Carbohydrate Metabolism during Long-Term Growth Hormone (GH) Treatment and after Discontinuation of GH Treatment in Girls with Turner Syndrome Participating in a Randomized Dose-Response Study

THEO C. J. SAS, SABINE M. P. F. DE MUINCK KEIZER-SCHRAMA, THEO STIJNEN, HENK JAN AANSTOOT, STENVERT L. S. DROP, AND THE DUTCH ADVISORY GROUP ON GROWTH HORMONE†

Department of Pediatrics, Division of Endocrinology, Sophia Children’s Hospital/Erasmus University (Th.C.J.S., S.M.P.F.M.K.-S., S.L.S.D.), and the Institute of Epidemiology and Biostatistics, Erasmus University (T.S.), 3015 GJ Rotterdam; and the Department of Pediatrics, Idselliand Hospital (H.J.A.), 2906 ZC Capelle, The Netherlands

ABSTRACT
To assess possible side-effects of GH treatment with supraphysiolog-ical doses on carbohydrate (CH) metabolism in girls with Turner syndrome (TS) during long term GH treatment and after discontinuation of GH treatment, the results of oral glucose tolerance tests and hemoglobin A1c measurements were analyzed in 68 girls with TS participating in a randomized dose-response trial. These previously untreated girls, aged 2–11 yr, were randomly assigned to 1 of 3 GH dosage groups: group A, 4 IU/m²day (−0.045 mg/kg/day); group B, first year, 4 IU/m²day; thereafter, 6 IU/m²day (−0.0675 mg/kg/day); group C, first year, 4 IU/m²day; second year, 6 IU/m²day; thereafter, 8 IU/m²day (−0.090 mg/kg/day). After the first 4 yr, girls 12 yr of age or older started with 5 μg/kg BW-day 17β-estradiol for induction of puberty. To assess the effects of long term high dose GH treatment on CH metabolism, the 7-yr data from the oral glucose tolerance tests in 9 girls of group C were evaluated (group C1). To determine whether the changes in CH metabolism during GH treatment would persist after discontinuation of GH treatment, the data for the 28 girls who were treated with GH for a mean (SD) period of 8.3 (13.3) months demonstrated that the GH-induced higher insulin levels decreased to values close to or equal to pretreatment values after discontinuation of GH treatment. Changes in CH variables were not significantly related to the GH dose. Hemoglobin A1c levels never showed an abnormal value. The prevalence of impaired glucose tolerance was low, and none of the girls developed diabetes mellitus.

In conclusion, long term GH treatment with dosages up to 8 IU/m²/day in girls with TS has no adverse effects on glucose levels, but induced higher levels of insulin, indicating relative insulin resistance. The increased insulin levels during long term GH treatment decreased after discontinuation of GH treatment to values close to or equal to pretreatment values. Although the reversibility of the effects of long term GH is reassuring, the consequence of long term hyperinsulinism is still unknown. (J Clin Endocrinol Metab 85: 769–775, 2000)
baseline (18). As these relatively young girls are being treated for many years until adult height is reached, the question arose as to whether the observed GH-induced insulin resistance would show a further increase during prolonged GH treatment. In addition, it is unknown whether the insulin resistance in girls with TS during long term treatment with supraphysiologic GH dosages would decrease to a pretreatment level after discontinuation of GH treatment.

The results of GH treatment on growth were described previously by our group (19). In the present study we investigated the effects of GH treatment on CH metabolism in a subgroup receiving GH in a dose up to 8 IU/m^2-day during 7 yr of GH treatment. In addition, in girls who had been treated with 4, 6, or 8 IU GH/m^2-day and had reached adult height, changes in CH metabolism were evaluated during GH treatment as well as after discontinuation of GH treatment.

**Subjects and Methods**

**Study group and treatment regimens**

Sixty-eight previously untreated girls with TS were enrolled in a multicenter GH-dose-response study in The Netherlands. The diagnosis was confirmed by lymphocyte chromosomal analysis. Inclusion criteria were chronological age between 2–11 yr, height below the 50th percentile for healthy Dutch girls (20), and a normal thyroid function. Exclusion criteria were associated endocrine and/or metabolic disorders, growth failure caused by other disorders or emotional deprivation, hydrocephalus, previous use of drugs that could interfere with GH treatment, and spontaneous puberty (21). Written informed consent was obtained from the girls and their parents or custodians. The study protocol was approved by the ethics committee of each participating center.

