Effect of growth hormone replacement therapy on plasma lecithin:cholesterol acyltransferase and lipid transfer protein activities in growth hormone-deficient adults

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Abstract The effects of growth hormone (GH) replacement on plasma lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and phospholipid transfer protein (PLTP), factors involved in high density lipoprotein (HDL) metabolism, are unknown. We carried out a 6 months study in 24 GH-deficient adults who were randomized to placebo (n = 8), low dose GH (1 U daily, n = 8), and high dose GH (2 U daily, n = 8), followed by a 6 months open extension study with high dose GH (1 drop-out). No significant changes in plasma lipoproteins, LCAT, CETP, and PLTP activities, cholesterol esterification (EST) and cholesteryl ester transfer (CET) were observed after placebo. After 6 months of GH (combined data, n = 24), very low + low density lipoprotein (VLDL + LDL) cholesterol ($P < 0.05$) and apolipoprotein B ($P < 0.05$) decreased, whereas HDL cholesterol and HDL cholesteryl ester increased ($P < 0.05$). Prolonged treatment showed comparable effects. Plasma apolipoprotein A-I and Lp[a] remained unchanged. Plasma LCAT ($P < 0.01$) and CETP activities ($P < 0.01$), as well as EST ($P < 0.01$) and CET decreased ($P < 0.01$) after 12 months of GH (n = 15), but PLTP activity did not significantly change. Changes in EST and CET after 12 months of treatment were independently related to changes in plasma LCAT ($P = 0.001$ and CETP activity ($P = 0.01$). In conclusion, GH replacement therapy improves the lipoprotein profile in GH-deficient adults. Chronic GH replacement lowers plasma LCAT and CETP activities, contributing to a decrease in cholesterol esterification and cholesteryl ester transfer. These effects may have consequences for HDL metabolism and reverse cholesterol transport.—Beentjes, J. A. M., A. van Tol, W. J. Sluiter, and R. P. F. Dullaart. **Effect of growth hormone replacement therapy on plasma lecithin: cholesterol acyltransferase and lipid transfer protein activities in growth hormone-deficient adults. *J. Lipid Res.* 2000.** 41: **925–932.**

Supplementary key words growth hormone deficiency • growth hormone replacement • HDL • LCAT • CETP • PLTP • cholesterol esterification • cholesteryl ester transfer

Epidemiological studies have demonstrated that cardiovascular mortality is increased in adult patients with hypopituitarism (1–3). Indeed, media thickness of the carotid artery intima is increased in hypopituitary patients, representing an early sign of atherosclerosis (4). It has been suggested that this increased cardiovascular risk is, at least in part, attributable to growth hormone (GH) deficiency (1, 5). In adults, GH deficiency is associated with unfavorable abnormalities in plasma lipoproteins, including high plasma total and low density lipoprotein (LDL) cholesterol concentrations, high plasma triglycerides, and low high density lipoprotein (HDL) cholesterol levels (5). Several studies have shown that GH replacement therapy is able to lower plasma total and LDL cholesterol, particularly in patients with adult-onset GH deficiency, without affecting plasma triglycerides (5, 6). In GH-deficient adults, HDL cholesterol increases, remains unchanged, or may even decrease after GH replacement (5–7).

HDL is crucially involved in the process of reverse cholesterol transport, whereby cholesterol is transported from peripheral cells back to the liver, where it is metabolized and excreted in the bile (8, 9). Apart from the action of lipases, intravascular HDL metabolism and remodelling is governed by lecithin:cholesterol acyltransferase (LCAT) and lipid transfer proteins (8, 10–12). Plasma LCAT esterifies free cholesterol to cholesteryl esters in HDL, whereas cholesteryl ester transfer protein (CETP) transfers these cholesteryl esters to very low density lipoproteins (VLDL) and LDL, thereby decreasing the HDL cholesteryl ester content (8–12). Another lipid transfer protein, phospholipid transfer protein (PLTP), transfers phospholipids between lipoproteins and is able to convert HDL into smaller and larger particles (13–15). During this process, small apolipoprotein (apo) A-I-rich, pre-β HDL particles

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; GH, growth hormone; HDL, high density lipoproteins; IGF-I, insulin-like growth factor I; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; Lp[a], lipoprotein [a]; PLTP, phospholipid transfer protein; VLDL, very low density lipoproteins.

