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Growth hormone (GH), IGF-I and insulin actions on spontaneous growth as well as during GH treatment

Doctoral Thesis

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Title: Growth hormone (GH), IGF-I and insulin actions on spontaneous growth as well as during GH treatment

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Preface

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1. **Jensen RB**, Thankamony A, O'Connell SM, Salgin B, Kirk J, Donaldson M, Ivarsson S-A, Söder O, Roche E, Hoey H, Dunger DB, Juul A, NESGAS Group. Baseline IGF-I levels determine insulin secretion and insulin sensitivity during the first year on growth hormone therapy in children born small for gestational age. Results from a North European Multicentre Study (NESGAS). *Hormone research in paediatrics* 2013;80(1):38–46.

2. **Jensen RB**, Thankamony A, O'Connell SM, Kirk J, Donaldson M, Ivarsson SA, Söder O, Roche E, Hoey H, Dunger DB, Juul A. A randomised controlled trial evaluating IGF1 titration in contrast to current GH dosing strategies in children born small for gestational age: The North European Small-for-Gestational-Age Study. *European Journal of Endocrinology* 2014;171(4):509–518.

3. **Jensen RB**, Thankamony A, Day F, Scott RA, Langenberg C, Kirk J, Donaldson M, Ivarsson S-A, Soder O, Roche E, Hoey H, Juul A, Ong KK, Dunger DB, Söder O, Roche E, Hoey H, Juul A, Ong KK, Dunger DB, Soder O, Roche E, Hoey H, Juul A, Ong KK, Dunger DB, Söder O, Roche E, Hoey H, Juul A, Ong KK, Dunger DB. Genetic Markers of Insulin Sensitivity and Insulin Secretion Are Associated With Spontaneous Postnatal Growth and Response to Growth Hormone Treatment in Short SGA Children: the North European SGA Study (NESGAS). *The Journal of Clinical Endocrinology & Metabolism* 2015;100(3): E503–E507.

4. Thankamony A, **Jensen RB**, O'Connell SM, Day F, Kirk J, Donaldson M, Ivarsson SA, Soder O, Roche E, Hoey H, Ong KK, Dunger DB, Juul A, Söder O, Roche E, Hoey H, Ong KK, Dunger DB, Juul A. Adiposity in children born small for gestational age is associated with β-cell function, genetic variants for insulin resistance, and response to growth hormone treatment. *The Journal of Clinical Endocrinology and Metabolism* 2016;101(1):131–142.

5. Wegmann MG, Thankamony A, Roche E, Hoey H, Kirk J, Shaikh G, Ivarsson SA, Söder O, Dunger DB, Juul A, **Jensen RB**. The exon3-deleted growth hormone receptor gene polymorphism (d3-GHR) is associated with insulin and spontaneous growth in short SGA children (NESGAS). *Growth Hormone and IGF Research* 2017;35(July):45–51.

6. **Jensen RB**, Thankamony A, Holst KK, Janssen JAMJL, Juul A, Dunger D, Poulsen P, Scheike T. Genetic influence on the associations between IGF-I and glucose metabolism in a cohort of elderly twins. *European Journal of Endocrinology* 2018;178(2):153–161.

7. Wegmann MG*, **Jensen RB***, Thankamony A, Frystyk J, Roche E, Hoey H, Kirk J, Shaikh G, Ivarsson SA, Söder O, Dunger DB, Juul A. Increases in Bioactive IGF do not Parallel Increases in Total IGF-I During Growth Hormone Treatment of Children Born SGA. *The Journal of clinical endocrinology and metabolism* 2020;105(4):1–8. *joint first authorship

8. Cleemann Wang A, Hagen CP, Nedaeifard L, Juul A, **Jensen RB**. Growth and Adult Height in Girls with Turner Syndrome Following IGF-1 Titrated Growth Hormone Treatment. *The Journal of Clinical Endocrinology and Metabolism* 2020;105(8):1–9.

9. **Jensen RB**, Boas M, Nielsen JE, Maroun LL, Jørgensen A, Larsen T, Main KM, Juul A. A common deletion in the growth hormone receptor gene (d3-GHR) in the offspring is related to maternal placental GH levels during pregnancy. *Growth hormone & IGF research official journal of the Growth Hormone Research Society and the International IGF Research Society* 2020 Dec; 55:101360

10. **Jensen RB,** Bytoft B, Lohse Z, Johnsen SK, Nielsen MF, Oturai PS, Højlund K, Damm P, Clausen TD, Jensen DM**.** Impact of Lean Body Mass and Insulin Sensitivity on the IGF-1-Bone Mass Axis in Adolescence: The EPICOM Study. *The Journal of Clinical Endocrinology and Metabolism* 2021;106(2):E772–E781.

English summary

Growth hormone (GH), Insulin-like growth factor-I (IGF-I) and insulin are closely linked peptide hormones acting in synergy as important regulators of protein synthesis and cell proliferation. In addition, GH, IGF-I and insulin play an integral role in maintaining glucose homeostasis by coordinating the response during the fasting state and after food intake. In twin studies, we and others found a strong heritability of IGF-I and insulin secretion whereas the heritability for insulin sensitivity was non-significant in our study. Results from large population-based cohort studies promoted IGF-I as a biomarker of diseases occurring later in life such as cancer, cardiovascular disease and diabetes, and a large meta-analysis found a U-shaped association between IGF-I and all-cause mortality.

The aims of this thesis were to explore the common processes of GH, IGF-I and insulin that influence growth in fetal life, in childhood and in puberty as well as during treatment with recombinant human GH (rhGH). A detrimental fetal environment may have long-lasting consequences due to the programming effects on GH, IGF-I and insulin resulting in an increased risk of disease later in life, especially in those subjects with rapid postnatal catch-up growth. However, around 10% of children born small for gestational age (SGA) do not have catch-up growth and end up with a decreased adult height. However, initiation of rhGH treatment in childhood markedly improves adult height, but there is a wide variation in the individual growth response, because the etiology of SGA is heterogenous. In the North European SGA study (NESGAS), we found that higher doses of rhGH gave a better short-term growth response, but other studies concluded that gain in adult height was not dose dependent. Furthermore, we showed that SGA children with lower IGF-I levels before start of treatment had a better insulin sensitivity and an increased growth response during rhGH treatment compared to the children with higher baseline IGF-I. The association between insulin sensitivity and growth was confirmed as causal in the NESGAS cohort using the Mendelian randomization approach where multi-allele scores associated with insulin sensitivity was directly linked to growth response in rhGH treated SGA children. In addition, other genetic variants such as a common polymorphism in the GH receptor gene (d3-GHR isoform) was associated with a better growth response during rhGH treatment than carriers of the full-length isoform. In contrast, we and others found decreased fetal growth and birth weight in carriers of the d3-GHR isoform.

Since the GH/IGF-I axis is involved in cell proliferation and has been mentioned as a biomarker of cancer later in life the safety of rhGH treatment has been explored. Importantly, data from a large European cohort found no support for a carcinogenic effect of rhGH treatment, but supraphysiological IGF-I levels and potential serious long-term adverse effects remain an issue. Consequently, consensus guidelines on rhGH treatment of children without GH deficiency (non-GHD) advocate for keeping IGF-I levels close to the normal reference for safety reasons. However, we found that titration of the rhGH dose to lower the supraphysiological IGF-I levels children born SGA and in girls with Turner syndrome, proved less effective in terms of height gain compared to current dosing regimens. Though, total IGF-I levels may not entirely reflect IGF-I bioactivity and

the associated activation of the IGF-I receptor. In fact, measurement of bioactive IGF in the NESGAS study revealed that this may be superior to total IGF-I as a marker of the biological active IGF-I.

In conclusion, treatment of children with rhGH greatly influences both growth and metabolic functions and this is highly influenced by environmental and genetic factors. Thus, it is important to understand the anabolic and metabolic processes influenced by the interaction between GH, IGF-I and insulin to improve safety, efficacy and cost-effectiveness of rhGH treatment in childhood.

Dansk resumé

Væksthormon (GH), insulinlignende vækstfaktor-I (IGF-I) og insulin er peptidhormoner, der er tæt forbundne, og fungerer som vigtige regulatorer for proteinsyntese og celleproliferation. Derudover spiller GH, IGF-I og insulin en vigtig rolle for opretholdelsen af et stabilt blodsukker ved hjælp af et koordineret respons både under faste og efter fødeindtagelse. Gennem tvillingestudier har vi og andre vist, at arveligheden for IGF-I og insulinsekretion var stor, mens arveligheden for insulinfølsomhed var ikke-signifikant. Store populationsbaserede studier har vist, at IGF-I niveauet er en biomarkør for senere sygdom såsom kræft, hjertekarsygdom og diabetes. En stor metaanalyse fandt en U-formet sammenhæng mellem IGF-I og dødelighed, hvilket betyder at både høje og lave IGF-I niveauer er relateret til en højere dødelighed.

Formålet med denne afhandling var at kortlægge interaktionen mellem GH, IGF-I og insulin, der påvirker væksten i fosterlivet, i barndommen og i puberteten såvel som under behandling med væksthormon. Et skadeligt fostermiljø kan have langvarige konsekvenser, idet der kan ske en 'programmering' af GH, IGF-I og insulin, som dermed kan føre til øget risiko for overvægt og metabolisk sygdom senere i livet. Det er især individer, der havde en lav fødselsvægt efterfulgt af en hurtig vækst i barndommen, som er i risikogruppen. Omkring 10% af børn med lav fødselsvægt (small for gestational age, SGA) vokser fortsat langsomt i barndommen og ender derfor med en lav sluthøjde, men behandling med væksthormon kan hos de fleste øge sluthøjden. Gruppen af børn født SGA er heterogen, og effekten af væksthormon varierer derfor meget. I NESGAS-studiet fandt vi, at højere doser af væksthormon gav et bedre kortvarigt vækstrespons, mens andre studier fandt at effekten på sluthøjden ikke var afhængig af væksthormondosis. Derudover fandt vi en sammenhæng mellem et lavere niveau af IGF-I før behandlingsstart, en højere insulinfølsomhed og et øget vækstrespons under væksthormonbehandling. Denne sammenhæng mellem insulinfølsomhed og vækst blev bekræftet som kausal, da genetiske variationer associeret med insulinfølsomhed var direkte associeret til vækstrespons hos de væksthormonbehandlede SGA-børn. Der er mange andre genetiske faktorer, der kan påvirke vækstresponset under væksthormonbehandling og studier af en genetisk polymorfi i væksthormonreceptorgenet (d3-GHR) har vist at bærere af d3-GHR-isoformen havde et bedre vækstrespons under væksthormonbehandling. I modsætning til den øgede vækst i barndommen, så fandt vi og andre, at fostervækst og fødselsvægt var reduceret hos dem, der var bærere af d3-GHR-isoformen.

Store befolkningsstudier har vist, at IGF-I er en biomarkør for kræft senere i livet, hvilket har betydet et øget fokus på sikkerheden ved væksthormonbehandling. I en stor europæisk kohorte fandt man dog, at risikoen for udvikling af kræft efter behandling med væksthormon ikke var øget i forhold til baggrundsbefolkningen. Af sikkerhedsmæssige årsager lægger de kliniske retningslinjer for væksthormonbehandling af børn dog stadig stor vægt på, at IGF-I-niveauerne skal være indenfor det normale referenceområde. Vi og andre har vist, at hvis væksthormondosis titreres, så IGF-I niveauerne ligger inden for det normale referenceområde, så er behandlingen mindre effektiv i forhold til øgning af højdetilvæksten både hos SGA-børn og hos piger med

Turner syndrom. De totale IGF-I-niveauer afspejler imidlertid ikke aktiveringen af IGF-I receptoren, hvorimod måling af bioaktiv IGF, som vi foretog i NESGAS-studiet, afslørede at dette formentlig bedre repræsenterer det biologisk aktive IGF-I.

Væksthormonhandling af børn har stor indflydelse på væksten og den metabolisk funktion, hvilket er påvirket af genetiske såvel som miljømæssige faktorer. Det er således vigtigt at forstå de anabolske og metaboliske processer, der er påvirket af interaktionen mellem GH, IGF-I og insulin for at forbedre sikkerheden og effektiviteten samt nedsætte omkostningerne i forbindelse med væksthormonbehandling i barndommen.

Introduction

Growth hormone (GH), Insulin-like growth factor-I (IGF-I) and insulin are intimately linked, and they are important regulators of protein synthesis and cell proliferation. Furthermore, GH, IGF-I and insulin play an integral role in maintaining glucose homeostasis by coordinating the response during the fasting state and after nutrient intake in order to break down and utilize fat to meet the energy needs and prevent hypoglycemia as well as contributing to the anabolic response(1).

IGF-I and insulin are ancestrally related and thereby share structural similarities. Although their receptors are different in many ways, they also share homology and the signaling processes are in many ways alike. GH, IGF-I and insulin actions have a significant impact on final height through the effects on fetal, childhood and pubertal growth as well as during GH treatment. These processes are influenced by genetic and epigenetic factors and numerous environmental aspects and the effects on growth and metabolism may have long-lasting consequences for development of disease later in life.

The aim of this review was to explore the interplay between growth and metabolic functions from fetal life until adult height with special emphasis on the impact of GH, IGF-I and insulin on spontaneous pre- and postnatal growth and particularly during growth hormone treatment. Additionally, the influence of the genetic susceptibility on growth and metabolic function during childhood will be explored and the influence of the programming effect, following an adverse intrauterine environment, on risk of disease later in life will be reviewed.

Homology between IGF-I and insulin

IGF-I, IGF-II and proinsulin evolved from a single precursor molecule more than 60 million years ago with the function of sending signals to ensure adequate nutrition for basal metabolic needs as well as for cell proliferation. IGF-I and insulin still share significant homology, but there are great variances in amino acids and thereby receptor affinity (reviewed by Clemmons(2)). In 1957 Salmon and Daughaday explored that a factor called the the sulfation factor or somatomedin mediated the mitogenic effect of GH on the growth plate(3). Another group of researchers had isolated a fraction they called non-suppressible insulin-like activity (NSILA) from human serum with insulin-like activity, but this fraction was not inhibited by insulin-specific antibodies(4). Later studies found that these factors were identical, so they were commonly termed somatomedin until the complete amino acid sequences of IGF-I and IGF-II were discovered in 1978(5, 6). The sequencing demonstrated obvious homology between IGF and proinsulin, and due to the number of differences in amino acid positions between IGF-I and insulin the authors concluded that this duplication of the gene happened before the time of appearance of the vertebrates(5).

IGF-I and insulin share common downstream cellular signaling processes, but major differences in amino acids in the primary domains determine the receptor binding and thereby the difference in affinity for the respective

receptors(7) (Figure 1). Both the IGF-I receptor (IGF1R) and the insulin receptor (IR) are tyrosine kinase containing receptors with a 48% amino acid homology between the receptors(8). However, the ligand binding specificity is still strict, and there is a wide variety in receptor density between the different tissues and cell types. The structural similarity between IGF1R and IR explains the formation of the IR/IGF1R hybrid receptors in cells that express both receptors such as myocytes and pre-adipocyte(9) (Figure 1). Thereby, stimulation of glucose transport into the muscle is mediated by IGF-I either directly through the IGF1R or the IR/IGF1R hybrid receptors, although it requires very high concentrations of free IGF-I. The multiple signaling pathway of the IGF-I and IR receptors are in many ways similar leading to proliferation, differentiation, metabolic functions and lipid and protein synthesis.

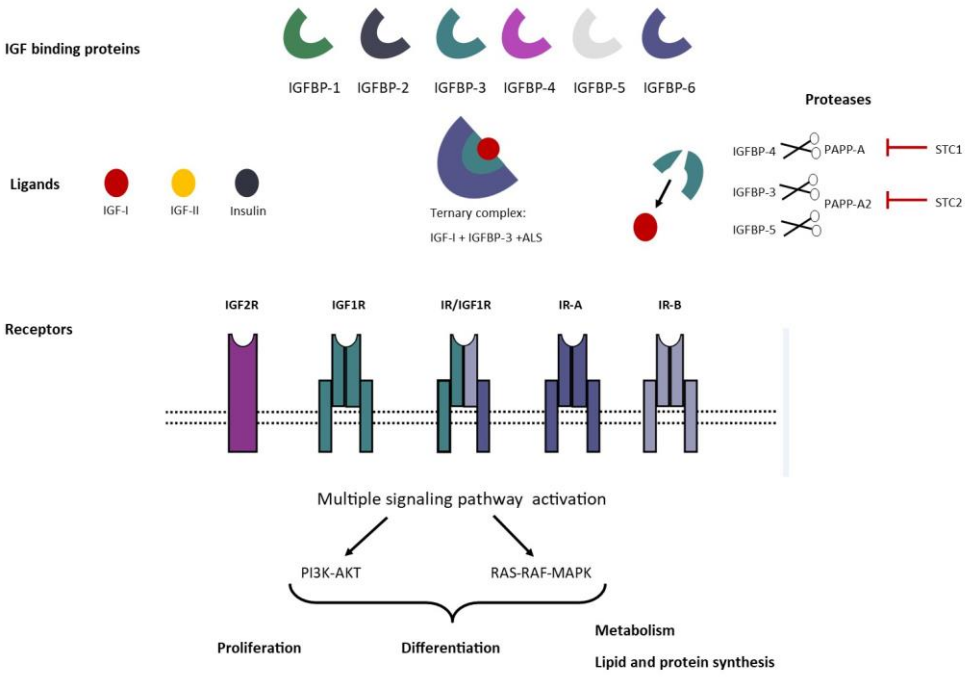

Figure 1: The ligands IGF-I, IGF-II and insulin and their respective receptors (IGF1R, IGF2R, IR-A and IR-B) and the hybrid receptor IGF-I/IR. The six IGF binding proteins (IGFBP-1-IGFBP-6), the ternary complex (IGF-I+IGFBP-3 and acid label subunit (ALS)), the proteases PAPP-A and PAPP-A2 capable of cleaving the IGF binding proteins and the modifiers STC1 and STC2.

IGF-I and IGF-binding proteins

Circulating IGF-I concentrations vary widely among healthy subjects and the concentrations are influenced by sex, age, and body mass index (BMI). Serum IGF-I concentrations are produced in the hepatocytes predominately stimulated by the pulsatile GH secretion pattern from the pituitary gland in the presence of adequate nutrients. However, IGF-I is also expressed in many other tissues such as fat, muscle, and the growth plate where it acts in an endocrine, paracrine and autocrine manner to promote growth. The levels of IGF-I are low at birth, relatively stable during childhood, increase rapidly during puberty and thereafter decrease progressively with the greatest decline during second and third decade (Figure 2). IGF-I concentrations are

higher in females than in males throughout life and the peak during puberty is earlier in girls than in boys corresponding to the earlier pubertal onset and peak height velocity seen in girls compared to boys(10, 11).
Most of the circulating IGF-I in the bloodstream is bound to IGF-binding proteins (IGFBPs) while only approximately 1% circulates as unbound, free IGF-I. IGF-I and IGF-II are bound to six different IGFBPs (IGFBP-1 to IGFBP-6) regulating the bioavailability of circulating IGF and thereby inhibiting as well as stimulating IGF mediated effects at the cellular level (Figure 1) (reviewed by Juul(12)). In 1975 Froesch and coworkers demonstrated that NSILA was found in serum in complexes of high molecular weight(13). The binding proteins solely bind IGF-I and IGF-II and not insulin or proinsulin as could be expected due to their structural similarities. The IGFs are predominantly bound in large ternary complexes with IGFBP-3, IGFBP-5 and acid labile subunit (ALS) that are saturated and GH dependent(14) (Figure 1). Approximately 75% of all IGF is bound in the ternary complex consisting of IGFBP-3 and ALS. This prolongs the half-life of IGF-I from a few minutes to more than 12 hours, and thereby increases the concentration of total serum IGF-I. Binding of IGF-I to IGFBP-1 is not saturated, and the hepatic production of IGFBP-1 is inversely regulated by the portal supply of insulin(15), which means that IGFBP-1 levels fluctuate according to insulin levels during the day and thereby regulates the bioavailability of IGF-I in relation to meals. There is increasing evidence that IGFBPs have IGF-independent functions and that they are more than just modulators of IGF bioavailability.

The ability of IGF-I to stimulate the IGF-1R is not only dependent on the binding proteins but also on the IGFBP proteolysis. Cleavage of IGFBPs lower their ligand affinity, resulting in liberation of IGF-I which is thereby accessible for the IGF1R. IGFBP proteolysis is determined by the IGFBP-proteases (pregnancy associated plasma protein A and A2 (PAPP-A and PAPP-A2)) as well as modifiers of IGFBP protease activity (stanniocalcin 1 and 2 (STC1 and STC2)) that can affect the activity of the proteases and thereby the bioactivity of IGF-I (Figure 1). IGFBP-3 and IGFBP-5 are cleaved by PAPP-A2 which is regulated by STC2. IGFBP-4 and to some extend IGFBP-2 are cleaved by PAPP-A which is regulated by STC1. Recent studies have discovered that IGFBP-4 play an independent role for growth and bone formation but probably also for the risk cardiometabolic disease, however, more experiments are needed to elucidate this field(16).

Measurements of total IGF-I concentrations by immunoassay, whereby IGF-I is released from the IGFBPs, do not consider the modifying effects of IGFBPs and IGFBP-proteases. Direct measurements of the biological active amount of IGF-I (i.e. the bioactive IGF) may be determined by the IGF-I kinase receptor activation assay (KIRA) measuring the ability of serum IGF-I to phosphorylate and activate the IGF1R(17). Nevertheless, it is still being debated whether this artificial activation of the IGF1R in transfected cells can represent the endogenous activation of the IGF1R and whether this responds to a biological response in cells *in vivo*.

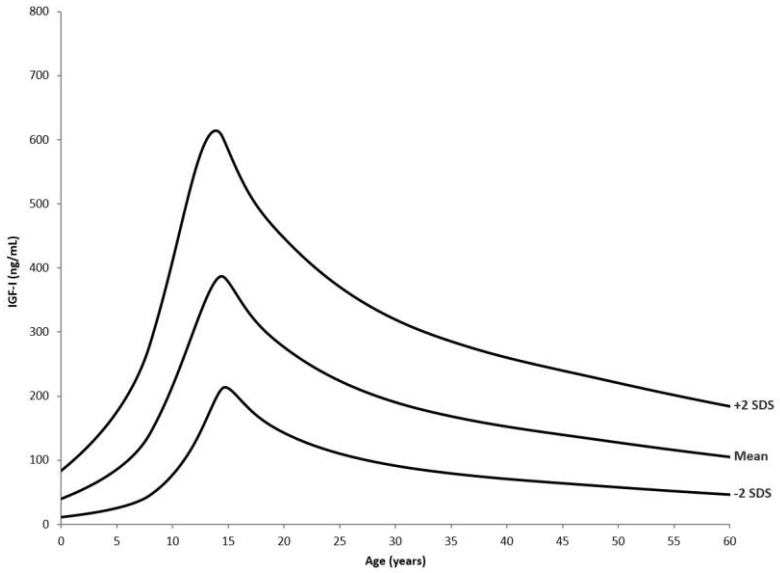

Figure 2: Insulin-like growth factor-I levels from birth to 60 years of age. Lines represent the reference range of a normal population from -2 standard deviation score (SDS) to +2 SDS.

Modified from Juul & Skakkebaek, 2019, *J Clin Endo Metab*, 104;7: 2770–2776

Summary: *IGF-I and insulin share significant homology and common downstream cellular signaling processes but the affinity to the receptors differ due to differences in the receptor binding domains. Whereas circulating insulin is unbound, almost all circulating IGF-I is bound to six different binding proteins. In order to activate the receptor, IGF-I needs to be liberated from the binding proteins and this process is regulated by IGFBP proteases and modifiers of these. Measurement of bioactive IGF is believed to determine the ability of IGF-I to activate the IGF-I receptor.*

GH, IGF-I, and insulin actions on metabolic function

The interaction between GH, IGF-I and insulin plays an important role in the response to nutrient intake and initiation of the appropriate metabolic response. Food intake is followed by a rise in insulin levels in healthy subjects and in the presence of adequate nutrient supply the systemic and portal rise in insulin is an important promoter of hepatic IGF-I secretion. This promotion of IGF-I synthesis and secretion by insulin was shown in former studies on cultured rat hepatocytes(18), and the same was found in pituitary-intact rats whereas there was no effect in hypophysectomised rats suggesting that insulin increases GH sensitivity in the hepatocytes(19). In healthy subjects increased insulin levels will decrease IGFBP-1 concentration resulting in increased levels of "free" or bioactive IGF and thereby a negative feedback to suppress GH secretion. Insulin and IGF-I are important for anabolic storage of glycogen reserves, and growth of lean mass by stimulating protein synthesis and inhibiting protein breakdown in the fed state. Furthermore, preadipocyte differentiation

is stimulated by IGF-I, but in mature adipocytes IGF-I receptors are reduced and insulin receptors are upregulated. Insulin has a lipogenic effect and stimulates lipid synthesis in liver, fat and skeletal muscle after feeding (reviewed by Moeller et al.(1)) (Figure 3).

During fasting or stress GH secretion increases and stimulates lipolysis by release and oxidation of free fatty acids (FFA) from mature adipocytes which results in changing the utility of energy from carbohydrate and protein utilization to lipid oxidation. The increased flux and oxidation of FFA induces insulin resistance and a glucose-FFA substrate competition inhibits insulin-stimulated glucose uptake in the muscle. However, studies have shown that insulin resistance following GH exposure occur before elevation of FFA indicating that GH may also influence insulin sensitivity independent of FFA(20). Thus, GH is a counterregulatory hormone that antagonizes both the hepatic and peripheral effect of insulin on glucose metabolism which is a defense against hypoglycemia during the fasting state. IGF-I regulates metabolism by reducing the flux of FFA through the liver and by stimulating FFA uptake and oxidation in skeletal muscle directly but also through suppression of GH by the negative feedback control (reviewed by Moeller et al.(1)). In a study of healthy young men the individuals with IGF-I levels in the lowest tertile of the reference range had reduced insulin secretion, increased hepatic insulin sensitivity and enhanced fat metabolism compared to a group of young men with IGF-I in the highest tertile(21). Thus, IGF-I plays an important role for β-cell function and thereby insulin secretion, which was confirmed by former studies on β-cell–specific IGF-I receptor knockout (KO) mouse models that documented a vital role of IGF-1 signaling in control of β-cell function(22). However, this study cannot reject reverse causation with lower insulin secretion due to a better insulin sensitivity caused by lower GH levels and thereby lower IGF-I concentrations.

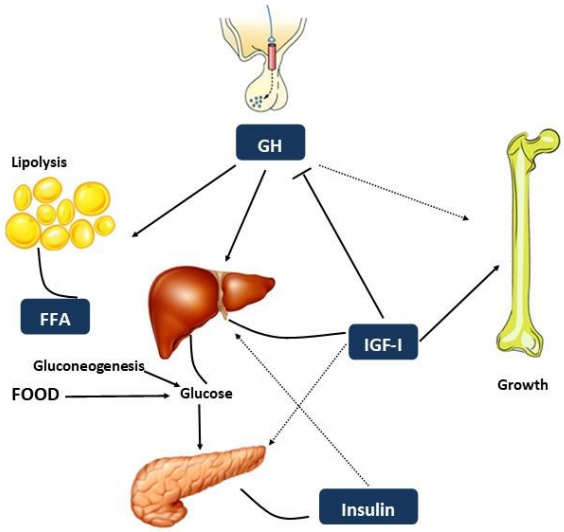

Figure 3: Interactions between GH, IGF-I and insulin

Summary: *GH, IGF-I and insulin act together as important regulators of protein synthesis, cell proliferation and lipogenesis after nutrient intake and maintain glucose homeostasis during the fasting state by changing the utility of energy from carbohydrate and protein utilization to lipid oxidation.*

GH, IGF-I, and insulin actions on spontaneous growth

Growth is controlled by multiple factors including genes, nutrition and hormones, and especially the GH/IGF-I axis, insulin, thyroid hormones and sex steroids play a crucial role. Normal growth can be divided into four phases(23). The fastest growth occurs during fetal life, especially during second trimester and this is followed by a fast but declining growth during infancy. From two years of age and during childhood the growth rate is stable until the growth spurt in puberty where peak height velocity occurs around the age of 12 years in girls and of 14 years in boys. Regulation of growth during these phases is a multifaceted interplay between many different factors, but GH, IGF-I and insulin are key components during all the phases.

Fetal Growth

Fetal growth is a complex process influenced by environmental factors, including maternal health, nutrition and lifestyle as well as genetic factors of both the mother and the fetus. Placental secretion of the human placental growth hormone variant (hGH-V) that enters maternal circulation from the villous syncytiotrophoblast and extravillous trophoblast layers of the placenta regulates fetal growth. Serum concentrations of hGH-V increase during pregnancy and maternal pituitary GH secretion is inhibited, in that way hGH-V acts as the key regulator of maternal IGF-I levels(24, 25, 26). Insulin and glucose levels in the maternal circulation are inversely associated with hGH-V, assuring nutrient availability to the fetus either directly or indirectly via IGF-I. The concentration of hGH-V in the maternal circulation is positively associated with fetal growth(24, 25) and hGH-V was decreased in pregnancies with intrauterine growth restriction (IUGR) and increased in pregnancies with macrosomia(26). Immunohistochemical analysis showed that both hGH-V and the growth hormone receptor (GHR) were expressed concomitantly in the placenta. Expression of both hGH-V and GHR were localized to the cytoplasm and mainly found in villous syncytiotrophoblasts, but with some expression also detected in extravillous trophoblasts, decidual cells and smooth muscle cells in chorionic vessels(27). Expression of both hGH-V and GHR in the same compartments of placenta indicates interaction between maternal hormones and receptors of fetal origin.

In the fetal compartment insulin is one of the major signals of nutrient availability acting directly via stimulation of cellular glucose uptake and indirectly via stimulation of the IGF secretion(28). The Pedersen hypothesis formulated more than 50 years ago emphasized the effect of glucose and insulin on fetal growth. This hypothesis proposed that fetal overgrowth seen in pregnancies with maternal diabetes and obesity was linked to an increased transfer of glucose from the maternal to the fetal compartment, stimulating the fetal β-cell to an increased insulin secretion and subsequent being born large for gestational age (LGA)(29). Insulin

concentrations in cord blood samples are positively correlated with fetal growth in both human and animal studies.

The importance of the fetal IGF system in endocrine and paracrine regulation of fetal growth has been illustrated by severe IUGR in *IGF1* and *IGF2* KO mice(30). Fetal IGF-I and IGF-II concentrations increase with gestational age and correlate positively with fetal weight estimated by ultrasonography(31). IGF-II is an important growth factor during fetal life but the effect on postnatal growth is not fully explored. Insulin and IGF-I in cord blood correlated positively with birth weight (BW), birth length, placental weight and gestational age, whereas there was an inverse correlation between BW and IGFBP-1(32, 33). IGFBPs are important for fetal growth and the protease PAPP-A cleaving IGFBP-4 and -5 is highly increased during pregnancy leading to increases the bioavailability of IGF and thereby mediates trophoblast invasion, and glucose and amino acids transport in the placenta(34). Low PAPP-A levels are associated with IUGR and small for gestational age (SGA). Being born SGA is not a diagnosis but a statistical cut-off including children with a BW below -2 SDS. Thus, the group of children born SGA is heterogeneous and the etiology of decreased fetal growth could represent extremes of adverse intrauterine exposures, unrecognized genetic defects, or reprogramming of metabolism through functional changes or epigenetic adaptations.

Fetal GH plays a minor role for fetal growth, but decreased levels of fetal GH and increased IGF-I levels during third trimester suggested that GH is regulated by a negative feedback mechanism of IGF-I already *in utero*. In addition, GH plays a role in prenatal growth demonstrated by decreased birth lengths of newborns with growth hormone deficiency (GHD) (35, 36), but children with *Igf1* gene defects suffer from more severe growth restriction(37). However, the metabolic effects of GH on lipolysis are crucial to prevent hypoglycemia and therefore newborn children with GHD often present with hypoglycemia during the neonatal period.

SGA and catch-up growth

The intrauterine growth pattern is an important marker of childhood growth. Most children born SGA have a postnatal catch-up growth during the first two years, but 10-15% of children born SGA do not show catch-up growth and thereby end up with a final height below -2 standard deviation scores (SDS) (38, 39, 40). The mechanisms inducing accelerated growth in some children and not in others may be the result of an impaired action of the GH/IGF-I axis in short SGA children (41, 42). Specifically, impairments in GH signaling, hepatic IGF-I generation, and IGF-I receptor signaling have been demonstrated in experimental IUGR animal models and in humans(43, 44). IGF-I levels were inversely correlated with BW in two large population-based cohorts of 4-8 year old children(45, 46). Furthermore, many short children born SGA have reduced appetite and food intake resulting in decreased body fat compared to children born appropriate for gestational age (AGA). Animal studies on IUGR suggested an altered development of the adipose tissue probably associated with altered adipokine signals to the brain and neuroendocrine regulation of appetite(47, 48).

Spontaneous catch-up growth in children born SGA was followed by an increased insulin secretion(49) whereas those without catch-up growth had reduced insulin sensitivity(50, 51). In a Chilean cohort of SGA children, IGF-I levels increased rapidly and were related to β-cell function during catch-up growth whereas at 3 years of age when catch-up was completed, IGF-I levels were related to BMI and insulin resistance(52). In a smaller French cohort of SGA children with catch-up growth the insulinogenic index was lower at four years of age compared to those born AGA suggesting impairment of β-cell function(53). Metabolic changes already early in life are strongly related to a rapid postnatal weight gain and could indicate a tendency to central fat deposition. Higher IGF-I levels in children born SGA with spontaneous catch-up growth could reflect a relative IGF-I resistance associated with insulin resistance.

Childhood growth

During the first six to twelve months of life IGF-I levels are largely independent of GH but are closely related to nutrition and insulin secretion. Many infants experience increased or decreased growth during this period leading to "catch-up" or "catch-down" growth which is part of the normal growth pattern. IGF-I and IGFBP-3 levels increase steadily throughout childhood and the rise during the first years of life reflects onset of GH action (54, 55) (Figure 2). In population-based cohorts of healthy children IGF-I levels were positively associated with postnatal weight gain and increase in lean mass(45, 56). Higher IGF-I levels were associated with greater height gain in healthy normal-weight children and higher levels of insulin secretion for the degree of insulin sensitivity(57).

Together with sex steroids, the GH/ IGF-I axis is an important factor in acquisition of bone mass (58), and there is substantial evidence that IGF-I regulates osteoblast and osteoclast cell proliferation(59, 60). However, the link between IGF-I and bone mineral content (BMC) has been proposed to be indirectly by the effect on skeletal muscle due to the increased mechanical load to which the bone adjusts its structure and mass (60). In a longitudinal cohort of 258 girls followed for seven years IGF-I was indirectly linked to bone mass accrual through stimulating muscle growth (61) and similarly more studies found that lean mass was an intermediary factor in the IGF-I bone relationship(62, 63). Insulin sensitivity was shown to have a moderating effect on the association between IGF-I and lean mass which was confirmed by a path analysis in girls aged 9-11 years(62), but not in adolescent offspring of mothers with type 1 diabetes in the EPICOM cohort (63). Furthermore, obesity and insulin resistance during childhood has been suggested to negatively influence bone mass and bone density. In the ALSPAC cohort a positive association between fat mass and BMC and BMD was found but insulin was inversely associated with BMD and periosteal circumference when adjusted for body composition(64, 65). However, data in this field are divergent proposing that overweight could augment bone strength by the extra mechanical load, but this link may disappear when adjusting for lean mass, and the metabolic impairments that accompanies obesity could be detrimental to bone strength(66, 67).

Childhood obesity is associated with a higher growth velocity and advanced bone age compared to lean subjects(68). However, the tendency towards an increased height during childhood tends to gradually disappear during puberty since children with obesity enter puberty at an earlier age often show a reduced growth spurt compared to lean subject as was seen in a large Swedish population-based longitudinal cohort(69). The hormonal regulation of the accelerated growth among obese children is not fully understood. Obese subjects have reduced half-life of GH and lower daily production rate of GH but normal IGF-I levels(70) which is partly explained by the augmented hepatic GH sensitivity due to portal hyperinsulinemia and a higher IGF-I bioavailability due to suppression IGFBP-1. Another speculation on the increased growth among obese subjects has been the anabolic effect driven by an increased insulin action on the IGF1R. Some of these speculations could also explain the increased growth seen in some children after surgery for craniopharyngioma despite of verified complete GHD but due to hyperphagia (71).

Pubertal Growth

Circulating levels of IGF-I and IGFBP-3 have a steep incline at the start of puberty reaching acromegalic levels at mid-puberty (Tanner stage 4) and decrease at the end of puberty (Tanner stage 5)(10, 11) (Figure 2). The rise in IGF-I concentration is important for the pubertal growth spurt, but the concentration peaks almost two years after peak height velocity which also indicates another impact of IGF-I than growth which could be sexual maturation(72). Sex steroids were shown to stimulate pulsatile secretion of GH most likely by decreasing pituitary and hypothalamic sensitivity to the negative feedback of IGF-I(73). Furthermore, local secretion of IGF-I in the ovaries and testes is central for testicular production of testosterone and spermatogenesis(74, 75) and in selection and growth of the primary follicle, estradiol production and ovulation in the ovary(76).

Fluctuation of insulin sensitivity occurs during pubertal development reflecting the interplay between GH, IGF-I, insulin, sex steroids and BMI(77). The changes of insulin sensitivity associated with puberty was first shown in a study by Amiel *et al*. in 1986 revealing that both hepatic and peripheral insulin sensitivity was lower in pubertal children compared to prepubertal children(78). Further studies have shown a decrease in insulin sensitivity early in puberty, reaching the lowest levels at Tanner stages 3 to 4 and increasing hereafter(77, 79). Girls had a more marked reduction in insulin sensitivity, but this can only partly be explained by increasing adiposity(80), the GH/IGF-I axis is also central. In boys the increase in lean mass and decrease in fat mass during normal pubertal development is followed by a rapid decrease in insulin sensitivity(81). A longitudinal study of 235 healthy children followed during puberty revealed a decrease in insulin sensitivity before physical signs of puberty and before increases in sex steroids were detectable(80). Increased adiposity before puberty only partly explained the decrease in insulin sensitivity but moreover this was explained by the activation of the GH/IGF-I axis as development of transient insulin resistance follows the same pattern as the GH/IGF-I axis with a peak in mid-puberty(77, 79, 80, 82).

Children born SGA are more likely to start puberty early than those born AGA where a more rapid bone maturation and an earlier and shorter peak height velocity result in faster progression through puberty and earlier menarche (89). The faster progression through puberty is a matter of concern in the recombinant human GH (rhGH) treated children born SGA where a good growth response at the start of treatment is followed by an advancement of bone age resulting in an earlier fusion of growth plates and thereby lower pubertal height gain and a lower effect of the treatment. Long-term longitudinal data on the pubertal progression in rhGH treated SGA children are scarce and more studies are needed.

Age at pubertal onset is declining world-wide and the rapid increase in childhood obesity has been suggested as one of the reasons for that. The NHANES III study, a US population-based study, revealed that increased BMI is related to earlier pubertal onset in both girls and boys(83). Children who are obese when they enter puberty have a lower insulin sensitivity than lean peers, and insulin sensitivity declines during puberty independent of obesity. Several studies suggest that obese youth do not recover insulin sensitivity at the end of puberty meaning that the decrease in insulin sensitivity is low throughout puberty(63, 84, 85). In addition, girls with central precocious puberty (CPP) had increased insulin resistance at the time of diagnosis compared with girls with normally timed puberty and these differences could not solely be explained by higher adiposity found in girls with CPP(86). Interestingly, insulin resistance increased after one year of treatment with gonadotropin releasing hormone agonist (GnRHa) despite complete gonadal suppression probably due to the substantial increase in fat mass observed in some girls during GnRHa treatment(86).

Summary: *Fetal growth is mainly regulated by placental growth hormone assuring nutrient availability to the fetus either directly or indirectly via IGF-I. Fetal IGF-I and insulin levels are important for fetal growth and size at birth whereas GH is less important. Most children born SGA have a rapid growth in infancy, but 10% do not demonstrate catch-up growth. Lack of catch-up growth is believed to be the result of a decreased GH/IGF-I action and altered regulation of appetite. During the first year of life growth is more dependent on nutrition than GH. IGF-I and insulin secretion are positively associated with weight and accrual of both muscle and bone mass during childhood. Obesity in childhood leads to increased growth but also earlier puberty and thereby earlier cessation of growth. IGF-I levels increase rapidly at pubertal onset reaching acromegalic levels at mid-puberty. Simultaneously, a decrease in insulin sensitivity is seen in puberty which is partly triggered by the increasing adiposity before puberty but moreover by the activation of the GH/IGF-I axis.*

GH, IGF-I and insulin actions and long-term health consequences

Fetal origins of adult disease

Long-term health consequences associated with the GH, IGF-I and insulin actions may be a result of the 'programming' effect caused by a deleterious fetal environment. In 1977 Forsdahl *et al.* found a positive correlation between infant mortality and later atherosclerotic heart disease in Norway(87). Around ten years later Barker and colleges did similar observations in England and Wales(88, 89, 90), which resulted in proposal of the 'Fetal Origins of Adult Disease' hypothesis suggesting that harmful events during fetal life may have long-term health consequences(91). Programming describes the effect of environmental stimuli during a critical period of early life, which may result in permanent physiological changes leading to increased disease susceptibility later in life(92). Numerous studies have shown this relationship between low BW and the risk of cardio-metabolic disease later in life(93, 94) and many studies indicated that this relationship was primarily due to the rapid postnatal weight gain seen in many children born SGA(95, 96). Hales and Barker proposed the thrifty phenotype hypotheses(97) explaining that poor fetal growth caused by poor nutrition *in utero* resulted in permanent metabolic changes including insulin resistance and reduced capacity for insulin secretion. Thus, low birth weight followed by a rapid catch-up growth may lead to development of obesity, T2D and cardiovascular disease later in life(98). Naturally, the risk of cardio-metabolic disease observed in children born SGA after catch-up growth has raised concern about the effect of GH treatment in SGA children with persistent short stature, but so far long-term longitudinal follow-up studies on the effects of GH therapy on metabolic risk in these patients are scarce.

McCance *et al.* found that the association between BW and the prevalence of T2D was U-shaped in the Pima Indians(99) suggesting that both being born SGA and LGA are risk factors for later cardio-metabolic disease. The association between LGA and later disease was related to maternal diabetes. It is well-known that offspring of mothers with diabetes may display excess fetal growth resulting in fetal hyperinsulinemia and thereby macrosomia(100). Studies have shown that offspring of mothers with gestational diabetes T2D during pregnancy have an increased risk of obesity, hypertension and insulin resistance already in young adulthood(101) and similar findings were shown for offspring of mothers with type 1 diabetes(102, 103, 104, 105).

The relationships between early growth patterns and later risk of metabolic disease are well described, but the mechanisms are poorly understood. Common genetic mechanisms have been suggested to link BW and risk of disease(106), but there is inconsistent evidence linking SNPs related to insulin sensitivity, T2D, or obesity to risk of SGA at birth(107, 108). However, several candidate mechanisms have been proposed and over the recent years there is growing evidence that epigenetic modifications including DNA methylation, histone modifications, chromatin remodeling and/or regulatory feedback by microRNAs can promote the metabolic syndrome phenotype by modulating gene expression (109). Changes of epigenetic patterns in the GH-IGF1

axis during the fetal period may lead to conditions as diverse as short stature, hypertension, T2DM, and cardiovascular disease(110) but so far data in this field of research are limited.

IGF-I as a biomarker of disease

Adult height (AH) may be a predictor of later disease(111) and the insulin and IGF-I signaling pathways are involved in this association. Large epidemiological studies found that IGF-I concentrations both in the lower and higher end of the reference range may be a biomarker of development of cardiovascular disease(112, 113, 114, 115), diabetes(116, 117, 118, 119) and cancer(120, 121, 122). A U-shaped association between IGF-I and all-cause mortality was found in a large meta-analysis(123) indicating that both low and high concentrations of IGF-I are associated with an increased risk of disease. In addition, the IGF-I levels are thought to follow a trajectory throughout life which is underlined by a high heritability of IGF-I(124). A recent study found that stability of the IGF-I trajectory in older individuals was associated with a lower mortality compared to fluctuating IGF-I levels(125). The biologic mechanism behind this complex link between IGF-I and morbidity and mortality later in life still needs to be elucidated, but the interaction between IGF-I and insulin undoubtedly plays a role. A large population-based cohort study also found a U-shaped association between IGF-I levels and insulin sensitivity(126) and in patients with T2D the range of IGF-I concentrations is broad and multiple variables are interacting. Thus, all these variables combined may influence IGF-I concentrations and actions but not through a uniform pathway.

GH treatment and long-term risk of cancer

The role of IGF-I (and IGF-II) in development of cancer has been thoroughly explored both in *in vitro* studies and animal studies because both IGF-I and IGF-II influence cell growth and have anti-apoptotic effects. Even though the evidence of a cancerogenic effect in humans is low (127), long-term safety of rhGH treatment has been an ongoing concern since epidemiological cohort studies have linked higher IGF-I levels to an increased risk of cancer. Due to this concern a European cohort; The Safety and Appropriateness of Growth Hormone treatments in Europe (SAGhE) study was established to monitor mortality and risk of cancer in a large cohort of 24,000 people across Europe formerly treated with rhGH(128). The first results from the SAGhE study published in 2012 revealed that all-cause mortality including mortality from bone tumors and cardiovascular disease was increased among rhGH treated patients(128). These results prompted major concern among pediatric endocrinologists but the following results of the SAGhE study found no cancerogenic effect of rhGH treatment(128, 129, 130) which was supported by other studies(131, 132) including a meta-analysis (133). However, although the causal link between higher IGF-I levels and development of neoplasia has not been determined the current guidelines for rhGH treatment during childhood recommend that the IGF-I levels are kept within the normal range (below + 2 SD) for safety reasons(134, 135, 136).

Summary: *Studies have pointed at IGF-levels as a biomarker of later disease as both subjects with low and high concentrations of IGF-I have an increased risk of disease and death. The biologic mechanism behind this*

is complex, but the interaction between IGF-I and insulin undoubtedly plays a role. The evidence of a cancerogenic effect of elevated IGF-I levels in humans is weak, but the epidemiological findings have put focus on the effect of rhGH treatment on risk of cancer. A large European study had some contradictory results, but the overall conclusion did not generally support a carcinogenic effect of rhGH.

Treatment with recombinant human Growth Hormone

In 1958 a report by Raben quoted that a link between growth disorders and the pituitary gland was established, and that a highly favorable effect on growth with GH extracted from human pituitaries was seen(137). Subsequently, treatment with GH from human pituitaries was performed until 1985 where a patient who had received therapy with human GH died from Creutzfeldt-Jakob's disease. Coincidentally, pharmaceutical companies had succeeded in producing GH by inserting the gene controlling GH synthesis into bacteria and from around 1985 rhGH was manufactured and approved for children with GHD. During the following years, rhGH treatment was approved for several other conditions, including children with chronic renal insufficiency in 1993, Turner syndrome (TS) in 1996, Prader-Willi syndrome (PWS) in 2000, and children with idiopathic short stature (ISS) in 2003 (only in the US and Canada)(138). Approval of treatment with rhGH in these disorders without GHD (non-GHD) was guided by clinical observations, animal experiments and randomized controlled trials evaluating the effect of rhGH on height gain in children. These first studies showed an increase in height velocity during the first years of treatment, and AH gain around 1 SD (6–7 cm).

Treatment with rhGH of short SGA children was approved by the US Food and Drug Administration in 2001 and by the European Agency for the Evaluation of Medicinal Products in 2003. This approval was based on a few studies showing a significant height gain during short-term rhGH treatment(139, 140, 141), whereas studies including data on improved AH after rhGH treatment was not published until 2003(142, 143). One study found an improvement of AH by almost 2 SD after long-term treatment with rhGH but this study also included children with partial GHD which may have had an impact on the results(142). In contrast, a short-term study with a mean treatment duration of 2.7 years found an increase in AH of 0.6 SD in the treated group compared to the untreated group(143). The recommended dose for treatment of SGA children was put forward in two consensus statements from 2001 and 2007 recommending a higher start dose of 68 µg/kg/day and then after catch-up growth and during puberty the doses could be lowered to 34-68 µg/kg/day. Initial studies showed that higher doses of rhGH increased the growth response on the short-term but there was no difference in final height(139, 142). The rhGH doses applied to the non-GHD groups are in general about 1.5–2-fold higher than those used for rhGH replacement in GHD.