After stratification for chronological age and height SD score for chronological age girls were randomly assigned to group A (n = 23) receiving 4 IU/m^2-day body surface area (equivalent to 0.045 mg/kg/day), group B (n = 23) receiving 4 IU/m^2-day in the first year followed by 6 IU/m^2-day (~0.0675 mg/kg/day), or group C (n = 22) receiving 4 IU/m^2-day in the first year, 6 IU/m^2-day in the second year, and thereafter 8 IU/m^2-day (~0.090 mg/kg/day).

Biosynthetic human GH (Norditropin, Novo Nordisk A/S, Bagsvaerd, Denmark) was given sc once daily at bedtime using a pen injection system. Every 3 months the total GH dose was adjusted to the calculated body surface area. According to the study protocol, treatment was stopped when subjects had grown less than 1 cm over 6 months. However, when girls were satisfied with the height achieved, they elected to stop GH treatment before study criteria for the termination of treatment had been reached. In the first 4 yr of GH treatment, no estrogens for pubertal induction were given to the girls. After 4 yr of GH treatment, estrogen therapy was immediately started in the girls who were older than 12.0 yr of age. The younger girls started estrogen therapy at a yearly dose of 0.090 mg/kg BW (orally), in the third year they received 7.5 μg/kg day and thereafter 10 μg/kg day. After discontinuation of GH treatment, the dose of estrogens was further increased depending on the clinical signs of breast development.

**Study protocol**

Before the start of treatment (baseline) and every 3 months after the start of GH treatment, all children were seen at their local hospital for a physical examination, including measurements of standing height (H) and weight (W). Height was expressed as SD score, using references for healthy Dutch girls (20), as well as for children for Dutch-Swedish-Danish references for girls with TS (22). Body mass index (BMI; weight (kilograms)/height (meters)^2) was expressed as the SD score for sex and chronological age (20).

Glycosylated hemoglobin (HbA1c) was determined yearly. The girls of all 3 GH dosage groups who had reached adult height had undergone an OGTT at baseline, after 4 yr of GH treatment, and 6 months after discontinuation of GH treatment. Additional OGTTs were performed in a random sample of 10 girls from group C (group C1) at 6, 18, 30, 60, and 84 months after the start of GH treatment. A single team performed all OGTTs after 3 days of unrestricted diet supplemented with 100 g carbohydrate (Fantomalt) and after overnight fasting. Glucose (1.75 g glucose/kg BW; maximum, 50 g) was administered orally within 5 min. Blood samples were collected at 0, 30, 60, 90, 120, 150, and 180 min, and plasma glucose and insulin levels were measured.

To evaluate the overall responses to the oral glucose load, apart from the glucose and insulin levels at the various time points, the following variables were described: 1) impaired glucose tolerance (IGT) was defined according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (22): 2-h level more than 7.8 mmol/L (140 mg/dL) and less than 11.1 mmol/L (200 mg/dL); 3) 3-h area under the curve for time-concentration corrected for fasting levels during the OGTT (ALC(h), calculated using the trapezoidal rule; and 3) the ratio of insulin/glucose at 30 min and the ratio at 120 min, calculated as an index for relative insulin resistance.

**Assays**

The plasma glucose level was measured at the local hospital laboratories with automatic analyzers using a hexokinase catalyzed-glucose oxidase method. Plasma insulin was determined in one laboratory by RIA (Medgenix, Fleurus, Belgium). The intraassay coefficient of variation was 6–10% and the interassay coefficient of variation was 6–11% (fasting normal range, <20 mU/L). HbA1c levels were measured in one laboratory using an automatic high pressure liquid chromatography analyzer (DIAMAT, Bio-Rad Laboratories, Inc., Edgemont, CA). The upper normal assay limit is less than 6.6%.

