

EUR Research Information Portal

Effect of discontinuation of long-term growth hormone treatment on carbohydrate metabolism and risk factors for cardiovascular disease in girls with Turner syndrome

Published in:

Journal of Clinical Endocrinology and Metabolism

Publication status and date:

Published: 01/01/2002

DOI (link to publisher):

[10.1210/jc.2002-020789](https://doi.org/10.1210/jc.2002-020789)

Document Version

Publisher's PDF, also known as Version of record

Citation for the published version (APA):

van Pareren, YK., Schrama, S., Stijnen, T., Sas, T., & Drop, S. (2002). Effect of discontinuation of long-term growth hormone treatment on carbohydrate metabolism and risk factors for cardiovascular disease in girls with Turner syndrome. *Journal of Clinical Endocrinology and Metabolism*, 87(12), 5442-5448. <https://doi.org/10.1210/jc.2002-020789>

[Link to publication on the EUR Research Information Portal](#)

Terms and Conditions of Use

Except as permitted by the applicable copyright law, you may not reproduce or make this material available to any third party without the prior written permission from the copyright holder(s). Copyright law allows the following uses of this material without prior permission:

- you may download, save and print a copy of this material for your personal use only;
- you may share the EUR portal link to this material.

In case the material is published with an open access license (e.g. a Creative Commons (CC) license), other uses may be allowed. Please check the terms and conditions of the specific license.

Take-down policy

If you believe that this material infringes your copyright and/or any other intellectual property rights, you may request its removal by contacting us at the following email address: openaccess.library@eur.nl. Please provide us with all the relevant information, including the reasons why you believe any of your rights have been infringed. In case of a legitimate complaint, we will make the material inaccessible and/or remove it from the website.

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

YVONNE K. VAN PAREREN, SABINE M. P. F. DE MUINCK KEIZER-SCHRAMA, THEO STIJNEN, THEO C. J. SAS, STENVERT L. S. DROP, AND THE DUTCH ADVISORY GROUP ON GROWTH HORMONE

Department of Pediatrics, Division of Endocrinology, Erasmus Medical Centre/Sophia Children's Hospital (Y.K.v.P., S.M.P.F.d.M.K.-S., T.C.J.S., S.L.S.D.), and Institute of Epidemiology and Biostatistics, Erasmus University (T.S.), 3015 GJ Rotterdam, The Netherlands

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/m²·d (~0.045 mg/kg·d), 6 IU/m²·d, or 8 IU/m²·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/m²·d (~0.045 mg/kg·d)], group B (1st yr, 4 IU/m²·d; thereafter, 6 IU/m²·d), or group C (1st yr, 4 IU/m²·d; 2nd yr, 6 IU/m²·d; thereafter, 8 IU/m²·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