IGF-I titration of rhGH dosing

Some of the non-GHD children (e.g. TS, PWS or SGA children) experience supraphysiological concentrations of IGF-I during rhGH treatment. Concern has been raised that the high levels of IGF-I in non-GHD children

could lead to an increased risk of disease because large population-based studies linked higher IGF-I levels to all-cause mortality(120, 122, 144). However, no studies have established a causal relation between higher IGF-I levels during rhGH treatment in childhood and increased long-term morbidity or mortality. Nevertheless, clinical guidelines for rhGH treatment of children with TS, PWS or short SGA children recommend to keep serum IGF-I concentrations below +2 or +3 SDS during rhGH treatment(134, 135, 136). The conventional rhGH dosing regimen is based on body size, but an alternative strategy is dosing by IGF-I concentrations, which leads an individualized rhGH dose to retain efficacy without exposing the subjects to high IGF-I levels. Experience in GHD and ISS children demonstrated an increased growth response, but also a higher average rhGH dose, in those with IGF-I concentrations titrated to the upper limit of the reference range compared to those titrated to achieve a mean IGF-I concentration (145, 146). Titration of rhGH dosing to keep IGF-I levels below +2SD in girls with TS proved less effective in terms of height gain than current dosing regimens and the doses were reduced compared to the current clinical guidelines(147). In the North European Small for gestational Age Study (NESGAS) 110 short SGA children were randomized into three different dosing regimens; a 'low dose' (35µg/kg/day), a 'high dose' (67µg/kg/day) or a rhGH dose titrated according to IGF-I levels(148). The high-dose group had an increased growth response compared to the other two groups during the two years of trial. IGF-I titration resulted in physiological IGF-I levels within the normal range, but it led to a wide range of rhGH doses and the IGF-I titration group had a poorer growth response(148).

The carcinogenic effect of rhGH treatment during childhood has not been shown and it could be speculated that continuous supra-physiological levels of IGF-I may be tolerated during rhGH treatment of some of the non-GHD children to maintain a good growth response. However, the supraphysiological levels of total IGF-I may not reflect the levels of bioactivity because almost all IGF-I is bound to the IGFBP's and only 1% is unbound 'free' IGF-I.

Measurements of bioactive IGF concentrations by the IGF-I KIRA determine the ability of serum IGF-I to phosphorylate and thereby activate the IGF1R, and hence IGF bioactivity(17). A discrepancy between bioactive IGF and total levels of IGF-I was reported in both adults(149) and children(150, 151). In adults, bioactive IGF concentrations correlated better with GHD than total IGF-I levels, and bioactive IGF was a better screening tool for GHD than total IGF-I with a sensitivity of 82% for bioactive IGF vs. 62% for total IGF-I(152). Studies of SGA and PWS children have shown that while total IGF-I levels increased to above +2 SDS during rhGH treatment, bioactive IGF stayed within the normal range for most of the children(150, 151). Among the rhGH treated SGA children total IGF-I concentrations correlated better with the growth response during the first year of high-dose rhGH treatment than bioactive IGF. However, bioactive IGF, and not total IGF-I, correlated with height and weight at baseline(151). These findings may suggest that bioactive IGF is a better marker of the biological active IGF-I regulated by the endogenous secretion of GH than total IGF-I

levels. On the other hand, only total IGF-I concentrations were associated with change in height during rhGH treatment(151).

Summary: *Treatment with recombinant human GH was initiated in the mid-eighties and since then several indications of rhGH treatment have been approved. Higher doses rhGH are associated with a better short-term growth response, but no difference in adult height gain. Supraphysiological IGF-I levels are seen in some of the rhGH treated non-GHD children. Clinical guidelines recommend keeping IGF-I levels below +3 SDS, but titration of rhGH dose according to IGF-I levels have less effective in terms of height gain compared to the weight-based dosing regimen. Determination of bioactive IGF could be a better marker of the bioactivity and ability to activate the receptor than total IGF-I concentrations.*

Growth hormone treatment and insulin sensitivity

Treatment with rhGH induces impaired hepatic and peripheral insulin sensitivity, and thereby diminishes insulin-dependent glucose disposal due to the increase in lipolysis and increase in flux and oxidation of FFA which leads to a glucose-FFA substrate competition. IGF-I response during rhGH treatment and insulin secretion are highly correlated which point towards an important role of IGF-I generation in maintaining appropriate β-cell function to produce a compensatory increase in insulin secretion in response to the rhGH induced insulin resistance. If this compensatory increase in insulin secretion is incomplete then disposition index will decrease, and this could lead to development of impaired glucose tolerance or even type 2 diabetes (T2D) (Figure 4).

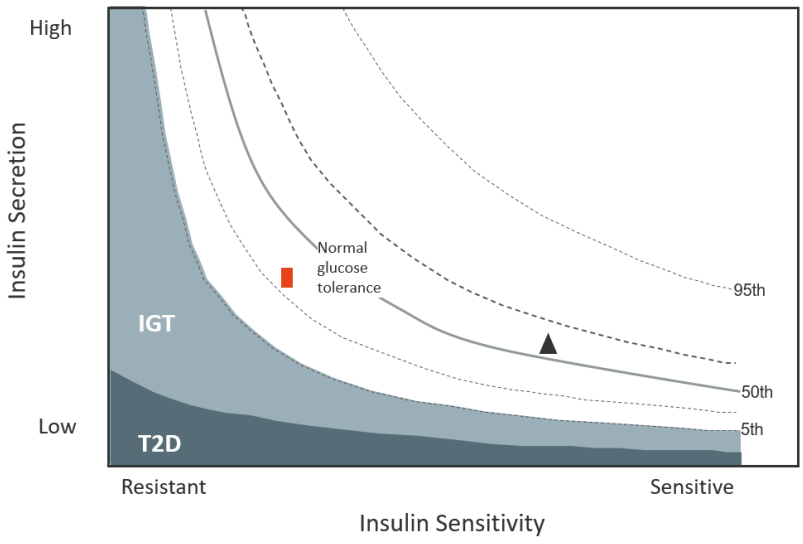

Figure 4: Association between insulin secretion and insulin sensitivity in normal subjects, in subjects with Impaired glucose tolerance (IGT) and in subjects with type 2 diabetes (T2D). The black triangle indicates values for a child before treatment with growth hormone and the red square a child during treatment with growth hormone.

Adapted from Kahn, et al. *Diabetes*. 1993;42: 1663-1672

Baseline serum IGF-I concentrations were inversely associated with growth response during the first year of rhGH treatment in SGA children(153, 154, 155). Studies on rhGH treatment of SGA children also found that lower IGF-I levels were associated with a higher insulin sensitivity and a better one-year growth response to rhGH treatment(153, 155, 156) and that changes in IGF-I (SDS) were related to a compensatory insulin secretion after one year of rhGH treatment(155). Reduced growth, IGF-I response and insulin sensitivity during rhGH treatment of SGA children with higher baseline IGF-I concentrations were suggested to indicate a relative hormone resistance or a defect common to insulin and IGF-I signaling(157). In a rat model of diet-induced insulin resistance a reduction in mRNA and protein expression of IGF-I in muscle and bone cells pointed towards a common component of the insulin/IGF-I mediated signaling process(158). Thus, it may be speculated that rhGH dosing in non-GHD children should be adjusted according to baseline IGF-I levels and insulin sensitivity and that in some children elevated IGF-I are necessary to overcome the relative hormone resistance (Figure 5).

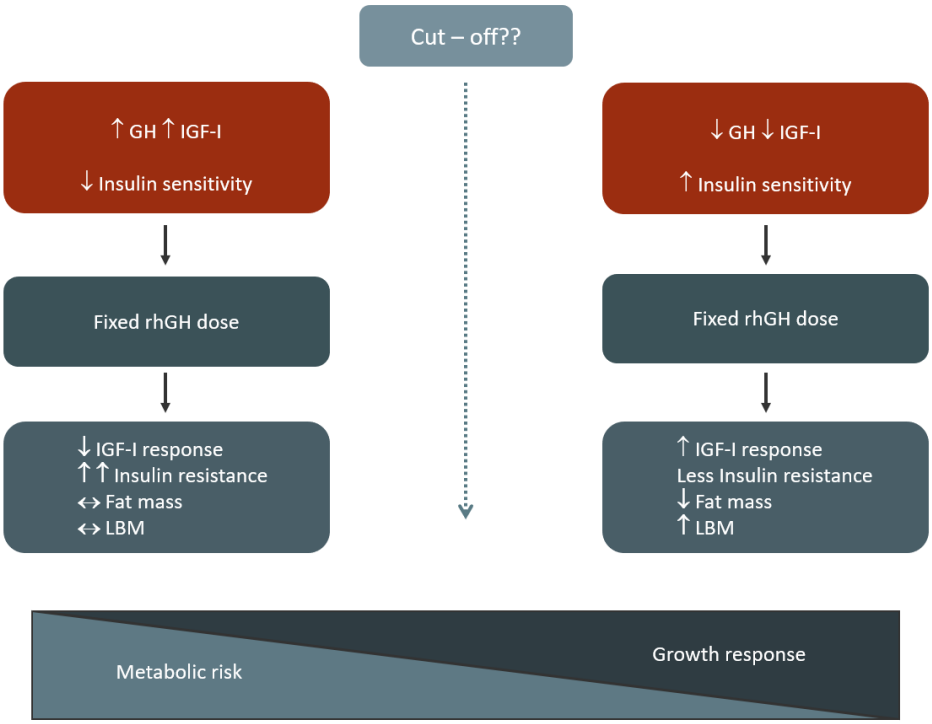

Figure 5: Risk and benefit during treatment with growth hormone of patients born small for gestational age (SGA) according to baseline levels of GH and insulin-like growth factor-I (IGF-I) (red box) and after rhGH treatment (blue box).

Potential adverse metabolic effects of rhGH treatment of SGA children who have an increased background metabolic risk has been a matter of concern. As expected, insulin sensitivity was markedly reduced during treatment of SGA children with rhGH(155, 159), and some studies also observed decreased disposition index and changes in fasting glucose and haemoglobin A1c, suggestive of an incomplete compensatory response to the decreased insulin sensitivity(155, 160, 161) whereas others found no changes(162). However, after

cessation of rhGH treatment of SGA children studies confirmed that these changes were reversal and that fat mass, insulin sensitivity and beta-cell function in rhGH-treated SGA adults were comparable were similar to untreated SGA controls(163, 164). These findings indicate that any favorable effects of rhGH on body composition and metabolic function were not sustained on cessation of treatment. In line with this, a study on insulin sensitivity in girls with TS found no deterioration in glucose homeostasis during seven years of rhGH treatment(165). Additionally, children with PWS improved their glucose homeostasis during the first three years of rhGH treatment probably due to the increase in lean mass(166). In contrast, the indication of many years of rhGH treatment in non-GHD children could be debated if treatment response according to height gain is widely variable and in some cases very low and no other beneficial effects are seen, but more longitudinal studies are needed to see the longstanding impact of rhGH treatment.

Interestingly, insulin sensitivity during rhGH treatment was not related to either IGF-I or growth responses in a cohort of SGA children suggesting differential effects of high-dose rhGH on pathways related to insulin signaling and those responsible for growth and IGF-I generation(155). Growth response and IGF-I generation are mediated through related pathways involving direct GH signaling, whereas effects on insulin sensitivity predominantly involve indirect mechanisms including alterations in lipolysis. Most short SGA children have significant deficits in body fat due to reduced appetite and lower nutrition. Greater adiposity in a cohort of rhGH treated short SGA children was associated with a better growth and IGF-I response as well as an increase in insulin secretion during first year rhGH treatment(167). This study also proposed a possible causal role of insulin resistance in mediating the link between lower adiposity and a lower GH sensitivity to exogenous rhGH(167). Thus, short children born SGA with higher IGF-I at baseline are more insulin resistant and have a lower adiposity which results in a decreased growth response and decreased generation of IGF-I and insulin as well as no change in adipose tissue during rhGH treatment. These children may be at higher risk of metabolic disease later in life due to the lack of a compensatory rise in insulin secretion (Figure 5). It could be speculated that treatment with an insulin sensitizer in addition to rhGH in short SGA children with increased IGF-I levels would improve IGF-I generation and growth response as seen in a former study of SGA girls with premature adrenarche(168, 169).

Summary: Treatment with rhGH induces insulin resistance but is followed by a compensatory increase in insulin secretion. Concern has been raised that children born SGA with a background metabolic risk would experience dysmetabolic function during rhGH treatment, but studies after cessation of treatment showed that the changes were reversal and that glucose metabolism in rhGH-treated SGA adults were similar to untreated SGA controls.

Genetic susceptibility

The impact of GH, IGF-I and insulin on pre- and postnatal growth as well as growth during rhGH treatment is influenced by both environmental and genetic factors. Traditionally, twin studies were used to reveal the importance of environmental and genetic influences for traits by comparing monozygotic and dizygotic twin pairs and thereby estimate heritability. Former twin studies revealed that the heritability estimates of IGF-I and IGFBP-3 concentrations were 63% and 60%, respectively, indicating the concentrations to be highly genetically determined(170, 171, 172). In contrast, heritability estimates for IGFBP-1 concentrations, suggested that environmental factors such as lifestyle controlled IGFBP-1 and insulin levels more than genetic factors(170, 172). A study of elderly twins confirmed the strong heritability of concentrations of IGF-I and IGFBP-3, as well as of insulin secretion and disposition index, whereas heritability for insulin sensitivity and IGFBP-1 concentrations were small and non-significant(124). Additionally, in the same cohort of twins, IGF-I levels were negatively associated with insulin sensitivity and there was no effect of zygosity on this relationship. Thus, the associations between IGF-I levels and abnormalities in glucose metabolism are mediated primarily by environmental rather than genetic factors(124).

Single gene

Single mutations in genes involved in the GH, IGF-I and insulin actions may have a major impact on growth and metabolism of the individual child. Complete lack of IGFR signaling is not compatible with fetal survival, whereas allelic haploinsufficiency may impair brain development and cause severe short stature. *IGF1* gene mutations result in severe fetal and postnatal growth failure along with mental retardation and the lack of negative feedback will result metabolic disturbances(173). *IGF2* gene mutations or imprinting disorders will result in pre- and postnatal growth failure but less effects on brain development which is seen in children with Silver Russell syndrome. Lack of insulin receptor signaling will cause Leprechaunism resulting in extreme fetal growth restriction and survival is only possible with treatment with recombinant IGF-I to substitute insulin receptor signaling. Absence of GH or absence of GH signaling will lead to a severely short AH and severe metabolic consequences (reviewed by Bang(174)).

Common Genetic polymorphisms

Single gene mutations are extremely rare, but more common gene polymorphisms may also have an impact on variations in insulin, GH and IGF-I signaling in the general population. Former studies hypothesized that common genetic polymorphisms influenced both pre- and postnatal growth and thereby challenged the "programming hypothesis" with the assumption that environmental factors solely modify the endocrine and metabolic alterations caused by a deleterious intrauterine environment. One of the proposed candidate polymorphisms was a common microsatellite polymorphism in the promoter region of the *IGF1* gene. Carriers of this allele had lower IGF-I levels, decreased height, and an increased risk of T2D compared to the carriers of the wild-type allele. Furthermore, absence of the wild-type allele was associated with a lower birth weight

in some studies (175, 176, 177, 178), but not in others (179, 180). Another candidate polymorphism in the insulin (*INS*) gene, i.e. the variable number of tandem repeats (*INS*-VNTR) locus, was suggested to link fetal growth with adult onset of disease(181, 182, 183), but other studies found that *INS*-VNTR was associated to insulin resistance in adulthood, but there was no link to birth weight(184, 185).

A common polymorphism in the GHR gene, found in approximately 50% of the European population, is a deletion of exon 3 (d3-GHR), which encodes a 22-amino acid residue sequence in the extracellular domain located away from the binding interfaces. This genetic variant has been extensively investigated. Since Dos Santos *et al.* showed in transfection experiments that the transduction of growth hormone signaling through d3-GHR homo- or hetero-isoforms was around 30% higher than through full-length GHR isoform(186). Furthermore, carriers of at least one d3-allele had an increased growth response after two years of rhGH in two cohorts of short SGA and ISS children(186). Following this study several clinical studies have investigated the growth response in rhGH treated children with GHD, SGA and girls with TS(187, 188, 189, 190, 191, 192). Although some controversy exists, two meta-analyses concluded that carriers of the d3-GHR isoform had a better growth response during rhGH treatment than carriers of the full-length isoform(193, 194). Interestingly, opposed to the increased growth response postnatally, some studies found a tendency towards decreased fetal growth and lower BW in the carriers of the d3 allele (27, 195, 196, 197). However, in a large cohort of SGA children the full-length isoform was the most prevalent(198) and in a cohort of healthy young men there was no association between BW and the GHR isoform(199). It has been suggested that a better GH signaling among the d3-GHR carriers could induce a decrease in insulin sensitivity due to the enhanced lipolytic effects and increase in FFA, but controversies exist. In a study of healthy children and adolescents the d3-GHR isoform was associated with a higher insulin secretion and DI after adjusting for age, gender, pubertal stage and insulin sensitivity(196) but other studies found no difference in insulin sensitivity between the different isoforms (198, 200). Among rhGH treated children born SGA in the NESGAS study no association between growth response and genotype was found, however, the carriers of the d3-GHR allele had lower insulin sensitivity but similar insulin secretion and DI at baseline compared to carriers of the full-length allele(192) which confirmed the results of the PREDICT study(191). In contrast, Audi *et al.* found no effect of the d3-GHR polymorphism on insulin sensitivity in a cohort of 219 rhGH-treated or untreated short SGA children(198). The mechanisms of action by which the common d3-GHR polymorphism influences prenatal and postnatal growth differentially and a possible metabolic effects remain largely unclarified and considering the fact that the polymorphism is found in half of the Caucasian population it is important to be aware of the risk of random findings.

Single nucleotide polymorphisms

Genome wide association studies (GWAS) have found numerous common single nucleotide polymorphisms (SNPs) throughout the genome associated with different genetic traits. As an example around 80% of the

variability of height is genetically determined and hundreds of single nucleotide polymorphisms at different loci were found to be involved in normal growth and thereby adult height, which is a highly heritable and classic polygenic trait(201, 202). Multi-allele gene scores of different traits were constructed through GWAS meta-analyses including large numbers of individuals with well-characterized phenotypes to provide insight into the underlying biological pathways(203).

The Mendelian randomization approach uses informative genotypes or allele scores of known functions as indicators of the likely causal effects of their target traits. Thereby, biological pathways found in epidemiological observational studies may be confirmed and the genetic findings can be used as biomarkers of later disease. In addition, a Mendelian randomization approach can be used as an alternative application of pharmacogenetics to inform the mechanisms of treatment response by considering genotypes or allele scores to examine the causal effect with less risk of reverse causation and confounding. In a study of short SGA children the clinical findings of a link between lower insulin sensitivity and a lower first year growth response to high-dose rhGH treatment(155) was proposed as a causal association since the insulin sensitivity allele scores from GWAS studies were associated with the growth response after one year(204) (Figure 6). Furthermore, the insulin secretion allele scores were associated with spontaneous postnatal linear growth which confirms the epidemiological findings in the large population-based ALSPAC cohort where insulin secretion was positively related to childhood height(57). Such causal interpretations of the interaction between GH, IGF-I and insulin depend on various assumptions and therefore requires further experimental validation; thus, future strategies could include the Mendelian randomization approach to individualize treatment as part of personalized medicine.

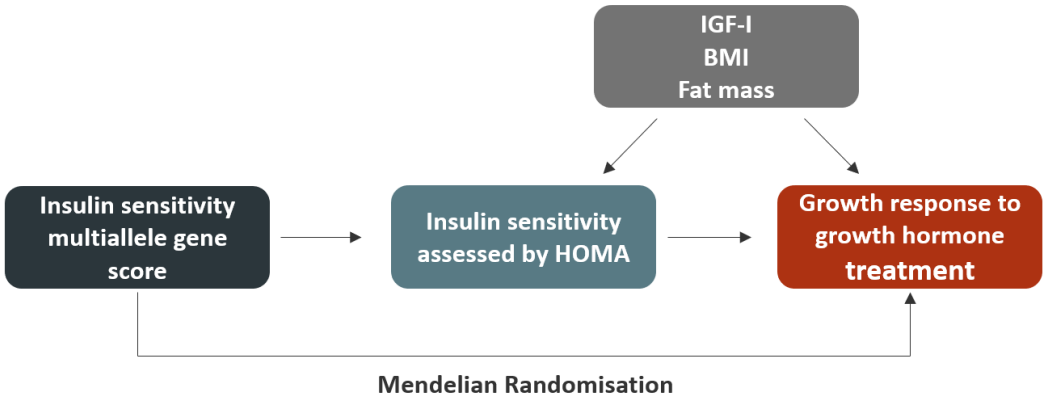

Figure 6: The Mendelian Randomization approach is used to assess the causal association of the genetic variant (black box) which acts as a proxy for modifiable biological traits (blue box) directly on the outcome (red box) bypassing the confounding effects (grey box).

Summary: *Concentrations of IGF-I are highly genetically determined whereas insulin concentrations are determined by environmental factors such as lifestyle more than genetic factors. Single gene mutations have severe phenotypes whereas common genetic polymorphisms and single nucleotide polymorphisms may be indicators of causal effects and thereby explain biological pathways. More knowledge of the effect of genetic and epigenetic variants on modifiable biological traits may enable us to individualize treatment to suit each individual person.*

Conclusions

In this review, the interactions between GH, IGF-I and insulin important for anabolic processes through pre- and postnatal life and during rhGH treatment, has been summarized. IGF-I and insulin are ancestrally related, and they play an integral role in maintaining glucose homeostasis as well as in regulating cell proliferation which have both short-term effects on growth and metabolic function and long-term health consequences.

The promising effects on growth with GH extracted from human pituitaries were discovered in 1958 and treatment with rhGH has been available since the mid-eighties and is now an approved indication for several patient groups including children with normal GH secretion (non-GHD). The non-GHD group includes children born SGA, children with PWS and girls with TS who have a background risk of later cardio-metabolic disease. It is well-known that growth hormone is a modulator of insulin sensitivity and potentially treatment with rhGH could worsen the background metabolic risk in non-GHD children, but no results indicated an increased risk for T2D in these patients. However, studies on children born SGA explored the impact of metabolic function on growth and found that insulin sensitivity and IGF-I levels were inversely related before start of rhGH treatment, and that baseline insulin sensitivity played a causal role for growth response during treatment.

Large epidemiological population-based cohort studies have explored IGF-I as a biomarker of cancer, cardiovascular and metabolic disease later in life. Many of the non-GHD children experience supraphysiological levels of IGF-I during rhGH treatment probably as a response to the decreased insulin sensitivity induced by rhGH. For safety reasons clinical guidelines recommend lowering the dose of rhGH if the IGF-I levels are elevated, but this approach led to a much lower growth response during rhGH treatment than the conventional treatment regimens in both SGA children and girls with TS probably. Thus, it may be necessary to accept elevated IGF-I during rhGH treatment of these patients to overcome the relative hormone resistance a thereby get a better growth response. In addition, novel treatment regimens are needed to optimize treatment to suit the individual child and improve efficacy of rhGH treatment, and titration of the rhGH dose according to the biological active form of IGF-I; bioactive IGF, instead of total IGF-I, could be proposed. Furthermore, improved knowledge of genetic and epigenetic variations and their impact on growth and metabolic function is a key step towards individualized medicine in the future.

The group of children born SGA is heterogeneous and the influence of rhGH therapy may vary according to the underlying etiology of their intrauterine growth failure. Most children born SGA have a spontaneous catch-up growth and the risk of metabolic dysfunction later in life is particularly related to a rapid gain in weight early in life. The minority of SGA children, who do not have catch-up growth, are eligible for rhGH treatment to reduce the height deficit. Treatment with rhGH also induces catch-up growth, but this is mainly an increase in lean mass more than fat mass and therefore may have beneficial effects on body composition. Treatment with rhGH has thereby been suggested to potentially reverse the adverse effects of intrauterine programming. The same beneficial effect of rhGH treatment has been proposed in patients with TS and PWS who phenotypically have decreased lean mass and increased fat mass before start of treatment. However, these data show short-term changes in potential surrogate markers for long-term metabolic health. Only a few long-term follow-up studies after cessation of treatment have been performed and they demonstrated that body composition, especially fat mass, insulin sensitivity and beta-cell function were comparable between previously rhGH treated subjects and untreated subjects born SGA. Thus, any favorable effects of rhGH on body composition and metabolic function were not sustained on cessation of treatment. Gain in height after treatment is highly variable in the rhGH treated non-GHD children and it could be debated whether this treatment given over many years should be available for all children with non-GHD. It is therefore of utmost importance that long-term longitudinal follow-up studies of all children receiving rhGH treatment, and especially those with non-GHD, are being performed to evaluate the safety, efficacy, and cost-effectiveness continuously.

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Appendix (Printed manuscripts)

Manuscript 1: Baseline IGF-I levels determine insulin secretion and insulin sensitivity during the first year on growth hormone therapy in children born small for gestational age. Results from a North European Multicentre Study (NESGAS). *Hormone research in paediatrics* 2013;80(1):38–46.

Manuscript 2: A randomised controlled trial evaluating IGF1 titration in contrast to current GH dosing strategies in children born small for gestational age: The North European Small-for-Gestational-Age Study. *European Journal of Endocrinology* 2014;171(4):509–518.

Manuscript 3: Genetic Markers of Insulin Sensitivity and Insulin Secretion Are Associated With Spontaneous Postnatal Growth and Response to Growth Hormone Treatment in Short SGA Children: the North European SGA Study (NESGAS). *The Journal of Clinical Endocrinology & Metabolism* 2015;100(3): E503–E507.

Manuscript 4: Adiposity in children born small for gestational age is associated with β-cell function, genetic variants for insulin resistance, and response to growth hormone treatment. *The Journal of Clinical Endocrinology and Metabolism* 2016;101(1):131–142.

Manuscript 5: The exon3-deleted growth hormone receptor gene polymorphism (d3-GHR) is associated with insulin and spontaneous growth in short SGA children (NESGAS). *Growth Hormone and IGF Research* 2017;35(July):45–51.

Manuscript 6: Genetic influence on the associations between IGF-I and glucose metabolism in a cohort of elderly twins. *European Journal of Endocrinology* 2018;178(2):153–161.

Manuscript 7: Increases in Bioactive IGF do not Parallel Increases in Total IGF-I During Growth Hormone Treatment of Children Born SGA. *The Journal of clinical endocrinology and metabolism* 2020;105(4):1–8.

Manuscript 8: Growth and Adult Height in Girls with Turner Syndrome Following IGF-1 Titrated Growth Hormone Treatment. *The Journal of Clinical Endocrinology and Metabolism* 2020;105(8):1–9.

Manuscript 9: A common deletion in the growth hormone receptor gene (d3-GHR) in the offspring is related to maternal placental GH levels during pregnancy. *Growth hormone & IGF research official journal of the Growth Hormone Research Society and the International IGF Research Society* 2020 Dec; 55:101360

Manuscript 10: Impact of Lean Body Mass and Insulin Sensitivity on the IGF-1-Bone Mass Axis in Adolescence: The EPICOM Study. *The Journal of Clinical Endocrinology and Metabolism* 2021;106(2):E772–E781.

Original Paper

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Baseline IGF-I Levels Determine Insulin Secretion and Insulin Sensitivity during the First Year on Growth Hormone Therapy in Children Born Small for Gestational Age. Results from a North European Multicentre Study (NESGAS)

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Key Words

Growth hormone treatment · Small for gestational age · Insulin-like growth factor-I · Insulin sensitivity · Insulin secretion · Disposition index · IGF-I resistance

Abstract

Objective: Developmental programming alters growth and metabolic outcome in children born small for gestational age (SGA). We explored insulin and glucose metabolism in SGA children treated with a fixed GH dose over 1 year. ***Methods:*** In the North European Small for Gestational Age Study (NESGAS), 110 short SGA children received GH at 67 μg/kg/day for 1 year. Insulin secretion was assessed by acute insulin response (AIR), insulin sensitivity (IS) by HOMA and disposition index (DI) by insulin secretion adjusted for IS. ***Results:*** First-year GH therapy led to increases in height and IGF-I standard deviation score (SDS), and reductions in IS ($p < 0.0001$). Compensatory increases in AIR ($p < 0.0001$) were insufficient and resulted in reduced DI ($p = 0.032$). Children in the highest IGF-I SDS tertile at baseline were the least insulin sensitive at baseline ($p = 0.024$) and 1 year ($p = 0.006$). IGF-I responses after 1 year were positively related to AIR ($r = 0.30$, $p = 0.007$) and DI ($r = 0.29$, $p = 0.005$). ***Conclusion:*** In SGA children treated with a high GH dose for 1 year, baseline IGF-I levels were related to IS whilst gains in height and IGF-I responses were associated with insulin secretion. Defining heterogeneity in IGF-I in SGA children may be useful in predicting growth and metabolic response.

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Introduction

Low birth weight is consistently associated with an increased risk of development of type 2 diabetes (T2D) and cardiovascular disease, and reduced adult stature in population studies [1, 2]. Developmental programming of endocrine axes, in particular the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis, may underlie the reduced linear growth and increased T2D risk observed in children born small for gestational age (SGA) [3, 4]. About 10% of SGA children do not demonstrate catch-up growth and remain short throughout life if left untreated [5–8]. GH therapy may be indicated in these children as it leads to improvement in adult height [9–12]. Potential adverse effects of GH on glucose metabolism in a group of children with an increased background metabolic risk are a matter of concern in in short SGA children treated with GH [13].

Although a heterogeneous group with regard to aetiology, alterations in the GH/IGF-I axis including abnormalities in GH secretory patterns and low IGF-I levels observed in SGA children may play an important role in the growth failure [14, 15] and potentially subsequent metabolic risks. Insulin sensitivity (IS) at baseline has been associated with baseline IGF-I levels, and may predict growth response [16, 17]. These findings suggest that variations in baseline IGF-I levels may be related to both growth and metabolic response to GH treatment. Exploring the links between these factors may help to identify a group of SGA children at a higher risk of metabolic decompensation and T2D.

The North European Small for Gestational Age Study (NESGAS) was set up to examine long-term efficacy and safety of childhood GH treatment in short SGA children. The GH treatment in the study involved a high-dose therapy during the first year to induce catch-up growth and to identify non-responders, followed by a randomised dose ranging and IGF-I-based dose titration studies. In this paper, we evaluate the relationship between baseline IGF-I levels, growth response and insulin and glucoregulatory responses during the first year when children are treated with a unified GH dose.

Patients and Methods

Patients

NESGAS is a randomised, parallel-group study of GH treatment in short prepubertal children born SGA and it involves seven investigating centres in four North European countries (UK, Ireland, Sweden and Denmark). The study population included children born SGA (birth weight and/or birth length ≤–2 standard deviation score (SDS)) at a gestational age of ≥28 weeks. The girls were aged between 4.00 and 8.99 years and the boys between 4.00 and 9.99 years. All had persistent short stature at 4 years of age with a height SDS (HSDS) ≤–2.5 SDS, a height velocity SDS <0 during the last 6 months before study entry, and a HSDS >1 SD below parental adjusted HSDS. They were prepubertal as defined by the largest testis volume <4 ml in boys and breast stage I in girls at the start of treatment, and all were naive to GH therapy at inclusion. Patients were excluded from the study if they had any suspected allergy to GH, had severe learning difficulties, previous or active malignancy, benign intracranial hypertension, diabetes and growth retardation due to chronic diseases, syndromes and chromosomal anomalies, with the exception of Silver-Russell syndrome.

Between September 2004 and April 2009, 117 children were recruited into the study. One patient had Silver-Russell syndrome. Seven patients were excluded from the study: 3 due to lack of compliance, 1 developed Legg-Calvé-Perthes disease prior to treatment, 1 was diagnosed with Turner syndrome after inclusion, 1 was excluded due to child protection issues, and 1 moved abroad.

Study Design

During the first 12 months of treatment, patients received a fixed dose of 67 μg/kg/day of recombinant human GH (Norditropin®, Novo Nordisk, Bagsværd, Denmark) given as a daily subcutaneous injection. The aim of high-dose treatment was to induce catch-up growth and identify non-responders (height gains <1 HSDS), and assess maximal effects on glucose and insulin homeostasis. Every 3 months the GH dose was adjusted according to the weight of the child. After 1 year of high-dose treatment, subjects were randomly allocated into three different dose regimens during years 2 and 3 (online suppl. fig. 1, www.karger.com/doi/10.1159/000353438). Poor responders were excluded from the study at 1 year. Only data from the first year of treatment are presented in this paper.

Study Assessments

Information on prenatal history, placental dysfunction, maternal disease, neonatal complications and retrospective growth data were collected and parental height and weight were measured at study entry. Data on birth weight, length, and head circumference were collected from the routine examinations at birth. Participants were assessed at study entry and then every 3 months when the following were measured: standing height on a wall-mounted stadiometer and weight by electronic scales by staff trained in auxological methods. At each visit, pubertal development was assessed by an experienced investigator using the Tanner criteria (breast development/testicular size and pubic hair) and serum IGF-I and IGFBP-3 levels were measured. The patients also underwent a short intravenous glucose tolerance test (IVGTT) and an oral glucose tolerance test (OGTT) at study entry on 2 separate days, and these were followed up with a second IVGTT at 1 year. The short IVGTT involved administering 0.3 g/kg of intravenous glucose over 3 min after an overnight fast, and measurement of blood glucose and insulin levels for the next 10 min (–15, –10, –5, 0, 1, 3, 5, 10 min) and C-peptide levels at 0 min. The OGTT involved administration of 1.75 g/kg glucose (maximum 75 g) dissolved in 250–300 ml water after an overnight fast and measurement of plasma glucose and insulin at 0, 60 and 120 min. HbA_{1c} was also measured at baseline and at 1 year. The WHO criteria were used to define abnormal glucose metabolism (WHO, 2011) [18].

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Laboratory Measurements

Plasma insulin and C-peptide levels were measured centrally in Cambridge, UK, by a DELFIA assay using kits B080-101 and B081-101, respectively (Perkin-Elmer Life Sciences, Turku, Finland). The inter-assay coefficients of variation (CVs) of insulin assay were 3.1% at 29 pmol/l and 2.1% at 79.4 pmol/l, and had a cross-reactivity of <0.5% with intact pro-insulin, 1% with 32–33 split pro-insulin and <0.1% with C-peptide. The inter-assay CVs for C-peptide assay were 4.0% at 190 pmol/l and 3.8% at 1125 pmol/l, and had a cross-reactivity of <0.1% with insulin and 60% with intact pro-insulin and 32–33 split pro-insulin at 400 pmol/l. Plasma glucose and HbA_{1c} were measured locally employing assays routinely used for clinical purposes.

Serum IGF-I and IGFBP-3 concentrations were determined centrally in Copenhagen using a solid-phase enzyme-labelled chemiluminescent immunometric assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, Calif., USA). Standards were calibrated towards the WHO NIBSC IRR 87/518. The detection limit for IGF-I was 20 ng/ml and inter- and intra-assay CVs were 5.93 and 2.02%, respectively. The detection limit for IGFBP-3 was 500 ng/ml and inter- and intra-assay CVs were 5.23 and 1.74%, respectively. IGF-I and IGFBP-3 SDS were calculated from our reference data based on serum samples from 1,729 healthy children (911 girls) using the same assays [19, 20].

Safety Parameters

Safety assessments were carried out at each visit. For serious adverse events, a serious adverse event form was completed and reported to the overall study coordinating investigator within 24 h of obtaining knowledge about the event. Adverse events were reported to the Health Authorities and Independent Review Boards/Independent Ethics Committees (IRBs/IECs) in accordance with national laws and regulations.

No serious adverse events were reported during the first year of GH treatment. The GH dose was well tolerated and only a few adverse events were considered related to GH treatment. The majority of adverse events were mild to moderate infections unrelated to the treatment. Adverse events that could be related to GH treatment predominantly included painful extremities, limping and scoliosis (n = 5), hypertrophy of adenoid tissue or tonsils (n = 4) and periorbital puffiness (n = 3). The majority of these reported symptoms are well-known adverse effects of GH treatment and resolved without any changes in treatment, and none of the patients developed T2D.

Calculations

SDSs were derived for birth weight, birth length, head circumference, height, weight and BMI using central country-specific reference databases [21–23]. Target HSDS was computed using the formula (maternal HSDS + paternal HSDS)/2. BMI was computed using the formula, weight (kg)/height^2 (m). IS was estimated from fasting glucose (average of –10, –5, and 0-min samples) and C-peptide values by homeostatic model (HOMA) using the HOMA 2 calculator [24] (http://www.dtu.ox.ac.uk/homacalculator/index.php). Fasting C-peptide rather than insulin levels were used for the calculations of IS as the HOMA model provided a linear output with C-peptide levels whereas fasting insulin levels were too low to fit in the model [24–26]. Acute insulin response (AIR) was calculated by the area under the curve of insulin response above the baseline during the first 10 min of IVGTT using the trapezoidal method and provides a measure of first-phase insulin secretion. Disposition index (DI) provided an estimate of insulin secretion adjusted for the degree of IS and was calculated as the product of IS and AIR.

Statistics

The variables were analysed for normal distribution using the Kolmogorov-Smirnov test and were log transformed to normality if necessary. The baseline and first-year data were compared by paired t tests. In further analyses, the children were divided into three groups (tertiles) according to IGF-I (SDS) levels at baseline. The groups were compared using an analysis of covariants (ANCOVA) model and were adjusted for gender and age. Statistical analyses were performed using the statistical package PASW, version 18 (SPSS, Inc., Chicago, Ill., USA). The normally distributed data are presented as mean (SD) and the transformed data as back-transformed geometric means (1 SD range) unless otherwise specified.

Ethical Considerations

The study (NESGAS EudraCT 2005-001507-19) was performed according to the Helsinki II Declaration and approvals by the ethics committee or institutional review board and national drug authorities were obtained at each study centre. Written informed consent was obtained from the parents or guardians of children included in the study.

Results

Baseline Characteristics

Longitudinal data were collected from 110 patients (69 males) (table 1). At baseline, mean chronological age was 6.28 (1.69) years, mean HSDS was –3.37 (0.76), mean weight SDS was –3.09 (1.03), and mean BMI SDS –1.20 (1.31). The mean IGF-I SDS at baseline was –1.10 (1.21) and IGFBP-3 SDS was –0.82 (1.18). At the start of treatment, no abnormalities in glucose metabolism were observed by HbA_{1c} levels or during the OGTT.

At baseline, age and IGF-I levels were inversely associated with IS ($r = -0.36$, $p < 0.0001$ and $r = -0.30$, $p = 0.004$, respectively), but were unrelated to AIR. Birth weight, gender, current height, weight and BMI were not associated with IS or AIR. Children in the highest tertile of IGF-I had increased levels of fasting insulin ($p = 0.040$), C-peptide ($p = 0.016$) and were the least insulin sensitive ($p = 0.024$) after adjustment for age and gender (table 1).

First-Year GH Treatment

As expected, high-dose GH therapy for 1 year resulted in marked increases in HSDS (+1.0 (0.48)) with large interindividual variability. Likewise, high-dose GH treatment increased IGF-I SDS (+3.72 (1.72)) with a similarly large variability between subjects, but was also associated

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Table 1. Baseline characteristics

	All patients	IGF-I tertiles			p trends	p trends*
		low	middle	high		
Number (boys)	110 (69)	37 (24)	36 (19)	37 (26)		
Birth						
Birth weight (SDS)	–3.18 (0.90)	–3.08 (0.89)	–2.98 (0.84)	–3.46 (0.92)	0.055	
Birth length (SDS)	–3.14 (1.62)	–2.58 (2.08)	–3.18 (1.17)	–3.60 (1.45)	0.100	
Gestational age, weeks	35.49 (3.88)	35.27 (3.52)	35.64 (4.68)	35.56 (3.46)	0.913	
Baseline						
Age, years	6.28 (1.69)	6.06 (1.91)	6.16 (1.50)	6.61 (1.64)	0.336	
Height (SDS)	–3.37 (0.76)	–3.60 (0.87)	–3.23 (0.68)	–3.27 (0.67)	0.076	0.126
Weight (SDS)	–3.09 (1.03)	–3.34 (1.10)	–2.94 (1.00)	–2.99 (0.95)	0.199	0.283
BMI (SDS)	–1.20 (1.31)	–1.37 (1.27)	–1.08 (1.34)	–1.16 (1.35)	0.619	0.650
Target height (SDS)	–0.90 (1.01)	–0.81 (0.93)	–0.93 (1.01)	–0.96 (1.10)	0.815	0.881
HSDS adjusted for TH	–2.45 (1.15)	–2.78 (1.28)	–2.26 (1.03)	–2.31 (1.07)	0.099	0.127
IGF-I (SDS)	–1.10 (1.21)	–2.37 (0.59)	–1.18 (0.29)	0.25 (0.66)	–	
IGFBP-3 (SDS)	–0.82 (1.18)	–1.76 (0.99)	–1.04 (0.62)	0.24 (0.87	<0.0001	<0.0001
Glucose metabolism						
Glucose, nmol/l	4.36 (0.68)	4.17 (0.72)	4.46 (0.62)	4.47 (0.67)	0.098	0.139
Insulin, pmol/l	16.14 (8.21–31.72)	12.42 (6.27–24.61)	18.25 (10.6 – 31.43)	18.74 (10.47–33.56)	0.012	0.040
C-peptide, pmol/l	196.96 (111.64–347.49)	160.25 (86.80–295.86)	211.39 (129.82–344.23)	243.51 (159.99–370.63)	0.005	0.016
HOMA, %	223.49 (138.58–360.43)	271.38 (170.57–431.77)	222.98 (134.14–370.67)	187.74 (125.91–279.93)	0.008	0.024
Acute insulin response ($10^2 \cdot$ pmol $\cdot$ min)	13.31 (6.89–25.68)	12.16 (6.83–21.63)	12.12 (5.22–28.12)	14.83 (9.15–24.02)	0.222	0.316
Disposition index ($10^4 \cdot$ pmol $\cdot$ min)	30.32 (16.48–55.75)	34.20 (20.18–57.95)	26.52 (12.19–57.71)	30.39 (18.76–49.24)	0.168	0.436

* Adjusted for gender and age, means (SD)/back-transformed geometric means (1 SD ranges).

with a conspicuous increase in fasting levels of insulin ($p < 0.0001$) and C-peptide ($p < 0.0001$) that reflected a corresponding decline in IS ($p < 0.0001$). There was a hyperbolic association between IS and AIR, and although a robust increase in AIR ($p < 0.0001$) was observed, the compensatory response was insufficient, resulting in a modest reduction in DI at 1 year of therapy ($p = 0.032$) (fig. 1, 2). These changes in insulin secretory dynamics were accompanied by moderate increases in fasting blood glucose ($p < 0.0001$) and HbA_{1c} ($p = 0.008$). None of the patients developed an impaired fasting glucose level or had HbA_{1c} >6.5%, but 3 patients had an HbA_{1c} of 6.0% at the end of 1 year.

Children in the lowest tertile of IGF-I at baseline had the greatest increases in HSDS ($p = 0.015$; fig. 1b) and IGF-I SDS ($p < 0.0001$; fig. 1a). However, the children in the highest tertile of baseline IGF-I maintained greater IGF-I SDS at 1 year ($p = 0.008$; fig. 1a). After 1 year of high-dose GH therapy, children in the highest IGF-I tertile at baseline had significantly higher levels of fasting insulin ($p = 0.015$) and C-peptide ($p = 0.009$), and reduced IS ($p = 0.006$) compared with other IGF-I tertiles (fig. 1c, 2; table 2). However, AIR and DI were similar in the IGF-I tertile groups after 1 year of GH treatment.

Overall, children with greater increases in IGF-I SDS during therapy had higher AIR ($r = 0.30$, $p = 0.007$) and DI ($r = 0.29$, $p = 0.005$) at 1 year, and these associations persisted when adjusted for baseline IGF-I SDS. The height gains were also favourably related to AIR ($r = 0.24$, $p = 0.022$) and DI at 1 year ($r = 0.21$, $p < 0.05$). However, in a linear regression model using both increases in height and IGF-I, only the increments in IGF-I remained associated with DI ($r = 0.23$, $p = 0.027$) after 1 year of GH therapy. In contrast, IS at 1 year was not related to IGF-I responses and height gains.

Discussion

In a large multicentre study of short children born SGA, treated over the first year with a fixed GH dose of 67 μg/kg/day, we found that higher baseline IGF-I levels were

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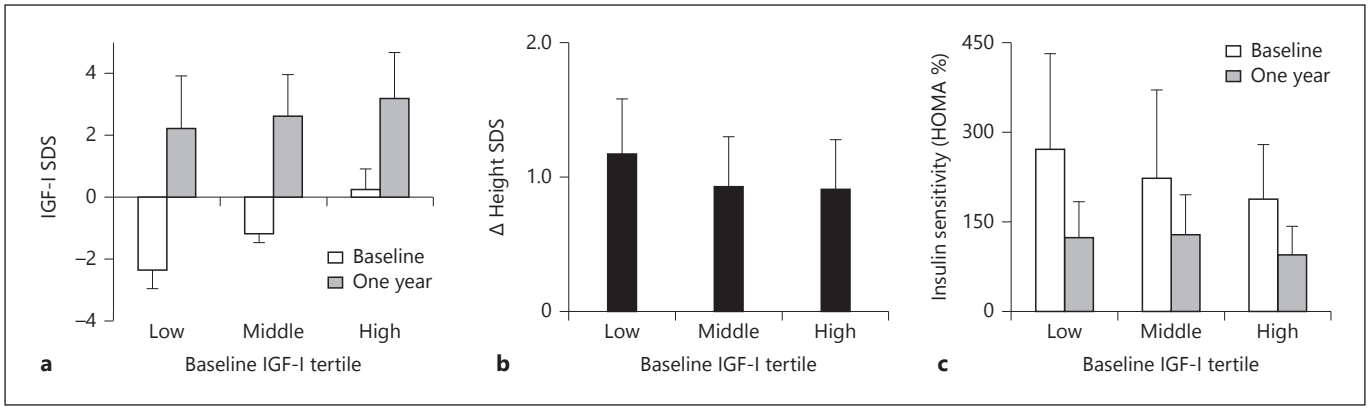

Fig. 1. a IGF-I SDS at baseline and after 1 year of high-dose GH in short SGA children according to baseline IGF-I tertile groups. **b** GH-induced changes in height according to baseline IGF-I tertile groups. **c** Mean IS at baseline and after 1 year of high-dose GH according to baseline IGF-I tertile groups.

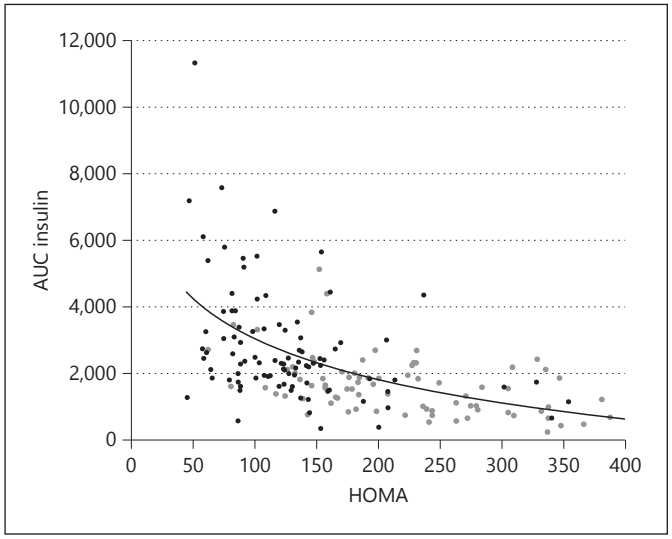

Fig. 2. IS determined by HOMA versus insulin secretion expressed as insulin AUC during a 10-min IVGTT (see Methods) before (grey dots) and after (black dots) treatment with high-dose GH for 1 year. Line represents logarithmic spline.

related to reduced IS both at baseline and at 1 year, but these subjects had the lowest increases in IGF-I SDS during treatment. Change in IGF-I SDS proved to be associated with compensatory insulin secretion and DI at 1 year.

The mean IGF-I SDS of –1.10 in our patients was similar to that observed in other studies of short SGA patients [27, 28], but as previously reported [29] is slightly greater than the levels described in unselected populations of idiopathic short stature patients with comparable degrees of short stature [30]. GH secretion was not routinely assessed in SGA children [31], since a growth prediction model concluded that stimulated GH levels did not predict growth response [32]. However, a more recent study concluded that including 24-hour GH secretion in a model made the prediction of growth response more accurate, and that the GH secretory status may be important in identifying the children who will benefit from GH treatment [33].

The use of a uniform high GH dose during the first year of treatment allowed us to evaluate the IGF-I responsiveness and its potential implications on growth and metabolism during the first year of treatment. We categorised the cohort according to baseline IGF-I tertiles to explore the heterogeneity of GH/IGF-I axis on growth and metabolic responses to GH treatment.