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are produced that may function as initial acceptors of free cholesterol from cell surfaces and provide the initial LCAT substrate (15, 16).

Current knowledge about the effect of GH on plasma LCAT and lipid transfer protein regulation is limited. In one study, plasma CETP activity was found to be increased in acromegalic patients (17). In a cross-sectional study including GH-deficient acromegalic and healthy male subjects, in contrast, we recently found a negative relationship between plasma insulin-like growth factor-I (IGF-I) and the plasma activity levels of LCAT, CETP, and PLTP (18). In view of these results it was hypothesized that GH replacement would decrease plasma LCAT and lipid transfer protein activity levels in GH-deficient adults. In 24 GH-deficient adults, we evaluated the effect of GH replacement on plasma LCAT, CETP, and PLTP activity levels, measured with exogenous substrates, in a 6-month placebo-controlled study, followed by a 6-months open extension period. Changes in plasma LCAT and lipid transfer proteins were the primary endpoints of the study. Furthermore, we documented the effect of this treatment on plasma cholesterol esterification and cholesteryl ester transfer between HDL and VLDL + LDL, measured with endogenous plasma substrates.

SUBJECTS AND METHODS

Subjects and study design

The study was approved by the medical ethics committee of the University Hospital Groningen, The Netherlands, and all patients provided written informed consent. Twenty-four adults (minimal age 21 years) with childhood or adult-onset GH deficiency were included. GH deficiency was defined as peak serum GH levels in response to insulin-induced hypoglycemia <5 μg/l (10 mU/l) with a venous blood glucose nadir ≤ 2.2 mmol/l and the presence of hypoglycemic symptoms. Regular insulin was intravenously administered in a dose of 0.15 to 0.20 U/kg body weight (19). The insulin tolerance test was performed within 3 months prior to the study. Causes of GH deficiency were nonfunctioning pituitary macroadenoma (n = 10), craniopharyngioma (n = 4), macroprolactinoma (n = 1), teratoma (n = 1), dysgerminoma (n = 1), and idiopathic (n = 7). Two patients had been previously treated with GH, which was stopped more than 5 years before entry in the present study. The median duration of GH deficiency before entry was 8.5 (range 2–46) years. Twenty-three patients were on stable l-thyroxin replacement therapy (125 to 175 μg daily), 17 received glucocorticoids (cortisone acetate 25 to 37.5 mg daily), and all men and 10 women used sex steroids (Sustanon® 250 mg intramuscular per 3 to 4 weeks or various combinations of estrogens and progestagens). Two postmenopausal women did not receive sex steroids. Six patients used desmopressin. In all participants, serum-free thyroxin and triiodothyronine levels remained within the reference range, and it was not necessary to adjust the l-thyroxin dose during follow-up in any of the patients. Moreover, substitution therapy with glucocorticoids and sex steroids was kept unchanged. Diabetes mellitus (fasting venous blood glucose ≥ 6.1 mmol/l), hypertension (systolic blood pressure >160 mmHg and diastolic blood pressure >95 mmHg), liver function abnormalities, kidney disease (elevated serum creatinine or proteinuria >300 mg/l), clinically manifest cardiovascular disease and intention to become pregnant were exclusion criteria.

The participating men and women were randomized separately to low dose GH replacement (n = 8, 1 U of Genotropin®, Pharmacia & Upjohn Inc., Sweden, subcutaneously per day at bedtime), high dose GH replacement (n = 8, 2 U of GH per day), or placebo (n = 8) during 6 months in a double blind fashion. Thus, there were 4 men and 4 women in each group. Thereafter, GH treatment was continued or started in a 6 months open extension period. GH and corresponding placebo administration was initiated in a dose of 1 U per day to avoid side effects, and increased after 4 weeks to 2 U per day in those patients allocated to the high dose treatment. As a consequence of the double blind study design, GH was also started in a dose of 1 U per day and then increased to 2 U per day in all patients in the extension study. One patient, initially allocated to low dose GH, dropped out in the extension period.

Body mass index (BMI) was calculated as weight divided by height squared. The waist/hip (W/H) ratio was measured as the ratio of the smallest girth between rib cage and iliac crest and the largest girth between waist and thigh (20). Bioelectrical impedance analysis (BIA) was measured with a tetrapolar BIA analyzer (RJL Systems, Detroit, MI). After application of an alternating current of 800 μA at 50 kHz, resistance and reactance were measured (21). Fat free mass was estimated with the manufacturer provided equation. Body fat mass was calculated by subtracting fat free mass from body weight.

