Carbohydrate and Lipid Metabolism During Various Growth Hormone Dosing Regimens in Girls With Turner Syndrome

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To analyze the effects of supraphysiological dosages of growth hormone (GH) on carbohydrate (CH) and lipid metabolism, we investigated 87 girls with Turner syndrome (TS) in two studies: (1) a 4-year GH dose-response (DR) study comparing three groups with stepwise GH dosage increases up to 8 IU/m^2/d in girls aged 2 to 11 years, and (2) a 2-year GH administration frequency-response (FR) study in girls aged 11 to 17 years, comparing once-daily (OD) and twice-daily (BID) injections of a total GH dose of 6 IU/m^2/d in combination with low-dose ethinyl estradiol (50 ng/kg/d orally). At baseline, impaired glucose tolerance (IGT) was present in 6% of the girls, and at the end of the studies, in 5%. In the DR study, the area under the curve for time-concentration (AUC_{ab}) for glucose after an oral glucose tolerance test (OGTT) showed no change over time and no significant difference between any of the study groups. However, in all three DR groups, the AUC_{ab} for insulin, fasting glucose, the insulinogenic index, hemoglobin A_{1c} (HbA_{1c}), and urinary C-peptide (uCp) were all significantly higher after 4 years compared with pretreatment ($P < .05$). In the FR study, group differences were not observed. Compared with healthy Dutch control subjects, the median baseline levels in relatively young girls in the DR study were similar for total cholesterol (TC) and lower for high-density lipoprotein (HDL) cholesterol. In contrast, the median TC levels of relatively older girls in the FR study were higher and HDL levels were similar. With increasing GH dosage in the DR study, median TC and low-density lipoprotein (LDL) levels decreased, whereas median HDL levels increased. The changes after 4 years were significant, including a decrease in the atherogenic index. GH treatment at the supraphysiological dosages used in this study did not increase the frequency of IGT or clinical diabetes. However, we observed an increased insulinogenic index indicative of insulin resistance. Therefore, long-term follow-up study is warranted in these otherwise healthy subjects. OD injection regimens changed the lipid profile toward a more cardioprotective direction with a significant reduction of the TC/HDL cholesterol ratio.

RECOMBINANT HUMAN growth hormone (GH) is widely used in girls with Turner syndrome (TS) to increase height velocity. Supraphysiological GH dosages are necessary to induce a positive nitrogen balance[1] in this disorder. However, such dosages given for many years may influence carbohydrate (CH) and lipid metabolism.

CH Metabolism

GH modulates tissue responses to insulin in man. GH deficiency (GHD) increases the sensitivity to insulin.[2] Supraphysiologic concentrations of GH in acromegalic patients[3] and in normal[4,5] and diabetic[6] adults caused a decrease in glucose sensitivity to insulin both in the liver and extrahepatic tissues. Diabetogenic effects only occur if compensatory mechanisms fail, eg, when insulin secretion is deficient.

CH intolerance and non–insulin-dependent diabetes mellitus are common in adult women with TS, and substantial concern has been expressed regarding possible detrimental effects of GH therapy. In girls with TS, a high frequency of diabetes has been reported,[7] and the reported frequency of impaired glucose tolerance (IGT) is 15%[8,9] to 43%[10] and is associated with a normal or increased insulin response.[11] Age, karyotype, family history of diabetes, and estrogen replacement can aggravate preexistent CH intolerance in TS. CH tolerance shows a relative improvement at puberty due to the almost complete absence of estrogen-progestagen secretion.[12] Results after 1 or 2 years of GH therapy in TS patients indicated that glucose homeostasis is maintained during GH therapy at the expense of a compensatory increase in the insulin response.[13-15]

Lipid Metabolism

In GHD adults, GH was found to have an intrinsic lipolytic activity, which improves after long-term GH administration.[14] This results in a reduction of adipose tissue and an increase in lean body mass.[15] Normalization of total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol has been observed.[16] Contradictory observations of GH therapy on lipid metabolism in GHD children have been reported. Two studies found a reduced atherogenic index.[17,18] Also, some studies showed a decrease in mean serum levels of TC[19] or an increase in high-density lipoprotein (HDL) cholesterol,[18] while others observed no changes in these serum levels.[20] Even a dosage increase from 0.3 IU/kg/wk to 0.9 IU/kg/wk (thrice-daily injections)[21] or an equally divided dose of 20 IU/m^2/wk in a thrice-daily regimen[22] did not have any effect on triglyceride (TG) and TC plasma levels. Acromegalic patients often show increased serum TG (but not TC), which return to normal levels after surgery.[23]

Girls with TS at adolescence had higher TC levels but similar TG levels when compared with age-matched controls (adjusted for age and obesity). Such findings are likely related to the absence of estrogens, possibly mediated by GH,[24] which decreases LDL and increases HDL.[25] In TS, no significant changes in TC or TG concentrations were observed after 1 or 2 years of GH administration,[8,26] although one group reported an increase in TG levels after 6 months GH therapy.[27]

In this study, we investigated the changes in CH and lipid

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Submitted August 30, 1996; accepted July 6, 1998.

Supported by Novo-Nordisk, Gentofte, Denmark.

