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CLINICAL STUDY

Atherogenic lipoprotein phenotype and low-density lipoprotein size and subclasses in patients with growth hormone deficiency before and after short-term replacement therapy

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Abstract

Objective: Patients with growth hormone deficiency (GHD) have increased cardiovascular risk and may show elevated triglyceride and reduced high density lipoprotein (HDL) cholesterol concentrations, two lipid abnormalities usually accompanied by increased small dense LDL in the 'atherogenic lipoprotein phenotype' (ALP). In the present study, we directly investigated (1) whether hypopituitary patients with GHD have increased small dense LDL; (2) whether growth hormone replacement therapy (GHRT) beneficially impact on such particles; (3) the prevalence of ALP in GHD and GHRT patients.
Design and methods: In 14 hypopituitary patients with GHD (44 ± 13 years, body mass index (BMI) 27 ± 3) before and after 4 months of GHRT, and in 11 healthy age- and BMI-matched controls we measured plasma lipids and LDL size and subclasses by gradient gel electrophoresis.
Results: Compared with controls, GHD showed increased triglycerides ($P = 0.0024$), similar total and LDL cholesterol levels and a tendency towards reduced HDL cholesterol concentrations ($P = 0.0894$). GHRT reduced total and LDL cholesterol levels ($P = 0.0303$ and 0.0120 respectively), but no effect was found on triglycerides and HDL cholesterol levels. LDL size was unchanged in GHD versus controls (269 ± 9 vs 274 ± 6 Å, P = ns), but LDL subclass analysis revealed a shift towards more dense particles ($P = 0.0046$). GHRT had no significant impact on LDL size and subclasses. The prevalence of ALP was 14% in GHD and 7% in GHRT.
Conclusions: In GHD patients, individual features of ALP (including increased small dense LDL) may be common, but complete ALP is relatively uncommon. Short-term replacement therapy seems to be ineffective on such lipid alterations, but the effect of a longer GHRT remains to be assessed.

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Introduction

Hypopituitarism with GH deficiency (GHD) is associated with increased cardiovascular morbidity and mortality due to premature and progressive atherosclerosis (1–2). Lipid metabolism alterations are common in hypopituitary patients with GHD (3, 4) and, besides increased total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations (5–8), these patients may present with elevated triglyceride and reduced high density lipoprotein (HDL) cholesterol concentrations (8, 9), two lipid abnormalities usually accompanied by increased small dense LDL in the so-called lipid triad or 'atherogenic lipoprotein phenotype' (ALP) (10–12). This form of dyslipidaemia represents a partially heritable trait and is associated with increased cardiovascular risk and the metabolic syndrome (13). Since more patients with coronary artery disease are found to have this trait compared with those with hypercholesterolaemia only, it has been suggested that the clinical importance of ALP probably exceeds that of LDL cholesterol (14, 15).

In fact, a number of indications suggest that besides the quantity, the quality of LDL exerts a direct influence on the cardiovascular risk (16). LDL comprises multiple distinct subclasses that differ in size, density, physicochemical composition, metabolic behaviour and atherogenicity, with at least four major subspecies: large LDL-I, medium LDL-II, small LDL-III and very small LDL-IV. Based on the measurement of peak particle diameter or ultracentrifugal density, individuals generally cluster into two broad subgroups, the majority with a predominance of larger or medium-sized LDL and a substantial minority with a higher proportion of smaller LDL particles (17–19). LDL size seems to be an important predictor of cardiovascular events and progression of coronary artery disease (CAD) (16, 20, 21) and the predominance of small dense LDL has been accepted as an emerging cardiovascular risk factor by

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the National Cholesterol Education Program Adult Treatment Panel III (22).

Whether growth hormone replacement therapy (GHRT) may beneficially impact on such particles is unknown. It has been reported that GHRT tends to correct the dyslipidaemia found in hypopituitary patients with GHD, mainly by reducing total and LDL cholesterol concentrations, whereas the effect on HDL cholesterol and triglycerides is less clear (23, 24). Thus, so far no studies have directly investigated: (1) whether hypopituitary patients with GHD have increased small dense LDL; (2) whether GHRT reduces levels of small dense LDL and (3) the prevalence of ALP in GHD and GHRT patients. We reasoned to perform a short-term (4 months) replacement therapy because it has been previously shown that plasma lipids may be beneficially altered even after a few months of GHRT (reviewed in (25)). Therefore, we investigated plasma lipid concentrations and LDL size as well as subclasses in hypopituitary patients with GHD before and after GHRT. Identical investigations were performed in a healthy age- and body mass index (BMI)-matched control population.

