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Effect of Discontinuation of Long-Term Growth Hormone Treatment on Carbohydrate Metabolism and Risk Factors for Cardiovascular Disease in Girls with Turner Syndrome

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GH treatment increases insulin levels in girls with Turner syndrome (TS), who are already predisposed to develop diabetes mellitus and other risk factors for developing cardiovascular disease. Therefore, in the present study, we investigated carbohydrate metabolism and several other risk factors that may predict development of cardiovascular disease in girls with TS after discontinuation of long-term GH treatment. Fifty-six girls, participating in a randomized dose-response study, were examined before, during, and 6 months after discontinuing long-term GH treatment with doses of 4 IU/m^2·d (~0.045 mg/kg·d), 6 IU/m^2·d, or 8 IU/m^2·d. After a minimum of 4 yr of GH treatment, low-dose micronized 17β-estradiol was given orally. Mean (SD) age at 6 months after discontinuation of GH treatment was 15.8 (0.9) yr. Mean duration of GH treatment was 8.8 (1.7) yr. Six months after discontinuation of GH treatment, fasting glucose levels decreased and returned to pretreatment levels. The area under the curve for glucose decreased to levels even lower than pretreatment level ($P < 0.001$). Fasting insulin levels and the area under the curve for insulin decreased to levels just above pretreatment level ($P < 0.001$ for both), although being not significantly different from the control group. No dose-dependent differences among GH dosage groups were found. At 6 months after discontinuation, impaired glucose tolerance was present in 1 of 53 girls (2%), and none of the girls developed diabetes mellitus type 1 or 2. Compared with pretreatment, the body mass index SD-score had increased ($P < 0.001$), and the systolic and diastolic blood pressure SD-score had decreased significantly at 6 months after discontinuation of GH treatment ($P < 0.001$ for both) although remaining above zero ($P < 0.001$, $P < 0.05$, and $P < 0.005$, respectively). Compared with pretreatment, total cholesterol (TC) did not change after discontinuation of GH treatment, whereas the atherogenic index [AI = TC/high-density lipoprotein cholesterol (TC/HDL-c)] and low-density lipoprotein cholesterol (LDL-c) had decreased; and both HDL-c and triglyceride levels increased ($P < 0.001$ for AI, LDL-c, and HDL-c; $P < 0.05$ for triglyceride). Compared with the control group, AI, serum TC, and LDL-c levels were significantly lower ($P < 0.001$ for all), whereas HDL-c levels were significantly higher ($P < 0.05$).

In conclusion, after discontinuation of long-term GH treatment in girls with TS, the GH-induced insulin resistance disappeared, blood pressure decreased but remained higher than in the normal population, and lipid levels and the AI changed to more cardio-protective values. (*J Clin Endocrinol Metab* 87: 5442–5448, 2002)

ONE OF THE main clinical features of Turner syndrome (TS) is short stature. Although girls with TS are not GH-deficient (1), GH treatment has been proven to lead to a considerable height gain in girls with TS in whom treatment with GH was started at a young age and were treated with supraphysiological dosages (2, 3). However, because GH treatment increases insulin levels, several authors have expressed their concern regarding the long-term effect of GH treatment in children with a predisposition for diabetes mellitus (DM) (4, 5).

Besides DM, girls with TS are also predisposed to develop cardiovascular disease (CVD). It has even been reported that CVD is the main cause of their reduced life expectancy (6, 7). In addition, risk factors for CVD, such as hyperlipidemia, hypertension, and insulin resistance, occur more often in TS (7–10).

In the present study, we investigate carbohydrate metabolism in girls with TS after discontinuation of long-term GH treatment with dosage up to 8 IU/m2·d (~0.090 mg/kg·d). Furthermore, we investigate several factors that may predict development of CVD, such as blood pressure (BP), body mass index (BMI), and blood lipid levels.

Subjects and Methods

Study group and treatment regimens

The study group comprised 56 girls with TS who were examined 6 months after discontinuation of GH treatment. Fifty-four children had an oral glucose tolerance test (OGTT) at 6 months after discontinuation of GH. All girls had been part of a multicenter GH dose-response study in The Netherlands, in which 68 girls were included. Inclusion criteria of the dose-response trial were described previously (11); in short: a chronological age between 2 and 11 yr, height below the 50th percentile for healthy Dutch girls, and a normal thyroid function. Of the original 68 girls, 6 girls were not examined at 6 months after discontinuation of GH treatment, and 6 girls were still treated with GH. Written informed

Abbreviations: AI, Atherogenic index; AUC, area under the curve; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DM, diabetes mellitus; HbA1c, glycosylated hemoglobin; HDL-c, high-density lipoprotein cholesterol; IGT, impaired glucose tolerance; LDL-c, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; TC, total cholesterol; TS, Turner syndrome.

