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For Peer Review

Metabolic outcome of GH treatment in prepubertal short children with and without classical GH-deficiency

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ABSTRACT

Context: Few studies have evaluated the metabolic outcomes of growth hormone (GH) treatment in idiopathic growth stature (ISS). Moreover, children with ISS appear to need higher GH doses than children with GH deficiency (GHD) to achieve the same amount of growth, and may therefore be at increased risk of adverse events during treatment. The individualized approach using prediction models for estimation of GH responsiveness, on the other hand, has the advantage of narrowing the range of growth response avoiding too low or high GH doses.

Design: Short prepubertal children with either isolated GHD (39) or ISS (89) participated in a 2-year randomized trial of either individualized GH treatment with six different GH doses (range, 17–100 $\mu\text{g}/\text{kg}/\text{day}$) or a standard dose (43 $\mu\text{g}/\text{kg}/\text{day}$).

Objective: To evaluate if individualized GH treatment reduced the variance of the metabolic measures as shown for growth response, and to compare changes in metabolic variables in children with ISS and GHD.

Hypothesis: Individualized GH dose reduces the range of metabolic outcomes, and metabolic outcomes are similar in children with ISS and GHD.

Results: We observed a narrower variation for fasting insulin (-34.2 %) and for HOMA (-38.9 %) after two years of individualized GH treatment in comparison to standard GH dose treatment.

Similar metabolic changes were seen in ISS and GHD. Delta (Δ) height SDS correlated with Δ insulin-like growth factor I (IGF-I), Δ leptin and Δ body composition. Principal component analysis identified an anabolic and a lipolytic component. Anabolic variables [Δ lean body mass (LBM) SDS and Δ IGF-I SDS] clustered together and correlated strongly with Δ height SDS and GH dose, whereas lipolytic variables [Δ fat mass SDS and Δ leptin] were clustered separate from the anabolic. Regression analysis showed GH dose-dependency in ISS, and to a lesser degree in GHD, for Δ LBM SDS and Δ height SDS, but not for changes in fat mass.

Conclusions: Individualized GH dosing during catch-up growth reduces the variance in insulin and HOMA and results in equal metabolic responses irrespective of the diagnosis of GHD or ISS.

List of Abbreviations

| | | |
|----|-------------------|--|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | AITT | arginine–insulin tolerance test |
| 6 | | |
| 7 | BMI | body mass index |
| 8 | | |
| 9 | Chol | cholesterol |
| 10 | | |
| 11 | CV | coefficient of variation |
| 12 | Δ | delta |
| 13 | | |
| 14 | FM | fat mass |
| 15 | DELFIA | dissociation-enhanced lanthanide fluorescence immunoassay |
| 16 | | |
| 17 | DPX-L | proper name of the Lunar pencil beam scanner |
| 18 | | |
| 19 | GH | growth hormone |
| 20 | | |
| 21 | GHD | growth hormone deficiency/ deficient |
| 22 | | |
| 23 | GH _{max} | maximum growth hormone secretion peak |
| 24 | | |
| 25 | GP-GRC | Gothenburg Pediatric Growth Research Center |
| 26 | | |
| 27 | HDL | high-density lipoprotein |
| 28 | | |
| 29 | HOMA | homeostasis model assessment |
| 30 | | |
| 31 | IGF-I | Insulin-like growth factor I |
| 32 | | |
| 33 | IGFBP-3 | Insulin-like growth factor-binding protein 3 |
| 34 | | |
| 35 | ISS | idiopathic short stature |
| 36 | | |
| 37 | iv | intravenous |
| 38 | | |
| 39 | LBM | lean body mass |
| 40 | | |
| 41 | LDL | low-density lipoprotein |
| 42 | | |
| 43 | Lp(a) | Lipoprotein (a) |
| 44 | | |
| 45 | MPH | mid-parental height |
| 46 | | |
| 47 | PCA | principal component analysis |
| 48 | | |
| 49 | PP | per-protocol |
| 50 | | |
| 51 | r | correlation coefficient |
| 52 | | |
| 53 | RIA | radioimmunoassay |
| 54 | | |
| 55 | SD | standard deviation |
| 56 | | |
| 57 | SDS | standard deviation score |
| 58 | | |
| 59 | SGA | small for gestational age |
| 60 | | |
| | TG | triglycerides |

INTRODUCTION

Growth hormone (GH) therapy has effects beyond the promotion of linear growth in children. It has effects on bone metabolism and bone mineral density, insulin sensitivity, protein and lipid metabolism, sodium homeostasis, and body composition in adults¹⁻³. However, although studies have investigated the metabolic effects of GH treatment in children⁴, many considered the metabolic changes that occur during GH treatment to be largely adverse effects that are secondary to the effects on growth^{5, 6}. Nevertheless, there are clear metabolic benefits in children receiving GH therapy. For example, an increase in lean body mass (LBM) during GH treatment has been reported in GH-deficient prepubertal children⁷ and in short children born small for gestational age (SGA) a significant increase in (and normalization of) LBM is found in comparison with untreated controls⁶, and an improvement in cardiovascular risk based on changes in the lipid profile⁸. Increases in serum insulin-like growth factor I (IGF-I) levels in response to GH treatment has been found to correlate with the 1-year growth response⁹, whereas leptin levels decrease as a consequence of the adipose tissue response to GH treatment¹⁰. In addition, insulin levels increase and correlate to growth response in some but not all children^{11,12}.

Few studies have evaluated the metabolic outcomes of GH treatment in children with idiopathic short stature (ISS)^{10, 13}. The metabolic outcomes are similar to these reported in children with GH deficiency (GHD) when conventional GH dosages such as 33 µg/kg/day are used. As children with ISS need a higher GH dose than children with GHD for significant growth response,¹⁴ they may be at greater risk of adverse events. In order to optimize both height and metabolic outcome in the clinical every day life, there is a need to study GH-dose algorithms in short children with a broad range in endogenous GH secretion.

We have previously reported that the observed variability in growth response can be reduced by individualizing GH treatment estimating GH responsiveness according to our multivariate non-linear prediction model^{14, 15}. This model, which is based on auxological variables and endogenous GH secretion, makes it possible to predict the growth response in individual children receiving GH therapy. Thus, tailoring of GH treatment implies that children who are predicted to respond poorly receive higher GH doses than those predicted to respond well¹⁴.

