Apolipoprotein B signal peptide and apolipoprotein E genotypes as determinants of the hepatic secretion of VLDL apoB in obese men

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Abstract **We aimed to examine the effect of genetic polymorphisms of apolipoprotein B-100 (apoB) signal peptide and apolipoprotein E (apoE) on the hepatic secretion of very low density lipoprotein (VLDL) apoB in 29 men with visceral obesity. We studied apoB secretion using a primed (1 mg/kg), constant (1 mg/kg/h) intravenous infusion of [1-^{13}C]leucine. The isotopic enrichment of VLDL apoB was determined using gas chromatography–mass spectrometry (GCMS). A multi-compartmental model was used to estimate the fractional turnover rate of VLDL apoB. Genotypes for the apoB signal peptide length polymorphism, 27 amino acid (SP27) and 24 amino acid (SP24), and apoE genotypes were determined using polymerase chain reaction. In subjects who were not apoE2 carriers and were homozygous for the SP27 of the apoB signal peptide, the hepatic secretion of VLDL apoB was significantly higher than in subjects who were not apoE2 carriers and were either heterozygous or homozygous for the SP24 allele (31.3 ± 11.8 mg/kg fat-free mass/day, n = 8 vs. 16.9 ± 12.2 mg/kg fat-free mass/day, n = 13, $P = 0.01$). In subjects who were not apoE4 carriers and were either heterozygous or homozygous for the apoB SP24 allele, the hepatic secretion of VLDL apoB was significantly lower than in subjects who were not apoE4 carriers and were homozygous for the SP27 allele (15.8 ± 12.9 mg/kg fat-free mass/day, n = 13 vs. 27.4 ± 11.5 mg/kg fat-free mass/day, n = 7, $P = 0.03$). The data suggest that in men with visceral obesity, the apoB signal peptide and apoE genotypes appear to be involved in the hepatic secretion of apoB.**—Riches, F. M., G. F. Watts, F. M. van Bockxmeer, J. Hua, S. Song, S. E. Humphries, and P. J. Talmud. **Apolipoprotein B signal peptide and apolipoprotein E genotypes as determinants of the hepatic secretion of VLDL apoB in obese men. *J. Lipid Res.* 1998.** 39: **1752–1758.**

Supplementary key words very low density lipoprotein • apoB-100 • genotype • visceral obesity • stable isotope

Apolipoprotein (apo)B is the principal protein moiety of the endogenously synthesized pro-atherogenic lipoproteins. Elevated plasma concentrations of apoB are an important risk factor for ischemic heart disease (1) and may explain the increased risk of atherosclerosis in subjects with visceral obesity. We have previously shown that hepatic secretion of apoB is elevated in men with visceral obesity compared with men who were not obese (2). Among the obese men there was wide variation in hepatic output of apoB and this may depend on allelic variations in genes that regulate lipid substrate supply to the liver and the rate of intrahepatic processing of apoB.

Accordingly, variation in the secretion of apoB may be due to genetic polymorphisms of apoE (3) and apoB signal peptide (4). Subjects with the apoE2 phenotype have been shown to have decreased hepatic secretion of very low density lipoprotein (VLDL) apoB in comparison with those with the apoE3 phenotype (3). Subjects with the apoE4 phenotype have an increased supply of lipid substrate to the liver which may elevate VLDL apoB secretion (5). Thus the effect of apoE4 on apoB transport may explain the increased risk of coronary heart disease in subjects with this genotype. A common length polymorphism exists in the apoB gene signal peptide sequence. The common allele encodes a 27 amino acid signal sequence (SP27 allele) and the rare allele encodes a 24 amino acid signal sequence (SP24 allele) (6). These naturally occurring apoB signal peptide variants have been associated with differences in lipid levels (7, 8) and/or increased coronary heart disease risk (9, 10). Specifically, the SP27/27 allele is associated with elevated serum cholesterol and

Abbreviations: apoB, apolipoprotein B-100; apoE, apolipoprotein E; BMI, body mass index; CV, coefficient of variation; FFA, free fatty acid; FFM, fat-free mass; GCMS, gas chromatography–mass spectrometry; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; L4, 4th lumbar vertebra; MRI, magnetic resonance imaging; SP, signal peptide; VLDL, very low density lipoprotein.

