Randomized GH Trial with 2 Different Dosages in Combination with a GnRH Analogue in Short SGA Children: Effects on Metabolic Profile, Serum Growth Hormone, IGF-I and IGFBP-3 Levels

Daniëlle van der Kaay\textsuperscript{1,2}, Boudewijn Bakker\textsuperscript{3}, Flip van der Hulst\textsuperscript{4}, Dick Mul\textsuperscript{5}, Jaap Mulder\textsuperscript{6}, Eelco Schroor\textsuperscript{7}, Denise van Elswijk\textsuperscript{1,2}, Inge Rowaan\textsuperscript{1,2}, Merel Willeboer\textsuperscript{1,2}, Maria de Ridder\textsuperscript{3,8}, Anita Hokken-Koelega\textsuperscript{1,2}

\textsuperscript{1}Department of Pediatrics, Division of Endocrinology, Erasmus Medical Center, Rotterdam; \textsuperscript{2}Dutch Growth Research Foundation, Rotterdam; \textsuperscript{3}Department of Pediatrics, Reinier de Graaf Guesthouse, Delft; \textsuperscript{4}Department of Pediatrics, Zaans Medical Center, Zaandam; \textsuperscript{5}Department of Pediatrics, Haga teaching Hospital and Juliana Children’s Hospital, Den Haag; \textsuperscript{6}Department of Pediatrics, Rijnstate Hospital, Arnhem; \textsuperscript{7}Department of Pediatrics, Isala Clinics, Zwolle; \textsuperscript{8}Department of Epidemiology and Biostatistics, Erasmus Medical Center, Rotterdam, The Netherlands.

**Short title:** Metabolic profile, GH, IGF-I and IGFBP-3 levels during GnRHa and GH treatment in short SGA children

**Key words:** Small for Gestational Age – Body Composition – Insulin Sensitivity – IGF-I Levels – GnRHa Treatment – GH Treatment

**Word count:** 3661; **Abstract:** 250; **Tables:** 3; **Figures:** 3

**Correspondence and reprint requests:**
Daniëlle van der Kaay, Dutch Growth Research Foundation, P.O. Box 23068, 3001 KB Rotterdam, The Netherlands (Telephone: +31102251533; Fax: +31102250133; E-mail: d.vanderkaay@erasmusmc.nl)

Trial registration number: ISRCTN18062389
ABSTRACT

Background. Gonadotropin releasing hormone analogue (GnRHa) combined with GH treatment has been proposed to increase adult height. Effect on metabolic profile, GH, IGF-I and IGFBP-3 levels in short SGA children is unknown.

Objective. To assess fat mass and lean body mass SDS, percentage trunk fat, blood pressure, insulin sensitivity (Si), β-cell function (disposition index (DI)), lipid profile, GH, IGF-I and IGFBP-3 levels during 2 years of combined treatment.

Subjects. Forty-one pubertal short SGA children, mean (± SD) age: 12.1 (± 1.0) years.

Design. Children received leuprolelde acetate depot 3.75 mg subcutaneously every 4 weeks, and were randomly assigned to receive 1 mg (group A) or 2 mg (group B) GH/m²/day.

Results. Percentage trunk fat increased in both groups, but to lower extent in group B. Lean body mass SDS increased only in group B. Changes in blood pressure, Si, DI and lipids were similar in both groups. Insulin sensitivity significantly decreased, but DI remained unchanged. Lipids remained normal. GH and IGF-I levels were significantly higher in group B.

Conclusion. Our study is the first to report that 2 years of combined treatment with a GnRH analogue and either 1 mg or 2 mg GH/m²/day does not adversely affect body composition and metabolic profile of short SGA children who come under medical attention at onset of puberty. There was a dose-dependent effect on fat mass SDS<sub>height</sub>, percentage trunk fat, lean body mass SDS<sub>height</sub>, GH and IGF-I levels in favour of treatment with GnRHa and the higher GH dose of 2 mg/m²/day.
INTRODUCTION

Gonadotropin releasing hormone analogue (GnRHa) treatment has long been used in children with central precocious puberty (CPP). Over the years, little attention has been paid to changes in metabolic profile during GnRHa treatment. Studies have focused on body composition and were performed in children with CPP only. Some studies reported an increase in fat mass or BMI SDS during GnRHa treatment with a return to baseline values after discontinuation\(^1\), whereas others reported no changes \(^3\) or even a decreased BMI SDS during GnRHa treatment \(^4\).