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3 The objective of the present study was to evaluate if individualized GH treatment reduced the range of
4 the metabolic measures as shown for growth response, and to compare changes in metabolic variables
5 in children with ISS and GHD. The two hypotheses were: Individualized GH dose reduces the range
6 of metabolic outcomes, and metabolic outcomes are similar in children with ISS and GHD.
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15 SUBJECTS AND METHODS

16 *Subjects and study protocol*

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18 The study was a 2-year prospective, randomized, open-label, multicenter trial of two GH treatment
19 regimens in short prepubertal children with either isolated GHD (n=39) or ISS (n=89) from five
20 pediatric endocrinology units in Sweden [Göteborg, Umeå, Uppsala, Malmö, and Halmstad]. In total
21 128 prepubertal children (38 girls, 90 boys) followed the protocol and formed the per protocol (PP)
22 population. All patients were of Caucasian origin and had a current height SDS below -2.0 ¹⁶, a
23 growth velocity < -1.0 SDS and had been born at a gestational age > 30 weeks. The maximum GH
24 secretion peak (GH_{max}) on an arginine–insulin tolerance test (AITT) and on the 24 hour profile was
25 used to separate children with GHD and ISS. **Classical GHD was defined as GH_{max} below 32 mU/L
26 using a polyclonal assay on either AITT or 24 hour profile.**
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39 Since the protocol allowed children who were considered “non-severe SGA” (birth weight or length
40 between -2 SDS and -2.5 SDS) to be included, ISS children were defined as being short without
41 having classical GHD. **Nine of these eleven SGA had a GH_{max} between 34.9 and 78 mU/L. Two out
42 of the eleven SGA had a GH_{max} between 25.3 and 31 mU/L (polyclonal DELFIA).**
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50 The preset treatment goal was to achieve a height near MPH SDS within 2 years of treatment. Patients
51 were randomized centrally to receive either a standard GH dose of 43 $\mu\text{g}/\text{kg}/\text{day}$ (one-third of the
52 children) or an individualized GH dose (two-thirds of the children) based on the following variables:
53 predicted change in height SDS, highest GH_{max} on the AITT or a 24-hour GH profile, height SDS at
54 GH start, age at GH start, sex, weight SDS at birth, height SDS 1 year before GH start and the
55 presence of channel-parallel growth, as previously reported¹⁴. Dose selection was based on the
56 predicted growth response and the predicted difference between current height and MPH SDS after 2
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3 years of receiving a standard GH dose of 33 $\mu\text{g}/\text{kg}/\text{day}$. The individualized GH dose was one of six
4
5 doses within the range of 17–100 $\mu\text{g}/\text{kg}/\text{day}$. The distribution of doses in the individualized-dose
6
7 group were 17 $\mu\text{g}/\text{kg}/\text{day}$ (n=3), 33 $\mu\text{g}/\text{kg}/\text{day}$ (n=27), 40 $\mu\text{g}/\text{kg}/\text{day}$ (n=10), 50 $\mu\text{g}/\text{kg}/\text{day}$ (n=26), 66
8
9 $\mu\text{g}/\text{kg}/\text{day}$ (n=14), and 100 $\mu\text{g}/\text{kg}/\text{day}$ (n=7): the mean GH dose in this group became 49 $\mu\text{g}/\text{kg}/\text{day}$.
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13 Growth and parts of the metabolic data (fasting insulin and IGF-I levels) have previously been
14
15 reported as safety measurements.¹⁴
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18 19 20 21 *Ethical consideration*

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23 The study protocol was approved by the Ethical Committees of the Universities of Göteborg (for
24
25 Göteborg and Halmstad), Umeå, Uppsala and Malmö and the Medical Product Agency of Sweden.
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27 Written informed consent was obtained from all parents and from children if possible. The study was
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29 performed in accordance with the Declaration of Helsinki and Good Clinical Practice.
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33 *Laboratory analyses*

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35 GH, IGF-I leptin and adiponectin assays were performed at the GP-GRC laboratory (accredited
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37 number 1899). **GH was analyzed using a dissociation-enhanced lanthanide fluorescence**
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39 **immunoassay (DELFLIA). The cut-off value for the diagnosis of classical GHD was <32 mU/L**
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41 **(polyclonal DELFLIA), corresponding to <24 mU/L (monoclonal DELFLIA) both using**
42
43 **International Reference Preparation 80/505, corresponding to <10 $\mu\text{g}/\text{L}$ cut-off value using**
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45 **International Reference Preparation 66/127**¹⁷.
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49 For IGF-I, an IGFBP-blocked radioimmunoassay (RIA) was used without extraction, and in the
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51 presence of IGF-II levels 250-fold in excess of normal (Mediagnost, Tübingen, Germany). IGFBP-3
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53 was measured by RIA as previously described¹⁸. IGF-I and IGFBP-3 levels were converted into SDS
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55¹⁹. The ratio of IGF-I/IGFBP-3 SDS was calculated for each sample^{19,20}.
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58 Serum leptin concentrations were measured by RIA (Linco Research, St. Charles, USA) with a range
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60 of 0.3–100.0 $\mu\text{g}/\text{L}$ and an intra-assay coefficient of variation (CV) of 7.0% at 2.4 $\mu\text{g}/\text{L}$, 5.2% at 6.6
 $\mu\text{g}/\text{L}$ and 4.9% at 14.0 $\mu\text{g}/\text{L}$. The interassay CVs were 12.5% at 1.4 $\mu\text{g}/\text{L}$, 7.6% at 6.7 $\mu\text{g}/\text{L}$ and 5.6%

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3 at 15.1 $\mu\text{g/L}$ ²¹. Adiponectin concentrations were measured in duplicate by an ELISA (R&D Systems
4 Inc., Minneapolis, Minn., USA). The assay has a lower detection limit of 1 $\mu\text{g/ml}$. All samples were
5 analyzed using the same assay batch, and all samples from each patient were analyzed using the same
6 assay. The assay had an intra-assay CV of 3.6% and an inter-assay CV of 6.9%. Total, high-density
7 lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterols, and triglycerides were analyzed at
8 the Sahlgrenska University Hospital in the department of laboratory medicine/ clinical chemistry using
9 an enzyme-based method (Modular P, Roche Diagnostics, Mannheim, Germany). CVs were 3%, 5%,
10 5%, and 4%, respectively.
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14 Serum insulin concentrations were measured by RIA (Pharmacia and Upjohn, Uppsala, Sweden)
15 expressed with an inter assay CV of 6–7%. Plasma glucose was measured using the hexokinase
16 method (Roche Diagnostics, Mannheim, Germany) with a 4% CV for sample concentrations between
17 5 and 15 mmol/L. Insulin resistance was measured by homeostasis model assessment (HOMA),
18 calculated as $[(\text{fasting serum insulin} \times \text{fasting plasma glucose})/22.5]$.
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21 *Measurement of body composition*