The GH/IGF-I axis has been extensively studied in SGA children and suggests varied mechanisms of growth failure, as there is evidence for both GH and IGF-I insensitivity [34–36]. The height gains achieved during the first year of treatment were similar to those documented in the literature, but considerable heterogeneity in height and IGF-I responses was observed [10–12, 37]. The individual increase in IGF-I is highly variable between subjects and increases in a large proportion of our prepubertal patients to levels similar to those observed during normal puberty which is clearly seen from figure 1. As previously reported, the growth and IGF-I responses to first-year GH treatment were significantly higher in children in the lowest IGF-I tertile group compared to other groups, suggesting that this group may have a lower GH secretion [16, 38]. However, increased growth and IGF-I response in those

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Table 2. Effects of GH on growth and glucose metabolism at 1 year by baseline IGF-I tertiles

	Baseline IGF-I tertiles			p trends	p trends*
	low	middle	high		
Height (SDS)	–2.43 (0.76)	–2.27 (0.71)	–2.35 (0.8)	0.745	0.856
Weight (SDS)	–2.32 (1.00)	–1.98 (1.13)	–2.09 (0.86)	0.343	0.365
BMI (SDS)	–1.12 (1.17)	–0.98 (1.31)	–1.01 (1.29)	0.543	0.507
IGF-I (SDS)	2.22 (1.70)	2.61 (1.34)	3.17 (1.51)	0.033	0.008
IGFBP-3 (SDS)	0.94 (1.23)	1.13 (0.78)	1.61 (1.20)	0.033	0.015
Δ HSDS	1.18 (0.4)	0.93 (0.37)	0.91 (0.37)	0.006	0.015
Δ IGF-I SDS	4.63 (1.80)	3.80 (1.33)	2.92 (1.50)	<0.0001	<0.0001
Glucose metabolism					
Glucose, nmol/l	4.64 (0.53)	4.68 (0.55)	4.81 (0.59)	0.400	0.543
Insulin, pmol/l	33.22 (21.28–51.87)	35.27 (19.21–64.78)	46.68 (29.84–73.01)	0.019	0.015
C-peptide, pmol/l	379.66 (258.49–557.63)	371.97 (258.01–536.27)	485.44 (329.70–714.74)	0.009	0.009
HOMA, %	122.96 (82.60–183.04)	128.69 (84.93–195.01)	95.11 (63.49–142.48)	0.007	0.006
Acute insulin response ($10^2 \cdot$ pmol/l $\cdot$ min)	23.38 (13.20–41.40)	20.42 (10.92–38.21)	27.48 (15.84–47.65)	0.124	0.289
Disposition index ($10^4 \cdot$ pmol/l $\cdot$ min)	28.98 (18.78–44.72)	26.28 (14.13–48.90)	26.14 (14.53–47.01)	0.721	0.565

* Adjusted for gender and age, means (SD)/back-transformed geometric means (1 SD ranges).

with low baseline IGF-I SDS compared to the other two groups might indicate a relatively higher IGF-I sensitivity in these children as compared to other groups. In contrast, children in the highest tertile of baseline IGF-I SDS had lower height gains, and the lowest gains in IGF-I SDS, indicative of IGF-I resistance. High baseline IGF-I levels were associated with lower IS both at baseline and during treatment with a supraphysiological GH dose. Our findings are supported by a smaller study where high baseline IGF-I levels and reduced IS were associated with lower growth and IGF-I responses to GH treatment [16]. Reduced growth, IGF-I response and IS observed in children in the highest baseline IGF-I tertiles may suggest relative resistance to multiple hormones, a proposed mechanism for developmental programming [39] or a defect common to insulin and IGF-I signalling. Furthermore, impairments in GH signalling pathways for hepatic IGF-I generation and downregulation of peripheral IGF-I receptor have been demonstrated in experimental intrauterine growth retardation animal models [16, 40, 41].

We found associations between IGF-I response to GH treatment and insulin secretion and DI at 1 year independent of height gains. These observations point to an important role of IGF-I generation in maintaining appropriate β-cell function in response to the GH-induced insulin resistance. The role of IGF-I signalling in maintaining β-cell function has been demonstrated in animal experiments in which knockout of the β-cell-specific IGF-I receptor led to reduced insulin secretion and glucose intolerance [42]. Interestingly, IS during therapy was not related to either IGF-I or growth responses, indicating differential effects of high-dose GH on pathways related to insulin signalling and those responsible for growth and IGF-I generation. While IGF-I generation and growth are mediated through related pathways involving direct GH signalling, effects on IS involve predominantly indirect mechanisms and include alterations in lipolysis [43]. One potential limitation of the study is the use of the HOMA model rather than whole-body measures of IS. However, the latter involve intensive evaluations and frequent sampling which are difficult to achieve in young children. Nevertheless, HOMA has been shown to be a good marker of hepatic IS.

As expected, 1 year of GH treatment resulted in marked reductions in IS [44]. We also observed decreases in DI and modest elevations in fasting glucose and HbA_{1c} suggestive of incomplete compensatory responses, but none of these were indicative of T2D. The significance of these observations is uncertain and further follow-up will be critical to determine the metabolic implications of these early findings. Our findings are in agreement with previous studies using varying GH doses showing increases in fasting glucose levels and HbA_{1c} in the first year [45–47]. However, de Kort et al. [47] did not find any changes in DI during GH treatment; use of varying doses of GH, but a different method for deriving DI in that study, may account for this discrepancy. Nevertheless, reports showing

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reversal of declines in DI, HbA_{1c} and fasting glucose in SGA adults once GH treatment is stopped, resulting in levels similar to those seen in untreated SGA controls, indicate that these changes may be reversible [11, 48]. Estimation of fasting IS from C-peptide levels using the HOMA model and DI from fasting IS have been useful in understanding glucose metabolism in adults [26, 49]. Although a lack of validation of these derived measures in children is a potential limitation, these less invasive methods may be helpful in exploring glucose metabolism in large cohorts of young children.

Our data are limited by a short period of observation and the use of relatively high GH doses. Nevertheless, the use of high-dose GH for 1 year may be important in enhancing catch-up growth, identifying non-responders and when combined with baseline IGF-I, may identify patients at greatest risk of development of T2D. These benefits need to be balanced against concerns that any exposure to high IGF-I levels for 1 year may carry other long-term risks although high IGF-I levels are common during puberty [50]. Further analysis of glucose metabolism in years 2 and 3 of the study where variable GH dose/IGF-I titration were used will help to elucidate these issues.

Recently, concern has been expressed about the long-term risks and mortality in patients treated with recombinant GH during childhood [51, 52]. The French part of the SAGhE study reported that the overall mortality rates were increased in this group of patients, particularly in those who had received the highest doses of GH. Not all types of cancer-related mortality increased, but bone tumour-related mortality in the treated group was significantly increased in 3 patients in the treated group compared to the expected 0.60 in the population [52]. By contrast, results from SAGhE in Belgium, the Netherlands and Sweden did not report an increased mortality from cancer or cardiovascular disease in the GH-treated group [51]. The results from these studies underline the necessity of further follow-up studies in order to monitor the long-term outcome in this group of patients.

Heterogenicity of somatotropic axis ranging from GH/IGF-I insufficiency to resistance seen in short SGA children may reflect variations in aetiology which include intrauterine exposures and putative genetic defects in IGF-I signalling pathways. However, our study indicates that the baseline IGF-I and IGF-I responsiveness are related to both growth and metabolic responses to GH treatment, and may be an important determinant of the risk for future development of T2D.

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Author Contributions

Rikke Beck Jensen: Dr. Jensen contributed substantially to the conception and design of this multicentre study, contributed on the acquisition of data and the analysis and interpretation of data, drafted the initial manuscript, and approved the final manuscript as submitted.

Ajay Thankamony: Dr. Thankamony contributed on the acquisition of data and the analysis and interpretation of data, drafted the initial manuscript, and approved the final manuscript as submitted.

Susan M. O'Connell: Dr. O'Connell contributed substantially to the conception and design of this multicentre study, contributed on the acquisition of data and the analysis and interpretation of data, drafted the initial manuscript, and approved the final manuscript as submitted.

Burak Salgin: Dr. Salgin contributed on the analysis and interpretation of data and approved the final manuscript as submitted.

Jeremy Kirk: Dr. Kirk contributed substantially to the conception and design of this multicentre study, contributed on the acquisition of data and approved the final manuscript as submitted.

Malcolm Donaldson: Dr. Donaldson contributed substantially to the conception and design of this multicentre study, contributed on the acquisition of data and approved the final manuscript as submitted.

Sten-A. Ivarsson: Dr. Ivarsson contributed substantially to the conception and design of this multicentre study, contributed on the acquisition of data and approved the final manuscript as submitted.

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Edna Roche: Dr. Roche contributed substantially to the conception and design of this multicentre study, contributed on the acquisition of data and approved the final manuscript as submitted.

Hilary Hoey: Prof. Hoey contributed substantially to the conception and design of this multicentre study, contributed on the acquisition of data and the analysis and interpretation of data, drafted the initial manuscript, and approved the final manuscript as submitted.

David B. Dunger: Prof. Dunger contributed substantially to the conception and design of this multicentre study, contributed on the acquisition of data and the analysis and interpretation of data, drafted the initial manuscript, and approved the final manuscript as submitted.

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A randomised controlled trial evaluating IGF1 titration in contrast to current GH dosing strategies in children born small for gestational age: the North European Small-for-Gestational-Age Study

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Abstract

Background: Short children born small for gestational age (SGA) are treated with a GH dose based on body size, but treatment may lead to high levels of IGF1. The objective was to evaluate IGF1 titration of GH dose in contrast to current dosing strategies.

Methods: In the North European Small-for-Gestational-Age Study (NESGAS), 92 short pre-pubertal children born SGA were randomised after 1 year of high-dose GH treatment (67 μg/kg per day) to three different regimens: high dose (67 μg/kg per day), low dose (35 μg/kg per day) or IGF1 titration.

Results: The average dose during the second year of the randomised trial did not differ between the IGF1 titration group (38 μg/kg per day, S.D. 0.019) and the low-dose group (35 μg/kg per day, S.D. 0.002; $P=0.46$), but there was a wide variation in the IGF1 titration group (range 10–80 μg/kg per day). The IGF1 titration group had significantly lower height gain (0.17 SDS, S.D. 0.18) during the second year of the randomised trial compared with the high-dose group (0.46 SDS, S.D. 0.25), but not significantly lower than the low-dose group (0.23 SDS, S.D. 0.15; $P=0.17$). The IGF1 titration group had lower IGF1 levels after 2 years of the trial (mean 1.16, S.D. 1.24) compared with both the low-dose (mean 1.76, S.D. 1.48) and the high-dose (mean 2.97, S.D. 1.63) groups.

Conclusion: IGF1 titration of GH dose in SGA children proved less effective than current dosing strategies. IGF1 titration resulted in physiological IGF1 levels with a wide range of GH dose and a poorer growth response, which indicates the role of IGF1 resistance and highlights the heterogeneity of short SGA children.

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Introduction

Small for gestational age (SGA) is a heterogeneous condition, which is a result of impaired foetal growth caused by multifactorial environmental factors *in utero* or as yet unidentified genetic disorders. Among the 10% of

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the SGA children who do not catch up during infancy, some have low insulin-like growth factor 1 (IGF1) levels suggesting alterations of the growth hormone (GH)/IGF1 axis (1, 2). However, the majority of short SGA children have sufficient GH secretion and some children have IGF1 levels above mean, which has been linked to a relative IGF1 resistance in some of the patients.

Randomised controlled trials have documented the beneficial effects of GH therapy on both short- and long-term growth in short children born SGA (3, 4, 5), and this indication for GH treatment was approved in 2001 in USA and in 2003 in Europe (6). However, the current recommended doses of GH used for short SGA children vary widely from 70 μg/kg per day in the US to 35 μg/kg per day in Europe, and the optimal GH dose regimen for children born SGA continues to be a matter of debate (7). Treatment with a higher dose of GH leads to an improved short-term growth response and a faster normalisation of height (8, 9), which we have recently confirmed in the North European Small-for-Gestational-Age Study (NESGAS) (10). While lower doses of GH may be equally effective in the long term, catch-up growth is less dramatic and may be variable, with some children requiring higher doses in the second year of treatment (8, 11, 12, 13, 14). Concern has been raised because both high doses as used in the USA and lower doses used in Europe can lead to unacceptably high levels of IGF1, which may have unknown long-term consequences. The basis of this concern relates to the finding of modest associations between higher circulating IGF1 and IGFBP3 levels and an increased risk of developing common cancers (15); however, this has not been evaluated in relation to SGA.

An alternative strategy to the conventional GH dosing regimen based on body size is dosing by IGF1 levels, which offers the opportunity to potentially tailor the GH dose to retain efficacy without exposing the subjects to high IGF1 levels. Experience in GH-deficient (GHD) and idiopathic short-stature (ISS) children demonstrated not only an increased growth response, but also a higher average GH dose, in those with GH titrated to the upper limit of normal IGF1 (SDS) levels compared with those titrated to achieve a mean IGF1 (SDS) or the conventional dose (16, 17, 18). IGF1 titration of GH doses in SGA children has not been explored previously.

In this study, after 1 year of high-dose GH treatment, the NESGAS patients were randomised to three groups: i) high-dose (67 μg/kg per day) GH, ii) low-dose (35 μg/kg per day) GH or iii) IGF1 titrated dose in order to explore the potential of IGF1 titration of GH dose in a well-characterised group of SGA children.

Patients and methods

Study population

NESGAS is a multicentre, randomised, parallel group study of GH treatment in short pre-pubertal children born SGA. Study design and first year data have been reported in detail previously (10). In brief, all children were treated with a uniform high dose (67 μg/kg per day) of GH for the first year of treatment in order to induce catch-up growth. All patients who had completed 1 year of high-dose (67 μg/kg per day) GH treatment had a height velocity of more than 1 SDS (ΔHVSDS > +1) and were randomised into one of the three groups (Fig. 1). The study (NESGAS EudraCT 2005-001507-19) was approved by the ethics committee or institutional review board and national drug authorities at each study centre and was performed according to the Helsinki II declaration. Written informed consent was obtained from guardians of each child before recruitment.

Intervention

The cohort was randomly assigned to three different dose regimens for 2 years (ratio 1:1:1). Allocation of patients was performed through minimisation (MINIM, Sealed Envelope, sealedenvelope.com) (19) to ensure equal distribution between study groups. Minimisation related to:

i) First-year growth response: HVSDS ≥ +2.5 (good responder) or HVSDS between +1 and +2.5 (medium responder).
ii) Gender.
iii) Age (4–6 years/6–9 years).
iv) Country.

Patients were randomised to one of the three dosing regimens of recombinant human GH (Norditropin, Novo Nordisk, Bagsvaerd, Denmark) given as a daily s.c. injection. The regimens included the high-dose regimen (67 μg/kg per day), low-dose regimen (35 μg/kg per day) and IGF1 titration regimen.

IGF1 titration of the GH dose

In the IGF1 titration group, the GH dose was adjusted every three months according to the IGF1 levels measured at each quarterly visit using an algorithm to maintain IGF1 SDS levels between 0 and +2 SDS (Supplementary Figure 1, see section on supplementary data given at the end of this article).

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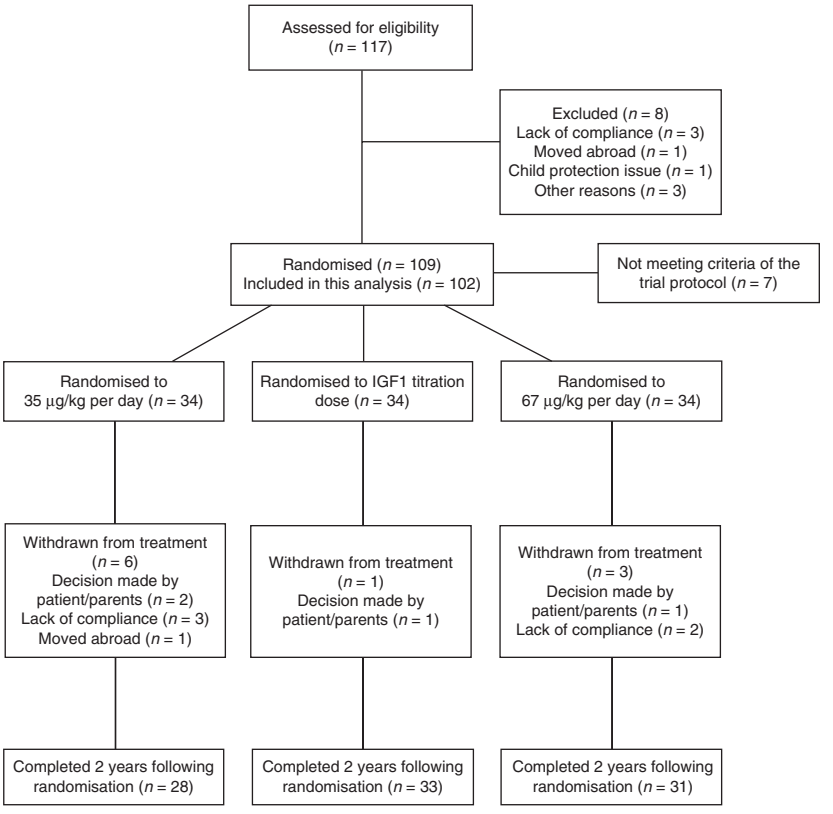

Figure 1

Flow diagram of the process through the phases of the parallel randomised trial.

Outcome measure

The primary outcome measure was the height gain (ΔHtSDS) during the second year of the trial. The secondary endpoints were changes in IGF1 levels (ΔIGF1) and changes in bone age (ΔBA).

Study assessments

Participants were assessed at study entry and at every 3 months, where the following were measured: standing height on a wall-mounted stadiometer and weight by electronic scales by staff trained in auxological methods. At each visit, pubertal development was assessed by an experienced investigator. Bone age was determined ad modum Greulich–Pyle.

Laboratory measurements

Serum IGF1 and IGFBP3 concentrations were determined centrally in Copenhagen using a solid-phase enzyme-labelled chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). Standards were calibrated towards the WHO NIBSC IRR 87/518. Detection limit for IGF1 was 20 ng/ml, and inter- and intra-assay coefficient of variation (CV) values were 5.93 and 2.02% respectively. The detection limit for IGFBP3 was 500 ng/ml, and inter- and intra-assay CV values were 5.23 and 1.74% respectively. IGF1 and IGFBP3 SDS were calculated from our reference data (20).

Calculations

SDS were derived for birth weight, birth length, height, weight, BMI, IGF1 and IGFBP3 using central country-specific reference databases (21, 22, 23). Target height SDS was computed using the formula (maternal HtSDS + paternal HtSDS)/2). For some of the analysis, the cohort was divided into tertiles according to the IGF1 levels before the start of treatment (IGF1 baseline). ΔBA was calculated: $BA_{2yr} - BA_{before\ randomisation}$. BA corrected for chronological age (CA) was calculated as BA − CA.

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Statistical analyses

The variables were analysed for normal distribution using the Kolmogorov–Smirnov test and were transformed to normality if necessary. Differences between groups were analysed using ANOVA or Student's *t* test where appropriate. The Pearson χ^2-test was performed to compare pubertal development between the groups. Statistical analyses were performed using the statistical package IBM SPSS statistics (version 21; SPSS, Inc.). Data are expressed as mean (S.D.) or back-transformed geometric mean (1 S.D. range) unless otherwise specified.

Based on power calculations for the primary outcome measure and assuming a 10% drop-out rate, recruitment of 112 patients was required to detect 0.25 S.D. increases in height SDS with 80% statistical power at a 5% significance level using a two-sided *t*-test.

Safety parameters

Safety assessments were carried out at each visit and recorded on a standard adverse event form. For serious adverse events (SAEs), serious adverse reactions (SARs) and suspected unexpected SARs (SUSARs), a form was completed and reported to the chief investigator. Adverse events were reported to the Health Authorities and Independent Review Boards/Independent Ethics Committees in accordance with national laws and regulations.

Results

Clinical characteristics

Longitudinal data were included from the 92 participants (61 males) who completed the 2 years of the randomised trial (Fig. 1). Clinical characteristics did not differ among the three groups at randomisation (Table 1).

Two-year randomised trial

As expected, the regimen of high-dose GH therapy for 2 years resulted in greater height gain (ANOVA $P<0.0001$) and weight gain (ANOVA $P=0.002$) during the last year of the trial compared with both the low-dose and IGF1 titration groups (Table 2). In the IGF1 titration group there was a trend towards a lower growth response (0.15, S.D. 0.16) during the last year of the trial when compared with the low-dose group (0.24, S.D. 0.18), although this was not significant ($P=0.17$; Fig. 2a).

The average GH dose in the IGF1 titration group during the first year of the randomised trial was significantly higher than that in the low-dose group (mean 49.2 μg/kg per day, S.D. 13.8 vs mean 35 μg/kg per day, S.D. 1.60, $P<0.0001$), whereas the average dose during the second year of the randomised trial did not differ between the IGF1 titration group (mean 38 μg/kg per day, S.D. 18.86) and the low-dose group (mean 35 μg/kg per day,

Table 1 Clinical characteristics at birth, before start of GH treatment and before randomisation. Results are expressed as mean (S.D.). Comparison was performed by ANOVA. If significance was reached, an additional comparison was performed by Student's *t* test between the low dose and the IGF1 titrated dose.

	GH dosing regimens			
	Low dose	IGF1 titration dose	High dose	***P* values**
n (Boys)	28 (17)	33 (21)	31 (23)	
Birth				
Birth weight (SDS)	−3.52 (1.12)	−3.64 (1.29)	−3.88 (1.33)	0.53
Birth length (SDS)	−3.34 (1.17)	−4.20 (2.06)	−3.38 (1.55)	0.17
Gestational age (week)	36.09 (3.69)	34.76 (4.54)	35.86 (3.51)	0.38
Target height (SDS)	−1.23 (1.19)	−1.25 (1.06)	−1.06 (1.02)	0.75
Before start of GH				
Age (year)	6.29 (1.59)	5.93 (1.60)	6.32 (1.68)	0.57
Height (SDS)	−3.47 (0.73)	−3.50 (0.87)	−3.27 (0.57)	0.41
Weight (SDS)	−3.14 (0.94)	−3.30 (1.03)	−3.18 (1.00)	0.80
BMI (SDS)	−1.22 (1.36)	−1.25 (1.23)	−1.42 (1.32)	0.82
IGF1 (SDS)	−1.12 (1.02)	−1.31 (1.11)	−0.86 (1.22)	0.26
IGFBP3 (SDS)	−1.05 (1.05)	−0.74 (1.13)	−0.57 (1.07)	0.30
Before randomisation				
Height (SDS)	−2.39 (0.82)	−2.47 (0.82)	−2.27 (0.70)	0.59
Weight (SDS)	−1.99 (0.98)	−2.37 (0.91)	−2.12 (1.03)	0.31
BMI (SDS)	−0.72 (1.23)	−1.13 (0.99)	−1.12 (1.32)	0.31
IGF1 (SDS)	2.57 (1.26)	2.84 (1.77)	2.69 (1.24)	0.77
IGFBP3 (SDS)	1.07 (0.74)	1.16 (1.23)	1.49 (0.81)	0.22

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Table 2 Effects of the three different dosing regimens on growth after 2 years of the trial. Results are expressed as mean (s.d.). Delta values show the change of the variables from before randomisation to the end of randomisation. Comparison was performed by ANOVA. If significance was reached, an additional comparison was performed by Students *t* test between the low dose and the IGF1 titrated dose. Statistical significance is marked with *.

	GH dosing regimens			
	Low dose (35 μg/kg per day)	IGF1 titration dose	High dose (67 μg/kg per day)	***P* values**
At the end of 2 years of randomisation				
n (Boys)	28 (17)	33 (21)	30 (23)	
Age (years)	9.33 (1.61)	9.07 (1.61)	9.38 (1.60)	0.73
Height (SDS)	−1.76 (0.94)	−1.95 (0.85)	−1.24 (0.91)	**0.008**
Weight (SDS)	−1.31 (1.02)	−1.70 (0.90)	−1.08 (1.04)	**0.045**
BMI (SDS)	−0.48 (1.35)	−1.04 (1.34)	−0.60 (1.30)	0.23
IGF1 (SDS)	1.76 (1.48)	1.16 (1.24)	3.04 (1.60)	**<0.0001**
IGFBP3 (SDS)	1.23 (1.09)	0.73 (1.17)	1.88 (0.86)	**0.001**
Pubic Hair (I, II, III)[a]	26/0/0	26/3/0	22/4/0	0.13
Breast (I/II/III)[a]	7/2/0	11/1/0	6/1/0	0.70
Change during the last year of randomisation				
ΔHeight (SDS)	0.23 (0.15)	0.17 (0.18)	0.46 (0.23)	**<0.0001**
ΔWeight (SDS)	0.27 (0.27)	0.16 (0.24)	0.42 (0.30)	**0.002**
ΔBMI (SDS)	0.13 (0.35)	−0.15 (0.86)	0.19 (0.39)	0.08
ΔIGF1 (SDS)	0.03 (1.14)	−0.69 (0.89)*	0.10 (1.21)	**0.02**
ΔIGFBP3	0.69 (1.18)	−0.07 (1.10)*	0.59 (0.27)	**0.04**

[a]The Pearson χ^2 test was used for comparison of pubertal development between groups.

s.d. 1.76) ($P=0.30$). Noticeably, there was a wide variation of the GH dose in the IGF1 titration group ranging from 10 to 80 μg/kg per day and also wide differences in growth response to GH therapy (Fig. 3). In the IGF1 titration group, 22 subjects (66%) achieved changes in HtSDS comparable to the low-dose group (ΔHtSDS 0.24±0.18; Fig. 4). In these subjects, this was achieved with a GH dose of 37 μg/kg per day (s.d. 17.00) and IGF1 levels of 1.53 (s.d. 0.86). In the remaining 11 subjects, gains in HtSDS (−0.03, s.d. 0.07) were lower than those observed in the low-dose group. Eight of these patients had IGF1 levels in the highest tertile at the start of GH treatment and their persistently higher IGF1 levels led to down-titration of GH doses to below 20 μg/kg per day (Fig. 4). This group had a significantly lower growth response during the second year of treatment compared with the subjects in the middle or low tertiles of baseline IGF1 levels (data not shown). By contrast, one patient had a poor growth response (ΔHtSDS −0.17) despite a GH dose of 60 μg/kg per day during the second year of the randomised trial. This patient had very low IGF1 levels at baseline (−3.21 s.d.) and the poor growth response may have been due to poor adherence. Overall comparisons between the three dosing regimens during the 2 years of the trial are shown in Table 2.

There were no significant differences among the low-dose, high-dose or IGF1 titration groups for changes either in bone age during the 2 years of the trial (ANOVA $P=0.38$) or bone age corrected for CA after 2 years of treatment (ANOVA $P=0.27$).

IGF1 levels

IGF1 levels were lower in the IGF1 titration group after 2 years of the trial (mean 1.16, s.d. 1.24) compared with the low-dose (mean 1.76, s.d. 1.48) and the high-dose (mean 2.97, s.d. 1.63) groups (Table 2 and Fig. 2b). IGF1 titration was associated with decreasing IGF1 levels during the first year of the trial, and IGF1 levels were titrated to levels between 0 and +2.5 SDS in 75% of patients ($n=24$) after 1 year of the trial. All of the patients in the IGF1 titration group had IGF1 (SDS) levels below +2.5 SDS after 2 years of the trial (Fig. 2) compared with only 64% ($n=16$) in the low-dose group and 40% ($n=8$) in the high-dose group. Some patients had continuously elevated IGF1 SDS levels up to +4.55 SDS and +5.63 SDS in the low-dose and high-dose groups respectively (Fig. 2).

Safety

During the 2 years of randomisation, eight SAEs were reported, but no SARs or SUSARs. There was no difference between the three groups of randomisation in reporting of

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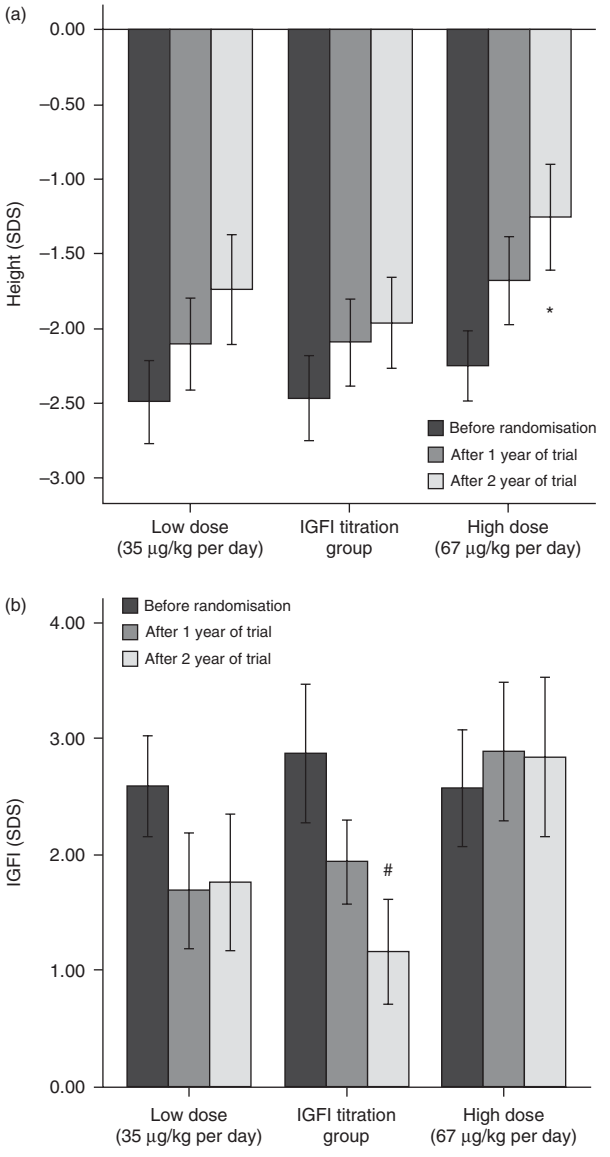

Figure 2

(a) Mean height (SDS) ±2 S.E. at 12 months (black bars), 24 months (dark grey bars) and 36 months (light grey) of GH treatment in the three groups of randomisation: low-dose (35 μg/kg per day) group, IGF1 titration group and high-dose (67 μg/kg per day) group. (b) Mean IGF1 (SDS) ±2 S.E. at 12 months (black bars), 24 months (dark grey bars) and 36 months (light grey) of GH treatment in the three groups of randomisation: low-dose (35 μg/kg per day) group, IGF1 titration group and high-dose (67 μg/kg per day) group.

SAEs: two SAEs in the low-dose group (one diagnosed with asthma, one with hypertrophy of the adenoids and adenoidectomy), three SAEs in the high-dose group (one with fracture of the radius after falling, one with torticollis and one girl, with cerebral palsy, who was diagnosed with epilepsy) and three SAEs in the IGF1 titration group (one developed scoliosis and was diagnosed with juvenile idiopathic arthritis, one had viral meningitis and one patient had a reoperation of hypospadias).

Discussion

In this randomised trial, growth response to the two established GH doses was, as expected, accompanied by high IGF1 levels, but the ability of the IGF1 titrated dose to mitigate these exposures was more variable. Personalising GH therapy in SGA to avoid high IGF1 exposures is attractive; however, this study emphasises that titration of GH dose from IGF1 levels alone may not result in the optimal growth response in short SGA children.

SGA is a heterogeneous condition and impaired postnatal growth in this group of patients may arise from a variety of effects on the GH/IGF1 axis including GH and IGF1 resistance. Variation in the responsiveness to GH therapy implies that individualised dosing of GH according to GH sensitivity may be required in SGA patients. Cohen *et al.* (16, 17) showed that targeting higher IGF1 levels by increasing the GH dose in GHD and ISS children resulted in increased change in height in the group of patients who had a GH dosage titrated to achieve IGF1 levels in the upper limit of the normal range compared with those who either had the fixed conventional dose or had received a dosage titrated to achieve IGF1 levels at the mean of a normal range. However, this study only included patients with low IGF1 levels (below −1 SDS), which may exclude those with potential IGF1 resistance. The algorithm used to titrate the GH dose in our study was different to that used in GHD and ISS patients. Mean IGF1 level after the first year of the randomised trial was +1.94 SDS (1.00 S.D.), whereas mean IGF1 level after 2 years of the trial was +1.16 SDS (1.24 S.D.) in the IGF1 titration group. This demonstrated that the algorithm worked in terms of achieving IGF1 levels between 0 and +2 SDS, but the lowering of GH dose according to IGF1 levels was prolonged as the algorithm only allowed a 15 μg/kg per day reduction in GH dose every 3 months. By contrast, the study on IGF1 titration in GHD and ISS children calculated the difference between measured and target IGF1 SDS using a 20% change in dose for each S.D. unit difference (16, 17, 18).

These data reflect the heterogeneity of SGA children. We and others have previously shown that SGA children with high baseline IGF1 levels show a poor response to GH therapy, a decreased IGF1 response and a lower

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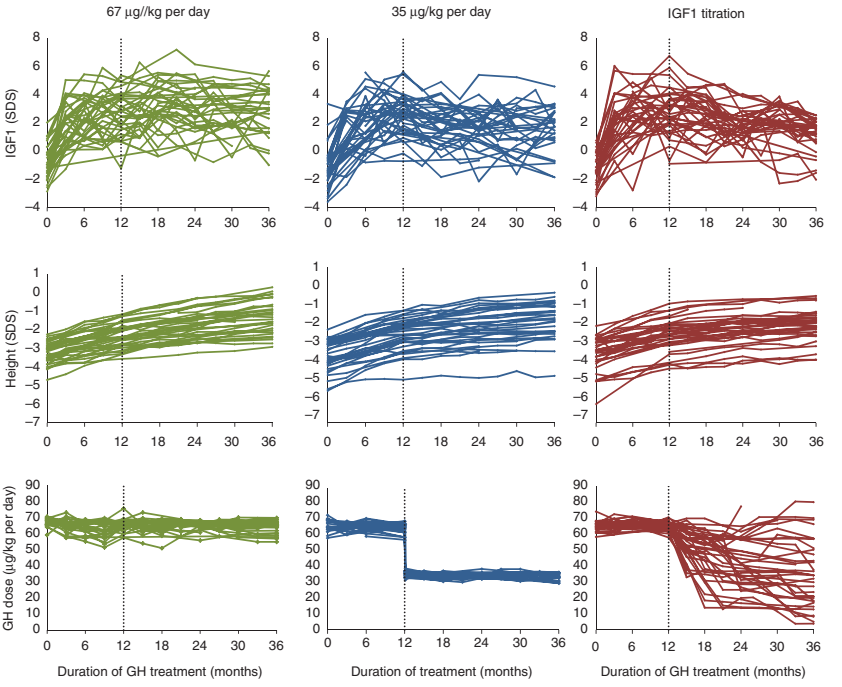

Figure 3

Individual longitudinal measurements of IGF1 (top row), height (middle row) and GH (bottom row) during 3 years of GH treatment in short SGA children according to three groups of randomisation: high dose (green), low dose (blue) and IGF1 titration (red).

insulin sensitivity (24, 25, 26), which indicates that some children have impaired hepatic IGF1 generation and potentially peripheral IGF1 resistance. Impairments in GH signalling pathways for hepatic IGF1 generation and down-regulation of peripheral IGF1 receptor have been demonstrated in experimental intrauterine growth retardation animal models (27, 28). As expected, we determined that, within the IGF1 titration group, eight out of ten patients with high baseline IGF1 levels had a poor growth during the second year of the randomised trial due to reductions in GH doses in response to persistently high IGF1 levels. Conversely, those with low IGF1 levels before the start of treatment were those with the best response, except one patient who responded poorly to a relatively high dose, where we suspected poor treatment adherence. Thus, IGF1 titration was underlining the importance of IGF1 resistance in this group of patients, indicating that some of these patients probably will need continuously maintained supra-physiological IGF1 levels in order to increase growth. The heterogeneity of the group of short SGA children calls for individualised GH therapy. Further studies may identify better predictors of GH response in order to enable a

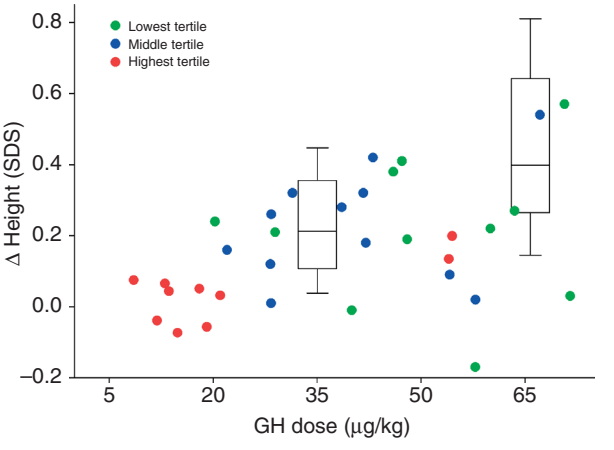

Figure 4

Box plots reflecting change in height (SDS) during the second year of the randomised trial in the low-dose and high-dose groups. The dots show the individual change in height per GH dose in the IGF1 titration group according to baseline IGF1 levels (red: highest tertile, blue: middle tertile, green: lowest tertile).

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more accurate personalised medicine approach including IGF1 levels, growth response and other possible biomarkers. However, from our study, IGF1 titration of the GH dose alone cannot be recommended in this population routinely, as it may lead to sub-optimal growth in some subjects.

One of the strengths of this study is the design, where all patients were treated with a uniform high dose of GH during the first year of therapy in order to induce catch-up growth. This was based on the knowledge that first-year growth response to GH treatment in SGA children is highly dose dependent, whereas the dose–response effect tends to level out during the following years of treatment (12). Thus, this study was designed to reduce the dose-dependent variation of growth response during the following years of treatment where the three different dosing regimens were explored.

There is an ongoing discussion about the long-term safety of GH treatment in relation to the development of cardiovascular and metabolic disorders and malignancies, but the results are inconsistent (29, 30). In adult populations, increased circulating concentrations of IGF1 have retrospectively been found to be related to an increased risk of development of cancer, but this relationship is not universally observed (15). Though, during puberty, the growth spurt is associated with exposure to high physiological levels of IGF1. In this study, we found no safety issues in the short term. Although high IGF1 levels could be a risk factor for later disease, this may not be true in a population such as those born SGA, where some of the patients will be IGF1 resistant and thereby require higher IGF1 levels to improve growth. On the other hand, although the short-term growth response in the IGF1 titration group was lower than the low-dose group, this may reflect a more physiological response to GH, which could be speculated to have beneficial effects on the long-term consequences of the treatment.

This randomised trial is the first to demonstrate the effects of an individualised dosing regimen by titration of the GH dose according to the IGF1 levels in short children born SGA. Theoretically, dosing based on IGF1 levels may not only mimic a more physiological growth response and potentially lower the long-term risk for adverse effects of the treatment, but may also be valuable from a cost–benefit point of view (31). However, our data of dose titration in SGA children proved to be less effective especially in those patients who had a degree of IGF1 resistance, as they are dependent on continuous supra-physiological IGF1 levels in order to grow. Although we cannot recommend IGF1 dose titration in this population, further studies of GH/IGF1 dose relationships and potentially adverse metabolic outcomes are required. Future studies using biomarkers and genetic markers influencing GH/IGF1 might improve understanding of the heterogeneity and individualisation of treatment.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-14-0419.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Dr R B Jensen contributed substantially to the conception and design of this multicentre study, the acquisition of data and the analysis and interpretation of data, drafted the initial manuscript, tables and figures and approved the final manuscript as submitted. Dr A Thankamony contributed to the acquisition of data and the analysis and interpretation of data, drafted the initial manuscript and approved the final manuscript as submitted. Dr S M O'Connell, Dr J Kirk, Dr M Donaldson, Dr S-A Ivarsson, Dr O Söder and Dr E Roche contributed substantially to the conception and design of this multicentre study and the acquisition of data, and approved the final manuscript as submitted. Prof. H Hoey, Prof. D B Dunger and Prof. A Juul contributed substantially to the conception and design of this multicentre study, the acquisition of data and the analysis and interpretation of data, drafted the initial manuscript and approved the final manuscript as submitted.

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MANUSCRIPT 3

Genetic Markers of Insulin Sensitivity and Insulin Secretion Are Associated With Spontaneous Postnatal Growth and Response to Growth Hormone Treatment in Short SGA Children: the North European SGA Study (NESGAS)

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Purpose: The wide heterogeneity in the early growth and metabolism of children born small for gestational age (SGA), both before and during GH therapy, may reflect common genetic variations related to insulin secretion or sensitivity.

Method: Combined multiallele single nucleotide polymorphism scores with known associations with insulin sensitivity or insulin secretion were analyzed for their relationships with spontaneous postnatal growth and first-year responses to GH therapy in 96 short SGA children.

Results: The insulin sensitivity allele score (GS-InSens) was positively associated with spontaneous postnatal weight gain (regression coefficient [B]: 0.12 SD scores per allele; 95% confidence interval [CI], 0.01–0.23; $P = .03$) and also in response to GH therapy with first-year height velocity (B: 0.18 cm/y per allele; 95% CI, 0.02–0.35; $P = .03$) and change in IGF-1 (B: 0.17 SD scores per allele; 95% CI, 0.00–0.32; $P = .03$). The association with first-year height velocity was independent of reported predictors of response to GH therapy (adjusted $P = .04$). The insulin secretion allele score (GS-InSec) was positively associated with spontaneous postnatal height gain (B: 0.15; 95% CI, 0.01–0.30; $P = .03$) and disposition index both before (B: 0.02; 95% CI, 0.00–0.04; $P = .04$) and after 1 year of GH therapy (B: 0.03; 95% CI, 0.01–0.05; $P = .002$), but not with growth and IGF-1 responses to GH therapy. Neither of the allele scores was associated with size at birth.

Conclusion: Genetic allele scores indicative of insulin sensitivity and insulin secretion were associated with spontaneous postnatal growth and responses to GH therapy in short SGA children. Further pharmacogenetic studies may support the rationale for adjuvant therapies by informing the mechanisms of treatment response. (***J Clin Endocrinol Metab*** **100: E503–E507, 2015)**

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Abbreviations: AUC, area under the curve; B, regression coefficient; BMI, body mass index; CI, confidence interval; GS-InSec, multiallele score for insulin secretion; GS-InSens, multiallele score for insulin sensitivity; HOMA, homeostasis model of assessment; HOMA-S, HOMA for insulin sensitivity; IGFBP-3, IGF binding protein-3; SDS, SD score; SGA, small for gestational age; SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

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Small for gestational age (SGA) at birth indicates impaired fetal growth due to a heterogeneous range of intrauterine conditions or in some infants by innate genetic defects. Approximately 10% of SGA children do not show spontaneous catch-up growth during the early postnatal years, and they are also short as adults if not treated with GH. Most short SGA children have sufficient GH secretion and show generally good responses to GH treatment, although there is considerable variation between patients.

Prediction models of the response to GH therapy in short SGA children have been generated to individually tailor treatment, to improve efficacy and safety, and to improve the cost-benefit ratio (1). The prediction model described by Ranke et al (1) explained 52% of the variance in the first-year growth response, with GH dose alone accounting for 35% of the variance.

We and others have reported that the growth response to GH therapy in short SGA children is associated with baseline insulin sensitivity and IGF-1 levels (2, 3). Children with the highest baseline IGF-1 levels had lower insulin sensitivity, lower height velocity, and lower IGF-1 responses after 1 year of GH therapy (3). Insulin secretion is diminished in SGA children, and this has been proposed as a possible factor in the failure to catch up in some infants (4). Furthermore, growth and IGF-1 responses to first-year GH treatment were related to insulin secretion in the North European SGA Study (NESGAS) (3). We hypothesized that genetic variation in insulin sensitivity or insulin secretion would be associated with interindividual variation in responses to GH in short SGA children.

Patients and Methods

Study population

The NESGAS is a multicenter, randomized, parallel group trial (EudraCT 2005–001507–19) of GH treatment in short SGA-born prepubertal children, which has been described in detail (3). Data included in the current analyses are related to the first year of high-dose GH treatment (67 μg/kg/d) in 96 NESGAS participants.

The study was performed according to the Helsinki II declaration and approved by the ethics committees. Written informed consent was obtained from parents of subjects.

Study assessments

Standing height was measured on a wall-mounted stadiometer, and weight was measured with electronic scales by staff. All children underwent a fasting blood sample and a short iv glucose tolerance test at baseline and at year 1 (3).

Plasma insulin and C-peptide concentrations were measured centrally by a DELFIA assay (Perkin Elmer Life Sciences). Interassay coefficients of variation were below 4% for both insulin and C-peptide. Serum IGF-1 and IGF binding protein-3 (IGFBP-3) concentrations were determined centrally using an Immulite 2000 assay (Diagnostic Products Corporation) with standards calibrated toward the World Health Organization's NIBSC IRR 87/518. Limit of detection and coefficient of variation were 20 ng/mL and 5.93% for IGF-1, respectively, and 500 ng/mL and 5.23% for IGFBP-3, respectively. IGF-1 and IGFBP-3 SD scores (SDS) were calculated from our reference data (5, 6). Plasma glucose and glycated hemoglobin (HbA1c) were measured locally.

Genotyping information

The cohort was genotyped using the Metabochip, a custom Illumina iSelect genotyping array that assays nearly 200 000 single nucleotide polymorphisms (SNPs) chosen on the basis of genome-wide association study meta-analyses (7).

In each individual, combined multiallele scores were generated comprising SNPs for insulin sensitivity (GS-InSens) or insulin secretion (GS-InSec), as recently described (8). The GS-InSens was calculated as a count of the insulin sensitivity-increasing alleles at 10 variants (Supplemental Table 2a). The GS-InSec was calculated as a count of the insulin secretion-increasing alleles at 18 of the 23 variants described by Scott et al (8) (for the remaining five variants, there were no suitable proxies genotyped) (Supplemental Table 2b). Both combined multiallele scores were recently validated in large population-based studies (8).

Calculations

Anthropometric measurements are presented as SDS using normal reference materials (9–11). Insulin sensitivity was estimated from the homeostasis model of assessment (HOMA) (http://www.dtu.ox.ac.uk/homacalculator/index.php). Acute insulin response was calculated as the iv glucose tolerance test area under the curve (AUC) of the insulin response. Disposition index was calculated as the product of insulin sensitivity and acute insulin response.

Statistics

Outcome variables were log10-transformed and standardized. Associations between genetic risk scores and these outcomes were assessed by fitting linear regression models adjusted for age and sex and either body mass index (BMI) or midparental height. Statistical analyses were performed using the statistical package IBM SPSS statistics (version 21; SPSS Inc).

The genetic allele scores were also added to a reported model for first-year predicted height velocity responses to GH therapy in short SGA children (1), which includes the variables age in years and weight SDS at the start of treatment, GH dose, and midparental height SDS.

Results

Associations with spontaneous growth

Clinical characteristics are presented in Supplemental Table 1. Birth weight (mean, −3.22 SDS), birth length (mean, −3.15 SDS), and gestational age (mean, 35.6 wk) were all unrelated to GS-InSens and GS-InSec (all $P > .24$; data not shown).

GS-InSens was unrelated to spontaneous growth (change in height [SDS] from birth to study baseline; $P = .24$) but positively associated with spontaneous weight gain (regression coefficient [B]: 0.12 SDS per allele; 95%

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confidence interval [CI], 0.01–0.23; $P = .03$). GS-InSec was positively associated with spontaneous growth (B: 0.15; 95% CI, 0.01–0.30; $P = .03$) and showed a similar trend with spontaneous weight gain ($P = .06$) (Table 1).

Height velocity and IGF-1 responses to GH therapy

GS-InSens was positively associated with height velocity (B: 0.18 cm/y per allele; 95% CI, 0.02–0.35; $P = .03$), weight (SDS) (B: −0.10 SDS per allele; 95% CI, −0.20 to −0.003; $P = .04$), and change in IGF-1 levels (0.17 SDS/y per allele; 95% CI, 0.00–0.32; $P = .03$) in response to GH therapy.

The variance in first-year height velocity in response to GH therapy predicted by the Ranke model ($R^2 = 0.17$) was lower than that in the original report, but the standard error (SE) (1.72 cm) was similar, likely reflecting the uniform GH dose used in our study. Addition of GS-InSens to this prediction model explained an additional 5% of the variance in the first-year height velocity response ($R^2 = 0.22$; SE, 1.71 cm; P value for R^2 change = .04).