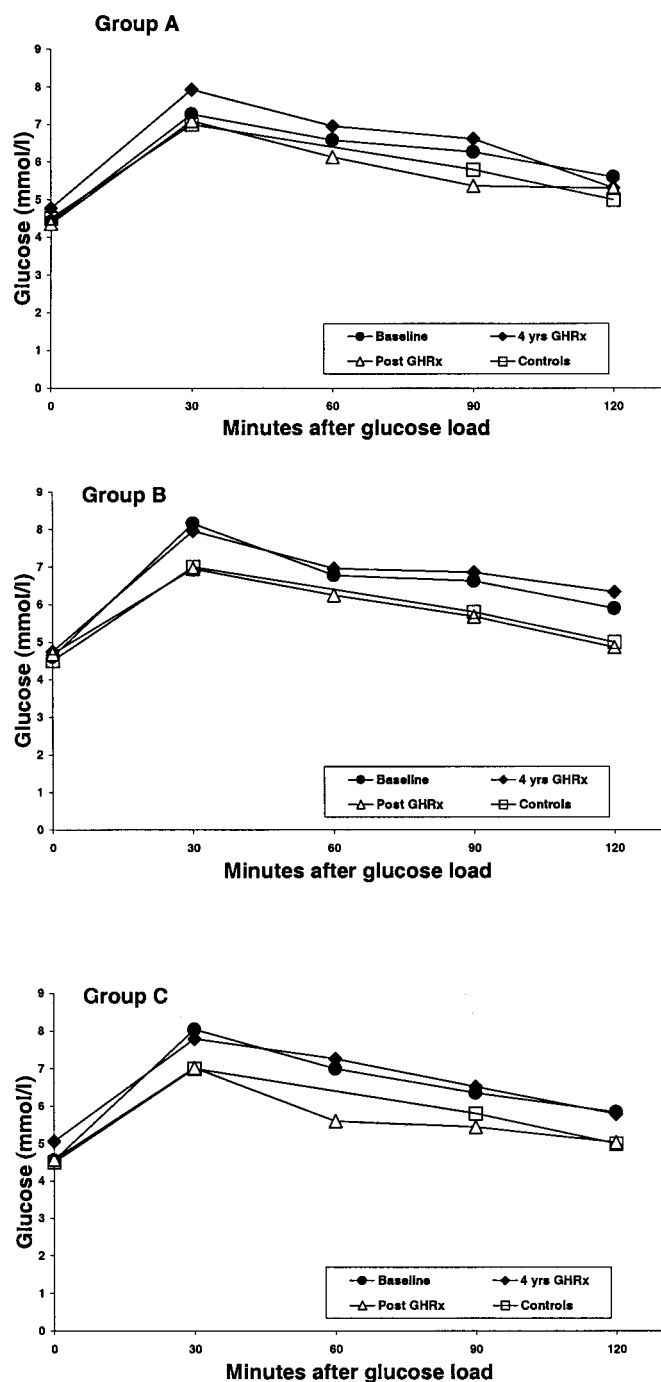


FIG. 1. Mean glucose levels during OGTT for group A (left) and group B (right) before treatment (black circles), at 4 yr of GH treatment (black diamonds), at 6 months after discontinuation of treatment (white triangles), and for the control group (white squares).