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24 Body composition was measured by dual energy X-ray absorptiometry (DEXA), for which a DPX-L
25 scanner (Lunar Co., Madison, WI) was used at each study centre. The DEXA measure results in a
26 three-compartment model consisting of fat mass (FM), lean body mass (LBM) and bone mineral
27 content (BMC). Manufacturer-supplied software (version 1.1 – 1.3z) was used. The difference in
28 software versions did not influence the examinations or the results. All analysis was made with the
29 extended analysis program for total body analysis. Quality assurance tests were made on a daily basis
30 and a phantom spine was examined once weekly, as recommended by the manufacturer. DEXA scan
31 reproducibility (coefficient of variation) has been reported to be 0.18–1.97% for total body
32 measurement and 0.96–6.91% for regional measurements ²².
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34

35 *Statistics*

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3 For all analyses, the assumptions of normality were assessed by analysis of skewness, kurtosis and
4
5 frequency histograms. A p-value of < 0.05 was considered to be statistically significant.
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8 Evaluations of the null hypothesis and differences between groups were made using a two-sample
9
10 independent *t*-test for independent variables. Paired-sample *t*-tests were performed to calculate
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12 differences in each variable during the 2 years of GH treatment. **One-way ANOVA was used to test**
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14 **differences of means with SGA as a covariate in the entire PP population and when comparing**
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16 **ISS with GHD or when comparing the two GH treatment regimes (standard with individualized**
17
18 **GH treatment).** Regression analysis was performed to demonstrate dependency of some metabolic
19
20 variables to growth response and GH dose.
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23 Principal component analysis (PCA) has been applied previously for the analysis of microarray
24
25 data and is a powerful descriptive and data-reduction technique when used to assess variables related
26
27 to a complex system such as the metabolic network²³. PCA is a 100-year-old mathematical technique
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29 credited to Karl Pearson. Its properties, as well as the interpretation of the components, have been
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31 investigated extensively. Rotation by the well-established 'Varimax' method was performed in order
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33 to focus on the most important interrelationships between metabolic variables. In the component
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35 figures of the PCA analysis we display graphically the correlations of the variables with the rotated
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37 components. Longer vectors have stronger correlations with one or two components. The cosine of
38
39 the angle between two long vectors approximates their mutual correlation. Therefore, clusters with
40
41 high-mutual positive correlations among the vectors are formed by vectors close together with small
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43 angles in between (0°, cosine=1). Negative correlations between two variables are visible if they
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45 point at opposite directions (180°, cosine=-1). Variables with an angle of 90° (cosine=0) have almost
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47 no correlation. All statistical methods were performed using SPSS version 17.0.
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RESULTS

Comparison of variation between individualized-dose and standard-dose treatment

No differences in **variance** were observed in any variable at start of treatment when comparing individualized and standard GH treatment. After 2 years of GH treatment, a reduced variance in the individualized-dose group compared with the standard-dose group was observed for insulin (-34.2%) and HOMA (-38.9%), $p < 0.05$ (**Figure 1** and **Table 1**). There was no reduction in **variance** in any other metabolic variable.

When the analysis was rerun excluding data from 11 children born SGA, the **SD** for insulin (-38.6%) and HOMA (-45.9%) was still significantly reduced ($p < 0.05$).

Comparison of variables at start and at 2 years of GH treatment in children with ISS and GHD

Auxological and metabolic variables at start of GH treatment were comparable between the ISS and GHD groups, except for weight, BMI, fat mass, and GH secretion. Children with GHD had a significantly higher weight, BMI, and fat mass than children with ISS (**Table 1**) but there were no differences in any variable after 2 years of GH treatment. Thus, the differences found at the start of treatment regarding the fat compartment, as measured by DEXA, disappeared during GH treatment. One-way ANOVA showed no differences of the mean of any variable with SGA as a covariate when considering the entire PP population ($n=128$). ANOVA was then repeated separately for ISS and GHD individuals still without showing any impact of being born SGA.

Comparison of changes in variables after 2 years of treatment in children with ISS and GHD

Figure 2 shows the mean levels of selected metabolic parameters at start, one and two years of treatment in children with ISS and GHD, respectively. IGF-I SDS levels increased at the same rate in both groups with a more pronounced increase during the first year of treatment ($p < 0.001$), as did insulin levels ($p < 0.001$).

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3 Adiponectin and leptin levels decreased significantly in both the ISS and GHD group during the 1 year
4 of treatment, but not further during the second year. Adiponectin levels were significantly lower at 2
5 years compared to start of treatment. A decrease in leptin levels was accompanied by a decrease in fat
6 mass SDS during the 1st year of treatment. At 2 years of treatment, fat mass was still decreased in
7 both groups, whereas leptin was exclusively decreased in the ISS group ($p < 0.01$), but not in the GHD
8 group. The ratio of LDL to HDL-cholesterol decreased over the two years only in the GHD group
9 ($p < 0.01$).

11 *Univariate relationship between growth response and the anabolic and lipolytic effects of GH*

12 There was a negative regression between Δ height SDS and Δ FM SDS in the ISS group, but such a
13 correlation was not found in the GHD. However, the absence of the negative regression is probably
14 due to fewer children in the GHD group. In contrast to Δ fat mass SDS, Δ LBM SDS showed a
15 significant positive regression in both groups (**Figure 3**).

17 *Relationship between anabolic and lipolytic effects of GH analyzed with PCA*

18 PCA showed clusters with high inter-correlations of anabolic variables (Δ LBM SDS, Δ height SDS,
19 Δ weight SDS, Δ IGF-I SDS, Δ IGFBP-3 SDS, and Δ BMC SDS). All anabolic variables were highly
20 correlated with GH dose (Figure 4). The lipolytic cluster consisted of Δ FM SDS, fat mass (kg), and Δ
21 leptin. It formed a tight cluster which was separated from the anabolic cluster. The angle between the
22 two clusters was approximately 90°, indicating no correlation between these clusters (**Figure 4** shows
23 the results for ISS group).

24 The same pattern of clusters was apparent in children with GHD; however, the correlations between
25 the components within each cluster were weaker, as for example for GH dose and the anabolic cluster.
26 The distances between the vectors were also larger in the GHD group than in the ISS group (data not
27 shown).

28 In children with GHD and ISS, Δ insulin and Δ HOMA, constituting an own vector cluster with high
29 inter-correlations, lay between the anabolic and lipolytic vector bundles at an angle of approximately
30 45°, **indicating a moderate positive correlation with both clusters (Figure 4).**

Dose-dependency of GH effects in children with ISS

In the ISS group a dose-dependency of GH effects on height SDS ($r=0.68$, $p<0.01$) and lean mass SDS ($r = 0.62$, $p<0.01$), but not for fat mass SDS (n.s.) was demonstrated by regression analysis (**Figure 5**). This dose-dependency was still the result when the group treated with $17 \mu\text{g}/\text{kg}/\text{day}$ was excluded. In contrast, no dose-response was seen in GHD children for either of the two DEXA variables.

