**Research Article**

# Metabolic Effects of Growth Hormone Treatment in Short Prepubertal Children: A Double-Blinded Randomized Clinical Trial

Anders Tidblad[a]   Jan Gustafsson[b]   Claude Marcus[c]   Martin Ritzén[a]
Klas Ekström[a]

[a]Department of Women's and Children's Health, Division of Pediatric Endocrinology, Karolinska Institutet, Stockholm, Sweden; [b]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden; [c]Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, Stockholm, Sweden

**Abstract**

***Introduction:*** Growth hormone (GH) is a central hormone for regulating linear growth during childhood and also highly involved in the metabolism of lipids, carbohydrates, and protein. However, few studies report on how treatment with GH during childhood influences metabolic parameters. Our aim was to investigate metabolic effects of different doses of GH in short children with GH peak levels in the low to normal range. ***Design:*** Thirty-five prepubertal short children ($<-2.5$ SDS), aged 7–10 years, with peak levels of GH between 7 and 14 µg/L during an arginine-insulin tolerance test, were randomized to 3 different doses (11/33/100 µg/kg/day) of GH treatment for 2 years. Auxological and metabolic investigations were performed. These included metabolites in blood and interstitial microdialysis fluid, dual-energy X-ray absorptiometry, frequently sampled intravenous glucose tolerance test (FSIVGTT), and stable isotope examinations of rates of glucose production and lipolysis. ***Results:*** At 24 months, the high-dose group (HD) had higher fasting insulin compared with the standard-dose (SD) and low-dose (LD) groups (HD: 111.7 vs. SD: 61.2 and LD: 46.0 pmol/L [$p < 0.001$]) and showed signs of insulin resistance (HOMA-IR, HD: 4.20 vs. SD: 2.17 and LD: 1.71 (LD) [$p < 0.001$]). The FSIVGTT also demonstrated higher acute insulin response ($p < 0.05$). Few other metabolic differences were found at 24 months, but a decreased insulin sensitivity index (Si) could already be seen at 12 months for both SD and HD compared with the LD group ($p < 0.05$). ***Conclusion:*** Treatment with GH resulted in a dose-dependent decrease in insulin sensitivity, demonstrated by higher levels of fasting insulin and signs of insulin resistance in both HOMA indices and FSIVGTT examinations.

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## Introduction

Growth hormone (GH) has a number of physiological effects, from regulation of linear growth during childhood to effects on the metabolism of lipids, carbohydrates, and protein during the entire life span [1–3]. Treatment with recombinant human growth hormone (rhGH) was introduced in 1985, and from a scarce amount of pituitary-derived GH used in cases of severe GH defi-

Anders Tidblad
Department of Women's and Children's Health, Karolinska Institutet and Division of Pediatric Endocrinology, Karolinska University Hospital
QB-83, Karolinska Vägen 37A, SE–171 76 Stockholm (Sweden)
anders.tidblad@ki.se

ciency (GHD), the unlimited production capacity made it possible to expand the treatment indications to include patients with short stature due to other reasons than poor endogenous GH secretion [4–6]. Treatment indications, at present date, differ somewhat globally but include, in addition to GHD, several other conditions with short stature, such as being born small for gestational age without catch-up growth, idiopathic short stature, short stature due to chronic renal failure, Turner syndrome, and Prader-Willi syndrome [5, 7].

Effects on height during treatment of different patient groups with rhGH have been extensively described during the 35 years of rhGH treatment [8–11], but effects on different metabolic parameters are less well known. In severe GHD, a phenotype of short stature as well as metabolic abnormalities can be seen [12, 13]. The rationale for treatment is thus not only improvement of height but also correction of these disturbances in patients with severe GHD. In a previous study, we investigated whether such disturbances also could be detected in short children in the normal to lower range of GH secretion but above the conventional threshold of GHD [14]. There were few differences in comparison with an age- and sex-matched healthy control group, but in a sub-analysis of those with lowest peak GH secretion, differences regarding insulin sensitivity and insulin-like growth factor I (IGF-I) could be demonstrated [14]. In this study, the same cohort of children was randomized to receive 3 different doses of rhGH for 2 years in order to investigate how rhGH treatment influences different metabolic parameters.

## Materials and Methods

### Study Design and Setting

The study was a multi-centre, double-blinded, randomized clinical trial in which 4 paediatric departments in Sweden participated between 2002 and 2010. The included patients were randomized to 3 different doses of rhGH: low (11 µg/kg/day), standard (33 µg/kg/day), or high (100 µg/kg/day) dose. The majority of the patients were recruited and investigated at Karolinska University Hospital. The participants were followed for 2 years with auxological measurements every 3 months and extensive in-patient examinations at baseline and 12 and 24 months.

### Study Population

The short prepubertal children eligible for the study met the following inclusion criteria: age between 7.0 and 9.9 years of age, height <−2.5 standard deviation scores (SDS) according to Swedish national reference [15], prepubertal (Tanner stage: B1, PH1 in girls and G1, PH1 in boys as well as testicle volume <4 mL), normal sitting height ratio (±2 SDS, according to Gerver et al. [16]), term delivery and normal birth weight for gestational age (within ±2

SDS according to Swedish birth reference [17]), bone age <8.5 years and <10 years in girls and boys, respectively, assessed by RUS/TW2, normal karyotype (girls), otherwise healthy, and a GH peak value ($GH_{max}$) between 7 and 14 µg/L during an arginine and insulin tolerance test. The exclusion criteria were preterm birth, any syndrome, any recognized disease that could compromise height, or any ongoing medical treatment except for well-controlled hypothyroidism.