Materials and methods

Subjects and study protocol

Patients with GH deficiency for at least 12 months and healthy control subjects matched for gender, age and BMI were recruited between October 2002 and July 2005 at the Division of Endocrinology and Diabetes of the University Hospital of Bern, Switzerland. GHD was defined as a peak GH of <3 mU/l during an insulin provocation test with nadir plasma glucose <2.2 mmol/l. Patients were included provided they had been under stable conventional hormone replacement therapies (glucocorticoid, thyroidal and gonadal) as needed for at least 6 months. Exclusion criteria were former or present adrenocorticotrophic hormone- or GH-secreting pituitary adenoma, abnormal liver or renal function, active neoplasia, severe cardiovascular disease (unstable coronary artery disease, heart failure NYHA III–IV), diabetes mellitus, haemophilia or therapy with drugs known to affect lipid metabolism.

Fourteen patients with GH deficiency and 11 control subjects were included in the present study, which was approved by the Ethics Committee of the University of Bern, and each subject gave written informed consent. All patients were GH-therapy naive adults. Height and weight were recorded and BMI was calculated as kilograms per square metre; obesity was defined as BMI equal or greater than 30. Patients were instructed in self-administration of GH using a pen device (Genotropin-Pen, Pfizer, New York, NYS, USA). Usual clinical care was provided (monthly visit with bioimpedance and insulin-like growth factor-I (IGF-I) measurements to adjust GH doses) and GH dose was gradually increased in order to obtain IGF-I concentrations in the upper half of the age-adjusted reference range (26). Target IGF-I levels were obtained after 2–3 months in all patients. After a total of 4 months of GH replacement therapy, weight maintaining diet and usual physical activity studies were performed.

Biochemistry

A blood sample was collected from each subject after 12–14 h overnight fast in sodium–EDTA tubes. Total cholesterol, triglycerides and HDL cholesterol were quantified by standard enzymatic-colourimetric methods (27–29). LDL cholesterol was measured enzymatically (Hitachi 917, Roche). In order to assess the prevalence of each individual component of ALP in both groups of subjects, we considered low HDL cholesterol levels those <1.03 mmol/l (e.g. <40 mg/dl) in men and <1.29 (e.g. <50 mg/dl) in women and elevated triglyceride concentrations those >1.69 mmol/l (e.g. >150 mg/dl) (22). Levels of small dense LDL were considered to be increased in those patients who presented with values greater than the mean ± 2 S.D. of the values of control subjects, as already described (30).

Non-denaturing polyacrylamide gradient gel electrophoresis

Non-denaturing polyacrylamide gradient gel electrophoresis of plasma was performed in Switzerland in the laboratory of KB at 10–14 °C in 2–16% polyacrylamide gradient gels for 12 patients with GHD (pre- and post-replacement therapy) and ten controls. All analyses were performed on frozen coded ('blinded') samples. Gels were subjected to electrophoresis for 24 h at 125 V in Tris-borate buffer (pH 8.3) as described elsewhere (31, 32). Gels were fixed and stained for lipids in a solution containing oil red O in 60% ethanol at 55 °C. Gels were placed on a light source and photographed with a Canon G3 digital camera. Migration distance for each absorbance peak was determined and the molecular diameter corresponding to each peak was calculated from a calibration curve generated from the migration distance of size standards of known diameter, which includes carboxylated latex beads (Duke Scientific, Palo Alto, CA, USA), thyroglobulin and apoferritin (HMW Std, Pharmacia, Piscataway, NJ, USA) having molecular diameter of 380, 170 and 122 Å respectively, and lipoprotein calibrators of previously determined particle size. LDL subclass distribution (LDL-I, LDL-IIA, LDL-IIB, LDL-IIIA, LDL-IIIB, LDL-IVA and LDL-IVB) as percentage of total LDL was calculated as previously described (33).

Statistical analysis

Statistical analyses were performed using Statview (Abacus Concepts, Inc., Berkeley, CA, USA) and SPSS 9.0 for PC (SPSS, Inc., Chicago, IL, USA). Univariate

analyses were performed using non-parametric Mann–Whitney test (for the differences between patients with GHD and controls) and non-parametric Wilcoxon test (for the differences between patients with GHD and GHRT) for numeric variables, while the differences in the prevalences for nominal variables were analysed by McNemar test. Correlation analyses were performed using the Spearman rank correlation method.

