PRELIMINARY REPORT

Effects of Growth Hormone Treatment on Very–Low-Density Lipoprotein Apolipoprotein B100 Turnover in Adult Hypopituitarism

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Adult hypopituitarism is associated with hyperlipidemia, mainly due to an increase of very–low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) levels. Recent studies have shown that such patients exhibit increased hepatic secretion of VLDL apolipoprotein B100 (VLDL apo B100). To examine the effects of growth hormone (GH) replacement on VLDL apo B100 turnover, 13 GH-deficient hypopituitary patients (8 women and 5 men; aged 47 ± 3 years, mean ± SEM; body mass index [BMI], 30 ± 2 kg/m^2) entered a double-blind placebo-controlled study for 6 months (GH 0.125 IU/kg/wk for 4 weeks, and then 0.25 IU/kg/wk). GH was subsequently used in all patients for a further 6 months. A 6-hour [1-^{13}C] leucine infusion was administered at baseline and at 6 months. The secretion rate of VLDL apo B100 was derived by kinetic analysis following quantitation of isotopic enrichment by gas chromatography/mass spectrometry. The GH-treated group (6 patients) demonstrated a similar fractional secretion rate (FSR) for VLDL apo B100 at 0 and 6 months. The pool size and absolute secretion rate (ASR) also were unaffected significantly by GH therapy. No significant changes were observed in the placebo group (7 patients). Treatment with GH for 6 months caused an increase in the high-density lipoprotein (HDL) cholesterol concentration (13 patients, 1.27 ± 0.13 *v* 1.16 ± 0.10 mmol/L, respectively, $P = .05$), whereas total cholesterol and triglyceride concentrations did not change. Nonesterified fatty acids (NEFAs) increased during GH therapy (471 ± 43 μmol/L at 6 months *v* 349 ± 49 μmol/L at baseline, $P < .0005$). The data suggest that GH does not affect VLDL apo B100 turnover in a significant way.

HYPOPITUITARY patients on conventional replacement therapy exhibit dyslipidemia,[1] the mechanisms of which are not fully clarified. The recent finding that hepatic production of very–low-density lipoprotein apolipoprotein B100 (VLDL apo B100) is increased[2] and reports of increased vascular mortality[3,4] led us to investigate the effects of growth hormone (GH) replacement in this group of patients. Stable-isotope studies have been used in the past to investigate lipoprotein and apolipoprotein metabolism, and have contributed to our understanding of the pathophysiology of dyslipidemic states such as type II diabetes[5] and obesity.[6] We therefore performed a 6-month stable-isotope study to measure the production rate of VLDL apo B100.

SUBJECTS AND METHODS

Patients

Thirteen adult hypopituitary patients (8 women and 5 men aged 47 ± 3 years; initial body mass index [BMI], 30 ± 2 kg/m^2, mean ± SEM) were recruited from St. Mary's endocrine clinic and adjacent hospitals. The hypopituitarism resulted from pituitary tumor treatment with surgery and/or radiotherapy. All patients had a maximal GH response of less than 6 mU/L on provocative testing (insulin-induced hypoglycemia or glucagon test). The patients were receiving conventional replacement therapy, which was optimized during outpatient appointments and remained unchanged during the study. Seven patients were allocated to receive placebo and 6 received GH treatment for 6 months. Clinical characteristics of the two groups are shown in Table 1.

The study was approved by the Parkside Health Authority Ethics Committee, and all patients provided informed written consent.

GH Therapy

The initial GH dose was 0.125 IU/kg/wk, which increased to 0.25 IU/kg/wk after 4 weeks of treatment. During the study period, adverse effects were recorded (mainly fluid retention and joint pain) and insulin-like growth factor-1 (IGF-1) levels were measured. One patient who was treated with GH during the first 6 months did not continue because of side effects (joint pain), and another patient opted to stop GH treatment at 6 months for reasons unrelated to the study.

GH and placebo vials were identical in presentation. The GH dose was adjusted if necessary to keep the circulating IGF-1 level in the age-related normal range.

