Very–Low-Density Lipoprotein Apolipoprotein B100 Kinetics in Adult Hypopituitarism

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Hypopituitarism is associated with hyperlipidemia, the mechanisms of which are not fully known. One possible mechanism is an increased hepatic secretion of very–low-density lipoprotein (VLDL) apolipoprotein B100 (apo B100). To investigate this, 13 hypopituitary patients (seven women and six men; age, 46 ± 3 years [mean ± SEM]; body mass index [BMI], 29 ± 2 kg/m^2) and 13 matched controls (seven women and six men; age, 43 ± 3 years; BMI, 28 ± 2 kg/m^2) were investigated in a stable-isotope study. [1-^{13}C]leucine (1 mg/kg body weight) was administered, followed by a continuous 6-hour infusion of [1-^{13}C]leucine (at a rate of 1 mg/kg/h). Patients had a similar fractional secretion rate (FSR) of VLDL apo B100 versus controls (0.37 ± 0.05 *v* 0.38 ± 0.06 pools/h, respectively), but they had a significantly larger pool size (3.4 ± 0.3 *v* 1.9 ± 0.3 mg/kg) and higher absolute secretion rate ([ASR] 27.8 ± 2.9 *v* 16.0 ± 2.5 mg/kg/d). The increase in hepatic VLDL production may explain the lipid abnormalities found in hypopituitarism. Fasting circulating nonesterified fatty acids (NEFAs) were decreased in the patients (284 ± 26 *v* 664 ± 92 μmol/L, $P < .001$) despite the increase in VLDL secretion. An inverse relationship was observed between the NEFA level and VLDL apo B100 FSR in the patients ($r_s = -.85$, $P < .005$).

HYPOPITUITARISM has long been known to be associated with abnormal circulating lipid profiles.[1,2] Most of the studies have shown increased levels of total and low-density lipoprotein (LDL) cholesterol,[3,4] together with increased triglyceride levels.[3] High-density lipoprotein (HDL) cholesterol concentrations have been reported as low[5] or normal.[6] The dyslipidemia in hypopituitarism occurs together with an increase in premature mortality. In some studies, the excess deaths have been from vascular disease,[7,8] namely cardiovascular[7] and cerebrovascular disease.[8]

The mechanism of the dyslipidemia is not understood. We therefore measured the production rates of very–low-density lipoprotein (VLDL) apolipoprotein B100 (apo B100) using stable isotopes. The apo B100 production rate in VLDL is a measure of particle synthesis, as each VLDL particle contains only one apo B100 molecule, which remains with the particle for its lifetime.

SUBJECTS AND METHODS

Subjects

Thirteen adult hypopituitary patients (seven women and six men) were recruited from St. Mary's endocrine clinic. Their age (mean ± SEM) was 46 ± 3 years and body mass index (BMI) 29 ± 2 kg/m^2. Thirteen normal matched controls (seven women and six men) were also recruited from hospital staff and the patients' friends. Their mean age was 43 ± 3 years and BMI 28 ± 2 kg/m^2. Hypopituitarism in the patients resulted from pituitary tumors treated with surgery and/or radiotherapy. Seven patients received an initial diagnosis of chromophobe adenoma, two macroprolactinoma, three Cushing's disease, and one craniopharyngioma. All patients had a maximal growth hormone (GH) response of less than 6 mU/L on provocative testing (insulin-induced hypoglycemia or glucagon test) Patients required conventional replacement therapy (hydrocortisone, n = 12; thyroxine, n = 10; sex steroids, n = 11; desmopressin, n = 4; and fludrocortisone, n = 1), which was optimized previously and remained unchanged during the study. Controls did not have any known metabolic disorders and were not under treatment with any medications. Clinical characteristics are shown in Table 1 for the patients and Table 2 for the controls.

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

Isotopes

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

Study Protocol

Patients and controls attended the Metabolic Day Ward at St. Mary's Hospital at 9 AM after a 10- to 12-hour overnight fast. They were previously advised to include at least 150 g carbohydrate in their diet during the preceding 3 days and to abstain from strenuous exercise and alcohol for the same period. Intravenous cannulae were positioned in each forearm, one for the infusion and the other for sampling. Fasting samples were taken for estimation of total cholesterol, triglycerides, and HDL cholesterol. During the study, patients rested quietly in bed and were allowed to drink only water. After the infusion, the patients were given a meal.