Alternatively, the addition of baseline IGF-1 SDS to the model also increased the explained variance in the first-year height velocity response ($R^2 = 0.26$; SE, 1.65 cm; P value for R^2 change = .009), and addition of both baseline IGF-1 and GS-InSens increased the explained variance, but this change in R^2 was not significant ($R^2 = 0.29$; SE, 1.63 cm; P value for R^2 change = .09).

Associations with insulin traits

Consistent with its expected functional role, GS-InSec was positively associated with disposition index, both before (B: 0.02 per allele; 95% CI, 0.00–0.04; $P = .04$) and 1 year after GH therapy (B: 0.03; 95% CI, 0.01–0.05; $P = .002$). However, the GS-InSens was unrelated to HOMA for insulin sensitivity (HOMA-S) or the disposition index at baseline and after 1 year of therapy (Table 2).

Discussion

In this study of short SGA-born children, validated genetic determinants of insulin sensitivity were associated with both height velocity and circulating IGF-1 level responses to GH therapy. The findings provide insights into the mechanisms that contribute to GH responses and also insights into the pathophysiology of poor spontaneous postnatal growth in SGA infants.

Pharmacogenetics considers the possible contribution of genetic factors to the prediction of individual treatment efficacy and/or risks of treatment-related adverse events and forms the basis for many putative strategies for stratified medicine (12). Prediction of individual growth responses to GH therapy has been suggested to optimize treatment in a range of childhood disorders. However, the reported prediction model for short SGA children was largely reliant on historical heterogeneity in the GH dose (1), which in current clinical practice is standardized. In our fixed GH dose study, inclusion of the insulin sensitivity allele score improved the explained variance by only

Table 1. Associations to Measures of Growth and Metabolism for GS-InSec

Measures of Growth and Metabolism for GS-InSec	Effect Size per Allele (B)	95% CI	*P* Value
Height (SDS) baseline[a]	0.02	−0.04 to 0.08	.49
Height (SDS) 1 y[a]	0.03	−0.04 to 0.09	.41
Δ Height (SDS) (baseline to 1 y)[a]	0.004	−0.03 to 0.04	.80
Δ Height (cm) (baseline to 1 y)[a]	−0.008	−0.14 to 0.13	.91
Weight (SDS) baseline[a]	0.06	−0.02 to 0.14	.17
Weight (SDS) 1 y[a]	0.04	−0.04 to 0.13	.30
Δ Weight (SDS) (baseline to 1 y)[a]	−0.02	−0.05 to 0.02	.30
Δ Weight (kg) (baseline to 1 y)[a]	−0.17	−0.49 to 0.15	.30
IGF-1 (SDS) baseline[a]	−0.03	−0.13 to 0.07	.54
IGF-1 (SDS) 1 y[a]	0.005	−0.11 to 0.12	.94
Δ IGF-1 (SDS) (baseline to 1 y)[a]	0.04	−0.09 to 0.15	.57
LogAUC insulin baseline[b]	0.02	−0.003 to 0.04	.09
LogAUC insulin 1 y[b]	0.03	0.005 to 0.05	**.02**
Log ΔAUC insulin (baseline to 1 y)[b]	0.007	−0.02 to 0.03	.53
LogHOMA-S baseline[b]	0.01	−0.01 to 0.03	.33
LogHOMA-S 1 y[b]	0.006	−0.01 to 0.02	.47
LogΔ HOMA-S (baseline to 1 y)[b]	−0.004	−0.1 to 0.003	.25
LogDisposition index baseline[b]	0.02	0.001 to 0.04	**.04**
LogDisposition index 1 y[b]	0.03	0.01 to 0.05	**.002**
Log ΔDisposition index (baseline to 1 y)[b]	0.01	−0.05 to 0.07	.73
Δ Height from birth to baseline[a]	0.15	0.01 to 0.30	**.03**
Δ Weight from birth to baseline[a]	0.09	−0.003 to 0.17	.06

[a] Corrected for age, sex, and midparental height.

[b] Corrected for age, sex, and BMI.

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Table 2. Associations to Measures of Growth and Metabolism for GS-InSens

Measures of Growth and Metabolism for GS-InSens	Effect Size per Allele (B)	95% CI	*P* Value
Height (SDS) baseline[a]	−0.05	−0.13 to 0.02	.17
Height (SDS) 1 y[a]	−0.08	− 0.15 to − 0.001	**.048**
Δ Height (SDS) (baseline to 1 y)[a]	−0.02	−0.06 to 0.02	.24
Δ Height (cm) (baseline to 1 y)[a]	−0.18	− 0.35 to − 0.02	**.03**
Weight (SDS) baseline[a]	−0.10	− 0.20 to − 0.005	**.04**
Weight (SDS) 1 y[a]	−0.10	− 0.20 to − 0.003	**.04**
ΔWeight (SDS) (baseline to 1 y)[a]	−0.01	−0.05–0.03	.63
Δ Weight (kg) (baseline to 1 y)[a]	−0.16	−0.56 to 0.23	.41
IGF-1 (SDS) baseline[a]	0.04	−0.080 to 0.170	.47
IGF-1 (SDS) 1 y[a]	−0.15	− 0.30 to − 0.002	**.047**
Δ IGF-1 (SDS) (baseline to 1 y)[a]	−0.17	− 0.32 to − 0.002	**.03**
LogAUC insulin baseline[b]	−0.006	−0.03 to 0.02	.63
LogAUC insulin 1 y[b]	−0.01	−0.04 to 0.01	.47
Log ΔAUC insulin (baseline to 1 y)[a]	−0.02	−0.04 to 0.02	.26
LogHOMA-S baseline[b]	−0.007	−0.03 to 0.02	.59
LogHOMA-S 1 y[b]	−0.004	−0.02 to 0.01	.64
Log ΔHOMA-S (baseline to 1 y)[b]	0.01	−0.006 to 0.009	.76
LogDisposition index baseline[b]	−0.01	−0.04 to 0.01	.30
LogDisposition index 1 y[b]	−0.01	−0.04 to 0.01	.27
LogΔ Disposition index (baseline to 1 y)[b]	−0005	−0034 to 0025	.75
Δ Height from birth to baseline[a]	−0.003	−0.19 to 0.18	.95
Δ Weight from birth to baseline[a]	−0.12	− 0.23 to − 0.01	**.03**

The regression coefficient (B) is the inverse of the insulin resistance score (IR score) described by Scott et al (8). An increase in multiallele score reflects a decrease in insulin sensitivity. Bold numbers indicate the significance level was $P < .05$.

[a] Corrected for age, sex, and midparental height.

[b] Corrected for age, sex, and BMI.

5%, from 17 to 22%, which is insufficient for such scores to have clinical utility in individual treatment prediction.

An alternative application of pharmacogenetics is to inform the mechanisms of treatment response by considering informative genotypes or allele scores as indicators of the likely causal effects of their target traits. Such inference forms the basis of the so-called "Mendelian randomization" approach (13). The independent association between the insulin sensitivity allele score and first-year height velocity responses supports observations in nongenetic studies of SGA infants where insulin resistance has been associated with poor response to GH therapy. IGF-1 resistance has also been implicated because of the close functional relationship between the insulin receptor and the type 1 IGF-1 receptor. We previously reported that children with relatively high baseline IGF-1 levels had lower insulin sensitivity and impaired IGF-1 generation in response to GH therapy (3). Our genetic associations support the possible causality of such associations and may allow a quantitative estimation of the relationship between insulin sensitivity and growth response. Such causal inference relies on various assumptions and therefore requires experimental validation, but it would support the rationale for the clinical testing of adjuvant insulin sensitization in combination with GH therapy (14).

The insulin secretion allele scores were associated with spontaneous postnatal growth in height and weight, whereas the insulin sensitivity allele scores were associated with weight gain. In the population-based Avon Longitudinal Study of Parents and Children cohort, insulin secretion was positively related to size at birth and to childhood height and IGF-1 levels (4). Similarly, in an earlier study of short SGA children, insulin secretion was positively related to height velocity (15). Thus, β-cell function appears to have a key role in spontaneous height growth, and this mechanism may underlie observed associations between shorter adult stature or lower IGF-1 levels and higher risk for type 2 diabetes (T2D) (16, 17). Common genetic mechanisms between early growth patterns and later risk of metabolic disease have been proposed; however, there is inconsistent evidence linking SNPs related to T2D or obesity to risk of SGA at birth (18–20). Our findings support common genetic mechanisms linking spontaneous postnatal height growth to disposition index, a marker of insulin secretory capacity, before and during GH treatment. The positive association between insulin sensitivity alleles and spontaneous postnatal weight gain is discordant with observed associations between rapid postnatal weight gain and insulin resistance (4) but is consistent with recent findings in adults (8) and likely indicates the positive anabolic effects of insulin signaling. Future studies should test the combination of the insulin sensitivity and insulin secretion allele scores for prediction of T2D in SGA-born or other high-risk groups.

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A limitation of this study is the relatively small population, although the cohort is well-characterized phenotypically. To increase statistical power, we examined combined allele scores rather than individual SNP genotypes. We are therefore unable to pinpoint individual variants or genes that regulate response to GH therapy; however, this approach allows broader support for a causal role of insulin sensitivity in general.

In conclusion, these novel data indicate causal influences of insulin secretion and insulin sensitivity on spontaneous postnatal height growth and growth responses to GH therapy, respectively, in short SGA-born children. The findings also support the relationship between insulin resistance and putative IGF-1 resistance, which may impair responses to GH therapy and potentially increase the risk of T2D. It will be interesting to examine whether similar mechanisms contribute to growth responses in patients with other conditions that warrant GH therapy, such as GH deficiency.

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Disclosure Summary: The authors have nothing to declare

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MANUSCRIPT 4

Adiposity in Children Born Small for Gestational Age Is Associated With β-Cell Function, Genetic Variants for Insulin Resistance, and Response to Growth Hormone Treatment

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Background: Genetic susceptibility to insulin resistance is associated with lower adiposity in adults. Insulin resistance, and therefore adiposity, may alter sensitivity to GH. We aimed to determine the relationship between adiposity, genetic susceptibility to insulin resistance or insulin secretion, and response to GH treatment in short children born small for gestational age (SGA).

Methods: In 89 short prepubertal SGA children (age, 6.2 ± 1.6 y; 55 boys) treated with GH for 1 year in a multicenter study, body fat percentage was estimated at baseline and 1 year using dual-energy x-ray absorptiometry. The main outcome measures were treatment-related changes in height, IGF-1 standard deviation score, insulin sensitivity, insulin secretion, and disposition index. Combined multiallele gene scores based on single nucleotide polymorphisms with known associations with lower insulin sensitivity (gene scores for insulin resistance [GS-InRes]) and insulin secretion (gene scores for insulin secretion [GS-InSec]) were analyzed for their relationships with adiposity.

Results: Mean percentage body fat at baseline was low compared to normative data ($P = .045$) and decreased even further on GH treatment (baseline vs 1-year z-scores, −0.26 ± 1.2 vs −1.23 ± 1.54; $P < .0001$). Baseline percentage body fat was positively associated with IGF-1 responses (p-trends = .042), first-year height gains (B [95% confidence interval], 0.61 cm/y [0.28,0.95]; $P < .0001$), insulin secretion at baseline (p-trends = .020) and 1 year (p-trends = .004), and disposition index at 1 year (p-trends = .024). GS-InRes was inversely associated with body mass index (−0.13 SD score per allele [−0.26, −0.01]; $P = .040$), body fat (−0.49% per allele [−0.97, −0.007]; $P = .047$), and limb fat (−0.81% per allele [−1.62, 0.00]; $P = .049$) at baseline. During GH treatment, GS-InRes was related to a lesser decline in trunk fat (0.38% per allele [0.16, 0.59]; $P = .001$) and a higher trunk-limb fat ratio at 1 year (0.04 per allele [0.01, 0.08]; $P = .008$). GS-InSec was positively associated with truncal fat (0.36% per allele [0.09, 0.63]; $P = .009$).

Conclusions: Adiposity in SGA children has favorable effects on GH sensitivity and glucose metabolism. The associations with multiallele scores support a causal role of insulin resistance in linking lesser body fat to reduced sensitivity to exogenous GH. **(*J Clin Endocrinol Metab* 101: 131–142, 2016)**

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Abbreviations: AIR, acute insulin response; APA, appropriate for gestational age; BMI, body mass index; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; GS-InRes, gene scores for insulin resistance; GS-InSec, gene scores for insulin secretion; HOMA, homeostasis model of assessment; SGA, small for gestational age; T2D, type 2 diabetes.

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Increased body fat, in particular central fat, is thought to have a major role in the development of metabolic risk factors in children born small for gestational age (SGA) (1). However, in contrast to most SGA children who undergo catch-up growth during infancy, short SGA children have significant deficits in body fat, mainly in the subcutaneous compartment, compared with children born appropriate for gestational age (AGA) (2, 3). The phenotype of low adiposity is not an expected consequence of GH deficiency or GH resistance (2), and therefore other mechanisms such as alterations in the neuroendocrine regulation of appetite and adipose tissue development may determine growth and body composition in these children (4). In short SGA children who fail to catch up, GH treatment is licensed to improve linear growth (5). GH is a crucial regulator of substrate metabolism during fasting, and its anabolic actions are tightly coupled with energy balance (6). Low adiposity in SGA children may reflect suboptimal energy balance and alter their sensitivity to GH.

Developmental programming of multiple endocrine axes has been hypothesized to underlie the increased risk for development of type 2 diabetes (T2D) in low-birthweight individuals (7). The close relationship between the actions of the GH/IGF-1 axis and glucose metabolism may explain the link between reduced statural growth and metabolic abnormalities in SGA children (6, 7). In addition, lower insulin sensitivity and insulin secretion are associated with reduced responses to GH treatment in SGA children (8, 9). We recently employed a Mendelian randomization approach to illustrate the likely causal link between insulin resistance and GH sensitivity in short SGA children; multiallele scores indicative of insulin resistance were associated with lower IGF-1 and height responses to GH treatment (10). In adults, the same multiallele score is associated with a lesser body fat, particularly in the gluteofemoral region and limbs (11). Furthermore, the multiallele score indicative of lower insulin secretion was associated with reduced spontaneous growth in SGA children and higher android fat in adults (10). Therefore, insulin resistance and/or insulin secretion could potentially link adiposity to GH-treatment responses in short SGA children.

The aim of the study was to test the hypothesis that variations in adiposity in short SGA children could be related to sensitivity to GH and to explore whether the gene polymorphisms indicative of insulin sensitivity or insulin secretion are also associated with body composition in these children.

Subjects and Methods

Study population

The subjects were from the North European Small for Gestational Age Study (NESGAS), a multicenter study of GH treatment in short prepubertal SGA children involving nine investigating centers in four North European countries (Denmark, Ireland, Sweden, and the United Kingdom) and has been reported in detail previously (12). Briefly, the study population included prepubertal SGA children with persistent short stature at 4 years of age; the girls were between 4 and 9 years of age, and the boys were ages 4 and 10 years. During the first year, children were treated with a uniform high dose of GH (67 μg/kg/d) to induce catch-up growth. The study (NESGAS EudraCT 2005–001507–19) was approved by the relevant ethics committees, institutional review boards, and national drug authorities at each study center and was performed according to the Helsinki II declaration. Written informed consent was obtained from parents of the children before any study activities.

Study assessments

The participants were assessed at study entry (baseline) and every 3 months when anthropometry and pubertal assessments were undertaken and serum IGF-1 levels were measured. They also underwent a short iv glucose tolerance test at baseline and at 1 year to evaluate insulin sensitivity and insulin secretion (8).

Dual-energy x-ray absorptiometry (DXA) scans

Body composition was assessed by DXA scans using the Hologic QDR-1000/W scanner (Hologic Inc) (three centers; n = 39) or the Lunar Prodigy DXA system (GE Medical Systems) (six centers; n = 50) at baseline and at 1 year. In one center, the Hologic scanner was replaced with a Lunar Prodigy system during the study period, and data from the children who were evaluated by two different scanners (n = 7) were transformed to Lunar Prodigy DXA values using a published method (13). These children were excluded when the changes in body composition from the baseline to 1 year were analyzed to avoid confounding by the type of scanner. Regional fat distribution was assessed using the default setting for segmental analysis in the scanners. The performance of the scanners was assessed using a phantom at the start of the study. The scanners showed a good level of agreement, and the difference in percentage body fat between centers was typically 1.5%, with a maximum of 2.1%. Of the 110 children who participated in the study, data on body composition were available from 89 children at baseline (incomplete data, four; scans not carried out, 17) and 85 children at 1 year (incomplete data, one; scans not carried out, 24).

Genotyping method

The cohort was genotyped using the Metabochip, a custom Illumina iSelect genotyping array that assays nearly 200 000 single nucleotide polymorphisms chosen on the basis of genome-wide association study meta-analyses as previously described (10, 11). In each individual, combined multiallele gene scores for insulin resistance (GS-InRes) or insulin secretion (GS-InSec) were generated as the count of the insulin sensitivity decreasing alleles at 10 variants and the insulin secretion decreasing alleles at 18 variants, respectively (Supplemental Table 1) (10). Both combined multiallele scores have been validated in large population-based studies (11).

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Assays

Serum levels of IGF-1, insulin, and C-peptide were assayed centrally as previously reported (8). Plasma glucose and fasting lipid profile were measured locally.

Calculations

Standard deviation scores (SDS) for height, weight, and body mass index (BMI) were derived using country-specific references (8). Insulin sensitivity was estimated from fasting glucose and C-peptide levels using the homeostasis model of assessment (HOMA) as previously reported (8). Acute insulin response (AIR) was calculated from the area under the curve of insulin response above the baseline during the first 10 minutes of the iv glucose tolerance test and provides a measure of the first-phase insulin secretion (14). The disposition index provides an estimate of insulin secretion adjusted for the degree of insulin sensitivity and was calculated as the product of the two (14).

To allow comparisons of adiposity of the subjects in relation to healthy children, we calculated z-scores of the percentage body fat using population-based age- and gender-specific normative data on Caucasian children (*z-scores*$_p$) (15) after appropriate transformations to adjust for the scanner types (13, 16). The limb fat was calculated as the sum of fat (in kilograms) in arms and legs, and the trunk-limb fat ratio was calculated by dividing the trunk fat by limb fat. We expressed the body fat as the percentage of total mass because it provided an estimate of adiposity independent of body size, and we calculated it using the formula: percentage fat in a region = fat mass of the region (kg) × 100/total mass of the region (kg).

Statistics

The variables for insulin and C-peptide levels, insulin sensitivity, AIR, and disposition index were log-transformed to normality. Although the percentage body fat *z-scores*$_p$ were derived using normative data, significant residual associations with age and gender were observed. Therefore, we derived "within-cohort" z-scores of percentage body fat at baseline (*z-scores*$_c$) as an estimate of adiposity independent of these factors from a linear regression model with percentage body fat as the dependent variable and age, gender, and type of DXA scanner as covariants. To determine the associations of baseline adiposity, the children were categorized into tertiles of percentage body fat *z-scores*$_c$. The effect of baseline adiposity in predicting first-year height velocity was assessed by including percentage body fat *z-scores*$_c$ in Ranke's height prediction model for SGA children (17), which includes variables of age, weight SDS at the start of treatment, GH dose, and midparental height SDS. Associations between adiposity and multiallele scores were explored using regression models that also included age and gender to reduce the variability in the data. Statistical analyses were performed using the statistical package IBM SPSS statistics (version 20; SPSS Inc). The data are presented as mean (SD) unless otherwise specified.

Results

The study included 89 Caucasian children (55 boys) with a mean age of 6.2 ± 1.6 years.

Baseline adiposity

At baseline, the children had a lower mean percentage body fat (*z-scores*$_p$, −0.26 ± 1.2; $P = .045$) and BMI (−1.29 ± 1.37 SDS; $P < .0001$) compared with healthy Caucasian children (12, 15) (Table 1 and Figure 1B). Although, percentage body fat *z-scores*$_p$ were derived using age and gender-specific normative data, it showed residual associations with age ($r = -0.21$; $P < .05$) and male vs female gender ($r = 0.66$; $P < .0001$). Percentage body fat and the *z-scores*$_p$ were not associated with height SDS. The tertile groups for baseline percentage body *z-scores*$_c$ were similar in age and height SDS (Table 2), but the highest tertile group had greater BMI SDS (p-trends = .04), percentage fat in trunk and limbs (all p-trends < .0001), and trunk-limb fat ratio (p-trends = .019). The tertile groups had similar levels of IGF-1, glucose, insulin, and C-peptide and insulin sensitivity; however, the highest tertile group had greater AIR (p-trends = .02) (Figure 2E).

Changes in body composition and glucose metabolism during GH treatment

During the first year of GH treatment, catch-up growth was accompanied by increases in lean body mass ($P < .0001$) and bone mineral content ($P < .0001$) (Table 1). Conversely, total body fat mass and limb fat mass declined (both, $P < .0001$), whereas trunk fat mass remained unchanged, resulting in an increased trunk-limb fat ratio at 1 year (Figure 1). The differential changes in fat mass compared to lean body mass and bone mineral content resulted in a markedly reduced percentage of fat in the whole body, limbs, and trunk (all $P < .0001$) (Figure 1). GH treatment led to considerable increases in height SDS, BMI SDS, IGF-1 SDS, and fasting insulin and C-peptide levels (Table 1). Insulin sensitivity decreased substantially; however, a compensatory increase in insulin secretion resulted in an unchanged disposition index. Triglyceride levels also increased, but no changes in total, low-density lipoprotein, or high-density lipoprotein cholesterol were observed.

Adiposity and response to GH treatment

Body composition

Children in the highest tertiles of percentage body fat *z-scores*$_c$ showed the greatest loss of percentage body fat in the whole body (p-trends = .005), trunk (p-trends = .0001), and limbs (p-trends = .002) (Table 2 and Figure 2). Nevertheless, the baseline differences in adiposity between the groups persisted at 1 year of treatment, with the highest tertile group still having the greatest fat percentage in the whole body (p-trends = .001), trunk (p-trends < .0001), and limbs (p-trends = .057).

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Table 1. Body Composition and Metabolism During First Year of GH Treatment

	Baseline	1 Year	*P* Value
Anthropometry			
Height SDS	−3.35 (0.74)	−2.31 (0.69)	<.0001
Weight SDS	−3.10 (1.03)	−2.12 (1.00)	<.0001
BMI, kg/m^2	14.16 (1.49)	14.68 (1.62)	<.0001
BMI SDS	−1.34 (1.38)	−0.96 (1.29)	<.0001
Body composition (DXA)			
Total lean mass, kg	11.5 (2.66)	15.6 (3.45)	<.0001
Bone mineral content, g	457 (166)	606 (188)	<.0001
Total body fat mass, kg	2.26 (1.06)	2.06 (1.12)	.007
Trunk fat mass, kg	0.68 (0.37)	0.72 (0.41)	.13
Limbs fat mass, kg	1.10 (0.68)	1.00 (0.67)	.0002
Total body fat, %	15.8 (5.80)	11.2 (4.70)	<.0001
Total body fat, % (z-score)[a]	−0.26 (1.21)	−1.23 (1.54)	<.0001
Trunk fat, %	10.6 (4.66)	8.63 (4.03)	<.0001
Limb fat, %	23.1 (9.70)	14.6 (7.70)	<.0001
Trunk-limb fat ratio	0.61 (0.20)	0.84 (0.32)	<.0001
Biochemistry			
IGF-1 SDS	−1.09 (1.28)	2.88 (1.52)	<.0001
Glucose, mmol/L	4.32 (0.66)	4.70 (0.55)	<.0001
Insulin, pmol/L (log)	1.19 (0.28)	1.59 (0.22)	<.0001
C-peptide, pmol/L (log)	2.30 (0.24)	2.61 (0.17)	<.0001
Insulin sensitivity (HOMA) (log)	2.38 (0.25)	2.06 (0.17)	<.0001
AIR (log)	3.13 (0.24)	3.39 (0.26)	<.0001
Disposition index (log)	5.51 (0.24)	5.46 (0.23)	.11
Total cholesterol, mmol/L	3.94 (0.72)	3.88 (0.70)	.38
LDL cholesterol, mmol/L	2.23 (0.63)	2.15 (0.58)	.11
HDL cholesterol, mmol/L	1.47 (0.35)	1.42 (0.33)	.070
Triglycerides, mmol/L	0.64 (0.33)	0.83 (0.40)	.001

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein. Data are presented as means (SD).

[a] Z-Scores for percentage body fat were calculated based on normative data (*z-score$_p$*).

Height and IGF-1 response

Increase in height SDS was positively associated with baseline percentage body fat *z-scores$_c$* (p-trends = .038). In this study, variance in the first-year height velocity on GH treatment predicted by Ranke's model (R^2 = 0.15) was relatively low because of the use of a fixed GH dose. The addition of percentage body fat *z-scores$_c$* explained a further 12% variance in the first-year height velocity ($P <$ .0001; R^2 = 0.27) (Table 3). We evaluated the associations of regional fat distribution on first-year height velocity by deriving z-scores for trunk and limb fat percentages at baseline (adjusted for age, gender, and scanner type). The addition of percentage limb fat z-scores explained a higher variance in the first-year height velocity (B [95% confidence interval (CI)], 0.77 cm/y [0.37, 1.17]; $P <$ .0001; R^2 = 0.25) compared with trunk fat z-scores (0.61 cm/y [0.24, 0.98]; P = .001; R^2 = 0.22) in the Ranke's model. Furthermore, percentage limb fat z-scores explained an additional 5% variance when added to the model with percentage trunk fat z-scores (R^2 increased from 0.22 to 0.27; P [R^2 change] = .031). Higher total body percentage body fat *z-scores$_c$* were associated with greater IGF-1 responses (p-trends = .042) and IGF-1 levels at 1 year (p-trends = .036). The addition of changes in IGF-1 SDS from baseline to 1 year further increased the explained variance in the first-year height velocity from 27 to 33% (P [R^2 change] = .013) (Table 3); however, the effects of the baseline percentage body fat remained significant. Reductions in body fat percentage during GH treatment were strongly associated with increased height gains independent of the baseline body fat (r = 0.47; $P <$ .0001), but they were not related to IGF-1 responses. Decreases in the limb fat percentage (r = 0.41; P = .001) were more strongly related to height gains compared with the decreases in the trunk fat percentage (r = 0.25; P = .053), independent of the corresponding fat percentages at baseline.

Glucose and lipid metabolism

During GH treatment, changes in glucose, insulin, and C-peptide levels and insulin sensitivity were similar across the tertile groups. However, children in the highest tertile group had greater increases in AIR during treatment (p-trends = .014) resulting in higher AIR (p-trends = .004) and disposition index (P = .024) at 1 year (Figure 2, E and F). No differences were observed in the changes in fasting lipids between the tertile groups (data not shown).

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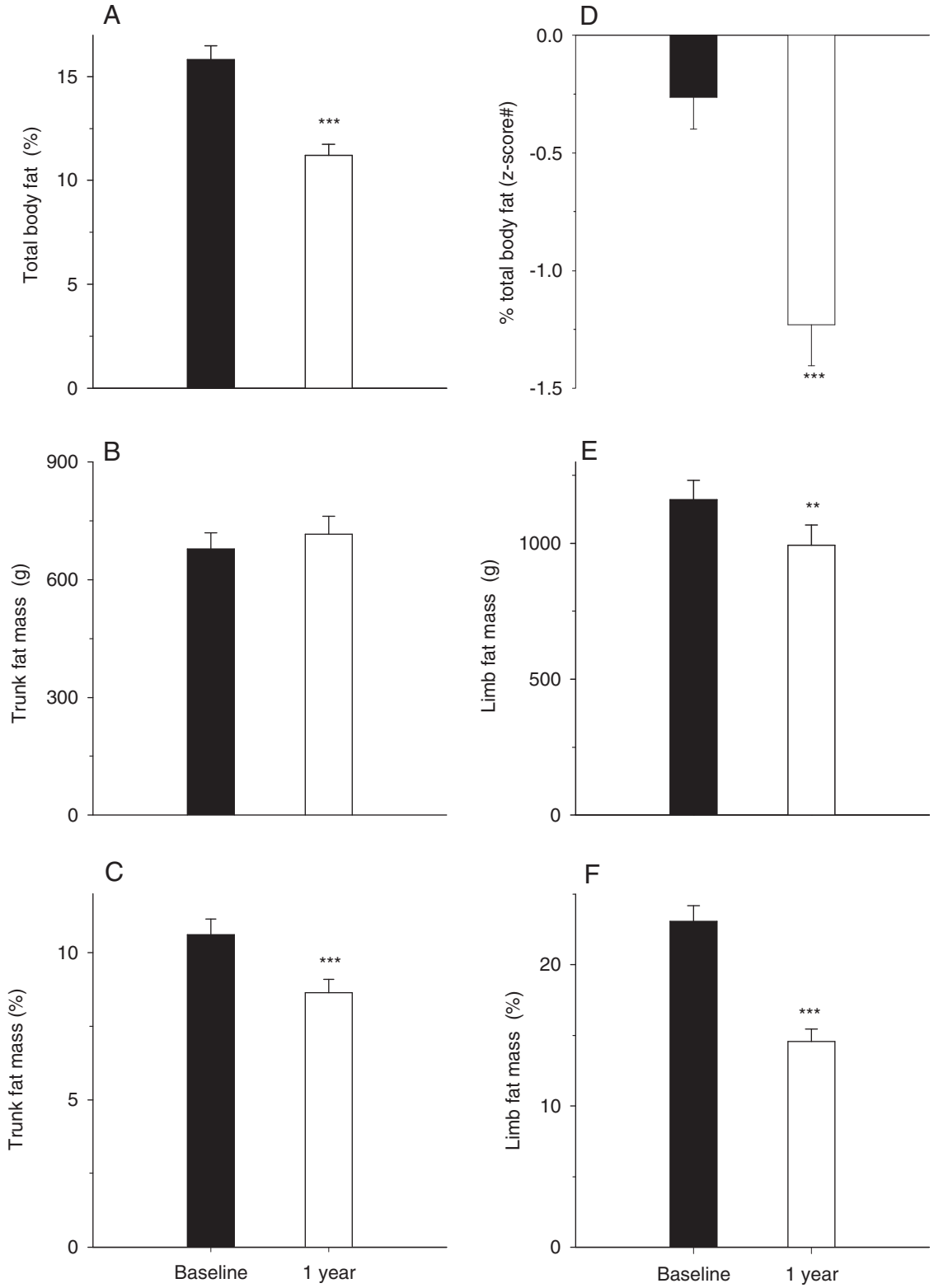

Figure 1. Changes in body fat during GH treatment. Total body fat percentage (A), z-scores for total body fat percentage (D), trunk fat mass in grams (B), and as percentage of total trunk mass (C), limb fat mass in grams (E), and as percentage of total limb mass (F). Bars represent means, and error bars represent the standard error of means. Black and empty bars represent measurements at baseline and 1 year, respectively. #, Z-Scores for total body fat percentage are based on normative data (*z-scores*$_p$). *P* values are from the comparison between baseline and 1-year measurements; **, $P < .001$; and ***, $P < .0001$.

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Table 2. Body Composition, Glucose Metabolism, and Response to GH Treatment in Patients Categorized by Tertiles of Z-Scores[a] for Total Body Fat Percentage at Baseline

	Tertiles of Baseline Total Body Fat			
	Low	**Middle**	**High**	***P* Trends**
Baseline				
No. of subjects (males)	30 (19)	29 (19)	30 (17)	NS
Age, y	6.04 (1.53)	5.95 (1.50)	6.50 (1.72)	.36
Height SDS	−3.30 (0.60)	−3.53 (0.85)	−3.26 (0.73)	.32
Weight SDS	−3.41 (0.79)	−3.24 (0.93)	−2.71 (1.23)	.025
BMI, kg/m^2	13.65 (1.12)	14.3 (0.91)	14.66 (2.06)	.035
BMI SDS	−1.73 (1.14)	−1.10 (0.91)	−0.89 (1.69)	.040
Total body fat, %	11.9 (4.90)	16.0 (4.20)	20.5 (4.80)	<.0001
Total body fat, % (*z-score*)[a]	−0.88 (0.36)	−0.17 (0.19)	0.89 (0.51)	<.0001
Trunk fat, %	7.27 (2.51)	9.72 (2.42)	15.8 (4.26)	<.0001
Limb fat (%)	17.8 (8.80)	24.0 (8.60)	28.0 (7.90)	<.0001
Trunk-limb fat ratio	0.59 (0.18)	0.56 (0.19)	0.70 (0.20)	.019
IGF-1 SDS	−1.12 (1.09)	−1.18 (1.35)	−1.00 (1.40)	.87
Glucose, mmol/L	4.17 (0.61)	4.34 (0.64)	4.47 (0.72)	.22
Insulin, pmol/L (log)	1.15 (0.26)	1.26 (0.23)	1.27 (0.26)	.14
C-peptide, pmol/L (log)	2.26 (0.24)	2.35 (0.23)	2.32 (0.23)	.41
HOMA insulin sensitivity, % (log)	2.42 (0.26)	2.33 (0.24)	2.36 (0.25)	.38
AIR (log)	3.04 (0.23)	3.18 (0.20)	3.21 (0.26)	.020
Disposition index (log)	5.46 (0.26)	5.51 (0.21)	5.57 (0.26)	.29
1 year				
Height SDS	−2.36 (0.56)	−2.42 (0.81)	−2.17 (0.70)	.36
Weight SDS	−3.41 (0.79)	−3.24 (0.93)	−2.71 (1.23)	.010
BMI, kg/m^2	14.1 (1.20)	14.6 (1.17)	15.3 (2.13)	.017
BMI SDS	−1.30 (1.16)	−1.04 (0.96)	−0.47 (1.69)	.12
Total body fat, %	8.97 (4.06)	11.1 (4.19)	13.8 (4.77)	.001
Trunk fat, %	6.13 (1.82)	8.73 (4.41)	11.1 (3.82)	<.0001
Limb fat, %	12.1 (7.60)	14.6 (7.70)	17.2 (7.30)	.057
Trunk-limb fat ratio	0.85 (0.38)	0.81 (0.31)	0.85 (0.26)	.87
IGF-1 SDS	2.57 (1.34)	2.63 (1.61)	3.46 (1.47)	.036
Glucose, mmol/L	4.62 (0.49)	4.64 (0.60)	4.78 (0.58)	.48
Insulin, pmol/L (log)	1.54 (0.23)	1.59 (0.20)	1.65 (0.21)	.19
C-peptide, pmol/L (log)	2.58 (0.16)	2.62 (0.17)	2.63 (0.19)	.59
HOMA insulin sensitivity, % (log)	2.09 (0.17)	2.05 (0.17)	2.03 (0.19)	.55
AIR (log)	3.27 (0.22)	3.45 (0.22)	3.48 (0.28)	.004
Disposition index (log)	5.36 (0.23)	5.50 (0.22)	5.52 (0.22)	.024
Changes from baseline to 1 year				
Δ Height SDS	0.94 (0.33)	1.04 (0.22)	1.14 (0.31)	.038
Δ Weight SDS	1.02 (0.40)	1.03 (0.34)	1.05 (0.49)	.96
Δ BMI, kg/m^2	0.46 (0.46)	0.47 (0.80)	0.72 (0.72)	.064
Δ BMI SDS	0.48 (0.45)	0.45 (0.46)	0.40 (0.54)	.84
Δ Total body fat, %	−2.94 (1.38)	−3.88 (1.61)	−5.30 (2.99)	.001
Δ Trunk fat, %	−0.90 (1.56)	−1.76 (1.79)	−3.61 (2.83)	<.0001
Δ Limb fat, %	−5.47 (2.90)	−7.80 (3.36)	−9.33 (4.88)	.003
Δ Trunk-limb fat ratio	0.27 (0.33)	0.18 (0.18)	0.11 (0.18)	.19
Δ IGF-1 SDS	3.69 (1.32)	3.80 (1.45)	4.17 (1.35)	.042
Δ glucose, nmol/L	0.45 (0.55)	0.30 (0.48)	0.38 (0.46)	.49
Δ insulin, pmol/L (log)	1.99 (0.09)	1.98 (0.09)	2.02 (0.08)	.27
Δ C-peptide, pmol/L (log)	2.81 (0.15)	2.73 (0.49)	2.84 (0.09)	.47
Δ HOMA insulin sensitivity, % (log)	3.21 (0.10)	3.24 (0.05)	3.23 (0.04)	.35
Δ AIR (log)	3.27 (0.29)	3.40 (0.21)	3.48 (0.18)	.014
Δ Disposition index (log)	5.74 (1.10)	5.99 (0.08)	5.96 (0.15)	.43

Abbreviation: NS, not significant. Data are presented as means (SD).

[a] Within cohort z-scores (*z-score*$_c$) for total body fat percentage at baseline adjusted for age, gender, and type of scanner.

Multiallele scores and body composition

Insulin sensitivity

At baseline, GS-InRes was inversely related to BMI SDS (B [95% CI], −0.13 SDS per allele [−0.26, −0.01]; P = .040) and percentage fat in the whole body (−0.49% per allele [−0.97, −0.007]; P = .047) and limbs (−0.81% per allele [−1.62, 0.00]; P = .049), but not in the trunk (Table 4). During GH treatment, a higher GS-InRes was associ-

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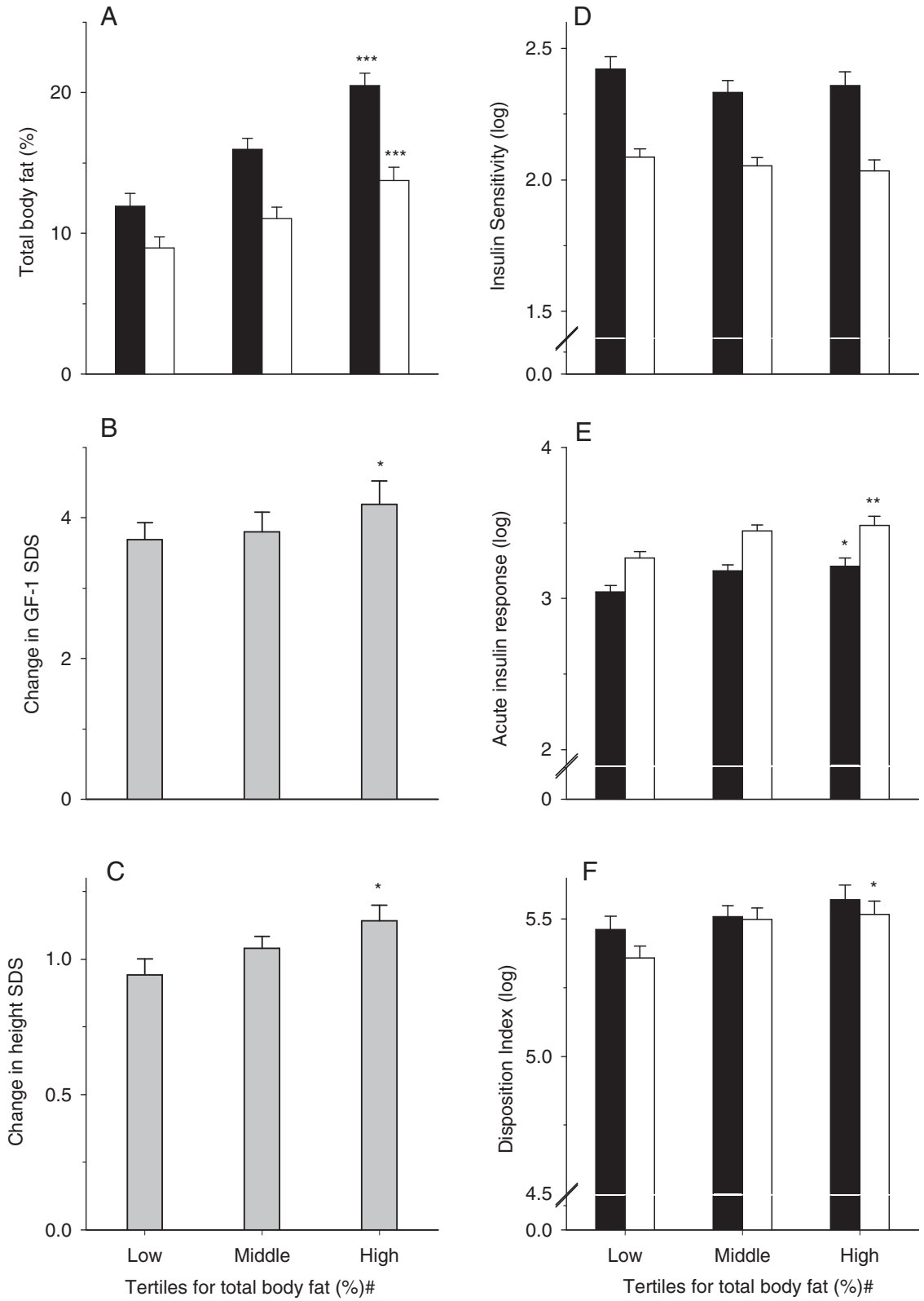

Figure 2. Changes in body fat, height, IGF-1, and measures of glucose metabolism in the tertile groups for percentage body fat z-scores at baseline during 1 year of GH treatment. A, Total body fat percentage; B, change in IGF-1 SDS; C, change in height SDS; D, insulin sensitivity as HOMA % (log); E, insulin secretion as log of AIR; and F, disposition index (log). Bars represent means, and error bars represent the standard error of the means. Black and empty bars represent measurements at baseline and 1 year, respectively; gray bars represent changes in measurements from baseline to 1 year. #, Tertiles of z-scores for total body fat percentage derived within the cohort ($z\text{-}score_c$). Asterisks represent p-trends across the tertile groups: *, $P < .05$; **, $P < .01$; and ***, $P < .001$. In y-axes with log-transformed values, a break has been introduced (E and F) to display the error bars and trends more clearly.

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Table 3. Effect of Baseline Total Body Fat on Ranke's Prediction Model for the First-Year Height Response in SGA Children

	B	95% CI	*P* Value	Partial Correlation	Collinearity (Tolerance)	R^2	*P* Value (R^2 Change)
Model 1							
Constant	13.7	11.5, 15.8	<.0001				
Age, y	−0.31	−0.54, −0.09	.008	−0.30	0.97		
Midparental height, SDS	0.46	0.07, 0.85	.022	0.26	0.96		
Weight at baseline, SDS	0.12	−0.26, 0.50	.52	0.07	0.93	0.15	.008
Model 2							
Constant	13.04	11.1, 15.0	<.0001				
Age, y	−0.29	−0.50, −0.08	.008	−0.31	0.97		
Midparental height (SDS)	0.47	0.11, 0.84	.012	0.29	0.96		
Weight at baseline, SDS	−0.04	−0.40, 0.33	.84	−0.02	0.88		
Baseline total body fat % (z-score)[a]	0.61	0.28, 0.95	<.0001	0.39	0.94	0.27	.001
Model 3							
Constant	10.9	8.36, 13.5	<.0001				
Age, y	−0.19	−0.41, 0.03	.096	−0.19	0.85		
Midparental height, SDS	0.45	0.10, 0.79	.012	0.28	0.95		
Weight at baseline, SDS	−0.13	−0.49, 0.23	.48	−0.08	0.83		
Baseline total body fat % (z-score)[a]	0.59	0.26, 0.92	.001	0.38	0.91		
Δ IGF-1 SDS (0 to 1 y)	0.30	0.07, 0.54	.013	0.28	0.82	0.33	.013

Abbreviation: B, unstandardized coefficient. Dependent variable is height velocity (cm/y). Model 1: Ranke's Model for prediction of first-year height velocity in SGA children; GH dose is not included in the model as a fixed dose was used in the study. Model 2: The effect of total body fat percentage on Ranke's Prediction Model. Model 3: Effect of the addition of change in IGF-1 SDS (0 to 1 y).

[a] Within cohort z-scores for total body fat percentage at baseline adjusted for age, gender, and type of scanner (*z-score*$_c$).

ated with lesser declines in total body fat (0.31% per allele [0.10, 0.51]; $P = .004$) and trunk fat (0.38% per allele [0.16, 0.59]; $P = .001$), and therefore increases in the trunk-limb fat ratio (0.03 per allele [0.01, 0.05]; $P = .003$). At 1 year, the GS-InRes was still inversely associated with percentage fat in the limbs (−0.81% per allele [−1.49, −0.13]; $P = .020$) and positively associated with the trunk-limb fat ratio (0.04 per allele [0.01, 0.08]; $P = .008$).

Table 4. Associations Between Multiallele Scores for Insulin Sensitivity and Body Composition[a]

	Effect Size per Allele (B)	95% CI	*P* Value[b]
Baseline			
BMI (SDS)	−0.13	−0.26, −0.01	.040
Body fat (%)	−0.49	−0.97, −0.01	.047
Limb fat (%)	−0.81	−1.62, 0.00	.049
Arm fat (%)	−1.19	−2.31, −0.06	.038
Leg fat (%)	−0.76	−1.55, 0.03	.060
Trunk fat (%)	−0.33	−0.77, 0.12	.16
Trunk-limb fat ratio	0.01	−0.01, 0.03	.49
1 year			
BMI (SDS)	−0.07	−0.22, 0.09	.40
Body fat (%)	−0.39	−0.81, 0.02	.064
Limb fat (%)	−0.81	−1.49, −0.13	.020
Arm fat (%)	−1.04	−1.95, −0.13	.026
Leg fat (%)	−0.59	−1.27, 0.09	.087
Trunk fat (%)	−0.03	−0.43, 0.37	.88
Trunk-limb fat ratio	0.04	0.01, 0.08	.008
Changes from baseline to 1 year			
Δ Body fat (%)	0.31	0.10, 0.51	.004
Δ Limb fat (%)	0.28	−0.11, 0.68	.16
Δ Arm fat (%)	0.18	−0.41, 0.78	.54
Δ Leg fat (%)	0.27	−0.16, 0.70	.22
Δ Trunk fat (%)	0.38	0.16, 0.59	.001
Δ Trunk-limb fat ratio	0.03	0.01, 0.05	.003

Abbreviation: B, unstandardized coefficient.

[a] Higher scores associated with lower insulin sensitivity.

[b] *P* values and B are derived from regression models with age and gender as covariants.

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Insulin secretion

GS-InSec was positively associated with percentage trunk fat at baseline (0.36% per allele [0.09, 0.63]; $P = .009$) and at 1 year (0.25% per allele [0.01, 0.50]; $P = .045$) (Supplemental Table 2). However, it was not associated with percentage fat in the whole body or limbs.

Discussion

In this study of short SGA children, higher pretreatment adiposity predicted greater height gains and IGF-1 response during GH treatment and increased β-cell function. Consideration of the baseline whole body and regional adiposity substantially improved the prediction of first-year height responses. Analysis of informative multiallele scores supported the likely causal role of insulin resistance in linking reduced body fat, particularly the peripheral body fat, to lower sensitivity to GH treatment.

In this large cohort, we confirmed the findings of reduced body fat in short SGA children (2, 3, 18). Previous studies using magnetic resonance imaging scans (3, 18) or skinfold thickness measurements (2, 19) have reported deficits in subcutaneous fat both in the trunk and limbs but similar visceral fat compared to AGA children (18). Alterations in adipose tissue development, adipokine signaling to the brain, and neuroendocrine regulation of appetite have been reported in animal models of intrauterine growth retardation associated with rapid catch-up growth (20, 21). Conversely, similar mechanisms may be relevant in short SGA children with no catch-up growth because they have a reduced appetite and food intake despite lower leptin levels, compared with AGA controls (22). Nevertheless, the low adiposity reflects suboptimal energy stores and is consistent with the low levels of insulin and IGF-1 in short SGA children compared with weight-matched AGA controls (6). Anabolic actions of GH are closely linked to overall energy balance, as shown by the increased IGF-1 responses in obesity and the low IGF-1 levels despite greater GH secretion during fasting (6, 23). Our findings of lower IGF-1 and growth responses in children with lesser adiposity suggest that reduced sensitivity to exogenous GH related to suboptimal energy stores contributes to a poorer treatment effect. Alterations in GH/IGF-1 axis ranging from relative GH deficiency to resistance may also explain these associations. However, the overall leanness of these children as a group and the fact that adiposity is unrelated to IGF-1 levels or insulin sensitivity suggest that alterations in GH/IGF-I axis are less likely to mediate the links between adiposity and response to GH treatment (2). Baseline adiposity predicted height gains independent of IGF-1 responses, which implies that pathways of GH action other than the hepatic IGF-1 generation are also influenced by the overall energy balance. The growth prediction models showed a substantial effect of baseline adiposity in promoting linear growth on GH treatment; however, the explained variance was insufficient (27%) for it to be used in clinical settings (24).

