CLINICAL STUDY

HDL cholesterol response to GH replacement is associated with common cholesteryl ester transfer protein gene variation (–629C>A) and modified by glucocorticoid treatment

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Abstract

Objective: GH replacement lowers total cholesterol and low-density lipoprotein cholesterol (LDL-C) in GH-deficient adults, but effects on high-density lipoprotein (HDL) cholesterol (HDL-C) are variable. Both GH and glucocorticoids decrease cholesteryl ester transfer protein (CETP) activity, which is important in HDL metabolism. We determined the extent to which the changes in HDL-C in response to GH replacement are predicted by the –629C>A CETP promoter polymorphism, and questioned whether this association is modified by concomitant glucocorticoid treatment.

Design and methods: A total of 91 GH-deficient adults (63 receiving glucocorticoids) were genotyped for the –629 CETP C>A polymorphism. Fasting serum lipids were measured before and after 1.2 ± 0.4 years of GH treatment (Genotropin, Pfizer Inc., Stockholm, Sweden).

Results: In the whole group, total cholesterol and LDL-C decreased ($P < 0.05$) after GH treatment, but the changes in HDL-C were not significant. In CC carriers receiving glucocorticoids ($n = 19$), HDL-C rose by 0.15 ± 0.25 mmol/l ($P = 0.02$; $P < 0.03$ from unchanged HDL-C in –629 AA + CA carriers on glucocorticoids and from CC homozygotes not receiving glucocorticoids). Multivariate regression analysis showed that individual changes in HDL-C were predicted by the CETP polymorphism (CC versus AA + CC, $P = 0.006$) in glucocorticoid users, independently of baseline HDL-C and other variables including apolipoprotein E4 carrier status; an opposite association with the CETP polymorphism was found in patients not receiving glucocorticoids ($P = 0.053$).

Conclusions: We suggest a common CETP variant-glucocorticoid treatment interaction concerning the effect of GH replacement on HDL-C. This may explain some of the reported variation in the HDL-C response to GH.

European Journal of Endocrinology 162 227–234

Introduction

Cardiovascular morbidity and mortality are likely to be increased in patients with hypopituitarism (1). The increased cardiovascular risk in hypopituitary patients is in part attributable to GH deficiency, which is associated with an adverse cardiovascular risk profile, including abnormalities in body composition, high serum concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol (LDL-C), and triglycerides, as well as low high-density lipoprotein (HDL) cholesterol (HDL-C) (2). GH replacement therapy improves body composition, and favorably lowers serum total cholesterol and LDL-C (2–5), but inconsistent effects of GH replacement on HDL-C have been reported. Smaller studies have demonstrated an increase, no change or even a decrease in HDL-C in adults with GH deficiency

(2, 6–8). A surveillance report from the Hypopituitary Control and Complications Study (HypoCSS; Eli Lilly) showed an increase in HDL-C (3), whereas an analysis from KIMS (Pfizer International Metabolic Database) demonstrated a small decrease in HDL-C after 1 and 2 years of GH replacement in hypopituitary adults (4). A meta-analysis comprising placebo-controlled trials did not reveal a significant effect on HDL-C (9).

The serum total cholesterol and LDL-C response to GH therapy may be stronger in younger subjects and in patients with adult onset GH deficiency (9, 10), but the extent to which genetic factors may affect GH replacement effects on HDL-C is largely unknown. HDL metabolism is regulated by several processes, including cholesteryl ester transfer protein (CETP)-mediated neutral lipid transfer process (11, 12). CETP transfers cholesteryl esters from HDL toward

triglyceride-rich lipoproteins. Consequently, increased CETP activity results in a lower HDL-C (11–13). Common genetic variations have been identified in *CETP* (14, 15), among which the –629 *CETP* C>A promoter polymorphism (rs1800775) has been shown to regulate *CETP* transcriptional activity *in vitro* (16). The more frequent C allele is associated with higher circulating CETP mass and activity and a lower HDL-C (17, 18). Of interest, GH replacement decreases CETP mass and activity in hypopituitary adults (6, 7). Moreover, circulating CETP mass and activity are probably lowered by glucocorticoids as well. CETP activity is considerably reduced in GH-deficient adults receiving conventional glucocorticoid substitution therapy (19, 20), which is considered to be unphysiological (21). This may explain why HDL-C is not decreased, despite higher serum triglycerides in glucocorticoid receiving hypopituitary patients who are treated with GH (22). Steroid treatment also attenuates CETP activity in other patient groups (23, 24), probably contributing to high HDL-C (25, 26).