The participants were studied at baseline and after 2, 6, 8 and 12 months of follow-up. On each occasion they were studied after an overnight fast, while venous blood was obtained for measurement of lipid parameters and hormone levels and body composition was determined as described above.

Laboratory measurements

Venous blood was collected into ethylenediaminetetraacetic acid-containing tubes (1.5 mg/ml) and was directly placed on ice. Plasma was obtained within 30 min by low speed centrifugation for 15 min at 4°C. Samples were frozen at −70°C until analysis. All laboratory measurements were carried out in coded samples.

Lipids were measured in whole plasma and in the HDL-containing supernatant fraction after precipitation of apolipoprotein (apoB)-containing lipoproteins with polyethylene glycol-6000 (22). VLDL + LDL were calculated as the difference between whole plasma and the supernatant fraction. Total cholesterol was measured by gas chromatography. Free cholesterol was assayed by a modification of this method in which the hydrolysis step was omitted (23). Esterified cholesterol was calculated as the difference between total and free cholesterol. Triglycerides were measured enzymatically. ApoA-I and apoB were assayed by immunoturbidimetry (Boehringer Mannheim, Germany, cat. nos. 726478 and 726494, respectively). Lp[a] was assayed by enzyme-linked immunosorbent assay (Biopool AB, Umea, Sweden, cat. no. 610221).

The plasma LCAT activity level was assayed using excess exogenous substrate containing [^{3}H]cholesterol as described (24). Plasma LCAT activity varies linearly with the amount of plasma in the incubation system. Plasma CETP activity was determined, after removal of VLDL and LDL from each sample, using an isotope assay that detects the transfer of [1-^{14}C]oleate-cholesteryl ester from labeled exogenous LDL to an excess of unlabeled pooled normal HDL (24, 25). In the assay system LCAT is inhibited with dithiobis-2-nitrobenzoic acid. Plasma CETP was calculated as the bi-directional transfer between labeled LDL and HDL. The plasma LCAT and CETP activity levels so measured are strongly correlated with their mass concentration in plasma (26, 27). Plasma PLTP activity was measured in a liposome vesicles–HDL system as described (24, 28). Plasma samples were incubated with [^{3}H]phosphatidylcholine-labeled liposomes and excess pooled normal HDL. After the incubation the liposomes were precipitated with a mixture of NaCl, $MgCl_2$, and heparin in

final concentrations of 230 mmol/l, 92 mmol/l, and 200 U/ml, respectively. The plasma PLTP activity is linearly correlated with the amount of plasma used in the incubation system and is not influenced by the phospholipid transfer promoting properties of CETP (28).

Plasma newly synthesized cholesteryl ester transfer (CET) was assayed by a radioisotope method as previously described (29, 30). In short, [^{3}H]cholesterol complexed to albumin was equilibrated overnight at 4°C with plasma-free cholesterol. Subsequently, the plasma samples were incubated at 37°C for 3 h. VLDL + LDL were then precipitated by addition of phosphotungstate/$MgCl_2$. Lipids were extracted form the precipitate and the cholesteryl esters were separated on silica columns, followed by counting of radioactivity. The assay system is not influenced by cholesterol esterification on LDL (<4% of total) (29). CET is closely correlated with net cholesteryl ester mass transfer from HDL to VLDL and LDL under conditions of LCAT inhibition (31). CET can thus be regarded as an accurate estimate of cholesteryl ester mass transfer in plasma. Plasma cholesterol esterification (EST) was determined by measurement of [^{3}H]cholesterol esterification using the same incubation system as for the CET assay (29, 30). For measurement of EST, plasma was incubated at 37°C for 1 h. The cholesterol esterification rate is linear with time for 5 h, indicating an excess of unesterified cholesterol in the assay system (29). The plasma LCAT, CETP, and PLTP activity assays as well as CET and EST measurements were performed in duplicate using the same batches of substrate. Plasma LCAT, CETP, and PLTP activity levels were related to human pool plasma and are expressed in arbitrary units (AU), corresponding to the percentages of the activities in this pool plasma. Plasma CET and EST were expressed in nmol/ml per h. The within-assay coefficients of variation (CVs) of LCAT, CETP, PLTP, CET, and EST are 4.5%, 2.7%, 3.5%, 7.1%, and 5.4%, respectively.

