Kinetics of Very–Low-Density Lipoprotein Apolipoprotein B-100 in Normolipidemic Subjects: Pooled Analysis of Stable-Isotope Studies

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To further explore the physiology of very–low-density lipoprotein (VLDL) apolipoprotein B-100 (apoB), we performed a pooled analysis of 21 reports based on the intravenous administration of stable isotope–labeled amino acids in a total of 154 healthy normolipidemic subjects. Prandial status was the most significant independent predictor ($P < .001$) of the hepatic secretion of apoB, which was higher in the fed state compared with the fasted state (1,819 ± 188 *v* 1,046 ± 61 mg/d, $P < .001$). In the fed state, apoB secretion increased with age ($P = .003$) and tended to be higher in men compared with women ($P = .0065$). The fractional catabolism of VLDL apoB decreased with weight ($P = .0038$) and was lower in men versus women (8.38 ± 0.55 *v* 12.59 ± 1.65 pools/d, $P = .007$), as well as patients that were carriers of the E4 allele compared with those who were not carriers of this allele (5.52 ± 0.49 *v* 9.58 ± 0.87 pools/d, $P < .001$). The VLDL apoB concentration in both the fed and fasted states was dependent on both the rate of hepatic production and fractional clearance of apoB. Plasma cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol concentrations in the fasted state were principally determined by the fractional catabolism of VLDL apoB ($P < .005$). These findings suggest that under physiologic conditions in healthy individuals, the transport of VLDL apoB in plasma is predominantly determined by age, sex, body weight, apoE genotype, and prandial status.

APOLIPOPROTEIN B-100 (apoB) is a constitutively expressed glycoprotein that is synthesized exclusively in hepatocytes and secreted into plasma as very–low-density lipoprotein (VLDL).[1] The metabolic transport rate of apoB is a determinant of the concentration of plasma lipoproteins and hence the risk of atherosclerosis and coronary disease.[2,3]

In vitro studies have indicated that apoB is synthesized and assembled in a process that involves the cotranslational lipidation of a nascent polypeptide across the endoplasmic reticulum, followed by a second lipidation step that produces the mature VLDL particle.[4] Up to 70% of apoB synthesized in the liver is degraded prior to the formation and secretion of VLDL.[5] The assembly, degradation, and secretion of VLDL apoB is regulated by the availability of lipid substrates, by lipid transfer proteins in the endoplasmic reticulum, and by cytoplasmic molecular chaperones, in a series of processes that are under both genetic and hormonal control.[1,6] While much has been learned from in vitro studies, the factors that regulate both the hepatic secretion and clearance of apoB in humans are yet to be fully elucidated.

The introduction of endogenous labeling of apoB with isotopically labeled amino acids over a decade ago has produced a profusion of publications referring to human subjects.[7,8] While most individual studies have been of small sample size, a large number of normolipidemic healthy individuals have been studied as a whole, and this afforded the opportunity to examine some factors that may influence the kinetics of apoB under physiologic and free-living conditions.[9-29] Based on several previous sources of evidence,[30-36] we hypothesize in the present analysis that age, sex, weight, apoE genotype, and prandial status would be significant determinants of the transport of apoB in plasma.

SUBJECTS AND METHODS

We selected 21 stable-isotope studies of apoB kinetics published in peer-reviewed journals over the period 1988 to 1999 that specifically examined or included normolipidemic healthy subjects.[9-29] We focused our analysis on the kinetics of VLDL apoB since it had been the most widely reported. For a study to be included, the investigational protocols should have used standard methods for administering stable isotopically labeled amino acids, determining isotopic enrichment of apoB, measuring the plasma apoB concentration, and analyzing kinetic data. The published studies also should have reported sufficient data on individual subjects pertinent to the study hypothesis. The following information was obtained from individual subjects: age, sex, weight, body mass index (BMI), apoE genotype, prandial status, plasma total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and VLDL apoB; and hepatic secretion and fractional catabolic rate (FCR) of VLDL apoB. Details of the sample size, dietary composition, prandial challenges, labeled amino acid and methods for its intravenous administration, assay for apoB, and mathematical technique for deriving the kinetic data were also recorded.