The present study was initiated to test the hypothesis that the –629 *CETP* C>A polymorphism predicts the HDL-C response to GH replacement in hypopituitary adults. Since both GH and glucocorticoids are likely to affect the CETP pathway, we also determined whether such an association may be modified by concomitant glucocorticoid treatment for ACTH deficiency.

Subjects and methods

The study was approved by the medical ethics committees of the University Medical Centres Groningen and Utrecht, The Netherlands. All participants provided written informed consent, and GH deficiency was established according to the international consensus criteria, i.e. a peak GH in response to insulin-induced hypoglycemia <3 µg/l and/or an insulin-like growth factor 1 (IGF1) concentration >2 s.d.s below the age-adjusted reference value (4). The participants (aged ≥18 years) were recruited from the outpatient clinics of the Departments of Endocrinology from each University Medical Centre. Patients with adult-onset or childhood-onset GH deficiency were eligible. In case of childhood-onset GH deficiency, GH provocation tests were again performed before the (re)start of GH treatment in order to confirm persistent GH deficiency. Exclusion criteria were the use of lipid lowering drugs, severe illness necessitating clinical admission in the preceding year, pregnancy, and severe hypertriglyceridemia (fasting serum triglyceride level >5.0 mmol/l). All patients participated in the KIMS (Pfizer International Metabolic Database) protocol, and they were studied before and during GH (Genotropin, Pfizer Inc., Stockholm, Sweden) treatment, which was scheduled at 1 year, i.e. at the first time point at which protocolized data concerning serum lipids and body composition were obtained. If 1-year follow-up lipid data were not available, measurements

obtained after more prolonged GH treatment were used. Dose titration that was aimed at normalizing the IGF1 level was applied in both centers.

ACTH deficiency was diagnosed using an 0800 h serum cortisol or a peak cortisol threshold in response to insulin-induced hypoglycemia as described (27). If an insulin tolerance test was contraindicated, a metyrapone test was used. Deficiencies of other pituitary hormones were diagnosed as described (28). Hormonal substitution therapy with glucocorticoids, thyroid hormone, sex steroids, and vasopressin (ADH) was administered when indicated. Data concerning the underlying disorder responsible for GH deficiency, age at onset of GH deficiency and number of additional pituitary hormone deficiencies were also retrieved from the medical records. Body fat mass was assessed using bioelectrical impedance, according to the algorithm provided by the manufacturer (29). Waist circumference was measured as the smallest girth between rib cage and iliac crest. Body mass index (BMI, kg/m²) was calculated as weight divided by height squared.

Laboratory methods

Serum samples were stored at –20 °C until assay. Serum lipids and IGF1 were measured in a central laboratory, according to the KIMS protocol. Cholesterol and triglycerides were measured enzymatically using routine biochemical methods. HDL-C was measured in the supernatant fraction after precipitation of apolipoprotein B-containing lipoproteins with sodium phosphotungstate and Mg²⁺ (30). LDL-C was calculated by the Friedewald formula. Plasma glucose was measured in the laboratory of each center using standard clinical chemical methods.

Genotyping was performed at the University Medical Centre Groningen and at the Laboratory of Experimental Vascular Medicine, Academic Medical Centre Amsterdam, The Netherlands. DNA was extracted from whole blood using the Qiaamp mini kit (Qiagen). The –629C>A *CETP* promoter polymorphism (rs1800775) was genotyped as described (17). Apolipoprotein E (*ApoE*) genotypes (rs429358 and rs7412) were determined using allelic discrimination on a TaqMan 7500 Real Time PCR system, using the SNP genotyping mixes C-3084793-20 and C-904973-10 and Taqman Universal PCR mastermix No AmpErase (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The method has been validated against a previously described restriction isotyping procedure (31, 32).