In prepubertal short children born small for gestational age (SGA), growth hormone (GH) treatment induces catch-up growth\(^5\)\(^-\)\(^7\). Metabolic effects of GH treatment in prepubertal short SGA children include the development of relative insulin resistance\(^8\)\(^,\)\(^9\) with an improvement of blood pressure and lipid profile\(^10\). Fat mass SDS adjusted for gender and height declined significantly, whereas the increase in lean body mass SDS adjusted for gender and height reflected the normal increase as a result of the increase in height\(^11\). In a randomized clinical trial where prepubertal short SGA children were treated with 1 mg or 2 mg GH/m\(^2\)/day, no significant differences in BMI SDS, blood pressure, insulin levels and lipid profile were found between the 2 GH dosage groups\(^8\)\(^,\)\(^10\).

Pubertal short SGA children are frequently treated with GnRHa along with different dosages of GH to optimize adult height, despite the fact that no data are available about the GH dose effect of combined treatment on metabolic profile and GH, IGF-I and IGFBP-3 levels.

In the present randomized trial, we investigated the effect of 2 years of combined treatment with GnRHa and 2 different dosages of GH (1 mg vs. 2 mg GH/m\(^2\)/day) on body composition, blood pressure, insulin sensitivity, β-cell function and lipid profile in pubertal short SGA children. Furthermore, we measured serum GH, IGF-I and IGFBP-3 levels at start of GH treatment and after 1 year of combined treatment and associated levels with the metabolic profile.
MATERIALS AND METHODS

Subjects

The study group consisted of 45 short SGA children (29 girls) who were at the beginning of puberty. They were included in a randomized trial investigating 2 dosages of GH in combination with GnRHa treatment (leuprolide acetate depot 3.75 mg subcutaneously every 4 weeks). Children who met the following inclusion criteria were included in the study: 1) birth length and/or birth weight SDS below -2 for gestational age, 2) chronological age of 8 years or older at start of the study, 3) current height SDS below -2.5 or a predicted adult height below -2.5 SDS (calculated as height at start of puberty plus 20 cm for girls and plus 30 cm for boys, according to Dutch references), 4) early pubertal stage, defined as Tanner breast stage 2 or 3 in girls and a testicular volume of 4-8 ml in boys, and a GnRH agonist test result indicating central puberty (peak LH 10 IU/L or more). Exclusion criteria were: 1) a complicated neonatal period, with signs of severe asphyxia (defined as Apgar score < 3 after 5 minutes), 2) long-term complications of respiratory ventilation such as bronchopulmonary dysplasia, 3) endocrine or metabolic disorders, chromosomal defects, growth failure caused by other disorders (such as emotional deprivation, severe chronic illness and chondrodysplasia) or syndromes (except for Silver-Russell syndrome, no children with Silver-Russel syndrome were included in this study), 4) previous or present medication that could interfere with growth or GH treatment. The study was approved by the Medical Ethics Committee of the participating centers and written informed consent was obtained from parents or custodians and children aged 12 years or older.

Four children (2 in each GH dosage group) were excluded during the 2-year observation period. Two girls did not meet the response criteria, retrospectively. One girl and 1 boy stopped treatment because of non-compliance.

Study design

After 3 months of GnRHa treatment, children were randomized in two GH dosage groups after stratification for gender, pubertal stage (Tanner stage 2 or 3) and parental height.
SDS (one parent with height SDS below -2 or both parents with height SDS within the normal range) (Figure 1). Children were assigned to group A receiving 1 mg GH/m²/day ( ~ 0.033 mg/kg/day) or to group B receiving 2 mg GH/m²/day ( ~ 0.067 mg/kg/day) Genotropin® (Somatropin). GH was administered subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

Complete overnight GH profiles were determined in 33 children as previously described 16, one at start of GH treatment and one after approximately 1 year of combined treatment following a sc. GH injection at 20.00h. Height was measured using a Harpenden stadiometer and expressed as SDS for calendar age and sex 13. Systolic and diastolic blood pressures (BP) were measured with an automated device. The mean of 3 measurements was used for analysis. Since height is an important determinant of blood pressure, BP was expressed as SDS to adjust for height and gender 17. Fat mass and lean body mass were measured by Dual Energy X-ray Absorptiometry scans on one machine (DXA; type Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Fat mass and lean body mass were expressed as SDS to adjust for gender and height (SDSheight) 18. Percentage trunk fat was calculated as (trunk fat / total trunk mass) x 100.