Results

Patients with GHD and controls were matched for age, gender and BMI (Table 1). As shown in Table 2, in relation to controls, patients with GHD showed increased levels of triglycerides ($P=0.0024$) and a tendency for a decrease in HDL cholesterol concentrations ($P=0.0894$). The increment in levels of both total and LDL cholesterol did not approach the statistical significance. Compared with control subjects, GHD patients presented with significant reduced IGF-I concentrations ($P=0.0127$). Using a GH titration regime, the final GH dose was obtained after 2 months GHRT. GHRT led to a significant increase in IGF-I ($P=0.0010$) and to a decrease in total cholesterol ($P=0.0303$) and LDL cholesterol ($P=0.0120$) concentrations, with no effect on levels of triglycerides or HDL cholesterol levels. The number of smokers in GHD versus controls did not differ significantly; in fact there was only one smoker in each group (data not shown).

LDL size was unchanged in GHD patients in relation to controls (269 ± 9 vs 274 ± 6 Å, $P=$ns; Table 3), but LDL subclass analysis revealed that GHD patients had slightly increased LDL-IIB ($P=0.0749$) and more strongly LDL-IIIA particles ($P=0.0046$). Spearman correlation analysis (data not shown) performed in the group of GHD patients revealed that LDL-IIIA was not significantly correlated with age, BMI or plasma lipids; however, the correlation with triglycerides approached the statistical significance ($r=+0.516$, $P=0.0668$). GHRT did not restore LDL size and had no significant impact on LDL size or subclass.

The relationships among the three components of ALP in GHD and GHRT patients were specifically assessed (Figs 1 and 2). In GHD patients, LDL size was inversely correlated with triglyceride levels ($r=-0.701$, $P=0.0111$) and positively but not significantly with HDL cholesterol concentrations ($r=+0.555$, $P=0.0608$). In GHRT patients, LDL size was inversely correlated with triglyceride levels ($r=-0.886$,

Table 1 Clinical characteristics in patients and controls.

								Hormone deficiencies			
Subject no.	**Age** (year)	**Gender**	**BMI** (kg/m^2)	**Diagnosis**	**Surgery**	**DxRT**	**Duration hypopituitarism** (year)	G	T	A	D
Hypopituitary GHD subjects											
1	37	M	28.7	Rathke's cleft	+		7	+	+	+	+
2	60	F	31.5	Sheehan's syndrome			35	+	+		
3	57	M	26.4	Rathke's cleft	+		5	+	+	+	+
4	31	M	24.2	Meningioma	+	+	18			+	
5	36	F	26.7	Craniopharyngioma	+	+	5	+	+	+	+
6	32	F	22.5	Craniopharyngioma	+		16	+	+	+	
7	34	M	24.7	Traumatic hypopituitarism			26	+	+	+	
8	36	M	28.0	Non-functioning adenoma	+	+	16		+	+	
9	51	M	26.3	Non-functioning adenoma	+		3		+	+	
10	36	F	31.5	Macroprolactinoma	+	+	21			+	
11	58	M	29.6	Non-functioning adenoma	+	+	2	+		+	
12	24	M	25.0	Craniopharyngioma	+		1	+	+	+	+
13	62	F	24.9	Non-functioning adenoma	+		2		+	+	
14	45	M	29.2	Craniopharyngioma	+	+	2	+	+	+	
Mean (S.D.)	42.8 (12.5)	9M/5F	27.1 (2.8)				11.4 (10.7)				
Control subjects											
1	41	M	24								
2	43	M	23.5								
3	54	M	26.5								
4	34	M	23.9								
5	47	F	26.6								
6	24	M	23								
7	33	F	20.2								
8	32	M	29.2								
9	50	M	30.6								
10	28	F	22.8								
11	39	M	26.1								
Mean (S.D.)	38.6 (9.4)	8M/3F	25.1 (3.0)								

DxRT, pituitary radiation; hormone deficiencies: G, gonadal; T, T_4; A, adrenal; D, antidiuretic hormone.

Table 2 Biochemical characteristics (mean±S.D.) in patients with growth hormone deficiency before (GHD), after growth hormone replacement therapy (GHRT) and in control subjects.

	Controls ($n=11$)	**GHD** ($n=14$)	**GHRT** ($n=14$)	**GHD versus controls** ($P=$)	**GHRT versus GHD** ($P=$)
Total cholesterol (mmol/l)	4.8±0.8	5.3±1.0	5.0±1.2	ns	0.0303
Triglycerides (mmol/l)	0.7±0.4	1.5±0.7	1.6±1.0	0.0024	ns
HDL cholesterol (mmol/l)	1.7±0.7	1.3±0.2	1.3±0.3	ns (0.0894)	ns
LDL cholesterol (mmol/l)	3.2±0.6	3.7±0.9	3.4±1.0	ns	0.0120
IGF-I (ng/ml)	150.0±46.6	94.6±50.9	181.7±56.0	0.0127	0.0010

$P=0.0001$) and positively with HDL cholesterol concentrations ($r=+0.576$, $P=0.0499$).