The energy balance is probably important in other childhood disorders treated with GH and may explain the inclusion of weight in the height prediction models for GH-deficient patients (24). However, it is particularly relevant to SGA children who have low adiposity (17). Our observations of preferential loss of peripheral body fat during GH treatment support previous reports (2, 19, 25) and contrast the predominant effect on central fat in GH-deficient patients (6, 26). We postulate that the pattern of fat loss in SGA children results from further declines in energy stores because the limb depots are primarily related to long-term fat storage (27). A stronger relationship between growth response and limb fat at baseline compared to the trunk fat supports this hypothesis. Furthermore, we found strong associations between first-year height gains and declines in body fat, particularly in the limbs, which suggests that rapid growth occurs at the expense of energy stores. The reduction of percentage body fat in our study (29%) on a higher GH dose (67 μg/kg) was greater than that (21%) reported on the more common lower GH dose (35 μg/kg) and is consistent with dose-dependent effects of GH on growth and lipolysis (6, 17).

The findings of a relationship between lower adiposity, lesser insulin secretion, and disposition index before and during GH treatment could reflect a physiological adaptation to prevent hypoglycemia as seen during fasting and other suboptimal nutritional states (28, 29). These associations may be mediated through alterations in the IGF-1 generation, which is important for maintaining β-cell function (30). The reduced β-cell function associated with lower adiposity could have long-term implications because thinness during childhood is related to an increased risk for T2D (31).

After an initial marked decrease, body fat is reported to return to pretreatment ranges in subsequent years when growth velocity declines (25). However, young SGA adults, after stopping GH treatment, have a tendency for a lesser limb fat percentage despite a higher total body fat percentage compared to AGA adults (32). Recently, fat depots in limbs and the gluteofemoral region are shown to store triglycerides long-term more efficiently compared with the trunk fat and are linked to favorable metabolic outcomes (11, 27). The total number of adipocytes, which is fixed by late childhood, may also be a critical factor in determining the expandability of subcutaneous adipose tissue and metabolic decompensation in response to nu-

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trient excess (21, 33, 34). Based on our findings of a positive relationship between adiposity, responses to GH treatment, and β-cell function, conserving peripheral body fat could form the target for nutritional interventions to optimize energy balance in SGA children treated with GH.

Recent findings that common genetic variants for insulin resistance are related to lesser gluteofemoral and limb fat suggest an important role of expandability of regional subcutaneous adipose tissue in metabolic outcomes (11). We have observed for the first time the same relationship (with larger observed effect sizes) in a selected group of SGA children already present before GH treatment, which persisted at 1 year on treatment. The observed associations here, between lower adiposity and both genetic susceptibility to insulin resistance and lower growth response to GH treatment, complement our reported associations between the same alleles and lower growth and IGF-1 responses to GH treatment in the same cohort (10). Although Mendelian randomization analyses cannot formally model causal mediation, these findings support a causal role for insulin resistance in mediating the effects of lower adiposity on lesser GH action (Supplemental Figure 1). We speculate that these pathways could be linked to the hepatic IGF-1 generation and IGF-1 sensitivity. Reported effects of metformin treatment on improving linear growth despite lower IGF-1 levels in low-birth-weight girls with premature adrenarche support the latter hypothesis (35, 36). During treatment, the insulin resistance alleles were inversely related to reductions in body fat, further suggesting reduced sensitivity to GH. However, the alleles were related to lesser reductions in the trunk fat and, therefore, an increased trunk-limb fat ratio at 1 year. We speculate that these changes could be due to the reduced function of peripheral adipose tissue and preferential fat storage centrally when lipid turnover is increased by GH treatment (6). The association between insulin secretion-lowering alleles and higher trunk fat has been reported in adults (11). Although its significance is not clear, this association could provide a link between a phenotype resulting from prenatal growth restraint with a tendency for central fat deposition and an increased risk for T2D (27, 37).

Our study has some drawbacks. Although percentage body fat is a commonly used measure of adiposity in children, it is limited by the potential association with height (38). However, height was unrelated to adiposity in our selected group of lean subjects. The reasons for the residual associations between population derived z-scores for percentage body fat, age, and gender in the study were not clear (15). We speculate that comparisons to normative data from a different type of DXA scanner are an important reason and may underlie the higher pretreatment body fat percentage in our study compared to previous reports (z-scores, −0.26 vs −0.6 to −1.2) (19, 39). We used the within-cohort z-scores for percentage body fat in the calculations rather than further adjusting population derived z-scores for age and gender to avoid complex models in this modestly sized study. The associations between multiallele scores and body composition were modest; however, they were consistent when assessed at both baseline and 1 year and support similar findings in adults. Long-term illness may confound our observations; however, we excluded children with syndromes, severe learning difficulties, or other disorders that may influence growth (8). We did not measure adipokines; further studies evaluating these and epigenetic changes in adipose tissue will be valuable to delineate the pathways underlying our findings.

In conclusion, our findings suggest that greater adiposity has beneficial effects on responses to GH treatment and glucose metabolism in short SGA children. Mechanisms associated with insulin resistance link lower adiposity and reduced response to GH treatment in these children. Although the association between genetic susceptibility to insulin resistance and lower adiposity appears to be generalizable across adults and children, the conclusions linking these factors to GH treatment responses are limited to the population studied here.

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Clinical Trial Registration: North European Small for Gestational Age Study EudraCT 2005–001507–19.

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The exon3-deleted growth hormone receptor gene polymorphism (d3-GHR) is associated with insulin and spontaneous growth in short SGA children (NESGAS)

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ABSTRACT

Objective: The effect of a common polymorphism in the Growth Hormone (GH) receptor (d3-GHR) gene on growth, metabolism and body composition was examined in short children born small for gestational age (SGA) on GH treatment.

Design: In 96 prepubertal, short SGA children treated with high-dose GH (67 μg/kg/day) in the NESGAS study, insulin sensitivity (IS), insulin secretion and disposition index (DI) were determined during the first year of treatment. Body composition was analysed by DXA. The d3-GHR locus was determined by simple multiplex PCR.

Results: At baseline, children in the d3-GHR group (d3/fl (n = 37), d3/d3 (n = 7)) had significantly lower IS (median (25–75 percentile)) (223.3% (154.4–304.8)) vs. (269.7% (185.1–356.7)) (p = 0.03) and higher concentrations of glucose (mean (SD)) (4.4 mmol/L (0.6) vs. 4.2 mmol/L (0.7)) (p = 0.03), C-peptide (232.1 pmol/L (168.8–304.1) vs. 185.1 pmol/L (137.7–253.9)) (p = 0.04) and insulin (19.2 pmol/L (11.8–32.2)) vs. (13.7 pmol/L (9.3–20.8)) (p = 0.04) compared to children homozygous for the full length allele (fl/fl-GHR (n = 52)). There were no differences in DI or insulin secretion. Postnatal, spontaneous growth was significantly greater in the d3-GHR group compared to the fl/fl-GHR group (p = 0.02). There were no significant differences in growth response, body composition or metabolism after one year of GH therapy.

Conclusion: Short SGA children carrying the d3-GHR polymorphism had increased spontaneous growth, lower IS and a compensatory increase in glucose, C-peptide and insulin before GH therapy compared to children homozygous for the full-length allele.

1. Introduction

Growth hormone (GH) treatment of children born small for gestational age (SGA) has an overall beneficial effect on final height [1]. In addition to the growth promoting effects, which are mainly achieved through the effects of insulin-like growth factor-I (IGF-I), GH exerts direct (IGF-I-independent) lipolytic and anabolic effects and lowers insulin sensitivity (IS). In addition, there is increasing evidence that the GH/IGF-I axis plays a role in normal glucose homeostasis mainly due to the fact that IGF-I and insulin share significant structural homology and downstream pathways [2]. Previously, we and others have shown that the interaction between GH dose, IGF-I and insulin levels and body composition in GH-treated short SGA children is important for the growth response and metabolism during treatment [3,4].

Growth responses following GH therapy vary markedly in short statured children of varying etiologies including IGHD, Turner and SGA

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Table 1
Clinical characteristics.

	N	fl/fl (N = 52)	d3/fl (N = 37)	d3/d3 (N = 7)	P value *	P value #
					fl/fl vs. d3/fl vs. d3/d3	fl/fl vs. d3-allele
Baseline						
Birth weight (SDS)	95	− 3.1 (0.9)	− 3.3 (1.0)	− 3.6 (0.9)	0.37	0.20
Birth Length (SDS)	64	− 2.8 (1.9)	− 3.5 (1.1)	− 4.0 (1.8)	0.17	0.07
Age (year) baseline	96	6.1 (1.6)	6.1 (1.7)	6.2 (1.6)	1.00	0.99
Weight (SDS) baseline	96	− 3.2 (1.1)	− 3.2 (0.9)	− 2.9 (1.2)	0.70	0.99
Height (SDS) baseline	96	− 3.5 (0.8)	− 3.3 (0.7)	− 3.3 (0.5)	0.41	0.18
BMI (SDS) baseline	93	− 1.2 (1.3)	− 1.5 (1.3)	− 0.9 (1.5)	0.50	0.52
Total body fat percentage (%) baseline	84	17.3 (7.0)	15.3 (5.1)	16.0 (5.6)	0.37	0.17
Limb fat percentage (%) baseline	81	25.6 (14.5–32.6)	19.0 (15.9–28.8)	23.6 (11.9–34.5)	0.54	0.29
Trunk fat percentage (%) baseline	79	10.8 (4.8)	9.9 (3.4)	10.3 (4.3)	0.70	0.42
Change from birth to baseline						
Δ Weight (SDS) (Birth to baseline)	95	0.0 (1.2)	0.1 (1.0)	0.7 (1.7)	0.30	0.36
Δ Height (SDS) (Birth to baseline)	64	− 0.5 (2.0)	0.5 (1.2)	0.8 (1.8)	0.05	0.02
After one year GH therapy						
Age (year) 1 y	95	7.2 (1.6)	7.2 (1.6)	7.4 (1.8)	0.97	0.98
Weight (SDS) 1 y	92	− 2.1 (1.2)	− 2.3 (0.9)	− 1.7 (1.1)	0.53	0.69
Height (SDS) 1 y	95	− 2.4 (1.0)	− 2.3 (0.7)	− 2.4 (0.8)	0.92	0.74
BMI (SDS) 1 y	66	− 0.8 (1.2)	− 1.5 (1.2)	− 0.4 (1.7)	0.06	0.07
Total body fat percentage (%) 1 y	81	12.0 (6.0–16.0)	10.5 (8.1–13.0)	8.0 (6.0–12.0)	0.68	0.54
Limb fat percentage (%) 1 y	79	14.8 (6.9–22.0)	11.8 (9.6–15.9)	7.7 (6.6–11.1)	0.20	0.29
Trunk fat percentage (%) 1 y	80	6.9 (5.3–11.1)	7.6 (6.5–10.3)	6.6 (5.3–12.5)	0.76	0.60
Change from baseline to 1 year						
Δ Weight (SDS)	92	1.1 (0.5)	0.9 (0.4)	1.1 (0.5)	0.28	0.13
Δ Height (SDS)	92	1.1 (0.6)	1.0 (0.3)	1.0 (0.3)	0.45	0.21
Δ BMI (SDS)	64	0.4 (0.5)	0.2 (0.7)	0.6 (0.5)	0.34	0.35
Δ Total body fat percentage (%)	76	− 4.0 (− 6.2 to − 2.2)	− 3.0 (− 5.0 to − 2.4)	− 3.0 (− 12.0 to − 3.0)	0.55	0.34
Δ Limb fat percentage (%)	69	− 7.6 (− 10.6 to − 5.0)	− 6.9 (− 10.1 to − 4.6)	− 7.5 (− 20.3 to − 3.5)	0.83	0.63
Δ Trunk fat percentage (%)	71	− 2.3 (− 3.8 to − 0.6)	− 1.6 (− 3.7–0,3)	− 1.7 (− 3.2 to − 0.9)	0.55	0.28

Data are presented as mean (SD) or median (25–75 percentiles). Comparisons were analysed by ANOVA-test or Kruskal-Wallis test (*) and independent-samples *t*-test or Mann Whitney test (#) when appropriate.

[5]. Several factors may influence the efficacy of the treatment and statistical prediction models explain only 52% of the variance in the first-year growth response in short SGA children, with GH dose alone accounting for 35% of the variance [6]. The aetiology of being born SGA may explain some of the remaining variance and genetic factors may be of great importance. In a previous study we showed that inclusion of a multi-allele score reflecting IS improved the explained variance of first year growth response by 5% in SGA children treated with a fixed high dose of GH [7]. Identifying factors that influence the growth response to GH treatment may improve the efficacy, safety and cost-effectiveness of the therapy.

There are two isoforms of the growth hormone receptor (GHR) in humans; a full-length isoform (fl-GHR) and an isoform that lacks exon 3 (d3-GHR). The d3-GHR polymorphism was found to enhance the signal transduction of the GHR in exposure to GH in vitro compared to the full-length allele homodimer (fl/fl-GHR) [8]. Several studies have shown an association between d3-GHR and increased growth response to GH treatment in children born SGA [8–10], however controversy exists [11–15]. Two meta-analyses concluded that the d3-GHR polymorphism increased first year growth response to GH treatment in children with short stature [16,17] and argued that this reflected better GH sensitivity caused by the greater signal transduction. Possible metabolic and lipolytic effects of the d3-GHR polymorphism have only been investigated in a few studies and controversies exist. In a study of healthy children and adolescents the d3-GHR polymorphism was associated with a higher insulin secretion and disposition index (DI) after adjusting for age, gender, pubertal stage and IS [18]. Furthermore, studies on GH treated children showed increased insulin and increased insulin resistance among the d3-GHR genotype compared to the fl/fl-GHR genotype [19], whereas other studies found no effect of the genotype on metabolism [15,20].

Based on the suggested higher responsiveness to GH of the d3-GHR polymorphism compared to the fl/fl-GHR we hypothesised that the d3-GHR polymorphism influenced the phenotype and response to GH treatment in short children born SGA. The aim of the present study was to determine the effect of the d3-GHR polymorphism on growth, metabolism and the body composition before and during the first year of high-dose GH treatment of short, prepubertal SGA children.

2. Materials and methods

2.1. Study population

Ninety-six (57 males) children from the North European Small for Gestational Age Study (NESGAS, EuDRACT 2005-001507-19) were included in this study. NESGAS is a multicentre, randomised, parallel group study of GH therapy in prepubertal, short children born SGA, who received high-dose GH (67 μg/kg/day) during the first year of treatment. Both study population and design have previously been published in details [3,4,7,21]. Briefly, the study population included prepubertal children born SGA (BW and/or BL ≤ − 2 standard deviation score (SDS), according to country specific references) and gestational age ≥ 28 weeks. At the age of 4 years all participants had persistent short stature with a height SDS (HSDS) ≤ − 2.5 SDS (according to country specific references), a height velocity SDS < 0 during the 6 months prior to study entry and a HSDS < 1 SD below parental adjusted HSDS.

2.2. Study design

All children received a fixed high-dose (67 μg/kg/day) of recombinant human GH (Norditropin®, Novo Nordisk, Bagsvaerd, Denmark) given as a daily subcutaneous injection the first year of treatment in order to induce catch-up growth and identify non-

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Table 2
Measurements of metabolic parameters.

	N	fl/fl (N = 52)	d3/fl (N = 37)	d3/d3 (N = 7)	P value *	P value #
					fl/fl vs. d3/fl vs. d3/d3	fl/fl vs. d3-allele
Baseline						
Glucose (mmol/l) baseline	95	4.2 (0.7)	4.5 (0.6)	4.3 (0.2)	0.07	0.02†
Insulin, (pmol/l) baseline	88	13.7 (9.3–20.8)	19.2 (11.1–32.7)	18.1 (15.0–29.0)	0.10	0.06†
C-peptide (pmol/l) baseline	88	185.1 (137.7–253.9)	224.9 (154.4–291.1)	274.2 (174.1–475.2)	0.07	0.02†
Insulin sensitivity (HOMA-S, %) baseline	88	269.7 (185.1–356.7)	226.7 (157.6–319.9)	175.5 (101.2–279.7)	0.05	0.01†
Insulin secretion (AIR, μU/ml/min) baseline	85	1406.0 (807.2–1904.8)	1528.6 (1014.7–2151.7)	1606.5 (785.3–2118.6)	0.66	0.27†
Disposition index (10^4·pmol/l·min) baseline	84	35.1 (23.8–54.3)	32.4 (24.2–45.7)	27.0 (16.4–29.4)	0.18	0.32†
IGF-I (SDS) baseline	90	− 1.3 (1.3)	− 1.1 (1.1)	− 0.1 (1.4)	0.11	0.27
Total cholesterol (mmol/L) baseline	89	3.9 (0.7)	4.0 (0.7)	4.0 (0.8)	0.57	0.29
LDL cholesterol (mmol/L) baseline	78	2.2 (0.6)	2.3 (0.7)	2.2 (0.5)	0.91	0.68
HDL cholesterol (mmol/L) baseline	81	1.5 (0.4)	1.5 (0.3)	1.4 (0.3)	0.80	0.93
Non-HDL (mmol/L) baseline	81	2.4 (0.7)	2.5 (0.7)	2.6 (0.6)	0.72	0.43
Free fatty acid (μmol/L) 0 min baseline	74	769.9 (537.6–1091.5)	635.6 (472.6–924.8)	574.3 (433.3–1064.7)	0.51	0.77†
After one year GH therapy						
Glucose (mmol/l) 1 y	95	4.7 (0.5)	4.8 (0.6)	4.6 (0.5)	0.45	0.38†
Insulin (pmol/l) 1 y	86	37.9 (28.6–51.9)	43.2 (31.0–57.9)	46.2 (21.1–83.0)	0.70	0.34†
C-peptide (pmol/l) 1 y	86	405.9 (319.6–551.4)	393.6 (341.8–539.7)	460.0 (224.6–847.3)	0.92	0.77†
Insulin sensitivity (HOMA-S, %) 1 y	86	118.1 (84.5–145.8)	119.0 (85.8–135.4)	101.1 (55.0–228.9)	0.91	0.72†
Insulin secretion (AIR, μU/ml/min) 1 y	85	2217.5 (1609.7–2822.2)	2925.8 (1933.6–3505.8)	2184.9 (1160.1–5693.3)	0.24	0.11†
Disposition index (10^4·pmol/l·min) 1 y	85	25.8 (18.2–35.5)	31.9 (23.1–42.3)	27.4 (19.6–42.3)	0.28	0.16†
IGF-I (SDS) 1 y	92	2.7 (1.4)	2.8 (1.7)	3.6 (0.5)	0.39	0.50
Total cholesterol (mmol/L) 1 y	87	3.8 (0.7)	4.0 (0.7)	3.9 (0.6)	0.70	0.41
LDL cholesterol (mmol/L) 1 y	79	2.1 (0.5)	2.2 (0.7)	2.2 (0.4)	0.91	0.71
HDL cholesterol (mmol/L) 1 y	83	1.4 (0.3)	1.4 (0.4)	1.4 (0.3)	0.93	0.99
Non-HDL (mmol/L) 1 y	82	2.4 (0.7)	2.5 (0.7)	2.6 (0.5)	0.74	0.45
Free fatty acid (μmol/L) 0 min 1 y	83	546.4 (359.8–756.6)	598.4 (376.4–749.5)	772.1 (479.0–839.5)	0.59	0.61†
Change from baseline to 1 year						
Δ Glucose (mmol/l)	94	0.5 (0.6)	0.3 (0.5)	0.3 (0.4)	0.18	0.06
Δ Insulin (pmol/l)	84	23.3 (14.9–41.6)	23.7 (11.7–37.6)	28.2 (6.0–54.)	0.79	0.62
Δ C-peptide (pmol/l)	84	233.9 (123.3–365.7)	177.9 (122.9–290.7)	185.6 (50.5–372.1)	0.46	0.22
Δ Insulin sensitivity (HOMA-S, %)	84	− 210.9 (233.2)	− 135.6 (166.7)	− 47.9 (38.5)	0.09	0.05
Δ Insulin secretion (AIR, μU/ml/min)	80	942.1 (375.1–1561.4)	1412.0 (496.0–2067.5)	736.8 (287.0–2590.7)	0.43	0.22
Δ Disposition index (10^4·pmol/l·min)	79	− 11.2 (28.0)	− 2.9 (23.0)	− 5.2 (11.3)	0.19	0.09
Δ IGF-I (SDS)	88	3.9 (1.6)	3.8 (1.5)	3.7 (1.4)	0.95	0.80
Δ Total cholesterol (mmol/L)	82	0.0 (0.7)	− 0.1 (0.4)	− 0.1 (0.5)	0.92	0.70
Δ LDL cholesterol (mmol/L)	73	− 0.1 (0.4)	− 0.1 (0.4)	0.0 (0.2)	0.84	0.83
Δ HDL cholesterol (mmol/L)	78	0.0 (0.3)	− 0.1 (0.2)	0.0 (0.2)	0.40	0.30
Δ Non-HDL (mmol/L)	77	0.0 (0.6)	0.0 (0.4)	0.0 (0.3)	0.98	0.88
Δ Free fatty acid (μmol/L) 0 min	70	− 313.3 (536.6)	− 104.0 (439.7)	− 32.3 (348.9)	0.18	0.07

Data are presented as mean (SD) or median (25–75 percentile). Comparisons were analysed by ANOVA-test or Kruskal-Wallis test (*) and independent *t*-test or Mann-Whitney test (#) when appropriate. † represents p-trends adjusted for gender, age and BMI.

responders. The children were assessed at entry of the study (baseline) and every 3 months after that. After the first year the children were blindly randomised into three different dose regiments of treatment for two more years by a web-based system (Sealed envelope™.com). Only data prior to GH therapy and from the first year of high-dose GH therapy will be used in this study.

2.3. Anthropometric measurements

Information on birth weight (BW) and birth length (BL) were collected from the routine examinations at birth. The clinical examination included: standing height on a wall-mounted stadiometer, weight by electronic scales by staff trained in auxological methods. Pubertal development was evaluated by an experienced investigator using the Tanner criteria. Body composition was assessed at baseline and at year 1 by dual x-ray absorptiometry (DXA) scans using the Hologic QDR-1000/W scanner (Hologic Inc) (three centres) or the Lunar Prodigy DXA system (GE Medical Systems) (six centres). The regional fat distribution was assessed by using the default setting for segmental analysis in the scanner.

2.4. Laboratory measurements

Plasma insulin and C-peptide levels were measured centrally in Cambridge, UK by a DELFIA assay using kits B080-101 and B081-101, respectively (Perkin Elmer Life Sciences, Turku, Finland). The inter-assay coefficients of variation (CVs) of insulin assay were 3.1% (29 pmol/L) and 2.1% (79.4 pmol/L). Insulin had cross-reactivity with: intact pro-insulin of > 0.5%, 32–33 split pro-insulin of 1% and C-peptide of < 0.1%. The interassay CVs for C-peptide assay were 4.0% (190 pmol/L) and 3.8% (1125 pmol/L). C-peptide had cross-reactivity with: insulin of < 0.1%, intact proinsulin of 60% and 32–33 split proinsulin at 400 pmol/L. Plasma glucose and fasting lipid profile were locally measured. Serum IGF-I levels were determined centrally in Copenhagen using a solid-phase enzyme-labelled chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, LA, USA). Standards were calibrated towards the WHO NIBSC IRR 87/518. IGF-I detection limit was 20 ng/mL and inter- and intra-assay CVs were 5.93% and 2.02%, respectively. All children underwent a short intravenous glucose tolerance test (IVGTT) and had fasting blood samples taken at baseline and after one year of treatment. At the short IVGTT 0.3 g/kg of intravenous glucose was administrated over 3 min after an overnight fast. Blood glucose and insulin levels were measured the following 10 min (− 15. − 10, 0, 1,3, 5 and 10 min) and C-peptide

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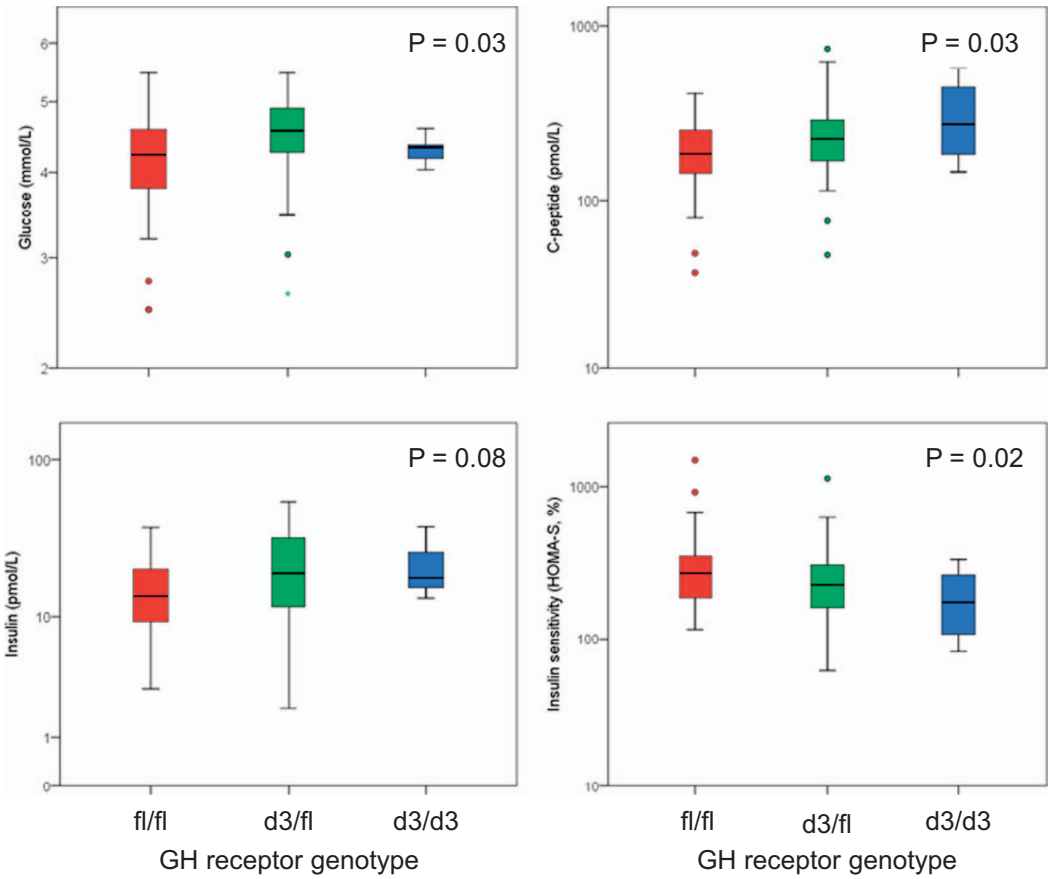

Fig. 1. Boxplots of metabolic parameters at baseline:
Boxplots of baseline glucose, C-peptide, insulin and IS in short SGA children according to the three genotypes: fl/fl (red), d3/fl (green) and d3/d3 (blue). Data are presented on a logarithmic scale. Error bars represent 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

levels were measured at 0 min. Free Fatty Acids (FFA) were measured centrally in Cambridge by a Roche Free Fatty Acid Kit (half-micro test) (kit code 11383175001). FFA detection limit was 50 µmol/L (in-house data), intra-assay CV was 12.5% at 122 µmol/L, 4.5% at 466 µmol/L & 5.0% at 1244 µmol/L.

2.5. Genotyping

The exon3-deleted GHR gene genotyping (fl/fl, d3/fl and d3/d3) were tested by simple multiplex PCR assay on isolated DNA with primers G1, G2 and G3 (GenBankTM accession number AF155912) as described by Pantel et al. [22]. Differentiation between the exon 3-retention and the 3-deletion isoforms were based on 935-bp band (fl-GHR) and 532-bp fragment (d3-GHR), respectively. The distribution of the d3-GHR genotypes did not deviate significantly from Hardy-Weinberg equilibrium.

2.6. Calculations

BMI was computed using the formula: BMI = weight (kg)/height^2 (m). SDS were derived for BW, BL, height, weight, BMI and IGF-I using normal reference materials [23–25]. Age and gender corrected SD-scores for IGF-I were calculated from our large reference dataset as previously published [26,27]. IS was estimated by the homeostatic model (HOMA, http://www.dtu.ox.ac.uk/homacalculator/index.php) from C-peptide values and fasting glucose (average of −10, −5, and 0 min samples) assessed by IVGTT. Insulin secretion was determined as first phase insulin secretion index (acute insulin response, AIR) and calculated as the area under the curve (AUC) of the insulin response and above the baseline in the first 10 min of IVGTT. Disposition index (DI) was provided as the product of IS and insulin secretion. Non-HDL cholesterol (mmol/L) was calculated as total cholesterol (mmol/L) minus HDL cholesterol (mmol/L).

2.7. Statistics

The variables were examined for normal distribution and log-transformed to normality if necessary. Data are presented as mean and standard deviation (SD) or median and interquartile range. The anthropometric variables are presented as SDS to evaluate males and females together. The three genotypes (d3/d3-GHR, d3/fl-GHR and fl/fl-GHR) were compared by an ANOVA *t*-test or Kruskal-Wallis test where appropriate. Children carrying at least one d3-allele were grouped together (d3-GHR group) and compared with children homozygous for the full-length allele (fl/fl-GHR group). Differences between the d3-GHR group and the fl/fl-GHR group were analysed by independent-samples *t*-test or non-parametric Mann Whitney test where appropriate. The metabolic measurements were adjusted for BMI (SDS), age and gender and only the adjusted p values were presented (P trends). Measurements of fat mass were adjusted for height (SDS), age and gender. P values < 0.05 were considered significant. All statistical analyses were performed using the statistical package PASW (version 22; SPSS Inc., Chicago, IL).

2.8. Safety parameters

Safety was evaluated on all children at each visit throughout the study and recorded on a “standard Adverse Event Form”. Adverse events were informed to the Health Authorities and Independent Review Boards/Independent Ethics Committees (IRBs/IECs) in

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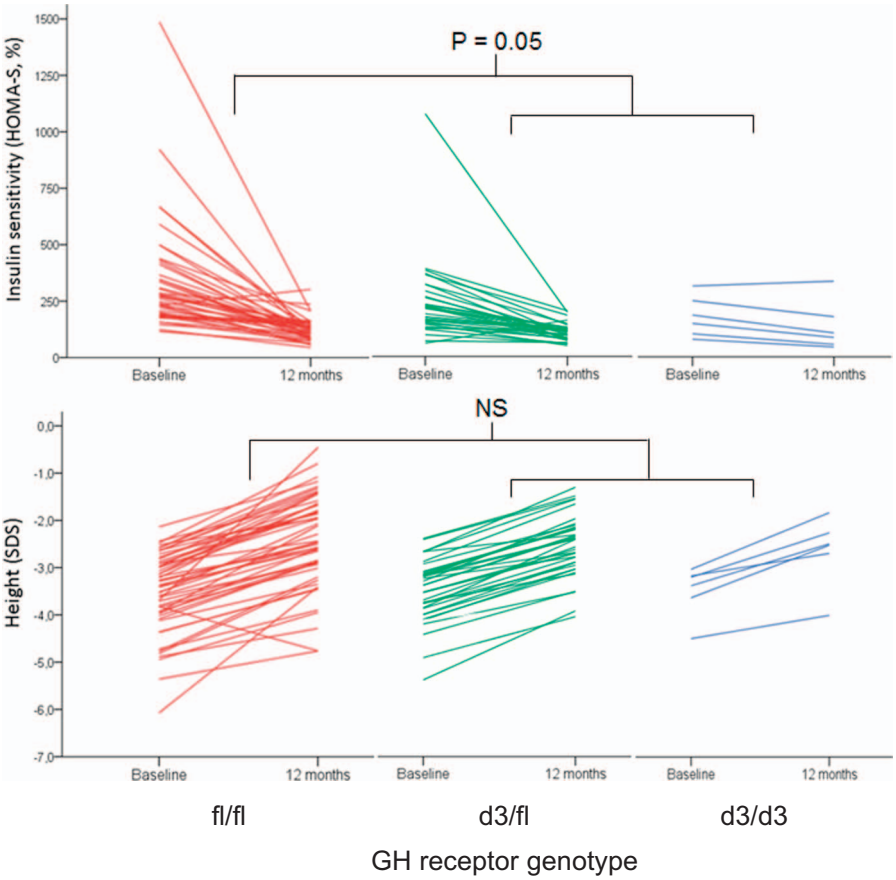

Fig. 2. Individual measurements of change in insulin sensitivity and height:
Individual measurements of insulin sensitivity (HOMA-S, %) (top row) and height (SDS) (bottom row) at baseline and after one year of high-dose GH therapy in short SGA children according to the three genotypes: fl/fl (red), green (d3/fl) and d3/d3 (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

accordance with national laws and regulations.

2.9. Ethical aspects

The study was performed according to the Helsinki II declaration. Approvals were obtained by the Ethical Committee or institutional review board and from national drug authorities at each study centre. Written informed consent was obtained from the parents or guardians of each child included in the NESGAS study.

3. Results

In the entire cohort (N = 96, 57 males) 52 (54.2%) children were homozygous for the full-length allele GHR (fl/fl), 37 (38.5%) were heterozygous for the d3-allele (d3/fl) and 7 (7.3%) were homozygous for the d3-allele (d3/d3) (Table 1).

At baseline carriers of the d3-allelehad significantly lower IS (median, 25–75 percentile) (d3/d3 (175.5%, 101.2–279.7), d3/fl (226.7%, 157.6–319.9)) compared to non-carriers (fl/fl 269.7%, 185.1–356.7) (Table 2, Fig. 1). The d3-GHR group had significantly higher concentrations of glucose (mean (SD)) (d3/d3 4.3 mmol/L (0.2) and d3/fl 4.5 mmol/L (0.6) vs. fl/fl 4.2 mmol/L (0.7)), C-peptide (d3/d3 274.2 pmol/L, 174.1–475.2 and d3/fl 224.9 pmol/L, 154.4–291.1 vs fl/fl 185.1 pmol/L, 137.7–253.9) (Table 2), and insulin (d3/d3 18.1 pmol/,15.0–29.0, d3/fl 19.2 pmol/L, 11.1–32.7 vs fl/fl 13.7 pmol/L, 9.3–20.8) (Table 2, Fig. 1). There were no differences in DI or insulin secretion between the genotypes (Table 2). Among children homozygous for the d3-allele (d3/d3) there was a trend towards higher IGF-I levels, but this did not reach statistical significance (Table 2). Total cholesterol, HDL-cholesterol, LDL-cholesterol, non-HDL cholesterol and FFA did not differ between the genotypes (Table 2).

The change in IS after one year of high-dose GH treatment was significantly larger in the fl/fl-GHR group (− 210.9% (232.2)) compared to the d3-GHR group (d3/d3–47.9% (38.5), d3/fl − 135.6% (166.7)) (Table 2, Fig. 2), resulting in similar levels of insulin sensitivity in both groups after one year of GH therapy (Table 2). There was a greater change in DI in the fl/fl-GHR group (p = 0.09), although the difference was not statistical significant (Table 2) after one year of GH therapy. The remaining metabolic measurements and lipid profiles did not differ between the genotypes after first year of GH therapy (Table 2).

Spontaneous growth from birth to baseline was significantly greater in the d3-GHR group (d3/d3 0.8 SDS (1.8), d3/fl 0.5 SDS (1.2)) compared to the fl/fl-GHR group (− 0.5 SDS (2.0)) (Table 1). BL and BW were lower in the d3-GHR group, although the differences were not statistical significant (Table 1). GA did not differ between the three genotypes (d3/d3 35.7 weeks (5.1). d3/fl 34.5 weeks (4.0) and fl/fl 35.8 weeks (4.1), p = 0.27). Following one year of high-dose GH treatment there were no significant differences in growth response (Fig. 2) or the other anthropometric measurements between the genotypes (Table 1). Total fat mass and the distribution of fat mass were similar among the genotypes at both baseline and after one year of GH therapy (Table 1). The metabolic variables were adjusted for BMI, age, gender and insulin concentration which did not alter the results (Table 2).

4. Discussion

In this cohort of short, prepubertal children born SGA treated with high-dose GH during the first year of therapy, we found that children carrying the common exon-3-deleted GHR gene polymorphism had spontaneous growth and lower insulin sensitivity resulting in increased levels of glucose, insulin and C-peptide at baseline compared to children homozygous for the full-length allele. During the first year of high-dose GH treatment there was a significantly larger change in IS among carriers of the full-length allele compared those carrying the d3-allele.

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There were no other significant differences in growth response, metabolism or body composition between the GHR genotypes after one year of GH therapy.

Growth, metabolism and fat mass are closely related both before and during GH treatment in short SGA children. The possible metabolic consequences of an increased intra-cellular signalling associated with the d3-GHR during GH treatment in children with short stature have only been investigated to a smaller extent. Two studies found that there was no significant difference in IS between the GHR genotypes in both untreated and GH treated SGA children [15,20]. In the current study we found that carriers of the d3-allele at baseline had significantly lower insulin sensitivity and increased levels of glucose and C-peptide reflecting a non-significant compensatory increase in insulin secretion. There were no significant differences in insulin secretion and fasting insulin between genotypes and the decrease in DI was not significant. In the PREDICT study children with mild GH deficiency (GHD) who were carriers of the d3-allele had significantly increased levels of insulin and insulin resistance during the first month of GH therapy compared to carriers of the full-length allele [19]. However, in the same study there was no influence of genotype among children with severe GHD. In the NESGAS study we found that the children in the d3-GHR group had significantly lower IS at baseline, but after one year of high-dose GH there were equal levels in IS between the genotypes because the fl/fl group had a greater decline. The changes in insulin sensitivity in children carrying the d3-GHR genotype may indicate that they have a greater sensitivity to the lipolytic effects of GH leading to an increased insulin resistance. However, the changes in glucose metabolism associated with the genotype were modest compared to effect of GH treatment. These findings are in line with studies on adult patients. In a cohort of patients with acromegaly the d3-GHR polymorphism was associated with decreased IS [28] and in another study diabetes was reported to be more prevalent among acromegalic patients carrying the d3-allele [29]. However, data are conflicting [30,31] and several studies of acromegalic and GHD adult patients have failed to find any association between the d3-GHR polymorphism and the prevalence of diabetes [30,32–34].

Former meta-analyses concluded that the d3-GHR polymorphism increased first year growth response to GH treatment in children with short stature [16,17]. Dos Santos et al. [8] demonstrated that children carrying the d3-alelle had an increased growth response to GH treatment during the first two years of treatment compared to children carrying the fl-allele homodimer. The increased growth response has been confirmed in other studies of children born SGA [9,10] but controversy exist [11–13,15]. In the current study we found no effect on the growth response after one year of high-dose GH therapy. The varying results of impact of the d3-GHR polymorphism on growth response to GH therapy may reflect the use of different GH doses. Several studies examining children treated with standard European GH dose (ranging from 30 to 40 μg/kg/day) confirmed the link between increased first year growth response and the d3-GHR polymorphism [9,10,35–37], while studies investigating cohorts of children treated with a higher dose GH (66 μg/kg/day) failed to confirm the association [13], including our current study (67 μg/kg/day). A meta-analysis of short children concluded that the association between genotype and growth response was more pronounced at lower doses of GH and at older age [17] but in a comparable meta-analysis the authors failed to observe similar effects of GH dose [16].

In the current study we found that spontaneous growth before start of GH treatment was increased among the children in the d3-GHR group. This is consistent with previous findings among healthy SGA children [38], but existing data are conflicting [10,11]. Furthermore, there was a trend towards higher IGF-I levels at baseline among children homozygous for the d3-allelle. Increased concentrations of IGF-I among the d3/d3-GHR carriers may also reflect an increased GHR signalling through the d3-GHR polymorphism as demonstrated by Dos Santos et al. [8]. The greater GHR sensitivity among d3-allele carriers has been confirmed in adult cohorts where acromegalic [33,39] and GHD patients [40] carrying the d3-allele required lower doses of the GHR antagonist pegvisomant and GH, respectively. However, the reported results are inconsistent [32,34] and a recent meta-analysis concluded that d3-GHR polymorphism did not affect pegvisomant response or dosing in acromegaly patients [41].

Since GH has an IGF-I independent lipolytic and anabolic effect it may be speculated that an increased GHR signalling could affect lipolysis. However, a Swedish study found that the d3-GHR polymorphism was associated with increased central adiposity in the general adult population [42]. In the present study, the d3-GHR polymorphism did not have a significant impact on total fat mass or the distribution of fat either before or after one year of high-dose GH treatment. Additionally, the d3-allele did not affect the lipid profiles in the current study, which is line with the findings in a cohort of acromegalic patients [28]. However, a study of children with GHD found that carriers of the d3-allele who had severe GHD had a significantly smaller decline in LDL-cholesterol compare to non-carriers after one month of GH therapy, whereas no significant differences between the genotypes were found among children with mild GHD [19]. We also did not find any difference in FFA levels, possibly because of the higher insulin levels in the d3-GHR group.

In this multicentre study of short, prepubertal SGA children treated with high-dose GH during the first year of therapy, we found that the d3-GHR polymorphism was associated with increased spontaneous growth, lower insulin sensitivity and increased concentrations of glucose, insulin and C-peptide at baseline compared to the full-length allele homodimer. These associations may reflect a greater responsiveness to GH among carriers of the d3-allele leading to effects on both growth and metabolism. However, the GHR genotype did not influence the GH-induced changes in height, but significantly affected changes in insulin sensitivity during high-dose GH treatment.

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MANUSCRIPT 6

Genetic influence on the associations between IGF-I and glucose metabolism in a cohort of elderly twins

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Abstract

Objective: IGF-I may be a marker of later metabolic and cardiovascular disease. The interactions between IGF-I and glucose metabolism are multifactorial, and there is potential confounding from several secondary effects. In this study, we examined the interaction between IGF-I and glucose metabolism in a large cohort of clinically well-characterized elderly twins.

Design: A total of 303 twin pairs of the same gender (606 twins) were included in the study; 125 monozygotic and 178 dizygotic twin pairs.

Methods: A clinical examination including a standard oral glucose tolerance test (OGTT) and anthropometric measurements was performed.

Results: The heritability estimates were high for IGF-I and IGFBP-3 (h_2: 0.65 (95% CI: 0.55–0.74) and 0.71 (0.48–0.94), respectively) and for insulin secretion ($h_2 = 0.56$, $P < 0.0001$), whereas the heritability estimates for insulin sensitivity were low ($h_2 = 0.14$, $P = 0.11$). In a multiple regression analysis (adjusting for age, gender and twin status), there was a negative association between IGF-I and insulin sensitivity (B: −0.13, SE 0.03, $P < 0.0001$) and IGF-I and disposition index (B: −0.05, SE 0.02, $P < 0.001$) in the entire cohort of 606 twins. The associations between IGF-I and both DI and HOMA-S did not differ between the DZ and MZ twins. Forty-five twin pairs were discordant for T2D, but the discordant twins had similar concentrations of IGF-I or IGFBP-3.

Conclusions: There was a high heritability for IGF-I and IGFBP-3, but a low heritability for insulin secretion and insulin sensitivity in a group of elderly twins. In addition, we found a strong negative relationship between IGF-I and insulin sensitivity, which did not seem to be strongly genetically determined.

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Introduction

There is emerging evidence that adult height may be a predictor of later disease (1). Around 80% of the variability of height is genetically determined and GWAS studies have found hundreds of common genetic variants that may determine height (2). However, several environmental factors such as the fetal environment and lifestyle factors also have a great impact on adult height. Insulin and insulin-like growth factor-I (IGF-I) signaling pathways have been suggested to be involved in the association between height and later disease. IGF-I mediates many

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of the actions of growth hormone (GH) on growth, development and cell differentiation, but in addition, IGF-I has distinct metabolic actions (3). Epidemiological studies suggested that lower concentrations of IGF-I may be a biomarker of development of cardiovascular disease (CVD) (4) and diabetes (5, 6, 7) and other studies found that higher levels of IGF-I were associated with risk of cancer (8). However, recent studies and a large meta-analysis suggest a U-shaped association between IGF-I and all-cause mortality (9, 10) as well as cancer (10), CVD (11) and diabetes (12, 13) meaning that both subjects with low and high concentrations of IGF-I may have an increased risk of disease. Hepatic insulin plays a role in promoting IGF-I generation (5, 6) and the U-shaped association between IGF-I and type 2 diabetes may be due to the changes of beta cell function and thereby insulin secretion over time. Thus, a person with insulin resistance have increased levels of insulin in the portal circulation, and this may drive an increase in hepatic IGF-I secretion, whereas at a later stage when this person develop type 2 diabetes, the insufficient beta cell function may have the reverse effect on IGF-I. Many of the large epidemiological studies include patients with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (14).

Former twin studies have revealed that the variation of IGF-I and the binding protein IGFBP-3 levels are highly genetically determined with heritability estimates of 63% and 60%, respectively (15, 16, 17). Studies of elderly twins showed similar IGF-I levels in the twin pairs (18), which suggests that IGF-I concentrations in each individual may follow a genetically determined trajectory throughout life. In contrast, heritability estimates for the binding protein IGFBP-1, which is regulated by insulin levels, were 36% and 48% for insulin, which suggests that IGFBP-1 and insulin levels may be determined by environmental factors such as lifestyle more than genetic factors (15, 19).

There is increasing evidence that the GH/IGF-I axis plays a role in normal glucose homeostasis, which may be determined by common genetic pathways. In a population-based cohort, a polymorphism in the *IGF1* gene was associated with lower height, lower birth weight, lower serum levels of IGF-I and an increased risk of type 2 diabetes and myocardial infarction (20, 21), but controversy exists (22). In addition, a meta-analysis of genome-wide data found that a SNP near the *IGF1* gene, that may influence IGF-I expression levels, was associated with fasting insulin and insulin resistance (23).

The strong genetic influence on IGF-I levels throughout life and the associations between genetic variations in the *IGF1* gene and insulin could indicate that shared genetic influences are involved in the relation found between IGF-I and glucose metabolism. However, the interactions between IGF-I and insulin sensitivity and secretion are considered multifactorial, and there is potential confounding from numerous secondary effects.

The aim of this study was to assess genetic vs environmental influences on the association between IGF-I parameters, insulin secretion and insulin sensitivity in a large cohort of elderly twins.

Subjects and methods

Subjects

The twins who participated in this study were identified through the Danish Twin Register (24), details about the sampling of the cohort have been published in detail previously (25). A total of 303 twin pairs of the same gender (606 twins) were included in the study; 125 monozygotic (men: 62, women: 63) and 178 dizygotic twin pairs (men: 86, women: 92) participated in the clinical examination including a standard oral glucose tolerance test (OGTT) and anthropometric measurements (previously reported in detail 25, 26, 27, 28). The mean age among the twin sample was 67.0 years (range 55–74 years). Zygosity status was established by the similarity method where twins were asked about physical similarity and mistaken identity (29).

As previously described, 79 subjects had T2D defined by either 120-min glucose >11.1 mmol/L or history of diabetes diagnosed at 40 years or older who did not receive insulin treatment within the first year of onset. Of these 79 subjects, 43 (54%) were diagnosed in the study and 36 had known T2D (mean duration of T2D was 6 year (range: 1–14) for DZ and 8 year (range: 1–19) for MZ). A total of 71 subjects had a 120-min glucose >11.1 mmol/L in the study. In addition, 129 subjects had impaired glucose tolerance (IGT) defined by 120-min glucose >7.8 and <11.1 mmol/L and 398 subjects had a normal glucose tolerance (NGT) defined by 120-min glucose <7.8.

The protocol was approved by the regional ethics committees and the study was conducted according to the principles of the Helsinki declaration.

Methods

Weight and height were measured with the subject in light clothing without shoes, and body mass index (BMI) was calculated (weight (kg)/height (m^2)). Waist

circumference was measured using a soft tape midway between the lower ribs and the iliac crest on the standing subjects. Hip circumference was measured over the widest part of the gluteal region, and the waist/hip ratio (WHR) was calculated accordingly.

Subjects underwent a standard 75 g oral glucose tolerance test (OGTT) after a 10- to 12-h overnight fast. Peripheral venous blood was taken before oral glucose ingestion and 30 min and 120 min later.

Plasma glucose concentrations were analyzed by the glucose dehydrogenase oxidation method. Plasma insulin concentrations were measured using a two-site, two-step, time-resolved immunofluoremetric assay (DELFIA) as described previously (26, 28). Intra-assay coefficients of variation were 3.6–4.3% and the inter-assay coefficients of variation were 1.7–3.4% for plasma insulin.

Plasma insulin-like growth factor-I (IGF-I) and the binding proteins IGFBP-1 and IGFBP-3 were analyzed using the commercially available immunoradiometric assays (Diagnostic Systems Laboratories, Webster, TX, USA). The inter-assay coefficients of variation for total IGF-I, and IGFBP-3 were 8.2 and 1.9%, respectively and the intra-assay coefficients of variation for total IGF-I and IGFBP-3 were 3.4 and 3.9%, respectively. For IGFBP-1, the intra-assay coefficient of variation was 3.4% while the inter-assay coefficient of variation was 8.1%.