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 of infusion, and half-hourly for the last hour of infusion. Blood (5 mL) was drawn into heparinized tubes, and the plasma was immediately separated by low-speed centrifugation at 4°C for 30 minutes and stored at −70°C for analysis of ^{13}C enrichment of plasma α-ketoisocaproic acid (α-KIC), the deamination product of leucine. An additional 10 mL blood was collected at the same time points, into tubes to which 120 μL 10% EDTA was added, for VLDL apo B100 isolation and analysis. These samples were also separated immediately by low-speed centrifugation at 4°C for 30 minutes. An equal mixture (50 μL) of 5% NaN_3 and

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Table 1. Patient Characteristics

Patient No.	Age (yr)	Sex	BMI (kg/m²)	Cause of Hypopituitarism	Duration of Hypopituitarism (yr)	Treatment	Replacement Medication
1	33	F	25	Craniopharyngioma	23	TC, R	HC, T, SS, D
2	50	F	32	Chromophobe adenoma	4	TS, R	HC, T, SS
3	47	M	35	Prolactinoma	20	TC, R	HC, T, SS
4	52	M	35	Chromophobe adenoma	5	TS, R	HC, T, SS, D
5	52	M	31	Chromophobe adenoma	1	TS, R	HC, T, SS
6	44	M	26	Chromophobe adenoma	4	TS, R	HC, SS, D
7	29	F	23	Cushing's disease	5	TS, R	HC, T, FI
8	38	M	29	Chromophobe adenoma	1	TC, R	HC
9	48	M	22	Prolactinoma	3	TS, R	HC, T, SS
10	66	F	30	Chromophobe adenoma	32	TC, R	HC, T, SS
11	54	F	27	Chromophobe adenoma	9	TS, R	SS
12	45	F	42	Cushing's disease	18	R	HC, T, SS
13	38	F	27	Cushing's disease	6	TS, R	HC, T, SS, D
Mean ± SEM	46 ± 3		29 ± 2		10 ± 3		

Abbreviations: F, female; M, male; TC, transcranial hypophysectomy; TS, transsphenoidal hypophysectomy; R, radiotherapy; HC, hydrocortisone; T, thyroxine; SS, sex steroids; D, desmopressin; FI, fludrocortisone.

5% gentamycin was added to each of these plasma samples after separation.

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 (Sweden) 2330 ultraspin centrifuge and an SRP (LKB Bromma) 50 AT rotor according to the method of Havel et al,[9] and delipidated using a mixture of ether/methanol (3:1 vol/vol). VLDL protein (50 μg) was subjected to 5% to 15% gradient polyacrylamide gel electrophoresis; the apo B band was excised and hydrolyzed in 2.0 mL 6-mol/L HCl at 110°C for 24 hours with 1 μg norleucine as the internal standard. The hydrolysate was dried under nitrogen, reconstituted in 0.5 mL 50% acetic acid, and transferred to a freshly prepared AG 50W-X8 cationic resin column (BioRad Laboratories, CA). After washing with deionized water, the amino acids were eluted with three washes of 1 mL 3-mol/L 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)trifluouroacetamide to form the bis(*tert*-butyldimethylsilyl) derivative.[10]

Isotopic enrichment of α-KIC was determined by the method of Ford et al.[11] α-Ketovaleric acid internal standard solution (50 μL) was added to 100 μL plasma; this was deproteinized with 1 mL 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 then 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. 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.[10]

Table 2. Control Characteristics

Control No.	Age (yr)	Sex	BMI (kg/m²)
1	30	F	25
2	31	F	24
3	41	F	30
4	37	M	24
5	38	M	21
6	30	M	22
7	37	M	22
8	28	M	26
9	54	F	24
10	61	F	44
11	52	F	30
12	52	F	35
13	65	M	33
Mean ± SEM	43 ± 3		28 ± 2

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,

$$\text{enrichment (APE)} = ([IR_t - IR_0]/[IR_t - IR_0 + 100]) \times 100,$$

where IR_t is the isotope ratio of sample at time t and IR_0 is the isotope ratio of sample at time 0 (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.[12] The raw APEs of plasma leucine and α-KIC enrichments were converted to mole percent excess (MPE) by application of the calibration enrichment-curve slopes obtained by regression analysis of the plot of theoretical MPE against observed APE.