DISCUSSION

In agreement with previous studies, we found that fasting insulin increased during GH treatment in both standard and individualized GH treated children. However, we observed a more narrow variation for fasting insulin and HOMA after two years of individualized GH treatment in comparison to standard GH dose treatment. This is in line with our previously finding of a reduction in the variation of the growth response to target height after two years of individualized GH treatment¹⁴. Additionally, no excess values of insulin and HOMA have been found, with lower maximum values after individualized GH treatment, despite of the same values at treatment start. We speculate that the reduction in variation observed for insulin and HOMA in children treated on an individual bases was reached by predicting the metabolic GH responsiveness at the same time.

Moreover, no differences of variance and no excessive values in IGF-I SDS and the LDL-HDL ratio were observed between the standard and the individualized-dose treatment groups, indicating that individualized GH treatment based on the estimation of GH responsiveness is safe, despite three times higher GH dose for some children. By adapting the GH dose it is possible to take into account individual estimated GH sensitivity of each child and indirectly also influencing to a less degree the metabolic adverse outcome such as the insulin resistance.

The present work confirms previous studies in which no major differences in response to GH treatment have been found between children with short stature as a result of GHD or ISS^{9, 10, 12, 15, 24-26}. ISS children were found to increase fasting insulin and IGF-I SDS in the same magnitude as did GHD children. This is despite higher mean GH doses in the former group.¹⁴

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3 The increase in fasting insulin may be a consequence of a decrease in insulin sensitivity, but the
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5 insulin resistance is counteracted by an increase in lean body mass and a decrease in body fat mass in
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7 both ISS and GHD^{8, 13, 27} 7. This could be the case, as the change in height correlated to the change in
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9 insulin but also strongly to the positive changes in the anabolic outcome as measured by lean mass as
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11 well as negatively with changes in fat mass (although to a less extent in the GHD group due to fewer
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13 children).

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18 The regression line associated with the anabolic effect of GH was quadratic indicating a steeper slope
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20 in lower GH dose range and a less marked slope in the higher dose range. In patients receiving GH
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22 therapy individualized according to their estimated GH responsiveness, there was dissociation between
23
24 the anabolic and lipolytic effects of GH treatment, with a dose dependency for the anabolic effects.
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26 This was also supported by the PCA data, where the dose correlated to growth, increase in IGF-I,
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28 IGFBP-3 and lean body mass but not to the lipolytic effect. However, a genuine effect of dosage
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30 cannot be determined in this study, as the current dose approach was dependent on auxology, GH
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32 secretion capacity and the distance to target height. Thus, the “real” dose effect would be in part
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34 obscured by this study design.
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41 The dissociation between lipolytic and anabolic GH effects found may be an effect of the fact that the
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43 dose-threshold for lipolytic effect has been passed, while there was still a dose effect on anabolism and
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45 growth within the dose range applied in our study. This is supported by the finding that the GH dose
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47 effective for changes of body composition and especially on lipolysis in adult GHD patients is much
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49 lower than the dose used in children with GHD. Johannsson²⁸ compared hypopituitary adults given a
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51 high dose GH treatment regimen (12 µg/kg/day) with those given low dose individually titrated GH
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53 treatment (2.5 - 5 µg/kg/day). The two groups had similar responses regarding changes of body
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55 composition, indicating that the GH dose range between 2.5 and 12 µg/kg/day is mostly effective on
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57 lipolysis. However, the two dose categories used in our study may be not suitable to study dose-
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59 response curves.
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CONCLUSIONS

By individualizing the GH dose based on auxological growth data and spontaneous GH secretion using a growth prediction model, the anabolic response can be predicted with minimal variance despite given high GH doses in children with ISS, as well as in children with GHD. Furthermore, the variance in insulin-sensitivity can be reduced.

Validated prediction models that indirectly take into account differences in GH secretion and GH sensitivity associated with diagnoses of short stature such as ISS or GHD have been found useful for the selection of the GH dose for each individual child not only to achieve an optimal growth response but also for metabolic outcome.

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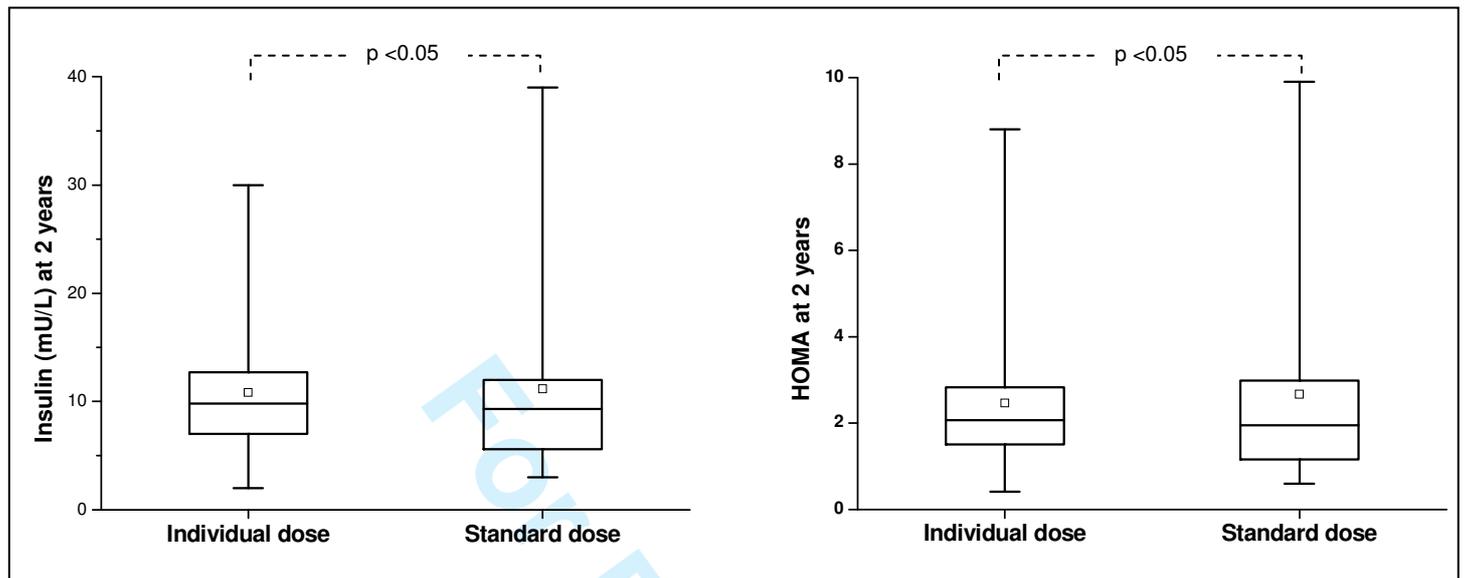
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Figure 1 Distribution of insulin and HOMA in the standard and the individualized GH dose group