Statistical analysis

Statistical analyses were carried out using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Data are given in mean ± s.d., median (interquartile ranges), or in percentages. Between-group differences in variables

were evaluated using unpaired *t*-tests, Mann–Whitney *U* tests, or ANOVA with subsequent Bonferroni correction for multiple comparisons. Between-group differences in (genotype) prevalences were determined using χ^2 analysis. Changes in variables were tested with paired *t*-tests or Wilcoxon tests where appropriate. Multiple linear regression analysis was performed to disclose the independent relationship between variables. Assuming a mean change in HDL-C of 0.12 ± 0.06 mmol/l after GH administration (derived from Leese *et al.* (6)), it was estimated that a between-group difference in HDL-C change of 0.06 mmol/l could be demonstrated in 11 patients per group with a power of 0.80 and a two-sided *P* value of 0.05. A two-sided *P* value <0.05 was considered to be significant.

Results

Ninety-one patients participated in the study (mean age 43 ± 14 years; 47 men, 44 women). Twenty-eight patients were classified as ACTH sufficient (mean age 36 ± 14 years; 11 men, 17 women), and 63 patients received glucocorticoids for ACTH deficiency (mean age 46 ± 13 years, $P < 0.001$; 36 men, 27 women, $P = 0.36$), of whom 58 were treated with cortisone acetate and five with hydrocortisone. In most cases, glucocorticoids were administered in two daily doses. The mean glucocorticoid dose (in hydrocortisone equivalents) was 22 ± 6 (range 6.0–30.0) mg/day, corresponding to 11.0 ± 2.9 (range 3.0–17.2) mg/m² of body surface area. In all participants, glucocorticoid dose remained unchanged during follow-up. Twenty-three patients (25.3%) had childhood-onset GH deficiency, which was present in 32.1% of ACTH-sufficient patients and in 22.2% of patients receiving glucocorticoids ($P = 0.46$). In the whole group, 59 patients (64.8%) had TSH deficiency, 69 (75.8%) had gonadotropin deficiency, and 18 (19.8%) had diabetes insipidus

(32.5%). TSH deficiency (84.1 vs 21.4%, $P < 0.001$), gonadotropin deficiency (90.5 vs 42.5%, $P < 0.001$), and ADH deficiency (28.6 vs 0%, $P = 0.004$) were more prevalent in glucocorticoid receiving patients than in ACTH-sufficient patients. In all thyroid hormone-treated patients, serum free thyroxine levels were within the reference range before and during GH replacement. All hypogonadal men used testosterone supplementation. All hypogonadal women aged <50 years used oral estrogen preparations, but sex steroids were only used by one of the eight (13%) women aged >50 years. Transdermal estrogens were not used. The percentage of hypogonadal women which used estrogen supplementation was not different between ACTH-sufficient and glucocorticoid-treated patients ($P = 0.18$). Baseline blood pressure was 127 ± 14 mmHg systolic and 83 ± 10 mmHg diastolic in the whole group with higher systolic ($P = 0.07$) and diastolic ($P = 0.03$) values in glucocorticoid-treated patients. Five patients had diabetes mellitus of whom four were treated with glucocorticoids ($P = 0.96$). Fasting plasma glucose (4.8 ± 1.2 mmol/l in the whole group) was not different between ACTH-sufficient and glucocorticoid-treated patients ($P = 0.84$).

The participants were studied before and after 1.2 ± 0.4 (range 0.9–2.8) years of GH replacement. Duration of follow up was not different between ACTH-sufficient and glucocorticoid-treated patients ($P = 0.14$). GH dose at follow-up was 0.40 ± 0.23 (range 0.20–1.60) mg/day in the whole group, and was not different between ACTH-sufficient patients (0.40 ± 0.20 mg/day) and patients receiving glucocorticoids (0.40 ± 0.24 mg/day, $P = 0.89$).