All data were entered into an SPSS version 9 database (SPSS, Chicago, IL) and analyzed by the corresponding statistical programs. Univariate and multiple linear regression methods were used to examine associations between kinetic estimates of VLDL apoB and the other variables, with skewed data being logarithmically transformed to normalize the distribution. Sex, prandial status, and apoE genotype were coded as binary variables as follows: 1 = female and 2 = male; 1 = fasted state and 2 = fed state; and 1 = E2/2, E3/2, E3/3 and 2 = E3/4, E4/4. Variables were entered into the same regression model if they could be either potentially causally related to apoB transport (eg, sex and prandial status) or a consequence of apoB transport (eg, plasma triglyceride and HDL cholesterol). To minimize confounding factors, regression models were adjusted for differences among studies in sample size, type of apoB assay, and method of kinetic analysis. Group

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Submitted November 4, 1999; accepted March 20, 2000.

Supported by the Raine Foundation, the University of Western Australia, Parke-Davis/Pfizer, Merck Sharp & Dohme, Servier Laboratories, the Medical Research Foundation of Royal Perth Hospital, the National Heart Foundation, the National Health and Medical Research Council, and the National Institutes of Health (RR 12609).

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0026-0495/00/4909-0020$10.00/0

doi:10.1053/meta.2000.8621

comparisons were made using unpaired *t* tests. Results are presented as the mean ± SE. Statistical significance was defined at the 5% level.

RESULTS

Table 1 lists the studies included in the analysis. ^{13}C-leucine was the isotopically labeled amino acid in 11 studies, deuterated leucine in 7 studies, ^{15}N-glycine in 3 studies, and ^{13}C-valine in 1 study. The isotopes were administered by primed constant infusion in 18 studies and bolus injection in 3 studies. Seventeen studies were performed in the fasted state and 4 in the fed state. VLDL apoB kinetic data were derived by monoexponential analysis in 10 studies, multicompartmental modeling in 9 studies, and linear regression in 4 studies. The dietary protocol in 3 of the fed studies used small hourly feeds with 15% of calories as protein, 49% carbohydrate, and 36% fat (15% saturates, 15% monounsaturates, and 6% polyunsaturates) with 180 g cholesterol/1,000 kcal. In the study by Zulewski et al,[29] individuals ingested a liquid formula diet consisting of 15% protein, 55% carbohydrate, 30% fat, and 0% cholesterol.

Table 2 shows the demographic and biochemical characteristics of the subjects. A total of 154 individuals were included in the analysis, of whom 116 were men and 38 women. On average, the subjects were middle-aged and of desirable body weight and had fasting plasma lipid and lipoprotein levels considered desirable by National Cholesterol Education Program criteria.[30] ApoE genotype data were available in 72 subjects, 19 carriers of the E4 allele and 53 noncarriers of this allele. Subjects in the fed studies were, on average, 11 years older than those in the fasted studies (51 ± 2.8 *v* 40 ± 1.1 years, $P < .001$). However, there were no significant differences in the sex distribution, weight, BMI, plasma lipid levels, and apoE genotype between the fed and fasted studies.

Table 3 shows the kinetic variables for VLDL apoB metabolism. There was a wide dispersion in the plasma concentration, pool size, hepatic secretion rate, and FCR of VLDL apoB, with 8-fold, 5-fold, 6-fold, and 5-fold differences between the highest and lowest values, respectively. The variation in plasma apoB concentration in the fasted state was not related to the different methods used for its measurement. In addition, kinetic data did not differ significantly according to the stable isotope, method of administration, or method of kinetic analysis used in the various studies.

Table 4 shows the univariate associations of hepatic VLDL apoB secretion for all studies combined and for studies performed in the fasted and fed states. A maximum of 142 studies were used in this analysis because data were not reported in some studies. Overall, there was a significant positive correlation between hepatic apoB secretion and increasing age ($P = .049$), fed state ($P < .001$), plasma VLDL apoB ($P < .001$), and plasma triglyceride ($P = .004$); there were no significant associations with sex, weight, BMI, apoE genotype, or plasma cholesterol. In multivariate regression including age, sex, and weight (or BMI), prandial status was the only significant predictor of VLDL apoB secretion (regression coefficient = 1,014 ± 169, β coefficient = 0.473, $P < .001$, $R^2 = 23.3\%$ for model). Figure 1 shows that hepatic secretion of apoB was higher in the fed state compared with the fasted state (1,819 ± 188 *v* 1,046 ± 61 mg/d, $P < .001$), with the difference remaining significant after adjusting apoB secretion for BMI, body weight, or body surface area. In the fasted state, hepatic apoB secretion was only significantly correlated with VLDL apoB ($P < .001$), with a negative association with age just failing to reach statistical significance ($P = .056$) (Table 4). In multivariate regression including age, sex, and weight, age was an independent predictor of apoB secretion (regression

