Effects of Short- and Long-Term Growth Hormone Replacement on Lipoprotein Composition and on Very–Low-Density Lipoprotein and Low-Density Lipoprotein Apolipoprotein B100 Kinetics in Growth Hormone–Deficient Hypopituitary Subjects

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In this study, we concurrently examined the effects of 8 and 40 weeks of growth hormone replacement (GHR) on lipids, lipoprotein composition, low-density lipoprotein (LDL) size, very–low-density lipoprotein (VLDL) apolipoprotein (apo)B kinetics and LDL apoB kinetics. Eight weeks of GHR did not alter lipid profiles. Forty weeks of GHR increased high-density lipoprotein-cholesterol (HDL-C) concentration ($P = .01$), nonsignificantly reduced LDL-C ($P = .06$), and reduced the HDL/LDL-C ratio ($P = .04$). Forty weeks of GHR increased HDL free cholesterol ($P = .03$), total cholesterol ($P = .01$), and cholesterol ester ($P < .01$) concentrations. No other significant changes in VLDL, LDL, or HDL composition or LDL size were noted at any time. Eight weeks of GHR reduced VLDL apoB absolute secretion rate (ASR, $P = .03$), with nonsignificant reductions in fractional secretion rate (FSR, $P = .09$) and pool size ($P = .09$). After 40 weeks of GHR, the VLDL apoB ASR, FSR, and pool size were not significantly different from baseline. Forty weeks of GHR increased both LDL apoB FSR ($P = .02$) and LDL apoB ASR ($P = .04$), with a small decrease in pool size. Thus, GHR may have important antiatherogenic effects; HDL-C increased, LDL-C was nonsignificantly reduced, the total/HDL-C ratio was reduced, VLDL apoB production was reduced, and LDL apoB turnover was increased.

THE INCREASED cardiovascular mortality demonstrated in hypopituitary subjects[1-3] may, in part, be attributable to the proatherogenic dyslipidemia associated with this condition. Most,[4-7] but not all studies,[8-11] have demonstrated elevated total and low-density lipoprotein (LDL) cholesterol concentrations. High-density lipoprotein-cholesterol (HDL-C) concentrations have usually been unchanged[5,7,10] or decreased.[4,9,11,12] Hypertriglyceridemia has been observed in most, but not all studies.[10] Growth hormone deficiency (GHD) may be etiologically important in this dyslipidemia, as suggested by the effect of growth hormone replacement (GHR). Most studies have demonstrated a reduction in total and LDL-C concentrations,[12-15] but some report no change.[11,16-18] HDL-C concentrations have been increased in some studies,[11,13,17,19,20] but unaltered in other[12,14,15,21,22] studies. GH has persistently failed to ameliorate hypertriglyceridemia, in all but one study,[21] perhaps suggesting an alternative etiology. Some studies[12,20,23] have suggested that the lipid-lowering effect of GHR is maximum in those subjects with the most pronounced dyslipidemia at baseline. However, interpretation of such studies is complicated statistically by regression to the mean.

The atherogenic potential of lipoproteins may be determined not only by their plasma concentrations, but also by their composition and size. Triglyceride enrichment of lipoproteins may confer an increase in atherogenicity.[24-29] Austin et al[30] demonstrated a 3-fold increase in the risk of myocardial infarction in subjects with small, dense (triglyceride-enriched) LDL. Such changes have been observed even in the presence of a normal lipid profile.[31] While very–low-density lipoprotein (VLDL) may be atherogenic per se, in that it may cross the vascular intima and act in a similar way to oxidized LDL,[32] triglyceride-enriched VLDL may be particularly atherogenic as, in addition to this, it may predispose to the formation of small, dense LDL.[31] The composition of lipoproteins in hypopituitarism has rarely been examined. Capaldo et al[33] reported LDL triglyceride-enrichment in childhood onset GHD, and we have previously reported triglyceride enrichment of VLDL in hypopituitary adult.[11] LDL particle size may also be reduced.[9] The effect of GHR on lipoprotein composition and size has rarely been assessed. Christ et al[22] reported a GH-induced reduction in VLDL cholesterol, but this was not substantiated by Chrisoulidou et al.[18] Webster et al[34] demonstrated a decrease in LDL phospholipid, total protein, total, and free cholesterol concentrations after 6 months of GHR, with a return to pretreatment levels by 12 months. Hew et al[35] reported increased LDL size after 1 year of GHR, while O'Neal et al[36] demonstrated a return to baseline after 2 years of GHR. The mechanisms underlying these observations remain unclear. Two groups of investigators[10,37] have used stable isotope studies to examine VLDL apolipoprotein (apo)B metabolism in GHD patients. Both[10,37] demonstrated increased VLDL apoB secretion. A similar technique was used by Christ et al[22] to examine the effects of 3 months of GHR on VLDL kinetics. Increases in both VLDL apoB secretion and clearance rates were observed with no net change in pool size. However, in our own studies,[18] 6 months of GHR did not induce any significant changes in VLDL apoB kinetics. The different duration of therapy may explain some of these discordant results. Stable isotope studies have been used to examine LDL kinetics in other conditions,[38,39] but not in hypopituitarism. The slow

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turnover of LDL necessitates prolonged isotope infusions, limiting the application of this technique. However, by increasing the concentration of stable isotope infused, the isotopic enrichment of LDL apoB can be increased to measurable levels with an 8-hour infusion. Using this technique, we examined the effect of GHR on LDL apoB kinetics in a subgroup of GHD patients. Thus, the studies examining the effects of GHR on lipid metabolism have been far from conclusive. Therefore, we aimed to simultaneously study the effect of GHR on plasma lipid concentration, lipoprotein composition, LDL particle size, VLDL, and LDL apoB kinetics in hypopituitary patients. To explore the hypothesis that the effects of GHR on lipid metabolism are, in part, determined by the duration of therapy, we have examined these parameters at 2 different time points.