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0026-0495/99/4801-0003$03.00/0

metabolism during two different GH treatment studies in girls with TS: a 4-year dose-response (DR) study and a 2-year frequency-response (FR) study.

Subjects and Methods

Study Groups

Only previously untreated girls with TS were enrolled in two nationwide multicenter trials. The diagnosis was confirmed by lymphocyte chromosomal analysis. Table 1 lists the baseline clinical characteristics. Inclusion criteria were height below the 50th percentile for chronological age (CA) for Dutch children[28] and normal thyroid function. Exclusion criteria were associated endocrine and/or metabolic disorders, growth failure due to other disorders or emotional deprivation, hydrocephalus, previous use of drugs that may interfere with GH therapy, and Tanner puberty stage of the breasts of at least B2.[29] The ethics committee of each participating center approved the study. Patients were enrolled after obtaining signed informed consent from both the parents (or custodians) and children. In both studies, biosynthetic human GH (Norditropin; Novo Nordisk, Gentofte, Denmark) was injected using a pen injection system (Nordiject 24).

DR Study

Sixty-eight girls aged 2 to 11 years participated in a 4-year GH DR study (Fig 1A). During this period, no estrogens were administered. The girls were randomized into three GH dosing groups with stratification according to CA and height standard deviation score for CA ($HSDS_{CA}$): A, n = 23, 4 IU GH/m^2 body surface (equivalent to 0.045 mg/kg/d) for 4 years; B, n = 23, 4 IU/m^2 in the first year, followed by 6 IU/m^2/d (equivalent to 0.0675 mg/kg/d) in the second through fourth year; and C, n = 22, 4 IU/m^2 in the first year, 6 IU/m^2/d in the second year, and 8 IU/m^2/d (equivalent to 0.09 mg/kg/d) during the third and fourth year.

In group B, one girl withdrew from the study due to noncompliance (after 24 months). In group C, a girl withdrew after 30 months due to a presumed increase of muscle mass and deterioration of school performance (according to the mother). One girl from group A withdrew after 36 months, as she wanted to initiate estrogen therapy before the end of the study.

FR Study

Nineteen girls with a CA of 11 years and a bone age (BA) (RUS [radius-ulna-short bones] score) of 13.5 years or less participated in a 2-year study on the effect of two administration frequency regimens (Fig 1B). Immediately prior to this study, all girls followed a 10-week crossover design as described previously.[30] In brief, after baseline measurements, ethinyl estradiol substitution (0.05 μg/kg/d) was started and continued throughout the study. After 4 weeks of ethinyl estradiol, GH was started for 2 weeks with either a once-daily (OD) or twice-daily (BID) frequency scheme (shown below). After a 2-week GH washout period, GH was resumed for 2 weeks but in the alternate administration frequency. Then, after a separate randomization procedure stratifying

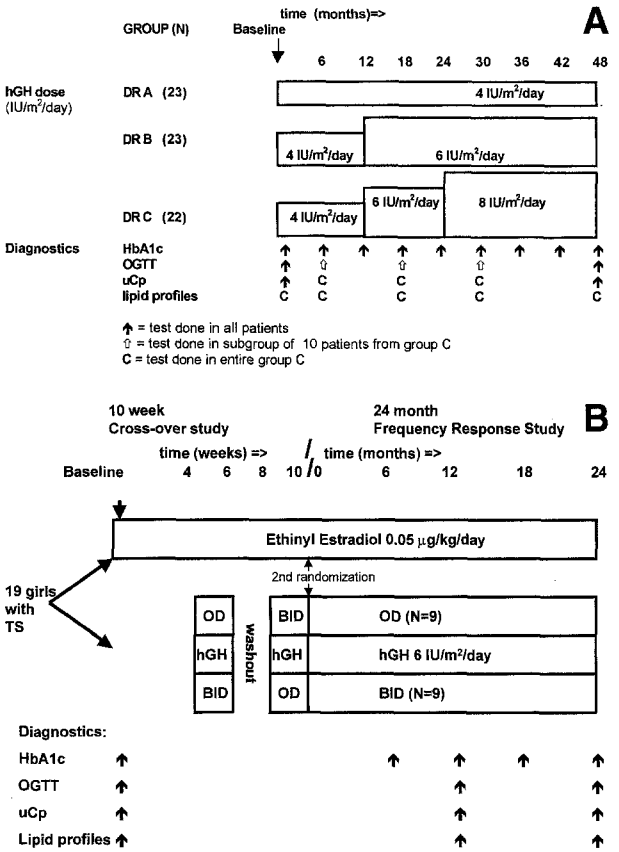

Fig 1. Schematic of the DR study (A) and FR study (B).

for BA (RUS score) and $HSDS_{CA}$, the girls were randomly divided into two GH injection frequency groups for 2 years: OD group (n = 9), 6 IU GH/m^2 body surface once daily; and BID group (n = 10), the same total GH dose divided into one third in the morning and two thirds at bedtime. In both FR study designs, identical ethinyl estradiol (0.05 μg/kg/d) and GH dosages were used.