Table 1. Experimental Protocols of Studies With Stable Isotopes Used in the Meta-analysis

First Author	Labeled Amino Acid	Method of Administration of Amino Acid	Method of Plasma ApoB Assay	Method of Kinetic Analysis	Prandial Status
Aguilar-Salinas[9]	1-^{13}C leucine	PCI	IM	Comp	Fasting
Arends[10]	1-^{13}C leucine	PCI	PL	Exp	Fasting
Bordin[11]	1-^{13}C leucine	PCI	ELISA	Exp	Fasting
Campos[12]	5,5,5-$^{2}H_3$ leucine	PCI	ELISA	Comp	Fasting
Cortner[13]	^{15}N glycine	PCI	PL	Exp	Fasting
Cryer[14]	^{15}N glycine	PCI	PL	LR	Fasting
Cummings[15]	1-^{13}C leucine	PCI	PL	Exp	Fasting
Cummings[16]	1-^{13}C leucine	PCI	PL	Exp	Fasting
de Sain[17]	1-^{13}C valine	PCI	IN	Comp	Fasting
Demant[18]	5,5,5-$^{2}H_3$ leucine	PCI, bolus	PL	Comp	Fasting
Lichtenstein[19]	5,5,5-$^{2}H_3$ leucine	PCI	ELISA	Exp	Feeding
Malmstrom[20]	3-$^{2}H_3$ leucine	Bolus	PL	Comp	Fasting
Malmstrom[21]	3-$^{2}H_3$ leucine	Bolus	PL	Comp	Fasting
Millar[22]	5,5,5-$^{2}H_3$ leucine	PCI	ELISA	Comp	Feeding
Parhofer[23]	1-^{13}C leucine, ^{15}N glycine	PCI, bolus	IT	LR, Exp, Comp	Fasting
Venkatesan[24]	1-^{13}C leucine	PCI	ELISA	LR	Fasting
Walsh[25]	5,5,5-$^{2}H_3$ leucine	PCI	ELISA	LR	Fasting
Watts[26]	1-^{13}C leucine	PCI	PL	Exp	Fasting
Watts[27]	1-^{13}C leucine	PCI	PL	Exp	Fasting
Welty[28]	1-^{13}C leucine	PCI	ELISA	Comp	Feeding
Zulewski[29]	1-^{13}C leucine	PCI	IN	Exp	Feeding

Abbreviations: PCI, primed constant infusion; IM, immunoturbidimetry; IN, immunonephelometry; ELISA, enzyme-linked immunosorbent assay; PL, apoB precipitation and Lowry; Comp, multicompartment model; Exp, exponential function; LR, linear regression model.

Table 2. Demographic and Biochemical Characteristics of the Subjects (mean ± SEM)