In the whole group, BMI and waist circumference remained unchanged after GH (Table 1). Fat mass decreased similarly in ACTH-sufficient and glucocorticoid-treated patients. Waist circumference decreased slightly in glucocorticoid-treated patients. Before GH treatment, serum IGF1 levels were lower in

Table 1 Clinical characteristics, insulin-like growth factor 1, plasma glucose and lipid levels in before and after GH treatment in 91 GH-deficient adults. Data in mean $\pm$ s.d. or in median (interquartile ranges).

	Whole group (<i>n</i> =91)		ACTH sufficient (<i>n</i> =28)		On glucocorticoids (<i>n</i> =63)	
	Before GH treatment	After GH treatment	Before GH treatment	After GH treatment	Before GH treatment	After GH treatment
BMI (kg/m ²)	28.3 ± 5.3	28.3 ± 5.3	28.6 ± 5.0	28.3 ± 4.5	28.2 ± 5.4	28.3 ± 5.6
Waist (cm)	97 ± 15	96 ± 14	93 ± 13	94 ± 13	98 ± 15	$97 \pm 15^*$
Fat mass (kg)	27.3 ± 11.3	$25.5 \pm 11.0^{\ddagger}$	29.4 ± 10.6	$27.1 \pm 8.5^*$	26.5 ± 11.7	$24.9 \pm 11.9^{\ddagger}$
IGF1 (μ g/l)	73 ± 45	$164 \pm 72^{\ddagger}$	98 ± 53	$180 \pm 71^{\ddagger}$	$62 \pm 36^{\S}$	$156 \pm 71^{\ddagger}$
IGF1 Z-score	-2.39 ± 1.32	$-0.16 \pm 1.34^{\ddagger}$	-2.20 ± 1.38	$-0.30 \pm 1.28^{\ddagger}$	-2.48 ± 1.29	$-0.09 \pm 1.37^{\ddagger}$
Total cholesterol (mmol/l)	5.46 ± 1.01	$5.33 \pm 0.93^*$	5.41 ± 0.96	$5.17 \pm 0.94^*$	5.48 ± 1.04	5.40 ± 0.12
LDL cholesterol (mmol/l)	3.50 ± 0.92	$3.30 \pm 0.82^{\ddagger}$	3.55 ± 0.85	$3.21 \pm 0.78^*$	3.48 ± 0.96	3.33 ± 0.85
HDL cholesterol (mmol/l)	1.16 ± 0.36	1.18 ± 0.34	1.22 ± 0.40	1.21 ± 0.35	1.14 ± 0.34	1.17 ± 0.33
LDL/HDL cholesterol ratio	3.29 ± 1.23	$3.03 \pm 1.17^{\ddagger}$	3.19 ± 1.17	$2.92 \pm 1.21^*$	3.34 ± 1.26	$3.08 \pm 1.16^*$
Triglycerides (mmol/l)	1.70 (1.30–2.10)	1.80 (1.20–2.30)	1.35 (1.10–1.80)	1.40 (0.95–1.98)	1.70 (1.30–2.10)	1.90 (1.50–2.40) [§]

BMI, body mass index; IGF1, insulin like growth factor 1; HDL, high-density lipoproteins. * $P < 0.05$; $^{\ddagger}P < 0.01$; $^{\ddagger\ddagger}P < 0.001$ from baseline; $^{\S}P < 0.05$; $^{||}P < 0.01$ from patients not using glucocorticoids.

Table 2 Baseline plasma lipids and apolipoprotein (apo) E4 carrier status according to $-629C>A$ cholesteryl ester transfer protein (*CETP*) promoter polymorphism in 91 GH-deficient adults. Data in mean $\pm$ s.d. or in median (interquartile ranges).