Isotopes

[1-^{13}C]leucine (99%) was obtained from Cambridge Isotope Laboratories (Woburn, MA). The leucine was dissolved in 150 mmol/L NaCl, packaged into 5-mL ampoules (10 mg leucine/mL) by the local pharmacy, and tested for sterility and pyrogenicity.

Study Protocol

This was a double-blind placebo-controlled study for 6 months. All but two patients remained on GH treatment for another 6 months after the study. Patients attended the Metabolic Day Ward at St. Mary's Hospital on 2 occasions during the study, at baseline (before initiation of treatment) and 6 months. The infusions started at 9 AM after a 10- to 12-hour overnight fast. Intravenous cannulae were inserted in each arm, one for the infusion and the other for sampling. At each visit (including at 12 months for patients who continued on GH therapy), fasting samples were also taken for estimation of total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, nonesterified fatty acids (NEFAs), VLDL cholesterol, and VLDL triglycerides. During the study, the patients rested quietly in bed and drank only water.

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Table 1. Patient Characteristics

Patient No.	Age (yr)	Sex	BMI (kg/m²)	Duration of HP (yr)	Replacement Medication
Placebo					
1	33	F	25	23	HC, T, SS, D
2	50	F	32	4	HC, T, SS
3	47	M	35	20	HC, T, SS
4	52	M	31	1	HC, T, SS
5	44	M	26	4	HC, SS, D
6	54	F	27	9	SS
7	45	F	42	18	HC, T, SS
Mean	46		31	11	
GH-treated					
1	52	M	35	5	HC, T, SS , D
2	49	F	41	21	HC, T, SS, D
3	29	F	23	5	HC, T, F
4	48	M	22	3	HC, T, SS
5	66	F	30	32	HC, T, SS
6	38	F	27	6	HC, T, SS, D
Mean	47		30	12	

Abbreviations: F, female; M, male; HP, hypopituitarism; HC, hydrocortisone; T, thyroxine; SS, sex steroids; D, desmopressin; F, fludrocortisone.

A 6-hour [1-^{13}C]leucine/saline infusion was administered (at a rate of 1 mg/kg body weight/h) after a priming dose of 1 mg/kg. Samples were collected before tracer infusion (fasting), hourly for the first 5 hours, and half-hourly for the last hour of infusion. Blood (5 mL) was taken using heparinized tubes for analysis of ^{13}C enrichment of plasma α-ketoisocaproic acid (α-KIC), the deamination product of leucine. The plasma was immediately separated by low-speed centrifugation at 4°C for 30 minutes and stored at −70°C until analysis. An additional 10 mL of blood was collected at the same time points in tubes containing 120 μL 10% EDTA for VLDL apo B100 isolation and analysis. Plasma from these samples was also immediately separated. An equal mixture of 5% solution of NaN_3 and gentamycin was added to each EDTA-treated plasma sample (10 μL per 1 ml plasma).

Analytical Procedures

VLDL was separated by ultracentrifugation at a density of 1.006 g/mL for 18 hours at 160,000× *g* with an LKB Bromma 2330 ultraspin centrifuge and an SRP 50 AT rotor (Jouan, Ilkeston, Derbys, UK), according to the method of Havel et al,[7] and delipidated using a mixture of ether/methanol (3:1 vol/vol). VLDL protein was subjected to gradient polyacrylamide gel electrophoresis; the apo B100 band was excised and hydrolyzed in 6 mol/L HCl at 110°C for 24 hours with 1 μg norleucine as an internal standard. The hydrolysate was dried under nitrogen, reconstituted with 50% acetic acid, and transferred to a freshly prepared Dowex AG-8X cationic resin column (Bio-Rad, Richmond, CA). After washing with deionized water, the amino acids were eluted with NH_4OH into glass reactivials (Wheaton, Mays Landing, NJ) and dried under nitrogen. The amino acid residues were reacted with acetonitrile and *N*-methyl-*N*-(*tert*-butyldimethylsilyl)triflouroacetamide to form the bis-derivative.[8]