Study Protocol

Before any treatment (baseline) and every 3 months after the start of continuous GH therapy, all girls were evaluated at their local hospital for a physical examination including measurement of standing height and weight. Hemoglobin A_{1c} (HbA_{1c}) was determined every 6 months. At baseline and at the end of study, urinary C-peptide (uCp) was determined in all girls during three 24-hour periods (day and night portions collected separately). The mean values of these three 24-hour collections were calculated. In addition, all girls from group C in the DR study collected urine 2 days before and during a 24-hour GH profile test

Table 1. Baseline Clinical Data (mean ± SD) for the GH Treatment Groups in Both Studies

Parameter	DR Study			FR Study	
	Group A	Group B	Group C	OD Group	BID Group
No. of girls	23	23	22	9	10
CA (yr)	6.3 ± 2.2	6.8 ± 2.4	6.5 ± 2.3	13.1 ± 1.7	13.6 ± 1.8
$HSDS_{CA}$ (DSD)	0.1 ± 1.0	0.3 ± 1.1	0.1 ± 1.1	0.2 ± 1.1	1.1 ± 1.3
BMI-SDS	0.2 ± 1.0	0.3 ± 1.3	0.2 ± 1.3	1.3 ± 1.2	1.2 ± 1.5
Karyotype (n)					
45,X	19	21	17	6	8
Other	4	2	5	3	2

Abbreviation: DSD, According to the Dutch-Swedish-Danish TS reference population.

at baseline and 6 months after initiation of GH, plus 6 months after each GH dosage increase. The urine was frozen during collections. All blood samples were stored on ice for no more than 3 hours. After centrifugation, the samples were frozen (−20°C) until assayed 1 to 3 weeks later. The urine samples were kept frozen at −20°C until assayed.

Oral Glucose Tolerance Test

All girls in the DR study underwent an oral glucose tolerance test (OGTT) at baseline and after 48 months. In addition, OGTTs were performed in a subgroup of 10 girls from group C every half-year after initiation and each GH dosage increment, ie, at 6, 18, and 30 months (Fig 1A). In all girls in the FR study (Fig 1B), an OGTT was performed pretreatment and after the first and second year of GH therapy (0, 12, and 24 months). A single team performed all OGTTs under standard conditions (3 days of unrestricted diet supplemented with 100 g carbohydrate [Fantomalt; Mutricia, Zoeterman, The Netherlands] and after overnight fasting, 1.75 g glucose/kg body weight [maximum, 50 g] within 5 minutes). The interval between the last GH injection and the OGTT was 11 to 13 hours. For girls from the BID group of the FR study, the interval was at least 2 hours. Blood samples were collected at 0, 15, 30, 60, 90, 120, 150, and 180 minutes and plasma glucose and insulin levels were measured.

To evaluate the overall response to the oral glucose load apart from the plasma levels at the various time points, the following variables were used. IGT for children was defined according to the criteria of the National Diabetes Data Group[31]: fasting venous plasma glucose less than 7.8 mmol/L and the 2-hour level greater than 7.8 mmol/L (140 mg/dL) and less than 11.1 (200 mg/dL). The 3-hour area under the curve for time-concentration corrected for baseline values (AUC_{ab}) during the OGTT was determined using the trapezoidal rule. The insulinogenic index, an indicator of insulin reserve capacity for the increase in blood glucose, was calculated as the ratio of the integrated increase of insulin over that of glucose.[32]

Lipid Fractions

Fasting (overnight) blood was collected from all girls from group C of the DR study at baseline and 6, 18, 30, and 48 months, and from all girls in the FR study at baseline and 12 and 24 months. The following parameters were tested: TC, LDL cholesterol, HDL cholesterol, and TG. The atherogenic index was calculated as the ratio of TC to HDL cholesterol.

Assays

The plasma glucose level was measured at the local hospital laboratories with automatic analyzers using a hexokinase-catalyzed glucose oxidase method. All other measurements were performed in one laboratory after storage at −20°C. Plasma insulin was determined by radioimmunoassay (RIA) (Medgenix, Fleurus, Belgium). The intraassay coefficient of variation (CV) was 6% to 10% and the interassay CV 6% to 11% (fasting normal range, <20 mU/L). uCp was measured with a RIA (Novo-Nordisk). Samples were diluted 20 times with phosphate-albumin buffer and preincubated with antiserum for 3 days at 2°C. Incubation with ^{125}I-C-peptide for 2 days was followed by a bound-free separation with ethanol. The intraassay CV was 5% to 6% and the interassay CV was 6% to 8% (normal range, 0.7 to 87 nmol/24 h). HbA_{1c} levels were measured using an automatic HPLC analyzer (DIAMAT, BioRad, Edgemont, CA). The upper-normal assay limit is less than 6.6%. Determination of the fasting serum level of the various lipid fractions was performed using the Kone Specific Analyzer (Kone Instruments, Espoo, Finland). Lipid analysis was subject to the quality-assessment program of the World Health Organization Regional Lipid Reference Center (Prague, Czech Republic). The TC level was measured using an automated enzymatic method[33] with the CHOD-PAP High Performance reagent kit (Boehringer, Mannheim, Germany). HDL and LDL cholesterol levels were measured by the same method after precipitation. For HDL cholesterol, the phosphotungstate method of Burstein was modified.[34] LDL cholesterol precipitation was performed with polyvinylsulfate (Boehringer). The overall CV for TC, HDL cholesterol, and LDL cholesterol was 2.9%, 3.7%, and 5.8%, respectively. TGs were determined after enzymatic hydrolysis with subsequent determination of liberated glycerol by calorimetry (Boehringer). No correction was made for serum free glycerol. The overall CV of this method did not exceed 3.2%.