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

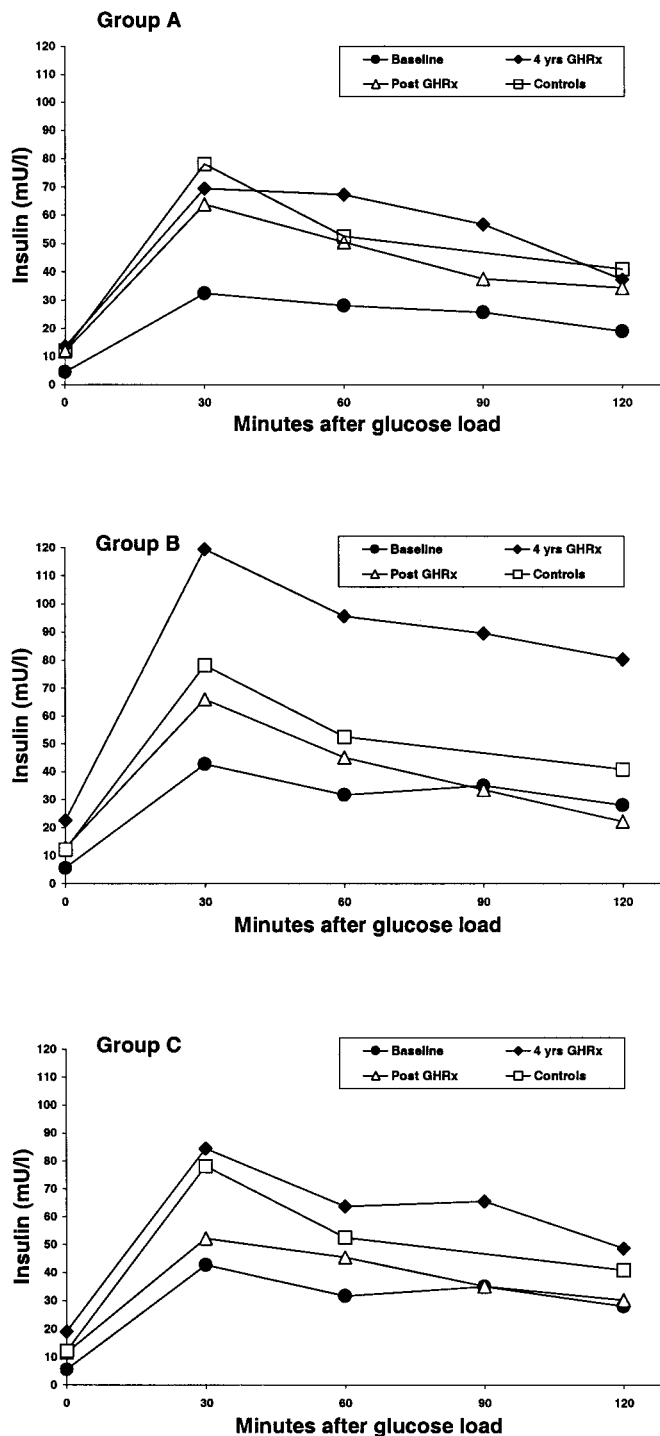


FIG. 2. Mean insulin levels during OGTT for group A (left), group B (middle), and for group C (right) before treatment (black circles), at 4 yr of GH treatment (black diamonds), at 6 months after discontinuation of treatment (white triangles), and for the control group (white squares).

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

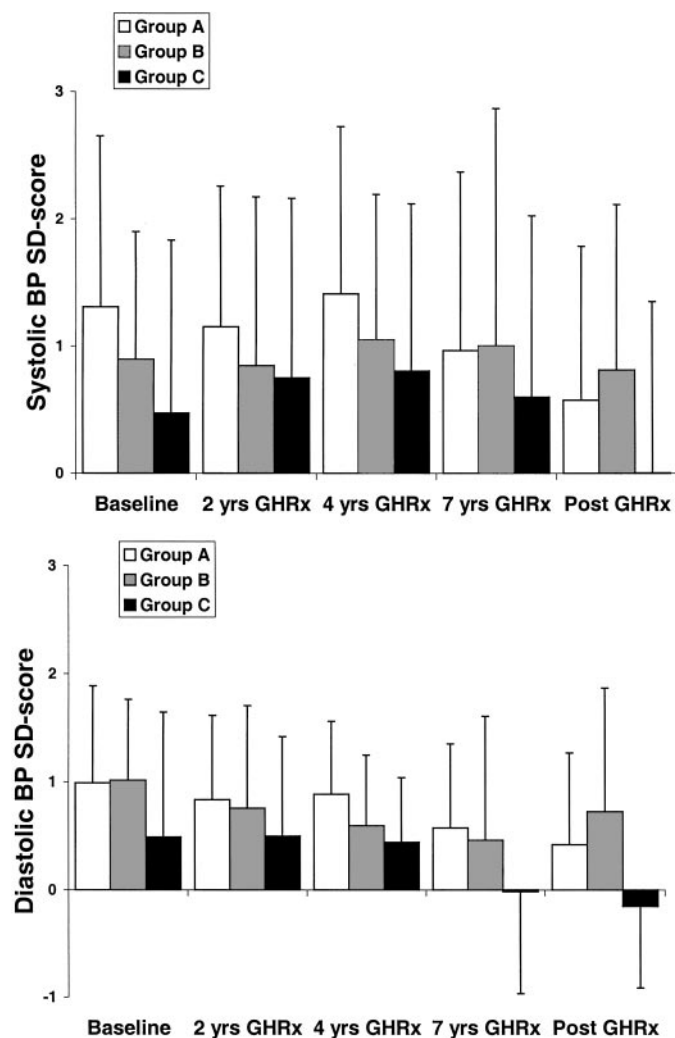


FIG. 3. Mean (SD) systolic BP SD-score (A) and diastolic BP SD-score (B), using age-matched reference values, before GH treatment; at 2, 4, and 7 yr of GH treatment; and at 6 months after discontinuation of treatment for group A (white bars), group B (gray bars), and for group C (black bars).