Blood glucose was analyzed on an APEC glucose analyzer (APEC, Danvers, MA). Plasma IGF-I was measured by radioimmunoassay after acid-ethanol extraction (Nichols Institute of Diagnostics, San Juan Capistrano, CA). The interassay CV of IGF-I is <8.5%. The reference range (−2 SD to +2 SD of logarithmically transformed values) is 16–89 nmol/l, 12–64 nmol/l, and 9–47 nmol/l at age 20, 40, and 60 years, respectively.

Statistical analysis

Parameters are given in medians (interquartile ranges) and changes in parameters are given in medians (95% confidence intervals). Between group differences in (changes in) parameters were evaluated by Kruskal-Wallis analysis of variances. Changes in parameters were evaluated by paired Wilcoxon tests and by Friedmans's two-way analysis of variance. Univariate relationships were evaluated by Spearmans' rank correlation analysis (R_s). Multiple regression analysis was carried out to evaluate the independent relationships between parameters. A two-sided P value <0.05 was considered to be significant.

RESULTS

As shown in **Table 1**, age, as well as BMI, waist/hip ratio, and fat mass at baseline were not significantly different in the three groups. BMI and waist/hip ratio did not change during active GH treatment (data not shown). In the low dose GH group, fat mass decreased after 6 and 12 months. The decrease in fat mass with high dose GH did not reach significance ($P = 0.12$ after 12 months), as a consequence of improved appetite and modest weight gain in two patients. Baseline systolic arterial pressure was slightly higher in the placebo group than in the high dose GH group. Fasting blood glucose was similar in the groups at baseline and did not change during GH treatment (data not shown). Baseline plasma IGF-I was not significantly different between the groups. As expected, plasma IGF-I remained unaltered after placebo and rose during GH treatment.

Baseline plasma (apo) lipoprotein parameters, LCAT, and lipid transfer protein activity levels, as well as plasma EST and CET are shown in **Table 2**. Baseline HDL cholesterol, the HDL/VLDL + LDL cholesterol ratio, and plasma triglycerides were lowest in the low dose GH group. As shown in **Table 3**, no significant changes in (apo)lipoprotein parameters were found after 6 months of placebo ad-

TABLE 1. Clinical characteristics, fasting blood glucose, and insulin-like growth factor-I (IGF-I) in growth hormone (GH)-deficient patients allocated to placebo, low dose GH (GH low) and high dose GH (GH high) replacement therapy at baseline and after 6 months of followup, followed by 6 months high dose GH replacement in all patients

Variable	Placebo (n = 8)	GH Low (n = 8)	GH High (n = 8)
Age (years)	52 (34–58)	37 (31–43)	42 (24–51)
Blood pressure (mmHg)			
Systolic	134 (128–147)[a]	127 (123–136)	125 (121–129)
Diastolic	82 (80–88)	78 (73–83)	80 (78–84)
Body mass index (kg/m^2)	27.1 (26.2–31.8)	25.6 (24.4–28.3)	27.1 (25.5–29.5)
Waist/hip ratio	0.95 (0.91–0.96)	0.90 (0.83–0.95)	0.93 (0.92–0.96)
Fat mass (kg)			
Baseline	25.8 (20.7–32.9)	22.1 (20.4–23.4)	29.1 (19.1–32.8)
6 months	25.0 (21.2–34.6)	19.4 (18.5–22.1)[c]	24.6 (16.1–32.2)
12 months	23.7 (16.5–30.1)[c]	18.9 (17.3–22.5)[c]	25.8 (14.7–29.6)
Blood glucose (mmol/l)	4.0 (3.5–4.5)	4.4 (3.6–4.6)	4.1 (3.8–4.4)
Plasma IGF-I (nmol/l)			
Baseline	11.5 (9.5–15.8)	5.8 (1.3–17.0)	14.8 (8.1–17.1)
6 months	11.4 (10.1–14.8)	24.0 (12.5–55.9)[c,d]	39.6 (37.1–45.6)[c,d]
12 months	35.3 (19.9–46.7)[b]	27.9 (12.5–56.9)[c]	43.2 (36.2–51.9)[c]

Data expressed as medians (interquartile ranges).
[a] $P < 0.05$ from high dose GH group; [b] $P < 0.05$, [c] $P < 0.01$ from baseline; [d] $P < 0.05$ from change with placebo.