Statistical Analysis

Results are expressed as the median (range) unless indicated otherwise. In both the DR and FR study, the nonparametric, two-sample Kruskal-Wallis or Wilcoxon test was applied to test differences between groups. Differences between points in time were tested by Wilcoxon's signed-rank test. The degree of obesity was expressed as the body mass index standard deviation score (BMI-SDS). Correlations were tested with the nonparametric Spearman's rank test. A p value less than .05 was considered significant.

RESULTS

CH Metabolism

OGTTs. When we compared the data at baseline and after 4 years of treatment from all participants in the DR study (Table 2), we found four girls (6%) with IGT at baseline. In two girls, 2-hour glucose data were lacking. After 4 years of GH therapy, only two of 63 girls, different from the four previously mentioned (3%; three girls withdrawn), showed IGT and both were in group B.

Table 2. Median (range) Value for CH Variables in Each Group Before and After 4 Years of GH Treatment in the DR Study

	Baseline			After 4 Years		
Variable	Group A	Group B	Group C	Group A	Group B	Group C
IGT (n)	2	1	1	0	2	0
AUC_{ab} glucose (min · mmol/L)	245 (−174-546)	213 (12-662)	272 (−227-584)	209 (29-426)	252 (−9-660)	224 (−159-474)
AUC_{ab} insulin (min · mU/L)	2,535 (−75-9,030)	2,805 (705-11,130)	2,580 (−75-5,700)	4,515* (2,145-13,575)	6,832* (1,770-48,000)	6,270* (2,715-12,165)
Fasting glucose (mmol/L)	4.6 (3.1-6.0)	4.6 (3.6-5.5)	4.4 (3.4-7.0)	4.8* (3.6-6.1)	4.7* (3.7-6.7)	5.0* (3.1-8.2)
Insulinogenic index	11.2 (0.4-20.3)	13.1 (3.6-928)	9.8 (0.3-24.2)	25.0* (12.5-77.2)	31.2* (−197-500)	24.5* (−201-220)
HbA_{1C} (%)	4.8 (3.6-5.5)	4.9 (4.0-6.1)	4.7 (4.0-6.2)	5.2* (3.1-6.0)	5.4* (3.7-6.2)	5.1* (4.3-6.1)
uCp (nmol/24 h)	6.4 (1.3-17.6)	9.0 (2.6-17.5)	8.2 (3.1-22.6)	16.5* (7.5-65.9)	25.9* (9.3-43.8)	21.1* (13.2-45.5)

*$P < .05$ v baseline.

Significant differences between groups were not found for any of the OGTT variables tested. This was true at baseline and after 4 years of GH treatment. We found higher but nonsignificant glucose values in group B patients after 4 years of GH at 120 and 150 minutes of the OGTT compared with group A ($P < .02$). In addition, 150-minute insulin levels tended to be higher in groups B and C compared with group A ($P < .02$). The insulin values in group B after 4 years of GH treatment were affected by the values for one girl with extremely high insulin levels but a normal OGTT.

Compared with baseline levels, all groups had a significantly higher median fasting glucose, AUC_{ab} for glucose and insulin, and insulinogenic index after 4 years of therapy. The baseline insulinogenic index and BMI-SDS were positively correlated ($r = .54$, $P = .01$).

The effects of GH dose increments in 10 girls of group C in the DR study are shown in Table 3 and Fig 2A and B.

IGT was present in one girl (4 and 6 IU GH/m^2/d). However, at baseline and during 8 IU GH/m^2/d, she had a normal OGTT. The median fasting glucose level and concomitant AUC_{ab} were similar at baseline and during the various GH dosages used. The dose-related increase of the median AUC_{ab} for insulin (significant from 18 months onward) and the insulinogenic index (significant from 6 months onward) cannot be solely explained by the effect of an age increase and may be GH-dependent. However, the variation observed was considerable.

The comparison between FR groups at baseline and after 2 years of GH therapy is shown in Table 4. When examining the three different time points (baseline, 1 year, and 2 years), IGT was found in one of 19 girls (5%). At baseline, this was a girl in the OD group, at 1 year of GH, another girl in the OD group, and at 2 years, a girl in the BID group. There were no significant differences between groups for any of the OGTT variables (Table 3). Within-group comparisons showed no significantly different AUC_{ab} values for glucose and insulin. The insulinogenic index was similar before and after GH treatment. At baseline, the insulinogenic index was correlated with BMI-SDS ($r = .61$, $P = .006$).

HbA_{1c}. In both studies, HbA_{1c} plasma levels never showed an abnormal value. All groups showed an increase of the mean HbA_{1c} level over the years of the study. In comparing baseline values with those at the end of the study in the OD group of the FR study, we found the differences to be statistically significant. This was not true for the BID group. In the DR study, there were no differences in HbA_{1c} between the different groups.

