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Adiposity in SGA children is associated with genetic variants for insulin resistance and response to GH treatment

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Abstract:	Background: Genetic susceptibility to insulin resistance is associated with lower adiposity in adults. Insulin resistance, and therefore adiposity, may alter sensitivity to Growth Hormone (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 (55 boys) short prepubertal SGA children (age,6.2±1.6years) treated with GH for one year in a multicentre study, body fat percentage was estimated at baseline and 1-year using DXA. The main outcome measures were treatment-related changes in height, IGF-1 standard deviation scores (SDS), insulin sensitivity, insulin secretion and disposition index. Combined multiallele gene scores based on single nucleotide polymorphisms with known associations with lower insulin sensitivity (GS-InRes) and insulin secretion (GS-InsSec) were analysed for their relationships with adiposity. Results: Mean percentage body fat at baseline was low compared to normative data

	(p=0.045), and decreased even further on GH treatment (baseline vs 1-year z-scores, -0.26±1.2 vs -1.23±1.54; p<0.0001). Baseline percentage body fat was positively associated with IGF-1 responses (p-trends=0.042), first-year height gains (B[95%CI]:0.61cm/year [0.28,0.95]; p<0.0001), insulin secretion at baseline (p-trends=0.020) and at 1-year (p-trends=0.004), and disposition index at 1-year (p-trends=0.024). GS-InRes was inversely associated with BMI (-0.13SDS per-allele [-0.26,-0.01]; p=0.040), body fat (-0.49% per-allele [-0.97,-0.007]; p=0.047), and limb fat (-0.81% per-allele [-1.62,0.00]; p=0.049) 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=0.001) and a higher trunk-limb fat ratio at 1-year (0.04 per-allele [0.01,0.08]; p=0.008). GS-InSec was positively associated with truncal fat (0.36% per-allele [0.09, 0.63]; p=0.009). Conclusions: Adiposity in SGA children has favourable effects on GH sensitivity and glucose metabolism. The associations with multiallele scores support a causal role of insulin resistance in linking lower body fat to reduced sensitivity to exogenous GH.
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Title: 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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Abstract:

Background: Genetic susceptibility to insulin resistance is associated with lower adiposity in adults. Insulin resistance, and therefore adiposity, may alter sensitivity to Growth Hormone (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 (55 boys) short prepubertal SGA children (age,6.2±1.6years) treated with GH for one year in a multicentre study, body fat percentage was estimated at baseline and 1-year using DXA. The main outcome measures were treatment-related changes in height, IGF-1 standard deviation scores (SDS), insulin sensitivity, insulin secretion and disposition index. Combined multiallele gene scores based on single nucleotide polymorphisms with known associations with lower insulin sensitivity (GS-InRes) and insulin secretion (GS-InsSec) were analysed for their relationships with adiposity.

Results: Mean percentage body fat at baseline was low compared to normative data (p=0.045), and decreased even further on GH treatment (baseline vs 1-year z-scores, -0.26±1.2 vs -1.23±1.54; p<0.0001). Baseline percentage body fat was positively associated with IGF-1 responses (p-trends=0.042), first-year height gains (B[95%CI]:0.61cm/year [0.28,0.95]; p<0.0001), insulin secretion at baseline (p-trends=0.020) and at 1-year (p-trends=0.004), and disposition index at 1-year (p-trends=0.024). GS-InRes was inversely associated with BMI (-0.13SDS per-allele [-0.26,-0.01]; p=0.040), body fat (-0.49% per-allele [-0.97,-0.007]; p=0.047), and limb fat (-0.81% per-allele [-1.62,0.00]; p=0.049) 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=0.001) and a higher trunk-limb fat ratio at 1-year (0.04 per-allele [0.01,0.08]; p=0.008). GS-InSec was positively associated with truncal fat (0.36% per-allele [0.09, 0.63]; p=0.009).

Conclusions: Adiposity in SGA children has favourable effects on GH sensitivity and glucose metabolism. The associations with multiallele scores support a causal role of insulin resistance in linking lower body fat to reduced sensitivity to exogenous GH.