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) ^e	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.

Acknowledgments

We acknowledge I. van Slobbe (research nurse) and A. van Teunenbroek, M.D., Ph.D., for contribution to this study.

Received May 22, 2002. Accepted August 26, 2002.

Address all correspondence and requests for reprints to: Y. K. van Pareren, M.D., Department of Pediatrics, Division of Endocrinology, Erasmus MC/Sophia Children's Hospital, Dr. Molewaterplein 60, 3015 GJ Rotterdam, The Netherlands. E-mail: vanpareren@zonnet.nl.

This work was supported by Novo Nordisk A/S.

The participating members of the Dutch Advisory Group on Growth Hormone were: M. Jansen, Wilhelmina Children's Hospital, Utrecht; B. J. Otten, Sint Radboud University Hospital, Nijmegen; J. J. G. Hoorweg-Nijman, Free University Hospital, Amsterdam; T. Vulsma, Academic Medical Center/Emma Children's Hospital, Amsterdam; J. Stokvis, Medical University Center, Leiden; C. W. Rouwé, Beatrix Children's Hospital, Groningen; H. M. Reeser, Juliana Children's Hospital, The Hague; W.-J. Gerver, Academic Hospital, Maastricht; J. J. Gosen, Rijnland Hospital, Leiderdorp; and C. Rongen-Westerlaken, Canisius-Wilhelmina Hospital, Nijmegen.