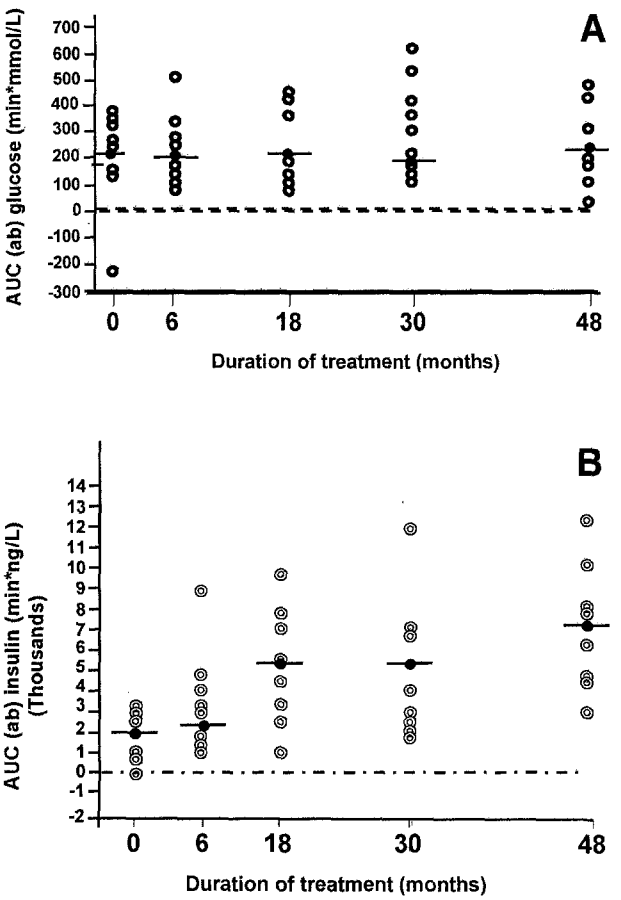

Fig 2. Integrated AUC for (○) glucose (A) and (◎) insulin (B). (—●—) Median. Values are from an OGTT in 10 girls of group C. OGTTs were performed at baseline and after 6, 18, 30, and 48 months of GH treatment at 4, 6, 8, and 8 IU/m^2/d, respectively.

uCp. At baseline, no significant differences in uCp were observed. Group C from the DR study showed higher uCp levels during higher GH dosages. This was found for daytime and nighttime collections (data not shown). At the end of both

Table 3. Median (range) Value for OGTT (n = 10), HbA_{1c}, uCp, and Lipid (n = 23) Variables in Group C at Baseline and During GH Treatment (6, 18, 30, and 48 months) in the DR Study

Variable	Baseline	6 mo	18 mo	30 mo	48 mo
IGT (n)	0	1	1	0	0
AUC_{ab} glucose (min · mmol/L)	215 (−227-383)	209 (92-527)	213 (86-440)	187 (86-615)	252 (33-474)
AUC_{ab} insulin (min · mU/L)	1,628 (−75-3,330)	2,363 (1,020-8,985)	3,855* (1,065-9,480)	5,168* (1,605-11,865)	7,245* (3,045-12,165)
Fasting glucose (mmol/L)	4.2 (3.4-7.0)	4.7 (4.3-4.9)	4.5 (3.4-7.0)	4.7 (2.2-5.2)	4.6 (4.3-5.1)
Insulinogenic index	6.9 (0.3-19.3)	15.5* (5.5-20.4)	18.5* (8.5-35.3)	22.8* (5.6-60.1)	31.9* (15.0-219.5)
HbA_{1c} (%)	4.6 (4.3-5.5)	5.0 (4.1-5.5)	4.8 (4.2-5.6)	4.9 (4.0-5.3)	5.1* (4.3-6.1)
uCp (nmol/24 h)	6.3 (3.9-20.9)	14.1* (8.2-37.9)	21.2* (7.5-51.4)	26.7* (12.0-52.7)	21.1* (13.2-45.5)
TC (mmol/L)	4.7 (3.0-6.4)	4.7 (3.5-5.8)	4.2* (3.0-4.9)	4.1* (2.9-5.0)	3.8* (3.2-6.6)
HDL cholesterol (mmol/L)	0.9 (0.6-1.4)	1.2* (0.7-2.1)	1.2* (0.8-1.8)	1.2* (0.7-1.9)	1.3* (0.9-1.9)
LDL cholesterol (mmol/L)	3.0 (1.4-4.7)	2.6 (1.4-4.0)	2.3* (0.7-3.4)	2.2* (1.7-3.3)	2.0* (1.4-4.8)
TG (mmol/L)	0.8 (0.3-2.2)	1.0 (0.5-1.7)	0.8 (0.6-2.4)	1.0 (0.5-2.0)	1.1* (0.6-2.5)

*$P < .05$ *v* baseline.