TABLE 2. Baseline plasma (apo)lipoproteins, lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP) activity levels, as well as plasma cholesterol esterification (EST) and cholesteryl ester transfer (CET) in growth hormone (GH)-deficient patients allocated to placebo, low dose GH (GH low), and high dose GH (GH high) replacement therapy

	Placebo (n = 8)	GH Low (n = 8)	GH High (n = 8)
Total cholesterol (mmol/l)	5.54 (4.85–7.05)	5.93 (5.56–7.23)	7.08 (5.85–7.51)
VLDL + LDL cholesterol (mmol/l)	4.28 (3.86–6.19)	5.41 (4.66–6.32)	5.77 (4.78–6.33)
HDL cholesterol (mmol/l)	1.02 (0.79–1.26)	0.80 (0.69–0.97)	0.98 (0.89–1.44)[a]
HDL/VLDL + LDL cholesterol	0.24 (0.17–0.27)[a]	0.14 (0.12–0.18)	0.20 (0.14–0.29)[a]
Triglycerides (mmol/l)	1.17 (1.02–1.69)	1.00 (0.93–1.14)	1.67 (1.51–1.79)[a]
ApoA-I (g/l)	0.93 (0.87–1.04)	0.90 (0.80–1.02)	1.08 (0.92–1.14)
ApoB (g/l)	0.97 (0.82–1.13)	1.05 (0.91–1.18)	1.20 (1.10–1.27)
ApoA-I/apoB	0.87 (0.76–1.29)	0.84 (0.77–1.03)	0.89 (0.76–0.96)
Lp[a] (mg/l)	72 (23–304)	45 (21–152)	121 (76–218)
LCAT activity (AU)	66 (46–94)	89 (53–99)	96 (41–106)
CETP activity (AU)	42 (25–76)	70 (50–79)	57 (35–66)
PLTP activity (AU)	70 (47–93)	86 (64–92)	62 (50–101)
EST (nmol/ml/h)	15 (6–79)	35 (22–84)	71 (20–108)
CET (nmol/ml/h)	9 (4–30)	13 (9–24)	23 (12–42)

Data in medians (interquartile ranges).
[a] $P < 0.05$ from low dose GH group.

ministration. The effects of 6 months GH replacement therapy on lipoprotein parameters are analyzed as the changes after 6 months of active treatment in the combined patients, representing the changes after 6 months of GH treatment in the low and high dose GH group and after 6 months active treatment in the extension period in the placebo group (n = 24, Table 3). The changes in (apo)lipoproteins after high dose GH never exceeded those after low dose GH. Table 3 also shows the changes in lipoprotein parameters after 12 months of active treatment compared to baseline in the combined low and high dose GH groups (n = 15). There were no differences in the changes in lipoprotein parameters after 12 months GH treatment compared to baseline between the patients initially assigned to low dose (n = 8) and high dose GH treatment (n = 7). Plasma total cholesterol did not significantly change after 6 months and decreased after 12 months of GH treatment. VLDL + LDL cholesterol and apoB levels decreased after 6 months as well as after 12 months of GH replacement, whereas HDL cholesterol tended to increase ($P < 0.05$ after 6 months of GH and $P = 0.10$ after 12 months). Plasma apoA-I remained unaltered. As a result, the HDL/VLDL + LDL cholesterol ratio and the plasma apoA-I/B ratio increased after 6 and 12 months of GH therapy. The increase in HDL cholesterol after 6 months of active GH treatment was due to a rise in the HDL cholesteryl ester (0.10, 95% confidence intervals 0.03 to 0.20 mmol/l, $P < 0.02$), whereas HDL free cholesterol did not significantly change (data not shown). The changes in HDL cholesteryl ester and free cholesterol after 12 months were not significant (data not shown). No effects of GH replacement on plasma triglycerides and on plasma Lp[a] were observed.