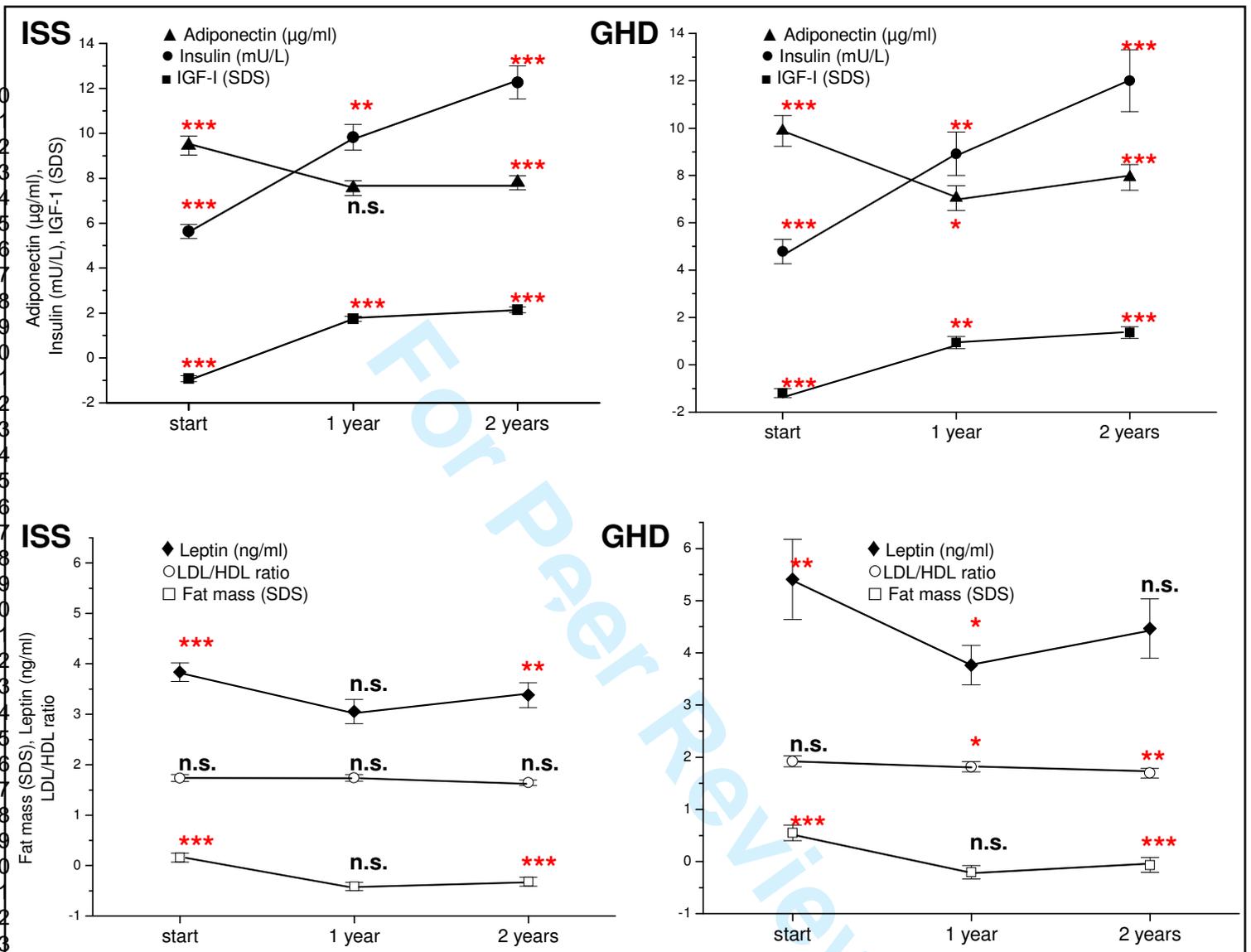
The distributions of values at 2 years for insulin and HOMA (homeostasis model assessment) according to randomization group are shown. The standard deviation around the mean is significantly narrower for insulin (34.2 %) and for HOMA (38.9 %) in the group receiving an individualized GH dose compared with the group receiving a standard dose. The *boxes* represent 50% of the study group (3rd and 1st quartile), with the *line* indicating the median (the *small box* indicating the mean); the *whiskers* indicate the maximum and minimum. Levene's test for equality of variances gives p-values at a 95 % confidence interval.

Review

Table 1 Metabolic and auxological parameters at study start and after 2 years of GH treatment