An informed consent from all included subjects and parents/guardians was obtained before the study. The patients could discontinue the study at any time by request of the patient and/or caregiver, in case of serious illness, serious adverse events, or in case of protocol violation.

### Randomization and Blinding

Randomization was performed as a block randomization by a computerized random allocation and was administered by the pharmacy at Karolinska University Hospital, masked and separated from the study investigators. The randomization blocks were created to ensure balanced allocation to the different treatment arms and created separately for girls and boys. The administered drug was only marked with the patient study number and not any information of dose. Thus, the allocated treatment was blinded for both study investigators and participants.

### Study Drug, Dosage, and Drug Administration

The rhGH used in the study was Humatrope® (Eli Lilly) which was commercially available in vials of 3 different concentrations (6, 12, and 24 mg) and packed in boxes containing substance and ampoules of saline diluent. The drug was given subcutaneously in the evening by assistance of an autoinjector provided by Eli Lilly. The treatment was delivered to the patients and families in unmarked packages to guarantee blinding of dose and recollected after use to assure compliance. All groups started with half of the randomized dose the first month and were evaluated every 3 months for height and weight measurements as well as adverse events. Weight changes were communicated to the pharmacist after each visit in order to adjust the dose to keep the allocated dose per weight and day. Measurements of IGF-I were carried out at each visit as a safety precaution, and if the levels were above +2 SDS, a step-wise dose reduction of 20% was made until the levels were within normal range (<+2 SDS) [18].

### Blood Samples and Metabolic Examinations

A detailed description of the different laboratory and metabolic examinations carried out during the study has previously been published [14]. In summary, the participants were evaluated every 3 months with clinical examination, auxological measurements, and fasting blood samples of glucose, insulin, IGF-I, and HbA1c. Calculations of insulin resistance were made using the homeostasis model assessment of insulin resistance (HOMA-IR) and the updated computerized version HOMA2-IR, an improved version regarding certain aspects such as handling variations in hepatic and peripheral insulin resistance [19, 20].

More extensive investigations were also performed at baseline and 12 and 24 months, with frequently sampled intravenous glucose tolerance test (FSIVGTT) and calculations of insulin sensitivity (Si), acute insulin response (AIR), and glucose effectiveness (Sg) by the Minimal Model computer program (MinMod Millennium) [21]. Body composition was analysed by dual-energy X-ray
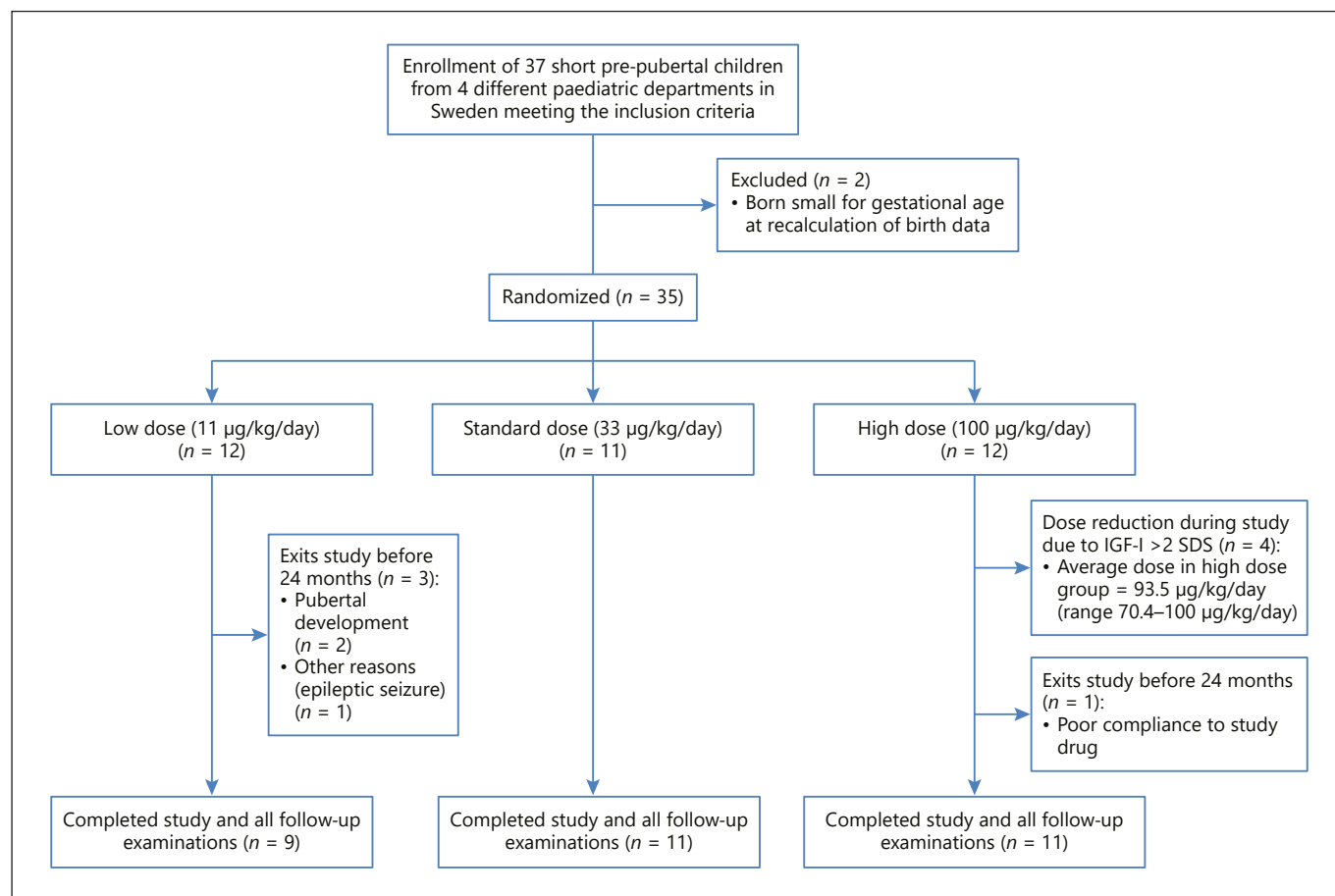
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**Fig. 1.** Flowchart of study inclusion, exclusion, and follow-up. Four patients in total ended the study before 24 months, 3 in the low-dose group and 1 in the high-dose group. All patients in the study were offered to continue with standard dose of recombinant human GH after the end of the study. GH, growth hormone; IGF-I, insulin-like growth factor I; SDS, standard deviation scores.