Isotopic enrichment of α-KIC was determined by the method of Ford et al.[9] Enrichment of both leucine and α-KIC was quantified using a Varian 3400 gas chromatograph/Finnigan Incos XL mass spectrometer (Thermoquest, Hemel Hempstead, UK) in electron-impact mode under computer control.[8]

Measurement of Stable Isotope Enrichment

Selective ion monitoring of the derivatized samples at *m/z* 302 for unlabeled leucine, *m/z* 303 for labeled leucine, *m/z* 232 for unlabeled α-KIC, and *m/z* 233 for labeled α-KIC was used to determine isotopic abundance. The atom percent excess (APE) enrichment was calculated using the formula enrichment (APE) = $(IR_t - IR_0)/(IR_t - IR_0 + 100)$, where IR_t is the isotope ratio of the sample at time t and IR_0 is the isotope ratio of the sample at time zero (before [1-^{13}C]leucine administration). The enrichment of plasma α-KIC (precursor pool) was obtained similarly. This method of calculation is equivalent to the method of Cobelli et al.[10] The raw APEs for plasma leucine and α-KIC enrichment were converted to moles percent excess (MPEs) by application of the calibration enrichment curve slopes, obtained by regression analysis of the plot of the theoretical MPE against the observed APE.

Calculation of Apo B100 Production

The fractional secretion rate (FSR) of VLDL apo B100 (pools per hour) was determined by fitting the monoexponential function to the enrichment data,[11] $E(t) = P(1 - e^{-k(t-d)})$, where E(t) is the enrichment at time t, P is the plateau enrichment (α-KIC precursor enrichment), k is the FSR of apo B100, and d is the intrahepatic delay time (Fig 1). Absolute secretion rates (ASRs) of VLDL apo B100 were calculated as

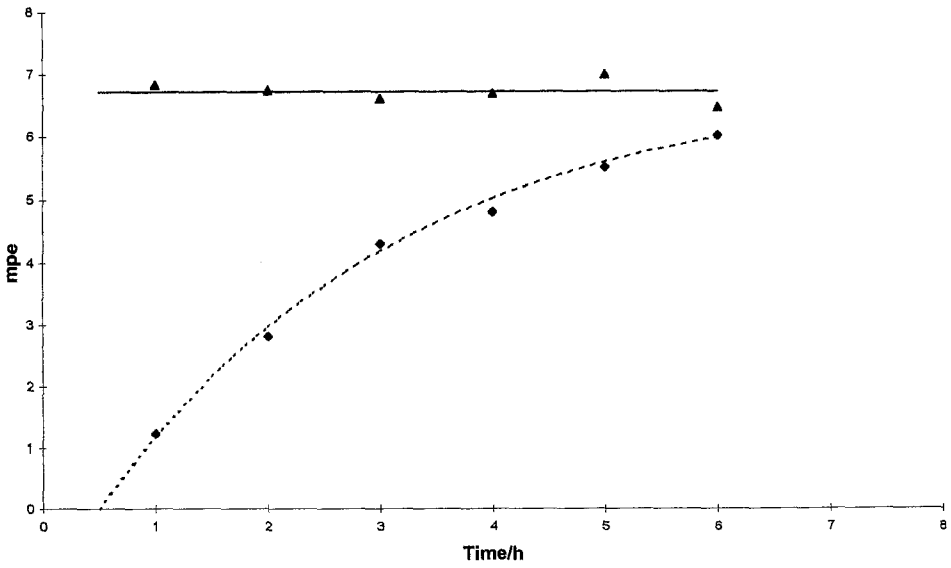

Fig 1. Time course of tracer leucine enrichment in VLDL apo B100 (◆) and α-KIC (▲).

the product of the FSR and pool size. Pool size was derived by multiplying the plasma volume (4.5% of body weight) and VLDL apo B100 concentration.[12] The VLDL concentration was measured by enzyme-linked immunosorbent assay using calibration serum (Boehringer Mannheim, Lewes, East Sussex, UK). All measurements were performed in triplicate, and the intraassay and interassay coefficient of variation was 5% and 7%, respectively.