Introduction:

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 the majority of 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 gestation (AGA) (2,3). The phenotype of low adiposity is not an expected consequence of Growth Hormone (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 licenced 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 hypothesised to underlie the increased risk for development of type 2 diabetes (T2D) in low birth-weight individuals (7). The close relationship between the actions of 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 randomisation 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 a 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.

Methods:

Study Population:

The subjects were from the North European Small for Gestational Age Study (NESGAS), a multi-centre study of GH treatment in short prepubertal SGA children involving 9 investigating centres in 4 North European countries (Denmark, Ireland, Sweden, and UK) 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 aged between 4 and 9 years and the boys between 4 and 10 years. During the first year, children were treated with a uniform high dose of GH (67µg/kg/day) 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 centre and 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 at every 3 months when anthropometry and pubertal assessments were undertaken and serum IGF-1 levels were measured. They also underwent a short intravenous glucose tolerance test (IVGTT) at baseline and at 1-year to evaluate insulin sensitivity and secretion (8).

DXA scans: Body composition was assessed by dual-energy X-ray absorptiometry (DXA) scans using Hologic QDR-1000/W scanner (Hologic Inc., Waltham, MA) (3 centres, n=39) or Lunar Prodigy DXA system (GE Medical Systems) (6 centres, n=50) at baseline and at 1-year. In one centre, 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 analysed 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 centres were 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: 4, scans not carried out: 17) and 85 children at 1-year (incomplete data: 1, 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 (SNPs) 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-1a and1b) (10). Both combined multiallele scores have been validated in large population-based studies (11).

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 BMI were derived using country-specific references (8). Insulin sensitivity was estimated from fasting glucose and C-peptide levels using the homeostatic model (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 IVGTT 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 by dividing the trunk fat by limb fat. We expressed the body fat as the percentage of total mass as it provided an estimate of adiposity independent of body size and calculated using the formula: percentage fat in a region = fat mass of the region (kg) x100/ 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 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 categorised 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 start of treatment, GH dose and midparental height SDS. Associations between adiposity and multiallele scores were explored using regression models which 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 lower mean percentage body fat (*z-scores*$_p$, -0.26±1.2, p=0.045) and BMI (-1.29±1.37 SDS, p<0.0001) compared with healthy Caucasian children (12,15) (Table-1, 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<0.05) and male vs. female gender (r=0.66, p<0.0001). 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=0.04), percentage fat in trunk and limbs (all p-trends <0.0001), and trunk-limb fat ratio (p-trends=0.019). 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=0.02) (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<0.0001) and bone mineral content (p<0.0001) (Table-1). Conversely, total body fat mass and limb fat mass declined (both p<0.0001) 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 fat in the whole body, limbs and trunk (all p<0.0001) (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, LDL or HDL- 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=0.005), trunk (p-trends=0.0001) and limbs (p-trends=0.002) (Table-2, 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=0.001), trunk (p-trends<0.0001) and limbs (p-trends=0.057).

Height and IGF-1 response: Increase in height SDS was positively associated with baseline percentage body fat *z-scores*$_c$ (p-trends=0.038). 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 ($p<0.0001$, $R^2=0.27$) (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 %CI]: 0.77cm/year [0.37, 1.17], $p<0.0001$, $R^2= 0.25$) compared with trunk fat z-scores (0.61cm/year [0.24, 0.98], $p=0.001$, $R^2= 0.22$) 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]=0.031). Higher total body percentage body fat *z-scores*$_c$ were associated with greater IGF-1 responses (p-trends=0.042) and IGF-1 levels at 1-year (p-trends=0.036). 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]=0.013) (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 ($r=0.47$, $p<0.0001$), but they were not related to IGF-1 responses. Decreases in the limb fat percentage ($r=0.41$, $p=0.001$) were more strongly related to height gains compared with the decreases in the trunk fat percentage ($r=0.25$, $p=0.053$) 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=0.014) resulting in higher AIR (p-trends=0.004) and disposition index (p=0.024) at 1-year (Figure-2E&2F). No differences were observed in the changes in fasting lipids between the tertile groups (data not shown).