Calculation of Apo B Production

The fractional secretion rate (FSR) of VLDL apo B (pools per hour) was determined by fitting the monoexponential function to the enrichment data,[13] $E(t) = P(1 - e^{-k(t-d)})$, where E(t) is the enrichment at time t, P is the plateau enrichment (α-KIC precursor enrichment), k is the FSR of apo B, and d is the intrahepatic delay time. The absolute secretion rate (ASR) for VLDL apo B was calculated as the product of FSR and pool size. Pool size was derived by multiplying the plasma volume (4.5% of body weight) and the VLDL apo B concentration.[14] VLDL concentrations were measured by enzyme-linked immuno-

sorbent assay. Briefly, appropriate dilutions of the VLDL isolates were prepared in a phosphate-buffered saline/T20 solution and incubated in immunoplates (Life Technologies, Paisley, UK) with anti-human apo B100 antiserum (Boehringer, East Sussex, UK) and anti-sheep IgG alkaline phosphate conjugate (Sigma, Poole, Dorset, UK). Following addition of *p*-nitrophenyl phosphate, the concentration was determined by interpolation of the standard calibration curve constructed from the results for apo B100 standards (Sigma) included in the assay. All measurements were performed in triplicate, and the intraassay and interassay coefficients of variation were 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; Roche Diagnostic, East Sussex, UK).[15] Nonesterified fatty acid (NEFA) levels were also measured with an enzymatic method on the centrifugal analyzer.[16]

Statistical Analysis

Data are presented as the mean ± SEM. Patients and controls were compared using the Mann-Whitney test. Spearman's correlation coefficient was used to examine relations between NEFA levels and the VLDL apo B100 FSR/ASR.

RESULTS

Patients and controls had similar total cholesterol levels (5.52 ± 0.28 *v* 5.21 ± 0.26 mmol/L; Table 3). The elevation in serum triglycerides (2.03 ± 0.52 *v* 1.05 ± 0.14 mmol/L) in the patients did not achieve statistical significance. Patients had lower HDL cholesterol than controls (1.07 ± 0.09 *v* 1.28 ± 0.08 mmol/L), but this also was not statistically significant. No significant differences between patients and controls were found for VLDL cholesterol (0.24 ± 0.10 *v* 0.32 ± 0.13 mmol/L, respectively) or VLDL triglyceride (0.33 ± 0.04 *v* 0.40 ± 0.08 mmol/L) (Table 4). Fasting NEFA levels were significantly decreased in the patients (284 ± 26 *v* 664 ± 92 μmol/L, $P < .001$).

Patients and controls had similar VLDL apo B100 FSRs (0.37 ± 0.05 *v* 0.38 ± 0.06 pools/h; Table 3). However, the pool size was significantly higher in the patients (3.4 ± 0.3 *v* 1.9 ± 0.3 mg/kg, $P < .005$). The ASR of apo B100 was also significantly higher in patients (27.8 ± 2.9 *v* 16.0 ± 2.5 mg/kg/d, $P < .05$). In the patient group, a significant relationship was found between NEFA levels and FSRs ($r_s = -.85$, $P < .005$; Fig 1).

Table 3. Fasting Lipid Profile and Kinetic Data in Patients and Controls

Subject No.	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	FSR (pools/hr)	Pool Size (mg/kg)	ASR (mg/kg/d)
Patients						
1	6.00	1.94	1 20	0.21	3.4	17.2
2	7.20	5.25	0.70	0.11	4.3	9.4
3	5.30	6.75	0.30	0.13	5.2	16.2
4	6.50	2.37	1.00	0.46	3.7	41.0
5	5.70	1.36	1.00	0.35	3.8	32.1
6	6.40	1.80	1.20	0.51	1.6	19.2
7	3.20	0.44	1.40	0.59	2.3	32.8
8	4.40	1.37	1.10	0.29	5.0	34.9
9	5.30	0.52	0.80	0.30	3.0	21.3
10	5.10	1.12	1.20	0.54	3.3	42.1
11	6.10	1.11	1 60	0.47	3.8	42.3
12	5.20	1.52	1.10	0.34	3.6	29.2
13	5.30	0.78	1.30	0.57	1.6	22.3
Mean ± SEM	5.52 ± 0.28	2.03 ± 0.52	1.07 ± 0.09	0.37 ± 0.05	3.4 ± 0.3	27.8 ± 2.9
Controls						
1	3.80	0.36	1.20	0.90	0.6	13.7
2	5.20	0.85	0 90	0.43	1.9	19.4
3	5.10	1.05	1.40	0.60	1.9	26.9
4	4.34	0.73	1.14	0.39	1.4	13.4
5	5.56	0.85	1.00	0.45	1.8	19.9
6	5.08	1.18	1.10	0.34	4.1	33.2
7	3.65	0.55	1.32	0.55	1.8	23.4
8	5 79	1.71	1.09	0.25	3.1	18.3
9	5.55	1.43	1.41	0.36	1.6	13.6
10	5.50	2.11	1.24	0.16	3.1	11.9
11	6 76	0.65	2.00	0.11	0.7	1.7
12	6.75	1.55	1.39	0.25	2.0	11.9
13	4.66	0.57	1.42	0.10	0.9	1.5
Mean ± SEM	5.21 ± 0.26	1.05 ± 0.14	1.28 ± 0.08	0.38 ± 0.06	1.9 ± 0.3†	16.0 ± 2.5*

Abbreviations. TC, serum total cholesterol; TG, serum triglycerides.