**Statistical analyses**

Results are expressed as the mean (sd) unless indicated otherwise. Differences in variables between time points were tested using paired Student’s t tests. Differences in the change in values between the GH dosage groups were first tested by a linear trend test to assess a possible dose-dependent effect. In case of a significant result, this was followed by comparisons between two groups with Student’s t tests. Correlations between variables were assessed with Pearson’s correlation coefficient. P < 0.05 was considered significant.

**Results**

Four girls (group A, n = 1; group B, n = 2; group C, n = 1) dropped out of the study long before reaching adult height because of noncompliance. In all 4 cases no problems with the CH tolerance were involved in the decision to discontinue the treatment. Data for these 4 girls were not used for the analysis of long term GH treatment. Table 1 lists the baseline clinical data of the remaining 9 girls of group C1 who have been followed for 7 yr as well as those of the 28 girls who have reached adult height. None of these 28 girls belonged to the subgroup C1. Although it is obvious that the 9 girls of

<table>
<thead>
<tr>
<th>TABLE 1. Mean (sd) baseline data for each treatment group</th>
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<tr>
<td>Group C1</td>
</tr>
<tr>
<td>No. of girls</td>
</tr>
<tr>
<td>Baseline age (yr)</td>
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<tr>
<td>Baseline HSDS_Ca</td>
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<tr>
<td>Baseline HSDS_Ca (RvW)</td>
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<td>Karyotype: 45,X</td>
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<td>Karyotype: other</td>
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Karyotype (45,X; other) is expressed in numbers of patients. HSDS_Ca, Height SD score for chronological age; RvW, Roede van Wieringen references for healthy Dutch girls; DSD, Dutch-Swedish-Danish references for girls with Turner syndrome.
group C1 were younger than the other 28 girls because of
sampling variability, there were no relevant differences in
baseline variables between the groups.

OGTTs during 7 yr of GH treatment in group C1

At baseline, none of the girls had an abnormal glucose
response during the OGTT. After 6 and 18 months of treat-
ment, in one girl IGT was found (glucose levels after 120 min,
8.4 and 9.8 mmol/L, respectively). Thereafter, she had nor-
mal OGTTs. None of the girls developed diabetes mellitus.
Figure 1 shows the mean glucose and insulin levels during
the OGTTs, respectively, at baseline and after 4 and 7 yr of
GH treatment. Table 2 lists the CH variables at the three time
points during the study period. Fasting glucose levels and
the AUCab for glucose did not significantly change during
treatment. Fasting insulin levels increased significantly dur-
ing the first 4 yr of GH treatment compared to baseline levels
\( P = 0.03 \) and had increased further after 7 yr of GH treat-
ment, resulting in levels significantly higher than those after
4 yr of GH treatment \( P = 0.014 \). The AUCab for insulin had
increased significantly in the first 4 yr of GH treatment \( P < 0.001 \) with no further increase thereafter. The insulin/
glucose ratio at 30 and 120 min, respectively, increased sig-
nificantly during the first 4 yr of GH treatment \( P = 0.003 \)
and \( P = 0.001 \), respectively) without significant changes
thereafter.

One of the girls started estrogen therapy after 6 yr of GH
treatment, and another girl had some spontaneous breast
development (Tanner breast stage II) after 57 months of GH
treatment. Excluding these two girls from the analysis, sim-
ilar patterns in the development in CH variables during GH

treatment. Excluding these two girls from the analysis, sim-
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 OGTTs at baseline, after 4 yr of treatment, and after
discontinuation of GH in 28 girls of groups A, B, and C

Figure 2 shows the mean glucose levels during the OGTTs
at baseline, after 4 yr of GH treatment, and 6 months after
discontinuation of GH treatment in groups A \( n = 9 \), B \( n = 10 \),
and C \( n = 9 \), respectively. These 28 girls have reached
adult height after a mean (sd) duration of GH treatment of
85.3 (13.3) months. Before the start of treatment, in 2 girls of
group C IGT was found (glucose levels after 120 min, 7.9 and
8.0 mmol/L, respectively). Both girls had no IGT during GH
treatment, but after discontinuation of treatment, IGT was
found again in 1 of these girls (glucose level after 120 min,
8.0 mmol/L). After 4 yr of GH treatment, another girl of
group B had IGT (glucose level after 120 min, 8.4 mmol/L);
however, after discontinuation of GH treatment the OGTT
results for this girl were normal. None of the girls developed
diabetes mellitus.