We also found (data not shown) in the group of GHD patients high triglyceride levels in six patients (43%), low HDL cholesterol levels in four patients (29%) and elevated small dense LDL in two patients (14%); the prevalence of ALP was 14%. After the replacement therapy, high triglyceride levels were found in seven patients (50%), low HDL cholesterol levels in three patients (21%) and elevated small dense LDL in two patients (14%); the prevalence of ALP was 7%. Only one subject showed high triglyceride levels in the control group.

Discussion

Beyond total LDL cholesterol concentrations, it seems that the quality of LDL exerts a direct influence on the cardiovascular risk (16). Several reasons have been suggested for the atherogenicity of small dense LDL. In relation to larger, more buoyant LDL, small dense LDL are taken up more easily by arterial tissue, have decreased sialic acid content and receptor-mediated uptake, as well as increased oxidative susceptibility and reduced antioxidant concentrations (16, 34, 35). The predominance of small dense LDL has been associated with an approximately threefold increased risk for CAD (20) and it has been accepted as an emerging cardiovascular risk factor by the National Cholesterol Education Program Adult Treatment Panel III (22). Since LDL size seems to be an important predictor of cardiovascular events and progression of CAD, screening for the presence of small dense LDL may potentially identify the subjects with higher vascular risk and may contribute in directing specific interventions of cardiovascular prevention (36).

Hypopituitarism with adult-onset growth hormone deficiency (GHD) bestows elevated cardiovascular risk, with increased cardiovascular morbidity and mortality due to premature and progressive atherosclerosis (1, 2). Lipid metabolism alterations are common in hypopituitary patients with GHD (5–9) and we confirmed in the present study that such patients have increased levels of triglycerides and slightly reduced HDL cholesterol concentrations, while the increment in levels of both total and LDL cholesterol was moderate. Differences in concomitant hormone replacement therapy as well as the small size of study groups may have been confounding factors and may partially explain the lack of significant changes in LDL cholesterol concentrations between GHD patients and controls.

We observed modifications in some parameters (i.e. total and LDL cholesterol) during GHRT which were not affected by GHD. Probably, this is a question of too small a number of subjects to reveal unpaired statistical significance. Total and LDL cholesterol were higher in GHD compared with controls, however, without reaching statistical significance in the unpaired test. Comparison between GHD and GHRT using paired test revealed an improvement in lipid levels, suggesting that patients with GHD were probably hyperlipidaemic for their individual reference range.

Using high-quality methodology, we found that LDL size was unchanged in GHD patients in relation to controls but LDL subclass analysis revealed a shift

Table 3 LDL size and subclasses in patients with growth hormone deficiency (GHD), growth hormone replacement therapy (GHRT) and controls.

	Controls ($n=10$)	**GHD** ($n=12$)	**GHRT** ($n=12$)	**GHD versus controls** ($P=$)	**GHRT versus GHD** ($P=$)
LDL size (Å)	274±6	269±9	270±9	ns	ns
LDL-I (%)	39±8	34±8	33±8	ns	ns
LDL-IIA (%)	21±4	20±3	20±4	ns	ns
LDL-IIB (%)	17±3	21±5	21±5	ns (0.0749)	ns
LDL-IIIA (%)	10±1	12±2	13±5	0.0046	ns
LDL-IIIB (%)	4±1	4±1	5±1	ns	ns
LDL-IVA (%)	7±4	6±2	6±1	ns	ns
LDL-IVB (%)	5±2	5±2	5±2	ns	ns

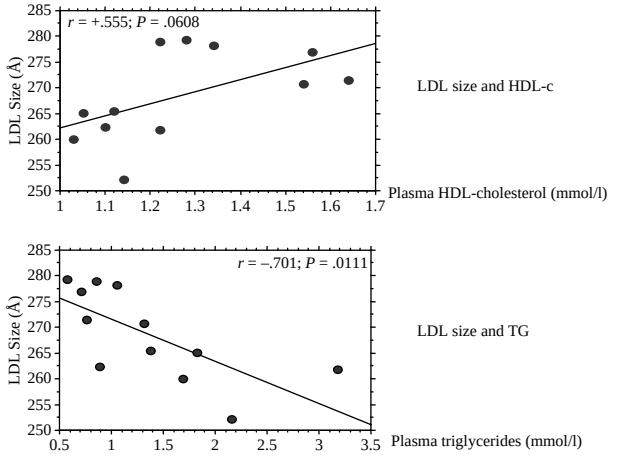

Figure 1 Spearman correlations between LDL peak particle size and plasma triglyceride and HDL cholesterol concentrations in patients with GHD.