METHODS AND SUBJECTS

Subjects

Sixteen GHD hypopituitary subjects were randomly recruited from the endocrine clinic at St. Mary's Hospital, London, UK. The same subjects were described in a previous report.[11] The clinical characteristics, etiology, and treatment of pituitary pathology are reported in Table 1. Fourteen subjects were ACTH-deficient. Hydrocortisone replacement was instituted in all cases and was assessed with a 5-hour cortisol profile measurement. Fourteen subjects received thyroxine (T_4) replacement, titrated to maintain the free T_4 concentration within the normal range. Eight males were hypogonadal, and all received testosterone replacement (6 intramuscularly, 2 transdermally). Six females were hypogonadal, 5 of whom received replacement. One postmenopausal female elected not to receive sex hormone replacement therapy. All patients had a stimulated GH response to hypoglycemia (performed in 15 subjects) or glucagon (performed in 1 subject) of less than 6 μ/L, in association with a low insulin-like growth factor (IGF)-1 concentration, confirming GHD. The study received ethical approval from the Parkside Health Authority, and all subjects gave written informed consent.

Clinical Procedures

Study design. The study consisted of 3 separate steps. The first stage comprised a "run-in" period of 8 weeks, during which subjects were encouraged to follow their normal daily routine. The first baseline stable isotope infusion study was performed at the end of this phase. The second stage consisted of an 8-week double-blind, placebo-controlled phase of GH therapy. Randomization was performed by the hospital pharmacy. Subjects who had received GH, but not those who had received placebo, underwent a second stable isotope infusion to assess the effects of 8 weeks of GH therapy. In the third open phase of the study, all patients received GH therapy, and a final stable isotope infusion was performed on completion of this phase, 40 weeks after initiation of GH therapy. Two patients did not complete the third phase (subject 8 died of a traumatic head injury and subject 6 left to pursue employment elsewhere).

GH administration. GH (Pharmacia-Upjohn, Milton Keynes, UK) was given by subcutaneous injection nightly into the thigh. Treatment began at a dose of 0.125 μ/kg/wk (0.04 mg/kg/wk) and was increased after 2 weeks to 0.25 μ/kg/wk (0.08 mg/kg/wk). Doses were then titrated twice weekly to maintain the IGF-1 levels in the third quartile of the sex- and age-matched normal range. The appearance of the placebo preparation (Pharmacia-Upjohn) was indistinguishable from that of GH. The mean GH dose required by men was 0.26 $\pm$ 0.1 mg/d or 0.7 $\pm$ 0.03 IU/d and by women, it was 0.38 $\pm$ 0.02 mg/d or 1.15 $\pm$ 0.05 IU/d. IGF-1 concentrations were measured 4 times weekly. One subject (subject 9) experienced peripheral edema, which resolved spontaneously without a reduction in GH dose. No other side effects were noted.

Anthropometric assessment. Body mass index (BMI, calculated as total body weight/height squared) and waist-to-hip ratio (WHR, measured as the narrowest waist/widest hip measurement) were assessed at baseline, after 8 and 40 weeks of treatment.

Blood sampling. Subjects were seen 4 times weekly to assess adequacy of endocrine replacement, to optimize GH dose, and to monitor side effects. On the day of the stable isotope infusions, blood was also taken for the estimation of fasting lipids, glucose, specific

Table 1. Cause and Treatment of Hypopituitarism

Case	Sex/Age	Diagnosis	TSS	R/T	HC (mg)	T_4 (μg)	Sex Hormone Replacement	Duration of GHD (yr)
1	M/50	Idiopathic hypopituitarism	N	N	30	100	Testosterone 25 mg IM 3/52	2
2	M/48	Craniopharyngioma	Y	Y	30	50	Testosterone 250 mg IM 3/52	4
3	M/37	Congenital isolated GHD	N	N	N	N	N	27
4	F/32	Chromophobe adenoma	Y	N	20	100	Levonorgestrel 75 μg Estrogen 625 μg po	3
5	M/34	Chromophobe adenoma	Y	Y	30	100	Testosterone patch 5 mg/d	2
6	F/64	Chromophobe adenoma	Y	Y	N	100	N	6
7	F/32	Idiopathic hypopituitarism	N	N	10	100	Levonorgestrel 75 μg Estrogen 625 μg po	7
8	M/63	Chromophobe adenoma	Y	Y	40	200	Testosterone 250 mg IM 3/52	18
9	F/33	Prolactinoma	Y	N	20	150	N	12
10	M/38	Pinealoma	Y	Y	20	100	Testosterone 250 mg IM 3/52	24
11	F/45	Congenital hypopituitarism	N	N	20	100	Levonorgestrel 75 μg Estrogen 625 μg po	45
12	M/40	Chromophobe adenoma	Y	Y	20	50	Testosterone patch 5 mg/d	2
13	F/52	Chromophobe adenoma	Y	Y	20	150	Levonorgestrel 75 μg Estrogen 625 μg po	32
14	F/31	Chromophobe adenoma	Y	N	15	175	N	3
15	M/68	Pituitary apoplexy	Y	N	30	100	Testosterone 250 mg IM 3/52	2
16	M/24	Chromophobe adenoma	Y	Y	20	N	Testosterone 250 mg IM 3/52	2

Abbreviations: TSS, transphenoidal surgery; R/T, radiotherapy; T/4, thyroxine (μg); HC, hydrocortisone (mg); po, oral.

insulin, nonesterified fatty acids (NEFAs), and plasma total apoB concentrations. Heparinized blood (12 mL) was additionally taken at baseline and after 40 weeks of GHR to assess HDL composition. Stable isotope studies were performed (as described below) in all patients (n = 16) at baseline, in 8 patients after 8 weeks of GH, and in 14 patients after 40 weeks of GHR. VLDL apoB kinetics have been determined from the stable isotope infusions in all cases. At present, LDL apoB kinetics have been determined in only 8 patients before and after 40 weeks of GH therapy and in 4 patients after 8 weeks of therapy. The remaining samples are stored for future analysis.