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triglyceride concentrations (8). Using a yeast reporter gene, Sturley et al. (11) showed that the apoB signal peptide variants influenced the secretion of the yeast invertase enzyme. In a rat hepatoma cell line, Benhizia et al. (4) confirmed these results and showed that compared to the SP27/apoB-17 fusion protein, secretion of a truncated apoB-17 was greatly reduced and degradation was enhanced when directed by SP24. Thus the link between apoB signal peptide genotypes and lipid and lipoprotein levels could be explained by the apoB isoforms influencing the intracellular degradation and secretion of apoB.

The purpose of this study was to investigate whether there was a relationship between the hepatic secretion of VLDL apoB and apoE and apoB signal peptide genotype in obese men. In addition we wanted to explore these associations in relation to variation in rates of cholesterogenesis, insulin resistance, and degree of visceral obesity.

METHODS

Subjects

Twenty nine male subjects (mean age 47.1 ± 9.2 years) with visceral obesity volunteered to participate in the study. Visceral obesity was defined as a body mass index (BMI) >28 kg/m^2, waist-to-hip ratio ≥1.0, and waist circumference ≥100 cm. The subjects were recruited from the community via newspaper advertisement. None of the subjects had diabetes mellitus, proteinuria, or other secondary causes of hyperlipidemia. They were not taking medication known to affect plasma lipid levels and none gave a family history of hyperlipidemia or premature coronary artery disease. The clinical protocol was approved by the Ethics Committee at Royal Perth Hospital and all subjects provided written consent.

Clinical protocol

Weight and height were measured without shoes and in light clothing. BMI was calculated as weight in kilograms divided by height^2 in meters. Waist circumference (cm) was measured at the point midway between the costal margin and iliac crest in the mid-axillary line, and hip circumference (cm) was measured at the widest point around the greater trochanter. Measurements of fat mass and fat-free mass (FFM) were estimated by a bioelectrical impedance method using a Holtain Body Composition Analyser (Holtain Ltd, Dyfed, Wales, UK).

After the men had entered the study, magnetic resonance imaging (MRI) scans were performed on all subjects. The 1.0T Picker MR scanner (Picker International, Cleveland, OH) used a T1 weighted fast spin echo sequence which gave a high fat-to-water signal ratio. Subjects lay in the magnet in a supine position, with arms placed straight above the head. Ten transverse axial images (field of view = 40–48 cm, 10 mm thick) at various intervertebral levels from T10 to the pubis were acquired for each subject. To reduce errors caused by respiratory movements, the scans were obtained from the subjects in suspended respiration after quiet exhalation. The data for subcutaneous and visceral adipose tissue area from these intervertebral levels were calculated using software developed within the MRI department.

For the [1-^{13}C]leucine infusion, subjects were admitted to the metabolic ward after fasting for 14 h. They were studied in a semi-recumbent position and allowed water only. Venous blood was collected at the commencement of the study for measurements of plasma concentrations of total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, lathosterol, and determination of apoE and apoB genotype. [1-^{13}C]leucine (99.5% enrichment) (Tracer Technologies, Andover, MA) was administered by a primed (1 mg/kg), constant (1 mg/kg/h) intravenous infusion (10-h duration) via a cannula placed into a superficial vein of the left antecubital fossa. Venous blood was collected from a second cannula placed in the contralateral arm.

Dietary intake

All subjects consumed ad libitum diets. Dietary data were collected from each subject in the form of a 3-day food intake record. The diaries were subsequently analyzed for energy and major nutrients by a dietitian using DIET4 Nutrient Calculation Software (Xyris Software, Brisbane, Australia) based on the Australian Food Composition Database (NUTTAB 95, Australian Government Nutrient Database, Canberra, Australia).

Analytical methods

Isolation and measurement of isotopic enrichment of VLDL apoB. A 3-ml aliquot of plasma was overlayed with 1.6 ml sodium chloride solution (d 1.006 kg/L) and ultracentrifuged for 16 h at 147000 *g* (Centrikon T-1190, Kontron Instruments, Milan, Italy). ApoB was isolated from each sample by precipitation with isopropanol (12). In our hands, this technique is highly specific for isolating apoB and was positively associated with VLDL apoB concentration as measured by immunoturbidimetry ($r = 0.89$, $P < 0.001$, n = 27) (F. M. Riches and G. M. Watts, unpublished observations). The precipitated apoB was delipidated with ether–ethanol 1:3 (v/v), dried under nitrogen, and hydrolyzed overnight in 6 m hydrochloric acid; 50% acetic acid was added to the samples and the amino acids were extracted by cation-exchange chromatography.