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 [4 IU/m^2·d (~0.045 mg/kg·d)], group B (1st yr, 4 IU/m^2·d; thereafter, 6 IU/m^2·d), or group C (1st yr, 4 IU/m^2·d; 2nd yr, 6 IU/m^2·d; thereafter, 8 IU/m^2·d). Biosynthetic human GH (Norditropin; Novo Nordisk A/S, Bagsvaerd, Denmark) was given sc once daily. GH treatment was discontinued when height velocity was less than 1 cm/6 months or when satisfied with their attained height. After a minimum of 4 yr of GH treatment, micronized 17β-estradiol was given orally to the girls of 12.0 yr and older (5 μg/kg body weight·d in the first 2 yr; 7.5 μg/kg·d in the 3rd yr; and thereafter, 10 μg/kg·d). After 2 yr of estrogen treatment, a progestagen was added (5 mg Duphaston). The estrogen dose was gradually increased to adult level (2 mg) after discontinuation of GH treatment. Five of the 56 girls had a repaired coarctation without a residual gradient, left ventricle hypertrophy, or hypertension; 17 girls had a nonstenotic abnormal aortic valve; and none of the girls had a renal malformation that could influence BP. One of the 56 girls, after repair of multiple congenital cardiac malformations, had a remaining left ventricle hypertrophy, which could explain her higher BP during and after discontinuation of GH treatment.

Study protocol

At the start of GH treatment (pretreatment) and every 3 months after the start of GH treatment, all girls were seen, at their local hospital, for a physical examination. All underwent an OGTT after overnight fasting [in the previous 3 d, 100 g of carbohydrate (Fantomalt); oral glucose load of 1.75 g/kg body weight, maximum of 50 g] at pretreatment, after 4 yr of GH treatment, and 6 months after discontinuation of GH treatment. Blood samples were analyzed at 0, 30, 60, 90, 120, 150, and 180 min, for plasma glucose and insulin levels. In addition, 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 (12): the 2-h glucose level more than 7.8 mM (140 mg/dl) and less than 11.1 mM (200 mg/dl). 2) The 3-h area under the curve (AUC) for time-concentration for glucose and insulin was calculated using the trapezoidal rule (3). The ratio insulin/glucose at 30 min and the ratio at 120 min were calculated as an index for relative insulin resistance. Results were compared with the data of 24 normal adolescent girls, 14.7 (0.98) yr old, selected on the basis of postpubertal stage (Tanner breast stage 5) as described by Potau *et al.* (control group) (13). Height and BMI [kg BW/(height)^2] were expressed as a SD-score for sex and chronological age (14, 15). Systolic and diastolic BP was determined four times with a single Dynamap Critikon 1846SX in sitting position using a cuff size corresponding to arm size. BP was expressed as a SD-score, using age- and sex-specific reference values (16). A child was considered normotensive if BP was below the 90th percentile. Additional blood samples were taken at the start of the study and subsequently every year, for determination of glycosylated hemoglobin (HbA1c) levels. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) levels were determined after overnight fasting at the start, at 4 yr of GH treatment, and at 6 months after discontinuation of GH treatment. The atherogenic index (AI) was calculated as the ratio of TC to HDL cholesterol. TC, HDL-c and LDL-c levels, and AI were compared with those of a Dutch control group of the same age and sex (17). After centrifugation, all samples were frozen (−20 C) until assayed.

Assays

The plasma glucose level was measured at the local hospital laboratories, and plasma insulin was determined in one laboratory by RIA (Medgenix, Fleurus, Belgium) as described previously. Control samples were measured by a comparable RIA (R^2 = 0.988; y = 0.397 + 0.925x) (13). HbA1c levels and lipid levels were measured in one laboratory as described elsewhere (4, 11). Lipid levels for the control group were measured by the same assays in the same laboratory (17). All blood sample measurements were performed in the same laboratories during the whole study period.

Statistical analyses

Results were expressed as mean (SD), unless indicated otherwise. For continuous variables with a skewed distribution, a logarithm-transformation was used. Differences among the dosage groups were tested by linear regression analysis, with the variables being age at the start and two dummy variables for the dosage group. Differences in time among continuous variables were compared by paired two-sided *t* test for the whole group. To test whether variables expressed in SD-score were different from zero, a one-sample *t* test was performed. Differences between the whole TS group and the control group for the carbohydrate variables were tested by two-sided independent sample *t* test. All correlations were partial correlations, adjusted for GH dosage. A *P* value less than 0.05 was considered significant. All calculations were performed by software from SPSS, Inc. (version 9.0; Chicago, IL).

Results

In Table 1, pretreatment characteristics of the 56 children are shown. All GH dosage groups had similar pretreatment characteristics. Mean (SD) age at 6 months after discontinuation of GH treatment was 15.8 (0.9) yr. Mean duration of GH treatment was 8.8 (1.7) yr. Forty-four of the 56 girls were treated with GH for 7 yr or longer.