absorptiometry (DEXA) using GE Lunar Prodigy Advance DXA (GE Healthcare Lunar, Madison, WI, USA) with the enCORE software version 5.7–12.3 [22]. All proportions of specific tissue mass were calculated in relation to total body mass and presented as percentages. Microdialysis of subcutaneous adipose tissue was performed to evaluate levels of glucose and glycerol (as a marker of lipolysis). The procedure has been described in detail previously [23]. All dialysate samples analysed were collected in fasting state (from midnight until 4 am). Lastly, measurements of whole body glucose production and lipolysis were performed using stable isotope-labelled glucose and glycerol [14]. Production rates of glucose and glycerol were calculated from the ratios of isotopic $[6,6-2H_2]$-glucose and $[1,1,2,3,3-2H_5]$-glycerol versus unlabelled compounds during periods of approximate steady state [24]. Contributions of enteral glucose and glycerol were prevented by prolonged fasting before the examination.

*Statistical Analysis*

Summary statistics of baseline characteristics are presented as means and standard deviations for continuous variables and as frequencies and percentages for binary variables. To account for the dependence in the data, due to repeated measures at different time points of each individual, a multilevel mixed-effects linear regression model was used. The data were clustered in 2 hierarchical levels by design, the 3 treatment groups and the different repeated measures of the individual subjects within each treatment group. The fixed effect in the model concerns the treatment assignment and the random effect, the variation of each individual within the different treatment groups. The model contained a random intercept and was fitted with the restricted maximum likelihood option to reduce small-sample bias in estimates of random-effect variances. Pairwise comparisons and tests of the effect of treatment between the groups were made at each time point, as well as tests of effect of time within each treatment group. The effect of peak GH secretion levels ($GH_{max}$) above or below 10 µg/L on fasting insulin, Si, HOMA-IR, HOMA2-IR, and IGF-I was tested within each treatment group at 12 and 24 months with the 2-sample independent $t$-test for normally distributed variables and with the Wilcoxon rank-sum test for non-normally distributed variables. Test of equal variance was used and applied if appropriate for the