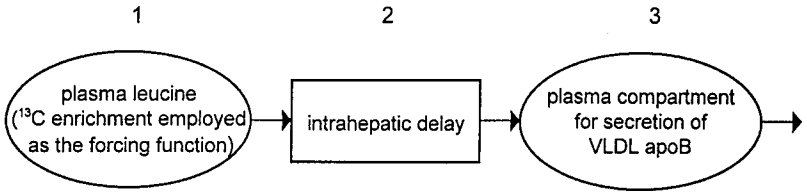
The samples were derivatized using N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide in acetonitrile and reconstituted in decane for gas chromatography-mass spectrometry (GCMS) analysis (Hewlett-Packard 5890, USA). Isotopic enrichment was determined by selected ion monitoring at a mass to charge ratio (m/z) of 303 and 302 and using electron-impact ionization. Leucine enrichment was calculated as:

$$E(t) = \frac{R_t}{R_t + 1} - \frac{R_0}{R_0 + 1} \times 100$$

where Rt is the ^{13}C/^{12}C ratio at time t and R_0 is ^{13}C/^{12}C ratio before the infusion of [1-^{13}C]leucine. The coefficient of variation of the method, assessed by taking five replicate samples at two time points in five of the studies, was less than 7% for isotopic enrichment of leucine.

Quantification of VLDL apoB and other analytes. During the infusion of [1-^{13}C]leucine, plasma samples were combined to yield three separate pooled samples per study. After precipitation of VLDL apoB by isopropanol, a modified Lowry method (13) was used to determine the VLDL apoB concentration in each pooled sample; CV < 4.0%.

Plasma lipid measurements were performed after a 12-h fast using conventional enzymatic methods. The Friedewald equation was used to calculate low density lipoprotein (LDL) cholesterol concentration in subjects with a triglyceride concentration <4.5 mmol/L. Three subjects had a triglyceride concentration >4.5 mmol/L and LDL cholesterol concentration was calculated as: LDL cholesterol = total cholesterol −(VLDL cholesterol + HDL cholesterol); VLDL cholesterol concentration was assayed directly in the VLDL fraction isolated by ultracentrifugation. Plasma insulin was measured by an immunoenzymometric assay using a Tosoh Automated Immunoassay analyzer (Tosoh Corporation, Tokyo, Japan); CV < 7.0%. Plasma glucose concentration was measured by an enzymatic hexokinase reaction method using a Technicon Axon analyzer (Bayer Diagnostics, Sydney, Aus-



Scheme 1. Multicompartmental model used to determine VLDL apoB kinetics.

tralia); CV < 3.1%. The concentration of plasma free fatty acids was determined by an enzymatic colorimetric assay; CV < 3.0%. Plasma lathosterol concentration was assayed as described by Mori et al. (14) using GCMS (Hewlett-Packard 5890); interassay CV 6.0%. ApoE genotypes were determined as described by Hixson and Vernier (15). ApoB signal peptide genotypes were determined as described by Xu et al. (7).

Calculation of VLDL apoB turnover rate. The data were converted to tracer/tracee mass ratios (Z(t)) using the method of Cobelli, Toffolo, and Foster (16). This was derived according to the equation:

$$Z(t) = [E(t)/\{E(I) - E(t)\}]$$

where E(t) is the isotopic enrichment of VLDL apoB at time t and E(l) is the isotopic enrichment of the infusate. A model consisting of three compartments (**Scheme 1**) was used to analyze the tracer/tracee data.

Compartment 1 is a plasma leucine compartment in which ^{13}C enrichment of plasma leucine was used as the forcing function for the precursor pool in the liver. Compartment 2 is an adjustable delay compartment for the synthesis of apoB and assembly of lipoproteins and compartment 3 is a plasma compartment for VLDL apoB secreted by the liver. SAAM-II (SAAM Institute, Seattle, WA) was used to fit the model to the observed data and the fractional secretion rate, equivalent to the fractional catabolic rate at steady state concentration, was determined for each subject. In interpreting these stable isotope studies, the model is based on the following assumptions: *i*) steady state conditions were maintained throughout the study period; *ii*) the shape of the plasma leucine curve reflected leucine enrichment in the precursor pool; *iii*) all apoB enters the plasma via VLDL particles; and *iv*) the delay time for the synthesis and secretion of VLDL apoB is between 10 and 50 min (17).