Glucose homeostasis

At start of GnRHa treatment and after 1 year of combined treatment, a modified frequently sampled intravenous glucose tolerance test (FSIGT) with Tolbutamide was performed as previously described 19. Insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR) and disposition index (DI) were calculated using Bergman’s MINMOD MILLENIUM software 20. Si quantifies the capacity of insulin to stimulate glucose disposal and Sg reflects the capacity of glucose to mediate its own disposal. The AIR, an estimate of insulin secretory capacity, was measured as the area under the curve from 0 to 10 minutes corrected for baseline insulin levels. DI is calculated as AIR*Si and is an estimate of β-cell function.
**Hormone assays**

Fasting insulin levels during FSIGTs were measured in one laboratory (IRMA; Medgenix, Biosource Europe, Nivelles, Belgium). The intra-assay coefficient of variance (CV) was 1.9% and the interassay CV was 6.3%. Fasting insulin levels at 3 months after stop of GnRHa treatment were measured using a chemoluminescent assay on an Immulite 2000 analyzer (Diagnostic Products Corporation, Los Angeles, CA). Both methods were highly correlated ($r^2 = 0.98$), using the following formula: $Y$ (Immulate) = 0.6922X (IRMA) - 0.1761. A conversion factor of 6.89 was used to transform data from mU/L (IRMA) to pmol/l (Immulate). HOMA insulin resistance index (HOMA-IR) was calculated using a computer model. Total cholesterol (TC), LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c), non-esterified fatty acids (FFA) and triglycerides (TG) were determined as previously described. Apolipoprotein A-1 (Apo-A1), Apolipoprotein B (Apo-B) and lipoprotein (a) (lp(a)) were determined by rate nephelometry on the Immage Immunochemistry system, according to manufacturers’ instructions (Beckman Coulter, Mijdrecht, The Netherlands). Between-run CVs were 4.2%, 2.8% and 6.9% for the lipoproteins at levels of 0.94, 0.53 and 0.35 g/l respectively. GH, IGF-I and IGFBP-3 levels were measured as previously described.

**Statistics**

Results for body composition, blood pressure, insulin sensitivity, β-cell function and lipid profile are presented as mean (± SD). Percentage trunk fat, AIR, DI, insulin levels, HOMA-IR, triglycerides and lp(a) levels were log transformed before analysis, in order to have a Gaussian distribution. SD-scores were compared with population means (zero SDS) using one-sample t-tests. To correct for multiple testing and some missing data, the changes over time and differences between groups A and B were analyzed using repeated measurements analysis with a categorical effect for time and an interaction term for time and GH dose (SAS 9.1 (SAS Institute Inc., Cary, nC, USA)).

GH profiles were analyzed using the Pulsar program. Mean and maximum GH levels were derived from this program. Analyses were performed using the statistical package...
SPSS (version 11.0; SPSS Inc., Chicago, IL) for Windows and results for GH profiles, IGF-I and IGFBP-3 levels are expressed as median (interquartile range (IQR)). The Mann-Whitney test was used for differences between groups. The Wilcoxon signed rank test was used to determine differences between points in time within groups. To test for linear relationships, partial correlations were determined for group A and B together, with adjustment for GH dosage. A P-value < 0.05 was considered significant.
RESULTS

Clinical characteristics

Table 1 lists the clinical data. At start of GH treatment, all children had clinical suppression of puberty and prepubertal overnight LH and FSH profile patterns, as we previously reported\(^{25, 26}\). No significant differences were found between groups A and B or between boys and girls.

Metabolic profile during combined treatment

All analyses were adjusted for gender and Tanner stage at start of GnRHa treatment, since body composition, insulin sensitivity and several lipid parameters were significantly different between boys and girls or between children with Tanner stage 2 and stage 3.

Body composition

At start of GnRHa treatment, mean fat mass SDS\(_{\text{height}}\) was significantly lower than the population mean in both groups (P<0.0001) (Figure 2). After 1 year of combined treatment, fat mass SDS\(_{\text{height}}\) had decreased significantly in group B only (P<0.0001). At 3 months after stop of GnRHa treatment, fat mass SDS\(_{\text{height}}\) had significantly increased in both groups (P=0.0001). In group A, fat mass SDS\(_{\text{height}}\) was significantly higher compared to baseline (P<0.0001) and similar to the population mean. In group B, fat mass SDS\(_{\text{height}}\) returned to values comparable to those at baseline, remaining significantly lower than the population mean (P=0.03).