References

- Wit JM, Massarano AA, Kamp GA, Hindmarsh PC, van Es A, Brook CG, Preece MA, Matthews DR 1992 Growth hormone secretion in patients with Turner's syndrome as determined by time series analysis. *Acta Endocrinol (Copenh)* 127:7–12
- Carel JC, Mathivon L, Gendrel C, Ducret JP, Chaussain JL 1998 Near normalization of final height with adapted doses of growth hormone in Turner's syndrome. *J Clin Endocrinol Metab* 83:1462–1466
- Sas TC, de Muinck Keizer-Schrama SM, Stijnen T, Jansen M, Otten BJ, Hoorweg-Nijman JJ, Vulsma T, Massa GG, Rouwe CW, Reeser HM, Gerver WJ, Gosen JJ, Rongen-Westerlaken C, Drop SL 1999 Normalization of height in girls with Turner syndrome after long-term growth hormone treatment: results of a randomized dose-response trial. *J Clin Endocrinol Metab* 84:4607–4612
- van Teunenbroek A, de Muinck Keizer-Schrama SM, Aanstoot HJ, Stijnen T, Hoogerbrugge N, Drop SL 1999 Carbohydrate and lipid metabolism during various growth hormone dosing regimens in girls with Turner syndrome. Dutch Working Group on Growth Hormone. *Metabolism* 48:7–14
- Cutfield WS, Wilton P, Bennmarker H, Albertsson-Wikland K, Chatelain P, Ranke MB, Price DA 2000 Incidence of diabetes mellitus and impaired glucose tolerance in children and adolescents receiving growth-hormone treatment. *Lancet* 355:610–613
- Price WH, Clayton JF, Collyer S, De Mey R, Wilson J 1986 Mortality ratios, life expectancy, and causes of death in patients with Turner's syndrome. *J Epidemiol Community Health* 40:97–102
- Gravholt CH, Juul S, Naeraa RW, Hansen J 1998 Morbidity in Turner syndrome. *J Clin Epidemiol* 51:147–158
- Ross JL, Feuillan P, Long LM, Kowal K, Kushner H, Cutler Jr GB 1995 Lipid abnormalities in Turner syndrome. *J Pediatr* 126:242–245
- Gravholt CH, Naeraa RW, Nyholm B, Gerdes LU, Christiansen E, Schmitz O, Christiansen JS 1998 Glucose metabolism, lipid metabolism, and cardiovascular risk factors in adult Turner's syndrome. The impact of sex hormone replacement. *Diabetes Care* 21:1062–1070
- Elsheikh M, Conway GS 1998 The impact of obesity on cardiovascular risk factors in Turner's syndrome. *Clin Endocrinol (Oxf)* 49:447–450
- Sas TC, de Muinck Keizer-Schrama SM, Stijnen T, Aanstoot HJ, Drop SL 2000 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. Dutch Advisory Group on Growth Hormone. *J Clin Endocrinol Metab* 85:769–775
- 1997 Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197
- Potau N, Ibanez L, Rique S, Carrascosa A 1997 Pubertal changes in insulin secretion and peripheral insulin sensitivity. *Horm Res* 48:219–226
- Karlberg J, Albertsson-Wikland K, Naeraa RW, Rongen-Westerlaken C, Wit JM 1992 Reference values for spontaneous growth in Turner girls and its use in estimating treatment effects. In: Hibi I, Takano K, eds. *Basic and clinical approach to Turner syndrome: 3rd International Symposium on Turner Syndrome*; July 8–10, 1992. Chiba, Japan: Elsevier Science Publishers B. V.; 83–92
- Roede MJ, van Wieringen JC 1985 Growth diagrams 1980. Netherlands third nation-wide survey. *Tijdschr Soc Gezondh* 63(Suppl):1–34
- Task Force on Blood Pressure Control in Children 1987 Report of the Second Task Force on Blood Pressure Control in Children National Heart, Lung, and Blood Institute, Bethesda, Maryland. *Pediatrics* 79:1–25
- Uiterwaal CS, Witteman JC, de Bruijn AM, Hofman A, Grobbee DE 1997 Families and natural history of lipids in childhood: an 18-year follow-up study. *Am J Epidemiol* 145:777–785
- Lesage C, Walker J, Landier F, Chatelain P, Chaussain JL, Bougneres PF 1991 Near normalization of adolescent height with growth hormone therapy in very short children without growth hormone deficiency. *J Pediatr* 119:29–34
- Joss EE, Zurbrugg RP, Tonz O, Mullis PE 2000 Effect of growth hormone and oxandrolone treatment on glucose metabolism in Turner syndrome. A longitudinal study. *Horm Res* 53:1–8
- Bloch CA, Clemons P, Sperling MA 1987 Puberty decreases insulin sensitivity. *J Pediatr* 110:481–487
- Cutfield WS, Bergman RN, Menon RK, Sperling MA 1990 The modified minimal model: application to measurement of insulin sensitivity in children. *J Clin Endocrinol Metab* 70:1644–1650
- Reaven GM, Moore J, Greenfield M 1983 Quantification of insulin secretion and *in vivo* insulin action in nonobese and moderately obese individuals with normal glucose tolerance. *Diabetes* 32:600–604