The hepatic secretion rate of VLDL apoB was calculated as the product of fractional secretion rate (or fractional catabolic rate) and pool size. Pool size was derived by multiplying plasma volume and the mean plasma VLDL apoB concentration. Plasma volume was calculated by multiplying body weight (kg) by 0.045 (18). A correction factor was applied to adjust for the decrease in relative plasma volume associated with an increase in body weight (19).

Statistical analysis

Skewed variables (plasma triglyceride, VLDL triglyceride, VLDL cholesterol, VLDL apoB concentration, pool size, fractional catabolic rate, and the hepatic secretion of VLDL apoB) were examined after log transformation. Binary variables were used to describe apoE genotype and apoB signal peptide variables. Subjects were divided according to whether they were or were not a carrier of apoE2 (i.e., 0 = E3/E3, E4/E3, E4/E4 and 1 = E3/E2, E4/E2) and whether they were or were not carriers of the SP24 allele (i.e., 0 = SP27/27 and 1 = SP24/27, SP24/24), resulting in four groups. A Student's *t*-test was used to compare groups. A similar analysis was performed after subjects were divided according to whether they were or were not carriers of the apo E4 allele. Associations between the hepatic secretion of VLDL apoB and other variables were examined using simple and multiple linear regression methods. In multiple linear regression analyses, a dummy variable was used to code apoE genotype and apoB signal peptide polymorphisms (e.g., when subjects were divided according to whether or not they had the apoE 2 allele and the SP27 allele, 0 = E3/E3, E4/E3, E4/E4 and SP27/27 and 1 = all other combinations). To test for a genetic interaction, the data was analyzed by ANOVA with both apoE and apoB as between-subject factors.

RESULTS

Table 1 shows the clinical characteristics of the obese subjects studied. The men were middle aged and all had a BMI >28 and waist circumference ≥100 cm. Dietary intake of the obese subjects was: energy 10261 ± 2233 kJ, fat 103 ± 31.1g, carbohydrate 219 ± 62.2 g, protein 122 ± 22.9 g, alcohol 25 ± 26.3 g, and cholesterol 423 ± 147.1 mg.

Figure 1 shows the tracer-to-tracee curve for VLDL apoB. In all subjects, the tracer-to-tracee ratios of plasma leucine reached steady state within 30 min, and this was sustained throughout the entire infusion period.

The kinetic parameters for VLDL apoB metabolism, apoE genotype, and apoB signal peptide polymorphisms are shown in **Table 2**. Fourteen subjects were apoE3/E3 homozygotes, six were E4/E3 heterozygotes, six were E3/E2 heterozygotes, two were E4/E2 heterozygotes, and one was E4/E4 homozygote (allele frequency apoE3 0.71; apoE4 0.17, apoE2, 0.12). Ten subjects were homozygous for the SP27 allele, 15 were heterozygous for the SP24 allele, and 4 were homozygous for the SP24 allele (allele frequency SP27, 0.6; SP24, 0.4).

The secretion of VLDL apoB was significantly correlated with the amount of visceral fat at the L4 vertebra (r = 0.41, P = 0.03) and with the concentrations of plasma triglyceride (r = 0.47, P = 0.01), VLDL cholesterol (r = 0.54, P = 0.002) (data not shown), and VLDL triglyceride (r = 0.54, P = 0.002) (data not shown), but not peripheral plasma concentration of insulin, lathosterol, or free fatty acids. No other significant correlations were present between the hepatic secretion of VLDL apoB and the anthropometric, biochemical, or dietary variables measured.

There was no significant difference between the hepatic secretion rate of VLDL apoB in subjects with or without the apoE2 allele (22.2 ± 17.9 mg/kg FFM/day, n = 8 vs. 22.4 ± 13.8 mg/kg FFM/day, n = 21, respectively, P = 0.70), in subjects with or without the apoE4 allele (27.8 ± 16.8 mg/kg FFM/day, n = 9 vs. 19.9 ± 13.4 mg/kg FFM/day, n = 20, respectively, P = 0.22), or in subjects with the