	AA+CA	AA	CA	CC
Whole group ($n=91$)	$n=61$	$n=18$	$n=43$	$n=30$
Apo E4 carriers (no/yes)	44/17	8/10	36/7	23/7
Total cholesterol (mmol/l)	5.46 ± 1.04	5.58 ± 0.78	5.40 ± 1.09	5.46 ± 1.04
LDL cholesterol (mmol/l)	3.47 ± 0.90	3.57 ± 0.71	3.42 ± 0.98	3.58 ± 0.96
HDL cholesterol (mmol/l)	1.19 ± 0.39	1.21 ± 0.35	1.18 ± 0.41	1.10 ± 0.28
Triglycerides (mmol/l)	1.60 (1.30–2.10)	1.50 (1.20–1.85)	1.80 (1.30–2.20)	1.70 (1.10–1.93)
ACTH sufficient ($n=28$)	$n=17$	$n=9$	$n=8$	$n=11$
Apo E4 carriers (no/yes)	11/6	4/5	7/1	8/3
Total cholesterol (mmol/l)	5.39 ± 0.92	5.76 ± 0.86	4.98 ± 0.84	5.45 ± 1.06
LDL cholesterol (mmol/l)	3.49 ± 0.80	3.77 ± 0.67	3.17 ± 0.86	3.65 ± 0.94
HDL cholesterol (mmol/l)	1.29 ± 0.46	1.31 ± 0.35	1.27 ± 0.59	1.10 ± 0.23
Triglycerides (mmol/l)	1.30 (0.95–1.65)	1.30 (1.10–1.75)	1.30 (0.80–1.48)	1.70 (1.10–2.00)
On glucocorticoids ($n=63$)	$n=44$	$n=9$	$n=35$	$n=19$
Apo E4 carriers (no/yes)	33/11	4/5	29/6	15/4
Total cholesterol (mmol/l)	5.48 ± 1.05	5.41 ± 0.69	5.50 ± 1.13	5.46 ± 1.06
LDL cholesterol (mmol/l)	3.46 ± 0.95	3.39 ± 0.74	3.48 ± 1.00	3.54 ± 1.00
HDL cholesterol (mmol/l)	1.15 ± 0.35	1.11 ± 0.34	1.17 ± 0.37	1.10 ± 0.31
Triglycerides (mmol/l)	1.85 (1.50–2.20)	1.50 (1.45–2.55)	1.90 (1.50–2.20)	1.70 (1.30–1.90)

HDL, high-density lipoproteins. All comparisons between the three genotype groups and between -629 *CETP* AA+CA carriers and CC homozygotes were not significant ($P>0.10$).

glucocorticoid-treated patients, but there was no difference in IGF1 when expressed as s.d. Z-scores. The IGF1 Z-scores attained after GH were similar in both groups. Before GH treatment, serum total cholesterol, LDL-C, and HDL-C were not different between patients with and without ACTH deficiency; triglycerides were higher in glucocorticoid-treated patients (Table 1). In the whole group, serum total cholesterol and LDL-C decreased after GH, but HDL-C and triglycerides remained unchanged. Consequently, the LDL/HDL-C ratio decreased. In ACTH-sufficient patients, the decrease in total cholesterol and LDL-C was significant. In glucocorticoid-treated patients, serum total cholesterol and LDL-C did not decrease significantly ($P=0.08$), although the LDL/HDL-C ratio decreased as well. HDL-C did not change in either ACTH-sufficient patients or in glucocorticoid-treated patients.

Table 2 shows baseline serum lipids according to the $-629C>A$ *CETP* polymorphism, which was in Hardy–Weinberg equilibrium in the whole group ($P>0.90$). The *CETP* genotype distribution was not different in

glucocorticoid-using patients compared to ACTH-sufficient patients ($P=0.36$). In the whole group and in patients not receiving glucocorticoids, HDL-C was lowest in CC carriers, but the *CETP* genotype effects were not significant ($P>0.20$). In glucocorticoid-treated patients, HDL-C was essentially similar across the *CETP* genotype groups. Serum total cholesterol, LDL-C, and triglycerides did not vary according to the $-629C>A$ *CETP* polymorphism (Table 2). Fifty-nine patients had the *ApoE3/E3* genotype, and eight were *ApoE2/E3* carriers (non-*ApoE4* carriers, $n=67$). Three patients were *ApoE2/E4* carriers, 19 were *ApoE3/E4* carriers, and two were *ApoE4/E4* homozygotes (*ApoE4* carriers, $n=24$). The presence of the *ApoE4* allele was not different between -629 *CETP* AA+CA subjects using glucocorticoid or not, nor between -629 *CETP* CC carriers using glucocorticoid or not (Table 2, $P>0.50$ for both comparisons). In the whole group and in glucocorticoid-treated patients separately, baseline serum cholesterol, LDL-C, HDL-C, and triglyceride levels were not significantly different between the

Table 3 Changes in plasma lipids in response to GH treatment according to $-629C>A$ cholesteryl ester transfer protein (*CETP*) promoter polymorphism in 91 GH-deficient adults. Changes in mean $\pm$ s.d. or in median (interquartile range).