Other Assays

Total cholesterol, triglyceride, and HDL cholesterol levels were measured enzymatically using an Olympus AU 5200 analyzer (Eastleigh, Hants, UK). Fasting VLDL cholesterol and VLDL triglyceride levels were measured enzymatically using a centrifugal analyzer (COBAS-BIO, Welwyn, Garden City, UK).[13] NEFA levels were also measured with an enzymatic method on the centrifugal analyzer.[14]

Statistical Analysis

Data are expressed as the mean ± SEM or median (range) where appropriate. Results at baseline and during treatment were compared using Student's paired *t* test. Comparisons of the placebo and GH groups during the first phase were performed using the Mann-Whitney test.

RESULTS

At the end of 6 months, the mean GH dose was 2.5 IU/d. IGF-1 levels significantly increased in the GH-treated group (from 8.6 ± 7.0 nmol/L at baseline to 31.2 ± 10.8 nmol/L at 6 months, $P < .01$), within the normal range for age, and remained unchanged in the placebo group (from 9.0 ± 5.4 to 9.3 ± 5.0 nmol/L, respectively).

In both the placebo and GH-treated groups, concentrations of total, VLDL, and HDL cholesterol were not significantly different during the 6-month period (Table 2). VLDL triglycerides showed a small but significant decline ($P = .04$) in the placebo group, but total plasma triglyceride levels were unaffected. No significant changes were observed in plasma NEFAs in the placebo group, but a significant increase was noted in the GH-treated group ($P < .005$). No significant changes were found in the kinetic parameters in the placebo-treated group (Table 2). The FSR, ASR, and pool size also were not significantly different in the GH-treated group during the 6-month period. When the GH-treated and placebo groups were compared, no significant differences were observed for any parameter except plasma triglycerides ($P = .04$ for differences between groups).

When the placebo-controlled and open-phase data were combined to show the effects of GH in all 13 patients following treatment for 6 months, HDL cholesterol increased significantly on GH therapy (1.27 ± 0.13 *v* 1.16 ± 0.10 mmol/L, $P = .05$; Table 3). NEFA concentrations also increased significantly (471 ± 43 μmol/L at 6 months *v* 349 ± 49 μmol/L at baseline, $P < .0005$). VLDL cholesterol and VLDL triglycerides did not change significantly during GH treatment, although VLDL triglyceride showed a tendency to increase (0.36 ± 0.06 mmol/L at 6 months *v* 0.26 ± 0.02 mmol/L at baseline, $P = .08$; data not shown).

DISCUSSION

In this study, total and LDL cholesterol concentrations did not change during GH treatment, in accordance with some studies,[15] although most reported studies have shown reductions in both total and low-density lipoprotein (LDL) cholesterol.[16,17] Although they were not selected on the basis of lipid status, the patients were not markedly dyslipidemic at the outset and this may have contributed to the lack of changes. Patients were not separated according to the degree of dyslipidemia, as their number was small and individual variation was not wide. There was a small GH-induced, statistically nonsignificant increase in serum triglyceride levels, in accordance with other studies.[16,17] There was a small statistically significant decrease in VLDL triglycerides with placebo, but the change over 6 months was not significantly different in the GH-treated and placebo groups. The increase of HDL cholesterol observed in the current study, although not a consistent finding,[16] has been reported previously.[15,17] Baseline NEFA levels were lower than the values in the normal population, as observed in other studies of hypopituitary patients,[18] and the NEFA increase with GH therapy is in keeping with the lipolytic effects of GH.[19] Circulating NEFAs are a substrate for VLDL triglyceride synthesis. However, despite the GH-induced increase in substrate supply, VLDL secretion was not affected, as indicated by the similar VLDL apo B100 ASR throughout the study period.