After 1 year of combined treatment, percentage trunk fat had increased significantly in group A only (P=0.002). At 3 months after stop of GnRHa treatment, percentage trunk fat had significantly increased in both groups, but to a lower extent in group B. Percentage trunk fat was significantly higher compared to baseline in both groups (P<0.001).

At start of GnRHa treatment, lean body mass SDS\(_{\text{height}}\) was significantly lower than the population mean in both groups (P<0.0001) (Figure 2). At 3 months after stop of GnRHa treatment, lean body mass SDS\(_{\text{height}}\) had significantly increased in group B only (P=0.007). In
both groups, lean body mass $\text{SDS}_{\text{height}}$ remained significantly lower compared to the population mean.

During the study period, there was a significant GH dose effect on fat mass $\text{SDS}_{\text{height}}$ ($P=0.01$) and percentage trunk fat ($P=0.03$). The GH dose effect on lean body mass $\text{SDS}_{\text{height}}$ showed a trend towards significance ($P=0.07$).

**Blood pressure**

At start of GnRHa treatment, mean systolic BP was significantly higher than the population mean ($P<0.0001$) (Table 2). A mean systolic BP SDS above the normal range (> +2 SDS) was found in 27% of study subjects.

Systolic BP SDS did not significantly change during the study period and was similar in groups A and B. Diastolic BP SDS had significantly increased after 1 year of combined treatment and remained higher at 3 months after stop of GnRHa treatment, albeit still within the normal range. All values were comparable between groups A and B.

**Insulin sensitivity and β-cell function**

$S_{i}$ had significantly decreased and $A_{IR}$ had significantly increased after 1 year of combined treatment. $S_{g}$ and $D_{1}$ remained comparable to baseline (Table 2). After 1 year of combined treatment, fasting insulin levels and HOMA-IR had significantly increased as well.

At 3 months after stop of GnRHa treatment, insulin levels and HOMA-IR remained similar to levels after 1 year of combined treatment. All values were comparable between groups A and B.

**Lipid profile**

Mean serum lipid levels remained within the normal range during the study period and were similar in groups A and B. At start of GnRHa treatment, $l_{p(a)}$ levels were above the normal range (>0.3 g/l) in 27% of children. During the study period, percentages were 34%
and 24% after 1 year of combined treatment and at 3 months after stop of GnRHa treatment, respectively.

**Overnight GH profiles during combined treatment**

At start of GH treatment (3 months after start of GnRHa treatment), mean and maximum GH levels were comparable for groups A and B and for boys and girls.

After 1 year of combined treatment, mean and maximum GH levels significantly increased in both groups and levels were significantly higher in group B (Table 3). Following the sc. GH injection at 20.00h, GH levels remained significantly longer above 40 mU/L and 20 mU/L in group B, compared to group A (P<0.0001) (Figure 3). No correlations were found between GH profile characteristics and age, bone age, gender, pubertal stage, peak LH level during a GnRH agonist test and fat mass SDS.

**IGF-I and IGFBP-3 levels during combined treatment**

At start of GH treatment (3 months after start of GnRHa treatment), IGF-I and IGFBP-3 levels were significantly lower than the respective population means (P<0.0001) (Table 3). IGF-I levels were comparable for groups A and B and for boys and girls. IGFBP-3 levels were significantly lower in group A, but comparable for boys and girls. IGF-I levels correlated with IGFBP-3 levels (r=0.62, P<0.0001) and mean GH levels (r=0.36, P=0.04). IGFBP-3 levels correlated with mean GH levels (r=0.39, P=0.03).

After 1 year of combined treatment, IGF-I levels increased to levels significantly higher than the population mean (P<0.0001) in both groups. IGF-I levels were significantly higher in group B. In group B, 88.9% of the children had IGF-I levels in the highest quintile (>0.84 SDS) and 27.8% had IGF-I levels above 2 SDS, compared to 43.8% (P=0.005) and 6.3% (NS) of children in group A, respectively. IGF-I levels were comparable for boys and girls.
IGFBP-3 levels increased significantly and similarly in both groups. Compared to the population mean, IGFBP-3 levels remained only significantly lower in group A (P = 0.01). Levels were comparable for boys and girls.