	Whole group		ACTH sufficient		On glucocorticoids	
	AA+CA ($n=61$)	CC ($n=30$)	AA+CA ($n=17$)	CC ($n=11$)	AA+CA ($n=44$)	CC ($n=19$)
Total cholesterol (mmol/l)	-0.14 ± 0.63	-0.10 ± 0.73	-0.17 ± 0.85	-0.36 ± 0.32	-0.13 ± 0.54	0.05 ± 0.86
LDL cholesterol (mmol/l)	-0.18 ± 0.63	-0.27 ± 0.63	-0.28 ± 0.66	-0.44 ± 0.31	-0.14 ± 0.63	-0.17 ± 0.75
HDL cholesterol (mmol/l)	0.00 ± 0.07	-0.07 ± 0.25	0.02 ± 0.17	-0.06 ± 0.19	-0.01 ± 0.18	$0.15 \pm 0.25^{*†}$
Triglycerides (mmol/l)	0.10 (–0.40 to 0.40)	0.20 (–0.23 to 0.63)	0.00 (–0.40 to 0.25)	0.20 (–0.20 to 0.60)	0.10 (–0.48 to 0.48)	0.20 (–0.30 to 0.70)

HDL, high-density lipoproteins. $^{*}P=0.02$ from baseline; $^{\dagger}P<0.03$ from -629 *CETP* AA+CA carriers on glucocorticoids and from *CETP* CC homozygotes not using glucocorticoids.

Figure 1 Individual changes in high-density lipoprotein (HDL) cholesterol in response to GH replacement according to -629 *CETP* C>A promoter polymorphism and concomitant glucocorticoid treatment. * $P=0.02$ from baseline; ** $P<0.03$ from -629 *CETP* CA+AA carriers receiving glucocorticoids, and from ACTH sufficient -629 *CETP* CC.

non-*ApoE4* carriers and the *ApoE4* carriers ($P>0.30$ for all comparisons; data not shown). Among patients not receiving glucocorticoids, baseline serum total cholesterol and LDL-C were higher in *ApoE4* carriers ($n=9$; 6.14 ± 0.58 and 4.18 ± 0.49 mmol/l respectively) than in non-*ApoE4* carriers ($n=19$; 5.07 ± 0.91 mmol/l, $P=0.003$ and 4.18 ± 0.49 mmol/l, $P=0.005$ respectively); there were no further differences in baseline serum lipids according to *ApoE4* carrier status in this group (data not shown).

The changes in serum total cholesterol, LDL-C, and triglycerides in response to GH replacement did not differ significantly according to the -629 C>A *CETP* polymorphism (Table 3). Of note, HDL-C increased in response to GH replacement in glucocorticoid receiving patients with the -629 CC *CETP* genotype ($P=0.02$); this increase was different from the changes in HDL-C in -629 *CETP* AA+CA glucocorticoid-treated patients ($P<0.03$) and from -629 *CETP* CC patients with

ACTH sufficiency ($P<0.03$; Table 3, individual changes in HDL-C are shown in Fig. 1). The changes in serum lipids after GH did not differ according to *ApoE4* carrier status (data not shown).