TABLE 3. Changes in plasma (apo)lipoproteins, lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP) activity levels, as well as plasma cholesterol esterification (EST) and cholesteryl ester transfer (CET) after 6 months placebo administration, after 6 months active GH treatment, and after 12 months active GH treatment

	Changes in Parameters		
	After 6 Months Placebo (n = 8)	After 6 Months GH (n = 24)	After 12 Months GH (n = 15)
Total cholesterol (mmol/l)	−0.06 (−0.53 to 0.32)	−0.39 (−0.81 to 0.09)	−0.21 (−1.05 to −0.03)[b]
VLDL + LDL cholesterol (mmol/l)	+0.04 (−0.43 to 0.36)	−0.46 (−0.91 to −0.05)[a]	−0.49 (−1.12 to −0.09)[a]
HDL cholesterol (mmol/l)	−0.08 (−0.13 to 0.01)	+0.12 (0.01 to 0.22)[a]	+0.09 (−0.11 to 0.23)
HDL/VLDL + LDL cholesterol	−0.01 (−0.04 to 0.02)	+0.05 (0.01 to 0.08)[a]	+0.04 (0.01 to 0.10)[a]
Triglycerides (mmol/l)	+0.04 (−0.48 to 0.79)	+0.13 (−0.05 to 0.31)	+0.02 (−0.10 to 0.21)
ApoA-I (g/l)	+0.04 (−0.06 to 0.24)	+0.05 (−0.03 to 0.14)	+0.02 (−0.03 to 0.07)
ApoB (g/l)	+0.05 (−0.08 to 0.20)	−0.12 (−0.22 to −0.01)[a]	−0.12 (−0.29 to −0.01)[b]
ApoA-I/apoB	−0.02 (−0.12 to 0.05)	+0.13 (0.07 to 0.22)[c]	+0.16 (0.02 to 0.32)[c]
Lp[a] (mg/l)	+40 (−7 to 111)	+21 (−9 to 39)	+16.5 (−14.0 to 69.2)
LCAT activity (AU)	+0.6 (−30.3 to 15.6)	−15.9 (−34.6 to 7.4)	−33.7 (−53.7 to −13.4)[c]
CETP activity (AU)	−1.7 (−20.5 to 9.5)	−11.9 (−23.7 to 2.5)	−22.7 (−37.0 to −7.5)[c]
PLTP activity (AU)	−4.0 (−16.1 to 5.1)	+3.3 (−10.4 to 10.1)	−11.6 (−28.8 to 1.7)
PLTP/CETP activity	+0.02 (−0.14 to 0.24)	+0.33 (−0.03 to 0.65)	+0.49 (0.10 to 0.84)[a]
EST (nmol/ml/h)	+1.1 (−38.6 to 7.3)	−13.9 (−41.9 to 1.9)	−31.8 (−61.4 to −10.7)[c]
CET (nmol/ml/h)	+0.0 (−12.9 to 2.8)	−2.8 (−12.0 to 3.4)	−12.0 (−18.7 to −3.8)[c]

Data in medians (95% confidence interval).
[a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$ from before GH.

Baseline plasma LCAT, lipid transfer protein activity levels, EST, and CET were not significantly different among the three groups at baseline (Table 2). Changes in these parameters with placebo and during active GH replacement therapy were analyzed as described above for (apo) lipoprotein parameters. No significant changes in these parameters were found after placebo. As shown in Table 3, the changes in plasma LCAT ($P = 0.18$), CETP ($P = 0.09$), EST ($P = 0.10$), and CET ($P = 0.24$) did not reach significance after 6 months GH replacement (n = 24), whereas plasma PLTP activity remained unchanged ($P = 0.72$). After 12 months of GH treatment, plasma LCAT and CETP activity levels as well as plasma EST and CET decreased. Plasma PLTP activity did not significantly change ($P = 0.07$). As a consequence, the ratio of plasma PLTP/CETP activity increased after 12 months. The observed effects of GH treatment on (apo)lipoproteins, plasma LCAT, and lipid transfer protein activity levels as well as on plasma EST and CET were essentially similar when the averaged data at 2 and 6 months and at 8 and 12 months were used in the analyses (data not shown).