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=0.040) and percentage fat in the whole body (-0.49% per-allele [-0.97, -0.007], p=0.047) and limbs (-0.81% per-allele [-1.62, 0.00], p=0.049), but not in trunk (Table-4). During GH treatment, a higher GS-InRes was associated with lesser declines in total body fat (0.31% per-allele [0.10, 0.51], p=0.004) and trunk fat (0.38%

per-allele [0.16, 0.59], p=0.001), and therefore increases in trunk-limb fat ratio (0.03 per-allele [0.01, 0.05], p=0.003). At 1-year, GS-InRes was still inversely associated with percentage fat in the limbs (-0.81% per-allele [-1.49, -0.13], p=0.020) and positively associated with trunk-limb fat ratio (0.04 per-allele [0.01, 0.08], p=0.008).

Insulin secretion: GS-InSec was positively associated with percentage trunk fat at baseline (0.36% per-allele [0.09, 0.63], p=0.009) and at 1-year (0.25% per-allele [0.01, 0.50], p=0.045) (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 pre-treatment 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 MRI 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 signalling 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, as 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-I axis ranging from relative GH deficiency to resistance may also explain these associations. However, overall leanness of these children as a group and that adiposity is unrelated to IGF-1 levels or insulin sensitivity suggest that they are less likely to have a primary role (2). Baseline adiposity predicted height gains independent of IGF-1 responses, which imply 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 GHD 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 GHD patients (6,26). We postulate that the pattern of fat loss in SGA children results from further declines in energy stores as 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 support 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 (67µg/kg) was greater than that (21%) reported on the more common lower GH dose (35µg/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 hypoglycaemia 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 as thinness during childhood is related to an increased risk for T2D (31).

Following an initial marked decrease, body fat is reported to return to pre-treatment 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 gluteofemoral region are shown to store triglycerides long-term more efficiently compared with the trunk fat and linked to favourable 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 nutrient 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 optimise 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 Randomisation 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 (Supplementary 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 this 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 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, and age and gender in the study were not clear(15). We speculate that comparisons to normative data from a different type of DXA scanner is an important reason and may underlie the higher pre-treatment 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 at 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. While 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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Figure Legends

Figure-1: Changes in body fat during Growth Hormone 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 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 ($z\text{-}scores_p$); p-values (*) are from the comparison between baseline and 1-year measurements; **, $p<0.001$ and ***, $p<0.0001$;

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 Growth Hormone treatment.

Total body fat percentage (A), change in IGF-1 SDS (B) and height SDS (C), insulin sensitivity as HOMA % (log) (D), insulin secretion as log of acute insulin response (E) and Disposition Index (log) (F)**.** Bars represent means and error bars the standard error of the means. Black and empty bars represent measurements at baseline and 1 year respectively; grey bars represent changes in measurements from baseline to 1 year. # tertiles of z-scores for total body fat percentage derived within the cohort ($z\text{-}score_c$), * represents p-trends across the tertile groups; *, $p<0.05$, **, $p<0.01$ and ***, $p<0.001$. In y-axes with log-transformed values, a break has been introduced (Figures E-F) to display the error bars and trends more clearly.

Table -1: Body composition and metabolism during first year of Growth Hormone treatment