Multiple regression analysis was performed to determine whether the association of the GH-induced changes in HDL-C with the -629 *CETP* C>A polymorphism was independent of clinical factors, baseline HDL-C, changes in triglycerides and in LDL-C as well as *ApoE4* carrier status (Table 4). In glucocorticoid-receiving patients, the changes in HDL-C were positively predicted by the -629 *CETP* genotype (CC versus AA+CA; $P=0.006$), independently of baseline HDL-C and age. This *CETP* gene effect was also independent of glucocorticoid dose (expressed per m² of body surface area, $P=0.92$). In patients not receiving glucocorticoids, in contrast, the individual changes in HDL-C were negatively predicted by the -629 *CETP* genotype (CC versus AA+CA; $P=0.053$). In both groups, these age-adjusted associations remained essentially similar (glucocorticoid receiving patients: $P<0.014$ in all analyses; no glucocorticoid receiving patients: $P<0.075$ in all analyses) after adjustment for either gender, childhood-onset versus adult-onset GH deficiency, treatment for other pituitary hormone deficiencies, *ApoE4* carrier status, changes in IGF1, in waist circumference, in triglycerides, and in LDL-C (data not shown). When the -629 *CETP* polymorphism was divided into three allelic groups (CC, CA and AA), again opposing trends were found between the association of baseline HDL-C-adjusted changes in HDL-C with the -629 *CETP* polymorphism in glucocorticoid-treated patients ($\beta=0.225$, $P=0.059$) compared to patients not receiving glucocorticoids ($\beta=-0.368$, $P=0.036$). When glucocorticoid treatment (either as a categorical variable or as glucocorticoid dose per m² of body surface area), the -629 *CETP* genotype (CC versus AA+AC), and the interaction of these variables were forced in a multivariate regression model, a positive interaction between the -629 *CETP* C>A genotype and glucocorticoid treatment on the HDL-C response to GH was found in all patients combined ($P=0.002$ and $P<0.001$ respectively), again independently of age ($P>0.40$) and baseline HDL-C ($P=0.001$).

Table 4 Association of changes in high-density lipoprotein (HDL) cholesterol in response to GH therapy according to -629 C>A cholesteryl ester transfer protein (*CETP*) promoter polymorphism by multiple linear regression analysis.

	ACTH sufficient ($n=28$)		On glucocorticoids ($n=63$)	
	β	P value	β	P value
Age (years)	-0.127	0.46	-0.054	0.66
HDL cholesterol	-0.521	0.006	-0.360	0.005
-629 C>A <i>CETP</i> polymorphism CC versus CA+AA	-0.345	0.053	0.323	0.006

β , Standardized regression coefficient; -629 CC, CA, AA: *CETP* -629 C>A promoter polymorphism.

Discussion

The present study has shown that HDL-C does not change in response to GH replacement in hypopituitary patients with and without an intact pituitary–adrenal axis. In the whole group, the individual changes in HDL-C were also not significantly associated with the –629 *CETP* C>A polymorphism. Remarkably, HDL-C increased in glucocorticoid-treated patients with the –629 *CETP* CC genotype, contrasting unchanged HDL-C levels in glucocorticoid patients with the –629 *CETP* AA+CA genotype and –629 *CETP* CC carriers with intact ACTH secretion. Furthermore, multivariate regression analysis showed that the presence of the –629 *CETP* C allele predicted an increase in HDL-C even independently of its baseline concentration in glucocorticoid-treated patients, whereas an opposite association was observed in ACTH-sufficient patients. Thus, our findings agree with the contention that there is an association of the HDL-C response to GH administration with this common *CETP* gene variation, which is modified by concomitant glucocorticoid administration.

Current conventional glucocorticoid replacement regimens often exceed the endogenous cortisol production rate in adults with intact adrenal reserve, which approximates 6–10 mg/m² per day (33, 34). In the present cohort, the mean glucocorticoid substitution regimen (in hydrocortisone equivalents) was 11.0 mg/m² per day; 60% of patients used a dose higher than 10 mg/m² per day. Thus, despite some expected acceleration of peripheral cortisol metabolism during GH replacement (35, 36), glucocorticoid substitution doses should still be regarded to be high in many patients. Importantly, an increased risk of stroke and coronary heart disease may be related to concomitant glucocorticoid substitution treatment in GH-replaced hypopituitary patients (37). Taken together, these considerations provide a clinical rationale to elucidate whether effects of GH replacement on serum lipids is modified by concomitant glucocorticoid administration. It is obvious that the possible cardiovascular benefit of the increase in HDL-C in response to GH, averaging 0.15 mmol/l in glucocorticoid receiving hypopituitary patients who were homozygous for the –629 *CETP* C allele remains uncertain. A meta-analysis comprising prospective observational studies has demonstrated that mortality from ischemic heart disease is 33% lower for each 0.33 mmol/l higher HDL-C (38). Extrapolating these data (38) to the present findings would translate into a 15% risk reduction in this subgroup of patients, besides expected benefit from GH-induced LDL-C lowering.