Calculations

BMI was computed using the formula, weight (kg)/height (m^2).

Insulin sensitivity (IS) was estimated from fasting glucose (average of −10, −5 and 0-min samples) and insulin values by homeostatic model (HOMA) using the HOMA 2 calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php). Insulinogenic index was calculated from the OGTT as the ratio of the increment in insulin concentration to the increment in glucose after 30 min (ins30′-ins0′/glu30′-glu0′). Disposition index (DI) provided an estimate of insulin secretion adjusted for the degree of IS and was calculated as the product of IS and insulinogenic index. Matsuda index provided an approximation of whole-body insulin sensitivity from OGTT using fasting glucose and insulin and mean values of glucose and insulin ($ISI_{Matsuda} = 1000/\sqrt{G_0 I_0 G_{mean} I_{mean}}$).

Impaired glucose intolerance (IGT) was defined according to the current WHO criteria: fasting venous plasma glucose concentration <7.8 mmol/L and a 120 min post OGTT plasma glucose between 7.8 and 11.1 mmol/L (ref). Type 2 diabetes (T2D) was defined by either (1) diagnosis of diabetes at the age of 40 years or older and current treatment with antidiabetic agents or diet or (2) meeting the WHO criteria; a fasting venous plasma glucose concentration ≥7.8 mmol/L and/or 2 h post OGTT venous glucose concentration ≥11.1.

Statistical analysis

Heritability (h^2) expresses the proportion of total variation of a trait attributable to genetic variation and can be estimated by comparing the correlation of a given phenotype within monozygotic twin pairs with the similarity within dizygotic twin pairs. To calculate interclass correlations we computed Pearson correlations for all pairs of variables that were reasonably approximated by the normal distribution and also compared with Spearman rank correlations.

To study this relationship between variables, we used a marginal linear regression using generalized estimating equations for MZ and DZ twins separately and combined the estimates to adjust for the possibility that MZ and DZ twins have different correlations. When combining the estimates for MZ and DZ twins, we first checked that estimates were not significantly different; this test is equivalent to comparing the intraclass correlation between the considered variables and is a test for a shared genetic component between the variables.

We used R and the Mets package for twin modeling.

Results

Interclass correlations

Clinical characteristics of the entire cohort divided according to gender are presented in Table 1. Males had significantly higher IGF-I and IGFBP-3 levels ($P < 0.0001$), which is in accordance with former studies (Table 1). Males had significantly lower IGFBP-1 levels ($P < 0.001$) and fasting glucose was significantly higher ($P < 0.0001$), whereas there was no difference in fasting insulin levels or insulin sensitivity determined by HOMA-S (Table 1).

All examined twin pairs (MZ 125; DZ 178) were included in the calculation of interclass correlations. The interclass correlations for IGF-I were $r = 0.65$ for MZ and $r = 0.33$ for DZ (Fig. 1A and Table 2) and the interclass correlations for IGFBP-3 were $r = 0.83$ for MZ and $r = 0.47$ for DZ (Fig. 1B and Table 2). The difference in interclass

Table 1 Clinical characteristics of the 606 twins divided according to gender. Data are presented as median (25–75th percentile)

	Women ($n=309$)	**Men** ($n=292$)
Age (years)	67.3 (65.6–69.8)	67.3 (65.0–69.4)
BMI (kg/m^2)	25.5 (22.5–28.6)	25.8 (23.8–28.0)
Waist-to-hip ratio	0.80 (0.77–0.85)	0.94 (0.90–0.98)
Fasting glucose (mmol/L)	5.6 (5.2–6.1)	5.8 (5.5–6.3)
Fasting insulin (pmol/L)	39.0 (26.0–55.0)	38.5 (28.0–54.3)
HOMA-S (%)	132.5 (94–200)	132.9 (91–186)
Disposition index (10^4*pmol*min)	8397 (5090–14359)	8053 (5209–117599)
IGF-I (nmol/L)	16.5 (13.1–21.0)	18.9 (15.5–23.9)
IGFBP-3 (mg/L)	3.1 (2.6–3.6)	3.4 (2.9–3.9)
IGFBP-1 (ng/mL)	36.5 (26.2–50.3)	32.6 (21.8–42.9)

correlation for IGF-I and IGFBP-3 between the two zygosities were highly significant ($P<0.0001$) and the heritability estimates were very high; $h_2=0.65$ for IGF-I and $h_2=0.71$ for IGFBP-3 (Table 2). There was also high heritability estimates for insulin secretion determined by insulinogenic index $h_2=0.56$, $P<0.0001$ (Table 2). In contrast, no significant differences in interclass correlations were found for IGFBP-1 (Fig. 1C and Table 2) and insulin sensitivity (HOMA-S) (Fig. 1D and Table 2) and indicating a relatively small genetic contribution to variation of these metabolic variables. The interclass correlations for the interaction between insulin secretion and insulin sensitivity determined by DI were $r=0.44$ for MZ and $r=0.22$ for DZ ($P<0.0001$) and the heritability estimate was $h_2=0.44$ (Table 2).

Birth weight (BW) was available in 123 twin pairs (52 MZ and 71 DZ twins), but there was no information on gestational age. The interclass correlations for BW were $r=0.66$ for MZ and $r=0.55$ for DZ leading to a low heritability ($h_2=0.21$).

All calculations of heritability were adjusted for age and gender for all variables, this did not alter any of the results and the unadjusted results are therefore presented.

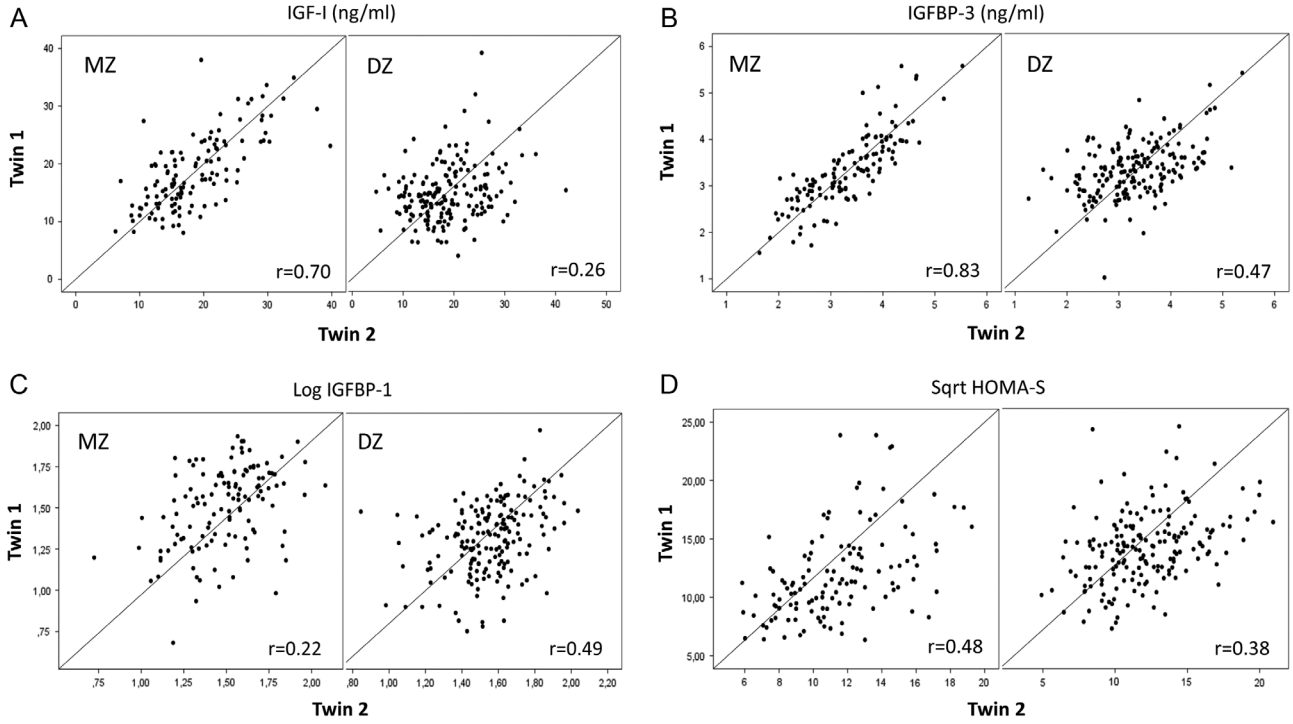

Figure 1
Correlations in serum concentrations of IGF-I (A), IGFBP-3 (B), IGFBP-1 (C) and HOMA-S (D) for MZ and DZ pairs of twins.

Table 2 Interclass correlations and heritability estimates for anthropometric and metabolic variables in monozygotic ($n=125$) and dizygotic ($n=178$) twin pairs. Data are presented as interclass correlation or heritability estimates (95% confidence interval).

Phenotype	Interclass correlation		*P* value	Heritability
	MZ	DZ		2 (rmz-rdz)
IGF-I	0.65 (0.54–0.73)	0.33 (0.27–0.37)	<0.0001	0.65 (0.55–0.74)
IGFBP-3	0.83 (0.77–0.87)	0.47 (0.35–0.57)	<0.0001	0.71 (0.48–0.94)
IGFBP-1	0.38 (0.24–0.31)	0.38 (0.23–0.50)	0.85	0.02 (−0.34–0.37)
Insulin secretion	0.56 (0.43–0.68)	0.28 (0.22–0.34)	<0.0001	0.56 (0.44–0.68)
Insulin sensitivity (HOMA-S)	0.47 (0.34–0.54)	0.40 (0.27–0.32)	0.11	0.14 (−0.19–0.47)
Disposition index	0.44 (0.30–0.56)	0.22 (0.15–0.28)	<0.0001	0.44 (0.31–0.57)

Concordance

In the entire cohort of MZ twins (125 twin pairs), 45 twin pairs ($n=90$ twins) were discordant for T2D (i.e. one twin with T2D and the other twin with non-diabetic glucose tolerance). As expected, there were significant differences in the parameters related to glucose metabolism outcomes between twin pairs discordant for T2D (data not shown). In contrast, there were no significant differences in IGF-I concentrations; median 16.65 (25–75% range; 14.9–22.3) vs 17.17 (14.0–25.1) $P=0.84$ and IGFBP-3 concentrations; median 3.33 (2.9–3.9) vs 3.29 (2.7–3.9) between those with T2D and the non-diabetic twins, respectively. The group of non-diabetic twins included twins with IGT, however, excluding the group with IGT did not alter the results.

Associations between IGF-I and glucose metabolism

In the entire cohort of twins, IGF-I was positively correlated to fasting insulin (β: 0.20, SE 0.23, $P<0.0001$) and fasting glucose (β: 0.14, SE 0.08, $P<0.0001$) and negatively correlated to insulin sensitivity (HOMA-S) (β: −0.21, SE 0.23, $P<0.0001$) (Fig. 2) and DI (β: −0.14, SE 0.41, $P=0.001$), whereas there was no correlation between IGF-I and insulin secretion (insulinogenic index) (β: −0.03, SE 0.38, $P=0.52$). In addition, IGF-I was positively correlated to IGFBP-3 (β:0.51, SE 0.67, $P<0.0001$) and negatively correlated to IGFBP-1 (β: −0.38, SE 0.21, $P<0.0001$).

When adjusting the analyses for age, gender and twin status in a multiple regression analysis IGF-I remained negatively associated to insulin sensitivity determined by HOMA-S (B: −0.13, 95%CI SE 0.03, $P<0.0001$), the Matsuda index (B: −0.11, SE 0.03, $P<0.0001$) and DI (B: −0.05, SE 0.02, $P<0.001$) in the entire cohort of 606 twins. In contrast, there was no association between IGF-I and insulin secretion determined by insulinogenic index ($P=0.45$). The associations between IGF-I and insulin, glucose, HOMA-S and DI did not differ between the DZ and MZ twins. All the results were consistent after excluding the subjects with IGT and T2D.

Discussion

In this large cohort of elderly twins, we confirmed a strong heritability of IGF-I and IGFBP-3 as found in former studies on both newborn and elderly twins (15, 16, 30) and a strong heritability for insulin secretion and disposition index, whereas the heritability for insulin sensitivity and IGFBP-1 were small and non-significant. In the 45 twin pairs, who were discordant for type 2 diabetes, metabolic parameters varied greatly, but the concentrations of IGF-I were similar among the twin with T2D and the twin without. However, in the entire cohort of twins, IGF-I was negatively associated with insulin sensitivity (adjusted for age, gender and twin status), which did not differ between MZ and DZ twins. The lack of effect of zygosity on this relationship could indicate that alterations in IGF-I levels associated with abnormalities in glucose metabolism may be mediated primarily by environmental rather than genetic factors. However, a limitation of our twin study is that the study was a cross-sectional study and therefore cannot give evidence of causality but can generate and confirm hypotheses. Furthermore, our study included participants with defined differences in glucose metabolism at the time of the study (NGT, IGT and T2D twins). The relation between levels of IGF-I and insulin sensitivity does not necessarily imply causation, it may instead be a possible 'reverse causation' in which the levels of IGF-I may reflect alterations caused by insulin resistance or by a compensatory mechanism.

IGF-I and insulin share significant structural homology and downstream pathways, their receptors are homologous and form heterodimers, which can bind both ligands. IGF-I secretion is mainly regulated by GH stimulation and nutrition, but findings in prospective studies determined that hepatic insulin plays a role in promoting IGF-I generation (5, 6, 31, 32). However, in

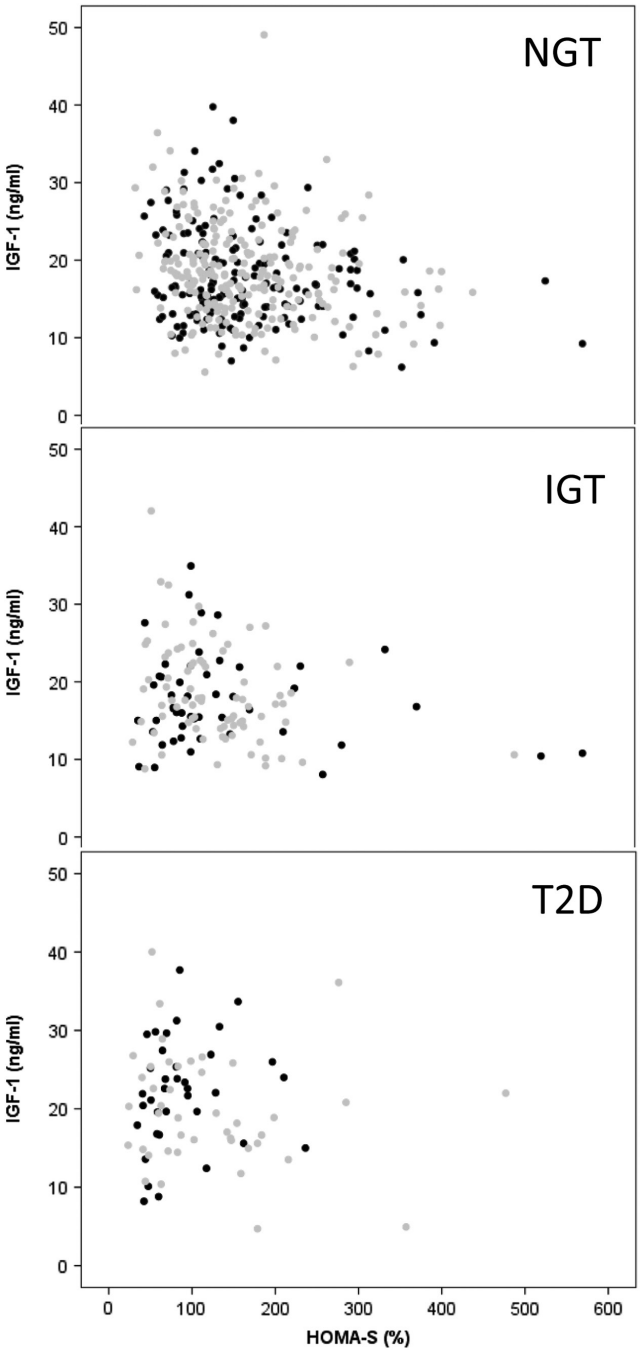

Figure 2
Association between IGF-I and HOMA-S in twins with normal glucose tolerance, NGT (top panel), impaired glucose tolerance, IGT (middle panel) and type 2 diabetes, T2D (lower panel). Black dots are monozygotic twins and gray dots are dizygotic twins.

this cohort of twins, there were no significant differences in IGF-I concentrations between twins discordant for type 2 diabetes and the positive association between IGF-I and insulin sensitivity remained even after excluding twins with IGT and type 2 diabetes. Furthermore, IGF-I circulates in the blood bound to six high-affinity binding proteins (IGFBPs). Insulin controls the concentration of IGFBP-1 because it downregulates the hepatic production of IGFBP-1 (33) and therefore increased insulin levels due to insulin resistance will reduce IGFBP-1 levels and thereby increase IGF-I bioavailability. In the current twin study, we found a positive association between IGF-I and fasting insulin but no association between IGF-I and insulin secretion. In contrast, IGF-I was negatively associated with insulin sensitivity, which was somewhat controversial as former studies have shown a positive association between the IGF-I concentration and insulin sensitivity (32, 34). However, a recent cross-sectional study in a large population discovered a U-shaped association between serum IGF-I levels and insulin resistance (14). In addition, analysis of two prospective cohort studies including more than 7000 non-diabetic subjects revealed an association between low IGF-I at baseline and an increased incidence of diabetes at follow-up, but this association became insignificant after adjustment for metabolic markers such as abdominal obesity, hypertension, glucose and dyslipidemia (35). Thus, the interaction between IGF-I and insulin sensitivity is complex and many of the large cohort studies are confounded by including subjects over a large span of age, BMI and metabolic status.

The physiological role of IGF-I on insulin sensitivity, observed from studies on IGF-I therapy revealing that IGF-I improves insulin sensitivity in normal subjects as well as in patients with GHD (36), could be another possible mechanism explaining the negative relation between IGF-I and insulin sensitivity found in our study. Treatment with rh-IGF-I in patients with diabetes improves insulin sensitivity significantly, insulin requirements are reduced and control of glucose and dyslipidemia is generally improved (37, 38). In addition, a former study on healthy non-obese male volunteers selected by IGF-I levels showed that those with IGF-I levels in the lowest quartile of normal distribution had lower fasting insulin levels and greater hepatic insulin sensitivity compared to those in the highest quartile (39). Furthermore, we and others have shown that children born small for gestational age (SGA) who had higher IGF-I concentrations had a lower insulin sensitivity and a poor growth response to rhGH treatment (40, 41). To confirm this association, we used the Mendelian randomization approach in a group of SGA children and found an independent association between alleles coding for insulin sensitivity and first year height velocity and

IGF-I responses to rhGH suggesting a causal link between insulin sensitivity and IGF-I (42).

Metabolic markers may be part of the causal pathway in the relation between IGF-I and diabetes, but it is also possible that IGF-I is truly confounded by obesity because obesity has a major blunting effect on the GH secretion, whereas circulating IGF-I levels will stay unaffected (43). Patients with T2D have a broad range of serum IGF-I concentrations and multiple variables may interact to regulate IGF-I levels such as inflammatory cytokines, decreases in hepatic insulin action due to insulin resistance, concomitant changes in IGF-binding proteins and the effects of obesity on GH secretion. In the current study, we found higher levels of IGF-I in the entire group of twins with T2D compared to those with normal glucose tolerance, whereas there was no difference in IGF-I levels between those with IGT and normal glucose tolerance. When comparing the monozygotic twin pairs discordant of IGT and T2D, they had similar IGF-I levels and there was no difference in BMI between the groups. A large meta-analysis showed a high heritability of 72% for type 2 diabetes (44). However, a recent study of more than 4000 monozygotic twin pairs discordant for BMI revealed that twins with higher BMI had an increased risk of onset of type 2 diabetes, which suggests that environmental factors are important as well (45).

Adult height has been proposed as a predictor of later disease and a large meta-analysis among more than one million people found that increased height was associated with increased risk of cancer but decreased risk of cardiovascular disease (1). The variability of height is mainly genetically determined (2), but several environmental factors such as the intrauterine environment, nutrition and other lifestyle factors may have a great impact on adult height. Insulin and IGF-I signaling pathways have been suggested to play an important role in the relation between height and later disease and previous findings suggest that each individual may follow a genetically determined trajectory throughout life. In our twin study, we confirmed that the high heritability of IGF-I, IGFBP-3 and insulin secretion persist into late in life, which confirms that the circulating levels of IGF-I and insulin may maintain relatively constant throughout life. A better way to study the difference in genetic and environmental factors is to include twin pairs reared apart or together to determine non-shared environmental influences. A Swedish study on 248 pairs of middle aged and elderly twins found that the phenotypic association between IGF-I and insulin and IGFBP-1 were caused by environmental effects (30); however, this study did not examine insulin secretion or insulin sensitivity.

In conclusion, we confirm the high heritability of IGF-I, IGFBP-3 and insulin secretion, which suggests that the levels of IGF-I in each individual may follow a trajectory throughout life, whereas the genetic factors played a smaller role for insulin sensitivity. IGF-I and insulin sensitivity were negatively associated and zygosity did not influence this association, which suggest an important role of environmental factors driving the relationship, but long-term longitudinal studies are needed to explore this in detail.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Increases in Bioactive IGF do not Parallel Increases in Total IGF-I During Growth Hormone Treatment of Children Born SGA

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Background: Some children born small for gestational age (SGA) experience supra-physiological insulin-like growth factor-I (IGF-I) concentrations during GH treatment. However, measurements of total IGF-I concentrations may not reflect the bioactive fraction of IGF-I which reaches the IGF-I receptor at target organs. We examined endogenous IGF-bioactivity using an IGF-I kinase receptor activation (KIRA) assay that measures the ability of IGF-I to activate the IGF-IR in vitro.

Aim: To compare responses of bioactive IGF and total IGF-I concentrations in short GH treated SGA children in the North European Small for Gestational Age Study (NESGAS).

Material and method: In NESGAS, short SGA children (n = 101, 61 males) received GH at 67 µg/kg/day for 1 year. IGF-I concentrations were measured by Immulite immunoassay and bioactive IGF by in-house KIRA assay.

Results: Bioactive IGF increased with age in healthy pre-pubertal children (n = 94). SGA children had low-normal bioactive IGF levels at baseline (-0.12 (1.8 SD), increasing significantly after one year of high-dose GH treatment to 1.1 (1.4) SD, $P < 0.01$. Following high-dose GH, 68% (n = 65) of SGA children had a total IGF-I concentration >2SD (mean IGF-I 2.8 SDS), whereas only 15% (n = 15) had levels of bioactive IGF slightly above normal reference values. At baseline, bioactive IGF (SDS) was significantly correlated to height (SDS) (r = 0.29, $P = 0.005$), in contrast to IGF-I

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(SDS) (r = 0.17, *P* = 0.10). IGF-I (SDS) was inversely correlated to delta height (SDS) after one year of high-dose GH treatment (r = -0.22, *P* = 0.02).

Conclusion: In contrast to total IGF-I concentrations, bioactive IGF stayed within the normal reference ranges for most SGA children during the first year of GH treatment. (***J Clin Endocrinol Metab* 105: 1–8, 2020)**

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Insufficient catch-up growth in some children born small for gestational age (SGA) may result in a low final height. Growth hormone (GH) treatment has a well-documented overall beneficial effect on final height in children born SGA and was approved for treatment of SGA children with persistent short stature in the EU in 2004 (1, 2). Nevertheless, the growth response to GH treatment among SGA children is characterized by considerable variability (3), and some SGA children experience supra-physiological serum insulin-like growth factor-I (IGF-I) concentrations during treatment (4).

Long-term safety and mortality in patients treated with GH during childhood is an ongoing concern, but the debate has been characterized by conflicting data. Studies in large epidemiological cohorts of healthy adults have shown an association between elevated IGF-I levels and an increased risk of cancer and all-cause mortality (5–7). However, no studies have been able to establish a link between elevated IGF-I levels during GH treatment in childhood and increased morbidity or mortality later in life. Though, there is still a need for follow-up studies of long-term risk of disease after GH treatment in childhood. In the majority of clinical guidelines for the approved indications of GH treatment in childhood (eg, SGA, Turner Syndrome and Prader-Willi Syndrome) it is recommended that serum IGF-I concentrations are kept within the normal reference range during GH treatment (2, 8, 9).

In the bloodstream the majority of IGF-I circulates bound to IGF-binding proteins (IGFBPs) while approximately 1% circulates as unbound, free IGF-I (10). The IGFBPs, IGFBP-proteases (eg, pregnancy associated plasma protein A and A2) as well as modifiers of IGFBP protease activity (eg, stanniocalcin 1 and 2) affect the interaction between IGF-I and its IGFBPs, and thereby alter the bioactivity of IGF-I (11). Measurements of the concentration of total IGF-I by immunoassay, whereby IGF-I is stripped from the IGFBPs, do not take the modifying effects of IGFBPs and IGFBP-proteases into account. Therefore, we used the IGF-I kinase receptor activation (KIRA) assay, as this determines the ability of serum IGF-I to phosphorylate and thereby activate the IGF-I receptor (IGF-IR) (12, 13). We believe this gives a biologically relevant estimate of the ability of serum IGF-I to activate the IGF-IR, and hence IGF-bioactivity. Indeed, a discrepancy between bioactive and total levels of IGF-I has been reported in both adults (14) and children (15) and it was reported that in adults, bioactive IGF concentrations correlated better with the diagnosis of GH deficiency (GHD) than total IGF-I levels (16).

In the current study we hypothesized that increased concentrations of IGF-I did not reflect the concentration of bioactive IGF. Accordingly, the aim of this study was to determine a normal reference range for bioactive IGF based on a cohort of healthy children and subsequently to evaluate responses of bioactive IGF and IGF-I concentrations, respectively, with growth and metabolic responses in short GH treated SGA children in the North European Small for Gestational Age Study (NESGAS).

Materials and Methods

Study population and design

NESGAS is a multicenter, randomized, parallel group study (EudraCT2005-001507-19) of GH treatment in short prepubertal children born SGA. The study population and design has been described in detail in previous publications (15, 17–20). In brief, all children received a fixed dose of 67 µg/kg/day of recombinant human GH (Norditropin®, Novo Nordisk, Bagsværd, Denmark) given as a daily subcutaneous injection during the first year of therapy to induce catch-up growth and identify non-responders. Data regarding weight, height and IGF-I using the Immulite assay have previously been published (17). One hundred and one (61 males) children from the NESGAS study were included in the current study. Only data from study entry and during the first year of GH therapy were included. The NESGAS study was performed according to the Helsinki II declaration and approved by the Ethical Committee or Institutional review board and national drug authorities in each study center. Written informed consent was obtained from parents or guardians of each child participating in the NESGAS study.

Laboratory measurements

Serum IGF-I and IGFBP-3 concentrations were determined using a solid-phase enzyme-labelled chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, LA, USA). Standards were calibrated against the WHO NIBSC IRR 87/518. The IGF-I detection limit was 20 ng/mL, inter-and intra-assay coefficients of variation (CVs) were 5.93% and 2.02%, respectively. The detection limit for IGFBP-3 was 500 ng/mL and inter-and intra-assay CVs were 5.23 % and 1.74 % respectively. IGF-I and IGFBP-3 SDS were calculated from our reference data based on serum samples from 1729 healthy children (911 girls) using the same assays (21, 22).

IGFBP-1 and bioactive IGF were measured by in-house assays at Medical Research Laboratories, Aarhus University Hospital, Denmark. IGFBP-1 was measured by an in-house time-resolved immunofluorometric assay (TR-IFMA), with intra- and inter-assay CVs of 5 and 10%, respectively (23). Bioactive IGF was measured using the IGF-I KIRA assay, as described by Chen et al. (24) with modifications (23). The detection limit was 0.1 ng/mL, and the intra-assay CV of samples 12%. The long-term inter-assay CV of a control sample was 20%. Samples were analyzed against a serial dilution of the WHO IGF-I reference preparation 02/254, and results expressed in ng/mL. Care was taken to analyze samples from the same individual in the same assay run. Insulin has a negligible cross-reactivity, whereas IGF-II cross-reacts with 12% (12). To acknowledge this, the output of the KIRA assay has been designated "bioactive IGF."

Reference range for bioactive IGF in a cohort of healthy children

A subpopulation of 150 healthy children (75 males) aged 6 to 11 years from the COPENHAGEN Puberty Study (25, 26) were included. All children were healthy Caucasian, and prepubertal at evaluation. A single non-fasting blood sample was drawn from an antecubital vein between 8 and 12 o'clock. Blood was centrifuged and stored at -20 Celsius until analyses.

Other assays

Plasma insulin and C-peptide levels were measured by a DELFIA assay using kits B080-101 and B081-101 respectively (Perkin Elmer Life Sciences, Turku, Finland) as described in detail previously (4). Plasma glucose and HbA1c were measured locally employing assays routinely used for clinical purposes.

Statistics

Normal distributed data were presented as mean (SD), while non-normal distributed data were presented as median (interquartile range). Age and gender corrected SD-scores for IGF-I measured by Immulite were calculated from our reference data based on samples from 1729 healthy children, as previously published (21, 22). Age and gender corrected SD-scores for bioactive IGF were calculated using a normal reference population of 150 healthy children. Differences between the sexes were compared by independent sample t-test or Mann-Whitney test and ANOVA test or Kruskal-Wallis test when appropriate. A correlation matrix was completed using Spearman non-parametric correlations. *P*-values < 0.05 were considered significant. The statistical analyses were performed using statistical package PASW (version 22; SPSS Inc., Chicago, IL).

Results

Baseline concentrations of bioactive IGF in short SGA children were within the normal range of healthy children (Table 1, Fig. 1), although in the lower part of the reference ranges. Moreover, bioactive IGF concentrations at baseline were significantly lower in boys (-1.4 SDS (-2.7 to -0.2)) (median (25–75 percentile))

Table 1. Baseline and 1 Year Characteristics

					P value	
		All children (N = 101)	Female (N = 40)	Male (N = 61)	Mann-Whitney	T-test
Baseline	**N**					
Age (years)	101	6.2 (1.7)	5.8 (1.3)	6.5 (1.8)		0.06
Weight (SDS)	101	-3.2 (1.0)	-3.2 (1.0)	-3.1 (1.1)		0.30
Height (SDS)	101	-3.4 (0.8)	-3.5 (0.9)	-3.4 (0.7)		0.27
Bioactive IGF-I (µg/L)	94	1.6 (0.7)	1.8 (0.7)	1.5 (0.6)		0.03
Bioactive IGF-I (SDS)	94	-1.2 (1.8)	-0.5 (1.6)	-1.6 (1.8)		0.005
IGF-I (ng/mL)	95	81.9 (62.1–111.0)	91.5 (65.0–118.8)	79.0 (57.2–110.0)	0.25	
IGF-I (SDS)	95	-1.2 (1.2)	-1.2 (1.1)	-1.1 (1.3)		0.87
IGFBP-3 (ng/mL)	95	2870 (2380–3475)	2870 (2580–3560)	2780 (2235–3467)	0.23	
IGFBP-3 (SDS)	95	-0.92 (-1.5 to -0.01)	-0.92 (-1.37-0.12)	-0.93 (-1.73 to -0.04)	0.53	
IGFBP-1 (ng/mL)	95	239 (174–320)	258 (179–350)	233 (171–294)	0.27	
After 1 year of GH therapy						
Age (years)	99	7.3 (1.6)	6.9 (1.4)	7.5 (1.7)		0.10
Weight (SDS)	96	-2.2 (1.0)	-2.2 (0.9)	-2.1 (1.2)		0.70
Height (SDS)	99	-2.4 (0.8)	-2.5 (0.9)	-2.4 (0.8)		0.58
Bioactive IGF-I (µg/L) 1 year	94	2.9 (0.9)	3.0 (0.9)	2.9 (0.9)		0.60
Bioactive IGF-I (SDS) 1 year	94	1.1 (1.4)	1.1 (1.0)	1.1 (1.6)		0.99
IGF-I (ng/mL)	95	312.0 (225.0–394.0)	338.0 (282.8–453.5)	308.0 (217.0–359.5)	0.05	
IGF-I (SDS)	95	2.8 (1.5)	2.9 (1.5)	2.8 (1.5)		0.67
IGFBP-3 (ng/mL)	95	4555 (4055–5082)	4600 (4207–5275)	4475 (3975–5000)	0.13	
IGFBP-3 (SDS)	95	1.25 (0.65–1.86)	1.17 (0.79–1.94)	1.30 (0.62–1.86)	0.77	
IGFBP-1 (ng/mL)	95	165 (134–220)	173 (138–216)	160 (132–234)	0.83	

Data are presented as Mean (SD) or median (interquartile range). Comparison between the sexes was analysed by Independent T-test or Mann-Whitney test when appropriate.

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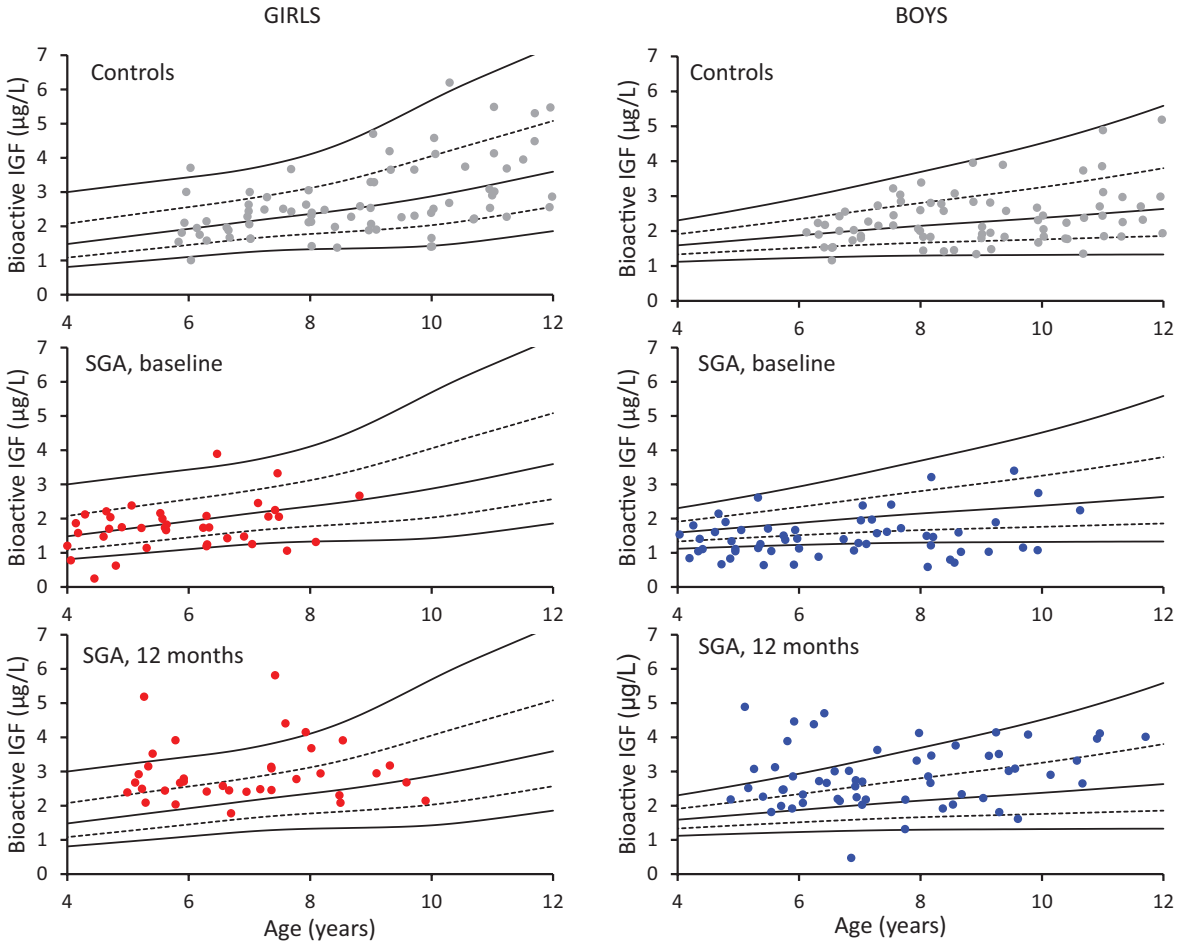

Figure 1. Bioactive IGF concentrations (µg/L), top row represents a normal reference population (grey dots), middle row represents baseline concentration and bottom row represents concentrations at 1 yr. Solid lines reflect mean ± 2 SD, dotted lines reflect -1 SD and +1SD.

compared to girls (-0.2 SDS (-1.4–0.4)) (P = 0.002) (Table 1, Fig. 1). In contrast, there were no significant differences in total IGF-I concentrations (SDS), weight (SDS) or height (SDS) between boys and girls at baseline (Table 1).

Bioactive IGF (SDS), weight (SDS) and height (SDS) did not differ between genders after one year of GH treatment (Table 1, Fig. 1) and thereby a significantly greater change was found in bioactive IGF among boys (+2.7 SDS (1.2–4.6)) than girls (+1.2 SDS (0.5–1.6)) (P = 0.004) after one year of GH treatment (Table 1, Fig. 2a). Changes in total IGF-I concentrations (SDS) (Fig. 2b) and height (SDS) (Fig. 2c) were similar in girls and boys.

After one year of GH treatment only 15% (n = 15) of the children in the NESGAS cohort had levels of bioactive IGF above 2 SD (Fig. 2a) whereas 68% (N = 65) of the children had concentrations of total IGF-I (SDS) above the normal range (>2SD) (Fig. 2b).

Bioactive IGF (SDS) correlated significantly with IGF-I (SDS) (r = 0.35, P = 0.001) and IGFBP-3 (SDS) (r = 0.36, P = 0.001) at baseline (Table 2). Bioactive IGF (SDS) was significantly correlated with height (SDS) and weight (SDS) at baseline (Table 2) but did not correlate to changes in height (SDS) after one year of GH treatment. In contrast, concentrations of IGF-I (SDS) and IGFBP-3 (SDS) were not associated with height (SDS) or weight (SDS) at baseline but correlated inversely with changes in height (SDS) after one year of treatment. Insulin sensitivity determined by HOMA-S were negatively correlated with bioactive IGF (r = -0.29, P = 0.007), IGF-I (r = -0.27, P = 0.01), IGFBP-3 (r = -0.33, P = 0.005) and insulin secretion (r = -0.47, P < 0.001). Furthermore, we observed a significant positive association between HOMA-S and IGFBP-1 as well as with change in height from baseline to 1 year (Table 2). IGFBP-1 was negatively correlated to delta height (SDS) after one year of high-dose GH treatment (Table 2).

The change in bioactive IGF (SDS) from baseline to 1 year was not associated with either height (SDS) at baseline (r = -0.16, P = 0.14) or change in height (SDS) during the first year of treatment (r = 0.12, P = 0.29). In contrast the change in IGF-I (SDS) was correlated with change in height (SDS) during the first year of treatment (r = 0.46, P < 0.0001).

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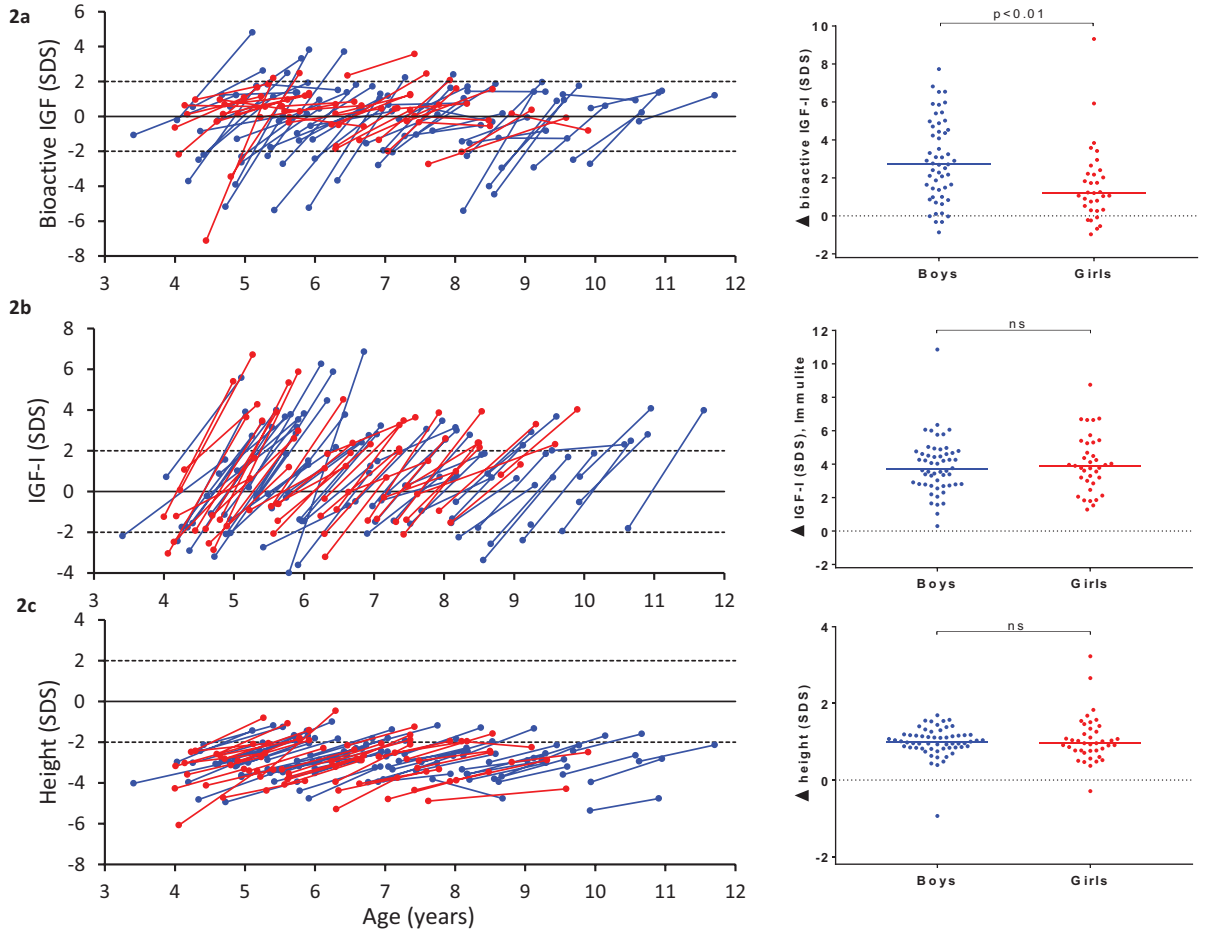

Figure 2. Blue lines are boys and red lines are girls. a: Changes in bioactive IGF during first year of growth hormone treatment, b: Changes in IGF-I SDS during first year of growth hormone treatment, c: Changes in Height SDS during first year of growth hormone treatment.

The molar ratio of IGF-I to IGFBP-3 has been suggested to reflect IGF-I bioavailability, and therefore we also determined IGFBP-3. However, we failed to observe correlations between changes in the IGF-I/IGFBP-3 ratio and changes in bioactive IGF (-0.03, P = 0.8). Furthermore, the ratio correlated neither to baseline height nor height changes (data not shown).

Discussion

In this cohort of SGA children treated for one year with GH, we show that the concentration of bioactive IGF was within the normal range in the majority of children, despite elevated total IGF-I concentrations. On the other hand, total IGF-I concentrations correlated better with the growth response during the first year of GH treatment than bioactive IGF, whereas only bioactive IGF correlated to height and weight at baseline. Insulin sensitivity was related to both bioactive IGF and total IGF-I concentrations as well as the binding proteins and growth response during the first year of treatment. To our knowledge this is the first study to explore bioactive IGF in a cohort of short GH treated SGA children, and we find it of interest that bioactive IGF stays within the normal range during the first year of treatment with GH.

The IGF-I response in vivo is controlled by the IGFBPs that can inhibit as well as stimulate IGF-I mediated effects at the cellular level. The ability of IGF-I to stimulate the IGF-IR is believed to be partly dependent on IGFBP proteolysis, as cleavage of IGFBPs lower their ligand affinity, causing IGF-I to become liberated and hence IGF-IR accessible (27). Many proteases have been identified, but the most thoroughly investigated enzymes as regards liberation of IGF-I and stimulation of growth include PAPP-A, which cleaves IGFBP-3 and IGFBP-5, and PAPPA, which cleaves IGFBP-4. The KIRA assay is a well-recognized assay for direct measurements of the biological active amount of IGF-I (12, 13). Nevertheless, it is still controversial whether activation of IGF-IR in transfected cells in an artificially environment is representative of the endogenous activation of the IGF-IR and whether it can be translated into a biological response in cells in vivo (24). However, our findings of a stronger

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Table 2. Correlation Matrix

	Bioactive IGF (SDS)	IGF-I (SDS)	IGFBP-3 (SDS)	IGFBP-1 (ng/mL)	Height (SDS)	Delta height (SDS)	Weight (SDS)	BMI (SDS)	Insulin sensitivity (HOMA-S)	Insulin secretion
Bioactive IGF (SDS)	1									
IGF-I (SDS)	0.35[c]	1								
IGFBP-3 (SDS)	0.62[c]	0.78[c]	1							
IGFBP-1 (ng/mL)	-0.18	-0.15	-0.19	1						
Height (SDS)	0.29[b]	0.17	0.15	-0.02	1					
Delta height (SDS)	-0.06	-0.22[a]	-0.35[b]	0.22[a]	0.004	1				
Weight (SDS)	0.37[c]	0.20	0.20	-0.03	0.59[c]	0.04	1			
BMI (SDS)	0.18	0.10	0.11	0.10	0.16	0.24[a]	0.80[c]	1		
Insulin sensitivity (HOMA-S)	-0.29[b]	-0.27[a]	-0.33[b]	0.42[c]	-0.08	0.28[b]	-0.22[a]	-0.09	1	
Insulin secretion	0.18	0.13	0.23	0.25[a]	0.17	0.06	0.07	-0.12	-0.47[c]	1

Spearman non-parametric correlations
[a]correlation is significant at the 0.05 level (two-tailed); [b]correlation is significant at the 0.01 level (two-tailed); [c]correlation is significant at the 0.001 level (two-tailed)
All variables are baseline values except Delta Height (SDS) (height (SDS) at 1 yr – height (SDS) at baseline)

correlation between bioactive IGF and height and weight before start of GH treatment suggest that bioactive IGF reflects the biological active IGF-I and the endogenous secretion of GH. On the other hand, the IGF-I concentration was associated with change in height during the first year of treatment with supra-physiological GH doses which may mirror the relation between IGF-I and insulin sensitivity.

In the current cohort the increase in bioactive IGF stayed within the normal range for most of the children whereas the IGF-I concentration was above the normal range in 68% of the children treated with GH for a year. In a Dutch study of GH treated children with Prader-Willi Syndrome, almost all the children had IGF-I SDS levels >2 SD, but only one child had a bioactive IGF concentration above the normal reference (28). That study also revealed that serum bioactive IGF concentrations correlated with neither duration of GH treatment nor GH dose. These findings align nicely with ours, even though the two bioassays are not strictly identical (15, 29). Bioactive IGF has been proposed to be a better screening tool in diagnosing GHD in adulthood than IGF-I concentrations, showing a sensitivity of 82% for bioactive IGF vs. 62% for IGF-I concentration (16). Based on the same cohort of adults with GHD, another study reported that the majority of GHD patients had subnormal bioactive IGF levels despite normalization of IGF-I concentrations during GH treatment and those with normalized bioactive IGF had significantly higher concentrations of IGF-I (14). Furthermore, the authors concluded that bioactive IGF in large part was independent of total IGF-I, as 70–75% of the variation in bioactivity was unexplained by total IGF-I (30). In our study, IGF-I concentrations explained 12% only and in conjunction the two studies indicate that the two measurement represent different entities of the IGF-system. Hence, these results suggest that GH dosing by titration of IGF-I concentrations is effective during physiological GH replacement of GHD children, but less so during pharmacological intervention with GH in non-GHD patients like short SGA children.

Among the present SGA children girls were found to have significantly higher baseline levels of bioactive IGF (SDS) as compared to boys, whereas boys had a significantly greater change in bioactive IGF-I SDS during GH therapy, leading to equal levels after one year. The same pattern was not reflected in the IGF-I concentrations or height. These findings are in accordance with previous findings in children with PWS (15). The gender difference in bioactive IGF during childhood could reflect differences in sensitivity to IGF-I and insulin between boys and girls born SGA and it may be speculated that these differences could influence timing of puberty in GH

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treated SGA children. However, opposed to our data, a former study reported that adult females with GHD appeared to have significantly lower levels of bioactive and total IGF-I compared to men (16), which is in agreement with the generally recognized fact that adult females with GHD are less sensitive to GH and therefore need larger doses of GH in order to normalize IGF-I levels.