**Table 1.** Baseline characteristics of the study cohort

|  | Low dose (n = 12) | Standard dose (n = 11) | High dose (n = 12) |
|---|---|---|---|
| Age, years | 8.42 (0.9) | 7.71 (1.0) | 8.30 (1.0) |
| Sex, n (%) |  |  |  |
|     Males | 7 (58.3) | 7 (63.6) | 8 (66.7) |
|     Females | 5 (41.7) | 4 (36.4) | 4 (33.3) |
| Height, cm | 116.2 (4.5) | 112.1 (5.6) | 115.6 (4.3) |
|     SDS | −2.81 (0.25) | −3.02 (0.28) | −2.90 (0.31) |
| Weight, kg | 20.9 (2.8) | 19.3 (2.8) | 20.1 (2.6) |
|     SDS | −2.00 (0.43) | −2.18 (0.63) | −2.22 (0.47) |
| Body mass index | 15.4 (1.1) | 15.3 (1.2) | 15.0 (1.0) |
|     SDS | −0.40 (0.64) | −0.42 (0.76) | −0.68 (0.59) |
| Birth characteristics |  |  |  |
|     Gestational age, weeks | 39.6 (1.1) | 40.0 (1.0) | 40.2 (1.0) |
|     Birth length, SDS | −0.99 (0.56) | −0.68 (0.87) | −0.53 (0.97) |
|     Birth weight, SDS | −0.42 (0.62) | −0.15 (0.82) | −0.03 (0.99) |
| Parental height |  |  |  |
|     Fathers' height, SDS | −1.07 (0.91) | −1.35 (0.86) | −1.22 (0.89) |
|     Mothers' height, SDS | −1.55 (0.69) | −1.38 (1.29) | −1.30 (0.96) |
|     Midparental height, SDS | −1.31 (0.59) | −1.35 (0.77) | −1.26 (0.79) |
|     Difference in height SDS and midparental SDS | −1.51 (0.56) | −1.66 (0.63) | −1.65 (0.78) |
| Bone age (TW2)[a] | 6.6 (1.3) | 5.9 (1.6) | 5.9 (1.8) |
| Difference in bone age and chronological age, years | 2.17 (1.14) | 2.06 (1.11) | 2.61 (1.42) |
| $GH_{max}$ (AITT)[b] | 10.3 (2.6) | 10.1 (2.0) | 10.0 (1.7) |
| Puberty development during follow-up, n (%)[c] |  |  |  |
|     At 12-month follow-up | 2 (17) | 0 | 0 |
|     At 24-month follow-up | 5 (42) | 2 (18) | 3 (25) |
| Actual mean daily dose of rhGH during study, μg/kg/day | 11 | 33 | 93.5 |

Data are given as mean (SD) except where indicated otherwise. SDS, standard deviation score; GH, growth hormone; rhGH, recombinant human growth hormone. [a] Bone age by Tanner-Whitehouse 2 (TW2). [b] $GH_{max}$, GH peak level during the arginine-insulin tolerance test (μg/L). [c] Defined as Tanner stage B2 for girls and testicle volume ≥4 mL for boys.

t tests. Spearman correlation tests were also performed for the same metabolic outcomes to analyse possible associations with $GH_{max}$ as a continuous variable. Due to start of pubertal development in some participants, additional analyses without these subjects were performed for all metabolic outcomes at the end of the study. All tests were 2-sided, and p values of <0.05 were considered statistically significant. All analyses were conducted using Stata version 14.2 (StataCorp, College Station, TX, USA).

## Results

Thirty-seven patients fulfilled the inclusion criteria and were enrolled in the study. Two individuals were excluded at a later stage due to being small for gestational age when SDS for birth weight was recalculated, leaving a total of 35 children (12 girls) in the study (Fig. 1). Basal characteristics of the 3 treatment groups are shown in Table 1.

Thirty-one patients (89%) completed the 2-year study and 4 ended the study prematurely; 3 in the low-dose group (2 due to pubertal development during the mid-study follow-up and 1 due to epileptic seizures) and 1 in the high-dose group due to poor compliance to the study drug (Fig. 1). A total of 4 individuals in the high-dose group needed dose reduction due to high IGF-I values. The actual average dose in the high-dose group over the 2-year period was 93.5 μg/kg/day (range: 70.4–100 μg/kg/day). Pubertal development was noted in 2 patients in the low-dose group at the 12-month visit and in 10 patients at the last visit after 24 months (5 in the low-dose group, 2 in the standard-dose group, and 3 in the high-dose group) (Table 1).

The main differences in metabolic outcomes between the treatment groups are presented in Table 2. After 12 months, the subjects in the high-dose group had higher

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**Table 2.** Comparison of metabolic outcomes at baseline and 12 and 24 months of follow-up between different treatment groups