Six months after discontinuation of GH treatment, fasting glucose levels for the whole group had decreased significantly, compared with 4 yr of GH treatment ($P < 0.01$), after a significant increase from pretreatment to 4 yr of GH treatment ($P < 0.01$), and returned to pretreatment levels (Table 2). Mean glucose levels during OGTT are depicted for groups A, B, and C in Fig. 1. The 180-min AUC for glucose at 6 months after discontinuation of GH treatment for the whole group decreased to levels even lower than pretreatment ($P < 0.001$), after a small nonsignificant rise from pretreatment to 4 yr of GH treatment. Fasting glucose levels and the 180-min AUC for glucose for the whole group were not significantly different among the GH dosage groups (Table 2). No significant differences were found between the whole TS group at 6 months after discontinuation of GH treatment and the control group in fasting glucose or 120 min AUC for glucose. Because the control group had a 120-min OGTT, the 120-min AUC in the TS group was used to compare data.

Mean insulin levels during OGTT are depicted in Fig. 2 for groups A, B, and C. Fasting insulin levels (Table 2) and the AUC for insulin at 6 months after discontinuation of GH treatment for the whole group had significantly decreased, compared with 4 yr of GH treatment ($P < 0.01$ after logarithm transformation, and $P < 0.001$, respectively), after a significant rise from pretreatment to 4 yr of GH treatment ($P <$

TABLE 1. Pretreatment variables

	Group A	Group B	Group C
Number of girls	19	17	20
Age at start of GH treatment	6.5 (1.9)	7.5 (1.9)	6.5 (2.4)
Height SD score[a] (normal girls)	−2.8 (0.9)	−2.7 (0.8)	−2.6 (1.0)
Height SD score[a] (TS girls)	0.01 (1.1)	0.2 (0.9)	0.19 (1.1)
Karyotype[b] 45,X	16	16	15
other	3	1	5

Data are expressed as mean (SD).

[a] Height SD score for sex and chronological age in normal girls (15) and in girls with TS (14) at start of GH treatment.

[b] Number of girls.

TABLE 2. Carbohydrate data before, during, and after long-term GH treatment

	GH	Group A (n = 19)	Group B (n = 16)	Group C (n = 19)	Whole group (n = 54)
Fasting glucose[a] (mM)	Start	4.4 (0.5)	4.6 (0.4)	4.6 (0.8)	4.5 (0.6)
	4 yr	4.8 (0.5)	4.7 (0.6)	5.1 (0.9)	4.9 (0.7)[c]
	Post	4.4 (0.5)	4.7 (0.4)	4.6 (0.7)	4.5 (0.5)[d]
AUC glucose[a] (mM × 180 min)	Start	1072 (184)	1118 (172)	1096 (181)	1095 (177)
	4 yr	1072 (122)	1154 (143)	1126 (188)	1116 (155)
	Post	953 (111)	975 (132)	962 (134)	963 (124)[c,e]
Fasting insulin[b] (mU/l)	Start	4 (8)	4 (10)	5 (13)	4.5 (2.2)
	4 yr	12 (23)	16 (38)	16 (45)	14.2 (3.3)[c]
	Post	11 (19)	11 (18)	11 (22)	10.7 (2.9)[c,d]
AUC insulin[a] (mU/l × 180 min)	Start	3863 (2411)	4858 (3284)	3941 (1626)	4205 (2482)
	4 yr	7798 (3355)	14369 (15123)	9733 (3385)	10533 (9094)[c]
	Post	6553 (2468)	5837 (3151)	5987 (2867)	6136 (2791)[c,d]
Ratio ins/glu[b] 30 min	Start	3.5 (10.7)	4.6 (10.2)	3.8 (6.6)	3.9 (7.4)
	4 yr	7.4 (13.8)	11.8 (37.6)	9.8 (20.6)	9.4 (21.2)[c]
	Post	7.8 (20.0)	7.1 (23.0)	6.5 (11.3)	7.1 (17.9)[c,d]
Ratio ins/glu[b] 120 min	Start	2.4 (7.5)	3.6 (9.6)	3.3 (6.4)	3.0 (7.5)
	4 yr	6.5 (13.2)	8.7 (16.5)	7.6 (13.8)	7.5 (13.5)[c]
	Post	5.6 (8.3)	3.8 (6.1)	4.8 (10.5)	4.7 (9.6)[c,e]
HbA1c[a] (% Hb)	Start	4.8 (0.5)	4.9 (0.5)	4.8 (0.5)	4.9 (0.5)
	7 yr	4.6 (0.5)	4.6 (0.7)	4.6 (0.4)	4.6 (0.5)[c]
	Post	4.3 (0.5)	4.3 (0.5)	4.3 (0.5)	4.3 (0.5)[c,e]

Data are expressed as [a] mean (SD) and [b] geometric mean (90th percentile). AUC, Area under the curve calculated with trapezoid rule; ins/glu, insulin/glucose.