Table 3 shows that fasting glucose levels had significantly
increased after 4 yr of GH treatment \( P = 0.003 \) and had
significantly decreased after discontinuation of GH treat-
ment to baseline levels \( P = 0.002 \). The AUCab for glucose
was significantly decreased compared to baseline \( P = 0.013 \)
after 4 yr of GH treatment, without a further significant
change after discontinuation of GH treatment. No significant
differences in the change in fasting glucose levels or AUCab
for glucose between the GH dosage groups were found.

Figure 3 demonstrated the mean insulin levels during the
OGTTs for groups A, B, and C, respectively. The fasting
insulin levels were increased after 4 yr of GH treatment \( P < 0.001 \)
and decreased after discontinuation of GH treatment
\( P = 0.002 \); Table 3). However, fasting insulin levels after
discontinuation of GH treatment were still significantly
higher than baseline levels \( P < 0.001 \). Although there seems
to be a trend toward higher fasting insulin levels with higher
GH dosages after 4 yr of GH treatment, this was not statis-
tically significant. The AUCab for insulin was significantly
increased after 4 yr of treatment \( P = 0.003 \) and significantly
decreased after discontinuation of GH treatment \( P = 0.001 \)
to pretreatment levels. No significant differences in the
change in AUCab for insulin between the GH dosage groups
were found.

The ratio of insulin to glucose at 30 min significantly in-
creased during GH treatment \( P < 0.001 \) and was followed by
a significant decrease after discontinuation of GH treat-
ment \( P = 0.009 \); Table 3). However, the ratio at 30 min after
discontinuation of GH treatment were still significantly
higher than baseline levels \( P = 0.010 \). The ratio at 120 min
was significantly increased after 4 yr of GH treatment com-
pared to baseline \( P < 0.001 \) and was followed by a signif-
icient decrease after discontinuation of GH treatment to base-
line levels \( (P = 0.001) \). None of the changes in the ratios was significantly different between the GH dosage groups.

As obesity is associated with insulin resistance, we analyzed the development of the \( \text{sd} \) scores of BMI in the 9 girls of group C1 during 7 yr of GH treatment and in the 28 girls of groups A, B, and C during and after discontinuation of GH treatment (Table 4). In the 9 girls of group C1, the baseline BMI \( \text{sd} \) score was not significantly different from zero. After 4 yr of GH treatment, the BMI \( \text{sd} \) score had increased significantly \( (P = 0.003) \) to values higher than zero \( (P = 0.036) \), without obvious changes thereafter. However, the increment in insulin levels during GH treatment were not correlated with the increment in BMI \( \text{sd} \) score. In the 28 girls, the baseline BMI \( \text{sd} \) score was significantly higher than zero \( (P = 0.002) \) and increased significantly during GH treatment \( (P = 0.043) \). After discontinuation of GH treatment, the BMI \( \text{sd} \) score was significantly higher than that after 4 yr of GH treatment \( (P = 0.002) \). Thus, the BMI \( \text{sd} \) score showed a different pattern than the insulin levels during GH treatment and after discontinuation of GH treatment.

\( \text{HbA}_{1c} \)

All individual \( \text{HbA}_{1c} \) levels in the total group of 65 girls were within the normal range. \( \text{HbA}_{1c} \) levels showed an overall significant decrease during 7 yr of GH treatment compared to baseline levels [mean \( \text{sd} \), from 4.8 (0.5) to 4.6 (0.5); \( P < 0.001 \)], without differences between GH dosage groups. In the girls who had discontinued GH treatment, the mean \( \text{sd} \) \( \text{HbA}_{1c} \) level in the last year of GH treatment [4.8 (0.4)] was not significantly different from baseline [5.0 (0.4)]. Compared to values in the last year of GH treatment, the \( \text{HbA}_{1c} \) levels had decreased significantly after discontinuation of GH treatment [from 4.8 (0.4) to 4.4 (0.4); \( P < 0.001 \)].