TABLE 1. Characteristics of the subjects with visceral obesity

Subject	Age	BMI	Waist	Total chol.	TG	HDL chol	Insulin	Glucose	FFA	Lathosterol	VAT at L4
	yrs	*kg/m²*	*cm*		*mmol/L*		*mU/L*	*mmol/L*		*μg/ml*	*cm²*
1	45	31.6	115.5	6.4	5.7	1.2	14.8	6.1	0.87	34.0	304
2	55	36.4	122.0	6.6	3.2	1.3	4.9	5.7	0.89	14.2	316
3	54	39.8	124.0	6.0	1.5	1.1	23.4	6.9	1.01	9.7	288
4	53	34.4	121.0	5.8	4.3	0.8	28.8	6.3	0.62	3.7	479
5	61	36.4	122.0	6.3	2.4	1.2	11.2	5.5	0.87	11.4	353
6	38	37.0	118.4	5.1	1.6	1.2	12.4	4.3	0.59	11.4	285
7	50	29.3	112.0	6.7	3.6	1.3	11.4	5.3	0.64	31.6	385
8	45	31.7	110.0	8.3	7.4	0.9	21.5	5.3	1.02	11.6	307
9	36	30.8	100.0	4.9	2.1	0.7	4.2	5.7	0.54	16.3	191
10	55	31.1	108.0	5.5	1.9	1.0	21.9	5.5	1.00	11.0	203
11	37	38.6	130.0	4.3	1.2	0.8	6.6	5.0	0.86	26.7	179
12	49	28.4	106.1	4.5	1.8	1.0	11.6	5.0	0.49	20.1	224
13	51	31.7	106.0	5.7	2.1	0.9	7.4	5.3	0.95	23.8	161
14	42	32.1	107.5	6.6	4.2	0.7	28.6	5.4	0.62	16.5	242
15	50	36.1	115.5	5.9	1.6	1.2	31.4	5.1	0.66	13.9	238
16	50	35.7	113.5	5.5	1.4	0.9	25.5	5.4	0.87	22.5	300
17	37	33.4	121.2	7.5	1.6	1.0	6.6	4.8	0.86	7.3	239
18	58	32.2	103.7	7.1	4.1	0.9	41.8	5.8	0.54	22.5	207
19	39	34.3	109.5	5.9	1.7	1.0	7.4	5.3	0.54	6.5	175
20	44	33.8	109.8	5.6	1.4	1.0	10.5	5.7	1.03	27.8	209
21	31	42.2	128.5	5.3	2.9	1.0	13.7	5.9	1.03	5.1	238
22	56	34.1	108.8	5.8	8.8	0.6	14.5	6.9	0.91	17.2	232
23	35	33.2	112.2	5.4	2.4	1.0	19.1	5.4	1.00	6.8	277
24	50	34.6	115.4	6.0	1.7	1.1	8.6	5.4	0.70	9.4	223
25	50	32.6	109.0	6.3	1.5	0.9	16.3	5.8	0.76	4.8	322
26	60	31.8	111.0	6.0	3.1	0.8	10.6	6.1	0.73	23.6	335
27	56	37.8	129.5	4.3	2.2	1.0	23.3	5.6	0.79	9.4	443
28	54	31.8	115.1	6.4	1.9	1.1	7.1	5.7	0.98	15.1	194
29	25	32.1	111.8	6.6	2.0	1.1	20.5	4.9	0.72	13.1	206
Mean ±SD	47.1 9.2	34.0 3.1	114.4 7.8	5.9 0.9	2.8 1.8	1.0 0.2	16.1 9.2	5.6 0.6	0.80 0.17	15.4 8.3	267.4 78.7

VAT, visceral adipose tissue; L4, 4th lumbar vertebra.

SP 27/27 allele compared with subjects with the SP 24/27 or SP24/24 allele (28.2 ± 12.6 mg/kg FFM/day, n = 10 vs. 19.2 ± 15.0 mg/kg FFM/day, n = 19, $P = 0.06$). However, men who were not carriers of the apoE2 genotype and who were homozygous for the SP27 allele had a significantly higher hepatic secretion of VLDL apoB than did subjects who were not carriers of the apoE2 genotype and were either heterozygous or homozygous for the SP24 allele (31.3 ± 11.8 mg/kg FFM/day, n = 8 vs. 16.9 ± 12.2 mg/kg FFM/day, n = 13, $P = 0.01$). The former also had a significantly higher hepatic secretion of VLDL apoB compared with all other subjects combined ($P = 0.02$). Furthermore, in subjects who were not apoE4 carriers and were either heterozygous or homozygous for the SP24 allele, the hepatic secretion of VLDL apoB was significantly lower than in subjects who were not apoE4 carriers and were homozygous for the SP27 allele (15.8 ± 12.9 mg/kg FFM/day, n = 13 vs. 27.4 ± 11.5 mg/kg FFM/day, n = 7, $P = 0.03$). The former also had a significantly lower hepatic secretion rate of VLDL apoB compared with all other subjects combined ($P = 0.01$). There was no significant difference in plasma triglyceride concentration between the groups. When the data (non-transformed) was analyzed by ANOVA with both apoE (absence and presence of apoE2) and apoB as between-subject factors, the two-way interaction only marginally failed to reach statistical significance ($P = 0.087$).