Table 4. Median (range) Value for CH and Lipid Variables in Each Group Pretreatment and After 2 Years of GH Treatment in the FR Study

Variable	Baseline		After 2 Years	
	OD Group	BID Group	OD Group	BID Group
IGT (n)	1	0	0	1
AUC_{ab} glucose (min · mmol/L)	315 (114-395)	283 (156-569)	203 (129-300)	264 (161-810)
AUC_{ab} insulin (min · mU/L)	4,395 (810-8,145)	5,835 (−960-8,445)	4,395 (1,035-4,815)	4,418 (1,680-18,390)
Fasting glucose (mmol/L)	4.8 (4.4-5.2)	4.8 (3.1-5.2)	5.0 (4.3-5.9)	4.1 (3.3-5.7)
Insulinogenic index	14.0 (7.1-30.2)	20.9 (−2.8-41.5)	18.4 (5.4-34.1)	15.8 (7.2-53.3)
HbA_{1C} (%)	4.9 (4.3-5.5)	4.9 (4.4-5.3)	5.1* (4.4-5.6)	5.0 (4.2-5.7)
uCp (nmol/24 h)	18.0 (12.5-67.2)	29.3 (7.6-63.5)	42.2* (17.4-89.2)	39.4* (13.5-86.0)
TC (mmol/L)	5.4 (3.4-6.4)	5.2 (3.8-6.5)	5.0 (3.4-6.0)	5.3† (4.0-7.0)
HDL cholesterol (mmol/L)	1.5 (1.2-1.7)	1.4 (1.0-1.7)	1.9* (1.2-2.3)	1.4† (0.9-1.7)
LDL cholesterol (mmol/L)	3.2 (1.1-4.6)	3.2 (2.5-4.6)	2.6 (1.5-3.5)	3.4† (2.1-4.9)
TG (mmol/L)	1.2 (0.6-1.8)	1.1 (0.6-2.5)	0.8 (0.5-1.7)	1.5 (0.5-2.8)

*$P < .05$ *v* baseline.

†$P < .05$, change from baseline between groups.

the DR and FR studies, uCp values were significantly increased compared with baseline levels. The change from baseline to the end of the study was not significantly different between groups.

Lipid Fractions

Group C of the DR study showed a decrease for the median serum levels of TC and LDL over time, whereas HDL and TG increased over time. The main effects can be observed after initiation of GH therapy, except for TG. After 4 years of GH treatment in the DR study, all lipid levels were significantly different from baseline. The atherogenic index (Fig 3) decreased significantly ($P = .0005$) from 4.8 (range, 3.6 to 7.2) at baseline to 3.2 (range, 2.0 to 6.0) after 4 years of GH treatment. Clinically relevant cutoff values for the normal levels of TC (≤5.0 mmol/L) and HDL (>0.9 mmol/L) were exceeded at baseline in six of 21 (28%) and 10 of 21 (48%) girls, respectively. The response to GH treatment as a percentage of baseline values was significantly higher in the low-HDL group compared with the normal-HDL group ($P = .01$).

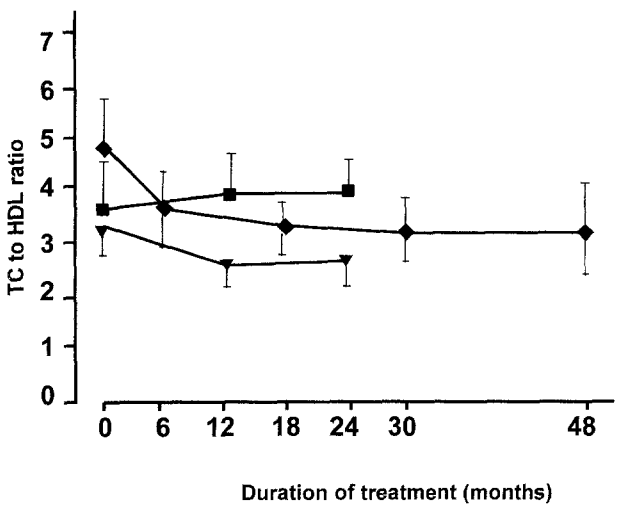

Fig 3. Ratio of TC to HDL cholesterol (mean ± SD) at baseline and after 6, 18, 30, and 48 months of GH treatment at 4, 6, 8, and 8 IU/m²/d for group C (◆, n = 22; DR study; duration, 4 years) and after 12 and 24 months of GH treatment at 6 IU/m²/d for the OD (▼; n = 9) BID groups (■; n = 10; FR study; duration, 2 years).

The median lipid levels in the OD group of the FR study were comparable to levels in the DR study for TC, HDL, and LDL. In contrast, median TG levels decreased marginally. After 2 years of GH in the FR study, only HDL levels in the OD group were significantly different from baseline. In the FR study, the change from baseline after 2 years of GH treatment was significantly different between the groups for TC, HDL, and LDL. The atherogenic index decreased significantly ($P = .008$) only in the OD group from 3.4 (range, 2.8 to 4.3) to 2.7 (range, 2.2 to 3.1). Levels in the BID group were stable (Fig 2). Clinically relevant cutoff values for normal levels of TC (<5.0 mmol/L) and HDL (>1.2 mmol/L) were exceeded at baseline by 12 of 19 (63%) and six of 19 (32%) girls, respectively. For both lipids, abnormal values were predominantly present in the BID group, seven of 12 and five of six girls, respectively. The response to GH treatment as a percentage of baseline values was not significantly different between the groups with normal/abnormal baseline levels.

DISCUSSION

CH Metabolism

The prevalence of IGT was low at baseline and during both the DR and FR studies. At the end of the 4-year DR study, even fewer patients had IGT. None of the girls developed diabetes, but one girl developed IGT from normal baseline values. The AUC_{ab} for glucose (Fig 2A) and its peak value showed no change over time and were never significantly different between any of the study groups, not even the study group treated at a maximal dose of 8 IU GH/m²/d. However, in all groups, the AUC_{ab} for insulin (Fig 2B), its peak value, fasting glucose, insulinogenic index, HbA_{1c}, and uCp were all significantly higher after 4 years compared with pretreatment.