Circulating CETP levels are highest in –629 *CETP* CC allele carriers (14, 16–18), whereas both GH and glucocorticoids are able to reduce serum CETP (7, 8, 20). The increase in HDL-C after GH replacement in glucocorticoid-treated hypopituitary patients

homozygous for the –629 *CETP* CC allele may thus be explained in part by assuming that GH elicits a more pronounced decrease in circulating CETP levels in these patients. Although we did not measure serum CETP mass or activity in the present study, it is plausible that the GH-induced increase in HDL-C is attributable to CETP lowering, resulting in decreased transfer of cholesteryl esters out of HDL particles (11–13). Notably, it is likely that other mechanisms also contribute to the effects of glucocorticoids on HDL metabolism. Glucocorticoids increase hepatic synthesis of apolipoprotein A–I, the major HDL protein constituent (39, 40), and reduce hepatic lipase activity (41), thereby maintaining or even increasing HDL-C. Several interdependent metabolic processes may, therefore, be involved in an effect modification of concomitant glucocorticoid treatment on the HDL-C response to GH replacement.

The association of HDL-C with common *CETP* variations can be modified by environmental factors, such as diet composition and alcohol (reviewed in Ref. (15)). Our present findings support the possibility of a *CETP* gene–glucocorticoid treatment interaction with respect to the HDL-C response to GH replacement. Gene–environment interaction has also been suggested for *ApoE* (42). For example, *ApoE* gene variation predicts the LDL-C response to cholesterol feeding (32), whereas *ApoE* may interact with circulating CETP mass on the HDL-C response to a cholesterol-rich diet (43). Of potential interest, one study in hypopituitary patients suggested that the HDL-C changes after GH replacement are dependent on the *ApoE* genotype (6), although no such effects of the *ApoE* phenotype were observed in another report (44). The *ApoE4* genotype did not predict changes in HDL-C response to GH replacement, and *ApoE4* carrier status did not modify the association of the changes in HDL-C after GH with the –629 *CETP* C>A polymorphism in the present study. It seems unlikely that *ApoE* gene variation has a major impact on GH replacement-induced changes in HDL metabolism. Our report did show expected (45) differences in baseline serum total cholesterol and LDL-C between *ApoE4* carriers and non-*ApoE4* carriers, which reached statistical significance in patients who were not treated with glucocorticoids. This supports the presence of *ApoE*–serum lipid relationships in hypopituitary patients.

Some other aspects of our study need to be considered. The –629 *CETP* C allele frequently amounted to 57% in this cohort of hypopituitary adults as compared to 52% in a population-based survey in The Netherlands (46). This suggests that this *CETP* polymorphism did not result in an important cardiovascular morbidity-related selection of hypopituitary patients participating in the current study. The present study was sufficiently powered to be able to demonstrate statistically effects on the HDL-C in response to GH, which was our primary outcome variable. Of note, the number of hypopituitary patients included in our

study was not large enough to demonstrate significant differences in HDL-C at baseline according to CETP genotype groups. Yet among patients not receiving glucocorticoids, the point estimates of the mean differences in HDL-C across CETP genotype groups were comparable to those reported recently (18).

In conclusion, this report suggests a common CETP variant-glucocorticoid treatment interaction concerning the effect of GH replacement on HDL-C. This may explain some of the reported variation in the HDL-C response to GH treatment, and could influence potential cardiovascular benefit.

Declaration of interest

The study is investigator driven, and the authors do not have any financial or other conflict of interest to declare.

Funding

This study is financially supported by Pfizer Inc., Stockholm, Sweden.

Acknowledgements

The help of Mrs Inge E Pop in data collection is greatly appreciated.

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Received 5 November 2009

Accepted 16 November 2009