IGF-I mediates the growth promoting actions of GH by stimulating cell proliferation and survival. Since IGF-I has mitogenic and anti-apoptotic effects in vitro, the role of IGF-I (and IGF-II) in cancer growth and development has been extensively investigated in both cellular and animal models, but the evidence of a cancerogenic effect in humans is weak (31). However, large epidemiological cohort studies of healthy adults have shown that IGF-I concentrations within the upper reference range is linked to an increased risk of cancer (5, 6). Therefore, the long-term safety and mortality in patients treated with GH during childhood is an ongoing concern and this was reinforced by the first results of a large cross-Europe cohort, the Safety and Appropriateness of Growth Hormone treatments in Europe (SAGhE) study, published in 2012. The SAGhE study was established to examine mortality risk and cancer incidence in a large register study including almost 24 000 people across Europe. The first results from the French register showed an increase all-cause mortality and increased mortality from bone tumors and cardiovascular disease (17). However, the following studies from other countries did not confirm this and the overall conclusion was that the results did not generally support a carcinogenic effect of GH (17–19). These findings have subsequently been supported by other studies (20, 25) as well as in a meta-analysis (26). Nevertheless, the uncertainty regarding IGF-I and risk of neoplasia has created a concern among treating physicians and generally guidelines for GH treatment of children recommend to keep serum IGF-I levels within the normal reference range (below 2SD) to increase safety of the treatment (2, 8, 9). However, we previously demonstrated in the NESGAS cohort that titration of the GH dose to keep IGF-I levels below 2SD proved less effective in terms of height gain than current dosing regimens for short SGA children (15). Thus, it has been speculated that some of these SGA children are less sensitive to IGF-I and that they may depend on continuously supra-physiological levels of IGF-I to maintain sufficient growth. In this context, we find it of interest that our study showed that the serum concentrations of bioactive IGF stayed within the normal range during high-dose GH treatment despite of elevated concentrations of IGF-I.

In conclusion, our results show for the first time that bioactive IGF levels are mainly kept within normal ranges despite elevated total IGF-I concentrations during one year of GH treatment of short SGA children. Titration of GH dose in SGA patients according to their total IGF-I concentration resulted in very low doses of GH and a low growth response in a previous study. Further studies are needed to investigate the potential clinical role of bioactive IGF-I in the monitoring of GH treated children.

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Additional Information

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Growth and Adult Height in Girls With Turner Syndrome Following IGF-1 Titrated Growth Hormone Treatment

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Context: Girls with Turner syndrome (TS) suffer linear growth failure, and TS is a registered indication for growth hormone (GH) treatment. GH is classically dosed according to body weight, and serum insulin-like growth factor-1 (IGF-1) concentrations are recommended to be kept within references according to international guidelines.

Objective: To assess the effect of long-term GH treatment in girls with TS following GH dosing by IGF-1 titration.

Design and setting: A retrospective, real-world evidence, observational study consisting of data collected in a single tertiary center from 1991 to 2018.

Patients: A cohort of 63 girls with TS treated with GH by IGF-1 titration with a median duration of 6.7 years (interquartile range [IQR]: 3.4-9.7 years).

Main outcome measures: Longitudinal measurements of height, IGF-1, and adult height (AH) following GH treatment were evaluated and compared between the different karyotypes (45,X, 45,X/46,XX, or miscellaneous).

Results: Using GH dose titration according to IGF-1, only 6% of girls with TS had supranormal IGF-1 levels. Median dose was 33 µg/kg/day (IQR: 28-39 µg/kg/day) with no difference between the karyotype groups. AH was reached for 73% who attained a median AH of 1.25 standard deviation score (SDS) for age specific TS references (IQR: 0.64-1.50 SDS), and a median gain in height (ΔHSDS: AH SDS minus baseline height SDS of TS references) of 0.50 SDS, equal to 3.2 cm (SD 7.68) for all karyotypes.

Conclusion: Our real-world evidence study suggested that titration of GH dose to keep IGF-1 levels within the normal range resulted in a lower AH gain than in studies where a fixed dose was used. (***J Clin Endocrinol Metab*** **105: 1–9, 2020)**

Key Words: Turner syndrome, adult height, IGF-1 titration, growth hormone

Turner syndrome (TS) is found in 1 per 2500 live born females (1), and this chromosomal abnormality is known to cause numerous clinical manifestations such as heart and kidney malformations, hearing loss, primary amenorrhea, and short stature. Girls with TS often suffer linear growth failure due to haploinsufficiency of the short stature homeobox-containing gene resulting in low adult height (AH) about 20 cm shorter than a normal reference population (2). TS is an approved indication of treatment with recombinant human growth hormone (GH) leading to a reported increase in AH of 5 to 8 cm at a dosage of 42 to 50 µg/kg/d, but with

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large individual variation in growth response (2-7). The present recommended GH dose for girls with TS is 45 to 50 µg/kg/day. An increase of the dose up to 68 µg/kg/day may be considered if AH potential is substantially compromised (8). However, dosage of GH in TS patients is still a matter of debate.

GH stimulates a direct production of insulin-like growth factor-1 (IGF-1), which mediates many of the growth-promoting actions of GH on linear growth. Most TS patients are not GH deficient, and GH treatment is given at supraphysiological levels, which may result in elevated concentrations of IGF-1 during treatment. Current international guidelines for GH treatment in girls with TS recommend to keep IGF-1 levels below +2 standard deviation scores (SDS) and to decrease GH dose if IGF-1 levels are above +3 SDS (8).

Large epidemiological studies of adults from the general population have shown that both low and high levels of IGF-1 concentrations were associated with increased cancer mortality and all-cause mortality (9,10). However, no studies have evaluated the morbidity or mortality in children with increased IGF-1 levels during GH treatment.

The use of serum IGF-1 values to adjust GH dosing has been debated (11). In a study comparing IGF-1 titration to weight-based dosing in both short prepubertal children and GH-deficient children, a significantly greater linear growth was found in the group of patients where IGF-1 was titrated to the higher level of the normal range compared to traditional weight-based dosing (12). IGF-1 titration of GH dose seems as a reasonable approach in terms of efficacy and safety (12, 13), especially in GH-deficient children.

To our knowledge IGF-1 titration of GH doses in girls with TS has not previously been investigated in detail. In this large single-center study, we evaluated for the first time growth and AH in 63 TS patients where GH doses were adjusted according to the IGF-1 concentrations.

Methods

Patients

Ninety-two patients with a TS diagnosis were identified from the patient registry at our department based on the *International Classification of Diseases 10* codes (Q96-Q96.9). The patients were followed in a single tertiary center (Department of Growth and Reproduction at Rigshospitalet, Copenhagen University Hospital, Denmark) from 1991 to 2018.

Of the 92 patients 16 were excluded either due to a male phenotype (n = 14) or missing medical notes (n = 2). Of the remaining 76 patients with TS, 63 patients were treated with IGF-1-titrated GH, and 13 did not receive GH therapy. Forty-six of the 63 patients (73%) treated with GH achieved an AH during the study period; near AH was defined as height velocity <2 cm per year.

Clinical examination, data, and medical history

This study was a retrospective analysis using the medical record files of the girls with TS. They attended routine clinical visits with a trained pediatric endocrinologist every 4 months during the period of treatment where the clinical and auxological progress was monitored. Standing height was measured to the nearest 0.1 cm with a wall-mounted Harpenden stadiometer (Holtain Limited, Pembrokeshire, UK), and weight on a Seca delta model 707 digital electronic scale (Seca, Hamburg, Germany) while wearing light clothes and no shoes, with a 0.1 kg precision. Pubertal development was evaluated by inspection and palpation according to Marshall and Tanner (14). Body mass index was calculated as weight (kg) divided by squared height (m^2). Target height was calculated as the mean of the height SDS of the mother and the father. The anthropometric measurements were expressed as SDS according to the Danish national reference (15). AH SDS is expressed in SDS for the end of growth 18+ years for both general population and age specific TS references. Bone age was determined according to the methods of Greulich and Pyle (16). Predicted AH was calculated using BoneXpert Adult Height Predictor (Visiana, Holte, Denmark). Projected AH was calculated based on the reference growth chart for northern European girls with TS (17) assuming that the TS girls would follow their baseline growth HSDS until final height without treatment. To assess the effectiveness of the GH treatment we used changes in height SDS (ΔHSDS) according to a TS reference (AH SDS minus height SDS at baseline), changes in height gain over projected AH (AH [cm] minus projected AH [cm]), and height gain over the predicted AH (AH [cm] minus predicted AH [cm]).

Karyotypes

The diagnosis of TS was validated and confirmed by a clinical geneticist by karyotyping using routine G-banding, including counting of at least 30 metaphases. All phenotypic female patients diagnosed with TS karyotypes were included. Phenotypic male patients with 45,X/46,XY were excluded. The included girls with TS were divided into 3 groups depending on their karyotype: 45,X (n = 25), Turner mosaicism 45,X/46,XX (n = 8), and miscellaneous (ie, 45,X/46,X,r(X) and 45,X/46,X,i(X)(q10)) (n = 29).

Analysis of insulin-like growth factor-1 hormone assays

Nonfasting blood samples were drawn between 8 AM to 5 PM from an antecubital vein, clotted, and centrifuged, and hormone analyses were performed. Serum IGF-1 was measured using 3 different assays during the study period. From 1991, a highly sensitive in-house radioimmunoassay, as previously described by Juul et al (18) was used, with an intra- and inter-assay coefficients of variation of 3.9% and 8.7%, respectively. From 2008, the IGF-1 levels were determined using IMMUNULITE 2000 IGF-1 conventional immunoassays (Siemens Healthcare Diagnostics, Los Angeles, CA, US), and the intra- and inter-assay coefficients were less than 4% and 9%, respectively (19). From 2013, the IGF-1 levels were determined using IDS-iSYS Multidiscipline Automated Analyser.

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The assays available for IGF-1 changed during the 27-year study period, and the assays were compared one by one before changing in 2008 and 2013, respectively.

IGF-1 titration

At the Department of Growth and Reproduction, Rigshospitalet, the treatment with GH of all patients was titrated using the IGF-1 (SDS) levels in serum. GH starting dose was 12.5 µg/kg/day for 4 weeks, and thereafter GH dose was increased to 25 µg/kg/day until first visit at 3 months. Thereafter, the dose was titrated up and down according to height changes and IGF-1 levels measured every 3 to 6 months. GH doses were titrated to obtain IGF-1 levels above 0 standard deviations (SD) and preferably to reach levels just below +2 SD in girls with reasonable growth responses. In cases of poor responses to GH, supranormal IGF-1 levels (up to +3 SD) have been accepted. GH doses have been increased or decreased with 0.1 to 0.2 mg to obtain levels of IGF-1 at the preferred levels.

Statistical analysis

The data are displayed as medians with interquartile ranges (IQR); the 25th to 75th percentile. Comparisons between the 3 groups of karyotypes was performed using a Kruskal-Wallis test. A multiple regression analysis was performed, expressing regression coefficients (B), standard error (SE). All statistical analyses were performed using SPSS software, version 22 (IBM Corporation, Armonk, NY, US). A *P*-value below 0.05 was considered statistically significant.

Ethical considerations

This retrospective study was based on patient record files, including clinical data and blood samples collected as part of the routine clinical follow-up. The use of data was approved by the Danish Health Authority (3-3013-2022/1) and the Danish Data Protection Agency (RH-2016-177, I-Suite number: 04732). Our clinical data from individual patients cannot be uploaded in any form to an open repository and shared according to GDPR and Danish law.

Results

Birth characteristics from all GH-treated girls with TS (n = 63) did not differ between karyotype subgroups (Table 1). Age at baseline was significantly different between karyotype subgroups; 6.0 years (5.2-7.6 years) in the 45,X group, 11.9 years (8.3-13.3 years) in the 45,X/46,XX group, and 9.4 years (5.4-13.5 years) in the miscellaneous group (*P* = 0.02) (Table 1).

Median IGF-1 at baseline was –0.47 SDS (–1.11to 0.33 SDS) for all patients, with a trend towards lower IGF-1 levels in the 45,X group, however, the difference was only borderline significant (*P* = 0.05) (Table 2). Throughout the period of GH treatment, a total of 923 measurements of serum IGF-1 were collected from the girls with TS. The IGF-1 concentration was below mean in 29% of the measurements (N = 264), between mean and +2SDS in 52% of the measurements (N = 484),

Table 1. Clinical characteristics at birth and at baseline in the 3 groups of karyotypes

	All Patients		Karyotypes				
	N		45,X	45,X/46,XX	Miscellaneous		
N	63	63	25	8	30		*P*-value
At birth							
Birth weight (SDS)	48	–0.82 (–1.66 to –0.13)	–0.92 (–1.26 to –0.20)	–0.29 (–1.91 to 0.34)	–0.86 (–1.73 to –0.16)		0.90
Birth length (SDS)	42	–0.33 (–1.21 to 0.47)	–0.09 (–1.21 to 0.28)	–0.09 (–1.21 to 1.03)	–0.52 (–1.21 to 0.33)		0.79
Gestational age (WK)	39	39 (38 to 40)	39.00 (38 to 40)	40	39 (38 to 40)		0.34
Mother's height (SDS)	62	–0.77 (–1.48 to 0.09)	–0.79 (–1.32 to –0.05)	–0.69 (–1.58 to 0.22)	–0.86 (–1.59 to 0.15)		0.91
Father's height (SDS)	60	–0.20 (–0.81 to 0.57)	–0.42 (–0.78 to 0.05)	–0.73 (–1.58 to 0.41)	–0.06 (–0.81 to 0.75)		0.17
Target height (SDS)[a]	62	–0.64 (–1.08 to 0.09)	–0.66 (–0.99 to –0.10)	–0.98 (–1.46 to 0.18)	–0.44 (–1.10 to 0.35)		0.64
At baseline							
Age (year)	63	7.59 (5.36 to 11.97)	5.97 (5.17 to 7.63)	11.86 (8.29 to 13.31)	9.41 (5.36 to 13.53)		**0.02**
Height (SDS) general population	60	–2.54 (–3.11 to –2.23)	–2.55 (–3.05 to –2.20)	–2.27 (–2.68 to –1.69)	–2.55 (–3.31 to –2.29)		0.10
Weight (SDS)	58	–1.36 (–2.06 to –0.57)	–1.31 (–2.16 to –0.85)	–0.89 (–1.25 to –0.10)	–1.53 (–2.24 to –0.52)		0.33
Body mass index (SDS)	58	0.38 (–0.26 to 1.00)	0.40 (–0.24 to 0.86)	0.14 (–0.23 to 0.78)	0.35 (–0.54 to 1.33)		0.16
Bone age (years)	29	7.83 (3.75 to 10.90)	6.00 (3.70 to 9.12)	11.20 (5.18 to 11.68)	9.15 (3.88 to 11.75)		0.28
Height (SDS) TS reference	60	0.50 (–0.25 to 1.25)	0.50 (–0.63 to 0.88)	1.38 (0.88 to 2.38)	0.50 (–0.25 to 1.00)		**0.02**

Data are presented as medians (interquartile range with 25th to 75th percentiles). Significant values are presented in bold.
[a]Target height: mean (paternal height SDS + maternal height SDS).

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Table 2. Clinical characteristics during growth hormone treatment in the 3 groups of karyotypes

	All Patients		Karyotypes			
	N		45,X	45,X/46,XX	Miscellaneous	P-value
N	**63**		**25**	**8**	**30**	
GH treatment						
Age at start of GH (years)	63	7.59 (5.36 to 11.97)	5.97 (5.17 to 7.63)	11.86 (8.29 to 13.31)	9.41 (5.36 to 13.53)	**0.02**
Age at cessation of GH (years)	46	15.54 (14.63 to 16.88)	15.59 (14.30 to 16.88)	15.53 (14.05 to 16.73)	15.49 (15.07 to 17.19)	0.53
Duration (years)	46	6.72 (3.36 to 9.72)	9.05 (6.98 to 10.20)	2.66 (1.97 to 3.41)	5.35 (1.71 to 8.87)	**0.001**
HV at baseline (SDS)	18	–1.15 (–1.90 to –0.50)	–1.00 (–1.31 to –0.60)	–1.00 (–3.45 to 1.45)	–1.53 (–2.01 to –0.92)	0.52
HV during GH (SDS)	31	0.44 (0.05 to 1.18)	0.08 (–0.17 to 0.38)	1.27 (0.48 to 2.05)	0.33 (–0.02 to 1.36)	0.71
AH (cm)[a]	46	153.7 (5.54)	153.67 (5.17)	156.30 (8.65)	152.98 (4.86)	0.73
AH (SDS) general population	46	–2.35 (–2.99 to –2.12)	–2.35 (–2.96 to –2.13)	–2.21 (–3.37 to –0.54)	–2.51 (–2.87 to –2.16)	0.73
AH (SDS) TS reference	46	1.25 (0.64 to 1.50)	1.25 (0.53 to 1.50)	1.38 (0.25 to 3.00)	1.00 (0.75 to 1.50)	0.67
ΔHeight (SDS) TS reference[a]	43	0.50 (–0.25 to 1.30)	0.75 (0.50 to 1.30)	–0.70 (–1.75 to 0.25)	0.50 (–0.37 to 1.62)	**0.03**
Predicted AH (cm)	18	150.22 (8.38)	148.90 (7.55)	164.95 (11.98)	147.71 (4.12)	0.14
Predicted AH (SDS)	18	–2.66 (–3.14 to –2.21)	–2.41 (–3.25 to –2.25)	–0.73 (–2.06 to 0.61)	–2.88 (–3.03 to –2.66)	0.08
AH (cm) – Predicted AH (cm)	18	3.63 (6.06)	6.33 (4.72)	–7.65 (0.89)	3.38 (4.55)	0.05
Projected AH (cm)	43	150.38 (9.06)	148.41 (6.84)	161.15 (9.16)	148.85 (8.88)	**0.01**
AH (cm) – Projected AH (cm)	43	3.20 (7.68)	4.97 (4.66)	–4.85 (6.98)	4.06 (8.83)	**0.02**
IGF-I						
IGF-1 at baseline (SDS)	57	–0.47 (–1.11 to 0.33)	–0.86 (–1.82 to –0.10)	0.08 (–0.70 to 0.62)	–0.33 (–1.01 to 0.83)	0.05
IGF-1 during GH (SDS)						
RIA	26	0.14 (–0.35 to 1.09)	–0.03 (–0.60 to 0.18)	0.87 (0.65 to 1.08)	0.37 (–0.12 to 1.12)	0.30
IML	33	1.37 (0.88 to 1.75)	1.36 (0.92 to 1.66)	0.02 (–0.73 to 0.20)	1.45 (1.19 to 2.13)	**0.03**
iSYS	18	1.46 (0.64 to 2.10)	0.99 (0.58 to 1.15)	0.78 (0.53 to 1.61)	2.03 (1.19 to 2.25)	0.09

Data are presented as medians (interquartile with 25th to 75th percentiles), except data measured in cm, which are represented as means (standard deviation). Significant values are presented in bold.
Abbreviation: HV, height velocity; RIA, radioimmunoassay.
[a]Adult height: Height velocity <2 cm/year.
[b]ΔHSDS: AH SDS (TS reference) – height SDS baseline (TS reference).

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and exceeded +2 SDS in 19% of the measurements (N = 175). In total only 6% of all IGF-1 measurements exceeded +3 SD during GH treatment (N = 51) (20). IGF-1 SDS during GH treatment divided into the 3 different assays showed the variabilities in the measurements throughout the period, (Table 2).

The median duration of treatment with GH was 6.7 (3.4-9.7) years, which differed according to karyotype: 45,X: 9.1 (7.0-10.2) years; 45,X/46,XX: 2.3 (2.0-3.4) years; and miscellaneous: 5.4 (1.7–8.9) years (P = 0.001) (Table 2). The median GH dose was 33 µg/kg/day (28-39 µg/kg/day). Dividing the cohort into tertiles according to received GH dose (median of tertiles: 41 µg/kg/day, 33 µg/kg/day, and 26 µg/kg/day, respectively) showed that AH (Fig. 1) and gain in HSDS (Fig. 2) during GH treatment were not related to average GH doses. However, a multiple regression analysis showed a significant positive association between GH dose and gain in height (SDS) even after adjustment for age at start and duration of treatment (B = 0.04, SE = 0.01, P = 0.009). A multiple regression analysis with AH as primary outcome (adjusted for age at start and duration of GH treatment) also showed a positive association but this did not reach statistical significance (B = 0.03, SE = 0.02, P = 0.06).

AH was reached in 73% of the girls with TS (n = 46), who attained a AH of –2.35 SDS (–2.99 to –2.12 SDS), and 40% of these patients (N = 18) attained a height within the reference range of the general population (greater than –2 SDS) (Fig. 3). Height velocity SDS at baseline was –1.15 SDS and 0.44 SDS during GH treatment for all karyotypes (Table 2). Median height gain for all karyotypes (ΔHSDS) was of 0.50 SDS (–0.25 to 1.30 SDS), with a significant difference between the 3 groups of karyotypes (P = 0.03). Height gain was largest for the girls with a 45,X or miscellaneous karyotype and lowest for those with a 45,X/46,XX karyotype (Table 2)

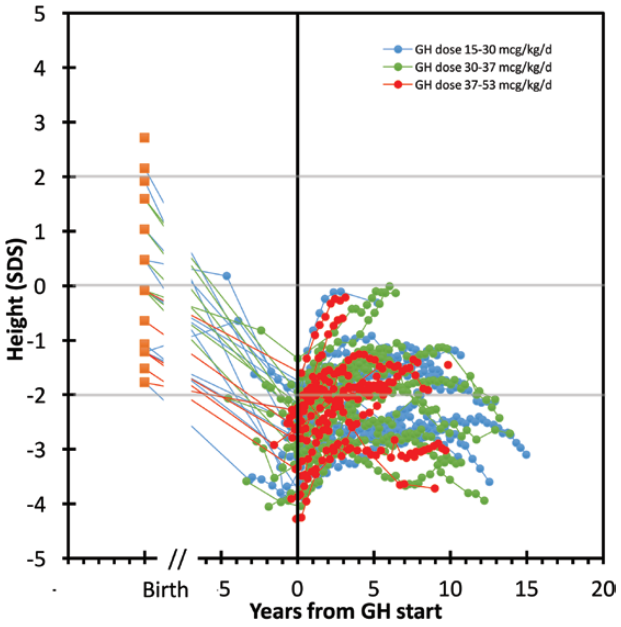

Figure 1. Height changes (SDS) before and during GH treatment, according to 3 different groups of mean GH doses.

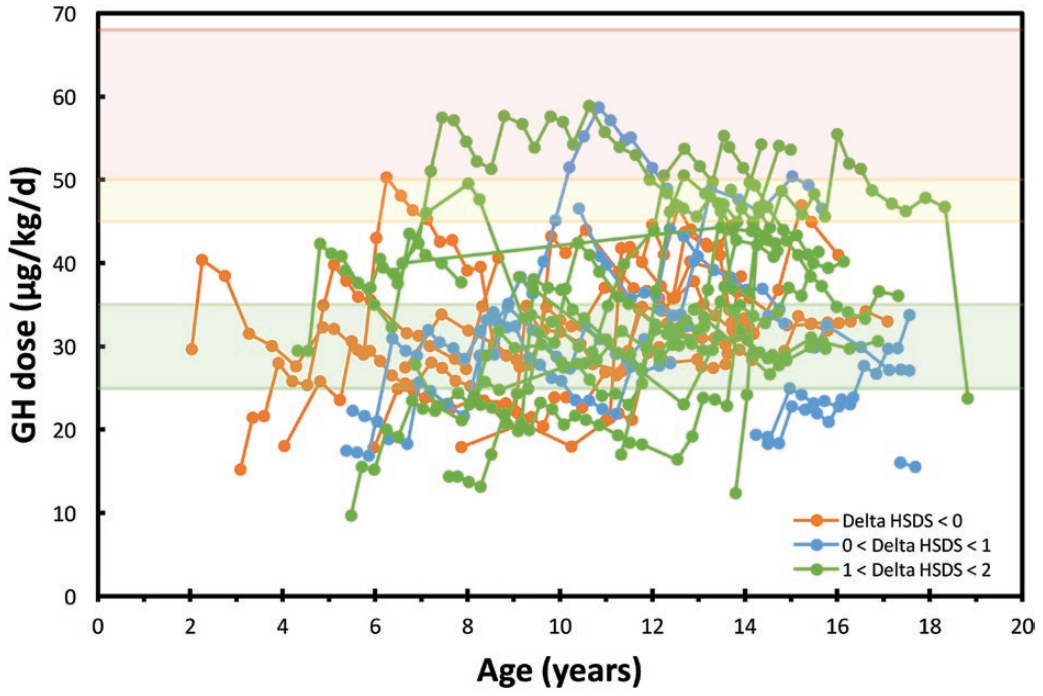

Figure 2. Gain in height changes (ΔHSDS for TS references) during treatment in tertiles, according to their received GH doses. Yellow area represents recommended dose of GH doses for girls with TS (45-50 µg/kg/day). Red area represents the highest recommended dose (up to 68 µg/kg/day). Green area represents recommended dose for patients with GH deficiency (25-35 µg/kg/day).

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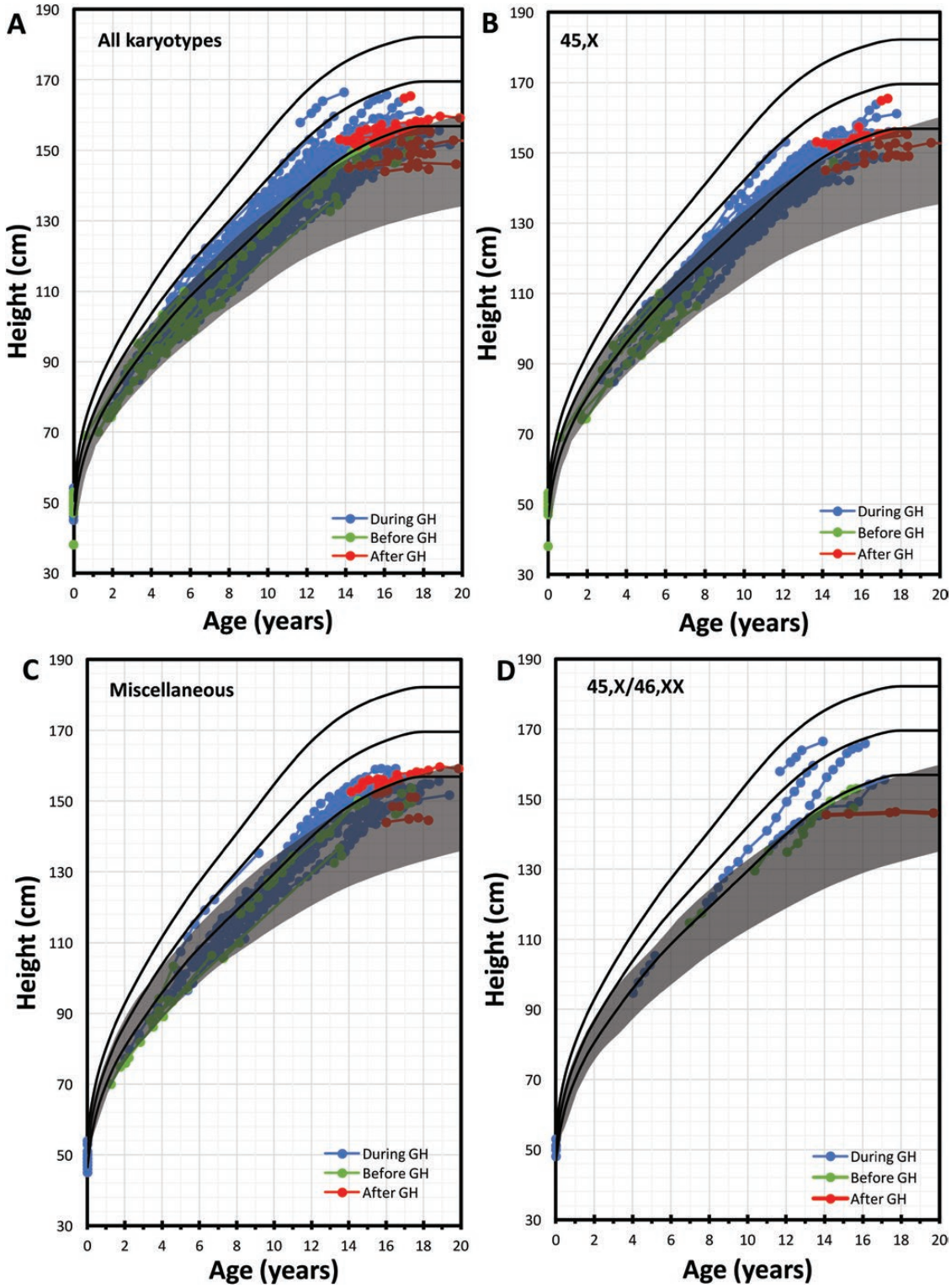

Figure 3. Height (cm) according to age (years) in TS patients treated with GH (blue lines: on treatment; green lines: before treatment; red lines: after treatment). (A) All patients, (B) karyotype 45,X, (C) karyotype 45,X/46,XX, and (D) miscellaneous karyotypes. Grey area represents height references of GH untreated TS girls ± 2 SD.

(Fig. 4), but this difference may be caused by the large difference in duration of treatment (Table 2). Height gain (cm) was assessed by AH (cm) minus projected AH (cm), which showed a mean increase of 3.20 cm (SD = 7.68) for all karyotypes, with a significant difference between the 3 karyotypes groups (P = 0.02) (Table 2). AH (cm) minus predicted AH (cm) showed a similar response in mean height gain of 3.63 cm (–0.250.30 to 7.45) for all karyotypes, but only with a borderline significant difference (P = 0.05). Among the untreated girls with TS, 3

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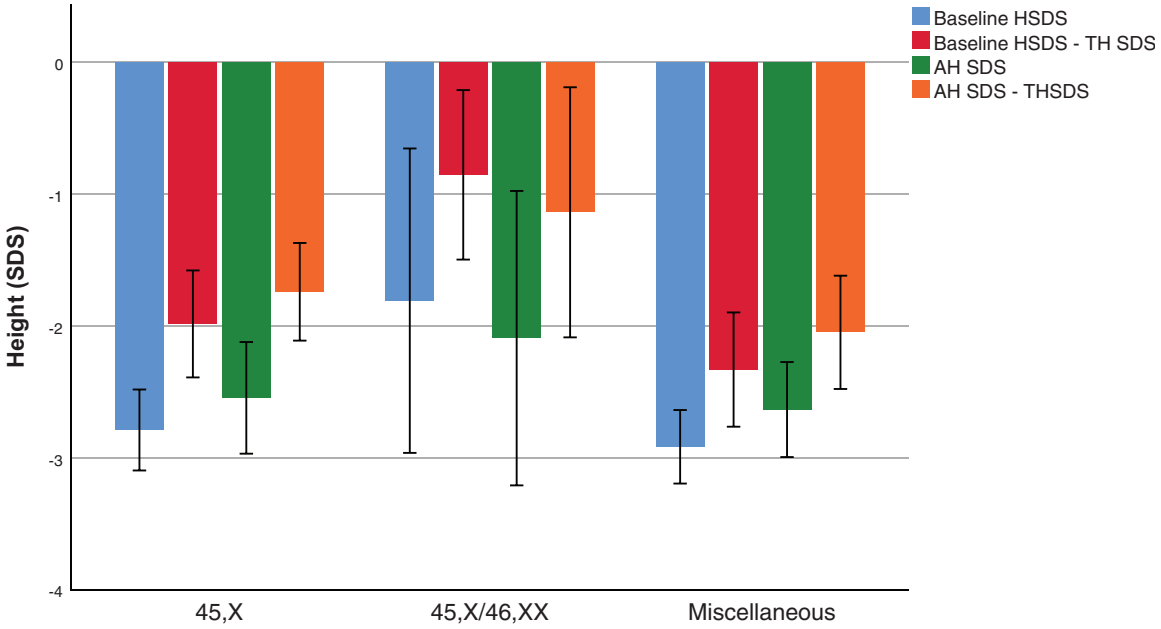

Figure 4. Height SD score during GH treatment divided by karyotype groups, at baseline (blue bars), at baseline minus target height (TH) (red bars), AH (green bars), and AH minus TH (orange bars). Bars represent mean ±2 standard error.

out of 13 untreated girls achieved an AH comparable to TS girls treated with GH (all 3 patients were miscellaneous: 45X/46XX/47XXX; 45X,46XY,idic(Y)(q11); and 45X,t(x;5)(q13;p15.3)dn). The remaining girls have not yet reached an AH and are not candidates for GH treatment due to their age-appropriate growth at present (21).

Forty-eight of the 59 (81%) girls above 10 years of age received estrogen treatment during treatment with GH. E2 was administered as transdermally (56%), orally (29%), or both (15%) (Table 3). The median age at start of estrogen treatment was 12.3 years (11.2-13.8 years; median of 4.7 years after initiation of GH) and did not differ between karyotypes (P = 0.12) (Table 3). The majority of patients experienced pubertal growth spurt with increased HSDS after initiation of E2 treatment (22). However, the effect of E2 varied considerably between patients during the first 2 years after initiation of E2 treatment, with a median gain in height of 0.3 SDS (0.07-0.53 SDS).

Discussion

In this large single-center study, we evaluated the effect on AH following long-term GH treatment with dose titration by IGF-1 levels in 63 girls with TS. We succeeded in attaining IGF-1 levels within the recommended target range in the majority of the girls using an average GH dose of 33 µg/kg/day. We report an AH gain of 3.20 cm in our real-world evidence study, which is below the findings observed in randomized controlled trials.

Improvement of AH in girls with TS treated with the traditional weight-based GH dosing regimen usually ranges between 5 and 8 cm at GH doses ranging from 42 to 50 µg/kg/d, although a Dutch study reported of gain in AH of 11 to 16 cm using much higher GH doses (45-90 µg/kg/d) (23). However, large interindividual variation in AH gain was apparent in all studies (3-7). In the current study, AH gain was evaluated using a reference material for untreated TS girls and the gain in height was slightly reduced using an IGF-1 titration regimen compared with the results reported in previous studies. There was a significant difference between the karyotypes as the mosaic group (45,X/46,XX) had lower gain in height following GH treatment. However, this group had a higher baseline height (SDS) according to the TS reference population (1.38 SDS) and thereby also a higher projected AH, which they reached. Predicted AH is determined by the height and bone age at baseline with a prediction model based on a normal reference population (24). This method has not been validated for TS girls, but in our study the predicted AH and the projected AH were quite similar, suggesting that the prediction model could give substantial information on the height potential in untreated TS girls.

The latest international clinical guidelines recommend to keep IGF-1 levels below +2 SDS and to decrease the GH dose if IGF-1 levels are above +3 SDS. Importantly, our present results showed that IGF-1 titration of the GH dose in girls with TS may lead to lower doses of GH than recommended (8) and that the gain in height in our study was lower than previously reported results. These findings underline the great variability in growth response to GH treatment in girls with TS and that numerous factors may influence the efficacy of the

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Table 3. Administration of estrogen in the 3 groups of karyotypes

	All Patients		Karyotypes			
	N		45,X	45,X/46,XX	Miscellaneous	
N			25	8	30	P-value
Age at start of E2 treatment	48	12.26 (11.22 to 13.78)	12.08 (11.08 to 13.17)	14.64 (14.25 to 15.03)	12.84 (11.30 to 14.60)	0.12
E2 treated, n (%)[a]	48	48 (81.36)	24 (100)	2 (25)	22 (81.48)	**<0.001**[b]
E2 TD administration, n (%)	27	27 (56.25)	14 (58.33)	1 (50)	12 (54.55)	
E2 PO administration, n (%)	14	14 (29.17)	7 (29.17)	1 (50)	6 (27.27)	
E2 both TD and PO, n (%)	7	7 (14.58)	3 (12.50)	0	4 (18.18)	

Data are presented as medians (interquartile with 25th to 75th percentiles). Significant values are presented in bold.
Abbreviations: E2, estrogen; PO, oral; TD, transdermal.
[a]Only girls above 10 years of age (n = 59).
[b]Calculated by Chi-Squared test.

treatment, such as early initiation of GH treatment (25). The dose of GH was decreased when IGF-1 levels exceeded +2 SD according to a normal reference. However, following the international recommended guidelines, decreasing GH doses when IGF-1 levels exceeded +3 SD might have resulted in a more significant effect of the titration regime. In a growth prediction model of girls with TS, GH dose was the most influential variable for first year growth response as well as an important factor for the growth response the following years on treatment (26). Girls with TS are not GH deficient, and many of them will have IGF-1 levels within the normal reference before start of GH treatment and may therefore experience a rise in IGF-1 to supraphysiological levels during GH treatment. We and others have previously shown that keeping IGF-1 levels below +2 SDS by titration of the GH dose was less effective in terms of height gain in small for gestational age children than current dosing regimens (27). Thus, it can be speculated that the effect of GH treatment in non-GHD children may depend on continuous supraphysiological levels of IGF-1 to maintain a sufficient growth response.

In the current study, the pubertal growth spurt was evident in most of the patients irrespectively of spontaneous or induced puberty. Administration of estrogen and GH and its combined effect on height is a matter of debate and has not yet reached consensus. One study reported that low-dose treatment with E2 in mid-childhood does not improve gain of near-AH in TS patients (28) whereas another study concluded that combining childhood ultralow-dose estrogen with GH may improve growth in girls with TS (5). Early treatment with low-dose estrogen combined with IGF-1 titration of GH in girls with TS has never been investigated.

This study has some limitations mostly because it is a retrospective study design, which did not allow us to compare our results with a control group. We therefore compared our cohort of GH-treated girls with TS to a previously published study on a group of untreated girls with TS. Another limitation of our study is the methodology of IGF-1 measurements changed throughout the period. However, compared to other studies, we have a large cohort of TS girls followed closely with many routine visits at a single tertiary center, assuring a uniform treatment strategy.

In this single-center study of GH treated girls with TS, we found that lower GH doses were adequate to obtain IGF-1 levels within the normal range as recommended in the clinical guidelines. However, our real-world evidence suggested that IGF-1 titrated GH dosing in girls with TS resulted in a lower AH gain compared to previous studies of weight-based GH dosing.

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Additional Information

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A common deletion in the growth hormone receptor gene (d3-GHR) in the offspring is related to maternal placental GH levels during pregnancy

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ABSTRACT

Background: A common growth hormone receptor polymorphism with deletion of exon 3 (d3-GHR) has previously been linked to increased postnatal growth on the one hand and decreased fetal growth on the other. Regulation of fetal growth is positively dependent on secretion of placental GH (hGH-V).
Objective: We explored the effect of the fetal d3-GHR genotype on maternal serum levels of hGH-V and fetal growth. The cellular localization of hGH-V synthesis and the GH receptors were determined in normal placentas.
Methods: 43 healthy mother-child pairs were examined during pregnancy with measurements of hGH-V during third trimester, and serial ultrasound measurements determined fetal growth rate. Birth anthropometrics were obtained. The GHR genotype of the child was analysed postnatally. Immunohistochemical (IHC) analysis was conducted on four placentas.
Results: The presence of the d3-GHR genotype was associated with a markedly reduced concentration of hGH-V in maternal serum (β −0.52, SE 0.24, $p = 0.04$) compared to those who had a fl/fl genotype. Accordingly, a tendency towards reduced fetal growth rate during third trimester (β −25.8, SE 12.7, $p = 0.05$) and a lower birth weight were found among carriers of the d3-GHR allele, but these associations did not reach statistical significance ($p = 0.08$). IHC analysis showed expression of placental GH and GHR in the villous syncytiotrophoblast, the extravillous trophoblast, and the decidual cells and smooth muscle cells in chorionic vessels.
Conclusions: The presence of the d3-GHR polymorphism in the fetus was associated with lower maternal serum levels of hGH-V, decreased fetal growth rate in third trimester and lower birth weight compared to the wildtype.

1. Introduction

Fetal growth is a complex process influenced by environmental factors, including maternal health, nutrition and lifestyle as well as genetic factors. Regulation of fetal growth is highly dependent on secretion of the human placental growth hormone variant (hGH-V) produced in the syncytiotrophoblast and extravillous cytotrophoblast layers of the placenta and entering the maternal circulation [1,2]. Serum concentrations of hGH-V increase during pregnancy, inhibit the pulsatile pituitary growth hormone (GH) secretion, and act as the key regulator of maternal insulin-like growth factor-I (IGF—I) levels. Placental GH is inversely associated with the levels of glucose and insulin in the maternal circulation, thereby assuring glucose disposal to the fetus by increasing nutrient availability either directly or indirectly via IGF-I [3]. Previous studies have shown a positive association between placental GH and fetal growth [4].

A common polymorphism in the GH receptor (GHR) with a deletion of exon 3 (d3-GHR), found in approximately 50% of the European population, has previously been found to influence signaling through the GHR in vitro [5]. The transduction of GH signaling was higher through the d3-GHR than the full-length homodimer [5]. Following this study several clinical studies have investigated the growth response in GH

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treated children with growth hormone deficiency, Turner syndrome and children born small for gestational age (SGA) [6–9]. Although some controversy exists, two metaanalyses concluded that carriers of the d3-GHR isoform had a better growth response during GH treatment than carriers of the full-length isoform [10,11] which may reflect a better GH sensitivity. Interestingly, in contrast to the increased growth response postnatally, we and others have previously shown a decreased fetal growth and lower birth weight in the carriers of the d3 allele in SGA children [12,13] as well as in healthy children [14,15]. However, in a large cohort of healthy young men there was no association between birth weight and GHR genotype [16]. The mechanisms of action by which the common d3-GHR polymorphism influences prenatal and postnatal growth differentially remain largely unclarified.

In the current study we analysed the effect of the d3-GHR genotype allele on maternal serum levels of placental GH and fetal growth in 43 healthy mother-child pairs. We hypothesised that carriers of the d3-GHR allele have an impaired fetal growth. In addition, we performed histological examinations of placentas from normal pregnancies to determine the cellular localization of placental GH and the GH receptors.

2. Materials and methods

2.1. Study participants and design

Pregnant women attending the first routine obstetric consultation (gestational weeks 6–12) at a university hospital in Copenhagen (Herlev Hospital) were invited to participate in a longitudinal study. All women were scheduled to have repeated ultrasound examinations and blood samples throughout pregnancy. Gestational age of the newborn child was determined from biparietal diameter (BPD) at the routine ultrasound scan at 18–20 weeks. A total of 151 women accepted to participate, 16 of whom dropped out before term, while three were excluded due to serious infectious and endocrinological disease. Data regarding hGH-V, IGF—I, fetal growth and thyroid hormones in this cohort of pregnant women has been reported in detail previously [1,17]. Blood samples for DNA isolation were obtained from 43 children and were included in the present study. A total of 326 blood samples were obtained from the 43 mothers during their pregnancy (mean number per woman (range): 6 (4–11)). Altogether, 111 ultrasound examinations were performed on 43 women and in 40 of these women fetal weight and individual fetal growth rates in third trimester could be estimated.

2.2. Laboratory methods

Blood samples were stored at −20 °C. HhGH-V and IGF-I levels were determined as previously described [1]. Genomic DNA was extracted from blood lymphocytes. The frequency of GHR transcript variants with retention (fl-GHR) or exclusion (d3-GHR) of exon 3 was tested by the multiplex PCR assay described by Pantel et al. [18]. This was performed with primers G1, G2, and G3 (GenBank accession no. AF155912). The distribution of the GHR genotypes did not deviate significantly from the Hardy-Weinberg equilibrium.

2.3. Statistical analyses

Gestation-specific standard deviation scores (SDS) for hGH-V were calculated using serum levels from all 132 women of the original cohort [19] in five week intervals throughout gestation (15–19 weeks; 20–24 weeks; etc.). For each individual woman, mean hGH-V SDS was calculated to obtain a representative value of the overall hGH-V level. Mean values of maternal IGF-I SDS levels were calculated accordingly.

From gestational week 27 to 42 fetal growth is approximately linear [20]. In order to describe the fetal growth rate in this period a linear regression model, fitted on data from every fetus over time (at least two fetal weight estimations after 27 weeks gestation) was applied to estimate the slope value representing the rate of change in fetal weight per unit of time (g/week).

The variables were examined for normal distribution and log-transformed if necessary. Data are presented as mean and standard deviation (SD) or back-transformed geometric mean (SD). The differences between the d3/d3-GHR, d3/fl-GHR and fl/fl-GHR groups were analysed by an independent-samples *t*-test comparing children carrying at least one d3-GHR allele with children homozygous for the full-length allele (fl/fl-GHR group). Multiple linear regression analyses were performed to investigate the associations between fetal growth and presence of the d3 allele as well as other confunders i.e. maternal BMI, maternal age, parity and fetal sex. Statistical analyses were carried out using SPSS, version 25 (IBM Corp., Armonk, NY).

2.4. Ethical aspects

The study was performed according to the Helsinki II Declaration and was approved by the local ethics committee and the Danish Registry Agency. All parents gave informed written consent.

2.5. Immunohistochemistry

Commercially available antibodies for placental growth hormone (hGH-V) (GenWay, Cat no 18–003-44,545), growth hormone receptor (GHR) (Abcam ab11380) and hCG (human Chorionic Gonadotropin) (DAKO A0231) were used. The immunohistocemical stainings (IHC) were performed using a standard indirect peroxidase method. Briefly, the dewaxed and rehydrated paraffin sections were heated in a microwave oven in TEG buffer (pH 9.0; Tris 6.06 g/5 L and EGTA) to unmask the antigen for hGH-V, while microwave treatment was not necessary for the hGH-V and hCG antibodies. Next, sections were incubated in 0.5% H_2O_2 to inhibit the endogenous peroxidase, followed by blockade for non-specific binding in diluted nonimmune goat serum (Zymed). Incubation with primary antibodies placental GH (1:50), placental GH (1:75) and hCG (1:3000) was carried out over night at 4 °C. Negative controls were performed on serial sections incubated without primary antibody in the dilution buffer (TBS). Subsequently, secondary biotinylated goat antimouse (against GHR) or biotinylated goat antirabbit (against placental GH and hCG) was applied (Zymed), followed by horseradish peroxidase-streptavidin complex (Zymed). Development was performed with AEC. The sections were washed in TBS after H_2O_2, and all second day treatments. Sections were counter stained with Mayer's hematoxylin. The IHC stainings were performed on one placenta at gestational week 31 (gemmelli), one at week 33 (IUFD) and three full term normal placentas. The hGH-V antibody was validated by Western blots by the manufacturer. The GHR antibody was unsuitable for western blots (Abcam) and hCG antibody showed a weak (4%) cross reactivity with LH (DAKO). The expression of GH and GHR antibodies was evaluated by conventional light microscopy and quantified by the intensity of the staining as follows: "- "= no staining, "+" = weak staining, "++" = moderate staining, and "+++" = strong staining.

3. Results

Clinical characteristics of the study population according to GHR genotypes are shown in Table 1. The distribution of GHR genotypes was 25 cases (58%) with fl/fl, 12 cases (28%) with d3/fl and 6 cases (14%) with d3/d3. There were no differences in maternal height, age or BMI before pregnancy between GHR genotype groups (Table 1). There were no significant differences in birth anthropometrics (Table 1).

Maternal serum levels of hGH-V hGH were significantly lower in pregnancies with fetal carriers of the d3-GHR allele (Fig. 1). A multiple regression analysis of hGH-V SDS adjusted for fetal sex and maternal BMI before pregnancy confirmed that serum levels of placental GH were lower in groups of fetuses carrying at least one d3 allele (β −0.52, SE 0.24, $p = 0.04$). Fetal growth velocity in third trimester was lower in the carriers of d3-GHR in a multiple regression analysis adjusting for

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Table 1
Clinical characteristics of the mother and fetus according to the d3-GHR genotypes.