During combined treatment, IGF-I levels only correlated with IGFBP-3 levels (r=0.50, P=0.003).
DISCUSSION

Our study is the first to report metabolic effects in pubertal short SGA children during combined treatment with a GnRH analogue and 1 mg or 2 mg GH/m²/day. Children treated with GnRHa and the higher GH dose of 2 mg/m²/day developed less fat mass SDSheight, less percentage trunk fat, and more lean body mass SDSheight. Blood pressure, insulin sensitivity, and lipid profile were similar in both GH dosage groups. As expected, insulin sensitivity significantly decreased, but the disposition index did not change in either group. Lipids remained within normal reference ranges. GH and IGF-I levels were significantly higher in children treated with GnRHa and 2 mg GH/m²/day.

At start of GnRHa treatment, fat mass SDSheight was significantly lower than the population mean in both groups, consistent with findings in prepubertal short SGA children. In children treated with GnRHa and GH 2 mg/m²/day, fat mass SDSheight remained significantly lower than the population mean. Percentage trunk fat increased in both GH dosage groups, but to lower values in children treated with GnRHa and 2 mg GH/m²/day. Several studies reported an increase in fat mass or BMI SDS during GnRHa treatment in children with central precocious puberty. This might be explained by lower GH levels during GnRHa treatment, as reported in children with GH deficiency. In contrast to GnRHa, GH has well-documented lipolytic effects. GH treatment in prepubertal short SGA children resulted in a significant decrease in fat mass SDSheight, especially in the first treatment year. The significant differences between the 2 GH dosage groups in our study can be explained by the fact that treatment with GH 2 mg/m²/day counteracts the effect of simultaneous treatment with a GnRH analogue, whereas treatment with GH 1 mg/m²/day is insufficient to prevent children from gaining fat mass during GnRHa treatment. Epidemiological studies have shown that low birth weight followed by catch-up in fat mass during childhood and adolescence, even when fat mass remained within the normal range, was associated with a higher risk of developing type 2 diabetes and cardiovascular disease. Follow-up until adult height is
required to investigate the long-term effects of changes in body composition and metabolic profile in short SGA children treated with GnRHα and GH.

At start of GnRHα treatment, lean body mass SDS_{height} was significantly lower than the population mean in both GH dosage groups. It was previously shown that older prepubertal short SGA children have a lower lean body mass SDS_{height} compared to younger ones. During the study period, lean body mass SDS_{height} increased significantly in children treated with GnRHα and 2 mg GH/m^{2}/day only. In contrast, in children with CPP lean body mass SDS adjusted for gender and age decreased significantly during GnRHα treatment. We therefore conclude that treatment with 2 mg GH/m^{2}/day, in combination with a GnRH analogue, results in an increase in lean body mass SDS_{height} in older short SGA children.

At start of GnRHα treatment, mean systolic BP SDS was significantly higher than the population mean. Higher blood pressure in childhood has been associated with an increased risk of developing hypertension in adulthood. Systolic BP SDS did not change significantly during the study period. This is compatible with previous findings, where a significant decrease in blood pressure SDS was found only after 3 years of GH treatment.

At start of GnRHα treatment, insulin sensitivity was lower and insulin secretion was higher in our pubertal short SGA children, compared to reported values in prepubertal short SGA children. This was expected, since healthy pubertal children have a physiologic insulin resistance with a compensatory increase in insulin secretion. During 1 year of combined treatment, Si significantly decreased and AIR significantly increased. The disposition index remained similar to baseline, reflecting that β-cells were able to compensate for the reduction in insulin sensitivity by increasing their insulin secretion. Si and AIR were comparable between both GH dosage groups. Since IGF-I levels were significantly higher during treatment with GnRHα and 2 mg GH/m^{2}/day, albeit within the normal range, this might be explained by the fact that the insulin-like effects of IGF-I counterbalance the insulin-antagonistic effects of.

At start of GnRHα treatment, mean serum lipid levels were within the normal range, although lp(a) levels were above the normal range (>0.3 g/l) in 27% of pubertal short SGA
children. High Lp(a) levels have been associated with an increased risk of developing cardiovascular disease in several studies\textsuperscript{36, 37}. Although some lipids showed a significant temporary increase (LDL-c, FFA, Apo-A1 levels), and others increased more steadily over time (TC and TG levels), the actual increase and subsequent decrease in levels was very small and lipids were similar in both groups. The clinical significance of these small changes in lipid levels is therefore considered negligible.

