 18-24 jaar; 169 prematuur en 136 aterm). We onderzochten het effect van zwangerschapsduur, grootte bij de geboorte en volwassen lichaamssamenstelling op de insulinegevoeligheid. In een meer gedetaileerde analyse, bestudeerden we dezelfde parameters in klinisch relevante subgroepen: small-for-gestational-age (SGA) zonder inhaalgroei, SGA met inhaalgroei en appropriate-for-gestational-age (AGA) personen, allen ofwel prematuur ofwel aterm geboren. In tegenstelling tot eerdere publicaties vonden wij geen bewijs voor nadelige effecten van prematuriteit op de insulinegevoeligheid in jong volwassenen. Rompvet was de belangrijkste determinant van insulinegevoeligheid, onafhankelijk van de grootte bij de geboorte of de zwangerschapsduur. Uit de subgroep analyse bleek dat alleen aterme SGA personen met spontane inhaalgroei tot een normale lengte een lagere insulinegevoeligheid hadden dan aterme AGA controles. Wanneer men echter de insulinesecretie in acht neemt, bleek de glucose homeostase in alle klinisch relevante subgroepen vergelijkbaar met die van aterme AGA controles. Samengevat, in tegenspraak met onze hypothese, was prematuriteit niet geassocieerd met een lagere insulinegevoeligheid in jong volwassenen.

Hoofdstuk 9

In de algemene discussie vergelijken we onze bevindingen met de huidige literatuur en sluiten af met algemene overwegingen en suggesties voor toekomstig onderzoek.

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Curriculum vitae of Ruben Willemsen

Ruben Willemsen was born in Leiden on July 6th, 1977. In 1995 he graduated from high school (Stedelijk Gymnasium Leiden) and started his medical training at the University of Leiden. During his studies he rowed at a national level and was a coach for the student rowing club A.L.S.R.V. Asopos de Vliet. He taught anatomy and neuro-anatomy to medical students from 1998 to 2000. From 1998 to 1999 he worked as a research assistant for several projects at the Centre for Human Drug Research (CDHR) in Leiden. In 2000 he participated in a research project investigating the release of T-helper 1 as well as T-helper 2 cytokines by peripheral blood mononuclear cells of melanoma patients and controls at the Department of Clinical Oncology of Internal Medicine at Leiden University Medical Centre (supervisors dr. M. Griffioen, dr. P. Schrier and dr. S. Osanto). From 2000 to 2002 he did his internships. His final internship of 3 months was spent at the Department of Pediatrics of the Diakonessenhuis Paramaribo, Surinam. After obtaining his medical degree in August 2002, he started to work as a resident at the Department of Pediatrics at the Albert Schweitzer Hospital in Dordrecht. In December 2003, he started as a research fellow at the Department of Pediatrics, Division of Endocrinology, Erasmus MC Sophia in Rotterdam (supervisor Prof.dr. A.C.S. Hokken-Koelega), which has resulted in the present thesis.

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