In multiple linear regression analysis, the hepatic secretion of VLDL apoB was independently associated with the degree of visceral fat at the L4 vertebral body ($P = 0.03$) and with apoE and apoB signal peptide genotypes, when 0 = absence of apoE2 and presence of SP27/27 and 1 = all other combinations ($P = 0.02$) (**Table 3A**). These associations remained significant after adjusting for plasma concentrations of insulin and lathosterol ($P = 0.03$ and $P = 0.03$, respectively) (Table 3B). In addition, the hepatic secretion of VLDL apoB was independently associated with the degree of visceral fat at the L4 vertebral body ($P = 0.014$) and with apoE genotype and apoB signal peptide polymorphisms, when 0 = absence of apoE4 and presence of SP24 and 1 = all other combinations ($P = 0.007$). These associations also remained significant after adjusting for plasma concentrations of insulin and lathosterol ($P = 0.018$ and $P = 0.006$, respectively).

DISCUSSION

This is the first study to demonstrate that the apoB signal peptide and apoE genotypes and the degree of visceral obesity appear to be involved in the hepatic secretion of VLDL apoB. The associations remained significant after adjusting for fasting insulin and lathosterol concentrations.

In the present study, we have demonstrated that the com-

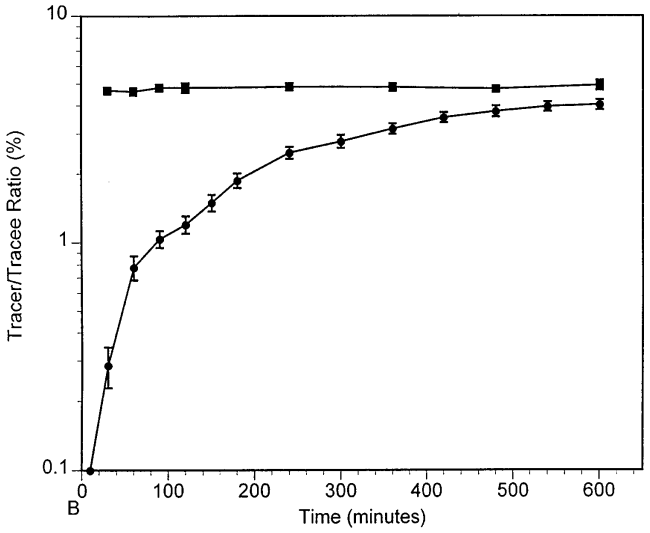


Fig. 1. Rates of 1-^{13}C enrichment of plasma leucine (■) and VLDL apoB (●) in 29 obese subjects during infusion of [1-^{13}C]leucine(mean ± SEM).

bination of an absence of the apoE2 allele and the presence of the SP27/27 allele significantly increases the hepatic secretion of VLDL apoB. We have also shown that the combination of an absence of the apoE4 allele and the presence of the SP24 allele results in a significant decrease in the hepatic secretion of VLDL apoB compared with subjects who were not apoE4 carriers and were homozygous for the SP27 allele. However, there was insufficient evidence to demonstrate that these genetic determinants by themselves affect VLDL apoB secretion. Demant and colleagues (3) have reported that subjects with the apoE2 phenotype have decreased secretion of the $VLDL_1$ apoB subspecies in comparison with the apoE3 phenotype. Individuals with the E4 allele have rapid removal of chylomicron and VLDL remnants (5) resulting in the expansion of the cholesteryl ester pool in the liver which may enhance VLDL apoB secretion (20, 21). By contrast, individuals with the apoE2 allele have a slower remnant uptake and thus the availability of intracellular cholesterol is reduced (5). Yeast expression studies suggest that the apoB signal peptide plays an important role in determining the intracellular handling of secreted proteins (11). Preliminary studies confirming this effect on apoB in a rat hepatoma cell line have recently been reported (4). These studies, therefore, suggest a functional role of the signal peptide in secretion and degradation of apoB, with the SP24 resulting in reduced secretion of apoB compared to SP27. The implications of this are that compared to SP27, SP24 would lead to a reduction in hepatic apoB-rich particles. This could account for the increased VLDL apoB output in subjects who are homozygous for the SP27 allele and are either apoE3/E3, apoE4/E3, or apoE4/E4 (i.e., absence of the apoE2 allele) and the decreased VLDL apoB output in subjects who are either heterozygous or homozygous for the SP24 allele and are either apoE2/E3 or apoE3/E3 (i.e., absence of the apoE4 allele). Further investigations are required to determine whether genetic