During combined treatment, mean and maximum GH levels were lower in our study group compared to levels in prepubertal SGA children treated with GH only\textsuperscript{38}. Moreover, maximum GH levels in children treated with GnRHa and 2 mg GH/m\textsuperscript{2}/day were similar to maximum GH levels in prepubertal children treated with GH 1 mg GH/m\textsuperscript{2}/day. In the latter study, GH levels were determined with the same assay in the same laboratory. We previously reported that GH levels significantly decreased during 3 months of GnRHa treatment\textsuperscript{16}, similar to reports in patients with precocious puberty treated with GnRHa\textsuperscript{39-41}. This is probably partly due to diminution of sex steroids\textsuperscript{42}. Thus, the lower GH levels in our study group might well be a result of simultaneous treatment with GH and a GnRHa analogue. Nonetheless, following a sc. GH injection at 20.00h, GH levels in short SGA children treated with GnRHa and 2 mg GH/m\textsuperscript{2}/day remained above 20 mU/L for almost 11 hours, demonstrating that these children have elevated GH levels for a great part of the day. GH and IGF-I levels increased significantly in both GH dosage groups, but to significantly higher levels in the 2 mg GH/m\textsuperscript{2}/day group. Dose-dependent rises have been described in prepubertal short children born SGA, GH-deficient adolescents and girls with Turner syndrome\textsuperscript{38, 43, 44}. The percentage of children with IGF-I SD-scores above 2 SDS was not significantly different between the groups. However, 27.8% of children treated with 2 mg GH/m\textsuperscript{2}/day had IGF-I levels above 2 SDS. Concern has been expressed about the association of high IGF-I levels during several years and long-term cancer risk\textsuperscript{45}. Although pubertal short SGA children will be treated for a relatively short period of time, it is important to monitor IGF-I levels during treatment.

Our study has some limitations. Since GH treatment in short SGA children was approved by the FDA in 2001 and the EMEA in 2003, our medical ethics committee did not
allow us to treat pubertal short SGA children solely with a GnRH analogue or to include a control group without any treatment. We did not include a study group of pubertal short SGA children treated with GH only, because this is investigated in another GH trial. In the future, it would be interesting to compare adult height results between these two studies. Since many clinicians started treating short SGA children with GnRHa and GH, despite the fact that there are no safety data, it is important to report safety data on metabolic profile in relation to GH and IGF-I levels, before adult height data will be available. Adult height data will have to be awaited before a definitive conclusion can be drawn whether combined treatment with GnRHa and either 1 or 2 mg GH/m²/day will result in an optimal adult height without adversely affecting metabolic outcome.

In conclusion, our study is the first to report that 2 years of combined treatment with a GnRH analogue and either 1 mg or 2 mg GH/m²/day does not adversely affect body composition and metabolic profile of short SGA children who come under medical attention at onset of puberty. There was a dose-dependent effect on fat mass SDS\text{height}, percentage trunk fat, lean body mass SDS\text{height}, GH and IGF-I levels in favour of treatment with GnRHa and the higher GH dose of 2 mg/m²/day. Blood pressure, insulin sensitivity and lipid profile were similar between the 2 GH dosage groups.
ACKNOWLEDGEMENTS

We thank all children and their parents for participating in this study. We very much appreciate the technical assistance of Mrs. Jolanda van Houten, research nurse. Participating physicians were: R.J.H. Odink and J.J.J. Waelkens, Catharina Hospital, Eindhoven, The Netherlands; Dr. W.M. Bakker-van Waarde, University Medical Center Groningen, Groningen, The Netherlands; Dr. C. Noordam, Radboud University Medical Center Nijmegen, Nijmegen, The Netherlands; Dr. C. Westerlaken, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; Dr. E.J. Sulkers, Walcheren Hospital, Vlissingen, The Netherlands. We would like to thank Dr. W.H. Hackeng for his GH assays and for analyzing the FSIGT tests. Mrs. J. Sluimer and Prof. E.P. Krenning, head of the department of nuclear medicine, are greatly acknowledged for the DXA facilities and equipment. Dr. I.M. van der Sluis is greatly acknowledged for her help in analyzing the height-adjusted Z-scores for the DXA parameters. We are grateful for the support of the nurses working on the Children’s Ward, Sophia Children’s Hospital. We appreciate the financial support of the Vereniging Trustfonds Erasmus Universiteit Rotterdam for conference visits.
DISCLOSURE