Paired *t* test for whole group: [c] $P < 0.01$ (*vs.* start); [d] $P < 0.01$, [e] $P < 0.001$ (*vs.* 4 yr).

0.001); but both remained increased, compared with pretreatment levels ($P < 0.001$ for both) (Table 2). No significant differences among GH dosage groups were found for change in time for fasting insulin levels and change in time for AUC for insulin. Compared with the control group, the AUC for insulin at 6 months after discontinuation of GH treatment for the whole TS group showed no significant difference.

The ratio for insulin to glucose at 30′ (30′ ratio) and 120′ (120′ ratio) for the whole group at 6 months after discontinuation of GH treatment decreased significantly, compared with 4 yr of GH treatment (both variables tested after logarithm transformation; 30′ ratio: $P < 0.01$; 120′ ratio: $P < 0.001$), after an increase in both ratios from pretreatment to 4 yr of GH treatment ($P < 0.001$ for both) (Table 2). Both the 30′ ratio and 120′ ratio at 6 months after discontinuation of GH treatment remained above pretreatment values ($P < 0.001$ for both). No significant differences among GH dosage groups were found for change in time for both ratio's.

At 6 months after discontinuation of GH treatment, IGT was present in 1 of 53 girls (2%). The IGT in this girl was not present before or during GH treatment. None of the girls developed DM type 1 or 2.

The HbA_{1c} values for the whole group at 6 months after discontinuation of GH treatment had significantly decreased, compared with 7 yr of GH treatment ($P < 0.001$), while showing no significant differences among GH dosage groups. Throughout the years, all individual HbA_{1c} levels remained within normal range.

From pretreatment to 7 yr of GH treatment, the BMI SD-score for the whole group had increased significantly [from −0.02 (0.88) to 0.90 (0.92), $P < 0.001$]. Compared with the mean BMI for the reference population (zero SD-score), the BMI SD-score was not significantly different at pretreatment, but it increased to values significantly above zero ($P < 0.001$) at 7 yr of GH treatment. At 6 months after discontinuation of GH treatment, the BMI SD-score had continued to increase slightly, compared with 7-yr values [1.13 (0.97), $P < 0.01$]. The BMI SD-score at 6 months after discontinuation of GH treatment and the change in time for BMI SD-score were not significantly different among GH dosage groups.

From pretreatment to 7 yr of GH treatment, systolic BP for the whole group did not change significantly, whereas diastolic BP showed a small decrease ($P < 0.01$), both remaining significantly higher than zero ($P < 0.001$, $P < 0.05$) (Fig. 3). At 6 months after discontinuation of GH treatment, the systolic BP SD-score had decreased significantly, compared with 7 yr of GH treatment ($P < 0.05$), while the decrease in diastolic BP SD-score did not reach significance. Compared with pretreatment, both systolic and diastolic BP SD-scores had decreased significantly at 6 months after discontinuation of GH treatment ($P < 0.001$ for both), although BP SD-scores after discontinuation of GH treatment remained significantly higher than zero ($P < 0.05$ for both). BP SD-scores at 6 months after discontinuation of GH treatment were not significantly different among dosage groups except for the diastolic BP SD-score between groups B and C (lower in group C: $P < 0.05$). The changes in time for BP SD-scores, however, were not significantly different among GH dosage groups. At pretreatment, 19 of 53 (36%) of the TS girls had a systolic and/or diastolic BP above +1.3 SD-score (~90th percentile for same age and sex); at 4 yr, 23 of 55 (42%); at 7 yr of GH treatment, 19 of 44 (43%); and after discontinuation of GH treatment, 14 of 53 (26%). Eleven of the 14 girls who had a BP above the +1.3 SD-score after discontinuation of GH treatment also had a BP above the +1.3 SD-score at pretreatment, and/or at 4 yr, and/or at 7 yr of GH treatment.

Serum TC and LDL-c levels after 4 yr of GH treatment for the whole group decreased significantly, compared with pretreatment ($P < 0.001$ for all), whereas HDL-c and triglyceride levels had increased ($P < 0.001$ for both) (Table 3). After