*$P < .05$.

†$P < .005$.

Table 4. NEFA, VLDL Cholesterol, and VLDL Triglyceride in Patients and Controls

Subject No.	NEFA (μmol/L)	VLDL-CH (mmol/L)	VLDL-TG (mmol/L)
Patients			
1	463	0.09	0.14
2	361	1.36	0.64
3	363	0.15	0.42
4	219	0.14	0.39
5	335	0.15	0.52
6	166	0.15	0.34
7	188	0.09	0.28
8	284	0.15	0.30
9	340	0.16	0.30
10	291	0.31	0.11
11	209	0.11	0.34
12	340	0.16	0.36
13	133	0.06	0.21
Mean ± SEM	284 ± 26	0.24 ± 0.10	0.33 ± 0.04
Controls			
1	477	0.04	0.08
2	352	0.25	0.43
3	744	0.17	0.33
4	231	1.83	0.18
5	476	0.25	0.40
6	487	0.20	0.81
7	422	0.05	0.05
8	476	0.28	0.86
9	748	0.16	0.22
10	1,303	0.41	0.81
11	1,052	0.02	0.08
12	1,176	0.42	0.70
13	681	0.17	0.28
Mean ± SEM	664 ± 92*	0.32 ± 0.13	0.40 ± 0.08

Abbreviations: VLDL-CH, VLDL cholesterol; VLDL-TG, VLDL triglycerides.

*$P < .001$.

DISCUSSION

In the group of patients reported herein, circulating lipid concentrations were not markedly deranged in that total cholesterol concentrations were similar in patients and controls. The elevation in triglyceride and the decrease in HDL cholesterol were not statistically significant. Nonetheless, hepatic secretion of VLDL apo B100 in hypopituitary patients was increased. This finding is in keeping with a previous report by Cummings et al.[17] They found additionally that the FSR was decreased in patients. At steady state, the FSR equals the fractional catabolic rate (FCR), and the data from Cummings et al[17] therefore suggested that the catabolism of VLDL apo B100 was decreased. The decreased FSR was not confirmed in the present study, suggesting that VLDL apo B100 catabolism was normal in our patients.

VLDL particle clearance is determined to a large extent by the postheparin plasma activity of lipoprotein lipase (LPL). An increase in LPL activity has been reported in adult hypopituitarism,[18] but there is no evidence to support increased VLDL particle clearance from either the present or the previous study. The different results for the FSR (and thus the FCR) between the current and the previous study[17] may reflect the more marked degree of dyslipidemia in the patients studied by Cummings et al,[17] and it is possible that a defect in VLDL particle clearance is also present in more severely dyslipidemic patients.

The mechanisms underlying the increased VLDL apo B100 production remain to be elucidated. In most insulin-resistant states, circulating NEFAs are elevated,[19] and the increase in NEFA substrate delivery to the liver has been proposed as a mechanism for increased hepatic VLDL triglyceride synthesis.[20] Although resistance to insulin action has been described in hypopituitarism,[21] NEFA levels are low throughout the night in these patients. This reduction in NEFA concentrations persists until breakfasttime,[21,22] and this has been demonstrated again in the present study. NEFA concentrations in control subjects in the current study are toward the upper end of the range for normal people, reflecting the fact that these controls were matched for body weight with hypopituitary patients, who tend to be overweight. In the hypopituitary patients, it is possible that the low NEFA concentrations despite the relative obesity of the subjects result from suppression of lipolysis due to low overnight cortisol levels,[23,24] which are observed with standard replacement regimens.[22] Overnight GH deficiency in these subjects, who did not receive GH replacement therapy, also