Confict of interest: There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding: This investigator-initiated study received a financial contribution by Pfizer Farma B.V., The Netherlands.
REFERENCES


24. Hokken-Koelega AC, Hackeng WH, Stijnen T, Wit JM, de Muinck Keizer-Schrama SM & Drop SL. Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe


44. van Teunenbroek A, de Muinck Keizer-Schrama SM, Stijnen T, Mouton JW, Blum WF, Mercado M, Baumann G & Drop SL. Effect of growth hormone administration frequency on 24-hour growth hormone profiles and levels of other growth related parameters in girls with Turner's syndrome. Dutch Working Group on Growth Hormone. *Clinical Endocrinology (Oxford)* 1993 39 77-84.

Table 1. Clinical characteristics of short SGA children. Data are expressed as mean (± SD).

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mg GH/m²/day</td>
<td>2 mg GH/m²/day</td>
</tr>
<tr>
<td>Number (female)</td>
<td>22 (13)</td>
<td>19 (13)</td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td>38.3 (± 2.4)</td>
<td>37.3 (± 3.5)</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>-2.0 (± 1.1)</td>
<td>-1.9 (± 0.83)</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>-2.7 (± 1.1)</td>
<td>-2.4 (± 0.72)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>12.1 (± 1.0)</td>
<td>12.1 (± 0.95)</td>
</tr>
<tr>
<td>Bone age (yrs)</td>
<td>11.4 (± 1.1)</td>
<td>11.1 (± 0.9)</td>
</tr>
<tr>
<td>Height SDS</td>
<td>-2.7 (± 0.68)</td>
<td>-2.8 (± 0.70)</td>
</tr>
<tr>
<td>Target height SDS</td>
<td>-0.65 (± 0.77)</td>
<td>-0.46 (± 0.75)</td>
</tr>
<tr>
<td>Tanner stage 2</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Tanner stage 3</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 2. Blood pressure, insulin sensitivity and lipids at start of GnRHa treatment, after 1 year of combined treatment and 3 months after stop of
GnRHa treatment in short SGA children. Data are expressed as model estimate (95% CI), after adjustment for gender and Tanner stage at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Start of GnRHa treatment (n=41)</th>
<th>1 yr of combined treatment (n=41)</th>
<th>3 mo after stop GnRHa treatment (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP SDS</td>
<td>1.59 (1.24-1.94)</td>
<td>1.26 (0.87-1.65)</td>
<td>1.39 (1.04-1.74)</td>
</tr>
<tr>
<td>Diastolic BP SDS</td>
<td>0.22 (0.00-0.45)</td>
<td>0.52 (0.27-0.78)</td>
<td>0.57 (0.36-0.79)</td>
</tr>
<tr>
<td>Si x 10⁻⁴/min⁻¹ (µU/ml)</td>
<td>7.38 (6.00-8.76)</td>
<td>4.61 (3.71-5.50)</td>
<td>ND</td>
</tr>
<tr>
<td>Sg x 10⁻²/min⁻¹</td>
<td>3.47 (2.98-3.96)</td>
<td>3.42 (2.94-3.89)</td>
<td>ND</td>
</tr>
<tr>
<td>AIR (mU/l)</td>
<td>421 (326-543)</td>
<td>790 (643-971)</td>
<td>ND</td>
</tr>
<tr>
<td>DI (AIR x Si)</td>
<td>2569 (2012-3279)</td>
<td>3105 (2514-3838)</td>
<td>ND</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>48.1 (41.7-55.5)</td>
<td>75.0 (63.8-88.1)</td>
<td>79.2 (66.7-94.0)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.91 (0.79-1.06)</td>
<td>1.39 (1.19-1.63)</td>
<td>1.43 (1.20-1.70)</td>
</tr>
<tr>
<td>TC (mmol/l) [3.0-5.5]</td>
<td>4.16 (3.99-4.33)</td>
<td>4.20 (4.00-4.40)</td>
<td>4.33 (4.13-4.53)</td>
</tr>
<tr>
<td>LDL-c (mmol/l) [1.3-3.4]</td>
<td>2.28 (2.11-2.46)</td>
<td>2.44 (2.25-2.64)</td>
<td>2.36 (2.19-2.52)</td>
</tr>
<tr>
<td>HDL-c (mmol/l) [0.9-1.9]</td>
<td>1.41 (1.31-1.52)</td>
<td>1.63 (1.51-1.76)</td>
<td>1.55 (1.44-1.66)</td>
</tr>
<tr>
<td>TG (mmol/l) [0.4-1.6]</td>
<td>0.76 (0.66-0.86)</td>
<td>0.79 (0.67-0.94)</td>
<td>0.92 (0.75-1.13)</td>
</tr>
<tr>
<td>FFA (mmol/l) [0.2-1.0]</td>
<td>0.52 (0.45-0.59)</td>
<td>0.69 (0.59-0.79)</td>
<td>0.51 (0.42-0.60)</td>
</tr>
<tr>
<td>Apo-A1 (g/l) [0.9-1.6]</td>
<td>1.39 (1.32-1.46)</td>
<td>1.56 (1.47-1.65)</td>
<td>1.45 (1.38-1.53)</td>
</tr>
<tr>
<td>Apo-B (g/l) [0.5-1.3]</td>
<td>0.71 (0.66-0.75)</td>
<td>0.73 (0.68-0.77)</td>
<td>0.72 (0.67-0.77)</td>
</tr>
<tr>
<td>Lp(a) (g/l) [≤ 0.3]</td>
<td>0.09 (0.06-0.13)</td>
<td>0.14 (0.09-0.21)</td>
<td>0.14 (0.09-0.22)</td>
</tr>
</tbody>
</table>