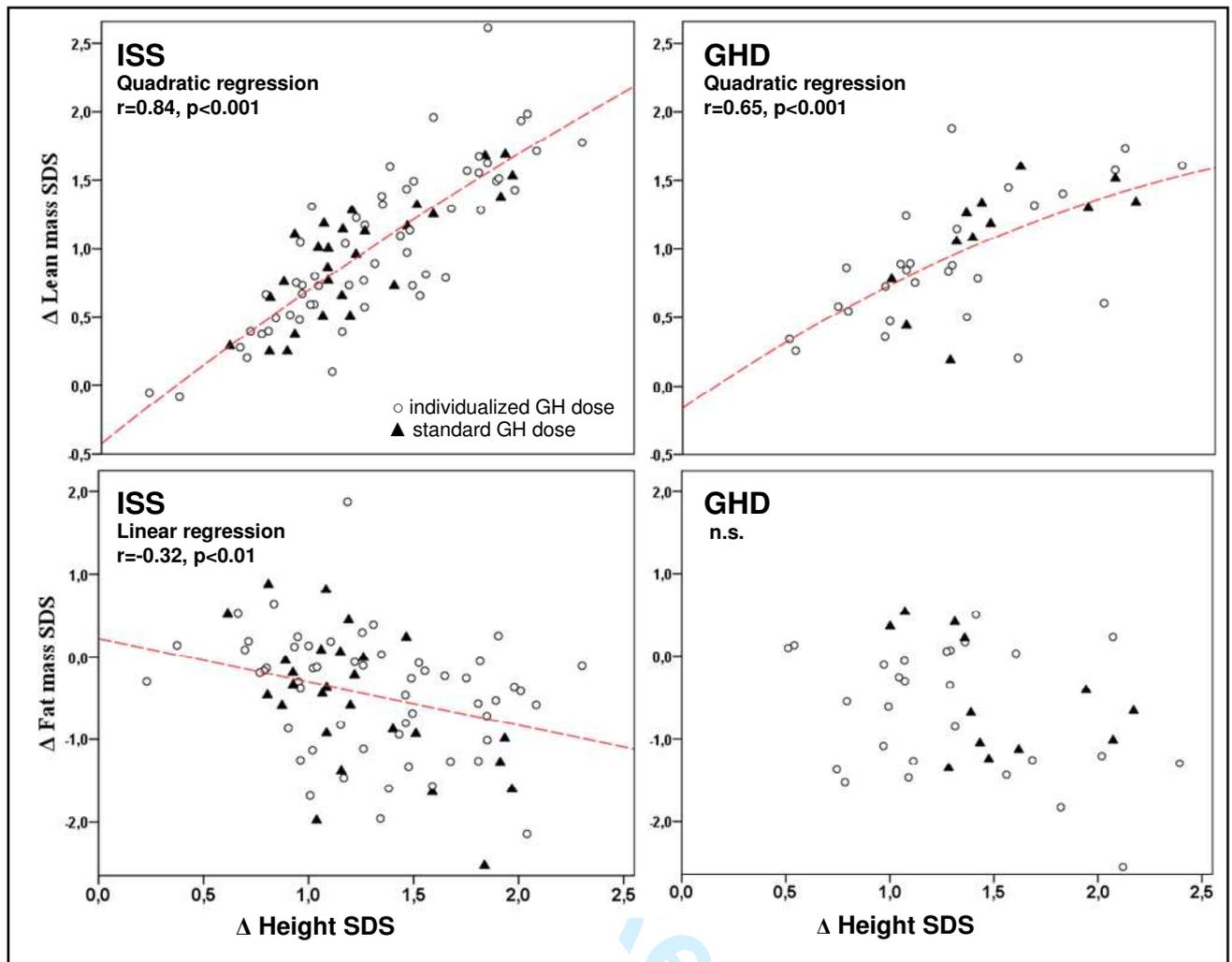
| Per-protocol population n=128 | ISS (Idiopathic short stature) | | GHD (Growth hormone deficiency) | | ISS vs. GHD | Individualized GH dose n=87 | Standard GH dose n=41 | Ind. vs. Std. |
|--|-----------------------------------|------------------------|------------------------------------|------------------------|-------------------|-----------------------------------|-----------------------------|---------------------|
| | n | Mean ± SD (min :max) | n | Mean ± SD (min : max) | p< | Mean ± SD | Mean ± SD | p |
| At study start | | | | | | | | |
| Girls/ boys | 25 / 64 | | 13 / 26 | | n.s. | 27 / 60 | 11 / 30 | n.s. |
| Age (years) | 89 | 7.4±2.0 (3.2 :11) | 39 | 7.1±2.0 (3.1 :10.9) | n.s. | 7.3 ± 2.1 | 7.2 ± 2.0 | n.s. |
| Height (SDS) | 89 | -2.7±0.5 (-4.1 :-1.8) | 39 | -2.7±0.4 (-3.7 :-2.0) | n.s. | -2.7 ± 0.5 | -2.7 ± 0.5 | n.s. |
| Weight (SDS) | 89 | -2.4±0.7 (-4.3 :-0.5) | 39 | -2.0±0.9 (-3.8 :0.3) | .05 | -2.3 ± 0.8 | -2.2 ± 0.9 | n.s. |
| Body mass index (SDS) | 89 | -0.6±1.0 (-3.4 :1.9) | 39 | -0.1±1.0 (-1.8 :2.1) | .01 | -0.5 ± 1.1 | -0.4 ± 1.0 | n.s. |
| GHmax 24h or AITT (mU/L) | 89 | 47.7±14.7 (32.4 : 90) | 39 | 23.5±7.4 (2.3 :31.9) | .001 | 40 ± 17.2 (5.4:89.9) | 41 ± 17.1 (2.3:72.4) | n.s. |
| IGF-I (SDS) | 89 | -1.1±1.1 (-3.3 :2.3) | 39 | -1.2±1.2 (-5.2 :0.9) | n.s. | -1.1 ± 1.2 | -1.1 ± 1.0 | n.s. |
| IGFBP-3 (SDS) | 89 | 0.0±1.3 (-3.3 :2.7) | 39 | -0.4±1.5 (-5.3 :1.6) | n.s. | -0.2 ± 1.3 | 0.1 ± 1.4 | n.s. |
| IGF-I/IGFBP-3 ratio (SDS) | 89 | -1.2±1.3 (-5.2 :1.3) | 39 | -1.2±1.1 (-5.2 :0.7) | n.s. | -1.1 ± 1.3 | -1.3 ± 1.2 | n.s. |
| Leptin (ng/mL) | 89 | 3.8±1.7 (1.9 :13) | 39 | 5.4±4.8 (2.1 :30.7) | n.s. | 4.1 ± 2.0 | 4.8 ± 4.6 | n.s. |
| Insulin (mU/L) | 86 | 4.7±2.6 (1.0 :17) | 38 | 4.8±3.2 (1.0 :20) | n.s. | 4.7 ± 2.7 | 4.7 ± 3.0 | n.s. |
| HOMA (mmol/mU) | 86 | 1.0±0.6 (0.1 :4.2) | 39 | 1.0±0.8 (0.2 :4.4) | n.s. | 1.0 ± 0.6 | 1.0 ± 0.7 | n.s. |
| Lean body mass (SDS) | 84 | -2.4±0.5 (-3.6 :-1.0) | 39 | -2.3±0.5 (-3.8 :-1.3) | n.s. | -2.4 ± 0.5 | -2.4 ± 0.5 | n.s. |
| Fat mass (SDS) | 84 | 0.2±0.8 (-2.2 :1.8) | 39 | 0.6±0.9 (-1.0 :2.4) | .05 | 0.2 ± 0.9 | 0.4 ± 0.9 | n.s. |
| Waist/hip (cm) ratio | 88 | 0.9±0.04 (0.8 :1.0) | 37 | 0.9±0.04 (0.9 :1.0) | n.s. | 0.9 ± 0.0 | 1.0 ± 0.9 | n.s. |
| After 2 years of treatment | | | | | | | | |
| Height (SDS) | 89 | -1.4±0.7** (-3.0 :0.2) | 39 | -1.3±0.6**(-2.4 :0.1) | n.s. | -1.4 ± 0.7** | -1.4 ± 0.7** | n.s. |
| Weight (SDS) | 89 | -1.3±0.9** (-3.9 :0.8) | 39 | -1.0±0.9**(-3.6 :0.7) | n.s. | -1.2 ± 0.9** | -1.2 ± 0.9** | n.s. |
| Body mass index (SDS) | 89 | -0.3±0.9** (-3.1 :1.9) | 39 | -0.03±0.9 (-2.1 :2.0) | n.s. | -0.2 ± 0.9** | -0.2 ± 0.9** | n.s. |
| IGF-I (SDS) | 89 | 1.6±1.0** (-0.7 :3.8) | 39 | 1.4±1.5**(-1.5 :4.2) | n.s. | 1.5 ± 1.2** | 1.5 ± 1.1** | n.s. |
| IGFBP-3 (SDS) | 89 | 1.9±0.9** (-0.1 :4.3) | 39 | 1.7±1.2**(-0.1 :4.4) | n.s. | 1.8 ± 1.1** | 2.0 ± 0.9 | n.s. |
| IGF-I/IGFBP-3 ratio (SDS) | 89 | 1.1±0.9** (-0.8 :3.1) | 39 | 0.8±1.2**(-2.2 :2.8) | n.s. | 1.0 ± 1.1** | 0.9 ± 1.0** | n.s. |
| Leptin (ng/mL) | 88 | 3.4±2.3* (1.4 :16) | 39 | 4.5±3.5 (0.9 :17) | n.s. | 3.5 ± 2.4** | 4.1 ± 3.4** | n.s. |
| Insulin (mU/L) | 89 | 10.5±6.1** (2.0 :34) | 38 | 12 ±8.2** (4.0 :39) | n.s. | 10.8 ± 5.7 | 11.2 ± 8.7** | n.s. |
| HOMA (mmol/mU) | 88 | 2.4±1.6** (0.4 :9.4) | 38 | 2.9±2.3** (0.8 :10.2) | n.s. | 2.5 ± 1.5** | 2.7 ± 2.4** | n.s. |
| Lean body mass (SDS) | 86 | -1.4±0.8** (-3.3 :0.9) | 39 | -1.3±0.5**(-2.8 :-0.1) | n.s. | -1.3 ± 0.7** | -1.4 ± 0.7** | n.s. |
| Fat mass (SDS) | 86 | -0.3±0.8** (-2.4 :1.6) | 39 | -0.1±0.9**(-1.8 :1.8) | n.s. | -0.3 ± 0.9** | -0.14± 1.7** | n.s. |
| Waist/hip (cm) ratio | 86 | 0.9±0.04** (0.9 :1.0) | 38 | 0.9±0.1** (0.7 :1.0) | n.s. | 1.0 ± 0.9** | 1.0 ± 0.9** | n.s. |
| GH _{max} , maximum GH secretion peak (24 hour GH profile; AITT, arginine-insulin tolerance test); IGF-I, insulin-like growth factor I; IGFBP-3, IGF-binding protein 3; SDS, standard deviation score; LDL/HDL ratio, ratio of low density lipoproteins/ high density lipoproteins. Comparison of mean between groups [ISS vs. GHD; individualized versus standard GH dose (Ind. vs. Std.)]: Two-samples independent t-test was used. Paired-samples t-test was performed to calculate differences in each metabolic variable between start and 2 years of GH treatment (marked by asterics. * p<0.05; ** p<0.01). | | | | | | | | |