After 4 years, the 120- and 150-minute median glucose levels from group B were significantly higher than in group A. Longitudinal follow-up study in 10 girls of group C showed that the increase of insulin expressed as the absolute level, AUC, or insulinogenic index was dose-related, albeit with large interindividual variation. Whereas the insulinogenic index was different from baseline after 6 months of GH treatment, the absolute insulin level and AUC were significantly different from baseline after 18 months of GH treatment, ie, after the second GH dose increment. Although higher insulin levels and insulin resistance

were observed, we found no induction of IGT or diabetes. Since this study lacks a control group of either normal or TS girls without GH treatment, we cannot prove that this is due to GH treatment. In untreated TS, hyperinsulinism and insulin resistance is a frequent finding.

The IGT prevalence at baseline was definitely lower in the present study versus other TS studies.[8,10,13,36,37] The disappearance of IGT in TS girls after initiation of GH treatment even at high dosages could support this lower prevalence, and indicates that the GH treatment strategy used is not related to severe CH disturbance. However, since the variability of OGTTs is substantial and since two girls developed IGT during our study, we suggest that careful CH evaluation be continued in future studies. Moreover, girls in the DR study represent a relatively young age group. In addition, the procedure used for the OGTT could have an effect, as we calculated the glucose load per kilogram of body weight instead of ideal body weight, and allowed a maximum glucose load of 50 instead of the 75 g advocated by the National Diabetes Data Group.[31]

Several studies[8,26,37-39] reported no significant change in HbA_{1c} levels for up to 3 years of GH treatment, whereas others observed a slight[9] or transient[13] increase. All values remained in the normal range. In the DR study, a small but significant increase in HbA_{1c} was found after 4 years of GH treatment. However, there was no difference between the different dosage groups.

In the FR study, no significant differences existed between the groups for any of the parameters tested. After 2 years of GH treatment, the AUC for glucose and insulin, as well as the insulinogenic index, were not significantly different from baseline, except for the OD group, which had significantly higher HbA_{1c} levels after 2 years of GH treatment. However, it should be emphasized that none of the girls in the present studies exceeded normal values. Higher HbA_{1c} values could well reflect the marginal increase observed at puberty in a normal population, concomitant with insulin resistance during puberty.[40]

Our data collected using relatively high GH dosages are in agreement with other studies, which have shown that after 1 to 4 years of GH treatment (dosages from 2 to 4 IU/m^2/d [5 to 15 mg/wk]) no significant changes are observed in fasting plasma glucose and in OGTTs.[8,9,26,37-39,41]

Some studies using relatively low dosages report no significant change in insulin levels after 1 to 2 years of GH.[8,9] Others found increased (fasting) insulin levels[13,37,38] without the development of glucose intolerance. In our DR study, the two highest GH dosages resulted in an increased AUC for insulin and insulinogenic index compared with the lowest dosage. Moreover, longitudinal follow-up study showed a dose-dependent increase of the insulin response. However, in this study, a positive correlation between the insulinogenic index and BMI-SDS was found at baseline, indicative of the known influence of body weight on the insulin requirement. Also, the number of girls with IGT remained low.

The AUC for insulin and the insulinogenic index in the FR study were higher at baseline than in the DR study. This at least partly reflects the age and weight dependency of insulin. After initiation of estrogen and GH treatment, these variables did not change significantly. This is partly due to the large interindividual variation. The BID group even showed a decrease of these insulin variables, in agreement with a Belgian study in which a 50/50 division of the daily dose was used.[39]

uCp levels reflect endogenous insulin secretion. At baseline and during GH therapy, we found higher values during the day versus the night, without significant differences between the different treatment groups of both studies. Comparison of 24-hour uCp levels with Dutch reference values[42] confirmed age dependency in TS girls before GH treatment ($r = +.35$, $P = .005$). Also, pretreatment values were consistent with reference values within the 95% limits for the proposed age classes, except for the youngest age class, in which three of six girls had higher values. After 4 years of GH treatment, 24-hour uCp values were higher than the 95% limit compared with the reference population in the 6- to 8-year age class (six of 10 girls) and in the age class 10 years or older (seven of 39 girls).

The consequence of chronic hyperinsulinism in otherwise healthy subjects is unknown. Induction of insulin-dependent diabetes may be possible in subjects who are genetically susceptible. However, cardiovascular effects, with an increased risk of hypertension and non–insulin-dependent diabetes,[43] are reversible in acromegalic patients even after long-term GH exposure.[23]

Lipid Fractions

We compared the results for girls treated with GH in this study with age-matched Dutch controls. Samples were measured in the same laboratory. The baseline median HDL level of girls aged 2 to 11 years (DR study) was substantially lower (0.9 *v* 1.5 mmol/L); in girls aged 11 to 17 years (FR study), it was similar (1.4 *v* 1.4 mmol/L).[44] In contrast, the baseline median TC level of girls in the DR study was similar (4.7 *v* 4.8 mmol/L). The FR study group showed significantly higher levels (5.3 *v* 4.7 mmol/L) compared with the control group. Similarly, untreated American girls with TS[45] only during adolescence showed higher TC levels compared with controls matched for age and obesity, although with systematically lower median values. This could be explained by the very low or absent levels of estrogens and consequent absence of a TC and HDL reducing effect[8,46] in girls with TS of that age. The findings may be confounded by the fact that in the FR study most girls were obese. Generally, obesity is associated with a decrease in HDL cholesterol.[47]