Determination of VLDL and LDL apoB Kinetics

Extension of the stable isotope method to examine LDL apoB kinetics. Previous investigators[39,40] examining LDL kinetics with 1 mg/kg leucine have found that infusions of 12 hours are required to obtain measurable LDL isotopic enrichment (IE). Arends et al[41] demonstrated a linear relationship between the concentrations of leucine infused (≈ 1 to 5 mg/kg) and the LDL apoB IE attained, suggesting that measurable LDL apoB IEs could be obtained with shorter infusions by increasing the concentration of infusate. In our preliminary studies, infusing leucine at a higher concentration of 2 mg/kg/h, achieved LDL apoB IEs of approximately 0.6 to 1.0 moles per excess (mpe) after 8 hours. Steady state conditions were maintained, as demonstrated by stable concentrations of apoB, ketoisocaproic acid (KIC), and leucine throughout the infusions. It has previously been suggested that infusing higher concentrations of leucine may have metabolic effects. However, leucine infusion rates resulting in up to 10% of total leucine rate of appearance do not to affect leucine metabolism,[41] while infusions resulting in 15% enrichment may increase leucine oxidation, with no effect on whole body protein synthesis or degradation.[42] An increase in whole body protein synthesis has only been demonstrated with much higher leucine infusion rates.[43] Arends et al[41] demonstrated no change in plasma glucose or insulin concentrations with leucine infusions of 2 mg/kg/h. In addition, the calculated leucine flux rates were similar irrespective of the concentration of leucine infused.[41] In our own validation studies, insulin and glucose concentrations remained constant throughout the infusions. Therefore, it seems unlikely that the increase of leucine to 2 mg/kg/hr will have any metabolic effect.

Stable isotope infusion protocol. 1-C^{13}-leucine (99%) was obtained from Cambridge Isotope Laboratories (Woburn, MA). It was dissolved in 150 mol/L NaCl, packaged in 5-mL ampules (10 mg leucine/mL) and tested for sterility and pyrogenicity. Subjects were admitted to St. Mary's Hospital at 7:30 AM after a 12-hour fast. A primed (2 mg/kg), constant (2 mg/kg) infusion of ^{13}C-leucine/saline was administered peripherally, with sampling at baseline and hourly thereafter for 8 hours. At each time point, 5 mL blood was drawn into heparinized tubes. Plasma was immediately separated by centrifugation at 900 *g* at 4°C for 30 minutes and stored at -70°C for analysis of ^{13}C-enrichment of α-ketoisocaproic (α-KIC), the deamination product of leucine and a measure of apoB precursor enrichment. At identical time points, 12 mL blood was drawn into tubes with 120 μL 10% EDTA for the isolation and analysis of VLDL apoB100. Plasma from these samples was separated as above, and an equal mixture (50 μL) of 5% NaN3 and 5% gentamicin was added. The plasma was immediately processed for the separation lipid fractions, as described below.

Laboratory Procedures

Isolation, purification, and derivatization of VLDL and LDL apoB. Plasma VLDL was separated by ultracentrifugation at a density of 1,006 g/mL for 18 hours at 160,000 *g* with a LKB (Bromma, Sweden) 2330 ultraspin centrifuge and a SRP (LKB) 50AT rotor according to the method of Havel et al.[44] VLDL was retrieved by tube splicing and was frozen at –70°C until required. The density of the infranatant plasma was adjusted to 1,063 g/mL with potassium bromide and centrifuged as described above. LDL was retrieved as above, subjected to dialysis, and frozen at –70°C until required. Aliquots of VLDL (50 μL) and LDL (20 μL) were later delipidated using a mixture of ether/methanol (3:1 vol/vol). The VLDL and LDL protein was subjected to gradient polyacrylamide gel electrophoresis (5% to 15%), the apoB band was excised and hydrolyzed in 2.0 mL of 6 N HCL at 110°C for 24 hours, with 40 μL norleucine as the internal standard. The hydrolysate was dried under nitrogen, reconstituted in 0.5 mL 50% acetic acid, and transferred to freshly prepared AG 50W-X8 cationic resin columns (BioRad Laboratories, Hercules, CA). After washing with deionized water, the amino acids were eluted with 3 mol/L NH_4OH into glass reactivials, and dried under nitrogen. The amino acid residues were reacted with acetonitrile and n-methyl-n-(tert-butidylmethylsilyl)-trifluoracetamide to form the bis (tert-butyldimethylsilyl) derivative, in preparation for mass spectrometry.

Derivatization of α-KIC. Isotopic enrichment of α-KIC was determined using the method of Ford et al.[45] α-Ketovaleric acid internal standard solution (50 μL) was added to 100 μL plasma and deproteinized with 1 mL of ethanol. After centrifugation, the supernatant was decanted into reactivials and evaporated to dryness under nitrogen. The residue was dissolved in phenylenediamine solution (0.2% wt/vol) and deionized water. The coupled ketoacids were extracted with ethyl acetate and dried over sodium sulfate. The dried residue was derivatized with 50 μL acetonitrile and 50 μL N,O-bis (trimethylsilyl)-trifluoroacetamide.

Measurement of stable IE. The IE of both leucine (derived from VLDL and LDL) and α-KIC was measured using a Varian 3400 gas chromatograph/Finnigan Incos XL mass spectrometer (Thermoquest, Hemel Hempstead, UK) in electron impact mode under computer control. 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 per excess (APE) enrichment of both leucine and α-KIC was calculated with a method similar to that of Cobelli et al,[46] using the formula:

$$IE\ (APE) = (IR_t - IR_0/IR_t - IR_0 + 100) \times 100$$

where IR_t = isotopic enrichment at time 't', IR_o = isotopic enrichment at baseline. The raw APEs of plasma leucine and α-KIC enrichments were converted to mole per excess (MPE) by the application of a calibration curve obtained by regression analysis of the plot of the theoretical MPE against observed APE.