	Baseline	1-year	P value
Anthropometry			
Height SDS	-3.35 (0.74)	-2.31 (0.69)	**<0.0001**
Weight SDS	-3.10 (1.03)	-2.12 (1.00)	**<0.0001**
BMI (kg/m^2)	14.16 (1.49)	14.68 (1.62)	**<0.0001**
BMI SDS	-1.34 (1.38)	-0.96 (1.29)	**<0.0001**
Body Composition (DXA)			
Total lean mass (kg)	11.5 (2.66)	15.6 (3.45)	**<0.0001**
Bone Mineral Content (g)	457 (166)	606 (188)	**<0.0001**
Total body fat mass (kg)	2.26 (1.06)	2.06 (1.12)	**0.007**
Trunk fat mass (kg)	0.68 (0.37)	0.72 (0.41)	0.13
Limbs fat mass (kg)	1.10 (0.68)	1.00 (0.67)	**0.0002**
Total body fat (%)	15.8 (5.80)	11.2 (4.70)	**<0.0001**
total body fat % (z-score) *	-0.26 (1.21)	-1.23 (1.54)	**<0.0001**
Trunk fat (%)	10.6 (4.66)	8.63 (4.03)	**<0.0001**
Limb fat (%)	23.1 (9.70)	14.6 (7.70)	**<0.0001**
Trunk-limb fat ratio	0.61 (0.20)	0.84 (0.32)	**<0.0001**
Biochemistry			
IGF- I (SDS)	-1.09 (1.28)	2.88 (1.52)	**<0.0001**
Glucose (mmol/l)	4.32 (0.66)	4.70 (0.55)	**<0.0001**
Insulin (pmol/l) (log)	1.19 (0.28)	1.59 (0.22)	**<0.0001**
C-peptide (pmol/l) (log)	2.30 (0.24)	2.61 (0.17)	**<0.0001**
Insulin sensitivity (HOMA) (log)	2.38 (0.25)	2.06 (0.17)	**<0.0001**
Acute Insulin Response (log)	3.13 (0.24)	3.39 (0.26)	**<0.0001**
Disposition Index (log)	5.51 (0.24)	5.46 (0.23)	0.11
Total Cholesterol (mmol/L)	3.94 (0.72)	3.88 (0.70)	0.38
LDL Cholesterol (mmol/L)	2.23 (0.63)	2.15 (0.58)	0.11
HDL Cholesterol (mmol/L)	1.47 (0.35)	1.42 (0.33)	0.070
Triglycerides (mmol/L)	0.64 (0.33)	0.83 (0.40)	**0.001**

Data presented as means (SD)
* Z-scores for percentage body fat calculated based on normative data (*z-score*$_p$)

Table-2: Body composition, glucose metabolism and response to Growth Hormone treatment in patients categorised by tertiles of z-scores* for total body fat percentage at baseline