| | Baseline | | | | 12 months[a] | | | | 24 months[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | low | standard | high | p value | low | standard | high | p value | low | standard | high | p value |
| *Blood samples* | | | | | | | | | | | | |
| F-glucose, mmol/L | 5.5 (0.7) | 5.4 (0.6) | 5.3 (0.4) | ns | 5.7 (0.6) | 5.3 (0.5) | 5.7 (0.5) | ns | 5.7 (0.4) | 5.4 (0.6) | 5.9 (0.6) | ns |
| F-insulin, pmol/L | 22.9 (14.3) | 22.2 (9.6) | 25.6 (18.6) | ns | 52.5 (36.4) | 43.5 (26.4) | 74.7 (36.9) | ns | 46.0 (22.9) | 61.2 (35.8) | 111.7 (52.9) | <0.001[c] |
| HbA1c, mmol/mol | 34.3 (3.4) | 33.1 (4.2) | 34.7 (2.6) | ns | 35.0 (2.5) | 34.3 (5.1) | 35.7 (3.2) | ns | 35.0 (1.5) | 34.5 (3.7) | 35.9 (2.2) | ns |
| IGF-I, µg/L | 107 (51) | 95 (38) | 107 (52) | ns | 197 (98) | 242 (62) | 352 (121) | <0.001[c] | 220 (106) | 291 (105) | 402 (114) | 0.007[d] <0.001[f] 0.002[e] |
| IGF-I, SDS | −1,79 (1.0) | −1,81 (1.0) | −1.67 (1.0) | ns | −0,55 (1.5) | 0.32 (0.9) | 1.48 (1.3) | 0.025[e] <0.001[f] | −0.42 (1.5) | 0.62 (1.2) | 1.81 (1.2) | 0.021[e] <0.001[f] |
| HOMA-IR | 0.79 (0.49) | 0.75 (0.33) | 0.86 (0.59) | ns | 1.99 (1.61) | 1.51 (0.95) | 2.77 (1.48) | 0.006[e] | 1.71 (0.93) | 2.17 (1.38) | 4.20 (1.94) | <0.001[c] |
| HOMA2-IR | 0.44 (0.25) | 0.43 (0.18) | 0.48 (0.33) | ns | 1.00 (0.70) | 0.83 (0.49) | 1.43 (0.72) | 0.012[e] | 0.89 (0.44) | 1.17 (0.69) | 2.13 (0.96) | <0.001[c] |
| *FSIVGTT* | | | | | | | | | | | | |
| Si, mU × L⁻¹ × min⁻¹ | 13.6 (5.3) | 15.4 (8.2) | 11.2 (4.2) | 0.022[e] | 10.1 (2.5) | 6.4 (1.7) | 5.4 (1.8) | 0.025[d] 0.003[f] | 7.9 (2.0) | 7.8 (2.9) | 5.2 (2.5) | ns |
| AIR, mU × L⁻¹ × min | 187 (110) | 271 (238) | 316 (182) | ns | 271 (203) | 395 (300) | 509 (264) | 0.026[f] | 348 (229) | 418 (337) | 667 (388) | 0.009[f] 0.035[e] |
| Sg, min⁻¹ | 0.028 (0.014) | 0.037 (0.016) | 0.031 (0.011) | ns | 0.024 (0.013) | 0.020 (0.012) | 0.028 (0.010) | ns | 0.036 (0.009) | 0.041 (0.052) | 0.024 (0.011) | ns |
| *DEXA* | | | | | | | | | | | | |
| Ab. fat mass, % | 13.5 (5.8) | 13.1 (5.2) | 13.2 (4.9) | ns | 15.2 (6.7) | 13.1 (6.2) | 10.6 (4.4) | NS | 15.2 (9.7) | 14.2 (6.4) | 12.5 (5.6) | ns |
| Tot. fat mass, % | 16.3 (5.2) | 16.2 (5.2) | 16.1 (5.3) | ns | 16.9 (6.2) | 14.2 (6.1) | 11.0 (4.8) | 0.035[f] | 17.2 (9.6) | 15.8 (6.3) | 12.9 (6.3) | ns |
| Lean body mass, % | 80.7 (4.8) | 80.7 (4.9) | 80.8 (5.0) | ns | 80.1 (5.8) | 82.8 (5.7) | 85.8 (4.6) | 0.030[f] | 79.6 (9.0) | 81.2 (5.9) | 83.9 (6.1) | ns |
| *Microdialysis (sc fat)* | | | | | | | | | | | | |
| Glucose, mmol/L | 5.5 (1.0) | 4.9 (1.0) | 5.5 (0.3) | ns | 4.9 (0.2) | 4.7 (0.3) | 5.0 (0.6) | ns | 4.8 (0.4) | 4.8 (0.4) | 4.8 (0.4) | ns |
| Glycerol, µmol/L | 145 (48) | 144 (68) | 171 (60) | ns | 133 (54) | 160 (85) | 128 (45) | ns | 137 (77) | 140 (66) | 210 (126) | 0.022[e] |
| *Isotope examination* | | | | | | | | | | | | |
| Glucose production | 5.2 (1.1) | 5.5 (0.6) | 4.9 (0.8) | ns | 5.0 (0.7) | 5.1 (1.2) | 5.3 (0.6) | ns | 4.7 (0.8) | 4.6 (0.6) | 4.6 (0.6) | ns |
| Glycerol production | 6.0 (3.7) | 6.3 (3.3) | 5.8 (2.8) | ns | 7.4 (6.3) | 7.2 (8.2) | 8.2 (4.4) | ns | 8.8 (10.3) | 4.9 (1.9) | 5.0 (3.0) | ns |

Data are given as mean (SD). ns, non-significant; SDS, standard deviation score; IGF-I, insulin-like growth factor I; HOMA-IR, homeostasis model assessment of insulin resistance; FSIVGTT, frequently sampled intravenous glucose tolerance test; Si, insulin sensitivity; AIR, acute insulin response; Sg, glucose effectiveness; DEXA, dual-energy X-ray absorptiometry. [a] At 12 months, low dose (*n* = 11, except for f-insulin, HOMA/-2-IR, and glucose production, where *n* = 11, except for f-insulin, HOMA/-2-IR, FSIVGTT, and isotope ex., where *n* = 10), standard dose (*n* = 11, except for f-insulin, HOMA/-2-IR, FSIVGTT, and isotope ex., where *n* = 10), and high dose (*n* = 11, except for isotope ex., where *n* = 10). [b] At 24 months, low dose (*n* = 9, except for IGF-I [*n* = 8], FSIVGTT [*n* = 7], and microdialysis [*n* = 6]), standard dose (*n* = 11, except for microdialysis [*n* = 10] and isotope ex. [*n* = 9]), and high dose (*n* = 11, except for microdialysis, where *n* = 7). [c] High versus low dose and high versus standard dose. [d] Standard versus low dose. [e] High versus standard dose. [f] High versus low dose.