Figure 2 Changes of selected metabolic variables during growth hormone treatment



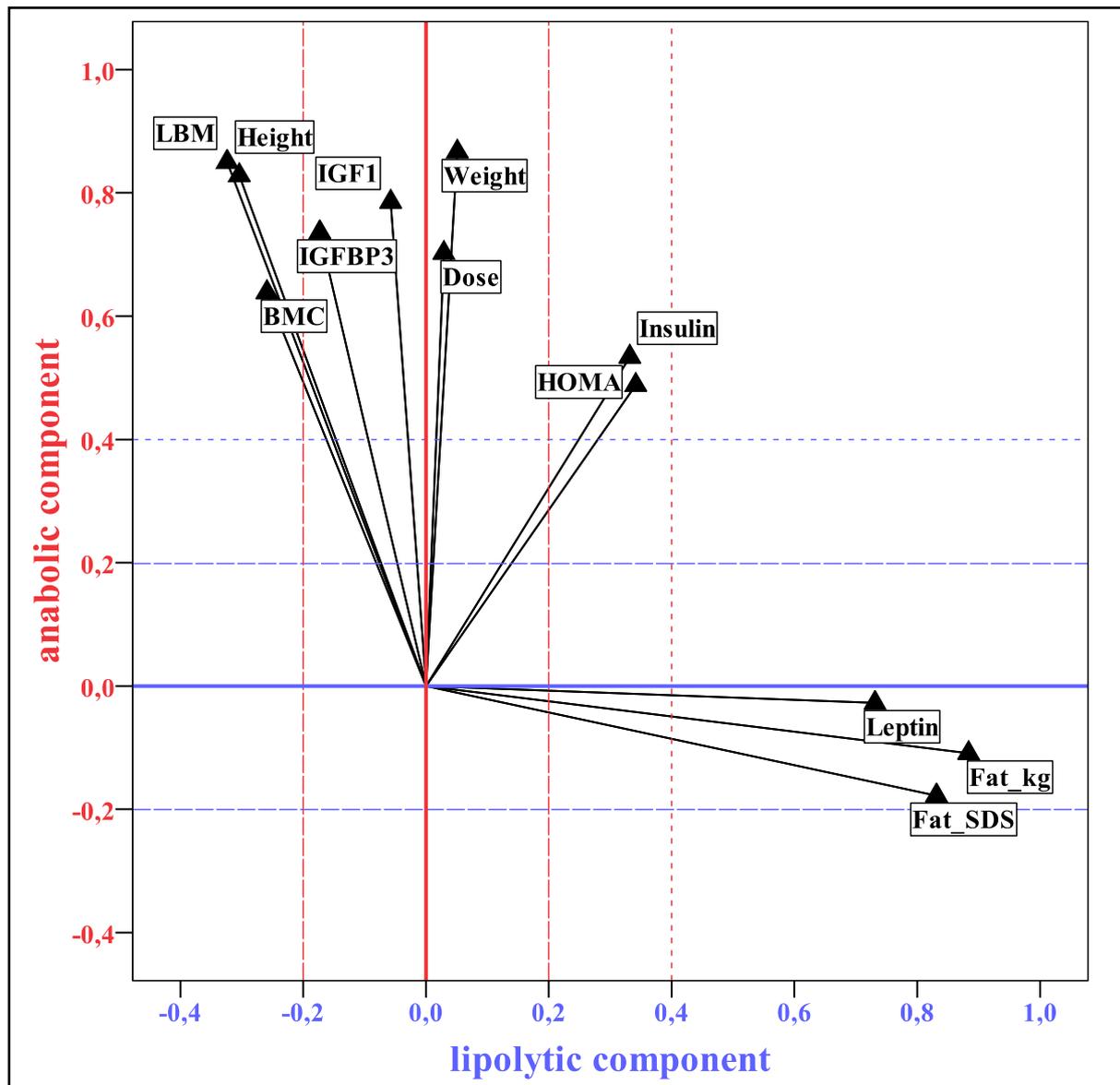
The lines show changes of mean \pm SEM during GH treatment in adiponectin, insulin, and insulin-like growth factor I (IGF-I) (upper panels) and leptin, LDL/HDL ratio, and fat mass (lower panels) in short prepubertal children with idiopathic short stature (ISS) (left panels) and GH deficiency (GHD) (right panels).