	Tertiles of baseline total body fat			P Trends
	Low	Middle	High	
Baseline				
n (male)	30 (19)	29 (19)	30 (17)	NS
Age (years)	6.04 (1.53)	5.95 (1.50)	6.50 (1.72)	0.36
Height (SDS)	-3.30 (0.60)	-3.53 (0.85)	-3.26 (0.73)	0.32
Weight (SDS)	-3.41 (0.79)	-3.24 (0.93)	-2.71 (1.23)	**0.025**
BMI (kg/m^2)	13.65 (1.12)	14.3 (0.91)	14.66 (2.06)	**0.035**
BMI (SDS)	-1.73 (1.14)	-1.10 (0.91)	-0.89 (1.69)	**0.040**
Total body fat (%)	11.9 (4.90)	16.0 (4.20)	20.5 (4.80)	**<0.0001**
Total body fat % (*z-score*)*	-0.88 (0.36)	-0.17 (0.19)	0.89 (0.51)	**<0.0001**
Trunk fat (%)	7.27 (2.51)	9.72 (2.42)	15.8 (4.26)	**<0.0001**
Limb fat (%)	17.8 (8.80)	24.0 (8.60)	28.0 (7.90)	**<0.0001**
Trunk-limb fat ratio	0.59 (0.18)	0.56 (0.19)	0.70 (0.20)	**0.019**
IGF-I (SDS)	-1.12 (1.09)	-1.18 (1.35)	-1.00 (1.40)	0.87
Glucose (mmol/L)	4.17 (0.61)	4.34 (0.64)	4.47 (0.72)	0.22
Insulin (pmol/L) (log)	1.15 (0.26)	1.26 (0.23)	1.27 (0.26)	0.14
C-peptide (pmol/L) (log)	2.26 (0.24)	2.35 (0.23)	2.32 (0.23)	0.41
HOMA Insulin sensitivity (%) (log)	2.42 (0.26)	2.33 (0.24)	2.36 (0.25)	0.38
Acute insulin response (log)	3.04 (0.23)	3.18 (0.20)	3.21 (0.26)	**0.020**
Disposition index (log)	5.46 (0.26)	5.51 (0.21)	5.57 (0.26)	0.29
1-year				
Height (SDS)	-2.36 (0.56)	-2.42 (0.81)	-2.17 (0.70)	0.36
Weight (SDS)	-3.41 (0.79)	-3.24 (0.93)	-2.71 (1.23)	**0.010**
BMI (kg/m^2)	14.1 (1.20)	14.6 (1.17)	15.3 (2.13)	**0.017**
BMI (SDS)	-1.30 (1.16)	-1.04 (0.96)	-0.47 (1.69)	0.12
Total body fat (%)	8.97 (4.06)	11.1 (4.19)	13.8 (4.77)	**0.001**
Trunk fat (%)	6.13 (1.82)	8.73 (4.41)	11.1 (3.82)	**<0.0001**
Limb fat (%)	12.1 (7.60)	14.6 (7.70)	17.2 (7.30)	0.057
Trunk-limb fat ratio	0.85 (0.38)	0.81 (0.31)	0.85 (0.26)	0.87
IGF-I (SDS)	2.57 (1.34)	2.63 (1.61)	3.46 (1.47)	**0.036**
Glucose (mmol/L)	4.62 (0.49)	4.64 (0.60)	4.78 (0.58)	0.48
Insulin (pmol/L) (log)	1.54 (0.23)	1.59 (0.20)	1.65 (0.21)	0.19
C-peptide (pmol/L) (log)	2.58 (0.16)	2.62 (0.17)	2.63 (0.19)	0.59
HOMA Insulin sensitivity (%) (log)	2.09 (0.17)	2.05 (0.17)	2.03 (0.19)	0.55
Acute insulin response (log)	3.27 (0.22)	3.45 (0.22)	3.48 (0.28)	**0.004**
Disposition index (log)	5.36 (0.23)	5.50 (0.22)	5.52 (0.22)	**0.024**
Changes from baseline to 1-year				
Delta Height (SDS)	0.94 (0.33)	1.04 (0.22)	1.14 (0.31)	**0.038**
Delta Weight (SDS)	1.02 (0.40)	1.03 (0.34)	1.05 (0.49)	0.96
Delta BMI (kg/m^2)	0.46 (0.46)	0.47 (0.80)	0.72 (0.72)	0.064
Delta BMI (SDS)	0.48 (0.45)	0.45 (0.46)	0.40 (0.54)	0.84
Delta total body fat (%)	-2.94 (1.38)	-3.88 (1.61)	-5.30 (2.99)	**0.001**
Delta trunk fat (%)	-0.90 (1.56)	-1.76 (1.79)	-3.61 (2.83)	**<0.0001**
Delta limb fat (%)	-5.47 (2.90)	-7.80 (3.36)	-9.33 (4.88)	**0.003**
Delta trunk-limb fat ratio	0.27 (0.33)	0.18 (0.18)	0.11 (0.18)	0.19
Delta IGF-I (SDS)	3.69 (1.32)	3.80 (1.45)	4.17 (1.35)	**0.042**
Delta glucose (nmol/L)	0.45 (0.55)	0.30 (0.48)	0.38 (0.46)	0.49
Delta insulin (pmol/L) (log)	1.99 (0.09)	1.98 (0.09)	2.02 (0.08)	0.27
Delta C-peptide (pmol/L) (log)	2.81 (0.15)	2.73 (0.49)	2.84 (0.09)	0.47
Delta HOMA- Insulin Sensitivity (%) (log)	3.21 (0.10)	3.24 (0.05)	3.23 (0.04)	0.35
Delta Acute Insulin Response (Log)	3.27 (0.29)	3.40 (0.21)	3.48 (0.18)	**0.014**
Delta Disposition Index (Log)	5.74 (1.10)	5.99 (0.08)	5.96 (0.15)	0.43