Calculation of apoB kinetic rates. The IEs of VLDL and LDL were represented graphically. The fractional synthetic rate (FSR) of VLDL apoB was then determined by fitting monoexponential VLDL IE curves, using:

$$E(t)_{VLDL} = P(1 - e^{-k(t-d)})$$

where E(t) = the enrichment at time t, P = α-KIC enrichment, d = intrahepatic delay time, k = FSR of VLDL apoB. The LDL apoB IE data were analyzed using the results of a simple, multiple pool, first order kinetics, cascade model. The synthesis of LDL was assumed to be a first order reaction depending on the initial concentration of VLDL. Hence, LDL apoB FSR was determined with a double exponential, using the equation:

$$E(t)_{LDL} = P\ (1 - \exp[-k(t - d)]/[1 - k/L] - \exp[-tL]/[1 - L/k])$$

where L is LDL FSR (other abbreviations, as above). These equations were fitted to the measured enrichment data using a simplex method to determine the 2 time constants with the smallest square error.[47] The use of a simple cascade model for the determination of VLDL and LDL apoB kinetics does not imply that the synthetic pathways are simple. The rate constants should instead be interpreted as the effective rate

constants over all synthetic pathways. In this model, the VLDL ASR represents VLDL hepatic secretion rate and the LDL ASR represents VLDL-dependent LDL production rate. This model assumes that LDL is derived only from VLDL, assumes unidirectional transfer of labeled leucine, and does not allow for recycling of leucine. However, the model does provide a good fit to the data and we cannot see any justification for introducing a more complex model to explain or describe our results. At steady state, the FSR of apoB equals the fractional catabolic rate (FCR). The absolute synthetic rates (ASR) of VLDL and LDL apoB were calculated as the products of FSR and pool size. The latter was determined as the product of the plasma volume and the apoB concentration. The plasma volume was estimated to be 4.5% of total body weight (kg).[48] The plasma volume is reduced by approximately 0.3 L in GHD. Errors incurred by this assumption will therefore be negligible.

Quantification of VLDL, plasma and LDL apoB concentrations. Plasma and VLDL apoB concentrations were determined in samples taken after 1, 3, and 5 hours of stable isotope infusions with a commercially available kit (Alpha Laboratories, Eastleigh, UK) using the COBAS MIRA S centrifugal analyzer. The LDL apoB concentration was calculated as the difference between the plasma and VLDL apoB concentration. The contribution of IDL apoB to the total plasma pool was on the order of 1% to 2% and was ignored.

Determination of lipoprotein composition and LDL particle size. The total cholesterol, cholesterol ester, triglyceride, and phospholipid concentrations of VLDL, LDL (obtained from the stable isotope infusion) and HDL (obtained from heparinized blood using the magnesium chloride/dextran sulphate precipitation method) were determined colorimetrically using commercially available kits (Wako, Alpha Laboratories, Neuss, Germany). Free cholesterol was calculated as the difference between the total and free cholesterol concentrations. As each molecule of VLDL and LDL contains 1 molecule of apoB, the molar ratios of lipids to apoB were used to reflect lipid enrichment of lipoproteins. LDL particle size was determined using a commercially available LFS Lipogel assay kit (Zaxia, Hudson, OH), which allowed the separation of lipoproteins in human plasma using a gradient polyacrylamide gel. This method has previously been validated by Krauss et al.[49]

Other assays. Plasma concentrations of total cholesterol, triglyceride, and HDL-C were measured enzymatically using an Olympus AU 5200 analyzer (Eastleigh, Hants, UK) by the Chemical Pathology Department. LDL-C concentration was estimated using the Friedwald equation. Fasting glucose concentrations were measured using the hexokinase method. Intact insulin concentration was measured using a specific 2-site antibody enzyme-linked immunosorbent assay (ELISA) method, developed within our own department.[50] Homeostatic models assessment (HOMA) was used to estimate insulin sensitivity (%S) and β-cell function (%B). Serum IGF-1 concentrations were measured using the Nichols Advantage Chemiluminescence immunoassay. Plasma NEFA[51] levels were determined with a enzymatic kit (WAKO, Alpha Laboratories).

Statistical Analysis

Normally distributed data (age, sex, BMI) are described using the mean and the standard error of the mean (SEM). All other data were nonparametric in distribution and are described using the median and interquartile range (IQR). As groups differed at each time point, analysis of covariance was not performed. Parametric data are compared using a matched student *t* test; nonparametric data are compared using the Wilcoxon Rank test. Spearman's rank correlation test was to determine the strength of relationship between nonparametric data. The study was powered to determine a 10% difference at 80% level. Statistical significance was assumed at a 5% level. The effects of 8 weeks of GH replacement on anthropometric data and plasma lipids were assessed by comparison with the placebo group and not compared with baseline. All other data obtained after GH administration were compared with baseline.

RESULTS

Effect of GHR on Anthropometric Indices

Eight weeks of placebo-controlled trial. At baseline, the placebo and GH group were well matched for age ($P = .26$), weight ($P = .78$), BMI ($P = .77$), and WHR ($P = .59$) (Table 2). After 8 weeks of therapy, weight ($P = .81$), BMI ($P = .81$) and WHR ($P = .63$) was similar in the placebo compared with the GH-treated group.

Forty weeks of GHR. Compared with baseline, no significant changes in weight, WHR, or BMI were observed ($P = .81$, .71, .72, respectively).