First Author	No. of Subjects (men/women)	Age (yr)	Weight (kg)	BMI (kg/m^2)	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL Cholesterol (mmol/L)	LDL Cholesterol (mmol/L)	ApoE Genotype (E3E2,E3E3/E3E4,E4E4)
Aguilar-Salinas[9]	7/1	33.5 ± 3.6	76.5 ± 3.9	25.6 ± 0.7	4.4 ± 0.2	0.9 ± 0.1	1.2 ± 0.1	2.9 ± 0.2	6/2
Arends[10]	6/0	23.3 ± 1.8	79.0 ± 2.3	24.2 ± 0.5	5.0 ± 0.4	1.0 ± 0.2	1.6 ± 0.1	3.1 ± 0.3	NR
Bordin[11]	10/0	32.3 ± 2.1	66.3 ± 2.2	23.0 ± 0.8	4.8 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	3.4 ± 0.2	NR
Campos[12]	0/8	53.4 ± 2.9	65.1 ± 3.9	23.5 ± 1.4	4.8 ± 0.3	0.8 ± 0.1	1.7 ± 0.1	2.8 ± 0.2	NR
Cortner[13]	4/0	43.3 ± 3.7	72.8 ± 4.8	NR	4.7 ± 0.4	1.0 ± 0.4	1.5 ± 0.2	2.8 ± 0.3	NR
Cryer[14]	4/1	38.8 ± 3.6	64.6 ± 4.1	NR	4.4 ± 0.4	0.7 ± 0.1	1.6 ± 0.1	NR	NR
Cummings[15]	4/2	55.7 ± 2.8	85.8 ± 5.6	28.7 ± 1.2	5.4 ± 0.3	1.2 ± 0.1	1.4 ± 0.1	NR	6/0
Cummings[16]	3/3	41.8 ± 3.7	68.2 ± 4.9	23.0 ± 1.5	4.5 ± 0.3	0.8 ± 0.2	1.3 ± 0.1	2.8 ± 0.3	5/1
de Sain[17]	3/4	32.7 ± 2.0	77.5 ± 5.5	24.3 ± 1.5	5.2 ± 0.3	1.3 ± 0.1	1.1 ± 0.1	3.0 ± 0.2	NR
Deman[18]	6/0	42.5 ± 5.9	78.0 ± 5.5	23.8 ± 1.1	5.9 ± 0.3	1.6 ± 0.2	1.2 ± 0.1	3.8 ± 0.3	3/2
Lichtenstein[19]	4/4	63.8 ± 2.3	71.3 ± 4.9	NR	5.4 ± 0.8	1.2 ± 0.4	1.2 ± 0.3	3.6 ± 0.8	7/1
Malmstrom[20]	7/0	51.3 ± 2.6	87.7 ± 4.2	26.3 ± 0.7	5.2 ± 0.2	1.4 ± 0.2	1.2 ± 0.1	NR	4/3
Malmstrom[21]	8/0	39.9 ± 3.7	87.1 ± 3.1	25.8 ± 0.9	4.9 ± 0.2	1.3 ± 0.1	1.2 ± 0.1	NR	NR
Millar[22]	16/0	48.4 ± 4.1	79.0 ± 2.3	25.5 ± 0.7	4.7 ± 0.2	1.2 ± 0.1	1.1 ± 0.1	NR	NR
Parhofer[23]	3/1	23.0 ± 0.7	67.8 ± 2.8	20.9 ± 0.6	3.8 ± 0.3	1.1 ± 0.2	NR	NR	NR
Venkatesan[24]	4/3	49.4 ± 4.8	64.7 ± 4.2	22.7 ± 0.9	4.8 ± 0.3	0.8 ± 0.1	NR	NR	NR
Walsh[25]	0/6	24.3 ± 0.7	60.3 ± 3.4	23.3 ± 0.9	3.8 ± 0.3	0.2 ± 0.1	1.2 ± 0.1	2.4 ± 0.2	NR
Watts[26]	3/1	44.0 ± 6.0	72.8 ± 6.0	24.6 ± 1.2	4.9 ± 0.5	0.8 ± 0.1	1.5 ± 0.2	3.1 ± 0.3	3/1
Watts[27]	17/0	45.5 ± 2.3	78.9 ± 1.9	25.1 ± 0.3	5.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	3.4 ± 0.1	11/6
Welty[28]	6/0	40.0 ± 2.3	76.3 ± 2.3	23.9 ± 0.7	4.6 ± 0.2	1.0 ± 0.1	1.1 ± 0.1	2.9 ± 0.2	3/3
Zulewski[29]	1/4	32.0 ± 6.0	61.3 ± 4.6	22.0 ± 1.0	5.3 ± 0.2	1.0 ± 0.3	2.0 ± 1.0	2.8 ± 0.3	5/0

Abbreviation: NR, not reported.

coefficient = −12.9 ± 4.9, β coefficient = −0.248, $P = .010$), with a trend for a significant negative association with male sex (β coefficient = −0.18, $P = .008$). In the fed state, apoB secretion was significantly related to increasing age ($P = .014$) and plasma VLDL apoB ($P = .005$). In multiple regression including age, sex, and weight, age remained a significant positive predictor of apoB secretion (regression coefficient = 41.3 ± 13.8, β coefficient = 0.525, $P = .006$) and there was a trend for a significant positive association with male gender (β coefficient = 0.339, $P = .095$). The aforementioned findings were also obtained after adjusting the regression models for differences in the sample size, apoB assay, and method of kinetic analysis.