With increasing dosages in the DR study and in agreement with some studies in GH-treated GHD children, the atherogenic index decreased[17,18] and the median serum levels of TC[19] and LDL decreased, along with a favorable increase in the median HDL level.[18] The main effects could be observed after initiation of GH therapy, in particular for HDL; for TC and LDL, they were found only after the first dosage increase. In GHD children, even a threefold GH dose increase from 0.3 IU/kg/wk to 0.9 IU/kg/wk (TIW injections),[21] or an equal division of the total GH dose (20 IU/m^2/wk) in a thrice-daily regimen, did not have an effect on TG and TC plasma levels after 6 to 12 months of treatment.

The GH effects over time in TS are interesting. In a Dutch reference group, a gradual increase of TC levels until the age of

10 to 11 years was found, whereas HDL cholesterol, after a temporary decrease at about age 9 years, showed a gradual decrease until the age of 16 to 17 years (to 1.3 mmol/L).[44] Both TC and HDL showed a progressive difference during GH therapy. Remarkably, the response to GH treatment was significantly higher in our group with low HDL baseline values compared with the normal HDL group.

Whereas in two other GH intervention studies in TS, no significant effects on lipids were found,[8,26] in our DR study, all lipid levels were significantly different from baseline after 4 years of GH treatment. The differences with the earlier findings may be due to the duration of GH treatment (4 years in the present study *v* 1 year in the other studies) and the use of incremental steps for the GH dose. Furthermore, using a single laboratory reduced the variability. Still, lipid measurements show large interindividual variation, and at short intervals, also a large biological intraindividual variation.[48] Therefore, the effects of intervention are considered clinically relevant only when the changes are greater than 15%. The effects of long-term GH treatment on the various cholesterol levels could have important beneficial implications for the possible development of cardiovascular disease also in TS.

Although median TG levels in group C of the DR study increased significantly by 48 months of GH therapy, they were still within the normal range. From studies with acromegalic and GHD patients, it was hypothesized that this could be due to reduced lipoprotein lipase and hepatic TG lipase activity and an increased TG production rate.[49]

It seems difficult to interpret the lipid data of the FR study, since the BID group at baseline consisted of more girls with abnormal TC levels. The treatment response, though, for TC and HDL was not different between normal/abnormal girls within each treatment group. Both groups in the FR study received an identical low dosage of ethinyl estradiol with a known hypocholesterolemic effect. After 2 years of GH therapy, the beneficial pattern of change in cholesterol fractions in the OD group of the FR study was similar to that of group C in the DR study. The absence of a decrease in the TC/HDL ratio of the BID group in the FR study (Fig 2) is in accordance with the absence of a decrease of TC in girls with TS when the GH dose was equally divided between two injections.[39]

In summary, the present studies did not show an increase of IGT, but there was an increase of insulin levels and insulin resistance. However, our studies were designed to test the dosing and frequency of GH administration, and for ethical reasons, we could not include a control group. Since an increase in insulin resistance also occurs with increasing age and BMI, a definite conclusion about the safety of GH treatment in terms of CH metabolism cannot be made. However, we can conclude from the absence of clinically significant effects that even at a supraphysiological dose of 8 IU/m^2/d, no measurable side effects occur. Since the consequences of chronic hyperinsulinism in otherwise healthy subjects is unknown, and considering the predisposition toward diabetes mellitus in TS, these data suggest that long-term follow-up evaluation CH metabolism is warranted in GH-treated TS patients. In the DR study, the lipid profile changed in a more cardioprotective direction, with a significant increase of HDL and significant decrease of TC and LDL, and thus a reduction of the TC/HDL cholesterol ratio.

ACKNOWLEDGMENT

The participating members of the Dutch Working Group on Growth Hormone are as follows: T. Vulsma, Amsterdam; G. Hoorweg and H. Delemarre-van de Waal, Amsterdam; H. Reeser, Den Haag; J. Waelkens, Eindhoven; C. Rouwe, Groningen; J.-M. Wit and G. Massa, Leiden; J. Gosen, Leiderdorp; W.-J. Gerver, Maastricht; B. Otten and C. Rongen-Westerlaken, Nijmegen; S. de Muinck Keizer-Schrama and S. Drop, Rotterdam; and M. Jansen, Utrecht, The Netherlands.

The authors are grateful to Anne van de Wiel, research nurse, for assistance in performing the studies and for the guidance of the girls and their parents. For their experienced laboratory assessments, we acknowledge Wil Hackeng (insulin and uCp), Praktijk voor Laboratorium Diagnostiek, Geervliet; Dr Souverijn (HbA_{1C}), Centraal Klinisch Chemisch Laboratorium, University Hospital, Leiden; and Anton de Bruijn (lipid fractions), Klinisch Biochemisch Laboratorium, Department of Epidemiology and Biostatistics, Erasmus University, Rotterdam, The Netherlands.

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