IGF-I compared with those in the standard- and low-dose group (352 vs. 242 and 197 µg/L, respectively [$p < 0.001$]). Furthermore, the high-dose group had signs of increased insulin resistance in HOMA-IR and HOMA2-IR compared with the standard-dose group (2.77 vs. 1.51, $p = 0.006$, and 1.43 vs. 0.83, $p = 0.012$, respectively). The decreased insulin sensitivity was also confirmed in the FSIVGTT, in which the high-dose group had a lower sensitivity index ($p = 0.003$) and an augmented acute insulin response ($p = 0.026$) compared to the low-dose group. The standard-dose group also showed signs of decreased insulin sensitivity with a lower Si compared to that of the low-dose group ($p = 0.025$). Total fat mass was lower in the high- compared to the low-dose group (Table 2). There were no differences between the groups regarding the microdialysis levels of glucose and glycerol or rates of glucose production and lipolysis studied by use of stable isotopes.

At the end of the study (24 months), the groups still differed in IGF-I levels, the high-dose group being higher than both the standard-dose group (+111 µg/L, $p = 0.002$) and the low-dose group (+182 µg/L, $p < 0.001$). The standard-dose group also had higher IGF-I values than the low-dose group (+71 µg/L, $p = 0.007$). Moreover, the high-dose group had higher fasting insulin than the standard- and low-dose groups (111.7 vs. 61.2 and 46.0 pmol/L, $p < 0.001$). HOMA-IR and HOMA2-IR were also higher in the high-dose group (4.20 vs. 2.17 and 1.71, $p < 0.001$, and 2.13 vs. 1.17 and 0.89, $p < 0.001$, respectively). However, Si did not differ between the groups; the high-dose group remained at a similar Si as that found at 12 months, and the standard dose only decreased marginally, but the low-dose group continued to decrease levelling out earlier differences (online suppl. Fig. 1c; for all online suppl. material, see www.karger.com/doi/10.1159/000513518). The AIR was, on the other hand, still different between the high-dose group and the other 2 groups, with a higher acute insulin response (+249 mU × L$^{-1}$ × min vs. the standard-dose group, $p = 0.035$, and +319 mU × L$^{-1}$ × min vs. the low-dose group, $p = 0.009$). Lastly, the microdialysis studies showed higher levels of glycerol for the high-dose group compared to the standard- and low-dose group, suggesting increased lipolysis, but only reached statistical significance in comparison with the standard-dose group (Table 2). No differences between the groups in body composition or rates of glucose production and lipolysis were seen at 24 months. Similar metabolic differences between the groups at 24 months were found when excluding the participants, in which pubertal development had started at that time (see online suppl. Table 1.

During the course of the 2-year study, several metabolic changes occurred in the groups. The most noticeable changes in the high- and standard-dose groups were those regarding fasting insulin and measures of insulin sensitivity (online suppl. Fig. 1a–d). For the high-dose group, there was also a significant increase in fasting glucose (+0.6 mmol/L, $p = 0.004$) and HbA1c (+1.4 mmol/mol, $p = 0.046$) from 0 to 24 months, but still within normal ranges. The changes from baseline to the end of the study period (delta [$\Delta$] values, 0–24 months) for selected outcomes are presented in Figure 2.

In the high-dose group, the average height SDS increase was 1.62 SDS, which differed from that of the standard-dose group which gained 1.12 SDS ($p < 0.001$) and that of the low-dose group which gained 0.57 SDS ($p < 0.001$) over the study period (Fig. 2a). There was a clear difference in variations of $\Delta$ values between the groups. Thus, there was less variation of $\Delta$ height SDS, $\Delta$ f-insulin, $\Delta$ HOMA-IR, and $\Delta$ total body fat in the low-dose group than the other 2 groups (Fig. 2a, d–f). Except for IGF-I in the high-dose group at 24 months (+143 µg/L, $p = 0.03$) in the GH$_{max}$ >10 versus <10 group, the GH peak levels (continuously or categorized as above or below 10 µg/L at baseline) did not correlate with the measured outcomes.

No severe adverse events occurred during the study. Four participants in the high-dose group needed dose reductions, as described above, due to high IGF-I levels (Fig. 1). One of these also developed sleep apnoea during the end of the study, which could be related to the treatment. Following adeno-tonsillectomy, there was a significant improvement in polysomnography registrations, and study completion was possible after dose reduction.