Effect of GHR on IGF-1 Concentrations

Eight weeks of placebo-controlled trial. There was no significant difference between the IGF-1 concentration of the placebo and GH groups at baseline ($P = .32$) (Table 3). After 8 weeks, IGF-1 concentrations were higher in the GH group compared with the placebo group (29.95 [8.4 to 40.95] *v* 10.65 [6.23 to 11.55] nmol/L, $P = .03$).

Forty weeks of GHR. IGF-1 concentration significantly increased from 9.65 (4.28 to 12.57) to 33.20 (30.25 to 46.13) nmol/L, $P = .001$.

Effect of GHR on Plasma Lipid and NEFA Concentrations

Eight weeks of placebo-controlled trial. At baseline, the plasma total, LDL, and HDL cholesterol concentrations were similar ($P = .60$, .67, .79, respectively) in the 2 groups, as were triglyceride and NEFA concentrations ($P = .75$, 0.75) (Table 4). Compared with placebo, 8 weeks of GH did not induce any changes in the triglyceride, total cholesterol, LDL, and HDL cholesterol concentrations ($P = .79$, .40, .60, .83, respectively) or in the LDL/HDL cholesterol ratio ($P = .16$).

Forty weeks of GHR. Compared with baseline, GHR induced a significant increase in HDL-C concentration (0.98 [0.82 to 1.18] to 1.02 [0.92 to 1.24] mmol/L, $P = .01$), with a nonsignificant reduction in LDL-C concentrations (3.16 [2.33 to 4.41] to 2.73 [1.86 to 3.59] mmol/L, $P = .06$). Total cholesterol concentration remained similar ($P = .98$), and the LDL/HDL cholesterol ratio was significantly reduced (3.18

Table 2. Effect of GHR on Anthropometric Data

	T = 0			T = 8 Weeks of GHR			T = 40 Weeks of GHR		
	TBW	BMI	WHR	TBW	BMI	WHR	TBW	BMI	WHR
Mean ± SEM	81 ± 5	28 ± 1	1.0 ± 0.1	80 ± 5	28 ± 1	1.0 ± 0.1	80 ± 5	29 ± 2	1.0 ± 0.1

Abbreviations: TBW, total body weight (kg); BMI, body mass index (kg/m^2); WHR, waist/hip ratio (mm Hg).

Table 3. Effect of GHR on IGF-1 Concentrations

	Median IQR (nmol/L)		
	Placebo	GH	Whole Group
T = 0 wk	8.90	11.75	9.65
	4.28-13.93	4.75-12.58	4.28-12.57
T = 8 wks	10.65	29.25*	
	6.23-41.55	8.4-60.95	
T = 40 wks			33.20†
			30.25-46.13

Abbreviation: IQR, interquartile range.
*P = .03 *v* placebo.
†P = .001 *v* baseline.

[2.25 to 4.03] to 2.88 [1.71 to 3.56], P = .04). Plasma triglyceride (P = .18) and NEFA (P = .30) concentrations were similar to baseline.

Effect of GHR on HOMA Measurements

Forty weeks of GHR. A significant reduction in %B (104.1 ± 55.52 to 74.6 ± 41.55, P = .01) and an increase in %S (101.0 ± 48.32 to 162.65 ± 78.46, P = .04) was observed (Table 5).

Effect of GHR on VLDL, LDL, and Plasma apoB Concentrations

Eight weeks of GHR. Compared with baseline, VLDL apoB concentration (P = .09) was nonsignificantly reduced, while plasma apoB (P = .31) and LDL apoB (P = .87) concentrations remained similar (Table 6).

Forty weeks of GHR. Compared with baseline values, no significant changes in plasma VLDL or LDL apoB concentrations were induced by GHR (P = .86, .30, .83).

Effect of GHR on VLDL apoB Kinetics

Eight weeks of GH. Compared with baseline, GHR significantly reduced VLDL apoB ASR (26.61 [19.64 to 28.05] to 13.85 [11.55 to 14.37] mg/kg/d, P = .03) (Table 7). The VLDL apoB pool (2.18 [1.72 to 3.53] to 1.93 [1.51 to 2.65] mg/kg, P = .09) and FSR (0.36 [0.28 to 0.55] to 0.30 [0.22 to 0.40] pools per hour [p/h], P = .09) were nonsignificantly reduced.

Forty weeks of GHR. A nonsignificant reduction in VLDL apoB ASR (26.61 [19.64 to 8.03] to 16.5 [14.84 to 25.58] mg/kg/d, P = .07) was observed. VLDL apoB FSR (0.34 [0.22 to 0.51] to 0.35 [0.26 to 0.42] p/h, P = .78) and pool size (2.18 [1.72 to 3.53] to 2.11 [1.79 to 2.46] mg/kg, P = .30) returned to baseline values.

Correlations. VLDL FSR correlated with VLDL triglyceride concentration (P = .01, Rs = -0.65), VLDL cholesterol concentration (P = .01, Rs = -0.66), and the VLDL cholesterol: apoB ratio (P = .0065, Rs = -0.69). VLDL ASR correlated with plasma triglyceride (P = .02, Rs = 0.59), HDL cholesterol (P = .03, Rs = -0.55), and measures of insulin resistances (fasting insulin concentration (P = .03, Rs = 0.58), %B (P = .04, Rs = 0.53) and %S (P = .02, Rs = -0.60). A nonsignificant inverse correlation with IGF-1 concentration was observed (P = .06, Rs = -0.51).

Effect of GHR on LDL apoB Kinetics

Eight weeks of GH. Sample size was too small at 8 weeks (n = 4) to make statistical comparisons. However, the LDL FSR was similar (0.012 [0.01 to 0.02) to 0.015 [0.00 to 0.03] pools/h, the LDL ASR was similar (14.59 [10.25 to 18.92] to 15.13 [4.05 to 23.16] mg/kg/d) and LDL plasma pool size was reduced (51.14 [40.18 to 56.08] to 41.99 [35.80 to 53.83] mg/kg) before and after treatment, respectively (Table 7).