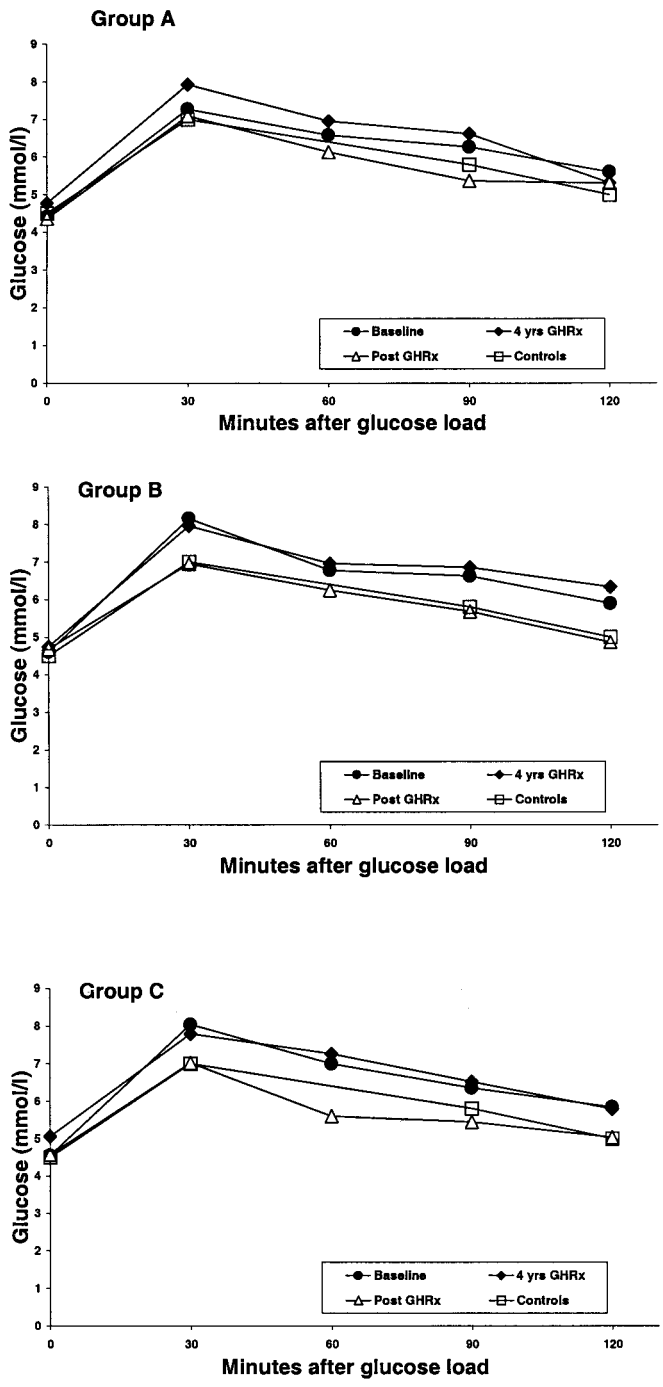

FIG. 1. Mean glucose levels during OGTT for group A (*left*) and group B (*right*) before treatment (*black circles*), at 4 yr of GH treatment (*black diamonds*), at 6 months after discontinuation of treatment (*white triangles*), and for the control group (*white squares*).