We have demonstrated previously that hypopituitary adult patients have an accelerated VLDL apo B100 ASR[20] despite

Table 2. Lipid and Kinetic Data From the First Phase of the Study

Parameter	GH-Treated (n = 6)		Placebo-Treated (n = 7)	
	Baseline	6 Months	Baseline	6 Months
Total cholesterol (mmol/L)	5.20 (3.20-6.50)	4.83 (3.65-6.29)	6.00 (5.20-7.20)	5.64 (4.62-6.67)
Triglycerides (mmol/L)	0.95 (0.44-2.37)	1.09 (0.71-2.19)	1.80 (1.11-6.75)	1.51 (0.68-5.21)†
HDL cholesterol (mmol/L)	1.10 (0.80-1.40)	1.15 (0.96-1.47)	1.10 (0.40-1.6)	1.12 (0.62-1.87)
VLDL cholesterol (mmol/L)	0.11 (0.06-0.14)	0.15 (0.06-0.30)	0.15 (0.09-1.36)	0.10 (0.07-0.48)
VLDL triglycerides (mmol/L)	0.29 (0.21-0.39)	0.29 (0.11-0.34)	0.36 (0.14-0.64)	0.26 (0.06-0.34)*
NEFAS (μmol/L)	254 (133-340)	390 (285-473)*	340 (166-463)	384 (228-688)
FSR (pools/h)	0.53 (0.30-0.59)	0.36 (0.32-0.63)	0.34 (0.11-0.51)	0.33 (0.15-0.67)
Pool size (mg/kg)	2.72 (1.63-3.71)	2.15 (0.76-9.15)	3.75 (1.57-5.18)	3.56 (1.27-12.57)
ASR (mg/kg/d)	31.9 (21.3-42.1)	25.3 (6.2-74.7)	19.2 (11.4-42.3)	30.0 (14.7-45.3)

NOTE. Values are the median (range).

*$P < .05$, 6 months *v* baseline.

†$P < .05$ for differences between groups.

Table 3. Results of 6 Months of GH Treatment for All 13 Patients

Parameter	Baseline	6 Months
Total cholesterol (mmol/L)	5.29 ± 0.28	5.46 ± 0.31
Triglycerides (mmol/L)	1.76 ± 0.46	2.17 ± 0.65
LDL cholesterol (mmol/L)	3.33 ± 0.24	3.21 ± 0.27
HDL cholesterol (mmol/L)	1.16 ± 0.10	1.27 ± 0.13*
NEFAS (μmol/L)	349 ± 49	471 ± 43†

NOTE. Values are the mean ± SEM.
*$P = .05$.
†$P < .0005$.

lower NEFA levels compared with normal control subjects. Other investigators have also demonstrated an elevated VLDL apo B100 ASR in hypopituitary adults.[2] The current data show that treating such patients with GH restores NEFA levels to normal but does not decrease VLDL apo B100 secretion, suggesting that GH alone is not responsible for the increased VLDL particle secretion of hypopituitarism.

GH administration has been demonstrated to induce hepatic LDL receptors,[21] although these studies were performed in normal subjects and the GH administered dose was high. There is no information on the number of hepatic LDL receptors and the effects of GH treatment in hypopituitary patients. A reduction in postheparin plasma lipoprotein and hepatic lipase activity has been noted in prepubertal children treated with GH.[22] Subsequent studies of GH therapy in GH-deficient adults showed a decrease in adipose lipoprotein lipase (LPL) activity, no effect on postheparin plasma LPL, and an increase in hepatic lipase activity.[23] Other studies have shown an increase in both postheparin LPL and hepatic lipase activities in adult hypopituitary patients.[24] Although GH is involved in the regulation of these enzymes, no effect was observed in the present study on the clearance of VLDL particles in vivo as reflected by the VLDL apo B100 FSR (at steady state, the FSR and catabolic rate are the same). However, the small number of patients may be a factor in the lack of statistical significance of the results.

In conclusion, we have shown that GH replacement in adult hypopituitarism appears not to affect VLDL apo B100 turnover significantly, and other causes for the increase in VLDL particle secretion need to be examined.

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