Forty weeks of GH. LDL apoB FSR was significantly increased (0.012 [0.010 to 0.020] to 0.032 [0.018 to 0.043] pools/h, P = .02), as was LDL apoB ASR (14.59 [10.25 to 18.92] to 3.49 [17.59 to 47.78] mg/kg/d, P = .04). LDL apoB pool size was unaltered (P = .67).

Correlations. LDL FSR correlated with LDL apoB pool size (P = .04, Rs = -0.66), plasma apoB concentration (P = .04, Rs = -0.62), and measures of insulin resistance (insulin concentration (P = .03, Rs = 0.65), %S (P = .04, Rs = -0.64). A nonsignificant correlation with LDL cholesterol concentration (P = .06, Rs = -0.57) was observed. LDL ASR correlated with plasma triglyceride (P = .04, Rs = -0.9) and measures of insulin resistance (fasting insulin concentrations (P = .005, Rs = 0.85), %B (P < .01, Rs = 0.72) and %S (P = .001, Rs = -0.83)).

Effect of GHR on Lipoprotein Composition

VLDL composition. Eight weeks of GH did not significantly alter VLDL total cholesterol (P = .50), free cholesterol (P = .40), phospholipid (P = .99), triglyceride (P = .87), cholesterol ester (P = .06), or apoB (P = .09) concentrations (Table 8). Forty weeks of GH did not induce any significant changes in VLDL total cholesterol (P = .31), free cholesterol (P = .25), cholesterol ester (P = .64), phospholipid (P = .47), triglyceride (P = .33), or apoB (P = .30) concentration.

LDL composition. Eight weeks of GHR did not induce any significant changes in LDL total cholesterol (P = .50), cholesterol ester (P = .87), phospholipid (0.87), triglyceride (0.87), apoB (P = .25), or free cholesterol concentration (P = .09). concentrations. Forty weeks of GHR did not significantly alter LDL total cholesterol (P = .18), free cholesterol (P = .12), cholesterol ester (P = .51), triglyceride (P = .09), phospholipid (P = .30), or apoB (P = .83) concentrations.

HDL composition. Forty weeks of GHR increased HDL free cholesterol concentration (0.20 [0.17 to 0.27] to 0.24 [0.22 to 0.25] mmol/L, P = .03), total cholesterol (1.02 [0.87 to 1.22] to 1.13 [1.05 to 1.35]) mmol/L, P = .01) and cholesterol ester

Table 4. Effect of GHR on Plasma Lipid Concentrations

	TC (mmol/L)	LDL (mmol/L)	Tg (mmol/L)	HDL (mmol/L)
T = 0 wk	5.23	3.16	1.64	0.98
	4.20-6.23	2.33-4.41	1.09-2.77	0.82-1.18
T = 8 wks	5.40	3.11	1.80	1.02
	4.10-6.00	2.20-4.17	1.51-2.83	0.84-1.20
T = 40 wks	5.12	2.73	2.88	1.02*
	4.24-5.96	1.86-3.59	2.29-3.7	0.92-1.24

Abbreviations: TC, total cholesterol; LDL, LDL cholesterol; Tg, triglyceride; HDL, HDL cholesterol.
*P = .01 compared with baseline.

Table 5. Effects of GHR on HOMA

	Glucose (mmol/L)	Insulin (pmol/L)	%B	%S
Mean ± SEM				
T = 0 wk	4.76 ± 0.17	44.91 ± 38.27	104.10 ± 55.52	101.00 ± 48.32
T = 40 wks	5.31 ± 0.30	27.40 ± 25.83	74.60 ± 41.55	162.65 ± 78.46
P value				
0 *v* 40 wks	.05	.01	.01	.04

Abbreviations: %B, measure of β-cell function; %S, measure of insulin sensitivity.

(0.82 [0.71 to 0.95] to 0.88 [0.83 to 1.12] mmol/L, $P = .005$) concentrations. No significant changes in HDL triglyceride ($P = .10$) or phospholipid ($P = .34$) concentrations were observed.

Effect of GHR on LDL Particle Size

The mean particle size before GH treatment was 26.6 [26.2 to 27.0] nm, which did not significantly alter with 40 weeks of GH replacement (26.6 [26.3 to 26.9] nm, $P = .81$).

Correlates. LDL particle size was correlated with plasma triglyceride ($P = .005$, rs = -0.70), HDL cholesterol ($P = .06$, rs = 0.51) and total cholesterol ($P = .09$, rs = 0.50) concentrations. LDL particle size also correlated with VLDL lipid content (cholesterol [$P = .003$, rs = -0.74], free cholesterol [$P = .004$, rs = -0.72], triglyceride [$P = .009$, rs = -0.67], phospholipid [$P = .004$, rs = -0.72]), but not VLDL apoB concentration ($P = .36$). No correlation was observed between LDL size and HDL composition (triglyceride, $P = .81$; cholesterol, $P = .13$; free cholesterol, $P = .52$, phospholipid, $P = .05$).

DISCUSSION

The effect of GHR on plasma lipid profiles, lipoprotein composition, LDL particle size, VLDL, and LDL apoB kinetics has not previously been simultaneously assessed. Therefore, we aimed to examine these parameters in a group of hypopituitary subjects with severe GHD. To explore the hypothesis that GHR exerts differential effects dependent on the duration of therapy, these parameters were assessed at 2 time points.