Data presented as means (SD). * within cohort z-scores (*z-score*$_c$) for total body fat percentage at baseline adjusted for age, gender and type of scanner;

Table- 3: Effect of baseline total body fat on Ranke's Prediction model for the first-year height response in SGA children

Models		B	95% CI	P value	Partial Correlation	Collinearity (Tolerance)	R^2	P value (R^2 change)
1	Constant	13.7	11.5, 15.8	**<0.0001**				
	Age (year)	-0.31	-0.54, -0.09	**0.008**	-0.30	0.97		
	Midparental height (SDS)	0.46	0.07, 0.85	**0.022**	0.26	0.96		
	Weight at baseline (SDS)	0.12	-0.26, 0.50	0.52	0.07	0.93	**0.15**	**0.008**
2	Constant	13.04	11.1, 15.0	**<0.0001**				
	Age (year)	-0.29	-0.50, -0.08	**0.008**	-0.31	0.97		
	Midparental height (SDS)	0.47	0.11, 0.84	**0.012**	0.29	0.96		
	Weight at baseline (SDS)	-0.04	-0.40, 0.33	0.84	-0.02	0.88		
	Baseline total body fat % (z-score)*	0.61	0.28, 0.95	**<0.0001**	0.39	0.94	**0.27**	**0.001**
3	Constant	10.9	8.36, 13.5	**<0.0001**				
	Age (year)	-0.19	-0.41, 0.03	0.096	-0.19	0.85		
	Midparental height (SDS)	0.45	0.10, 0.79	**0.012**	0.28	0.95		
	Weight at baseline (SDS)	-0.13	-0.49, 0.23	0.48	-0.08	0.83		
	Baseline total body fat % (z-score)*	0.59	0.26, 0.92	**0.001**	0.38	0.91		
	Delta IGF-1 SDS (0 to 1-yr)	0.30	0.07, 0.54	**0.013**	0.28	0.82	**0.33**	**0.013**

* within cohort z-scores for total body fat percentage at baseline adjusted for age, gender and type of scanner ($z\text{-}score_c$)
Dependent Variable: Height velocity (cm/year); B, unstandardized coefficient; CI, confidence interval
Model 1: Ranke's Model for prediction of first-year height velocity in SGA children; Growth Hormone 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-I SDS (0 to 1-year)

Table 4: Associations between multiallele scores for insulin sensitivity and body composition#

	Effect size per allele (B)	95 % CI	P value*
Baseline			
BMI (SDS)	-0.13	-0.26, -0.01	**0.040**
Body fat (%)	-0.49	-0.97, -0.01	**0.047**
Limb fat (%)	-0.81	-1.62, 0.00	**0.049**
Arm fat (%)	-1.19	-2.31, -0.06	**0.038**
Leg fat (%)	-0.76	-1.55, 0.03	0.060
Trunk fat (%)	-0.33	-0.77, 0.12	0.16
Trunk-limb fat ratio	0.01	-0.01, 0.03	0.49
1-year			
BMI (SDS)	-0.07	-0.22, 0.09	0.40
Body fat (%)	-0.39	-0.81, 0.02	0.064
Limb fat (%)	-0.81	-1.49, -0.13	**0.020**
Arm fat (%)	-1.04	-1.95, -0.13	**0.026**
Leg fat (%)	-0.59	-1.27, 0.09	0.087
Trunk fat (%)	-0.03	-0.43, 0.37	0.88
Trunk-limb fat ratio	0.04	0.01, 0.08	**0.008**
Changes from baseline to 1-year			
Delta body fat (%)	0.31	0.10, 0.51	**0.004**
Delta limb fat (%)	0.28	-0.11, 0.68	0.16
Delta arm fat (%)	0.18	-0.41, 0.78	0.54
Delta leg fat (%)	0.27	-0.16, 0.70	0.22
Delta trunk fat (%)	0.38	0.16, 0.59	**0.001**
Delta trunk-limb fat ratio	0.03	0.01, 0.05	**0.003**

higher scores associated with lower insulin sensitivity; B, unstandardized coefficient; CI, confidence interval
* P-values and B are derived from regression models with age and gender as covariants