- Travers SH, Jeffers BW, Bloch CA, Hill JO, Eckel RH 1995 Gender and Tanner stage differences in body composition and insulin sensitivity in early pubertal children. *J Clin Endocrinol Metab* 80:172–178
- Caprio S, Boulware S, Diamond M, Sherwin RS, Carpenter TO, Rubin K, Amiel S, Press M, Tamborlane WV 1991 Insulin resistance: an early metabolic defect of Turner's syndrome. *J Clin Endocrinol Metab* 72:832–836
- Polychronakos C, Letarte J, Collu R, Ducharme JR 1980 Carbohydrate intolerance in children and adolescents with Turner syndrome. *J Pediatr* 96:1009–1014
- Elsheikh M, Bird R, Casadei B, Conway GS, Wass JA 2000 The effect of hormone replacement therapy on cardiovascular hemodynamics in women with Turner's syndrome. *J Clin Endocrinol Metab* 85:614–618
- Sas T, Mulder P, Aanstoot HJ, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 2001 Carbohydrate metabolism during long-term growth hormone treatment in children with short stature born small for gestational age. *Clin Endocrinol (Oxf)* 54:243–251
- Nathwani NC, Unwin R, Brook CG, Hindmarsh PC 2000 The influence of renal and cardiovascular abnormalities on blood pressure in Turner syndrome. *Clin Endocrinol (Oxf)* 52:371–377
- Price DA, Clayton PE, Crowne EH, Roberts CR 1993 Safety and efficacy of human growth hormone treatment in girls with Turner syndrome. *Horm Res* 39:44–48
- Lanes R, Gunczler P, Palacios A, Villaroel O 1997 Serum lipids, lipoprotein lipase, and plasminogen activator inhibitor-1 in patients with Turner's syndrome before and during growth hormone and estrogen therapy. *Fertil Steril* 68:473–477
- Saenger P, Attie KM, DiMartino-Nardi J, Fine RN 1996 Carbohydrate metabolism in children receiving growth hormone for 5 years. Chronic renal insufficiency compared with growth hormone deficiency, Turner syndrome, and idiopathic short stature. The Genentech Collaborative Group. *Pediatr Nephrol* 10:261–263
- Johannsson G, Albertsson-Wikland K, Bengtsson BA 1999 Discontinuation of growth hormone (GH) treatment: metabolic effects in GH-deficient and GH-sufficient adolescent patients compared with control subjects. Swedish Study Group for Growth Hormone Treatment in Children. *J Clin Endocrinol Metab* 84:4516–4524
- Kuromaru R, Kohno H, Ueyama N, Hassan HM, Honda S, Hara T 1998 Long-term prospective study of body composition and lipid profiles during and after growth hormone (GH) treatment in children with GH deficiency: gender-specific metabolic effects. *J Clin Endocrinol Metab* 83:3890–3896
- Mijatovic V, Kenemans P, Netelenbos JC, Peters-Muller ER, van Kamp GJ, Voetberg GA, van de Weijer PH, van der Moeren MJ 1997 Oral 17 beta-estradiol continuously combined with dydrogesterone lowers serum lipoprotein(a) concentrations in healthy postmenopausal women. *J Clin Endocrinol Metab* 82:3543–3547
- van Stiphout WA, Hofman A, de Bruijn AM, Valkenburg HA 1985 Distributions and determinants of total and high-density lipoprotein cholesterol in Dutch children and young adults. *Prev Med* 14:169–180
- Hickman TB, Briefel RR, Carroll MD, Rifkin BM, Cleeman JI, Maurer KR, Johnson CL 1998 Distributions and trends of serum lipid levels among United States children and adolescents ages 4–19 years: data from the Third National Health and Nutrition Examination Survey. *Prev Med* 27:879–890
- Garden AS, Diver MJ, Fraser WD 1996 Undiagnosed morbidity in adult women with Turner's syndrome. *Clin Endocrinol (Oxf)* 45:589–593
- Landin-Wilhelmsen K, Bryman I, Wilhelmsen L 2001 Cardiac malformations and hypertension, but not metabolic risk factors, are common in Turner syndrome. *J Clin Endocrinol Metab* 86:4166–4170
- van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A, Effect of discontinuation of GH treatment on risk factors for cardiovascular disease in adolescents born small for gestational age. *J Clin Endocrinol Metab*, in press
- D'Agostino RB, Russell MW, Huse DM, Ellison RC, Silbershatz H, Wilson PW, Hartz SC 2000 Primary and subsequent coronary risk appraisal: new results from the Framingham study. *Am Heart J* 139:272–281
- Hoffman RP, Vicini P, Sivitz WJ, Cobelli C 2000 Pubertal adolescent male-female differences in insulin sensitivity and glucose effectiveness determined by the one compartment minimal model. *Pediatr Res* 48:384–388