## Discussion

In this randomized controlled clinical trial investigating metabolic effects of GH treatment in short prepubertal children, we found a dose-dependent relationship between GH dose and fasting insulin as well as indices of insulin sensitivity. All dose groups developed diminished insulin sensitivity measured by FSIVGTT. However, there was a difference in tempo since it occurred earlier in the high- and standard-dose groups (at 12 months) compared to the low-dose group (at 24 months). All groups also showed increased fasting insulin levels throughout the course of the study, but the levels differed between the groups, with the largest increase in the high-dose group compared to the standard- and low-dose groups. Effects on body composition could be seen in the
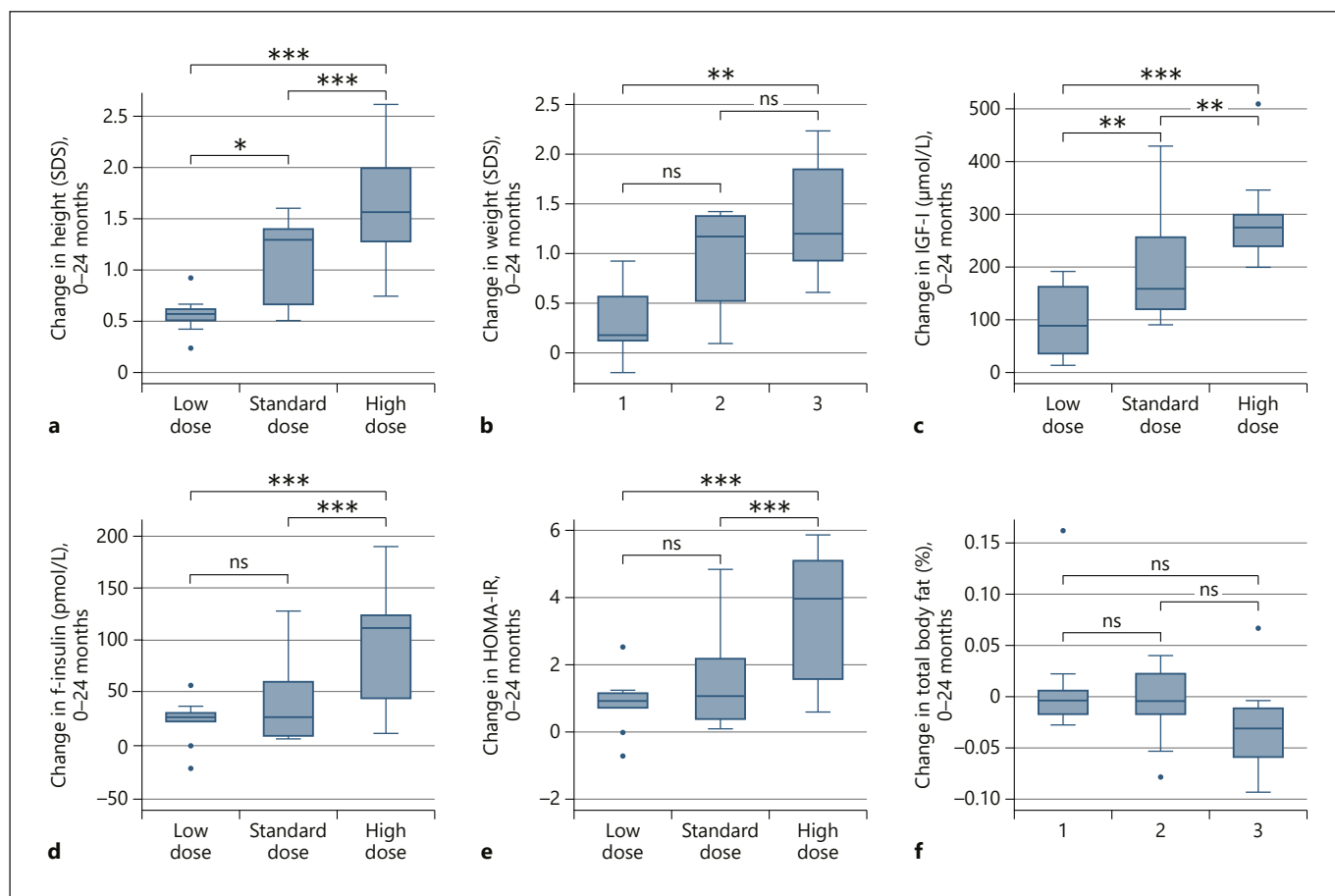
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**Fig. 2.** Changes in auxological and metabolic outcomes over the study period. The boxplots (**a–f**) show changes from baseline to the end of the study period (Δ values, 0–24 months) for the 3 treatment groups in different auxological and metabolic parameters. A clear difference in height gain (**a**) is seen between the groups, with less variation in the low-dose group compared to the other 2 treatment groups. Increase in weight SDS (**b**) and IGF-I (**c**) is also seen in all groups. Effects on fasting insulin (**d**) and the HOMA-IR (**e**) are most noticeable in the high-dose group as well as the effects on proportion (%) of total body fat (**f**) measured by DEXA. ns, not significant, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. SDS, standard deviation scores; IGF-I, insulin-like growth factor I; HOMA-IR, homeostasis model assessment of insulin resistance; DEXA, dual-energy X-ray absorptiometry.

high-dose group at 12 months, with decreasing amount of total body fat mass, but not in the standard- or low-dose group. Furthermore, the high-dose group had, as expected, the largest increase in IGF-I and the largest Δ height SDS gain of the groups. There were no differences over time or between the groups regarding microdialysis levels of interstitial glucose and glycerol or isotopic examinations of rates of glucose production and lipolysis.