Univariate regression analysis showed no relationships between the individual changes in serum free thyroxin and free triiodothyronine levels and the changes in plasma LCAT ($R_s = 0.25$, $P = 0.35$ and $R_s = -0.12$, $P = 0.65$, respectively, n = 15) and CETP activity levels ($R_s = 0.17$, $P = 0.52$ and $R_s = 0.19$, $P = 0.48$, respectively, n = 15) after 12 months of active GH treatment. Multiple regression analysis with the data obtained after 12 months of active GH treatment showed that individual changes in plasma EST were related to changes in plasma LCAT activity ($r = 0.75$, $P = 0.001$, n = 24) without significant contributions of changes in VLDL + LDL cholesterol ($P = 0.59$) and plasma triglycerides ($P = 0.79$). Changes in plasma CET were related to changes in plasma CETP activity ($r = 0.63$, $P = 0.01$, n = 24) again without significant contributions of changes in VLDL + LDL cholesterol ($P = 0.64$) and plasma triglycerides ($P = 0.41$).

DISCUSSION

In adult GH deficiency, several but not all trials have demonstrated a lowering in plasma total and LDL cholesterol as well as in plasma apoB in response to GH replacement therapy (6, 32–37), an effect which may persist for up to 10 years of treatment (38). These previous studies used a GH replacement dose of about 2.5 to 5 U/day. In the present study, GH doses of 1 and 2 U/day were given, resulting in median plasma IGF-I levels in the lower and higher physiological range, respectively (39). A decrease in VLDL + LDL cholesterol and apoB was observed after 6 and 12 months of GH replacement, whereas no such changes were found after 6 months of placebo administration. Moreover, HDL cholesterol increased after 6 months of treatment and tended to remain higher thereafter, resulting in an increase in the HDL/VLDL + LDL cholesterol ratio. The plasma apoA-I/B ratio also rose with GH replacement. These (apo)lipoprotein changes were not larger with high dose than with low dose GH, raising the possibility that a GH dose of 1 U/day could be sufficient to lower plasma apoB-containing lipoproteins. As expected (32–37, 40–43), no changes in triglycerides were found. Plasma Lp[a] did not significantly change after 6 and 12 months of GH in this study. For unknown reasons, plasma Lp[a] has been variably reported to remain unchanged or to increase in response to GH treatment (36, 40–42, 44). Taken together, the present observations confirm and extend the general view that GH substitution ameliorates the lipoprotein abnormalities associated with adult GH deficiency, thereby possibly lowering cardiovascular risk in this patient category.

A novel finding of the present study is that the plasma activity levels of LCAT and CETP, as a measure of their mass in plasma, decreased after 12 months of GH replacement. Although a potential drawback of our study is that the changes were only significant in the extension period compared to baseline, these observations are in accord with our hypothesis that GH, either directly or through an effect on IGF-I, has the ability to lower the plasma levels of these factors. No significant change in plasma PLTP activity was demonstrated. Consequently, the PLTP/CETP activity ratio rose after 12 months of GH replacement. In our observational study among GH-deficient, acromegalic, and healthy subjects, the negative correlation between plasma IGF-I with lipid transfer protein activity levels was stronger for CETP than for PLTP (18).

The mechanisms responsible for the effects of chronic GH replacement on plasma LCAT and CETP activity levels are unknown. LCAT is synthesized by hepatic tissue (45), whereas adipose tissue and liver are sources of CETP in human plasma (12, 46). GH administration stimulates LDL receptor expression (47) and increases VLDL apoB removal (48), probably contributing to its VLDL + LDL cholesterol-lowering effect. Plasma CETP levels are positively correlated with LDL cholesterol in normo- and hypercholesterolemic subjects (11, 49), and decrease in conjunction with LDL lowering in response to treatment with the HMG-CoA reductase inhibitor, simvastatin (50). Thus, the liver could represent a site responsible for CETP regulation by GH. Among other tissues, adipose tissue may also be involved in plasma CETP lowering, in view of the decrease in fat mass after GH replacement. As yet it is unknown whether GH administration could affect plasma LCAT and CETP indirectly via an effect on insulin sensitivity.