In this study, 8 weeks of GHR did not induce any changes in lipid profile. However, after 40 weeks of therapy, a significant increase in HDL cholesterol concentrations was observed, in keeping with some,[13,17-19] but not all,[12,14,15]studies. LDL cholesterol concentrations were moderately reduced, although not significantly so, as demonstrated in most previous studies.[12-15,52] The LDL to HDL cholesterol ratio was significantly reduced. Triglyceride and NEFA concentrations remained similar throughout. This may reflect the balance between GH-induced stimulation of lipolysis and the improvement in insulin sensitivity (as demonstrated by HOMA at 40 weeks). These observations were independent of changes in body habitus.

Table 6. Effect of GHR on apoB Concentrations

	apoB Concentration (mg/dL)		
	Plasma	VLDL	LDL
T = 0 wk	118.00	4.84	113.65
	93.75-132.75	3.81-7.84	89.3-124.51
T = 8 wks	95.00	4.28	93.31
	83.50-125.50	3.36-5.88	79.56-119.62
T = 40 wks	105.50	4.69	97.02
	86.25-137.88	3.98-5.47	85.24-131.96

NOTE. Values are medians and interquartile ranges.

Elevated plasma apoB concentrations may be an independent marker of atherogenic risk.[53] A beneficial GH-induced decrease in plasma apoB has been observed by some investigators,[13,15,40] but not all.[13,15,34] In this study, GHR induced a nonsignificant downward trend in plasma total apoB concentrations.

Abnormalities of lipoprotein composition and size may confer increased cardiovascular risk.[54] GHR altered VLDL and LDL composition in some,[22] but not all,[18,34]studies. In this study, VLDL and LDL composition were not significantly altered by GH therapy at any time point. O'Neal et al[9] reported reduced LDL peak particle size in hypopituitarism, which may[35] or may not[36] be ameliorated with GHR. In this study, LDL particle size was unaltered by GHR. This is, perhaps, as expected, as LDL particles were not small before treatment with GH and is consistent with the absence of any significant changes in LDL composition. LDL particle size correlated with measures of VLDL lipid content, such that lipid-enriched VLDL was associated with smaller LDL particles, as has previously been reported. The observed inverse correlation between LDL particle size and VLDL phospholipid, total, and free cholesterol is more difficult to explain. Phospholipid and free cholesterol are primarily surface lipids and have been used by other investigators as a measure of particle size. This relationship, therefore, may again simply suggest that larger VLDL particles are associated with smaller LDL particles. No correlation was observed between VLDL apoB concentration and LDL particle size, suggesting a stronger association between VLDL composition and LDL size, than with VLDL particle concentration. GHR was associated with a significant increase in HDL cholesterol ester, free cholesterol, and total cholesterol concentrations. This may represent reduced transfer of cholesterol ester (CE) from HDL to other lipoproteins, suggesting GH-induced suppression of cholesterol ester transfer protein (CETP) activity. Previous studies support this theory.[55-57] Accelerated plasma CETP may be associated with increased cardiovascular risk,[55,58] perhaps suggesting that suppression of CETP activity is beneficial.

The effects of GHR on VLDL apoB kinetics have been examined by 2 groups of investigators[18,22] with differing results. In this study, 8 weeks of GHR was associated with a significant reduction in VLDL apoB ASR. VLDL apoB FSR was also reduced, but to a lesser extent, resulting in a moderate decrease in VLDL apoB pool size. Forty weeks of GH therapy was associated with a return of VLDL apoB ASR, FSR and

Table 7. Effect of GHR on VLDL and LDL apoB Kinetics

		VLDL apoB			LDL apoB		
		FSR	Pool	ASR	FSR	Pool	ASR
T = 0 wk	M	0.34	2.18	26.61	0.01	51.14	14.59
	IQR	0.22-0.51	1.72-3.53	19.64-28.05	0.01-0.02	40.18-56.03	10.25-18.92
T = 8 wks	M	0.30	1.93	13.85*	0.02	41.99	15.13
	IQR	0.22-0.40	1.51-2.65	11.55-14.37	0.00-0.03	35.80-53.83	4.05-23.16
T = 40 wks	M	0.35	2.11	16.53	0.032*	45.46	32.49*
	IQR	0.26-0.42	1.79-2.46	14.84-25.58	0.01-0.04	37.46-59.75	17.59-47.78

Abbreviations: M, median; IQR, interquartile range; FSR, fractional synthetic rate (p/h); pool, apoB plasma pool (mg/kg); ASR, absolute synthetic rate (mg/kg/d).

*$P < .05$.

pool size to baseline values. VLDL apoB FSR correlated negatively with VLDL triglyceride content. Consistent with this, Packard and Shephard[56] demonstrated that triglyceride replete VLDL was preferentially catabolized via hepatic uptake rather than by the faster route of lipolysis. VLDL apoB ASR correlated with VLDL triglyceride concentrations, supporting the notion that VLDL apoB production is dependent on lipid availability. NEFA availability may also be important and may be the basis for the observed correlation with insulin resistance.

A differential effect of GH on VLDL apoB kinetics dependent on the duration of treatment has also been demonstrated. This may contribute to the discrepancies found in the literature. This biphasic response to GHR is seen in other tissues. A reduction in bone mineral density has been reported after 6 to 12 months of GHR,[57] followed by an increase after 12 months.[59] Similarly, Salomon et al[60] demonstrated an increase in insulin resistance after 1 month of GH therapy, with a return to normal after 6 months. Such biphasic responses may represent the attainment of new steady states.