AIRg, DI, insulin levels, HOMA-IR, triglycerides and lp(a) levels were log-transformed prior to analysis
The values between brackets represent reference ranges for healthy children; ND=not determined

§ P<0.0001 compared to the population mean (0 SDS)

a P<0.03: 1 year of combined treatment, compared to start of GnRHa treatment

b P<0.03: 3 mo. after stop of GnRHa treatment, compared to start of GnRHa treatment

c P<0.02: 3 mo. after stop of GnRHa treatment, compared to 1 year of combined treatment
Table 3. GH levels, IGF-I and IGFBP-3 SDS at start of GH treatment and after 1 year of combined treatment in short SGA children. Data are expressed as median (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Group A (1 mg GH/m²/day)</th>
<th>Group B (2 mg GH/m²/day)</th>
<th>P-value¹</th>
<th>P-value²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start of GH treatment</td>
<td>1 year of combined treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean GH (mU/L)</td>
<td>8.5 (4.7-11.3)</td>
<td>25.2 (19.7-31.4)</td>
<td>P=0.001</td>
<td></td>
</tr>
<tr>
<td>Max GH (mU/L)</td>
<td>39.3 (26.4-49.4)</td>
<td>60.0 (44.3-83.6)</td>
<td>P=0.001</td>
<td></td>
</tr>
<tr>
<td>GH&gt;40 mU/L (hrs)</td>
<td>2.8 (0.67-3.9)</td>
<td>7.2 (6.3-8.8)</td>
<td>P&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>GH&gt;20 mU/L (hrs)</td>
<td>7.8 (5.4-8.3)</td>
<td>10.8 (8.8-11.3)</td>
<td>P&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>IGF-I SDS</td>
<td>-0.93 (-2.2 to -0.11)</td>
<td>0.81 (0.55-1.1)</td>
<td>P&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Δ IGF-I SDS</td>
<td>1.7 (1.1-2.3)</td>
<td>-0.58 (-1.5 to 0.38)</td>
<td>1.5 (1.2-2.5)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.9 (1.1-3.0)</td>
<td>P=0.001</td>
</tr>
<tr>
<td>IGFBP-3 SDS</td>
<td>-1.5 (-2.0 to -0.83)</td>
<td>-0.55 (-0.74 to -0.14)</td>
<td>P=0.001</td>
<td></td>
</tr>
<tr>
<td>Δ IGFBP-3 SDS</td>
<td>1.0 (0.32-1.4)</td>
<td>-0.83 (-1.4 to -0.46)</td>
<td>0.26 (-0.04-0.77)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.1 (0.89-1.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹ During combined treatment, compared to start of GH
² Group B vs. group A: after 1 year of combined treatment with GnRH and GH
NS = not significant
Figure 1.

GnRHa treatment

Group A (1 mg GnRHa/day)

Group B (2 mg GnRHa/day)

Randomization

Start GnRHa  Start GH  1 w of combined treatment  Stop GnRHa  3 mo. after stop GnRHa

Months 0 3 15 24 27
Figure 3.

Mean GH level (mU/L)

GH injection

Time (hours)

254x190mm (72 x 72 DPI)