It is important to note that even at baseline, plasma LCAT and CETP activity levels were low in a considerable number of GH-deficient patients compared to previously reported data in healthy subjects (51, 52). This resulted in considerable variation in baseline plasma LCAT and CETP activity levels. The lowering of plasma LCAT and CETP activity levels by GH indicates that this treatment may accentuate relative deficiencies of LCAT and CETP in hypopituitary patients. In our cross-sectional study in GH-deficient, acromegalic, and healthy subjects (18), we also observed relatively low plasma LCAT and CETP activity levels in GH-deficient patients, despite a negative correlation with plasma IGF-I in the whole cohort. In a further study, it was

found that glucocorticoid replacement therapy is probably associated with low plasma LCAT and CETP activity levels (53). The majority of the presently studied hypopituitary patients received glucocorticoids, and almost all of them were treated for hypothyroidism and hypogonadism. This precludes a meaningful analysis of the effect of (treatment for) other pituitary hormone deficiencies on the plasma LCAT and CETP responses to GH replacement therapy. Nonetheless, an important effect of GH on plasma LCAT and CETP via an effect on thyroid hormones is unlikely, because no relationships were found between individual changes in thyroid hormone levels and the changes in plasma LCAT and CETP.

Plasma cholesterol esterification and cholesteryl ester transfer are governed by the plasma activity levels of LCAT and CETP as such, as well as by the concentration and composition of the lipoproteins involved in these processes (11, 54). In the current study, plasma EST and CET were significantly lowered after 12 months of GH replacement therapy. The changes in plasma EST and CET were strongly correlated with the changes in plasma LCAT and CETP activity, without independent contributions of changes in VLDL + LDL cholesterol and plasma triglycerides. This would indicate that GH replacement influenced the processes of cholesterol esterification and cholesteryl ester transfer predominantly by lowering the plasma levels of active LCAT and CETP, respectively.

The transfer of cholesteryl esters out of HDL is considered to affect HDL cholesterol and cholesteryl ester concentrations (11, 12, 30), as indicated by the increased HDL cholesteryl ester content after experimental inhibition of plasma CETP in the rabbit (55), and the elevated HDL cholesterol in humans with genetic CETP deficiency (56). The decrease in plasma CET may thus have contributed to the rise in HDL cholesterol and cholesteryl ester during GH replacement. Of note, cholesteryl ester transfer out of HDL and cholesterol esterification in HDL are thought to be integrated processes (57, 58), and the lowering in plasma CET could have induced a secondary decrease in plasma EST, even apart from the effect of GH replacement on plasma LCAT activity levels per se. It cannot be excluded that GH replacement may have affected cholesterol and other lipids in HDL by other mechanisms than plasma CET, including effects on lipases. Postheparin plasma hepatic lipase activity has been reported to decrease (59) or to remain unchanged (33) after treatment with GH. It is also possible that GH replacement affects cellular processes involved in peripheral cholesterol efflux and hepatic cholesterol uptake via HDL (60, 61).

Accelerated plasma cholesteryl ester transfer has been documented in dyslipidemic subjects without pituitary disorders in association with an increased cardiovascular risk (11, 12, 51, 54, 62). This could imply that the lowering in plasma CET in response to GH replacement represents an anti-atherogenic phenomenon. Plasma PLTP activity did not significantly change. This would raise the possibility that the ability of plasma to generate pre-β HDL particles, which are thought to be important in cholesterol removal from peripheral cells (15, 16, 63, 64), remains largely unaffected by GH replacement, although hepatic lipase and possibly CETP may also be involved in pre-β HDL formation (9, 65). On the other hand, decreases in plasma EST and CET indicate a diminished capacity of plasma to esterify cholesterol in HDL and to subsequently transfer cholesteryl esters from HDL to VLDL and LDL, and, hence, may imply an impairment in these steps of the process of reverse cholesterol transport after GH replacement. The consequences for atherogenesis of a decrease in plasma cholesterol esterification and cholesteryl ester transfer in response to GH could be dependent on the balance between the efficacy of hepatic metabolism of apoB-containing lipoproteins as opposed to direct non CETP-mediated delivery of peripheral cell cholesterol to the liver via HDL (49, 66). In this respect it is important that GH is able to up-regulate hepatic LDL receptor activity (47).

In conclusion, GH replacement therapy in hypopituitary adults lowers plasma cholesterol esterification and cholesteryl ester transfer in conjunction with decreases in plasma LCAT and CETP activity levels. These effects may have consequences for HDL metabolism and for reverse cholesterol transport.

This study was financially supported by Pharmacia & Upjohn Inc., Sweden. The technical assistance of P. van den Berg, T. van Gent, and L. M. Scheek is highly appreciated.

Manuscript received 11 November 1999 and in revised form 11 January 2000.

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