The effect of GHR on LDL apoB kinetics has not previously been described. We have extended the technique previously validated for the assessment of VLDL apoB kinetics to the assessment of LDL apoB kinetics. By increasing the concentration of infusate and applying a simple biexponential equation to the enrichment data, we were able to determine LDL apoB kinetics with an 8-hour infusion. No alteration in steady state was observed; leucine concentrations remained constant, as did insulin and glucose concentrations. Using this technique, we assessed the effect of GHR on LDL apoB kinetics in a subgroup of the hypopituitary patients. A significant increase in LDL turnover was observed after 40 weeks of GHR. This is consistent with the GH-induced upregulation of hepatic LDL receptors noted in hypophysectomized rats by Angelin and Rudling et al,[61] and also observed in normal and hepG2 cells by Parini et al.[62,63] LDL apoB production was significantly increased, resulting in a nonsignificant reduction in LDL apoB pool size. Such an increase in LDL particle turnover may be of benefit in reducing atherosclerosis, as a shorter residence time may reduce LDL oxidation. The LDL FSR, but not ASR, correlated negatively with the plasma LDL cholesterol concentration, LDL apoB, and plasma apoB pool size, suggesting that plasma LDL concentration was determined by its catabolic rates, rather than its production rates. LDL ASR correlated with VLDL apoB synthesis and measures of insulin resistance. Insulin resistance is associated with increased VLDL apoB production either secondary to increased NEFA concentration[64] or

Table 8. Effect of GHR on Lipoprotein Composition

	Lipoprotein		TC (mmol/L)	FC (mmol/L)	CE (mmol/L)	TG (mmol/L)	PL (mmol/L)	ApoB (mg/dL)
T = 0 wk	VLDL	M	0.51	0.31	0.23	1.48	0.46	4.83
		IQR	0.40-0.97	0.20-0.70	0.16-0.35	1.02-2.55	0.33-1.04	3.81-7.84
	LDL	M	3.12	0.74	2.09	0.20	0.76	113.65
		IQR	2.03-4.0	0.51-1.05	1.53-3.23	0.15-0.30	0.47-1.20	89.3-124.51
	HDL	M	1.02	0.20	0.82	0.06	0.78	NA
		IQR	0.87-1.22	0.17-0.27	0.71-0.95	0.05-0.07	0.70-0.93	
T = 8 wks	VLDL	M	0.58	0.45	0.22	1.34	0.51	4.28
		IQR	0.36-1.24	0.27-0.87	0.12-0.37	1.01-2.40	0.36-0.99	3.36-5.88
	LDL	M	2.21	0.58	1.75	0.18	0.58	93.31
		IQR	1.9-3.12	0.37-0.72	1.34-2.40	0.14-0.26	0.56-0.75	79.56-119.62
T = 40 wks	VLDL	M	0.78	0.41	0.34	1.77	0.68	4.69
		IQR	0.37-1.16	0.21-0.64	0.17-0.57	1.31-2.27	0.35-0.95	4.0-5.47
	LDL	M	2.56	0.45	1.87	0.25	0.60	97.02
		IQR	1.73-3.25	0.32-0.83	1.43-2.69	0.18-0.33	0.54-0.71	85.24-131.96
	HDL	M	1.13*	0.24†	0.88‡	0.07	0.79	NA
		IQR	1.05-1.35	0.22-0.25	0.83-1.12	0.06-0.07	0.69-0.90	

Abbreviations: M, median; IQR, interquartile range, TC, total cholesterol; FC, free cholesterol; CE, cholesterol ester; TG, triglyceride; PL, phospholipid; NA, not applicable.

*$P = .01$, †$P = .03$, ‡$P = .005$ when compared with baseline values.

due to diminished hepatic apoB degradation posttranslationally.[65] Increased VLDL apoB flux may explain the correlation between insulin resistance and LDL apoB production observed in this study.

However, the effect of GH deficiency is difficult to differentiate from the effects of conventional replacement. Most centers use twice daily hydrocortisone replacement regimes. However, these are far from physiologic, with low levels overnight[66] and often overreplacement during the day.[67] Glucocorticoid excess is associated with increased total and central fat mass, hypertriglyceridemia,[68] and glucose intolerance,[69] all of which may contribute to the dyslipidemia associated with hypopituitarism. Hypothyroidism is associated with hypercholesterolemia, hypertriglyceridemia, and weight gain.[70] The wide normal range of thyroid hormones and the inability to use thyroid-stimulating hormone (TSH) as a marker of replacement in the context of pituitary disease, make the adequacy of replacement difficult to assess. Estrogen deficiency may be associated with an increase in central fat deposition,[71] and androgen deficiency may cause central obesity. Sex hormone replacement therapy does not necessarily reduce these risks, as estrogen replacement may be associated with hypertriglyceridemia and testosterone replacement with lowered HDL-C levels. However, some information on the effect of GH on lipid metabolism can be gleaned from studies examining abnormalities in subjects with isolated GHD. De Boer et al[4] examined lipid profiles in subjects with isolated GHD and found a much lower incidence of hyperlipidemia (1 of 28 patients) in these subjects than in those with multiple endocrine deficiencies (13 of 38 patients) receiving conventional hormone replacement. This may suggest a minor role of GH deficiency in the role of hypopituitary-associated dyslipidemia. However, studies examining the effect of GH replacement on lipoprotein composition and kinetics suggest a more pivotal role for GH.

In summary, the effects of short and longer term GHR on plasma lipids, lipoprotein composition and size, VLDL, and LDL apoB kinetics have concurrently been assessed. Several important points have evolved. First, we have extended the stable isotope technique well validated for the study of VLDL apoB to examine LDL apoB kinetics within a shorter time scale, and have for the first time, demonstrated the effect of GHR on LDL apoB kinetics in hypopituitary subjects. Second, this study has allowed us to explore the relationship between lipoprotein composition and apoB kinetics, which may further our understanding of this lipid metabolism. Third, GHR may have antiatherogenic effects; HDL-C was elevated, LDL-C was nonsignificantly reduced, the total/HDL cholesterol ratio was reduced, insulin sensitivity was improved, the overproduction of VLDL apoB was reduced (albeit temporarily), and LDL apoB turnover was increased. Finally, we have demonstrated that the effects of GHR are dependent on the duration of therapy, and that this may explain some of the discordant results published in the literature.

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