Figure1

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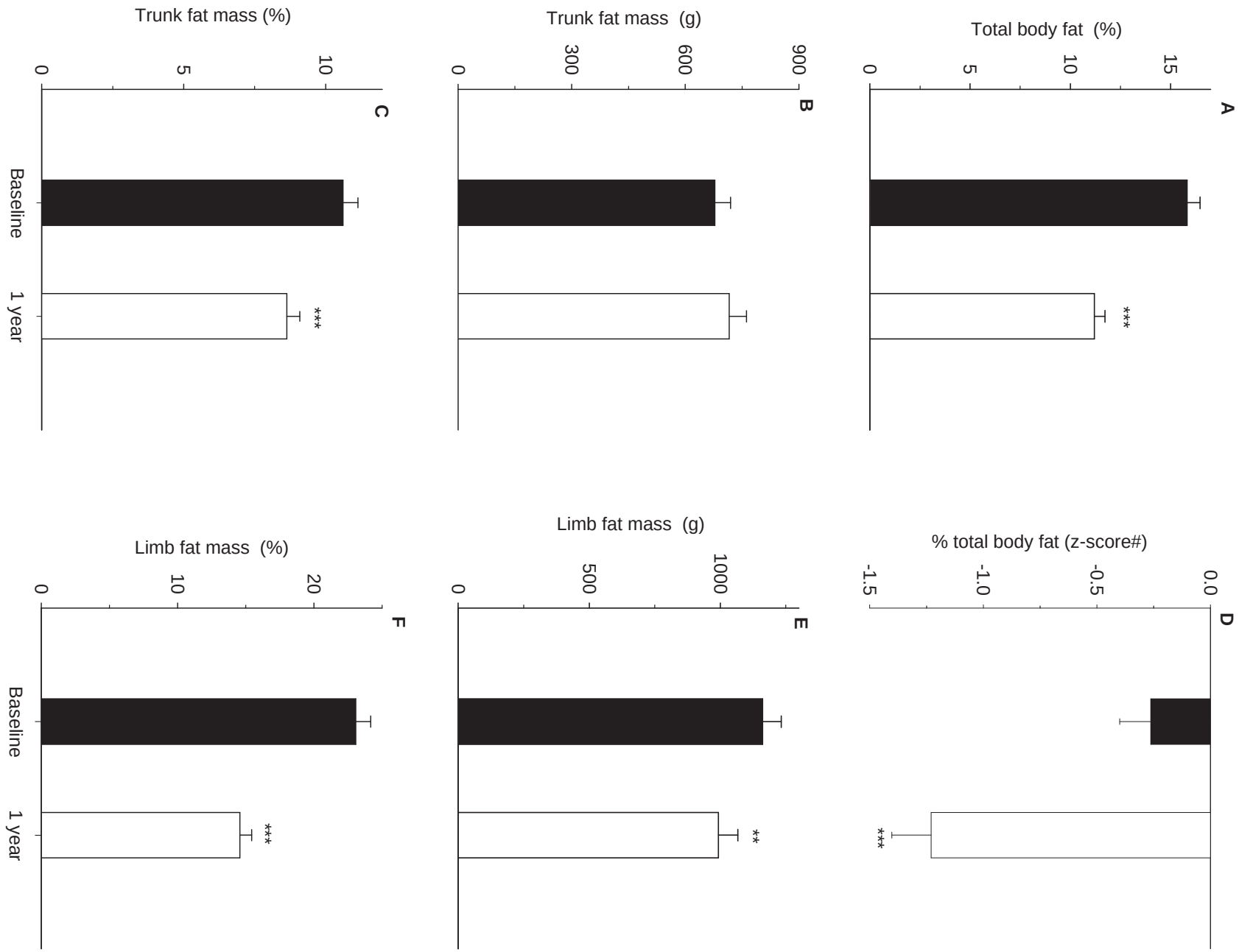

Figure2

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