Discussion

To our knowledge, this is the first report describing CH metabolism during long term GH treatment as well as after discontinuation of GH treatment in girls with TS, treated with dosages up to 8 IU/m²·day. Previous studies reported increased insulin levels with or without changes in glucose levels after relatively short periods of GH treatment (23, 24). To optimize the GH treatment in girls with TS in the present study, treatment was started at a younger age, and some of the girls received a higher GH dosage than that used in other studies. In a previous report we demonstrated that in most of these girls, GH treatment resulted in a normalization of height during childhood as well as a normalization of adult height. Although the growth response was higher in the groups receiving higher GH dosages, the difference in growth response was quite small in proportion to the difference in GH dose (19). The present study showed that 7 yr of treatment with a high GH dose in these girls did not negatively influence glucose levels, but induced higher levels of insulin. Although fasting insulin levels showed a sustained increase during GH treatment, the glucose-induced increase in insulin showed no further increase after 4 yr of treatment. The increase in insulin levels during GH treatment without marked changes in glucose levels indicated relative insulin resistance.

As our study did not include untreated girls with TS, other factors causing insulin resistance cannot be entirely ruled out. One may speculate that the increase in insulin levels was due to estrogens. However, similar patterns in insulin levels were seen in girls who remained prepubertal during the entire study period. As increased body mass is related to insulin resistance, the increase in insulin resistance during the study period could be explained by the increase in body mass. However, no relationships were found between the increase in insulin levels during GH treatment and the increment in BMI \( \text{sd} \) score. Therefore, we can conclude that GH treatment is the main cause of the observed relative insulin resistance.

In addition, our study demonstrated that the increased insulin levels during long term GH treatment with 4, 6, or 8 IU/m²·day in 28 girls with TS decreased after discontinuation of GH treatment to values close or equal to pretreatment values. These results are in line with reported preliminary short term data (25, 26). In a previous study by our group, GH treatment with 6 IU/m²·day in 18 relatively older girls with TS showed that the increased insulin levels during the entire GH treatment period had decreased significantly at 6 months after discontinuation of GH treatment (25). Saenger et al. found similar results in nine girls with TS (26).

Although fasting insulin levels decreased after discontinuation of GH treatment, these levels were still significantly higher than pretreatment levels. One can speculate that this is due to a partial irreversibility of the GH-induced insulin resistance. However, compared to the insulin levels during OGTTs in healthy girls reported previously by Potau et al. (27), the insulin levels after discontinuation of GH treatment in the girls in our study are quite comparable. Therefore, it is more likely that these higher fasting insulin levels after
discontinuation of GH treatment compared to pretreatment levels may be explained by the normal increase in insulin levels during childhood. Moreover, estrogen treatment and obesity are associated with insulin resistance. In the present study at the start of GH treatment and after 4 yr of GH treatment, none of the girls received estrogens, whereas after discontinuation of GH treatment, all girls were receiving estrogens.
estrogen treatment. Furthermore, the BMI sd score after discontinuation of GH treatment was considerably higher than that before the start of treatment. Therefore, rather than the GH treatment itself, estrogen treatment and the increased body mass may explain the higher fasting insulin levels after discontinuation of GH treatment compared to pretreatment levels.

Although it is reassuring that the effects of long term GH treatment on CH metabolism are reversible, the consequences of hyperinsulinism during most of the childhood period on the risk of CH intolerance in adulthood is unknown. Therefore, long term follow-up of these women is required. In addition, more sophisticated methods, such as glucose clamp or the more recent developed minimal model method, could lead to improved insights into the CH metabolism in these girls during and after long term GH treatment.

In conclusion, long term GH treatment with dosages up to 8 IU/m² day in girls with TS has no adverse effect on glucose metabolism, but induced higher levels of insulin, indicating relative insulin resistance. The increased insulin levels during long term GH treatment decreased after discontinuation of GH treatment to values close to or equal to pretreatment values. Although the reversibility of the effects of long term GH treatment is reassuring, the long term consequences of hyperinsulinism during childhood are still unknown.

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References