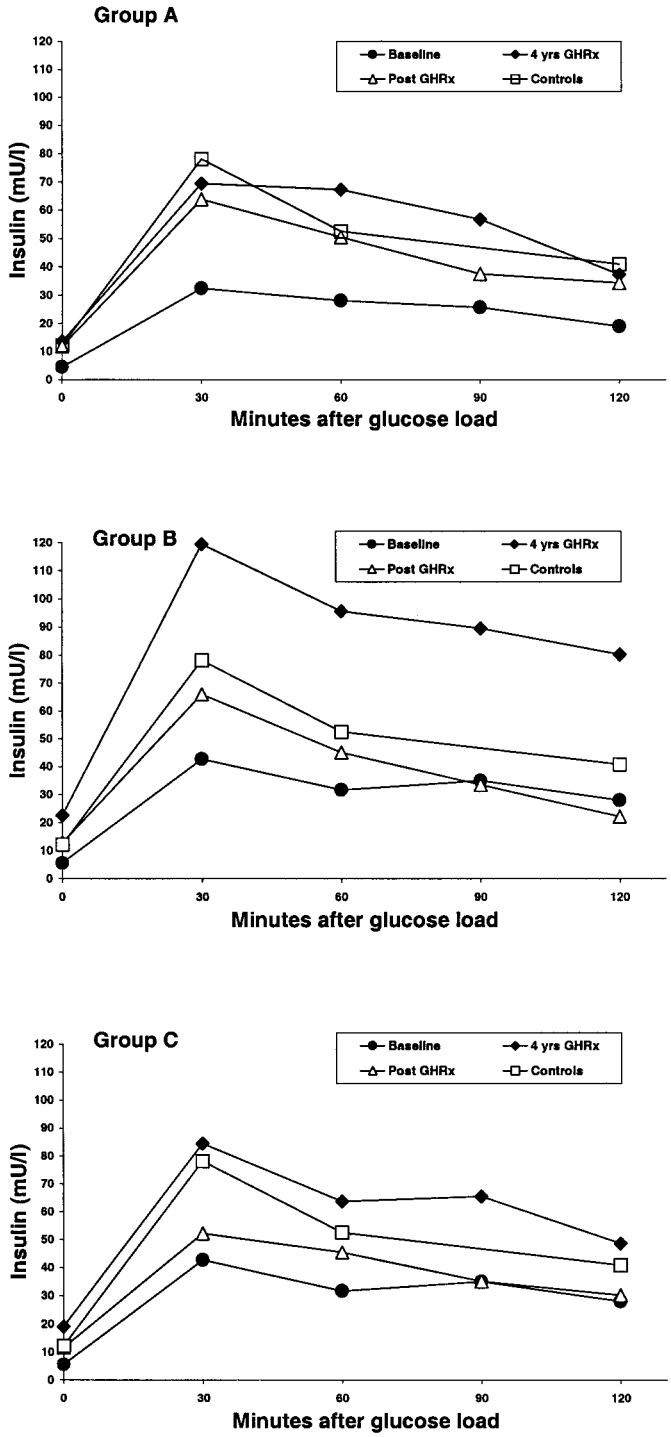

FIG. 2. Mean insulin levels during OGTT for group A (*left*), group B (*middle*), and for group C (*right*) before treatment (*black circles*), at 4 yr of GH treatment (*black diamonds*), at 6 months after discontinuation of treatment (*white triangles*), and for the control group (*white squares*).

discontinuation of GH treatment, TC, LDL-c, but also HDL-c levels had increased significantly, compared with 4-yr levels ($P < 0.001$ and $P < 0.01$, respectively), whereas triglyceride levels decreased significantly ($P < 0.05$). Compared with pretreatment, TC did not change at 6 months after discontinuation of GH treatment, LDL-c had decreased, and both HDL-c and triglyceride levels increased ($P < 0.001$ for LDL-c and HDL-c, $P < 0.05$ for triglyceride). Discontinuation of GH treatment resulted in a decrease in AI (TC/HDL-c), compared with 4-yr values ($P < 0.001$), but also compared with pretreatment ($P < 0.001$). The changes in serum lipid levels were not significantly different among the GH dosage groups, except for the change in time for group C (decrease

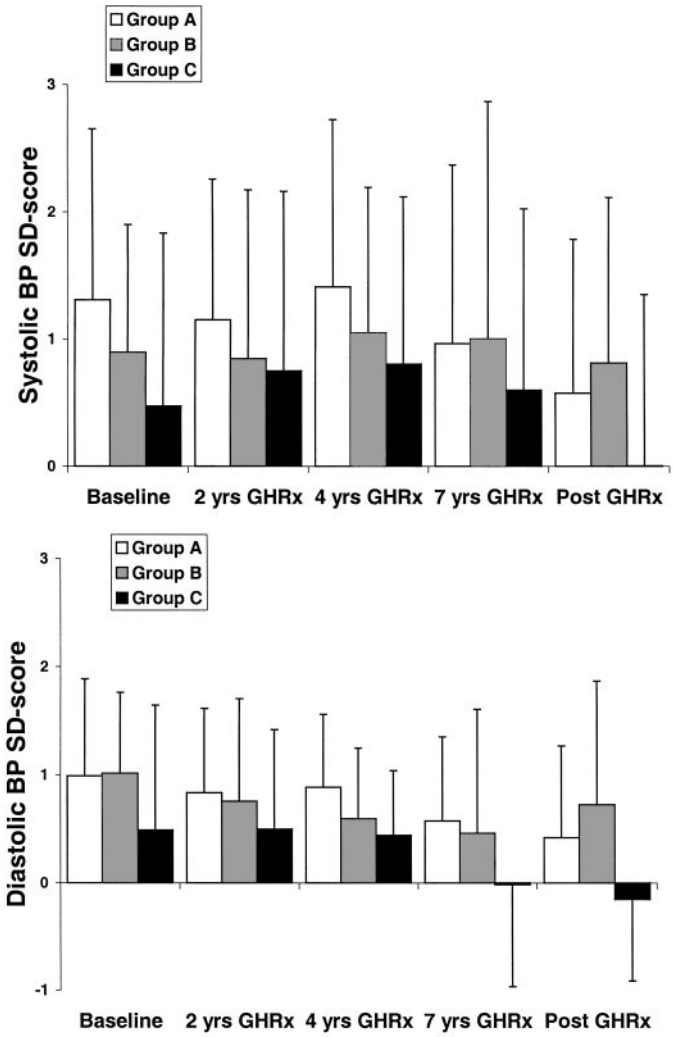

FIG. 3. Mean (SD) systolic BP SD-score (A) and diastolic BP SD-score (B), using age-matched reference values, before GH treatment; at 2, 4, and 7 yr of GH treatment; and at 6 months after discontinuation of treatment for group A (*white bars*), group B (*gray bars*), and for group C (*black bars*).

instead of increase) in TC from pretreatment to 6 months after discontinuation of GH treatment (group C *vs.* group A or B, $P < 0.01$ for both) and a smaller increase in LDL-c from 4 yr of GH treatment to 6 months after discontinuation of treatment (group C *vs.* group A or B, $P < 0.05$). Compared with the control group, serum TC and LDL-c levels were significantly lower in the whole TS group at 6 months after discontinuation of GH treatment ($P < 0.001$ for both), whereas HDL-c levels were significantly higher ($P < 0.05$). Furthermore, the AI was significantly lower at 6 months after GH treatment, compared with the control group (after log transformation, $P < 0.001$).