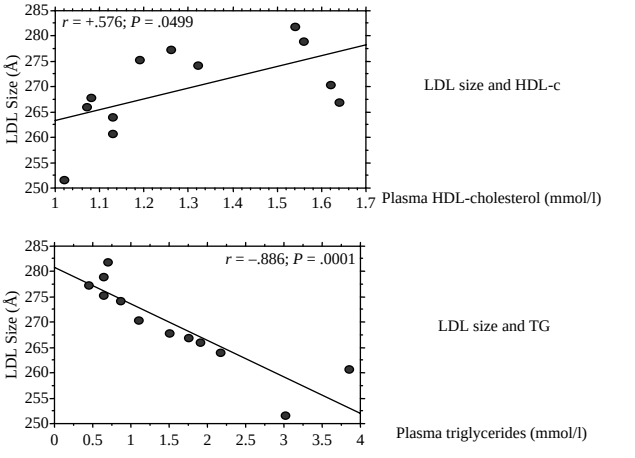

Figure 2 Spearman correlations between LDL peak particle size and plasma triglyceride and HDL cholesterol concentrations in patients with GHRT.

towards more dense LDL particles. The technique used for assessing LDL size is crucial; while, in fact, there is a general agreement on sizing based on electrophoretic mobility, the conclusions are far less clear for other techniques, such as those based on ultracentrifugation or, more so, on nuclear magnetic resonance (37). GHRT resulted in a decrease in total and LDL cholesterol concentrations but did not significantly influence triglycerides or HDL cholesterol concentrations, in keeping with previous results (23, 24). Since no effect was found on HDL cholesterol and triglyceride levels, it is no surprise that GHRT had no significant impact on LDL size or subclasses, since these variables are strongly metabolically related (16, 18). In fact, we showed in both groups of patients with GHD and GHRT that LDL size was inversely correlated with triglyceride levels and positively with HDL cholesterol concentrations. Yet the effect of a longer GHRT remains to be assessed. Since longer periods of treatment led to decreased triglyceride levels and increased HDL cholesterol concentration (38), it is likely that a longer GHRT may also lead to reduced levels of small dense LDL, but this need to be tested in further studies.

Elevated triglyceride levels and reduced HDL cholesterol concentrations constitute with increased small dense LDL the so-called lipid triad or ALP, a form of dyslipidaemia associated with increased cardiovascular risk and the metabolic syndrome (10–13). The metabolic syndrome is related to insulin resistance (22) and insulin resistance is a common feature of hypopituitary patients with GHD (23). The cut-offs for the definition of ALP are still ill-defined and, in order to assess the prevalence of each individual component of ALP, we considered low HDL cholesterol levels those <1.03 mmol/l (e.g. <40 mg/dl) in men and >1.29 (e.g. <50 mg/dl) in women and elevated triglyceride concentrations those >1.69 mmol/l (e.g. >150 mg/dl), as suggested by the National Cholesterol Education Program Adult Treatment Panel III (22). Regarding small dense LDL particles, increased values were defined as small dense LDL concentrations greater than the mean± 2 S.D. of the control values (30).

The present results indicate that individual features of ALP (e.g. high triglycerides, low HDL cholesterol, elevated small dense LDL) may be common (14–50%), but complete ALP is relatively uncommon (14% in GHD and 7% after GHRT). Therefore, it remains to be established whether quantitative and qualitative changes of lipids are the main cause for the increased cardiovascular mortality and morbidity found in hypopituitary patients with GHD (39, 40). Similarly, the lack of effect of GHRT on LDL size in the present study does not support the concept that a possible effect of GHRT on cardiovascular mortality, as recently suggested (41), is related to the effect of GH on lipid metabolism.

In conclusion, using high-quality methodology we found that LDL size was unchanged in hypopituitary patients with GHD in relation to age- and BMI-matched controls, but LDL subclass analysis revealed a shift towards more dense LDL particles. Short-term replacement therapy did not impact on LDL size or subclass, but the effect of longer GHRT remains to be assessed. In addition, we showed that individual features of ALP may be common in hypopituitary patients with GHD and GHRT, but complete ALP is relatively uncommon. However, it might be possible to individualise specific therapeutical interventions if more than the traditional lipids are taken into account. Particularly, the presence of atherogenic small dense LDL may help to improve the assessment of cardiovascular risk and adapt the treatment goals in hypopituitary patients with GHD.

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