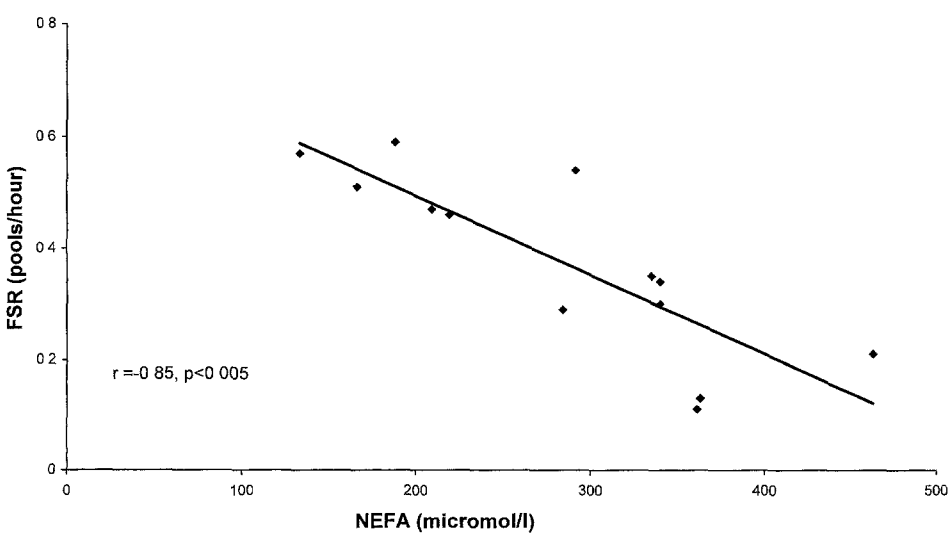

Fig 1. Correlation between NEFA level (μmol/L) and FSR of VLDL apo B100 (pools per hour) in the patients.

may have contributed to the relatively low NEFA levels observed.[22]

Thus. in hypopituitarism, increased VLDL particle synthesis occurs despite low circulating NEFA concentrations. The concentration of VLDL triglyceride was not different in patients and controls in our study, and this may indicate that VLDL particles are less enriched with triglycerides. Substrates for VLDL triglyceride synthesis, apart from NEFA, include triglyceride within other lipoproteins (such as HDL) that are catabolized by the liver. It is possible that triglyceride-enriched HDL is a major source of substrate for VLDL triglyceride synthesis in hypopituitarism. Another possibility is that hepatic fatty acid synthesis provides the substrate. However, it has been shown that the liver preferentially uses preformed fatty acids for VLDL triglyceride synthesis, even in situations where there is augmented de novo fatty acid synthesis in the liver, as occurs, for example. with high-carbohydrate diets.[25] Fatty acid kinetic studies would help to clarify this.

All 13 patients were GH-deficient, 12 were corticotropin (ACTH)-deficient, 11 were gonadotropin-deficient, and 10 were thyrotropin (TSH)-deficient. ACTH deficiency was treated with hydrocortisone and TSH deficiency with thyroxine, but no patient received GH replacement. Hydrocortisone replacement was previously optimized by measuring diurnal cortisol concentrations. and the patients were euadrenal clinically. However, all glucocorticoid replacement regimens are unphysiological (in addition to subnormal levels overnight, circulating cortisol levels are frequently supraphysiological in the period following tablet ingestion) and an inappropriate dosage could certainly contribute to the abnormalities of lipid metabolism.[26] In a similar fashion, thyroxine replacement is less precise in hypopituitarism versus primary hypothyroidism (adequacy may be gauged only by clinical assessment and circulating thyroid hormone levels, as TSH measurements are of limited value). Inappropriate thyroxine replacement (especially underreplacement) might well contribute to the abnormal lipid metabolism.[27]

Lipid abnormalities comprise an important feature of the adult GH deficiency syndrome.[28] GH replacement in adult hypopituitarism often causes a decline in total and LDL cholesterol.[29,30] However, the effects of GH on circulating triglyceride concentrations are minimal,[31,32] and the influence of GH deficiency on VLDL apo B100 kinetics remains uncertain.

Six of seven women and five of six men were on sex hormone replacement regimens, which were optimized previously by conventional clinical and biochemical methods. Although underreplacement, or inappropriate sex hormone replacement, may cause lipid and lipoprotein abnormalities,[33,34] previous studies have demonstrated that the lipoprotein disturbances of hypopituitarism are not confined to patients who are sex hormone–deficient.[6]

The abnormalities of lipid metabolism found in this study may therefore be related to standard pituitary hormone replacement regimens or to GH deficiency. The role of GH deficiency may be clarified following studies before and after GH substitution in hypopituitarism.

In conclusion, hypopituitary adults exhibit increased hepatic secretion of VLDL apo B100 compared with matched controls, in association with low circulating NEFA levels. The mechanisms remain to be elucidated.

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