A significant correlation was found for the whole group when fasting insulin levels (log transformed) and the AUC for insulin was correlated with BMI SD-score at 6 months after discontinuation of GH treatment, after correction for GH dosage group ($r = 0.58$, $P < 0.001$; $r = 0.35$, $P < 0.05$, respectively). No correlation was found for the whole group among the 30′ ratio, 120′ ratio, systolic or diastolic BP SD-score, TC, AI, or triglyceride levels and BMI SD-score at 6 months after discontinuation of GH treatment. The AI at 6 months after discontinuation of GH treatment, after correction for GH dosage, did not correlate with fasting insulin levels, AUC for insulin, or systolic or diastolic BP SD-score at 6 months after discontinuation of GH treatment.

Discussion

In this article, we describe the effect of discontinuation of long-term GH treatment on glucose and insulin levels, BMI, BP, and serum lipid levels in girls with TS. We show that both fasting and stimulated insulin levels, after an increase during GH treatment, returned to normal after discontinuation. In addition, we show that after discontinuation of GH treatment, both systolic and diastolic BP and the AI (TC/HDL-c) had fallen.

Fasting glucose levels increased during GH treatment and decreased after discontinuation of GH treatment, whereas stimulated glucose levels showed no change during GH treatment and decreased after its discontinuation. Furthermore, after ending GH treatment, insulin levels and the indices for relative insulin resistance (30′ and 120′ ratios for insulin/glucose) fell but only to a point above pretreatment levels. Similar results have been found previously in children with idiopathic short stature and in girls with TS after discontinuation of GH treatment (18, 19). Moreover, we show that stimulated insulin levels after discontinuation were comparable with normal postpubertal girls. Several studies have shown that insulin sensitivity decreased during puberty, resulting in an increase in stimulated insulin levels (13, 20, 21). Subsequently, in post puberty, although insulin levels were decreasing, they were still at a higher level than before puberty (20). These results might therefore imply that the reason why insulin levels and the indices for relative insulin resistance did not return to pretreatment positions was that our study group was in its postpubertal stage. Another explanation for the higher insulin levels and indices for relative insulin resistance after discontinuing GH might be the increase in BMI SD-score we found in our study group after discontinuation of GH treatment. Previous studies have shown a positive correlation between insulin levels and BMI in normal children and adults (22, 23). Supporting this explanation, in our study, we found a positive correlation between BMI SD-score and fasting and stimulated insulin levels after discontinuation of GH treatment. Several reports, however, have shown an increased prevalence of insulin resistance and IGT in untreated women with TS (24, 25). Therefore, the higher insulin levels might also be a result of having TS. The prevalence of IGT in our study, however, was low (1 girl).

After discontinuation of GH treatment, we found a decrease in BP SD-scores, compared with pretreatment. Because this decrease has been corrected for age, it is unlikely that age could explain this decrease. A possible explanation might be the initiation of estrogen treatment. Confirming this explanation, Gravholt *et al.* (9) showed that the start of supplementation of natural estrogens, in combination with progestagens in adult women with TS, decreased ambulatory BP. In another study on adult TS women, however, no change in

TABLE 3. Lipid levels before, during, and after GH treatment

	GH	Group A (n = 19)	Group B (n = 17)	Group C (n = 20)	Whole group (n = 56)	Controls[a] (n = 703)
TC[b] (mM)	Start	4.0 (0.7)	4.3 (0.8)	4.5 (0.9)	4.3 (0.8)	
	4 yr	3.8 (0.7)	4.1 (0.8)	4.0 (0.7)	4.0 (0.7)[e]	
	Post	4.1 (0.8)	4.5 (0.7)	4.1 (0.6)[j]	4.2 (0.7)[e]	4.7 (0.7)[l]
HDL-c[b] (mM)	Start	0.6 (0.1)	0.7 (0.1)	0.9 (0.2)	0.7 (0.2)	
	4 yr	1.0 (0.3)	1.0 (0.2)	1.2 (0.3)	1.1 (0.3)[e]	
	Post	1.3 (0.3)	1.4 (0.3)	1.5 (0.4)	1.4 (0.3)[e,h]	1.3 (0.3)[k]
LDL-c[b] (mM)	Start	2.6 (0.7)	2.9 (0.9)	2.9 (1.0)	2.8 (0.9)	
	4 yr	1.9 (0.7)	2.1 (0.6)	2.2 (0.7)	2.1 (0.7)[e]	
	Post	2.1 (0.6)	2.5 (0.6)	2.2 (0.6)[i]	2.3 (0.6)[e,g]	2.9 (0.7)[l]
Trigl[c] (mM)	Start	1.0 (1.5)	0.9 (1.5)	0.9 (2.1)	0.9 (1.6)	
	4 yr	1.3 (3.2)	1.2 (2.6)	1.2 (2.2)	1.2 (2.5)[e]	
	Post	1.3 (2.8)	1.0 (1.7)	0.9 (1.7)	1.1 (1.9)[d,f]	
Atherogenic index[c]	Start	7.1 (8.6)	6.4 (8.1)	4.9 (6.8)	6.0 (8.2)	
	4 yr	4.0 (5.6)	4.0 (5.6)	3.2 (4.6)	3.7 (5.5)[e]	
	Post	3.2 (4.0)	3.2 (4.3)	2.8 (3.8)	3.0 (4.0)[e,h]	3.6 (4.8)[l]