TABLE 2. Kinetic parameters for VLDL apoB metabolism in the obese subjects

Subject	VLDL ApoB	VLDL ApoB Pool Size	Fractional Catabolic Rate	Hepatic Secretion Rate	ApoE Genotype	ApoB Signal Peptide
	mg/L	*mg*	*pools/day*	*mg/kg fat free mass/day*		
1	155.6	529.0	4.9	47.7	E3/E3	ins/ins
2	111.1	400.0	2.5	14.6	E3/E2	ins/del
3	53.9	209.7	2.8	7.3	E3/E3	del/del
4	181.8	709.0	4.3	43.0	E3/E3	ins/del
5	108.9	409.5	3.8	21.4	E3/E2	ins/ins
6	34.0	120.7	12.9	25.4	E4/E3	ins/del
7	133.0	460.2	7.1	55.0	E4/E2	ins/del
8	384.5	1330.4	2.1	43.3	E3/E2	del/del
9	98.9	303.6	4.1	20.8	E4/E3	del/del
10	40.5	131.2	3.4	7.7	E4/E3	ins/del
11	45.3	170.3	13.2	31.3	E3/E3	ins/ins
12	43.3	161.5	4.2	10.8	E3/E3	ins/del
13	37.4	129.8	5.5	10.8	E3/E3	ins/del
14	33.4	111.2	5.3	9.4	E3/E3	ins/del
15	53.1	182.1	6.4	17.3	E3/E3	ins/del
16	74.5	260.8	6.1	29.0	E3/E3	ins/ins
17	54.5	211.5	3.2	11.3	E4/E3	ins/del
18	151.1	501.7	5.1	39.6	E4/E4	del/del
19	60.1	207.9	9.9	30.3	E3/E3	ins/ins
20	65.0	256.1	2.1	7.4	E3/E3	ins/del
21	98.9	351.1	3.5	19.7	E3/E2	ins/del
22	310.6	1052.9	2.9	42.6	E4/E3	ins/ins
23	73.4	264.2	2.3	10.5	E3/E3	ins/ins
24	58.9	221.5	1.0	3.2	E3/E2	ins/del
25	65.0	236.6	5.6	21.6	E3/E3	ins/ins
26	100.0	356.0	1.5	8.6	E3/E3	ins/del
27	98.3	377.5	5.8	37.7	E4/E3	ins/ins
28	48.3	171.9	6.5	10.0	E4/E2	ins/ins
29	53.9	191.9	7.2	10.3	E3/E2	ins/del
Mean	97.5	345.5	4.8	22.3		
±SD	80.1	275.9	3.0	14.7		

polymorphisms not examined in the present study contribute to variation in the hepatic secretion of VLDL apoB and its rate of catabolism. The microsomal triglyceride transfer protein (22), which is required to transfer triglycerides to apoB and plays a role in the formation of VLDL particles, apoB XbaI polymorphisms (23), adipsin-acylation stimulating protein (21), and the lipoprotein lipase gene (24) may also affect apoB metabolism.

The balance of evidence suggests that VLDL apoB production is increased in obese subjects compared with nonobese subjects (2, 25–27). However, none of these studies investigated visceral adipose tissue as determined by MRI, a method that is able to accurately quantify both subcutaneous and visceral fat. Visceral adiposity is closely related to impaired lipid metabolism (28), insulin resistance (29), and high blood pressure (30). In the present study, visceral fat at the L4 vertebrae, a surrogate measure for intra-abdominal fat volume (31), was associated with the hepatic secretion of VLDL apoB. It has been suggested that an increased secretion of this apolipoprotein in subjects with visceral obesity is a manifestation of increased portal flux of free fatty acids (FFAs) that increase lipid substrate availability (32). Although FFAs constitute the major precursor for hepatic triglyceride synthesis we did not find an association between plasma FFAs and the secretion of