Paired samples t-test was performed to calculate differences between start and 1 year (asterisk shown at start), 1 year and 2 years (asterisk at 1 year), and start and 2 years (asterisk at 2 years); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 3 Regression analysis of body composition in relation to growth response

Regression analysis of Δ lean mass SDS (upper panels) and Δ fat mass SDS (lower panels) on growth response (Δ Height SDS) in idiopathic short stature (ISS, left panels) and GH deficiency (GHD, right panels) is shown. The treatment groups are shown separately, individually GH treatment (open circles) and standard GH treatment (filled triangles). Quadratic and linear polynomials were used for regression lines. Correlation coefficients r and p -values are given; n.s. not significant.

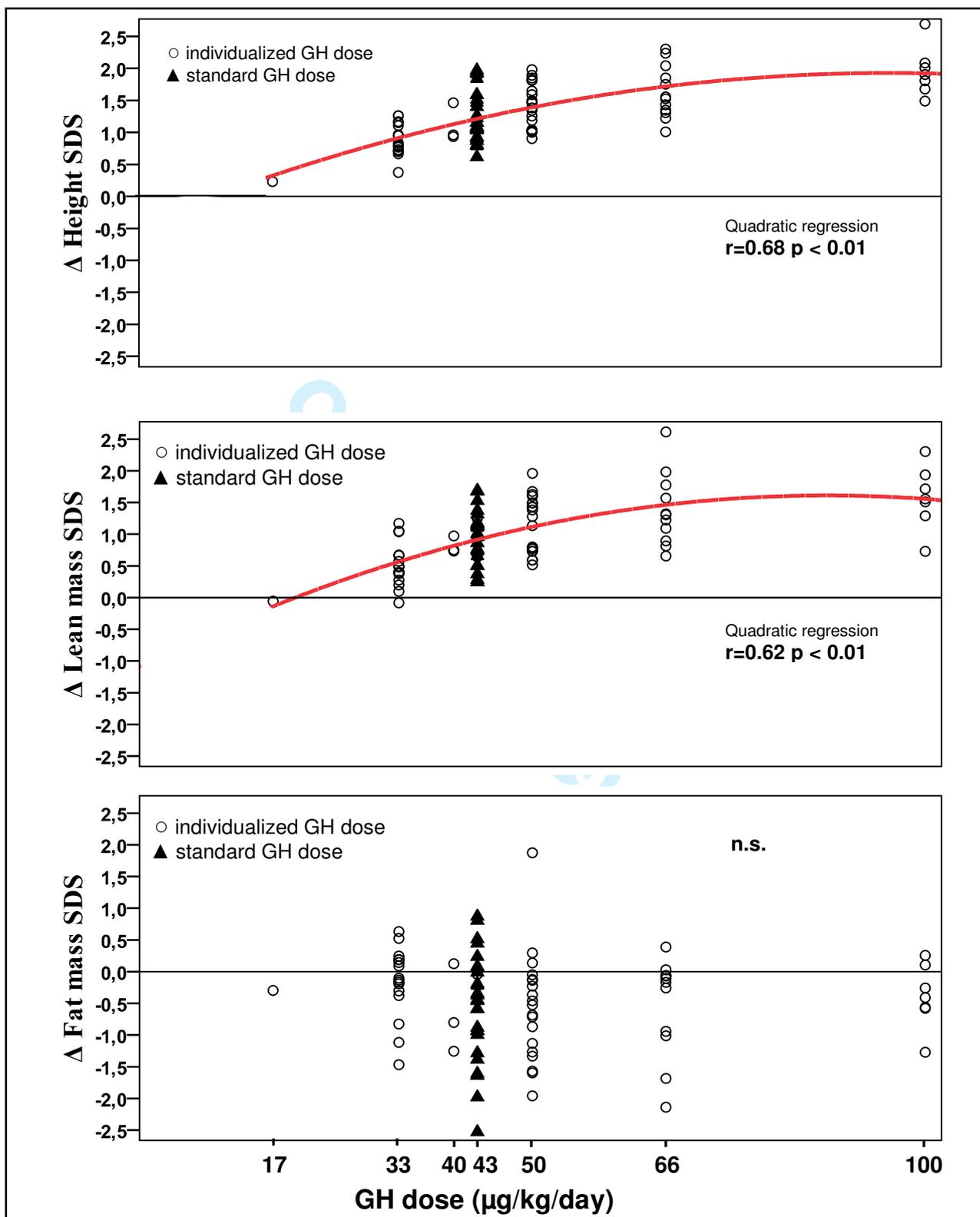
Figure 4 Two-dimensional principal component analysis (PCA) of data from the group of children with idiopathic short stature (ISS)



The vector for each variable consists of a correlation with an anabolic component (y-axis) and a lipolytic component (x-axis). This PCA analysis explains 61% of the total variance observed in all variables. **Vectors with close mutual angles form a vector bundle.**

Changes (Δ) in different variables during the two years on GH treatment are included in the analysis. All variables are shown as delta values between start and after 2 years of treatment. IGF1: insulin-like growth factor-I, IGFBP3: IGF-binding protein 3, LMB: lean body mass, BMC: bone mineral content, Fat_kg: fat mass (kg), Fat_SDS: fat mass (SDS), HOMA: homeostasis model assessment, Dose: GH dose.

Figure 5 Dose-dependency of height gain and lean body mass but not fat mass in ISS



Regression analysis of delta height SDS (upper panel), delta lean mass SDS (middle panel) and delta fat mass SDS (lower panel) on GH dose in idiopathic short stature is shown. Quadratic polynomials were used to show the relation between the dependent variables and the different GH dose groups (17, 33, 40, 43, 50, 66, and 100 $\mu\text{g/kg/day}$). Correlation coefficient r is given; n.s., not significant.