	Total, $n = 43$	fl/fl, $n = 25$	fl/d3, $n = 12$	d3/d3, $n = 6$	P value*
Maternal characteristics					
Parity (primi/multi)	18/25	7/18	7/5	4/2	0.06
Maternal height (cm)	168.3 (6.1)	168.7 (5.8)	166.2 (7.2)	171.2 (4.1)	0.65
Maternal BMI (kg/cm2)	24.5 (4.3)	24.3 (4.5)	25.2 (4.2)	24.3 (3.8)	0.66
Maternal age (years)	31.7 (3.3)	31.9 (3.5)	31.8 (3.2)	31.0 (3.4)	0.72
Smoking in pregnancy (n (%))	14 (33)	9 (36)	3 (25)	2 (33)	0.74
Maternal h hGH-V (ng/ml)	10.3 (7.9–13.8)	11.7 (7.9–14.9)	9.4 (7.6–11.3)	8.2 (6.6–14.7)	0.07
Maternal IGF-I (ng/ml)	227 (229–354)	309 (240–361)	263 (207–294)	253 (233–335)	0.22
Maternal IGFBP-3 (ng/ml)	4252 (3884–5026)	4251 (3426–4251)	4351 (3647–4971)	4212 (4152–5302)	0.65
Fetal characteristics					
Sex (male/female)	24/19	12/13	7/5	5/1	0.35
Gestational age at birth (days)	277 (16)	276 (17)	275 (18)	283 (8.6)	0.76
Birth weight (g)	3508 (704)	3621 (775)	3330 (553)	3609 (611)	0.23
Birth length (cm)	52.1 (2.2)	52.5 (2.0)	51.2 (1.9)	52.7 (3.2)	0.29
Birth weight (SDS)	- 0.01 (1.3)	0.30 (1.3)	−0.52 (1.1)	−0.20 (1.4)	0.08

Data are presented as numbers (percentage), mean (standard deviation (SD)) or median (25th -75th percentile). The groups are compared by Student's t-test, Fischers exact test or Mann-Whitney U test when appropriate.

* The p-value reflects the difference between the carriers of at least one d3-GHR allele (fl/d3, d3/d3) and those who were homozygous for the full-length allele (fl/fl).

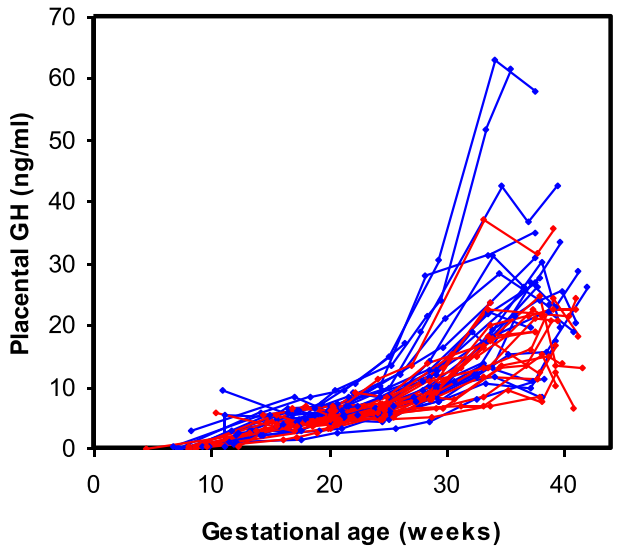

Fig. 1. Placental GH levels in maternal serum from 43 healthy pregnant women according to gestational age and GHR genotype in the offspring. Blue lines denote subjects carrying the homozygous full-length GHR gene (fl/fl), and red lines carriers of the exon-3 deletion in the GHR gene (both heterozygous (fl/d3) and homozygous (d3/d3)).

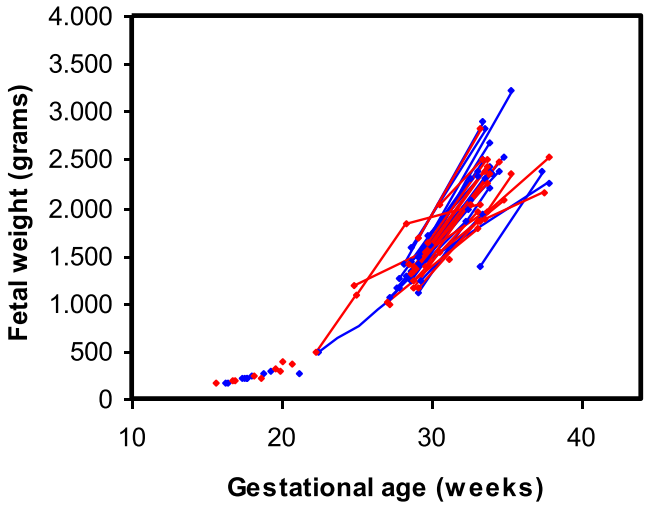

Fig. 2. Fetal weights estimated by ultrasound in 43 healthy pregnant women according to gestational age and GHR genotype in the offspring. Blue lines denote subjects carrying the homozygous full-length GHR gene (fl/fl), and red lines carriers of the exon-3 deletion in the GHR gene (both heterozygous (fl/d3) and homozygous (d3/d3)).

maternal age, parity and fetal sex, but this did not reach significance (β −25.8, SE 12.7, $p = 0.05$). Fig. 2 illustrates fetal weight gain for each individual fetus which is linear during third trimester (Fig. 2). There was no difference in birth weight between the children carrying the d3 allele and the fl/fl group. However, when taking gender and gestational age into account by calculating BW (SDS), there was a trend towards lower BW (SDS) in the carriers of at least one d3 allele, but this did not reach statistical significance ($p = 0.08$, Table 1).

Expression pattern of GH and GHR in human placenta.

Both hGH-V and GHR were expressed in all human placenta samples. To distuinguish between syncytiotrophoblast and cytotrophoblasts cells, staining for hCG was included and serial sections used. At term hCG is known to be expressed in syncytiotrophoblast cells but not in cytotrophoblasts. Expression of both hGH-V and GHR was localized to the cytoplasma. The hGH-V antibody stained the villous syncytiotrophoblast intensively (+++), the extravillous trophoblast mildly to moderately (+/++). Decidual cells and smooth muscle cells in chorionic vessels were moderately (++) positive. The GHR showed weaker and less well defined staining. The entire villous including syncytiotrophoblast, cytotrophoblast and villous stroma stained minimally to weakly (−/+). Weak to moderate positivity (+/++) was found in the extravillous trophoblast and in smooth muscle cells whereas the strongest staining (+++) was found in more than half the foetal and in some maternal erythocytes. The hCG showed intensive (+++) staining of the syncytiotrophoblast and focal weak (+) staining of extravillous cytotrophoblast (Fig. 3, Table 2).

4. Discussion

In this study of 43 mother-child pairs with normal pregnancies, we demonstrated that the presence of the d3-GHR genotype in the offspring was associated with a markedly reduced concentration of hGH-V and IGF-I in maternal serum. Furthermore, the d3-GHR genotype was associated with a decreased fetal growth rate during third trimester, and a lower birth weight, but these associations did not reach statistical significance. To our knowledge this is the first report on how a common genetic polymorphism in the offspring contributes to the regulation of growth promoting hormones such as hGH-V in the pregnant mother. In

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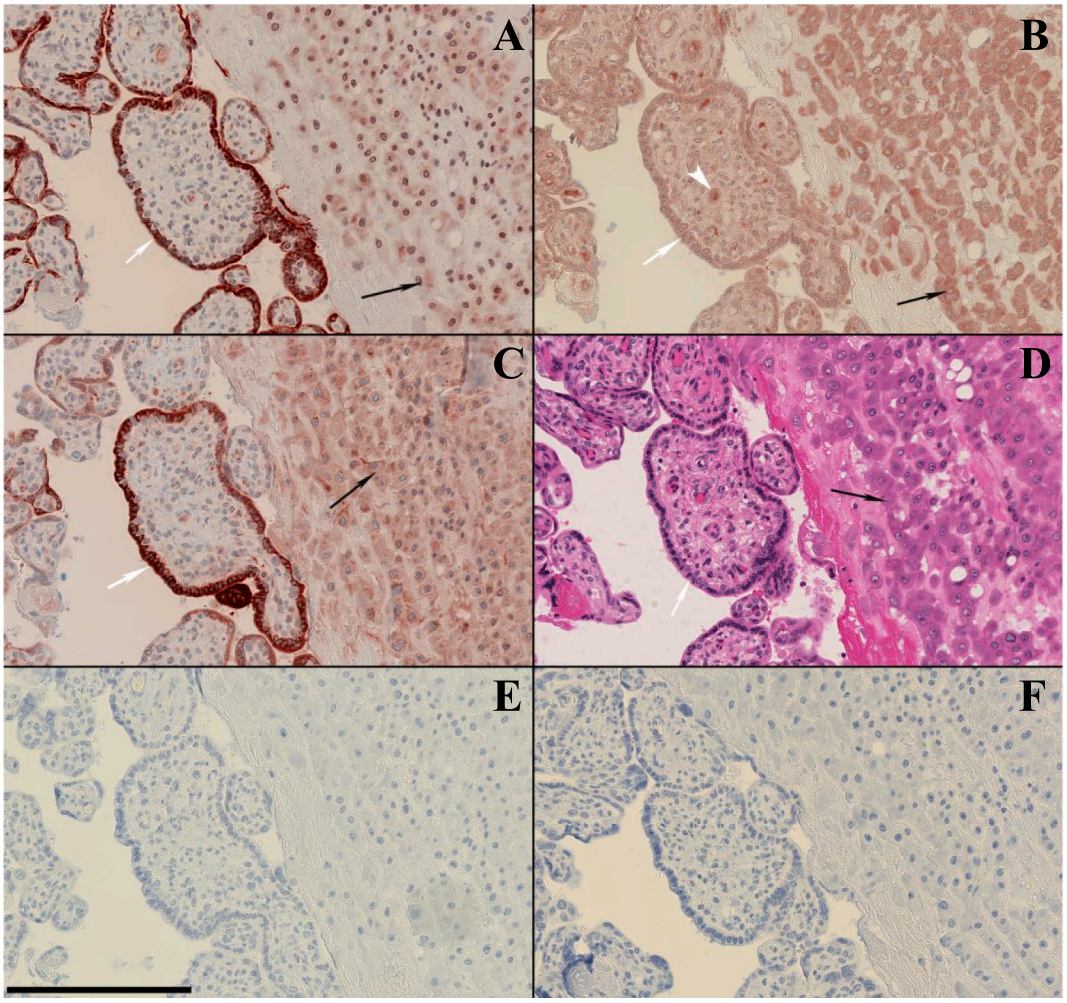

Fig. 3. Immunohistochemical staining of placenta samples for A) hGH-V: placenta growth hormone (IHC), B) GHR: growth hormone receptor (IHC), C) hCG: human Chorionic Gonadotropin (IHC), D) hematoxylin-eosin E) Control for rabbit antibodies (hGH-V and hCG), omission of primary antibody and F) control for mouse antibody (GHR). IHC and controls stained with Mayer's hematoxylin. Bar represents 100 μm.

Table 2
Immunohistochemical localization of hCG, placental GH (hGH-V) and GH receptor (GHR) in human placental tissues according to cellular compartments.

Antigen	hCG	HGH-V	GHR
Syncytiotrophoblast	+++	+++	−/+
Syncytial knots	+++	+++	−/+
Villous cytotrophoblast	−	−	−/+
Extravillous trophoblast	−/+	+/++	+/++
Decidua	−	−/+	−/+
Fibrin?	++	−	−
Foetal blood cells	−	−	++/+++
Villus stroma	−	−	−/+
Stromal muscle cells	−/+	++	+/++

+ denotes weak staining, ++ moderate staining, +++ strong staining.

addition, immunohistochemical analysis showed that both hGH-V and GHR were expressed concomittantly in the placenta. Expression of hGH-V was localized to the cytoplasm and mainly found in villous syncytiotrophoblasts, but with some expression also detected in extravillous trophoblasts, decidual cells and smooth muscle cells in chorionic vessels. Expression of GHR was found in the same compartments of placenta, although the pattern appeared less well-defined compared to hGH-V.

Several studies have linked the d3-GHR polymorphism to an increased postnatal growth since Dos Santos et al. showed an improved GH signaling of the d3-GHR allele in an in vitro study [5,13]. Interestingly, other studies found the carriers of the d3-GHR allele had a lower birth weight compared to those with the full-length allele [8,12,14,15]. In a cohort of healthy adolescents, we found a higher frequency of the d3 allele among children born SGA with known intrauterine growth restriction (IUGR) compared to those children born SGA without IUGR or children born appropriate for gestational age [12].

To our knowledge, no other studies have directly examined the associations of d3-GHR with fetal growth and maternal levels of placental GH and IGF—I. Former studies have revealed an increase in maternal serum hGH-V concentrations during pregnancy and that a rapid fall of hGH-V levels after parturition, positively associated to fetal growth in the third trimester [1,2,21,22]. In the current study we found that pregnant women carrying a fetus with the d3-GHR allele had significantly lower serum levels of placental GH and IGF—I, and that, accordingly, these fetuses had a reduced fetal growth rate in third trimester. This is in accordance with a study by Padidela et al. who found that carriers of the d3-GHR allele had lower birth weight (SDS) and a lower placental weight [15]. Former studies have concluded that this common d3-GHR polymorphism is related to increased postnatal growth possibly due to a better GH sensitivity. In contrast, the current study showed that the d3-GHR poymorphism affects placental secretion of hGH-V and, thereby, fetal growth negatively. These opposing results are preliminary and larger studies will be needed to confirm this.

The association between the d3-GHR polymorphism and maternal placental GH requires the presence of GHR in placental tissue. It is well

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known that GHR is present in abundance in hepatic tissue, but also in multiple non-hepatic tissues including fetal tissues. Our immunohistological study clearly showed that hGH-V and to a lesser extent GHR are expressed concomittantly in the syncytiotrophoblastic layer and both presented towards the maternal circulation. This suggests a role in the feedback mechanism between maternal hGH-V and placental function and thereby fetal growth. The observed localization and expression pattern of hGH-V in human placenta is in line with and extends the knowledge from previous reports. Thus, it has been shown that hGH-V mRNA expression was predominantly concentrated in the syncytiotrophoblast [23], and that the GHR is localized in the syncytiotrophoblast from 8 weeks of gestation [24]. Most likely, hGH-V binds to the GHR in the syncytiotrophoblast. The presence of the GHR towards the maternal circulation indicates the interaction between maternal hGH-V and placental function and thereby fetal growth. However, our study could not elucidate the role of the d3-GHR polymorphism in this interaction.

In has also been proposed that the d3-GHR polymorphism has metabolic and lipolytic effects. Insulin sensitivity decreases when GH secretion increases in normal physiology. Higher insulin secretion and disposition index was found in the carriers of the d3-GHR allele in a cohort of healthy children and adolescents [14]. Among GH treated children the insulin sensitivity was decreased among the d3-GHR carriers compared to the fl/fl-GHR [6,8], but other studies found no effect of the genotype on insulin metabolism [25,26]. Transplacental nutrient delivery promotes a rise in fetal insulin and IGF—I, which further promotes fetal growth. During third trimester insulin regulates glucose, but earlier in gestation insulin creates an anabolic environment in the fetus if nutient supply is optimal [27]. Thus, it could be speculated that the effect of insulin was decreased due to impaired sensitivity of the receptors among the carriers of the d3-GHR allele, which in turn could lead to impaired fetal growth.

In this preliminary study we found that the presence of the d3-GHR polymorphism in the fetus was associated with lower maternal serum levels of hGH-V and lower fetal growth rate in the third trimester. Furthermore, we demonstrated the presence of the GHR in the same placental compartments as hGH-V, which supports the hypothesis of an interaction between maternal hormones and receptors of fetal origin.

Disclosure

The authors have nothing to disclose.

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Clinical Research Article

Clinical Research Article

Impact of Lean Body Mass and Insulin Sensitivity on the IGF-1–Bone Mass Axis in Adolescence: the EPICOM Study

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Abbreviations: BIGTT-AIR, oral glucose tolerance test–derived index of acute insulin response; BIGTT-IS, oral glucose tolerance test–derived index of insulin sensitivity; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; DEXA, dual-energy x-ray absorptiometry; EPICOM, Epigenetic, Genetic and Environmental Effects on Growth, Metabolism and Cognitive Functions in Offspring of Women with Type 1 Diabetes; GH, growth hormone; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein 3; OGTT, oral glucose tolerance test; pQCT, peripheral quantitative computed tomography; T1D, type 1 diabetes.

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Abstract

Context: Insulin-like growth factor-1 (IGF-1) is involved in the growth of muscle and bone mass and contributes to glucose homeostasis. The offspring of mothers with diabetes during pregnancy have an increased risk of insulin resistance (IR).

Objective: We hypothesized that bone mass was decreased in the offspring of mothers with type 1 diabetes (T1D), and that the IGF-1–bone mass relationship would be negatively influenced by IR.

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Design: Data from the Epigenetic, Genetic and Environmental Effects on Growth, Metabolism and Cognitive Functions in Offspring of Women with Type 1 Diabetes (EPICOM) study performed from 2012 to 2013 were included.
Setting: This work is a follow-up study of a nationwide register study.
Patients: A total of 278 adolescent index offspring whose mothers had T1D and 303 matched controls were studied.
Main Outcome Measure: Bone mineral content (BMC) determined by a dual-energy x-ray absorptiometry scan and the interaction with IGF-1 and insulin sensitivity were measured.
Results: There was no difference in BMC, bone mineral density, height (SD score [SDS]), or BMC/height between index and control offspring. IGF-1 (SDS) did not differ between the groups but insulin-like growth factor-binding protein 3 (SDS) was higher in index boys compared to controls (B = .31 [95% CI, 0.06-0.57], $P = .02$). The statistical path analysis showed that IGF-1 predicted BMC/height (B = .24 [95% CI, 0.02-0.45], $P = .03$), but lean mass was a mediator of this. IGF-1 and the homeostatic model assessment of IR were positively associated (B = .75 [95% CI, 0.37-1.12], $P < .001$). There was no moderating effect of the interaction between IR and IGF-1 on lean mass in the entire cohort (B = .005 [95% CI, –0.03 to 0.04], $P = .81$) or when analyzing index cases and controls separately.
Conclusion: We found that lean mass was an intermediary factor in the IGF-1–bone mass relationship in a large cohort of adolescents, and this relationship was not moderated by IR.

Freeform/Key Words: insulin-like growth factor-1 (IGF-1), insulin sensitivity, bone mineral content

Fluctuation of insulin sensitivity occurs during pubertal development reflecting the interplay between insulin metabolism, the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis, and sex steroids. Insulin sensitivity decreases before physical signs of puberty and before increases in sex steroid levels are detectable (1). The decrease in insulin sensitivity may partly be explained by increasing adiposity before puberty but also by the physiological activation of the GH/IGF-1 axis, which contributes to a relative insulin resistance (1). IGF-1 levels increase through childhood with a steep incline during puberty until Tanner stage 4 is reached and decreases in Tanner stage 5 at the end of puberty (2). Changes in insulin sensitivity and IGF-1 concentrations during puberty follow the same pattern with a peak in midpuberty (2, 3).

IGF-1 is an important hormone for childhood growth involved in development, regulation, and cell proliferation of skeletal muscle and bone mass (4, 5). There is substantial evidence that IGF-1 plays an important role in osteoblast and osteoclast cell proliferation (6-8). However, it has also been suggested that IGF-1 promotes bone growth indirectly by the effect on skeletal muscle via the increased mechanical load to which the bone adapts its structure and mass. A study of transgenic mice that overexpressed IGF-1 in muscle found that the increased muscle mass was associated with increased cortical bone (9). Human studies of a longitudinal cohort of 258 girls followed through puberty found that IGF-1 was indirectly associated with bone mass accrual measured by peripheral quantitative computed tomography (pQCT) through stimulating muscle growth (10), and similarly Kindler et al concluded that lean mass was an intermediary factor in the IGF-1 bone relationship in 9- to 11-year-old girls (11).

Increasing evidence suggests that IGF-1 plays an important role in glucose metabolism (12). IGF-1 and insulin receptors share some homology and downstream signaling pathways, and insulin resistance may therefore have adverse effects on IGF-1–dependent processes. Studies have shown that obesity and increased insulin resistance during puberty may have a negative impact on bone mass and density in children (13-17). Insulin resistance has been proposed to be followed by "IGF-1 resistance" (18) and thereby it could be hypothesized that bone development is compromised both directly by the decreased proliferative effect of IGF-1 on osteoblasts (6) and also indirectly via suboptimal IGF-1–dependent muscle development in insulin-resistant children. One former study evaluated bone mass in the offspring of mothers with type 1 diabetes (T1D), who had a less favorable metabolic profile than controls, but they found no difference in areal bone mineral density (BMD) or volumetric BMD between offspring and controls (19).

The aim of the present study was to explore the association between IGF-1 and bone mineral content for height (BMC/height) including the mediating effect of

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lean mass and the moderating effect of insulin resistance in the Epigenetic, Genetic and Environmental Effects on Growth, Metabolism and Cognitive Functions in Offspring of Women with Type 1 Diabetes (EPICOM) cohort. In the EPICOM cohort, we studied 278 index offspring age 13.0 to 20.4 years whose mothers had T1D during pregnancy and an age-matched control group of 303 adolescents (20, 21). We have previously shown that the index cases had a less favorable metabolic profile and higher frequency of prediabetes than the control group. We therefore hypothesized that the index cases would have decreased bone mass due to lack of the anabolic effect of IGF-1 and insulin on osteoblast proliferation and that the IGF-1–bone mass relationship would be negatively influenced by insulin resistance.

Materials and Methods

A nationwide registry, with data on all pregnancies in women with T1D in Denmark from 1993 to 1999, was used to invite the offspring of mothers with T1D to participate in a follow-up study during 2012 to 2013. For the present study only singletons and only the first child per mother were included and 746 children of women with T1D (index children) from the original cohort were eligible for the follow-up examination and invited to participate in the study (reported in detail previously [20]). A total of 278 index offspring and 303 control individuals from the background population were included in the study. Maternal pregestational or gestational diabetes was an exclusion criterium in the group of control individuals. The protocol was in accordance with the Declaration of Helsinki and approved by the local ethics committee (M-20110239). The study was registered at Clinicaltrials.gov (ID: NCT01559181).

Clinical examination

The participants were examined in 3 university hospital settings in Denmark (Copenhagen, Odense, and Aarhus) from April 2012 until October 2013, and the participants had a mean age of 16.7 years (range, 13.0-20.4 years). Participants were studied after an overnight fast. Anthropometric measurements (height, weight, and waist circumference) were performed as previously described (20) and a standard 2-hour oral glucose tolerance test (OGTT) was performed after collection of fasting blood samples. Height, weight, and body mass index (BMI) SD scores (SDS) were calculated using normal Danish reference material (22), and pubertal development was evaluated by inspection and palpation according to Marshall and Tanner (23, 24).

Total body fat percentage, lean mass, BMC, and BMD were determined using dual-energy x-ray absorptiometry (DEXA). The DEXA scans were performed using a GE Healthcare Lunar Prodigy whole-body scanner (model DF+350646; GE Medical Systems) in Copenhagen; and a Hologic whole-body scanner model Discovery A (Odense) or Discovery W (Aarhus), as previously described (21). Bone mass increases during puberty mainly because of increased statural growth and thereby increase in bone size (25). We therefore used the size-adjusted bone mass: BMC divided by height (g/cm).

Biochemical analyses

Plasma glucose was measured using a hexokinase-glucose-6-phosphate dehydrogenase assay (Abbott Diagnostics). Serum insulin was measured by the enzyme-linked immunosorbent assay method using dual-monoclonal antibodies (ALPCO Diagnostics). Serum IGF-1 and insulin-like growth factor-binding protein 3 (IGFBP-3) concentrations were determined by chemiluminescence technology (Immunodiagnostics Systems [IDS]-iSYS IGF-I and IDS-iSYS IGFBP-3 assays, IDS Ltd) on the IDS-iSYS Multi-Discipline automated analyzer (IDS-iSYS). The level of detection for serum IGF-1 and IGFBP-3 were 10 ng/mL and 80 ng/mL, respectively. None of the measurements of IGF-1 and IGFBP-3 were below the level of detection in this cohort. IGF-1 (SDS) and IGFBP-3 (SDS) were calculated using a normal reference population (unpublished data). Insulin sensitivity was evaluated by the OGTT-derived model for assessment of insulin sensitivity index (BIGTT-SI0-30-120) (26) and the homeostatic model assessment of insulin resistance (HOMA-IR) (27). To assess β-cell function, we calculated the OGTT-derived index of acute insulin response (BIGTT-AIR-0-30-120) (26).

Statistical analyses

Histograms of all variables were evaluated for outliers and non-normal distribution. Nonnormal distributions were corrected by log (HOMA-IR, fat mass, lean mass) transformations. A linear model was fitted for each of the outcomes with index/control status as an independent variable reporting the differences between the groups as estimates with 95% CI and *P* values. Data were adjusted for sex, age (excluding SDS indices), and pubertal stage. Log-transformed data are presented as differences between the groups given as a percentage.

A path analysis (model 4 mediation as described by Preacher et al [28]) was performed to determine whether the significant association between IGF-1 and BMC/height

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was mediated through lean mass. Furthermore, we tested the moderating effect of HOMA-IR on the lean mass–mediated relationship between IGF-1 and BMC/height. BMC/height was regressed on lean mass, IGF-1, HOMA-IR, and IGF-1×HOMA-IR. Lean mass was regressed on IGF-I, HOMA-IR, and IGF-I×HOMA-IR. Pubertal staging was a covariate in all analyses. All *P* values less than .05 were considered statistically significant, and we used SPSS, version 25 (IBM Corp). Path analyses were performed using the SPSS PROCESS program (28).

Results

The entire cohort consisted of 581 participants (346 females): 278 index offspring and 303 control offspring. There was no difference in pubertal staging comparing the index cases to the controls (Pearson chi-square *P* = .49), and 90% of the participants were Tanner 4 or 5. BMC/height, lean mass, IGF-1, and HOMA-IR fluctuated throughout puberty (Fig. 1). BMC/height and lean mass increased from Tanner stage 2 to 5 (Fig. 1A), and the boys had higher BMC/height and lean mass than girls, especially in Tanner stages 4 and 5 (Fig. 1B). There was no difference for both sexes in height (SDS), BMC, BMC/height, or BMD between the index and control offspring adjusted for Tanner stage (Table 1). As previously shown, we found a higher weight (SDS), BMI (SDS), fat percentage, and HOMA-IR in the index group compared to controls (see Table 1), but this reached significance only among the girls. Insulin resistance determined by HOMA-IR was stable throughout puberty both in boys and girls, with a slightly higher HOMA-IR in girls than in boys (Fig. 1D). Insulin sensitivity determined by BIGTT-SI and insulin secretion determined by BIGTT-AIR were also stable throughout puberty for both sexes (data not shown), and BIGTT-SI was lower in the index group compared to controls (see Table 1). BIGTT-AIR was higher among the index cases, but this was significant only among the girls (see Table 1). Serum IGF-1 concentrations increased from Tanner 2 to 3 in girls and then declined during Tanner stages 4 and 5, with lower levels in girls than in boys (Fig. 1C). The boys had stable IGF-1 levels through the Tanner stages. IGF-1 (SDS) did not differ between the groups (see Table 1) but IGFBP-3 (SDS) was higher in index boys compared to controls (B = .31 [95% CI, 0.06-0.57], *P* = .02) (see Table 1). There was no difference in IGF-1 (SDS) and IGFBP-3 (SDS) between index and control girls.

In the path model, adjusting for pubertal stage and sex, IGF-1 (SDS) predicted lean mass (B = .008 [95% CI, 0.002-0.01], *P* = .01) in the entire cohort, which in turn predicted BMC/height (B = 19.6 [95% CI, 16.9-22.3], *P* < .0001) (Fig. 2). IGF-1 (SDS) predicted BMC/height adjusted for pubertal stage (B = .24 [95% CI, 0.02-0.45], *P* = .03) (see Fig. 2), but when testing lean mass as a mediator of this relationship, we found that the indirect effect of IGF-1 (SDS) on BMC/height was no longer significant (B = –.09 (–0.2 to 0.1), *P* = .60) (see Fig. 2). The path model was performed on the index group and controls separately. The findings were similar to the findings for the entire cohort with significant associations between IGF-1 and lean mass in each group, and in both groups the association between IGF-1 and BMC/height was mediated by lean mass (data not shown).

In the entire cohort, serum IGF-1 (SDS) was positively associated with insulin resistance (HOMA-IR) (B = .75 [95% CI, 0.37-1.12], *P* < .0001) and negatively associated with BIGTT-SI-0-30-120 (B = –.06 [95% CI, –0.08 to –0.03], *P* < .0001). IGF-1 (SDS) was positively associated with insulin secretion determined by BIGTT-AIR-0-30-120 (B = .59 [95% CI, 0.15-1.02], *P* = .009), reflecting that IGF-1 concentrations are negatively associated with insulin sensitivity and positively associated with insulin secretion. All analyses were adjusted for pubertal stage and sex. IGF-1 (SDS) was not associated with fat mass (adjusted for sex and pubertal stage) (B = .22 [95% CI, –0.15 to 0.07], *P* = .32). However, fat mass was associated both with BMC/height (B = 3.8 [95% CI, 2.9-4.7], *P* < .0001) and lean mass (B = 19.2 [95% CI, 16.7-21.7] *P* < .0001) (adjusted for pubertal stage and sex).

In the entire cohort, HOMA-IR was not significantly associated with BMC/height (B = –.30 [95% CI, –1.19 to 0.59], *P* = .51), and the results did not change when dividing the cohort according to sex. The moderating effect of the interaction between insulin resistance and IGF-1 (HOMA-IR×IGF-1) on lean mass was calculated (see Fig. 2). IGF-1 (SDS) and HOMA-IR (B = –.04 [95% CI, –0.08 to –0.01], *P* = .02) were both significantly associated with lean mass, but the interaction was not significant (B = .008 [95% CI, –0.02 to 0.03], *P* = .60), indicating that moderation was not present (Table 2). The moderating effect of HOMA-IR remained nonsignificant when dividing the cohort according to index and controls (data not shown).

Discussion

In this large cohort of offspring of mothers with T1D, we found no difference in serum IGF-1, bone mass, and muscle mass compared to controls, whereas fat mass was significantly increased among female offspring of women with T1D. In the entire cohort, we found a strong positive relation between IGF-1 and insulin resistance, but no effect of insulin resistance on bone mass. In contrast, IGF-1 levels were associated with BMC, and this association was

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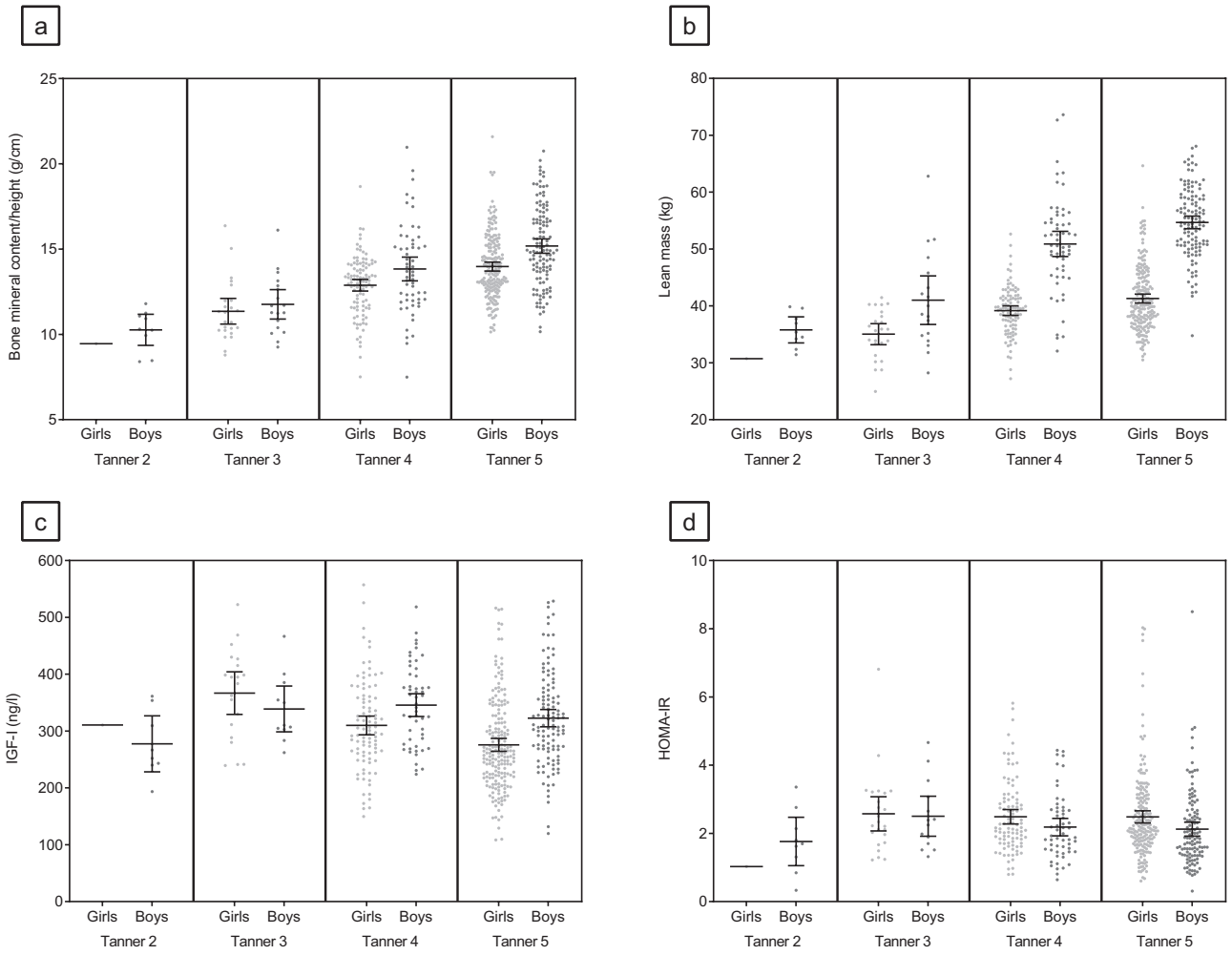

Figure 1. The cohort divided according to sex (girls: light gray dots, boys: dark gray dots) and Tanner stages 2 to 5: A, Bone mineral content/height (g/cm); B, lean mass (kg); C, insulin-like growth factor-1 (IGF-1) concentration (ng/L); and D, homeostatic model assessment of insulin resistance (HOMA-IR).

mediated by lean mass. In conclusion, lean mass was an intermediary factor in the IGF-1 bone relationship, which was not modulated by insulin resistance. Therefore, we can refute our primary hypothesis that insulin resistance negatively influences the muscle-dependent IGF-I–bone axis in neither the entire cohort nor when index cases and controls were analyzed separately.

During pubertal development transient insulin resistance follows the increased activity in the GH/IGF-I axis by an increase early in puberty, reaching the highest levels at Tanner stages 3 to 4 and decreasing thereafter (2, 3). The reduction in insulin sensitivity is more pronounced among girls, but can only partly be explained by increasing adiposity (1). In the present cohort of healthy adolescents, we found that IGF-1 levels especially among girls peaked at Tanner 3 and declined thereafter. Insulin resistance determined by HOMA-IR was stable throughout the pubertal stages both for boys and girls and did not follow the pattern of IGF-1. This may reflect that index cases and controls are both presented in the same figure. Our previous analyses on metabolism in this cohort showed that the difference in insulin sensitivity between the index cases and the controls increased with age (21), which thereby could explain the lack of decline in insulin resistance at Tanner 5 in the entire cohort. As reported previously, our cohort of offspring of mothers with T1D had a higher prevalence of components included in metabolic syndrome and prediabetes with reduced insulin sensitivity and relative insulin secretion deficiency compared with controls (20).

IGF-1 exerts anabolic effects on the skeleton by promoting osteoblastogenesis and inhibiting osteoblast apoptosis (29), increases osteoclastogenesis (30), bone resorption, and bone remodeling, and is key to acquisition of bone mass during adolescence (31). In the EPICOM cohort of adolescents, we found a significant correlation between IGF-1 and bone mass (BMC/height). However, in the causal path analysis, we found that this correlation disappeared when including lean mass, pointing toward lean mass as

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Table 1. Anthropometrics, metabolic characteristics and body composition in index and control offspring

	Female		Male		Female			Male		
	Control (n = 182)	Index (n = 164)	Control (n = 121)	Index (n = 114)	Diff B	95% CI	*P*	Diff B	95% CI	*P*
Age, y	17.0 (1.7)	16.9 (1.6)	16.6 (1.6)	16.5 (1.6)	–0.21	(–0.6 to 1.6)	.27	–0.01	(–0.45 to 0.42)	.96
Weight, SDS	0.29 (1.1)	0.76 (1.4)	0.04 (1.1)	0.36 (1.1)	0.14	(0.22 to 0.78)	< .001	0.22	(–0.08 to 0.53)	.15
Height, SDS	–0.06 (0.9)	–0.24 (1.1)	0.25 (1.0)	0.39 (1.1)	–0.14	(–0.35 to 0.08)	.22	0.17	(–0.11 to 0.45)	.24
BMI, SDS	0.51 (1.1)	1.06 (1.3)	–0.09 (1.1)	0.24 (1.0)	0.55	(0.29 to 0.81)	< .001	0.22	(–0.07 to 0.52)	.15
IGF-1, SDS	–0.52 (0.8)	–0.45 (1.0)	–0.32 (0.8)	–0.14 (0.8)	0.06	(–0.13 to 0.25)	.54	0.18	(–0.06 to 0.41)	.14
IGFBP-3, SDS	0.34 (1.1)	0.40 (1.19)	0.38 (1.0)	0.65 (0.8)	0.09	(–0.16 to 0.34)	.48	0.31	(0.06 to 0.57)	.02
HOMA-IR	2.13 (1.8 to 3.0)	2.36 (1.7 to 3.1)	1.85 (1.4 to 2.4)	2.10 (1.5 to 2.7)	5.8	(1.6 to 10.0)	.007	4.7	(–1.5 to 10.5)	.12
BIGTT-IS	9.4 (3.4)	7.9 (3.6)	9.67 (3.0)	8.29 (2.9)	–1.6	–2.4 to –0.8)	< .001	–1.23	(–2.1 to –0.4)	.004
BIGTT-AIR	1791(1453 to 2198)	1992 (1573 to 2645)	1682 (1431 to 2082)	1838 (1503 to 2421)	0.05	(0.004 to 0.09)	.03	0.03	(–0.02 to 0.08)	.26
BMC, g	2234 (369)	2261 (390)	2539 (552)	2577 (517)	–9.4	(–84.7 to 65.8)	.81	30.1	(–98 to 158)	.64
BMD, g/cm^2	1.10 (0.1)	1.09 (0.1)	1.12 (0.1)	1.12 (0.1)	–0.01	(–0.03 to 0.01)	.29	–0.003	(–0.03 to 0.03)	.86
BMC/height	13.3 (2.0)	13.6 (2.0)	14.2 (2.7)	14.4 (2.6)	0.03	(–0.37 to 0.43)	.88	0.11	(–0.53 to 0.76)	.73
Fat, %	31.4 (27 to 35)	34.8 (29 to 40)	16.8 (13 to 22)	17.2 (15 to 25)	7.2	(3.9 to 10.5)	< .001	3.6	(–2.4 to 9.5%)	.24
Lean mass, kg	39.5 (37 to 43)	39.8 (36 to 43)	52.4 (45 to 57)	53.8 (49 to 58)	1.0	(1.1 to 1.3)	.83	0.9%	(–0.8 to 2.7)	.29

Data are presented as means (SD) or as medians (25th-75th percentile) if skewed distribution. Differences between groups are reported as estimates from linear regression B with 95% CI and *P* values. Data with skewed distributions are log-transformed and differences are given as percentage difference. All variables except age are adjusted for sexual maturation.

Abbreviations: BMC, bone mineral content; BMC/height, bone mineral content for height; BMD, bone mineral density; BMI, body mass index; BIGTT-AIR, oral glucose tolerance test–derived index of acute insulin response; BIGTT-IS, oral glucose tolerance test–derived index of insulin sensitivity; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein 3; SDS, SD score.

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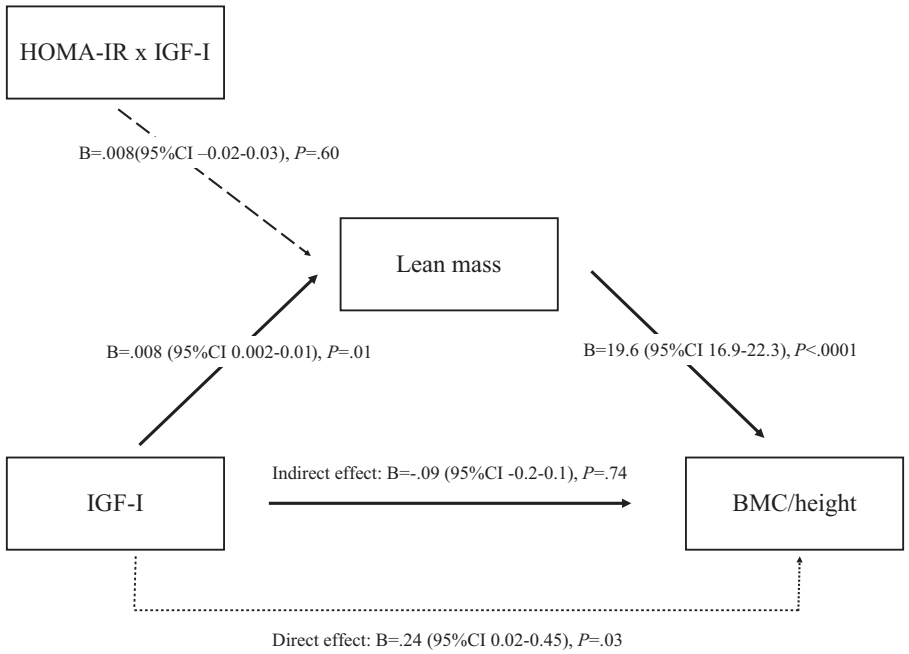

Figure 2. The dotted line represents the direct effect of insulin-like growth factor-1 (IGF-1) on bone mineral content for height (BMC/height) in the entire cohort. The solid lines represent the model with lean mass as a mediator of the relationship between IGF-1 and BMC/height. Unstandardized regression coefficients are presented as B (95% CI). All analyses include pubertal stage and sex as covariates. The broken line indicates the effect of the interaction between IGF-1 and homeostatic model assessment of insulin resistance (HOMA-IR) on lean mass.

Table 2. Linear regression analysis to determine the effect of interaction between insulin-like growth factor-1 and homeostatic model assessment of insulin resistance on lean mass

	B (95% CI)	SE	*P*
Constant	1.47 (1.42 to 1.52)	0.02	*P* < .0001
IGF-1, SDS	0.019 (0.01 to 0.03)	0.004	*P* < .0001
Log HOMA-IR	–0.04 (–0.08 to –0.01)	0.02	*P* = .02
IGF-1 (SDS)×log HOMA-IR	0.005 (–0.03 to 0.04)	0.02	*P* = .81

Data are reported as estimates from linear regression (B) with 95% CI, SE, and *P* values.

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor-1; SDS, SD score.

an intermediary factor in the IGF-1–bone relationship in line with former studies (10, 11). A study of girls age 9 to 11 years by Kindler et al found that the HOMA-IR by IGF-1 interaction negatively predicted lean mass, and this moderating effect of HOMA-IR was stronger in participants with a better insulin sensitivity (HOMA-IR < 4.0) (11). When testing the moderating effect of the interaction between insulin resistance and IGF-1 on lean mass in the EPICOM cohort, we found there was no effect of insulin resistance, indicating that the effect of IGF-1 on skeletal muscle is independent of the sensitivity to insulin in adolescents. Our cohort was older and included both sexes compared to the cohort studied by Kindler and colleagues. Age and sexual maturation may play a significant role for this moderating effect in addition to sex, but our analysis of the moderating effect was still nonsignificant when dividing the cohort according to sex. Our present study confirmed the results of a study by Mughal et al, who examined 67 offspring of mothers with T1D with DEXA and pQCT) and found that they had higher bone area and BMC compared to controls (19). However, areal BMD and volumetric BMD did not differ between offspring and controls, indicating that the offspring had larger bones compared to the controls but the mineral content per unit area or volume did not differ. This cohort were smaller than our cohort but, in many ways, was comparable as the offspring had a higher BMI and a significantly higher fat percentage than controls, but this study did not include data on insulin sensitivity.

In the present study, insulin resistance and IGF-1 were positively associated in the entire cohort. Insulin promotes hepatic IGF-1 production, and additionally IGF-1 shares structural homology with insulin; they can bind the same receptors, but with major differences in affinity, and they share downstream signaling pathways (32). In studies of GH treatment of children born small for gestational age, we and others found that insulin resistance and IGF-1 levels were positively associated and that growth response during GH treatment was negatively associated with insulin resistance (33-35). In the EPICOM study we found no difference in height (SDS) between the index cases and controls. However, among the boys the

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index cases had higher IGF-1 (SDS) and IGFBP-3 (SDS) levels compared to the controls, but only the difference in IGFBP-3 (SDS) reached significance. IGFBP-3 is 1 of 6 binding proteins that bind IGF-1 and thereby modulate IGF bioavailability. Traditionally, IGFBP-3 has been thought to be involved in metabolic regulation due to the binding of IGF-1, but recently studies have reported that IGFBP-3 may have a metabolic role independent of the IGF axis. However, the exact role of IGFBP-3 in glucose and lipid metabolism is still poorly understood. Overexpression of IGFBP-3 in transgenic mice resulted in fasting hyperglycemia, glucose intolerance, and insulin resistance (36), whereas data on IGFBP-3 knockout mice have been inconsistent. One study found that IGFBP-3 knockout mice who were fed a high-fat diet showed fasting hyperglycemia and hyperinsulinemia, indicating insulin resistance (37). Furthermore, in vitro studies have suggested that IGFBP-3 may lead to insulin resistance in adipocytes, and one study found that IGFBP-3 inhibited adipocyte differentiation (38). Adipocyte differentiation is required to mediate insulin sensitivity in adipocytes, and a possible inhibitory effect of IGFBP-3 on adipocyte differentiation could thereby lead to insulin resistance. Thus, the exact role of IGFBP-3 in metabolic regulation is not well understood, but it could be speculated that the increased concentrations of IGFBP-3 in the index boys in our cohort could play a role in adipocyte differentiation and thereby influence insulin sensitivity. Previously published results on adipokines from the EPICOM study showed that both male and female offspring of women with T1D had increased serum leptin and leptin/adiponectin ratio compared to controls, whereas serum adiponectin was reduced in females only. However, no direct association between maternal glycemic control during pregnancy and adiponectin and leptin levels or leptin/adiponectin ratio in the offspring was found (39).

Several studies have shown that obesity and insulin resistance during childhood may have a negative impact on bone mass and bone density (15, 16) and some reveal a sex difference (17), but many other studies find that overweight children have similar or even greater bone mass (13, 40). However, data are divergent and when adjusting for lean body mass, adiposity seems to be a negative predictor of bone mass during childhood (13, 40). However, in the present analyses we found that fat mass was positively associated with BMC/height even after adjusting for lean mass and pubertal stage. Detrimental effects on skeletal health in obese children may reflect a global health concern because the prevalence of childhood obesity is rapidly increasing worldwide. In addition, the EPICOM cohort represents a cohort of children whose mothers had T1D during pregnancy, which may have caused intrauterine hyperglycemia, hyperinsulinemia, and overgrowth. The effect of an adverse intrauterine environment on the risk of metabolic disease later in life is known as the *fetal programming effect*. In the EPICOM cohort we previously showed a programming effect on the metabolic risk as offspring of mothers with T1D had an adverse metabolic profile compared to controls (20, 21). However, the present data do not reveal a programming effect on skeletal health later in life, which confirms former studies suggesting birth weight and adult bone metabolism are unrelated when adjusting for size in adulthood (41).

A major strength of this study is that the cohort of adolescents is large and well characterized. We applied the statistical path analysis to explore the relationship between IGF-1 and bone mass and the modulating effect of insulin resistance. However, taking puberty into account a cross-sectional design is not optimal, and a longitudinal follow-up study through puberty would be necessary to explore the significant effects of puberty on the interaction between IGF-1, insulin sensitivity, and bone mass. Furthermore, the DEXA scan is a 2-dimensional measurement of bone mass, and a more thorough analysis of the microarchitecture of the bone using measurements such as pQCT would give more detailed information on the trabecular bone structure.

The findings in this cohort are considered to be generalizable to other cohorts of adolescents, but this cohort is homogeneous, consisting of White Danish children with a somewhat higher genetic height potential, which may theoretically affect the comparability of the cohort. However, we believe that taking height into consideration when evaluating bone mass will diminish this possible bias.

In conclusion, we here present that lean mass was an intermediary factor in the IGF-1 bone relationship in a large cohort of adolescents. We did not confirm previous findings in which the muscle-dependent relationship between IGF-1 and bone mass was found to be compromised by insulin resistance (11). The programming effect of a detrimental intrauterine environment was evident on insulin resistance and fat mass, but we found no difference in bone mass, IGF-1, or height between the adolescents exposed to T1D during pregnancy and the control participants. However, it may be speculated that the changes in insulin metabolism and adiposity in adolescence may have long-term harmful effects both on metabolic and skeletal health.

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Data Availability: The data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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