Few studies have investigated metabolic effects of GH treatment in short prepubertal children with GH secretion in the lower to normal range. Early small-scale studies on prepubertal non-GHD children demonstrated similar findings as those in the present study, with increasing levels of fasting insulin during GH treatment but with no

or only minor effects on fasting glucose and glucose tolerance [25–27]. A larger study on prepubertal children with idiopathic short stature by Saenger et al. [28] also demonstrated substantially increased fasting insulin levels, although starting at a low normal level and kept within normal range during the 5-year follow-up. However, only 20/121 (16.5%) of those entering that study had a measured fasting insulin level at the end of the study. Furthermore, the defined normal range was not based on a paediatric population [29]. Newly published [30] paediatric reference data for fasting insulin, glucose, HbA1c, and HOMA-IR show that our high-dose group was above the 95th percentile for fasting insulin, glucose, and HOMA-IR but not for HbA1c. On the other hand, the standard-

and low-dose groups were well within the normal ranges for these parameters, even with their markedly increased fasting insulin levels and HOMA-IR during the 2-year study period.

In the high-dose group, there was a significant effect over time on fasting glucose and HbA1c, rarely reported in earlier studies [31]. However, more recent data on GHD patients demonstrate similar effects on fasting glucose, in some reports associated with higher doses of GH [32] and in others regardless of GH dose [33–36]. Furthermore, the effects on fasting insulin and indices of insulin sensitivity, in line with our findings, have also been replicated in many studies on GH treatment to GHD patients [31, 37, 38].

Our results demonstrate that GH treatment has a dose-dependent effect on insulin and glucose metabolism. Several mechanisms on how GH affects carbohydrate metabolism and insulin sensitivity have been proposed [39], including effects on synthesis [40] and secretion [41], effects on antilipolytic signals in adipose tissue [42], and induction of peripheral [43, 44] and central insulin resistance [2, 45]. The exact mechanisms are, however, less simple to disentangle and probably include both effects on peripheral as well as central insulin sensitivity since higher fasting insulin was needed for glucose homeostasis without affecting the total body glucose production. However, the significant increase in fasting glucose without increased glucose production over time, in the high-dose group, could perhaps indicate a predominant effect on the peripheral insulin sensitivity in that dose group.

Interestingly, the variation in Δ values showed a clear difference between the groups, where the low-dose group had much less variation in most parameters, compared to the standard- and high-dose groups. The large variation observed on both growth and metabolic parameters is probably explained by the diverse underlying aetiologies for short stature in this group of patients [46–48]. Consequently, the lack of variation in the low-dose group could perhaps indicate that the low dose was insufficient to substantially affect the measured outcomes in this patient group, hence the lower variation in their response.

A major strength of the present study is the advanced methodology used to analyse the metabolic effects of different GH doses. Earlier studies have, to a large extent, solely relied on indirect measurements of insulin sensitivity with a few exceptions [37, 49]. In agreement with previous findings, we could also show effects on insulin sensitivity and acute insulin response measured by FSIVGTT and in body composition by DEXA, laborious methods seldom used in clinical practice but important in order to bring more insight into actual metabolic effects of GH treatment.

A limitation in the present study is the limited size which makes subgroup analyses underpowered. We have previously reported [14] differences in insulin sensitivity indices and IGF-I for subgroups of patients based on GH peak levels at a stimulation test before GH treatment, but found very small differences in this study. A possible explanation could be that the effect of treatment, independent of dose, overrides such subtle differences before treatment start. Another limitation is the loss of follow-up in 4 patients, but, in total, almost 90% of the included patients fulfilled the study, and the reasons for leaving the study were not considered to be associated with the treatment dose allocation. Finally, some of the patients entered puberty during the study period which could have an effect on several of the measured metabolic outcomes. However, the occurrence of pubertal signs was quite evenly spread between the groups and most frequent in the low-dose group which should have reduced the observed differences rather than enhanced them, if of any significance. The additional analyses presented in the supplement, excluding all participants that reached puberty during the follow-up, could also confirm this.

In conclusion, we found a clear dose-dependent effect of GH treatment on several metabolic parameters during the 2-year study period and particularly for the high-dose group regarding fasting insulin levels and indices of insulin resistance. There was a difference between the groups regarding IGF-I and height gain, where the high-dose group had the largest Δ height SDS. However, the standard-dose group also showed a clear effect on Δ height SDS but without the prominent effects on fasting insulin and insulin sensitivity as the high-dose group, and perhaps this could constitute a more acceptable trade-off between height gain and reduced insulin sensitivity. Nevertheless, all groups had a clearly decreased insulin sensitivity, even the low-dose group, confirming the close interplay of GH/IGF-I and insulin over the whole spectrum of administered doses in this study.

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## Statement of Ethics

All the participants and their parents or legal guardians provided written informed consent. The study was approved by the Regional Ethical Board at Karolinska Institutet, Stockholm (DNR 01-069) and conducted in accordance with the ethical principles of the Declaration of Helsinki [50].

## Conflict of Interest Statement

A.T. has received speaker's honorarium from Pfizer. The other authors (C.M., J.G., M.R., and K.E.) have no conflicts of interest to declare.

## Author Contributions

C.M. and M.R. conceived the study, K.E. conducted the study, J.G. performed the stable isotope examinations, and A.T. assembled the clinical data, performed the statistical analyses, and wrote the manuscript. All authors contributed to the interpretation of the data, critical revision of the manuscript, and approval of the final manuscript.

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