TABLE 3. Multiple linear regression analyses showing the association between the hepatic secretion of VLDL apoB (mg/kg FFM/day) and the degree of visceral fat at the L4 vertebra, and apoE genotype and apoB signal peptide polymorphisms (A) and after adjusting for insulin and lathosterol concentrations (B)

Predictor Variable	Regression Coefficient (SE)	β Coefficient	*P* Value
A:			
Visceral adipose tissue area at the L4 vertebra	0.003 (0.001)	0.38	0.028
ApoE genotype and apoB signal peptide polymorphisms[a]	−0.62 (0.26)	−0.39	0.024
$R^2 = 0.32$			
B:			
Visceral adipose tissue area at the L4 vertebra	0.003 (0.002)	0.38	0.033
ApoE genotype and apoB signal peptide polymorphisms[a]	−0.60 (0.26)	−0.38	0.026
Insulin (mU/L)	0.01 (0.01)	0.15	0.366
Lathosterol (μg/ml)	0.02 (0.01)	0.24	0.148
$R^2 = 0.39$			

[a]0 = apoE3/E3, E4/E3, E4/E4 and SP27/27; 1 = all other combinations.

VLDL apoB in the present study. This may have been due to the measurement of peripheral as opposed to portal vein free fatty acid concentration and that we did not measure free fatty acid turnover rates.

Numerous in vivo studies (33, 34) have demonstrated that in the non-diabetic state, the hepatic secretion of VLDL apoB is decreased by acute hyperinsulinemia. Therefore, in subjects who are insulin resistant (e.g., subjects with visceral obesity) apoB secretion is increased owing to loss of sensitivity to the normal insulin-mediated suppression of this apolipoprotein. Insulin may also regulate VLDL secretion by restricting the supply of FFAs, glycerol, and triglyceride to the liver (35) and increasing apoB degradation in the endoplasmic reticulum (36). That we did not find an association between peripheral plasma insulin concentration and the hepatic secretion of VLDL apoB does not exclude a role for insulin in the synthesis and secretion of apoB, particularly as we did not measure portal insulin levels. As expected, the hepatic secretion of VLDL apoB was associated with fasting plasma triglyceride and VLDL triglyceride concentrations. Wu and colleagues (37) have suggested that this association may be due to the direct effect of the hepatic uptake of triglyceride-rich VLDL that in turn regulates apoB output. However, other experimental (38) and human studies (39, 40) have demonstrated that the intrahepatic pool of cholesteryl ester is responsible for driving apoB secretion. In visceral obesity it is possible that both cholesterol and triglycerides are rate-limiting substrates. We studied the subjects on their habitual diet as we wished to examine the hepatic secretion of VLDL apoB over a wide range of nutrient intakes and while subjects were in a steady state of energy and nutrient consumption. However, there was no association between nutrient intake and the hepatic secretion of VLDL apoB. This is in spite of a previously reported effect of the polyunsaturated:saturated fat ratio (41) and dietary carbohydrate intake (42).

The analyses in the present study involve several assumptions. The multi-compartmental model assumes that plasma leucine is the source of the leucine that is incorporated into apoB, that apoB is attached to VLDL when it is secreted into the plasma, and that there is a delay time of between 10 and 50 min until labeled apoB enters the plasma. We have not examined the conversion of VLDL apoB to IDL apoB or LDL apoB but would anticipate an increase in the synthesis of these lipoproteins from VLDL in subjects who are either apoE3/E3, E4/E3 or E4/E4 and have the SP27 allele. We were also unable to make inferences regarding VLDL subfractions. Given the correlation between the hepatic secretion of VLDL apoB and VLDL triglyceride concentration, we cannot exclude the possibility that the supply of triglycerides to the liver controls the secretion of VLDL apoB. Finally, as an accumulation of visceral adipose tissue results in a cluster of metabolic changes, in our study design it is difficult to dissociate the independent effects of different lipid substrates on the hepatic secretion of VLDL apoB.

In conclusion, the data show that in subjects with visceral obesity, the combination of the apoE and apoB signal peptide genotype, as well as the amount of visceral adipose tissue, are important determinants of the hepatic secretion of VLDL apoB. This strongly supports the functional role of the apoB signal peptide in determining hepatic apoB secretion. Further studies are required to examine the effect of other genetic polymorphisms on the hepatic secretion of VLDL apoB and its rate of catabolism.

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