[a] Dutch control group of same age and sex (17).

Data are expressed as [b] mean (SD) and [c] geometric mean (90th percentile). Trigl, Triglycerides. Atherogenic index = TC/HDL-c.

Paired *t* test for change in time for whole group: [d] $P < 0.05$, [e] $P < 0.001$ (*vs.* start); [f] $P < 0.05$, [g] $P < 0.01$, [h] $P < 0.001$ (*vs.* 4 yr).

Linear regression analysis for change from 4 yr of GH: [i] $P < 0.05$ (group C *vs.* groups A and B, corrected for age at start); for change from start: [j] $P < 0.01$ (group C *vs.* groups A and B, corrected for age at start); for control group *vs.* whole TS group: [k] $P < 0.05$, [l] $P < 0.001$ (corrected for dosage group and age).

BP was found after hormone replacement therapy was initiated (26). Another possible explanation might be a positive effect of GH treatment on BP, which has been postulated to occur in children born small for gestational age (27). BP after discontinuation of GH treatment, however, remained slightly higher than in girls matched for age. The reason possibly lies in the fact that having TS is a predisposition for hypertension (7). The etiology of the predisposition, however, remains unclear (28). Because we found a decrease in BP, compared with pretreatment, it is unlikely that GH treatment was responsible for the higher BP.

During the first 4 yr of GH treatment, we show a decrease in AI, TC, and LDL-c and an increase in HDL-c and triglyceride. Previous studies, studying lipid levels during GH treatment in TS, showed either similar results during GH treatment (29, 30) or no effect (31). After discontinuation of GH treatment, compared with 4-yr lipid levels, TC and LDL-c levels had increased, compared with the decrease we found during treatment, whereas triglyceride levels had decreased slightly after an increase during GH treatment. Similar results were found in reports on the effect of discontinuation of GH in GH-deficient adolescents (32, 33), thus possibly implying a GH effect. HDL-c levels after discontinuation of GH treatment, however, showed a further increase. A possible explanation for this could be the induction of puberty with natural estrogens and dydrogestagen, which has been shown to lead to an increase in HDL-c (34). A second explanation for the increase in TC, LDL-c, and HDL-c after discontinuing GH might be the age effect, because it has been established that after puberty, TC, LDL-c, and HDL-c increase with age (35, 36). Interestingly, when we compared lipid levels after discontinuation of GH treatment, to a normal control group of similar age, we found that TC, LDL-c, and AI levels were lower and HDL-c levels were higher in our study group. This indicated that, whereas previous reports on lipid levels in untreated girls and women with TS have shown conflicting results regarding the prevalence of dyslipidemia (8, 10, 37, 38), in our study group, after long-term GH treatment, we found no evidence of dyslipidemia.

Because several studies have found that women with TS are predisposed to develop CVD (6, 7), and several risk factors for CVD (such as high BP, dyslipidemia, and abdominal obesity) have been found to be more prevalent in TS, we analyzed our data for clustering of these CVD risk factors by way of correlations. Although, after discontinuation of GH treatment, we found a positive correlation between BMI SD-score and insulin levels, we could not detect any other correlations among the risk factors. In contrast, in a study on the effect of discontinuation of GH treatment in children of similar age, but born small for gestational age, a positive correlation among the AI, BMI SD-score, systolic and diastolic BP SD-score, and fasting insulin was found (39). The lack of correlation among the cardiovascular risk factors in our group with TS, however, suggested that, in this group, no clustering of risk factors was present. Because a clustering of risk factors potentially increases the risk for CVD (40), not only follow-up of all risk factors but also evaluation of clustering should take place in the future. Though we did not find evidence of clustering, we did find a positive correlation between insulin resistance and BMI. Although this relationship is also found in normal children (23, 41), insulin resistance does predispose for the development of DM type 2. We would therefore urge clinicians to do their utmost to prevent further weight gain in girls and women with TS.

In conclusion, after discontinuation of long-term GH treatment in girls with TS, the GH-induced insulin resistance disappeared. BP decreased both during and after discontinuation of GH treatment, but remained higher than in the normal population, whereas lipid levels and the AI after discontinuation of GH treatment were more beneficial, regarding the development of CVD, than in a normal control group.

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