Table 5 shows the univariate associations of the fractional catabolism of VLDL apoB for all studies combined and for studies divided into the fasted and fed states. The FCR of apoB

Table 3. Kinetic Parameters of VLDL ApoB Metabolism for Individual Studies (mean ± SEM)

First Author	VLDL ApoB (mg/L)	VLDL ApoB Pool Size (mg)	FCR (pools/d)	Absolute Secretion Rate (mg/d)
Aguilar-Salinas[9]	52.9 ± 6.5	186.7 ± 29.5	9.5 ± 1.1	1,620.6 ± 180.0
Arends[10]	38.1 ± 9.5	138.3 ± 36.9	15.3 ± 4.7	1,549.3 ± 389.2
Bordin[11]	65.0 ± 11.1	190.5 ± 27.8	9.0 ± 0.9	1,537.9 ± 145.5
Campos[12]	38.4 ± 9.5	105.3 ± 23.4	20.5 ± 2.6	1,808.6 ± 326.1
Cortner[13]	24.9 ± 14.1	87.7 ± 54.3	13.0 ± 3.3	682.9 ± 167.7
Cryer[14]	NR	NR	9.2 ± 1.1	NR
Cummings[15]	34.9 ± 4.3	115.7 ± 18.7	8.6 ± 1.0	920.7 ± 115.3
Cummings[16]	16.6 ± 4.7	53.7 ± 17.2	14.7 ± 2.8	568.5 ± 63.1
de Sain[17]	42.1 ± 8.1	147.1 ± 27.1	6.4 ± 0.7	1,017.9 ± 241.9
Demant[18]	67.9 ± 11.4	239.2 ± 45.6	5.0 ± 0.7	1,083.3 ± 178.1
Lichtenstein[19]	131.3 ± 22.7	418.6 ± 73.2	5.4 ± 0.7	2,015.7 ± 275.4
Malmstrom[20]	63.5 ± 5.9	250.0 ± 27.2	4.1 ± 0.4	988.3 ± 91.2
Malmstrom[21]	32.6 ± 4.2	130.6 ± 20.1	8.2 ± 1.4	944.1 ± 97.4
Millar[22]	74.3 ± 15.5	261.6 ± 54.1	14.7 ± 2.5	2,237.7 ± 340.4
Parhofer[23]	37.4 ± 4.7	113.4 ± 15.5	8.9 ± 1.0	1,049.3 ± 248.0
Venkatesan[24]	24.3 ± 2.8	71.1 ± 10.3	4.2 ± 0.2	301.2 ± 44.2
Walsh[25]	58.2 ± 11.4	158.7 ± 30.3	NR	NR
Watts[26]	24.5 ± 3.5	82.2 ± 15.4	10.4 ± 1.2	823.5 ± 135.0
Watts[27]	31.4 ± 4.8	111.4 ± 16.5	7.9 ± 1.4	676.5 ± 93.4
Welty[28]	43.5 ± 5.2	141.6 ± 13.7	7.6 ± 0.6	1,052.8 ± 98.5
Zulewski[29]	58.4 ± 4.8	162.6 ± 20.5	6.9 ± 0.7	1,084.3 ± 117.9

Abbreviation: NR, not reported.

Table 4. Univariate Associations of the Hepatic Secretion Rate of VLDL ApoB for All Stable Isotope Studies Combined and Studies Divided Into Fasted and Fed States

	All Studies (N = 142)				Fasted Studies (n = 107)				Fed Studies (n = 35)			
Variable	R^2	Regression Coefficient	SE	*P*	R^2	Regression Coefficient	SE	*P*	R^2	Regression Coefficient	SE	*P*
Age	.028	10.51	5.29	.049	.034	−9.28	4.81	.056	.196	33.67	12.88	.014
Sex	.002	100.30	170.94	.558	.003	−88.58	148.62	.552	.073	306.11	436.94	.116
Weight	.004	4.41	5.86	.453	.004	3.19	4.98	.523	.024	15.00	16.68	.375
BMI	.005	20.72	26.48	.436	.007	18.02	21.76	.409	.001	−15.45	101.95	.881
Prandial status	.158	773.02	150.56	.000								
ApoE	.034	−245.33	157.03	.123	.013	−122.78	147.10	.408	.087	−497.42	390.91	.220
Cholesterol	.004	55.92	78.75	.479	.006	62.70	75.87	.410	.017	−105.77	328.63	.758
Triglyceride	.072	316.69	106.76	.004	.002	67.31	134.54	.618	.168	350.28	318.01	.313
HDL cholesterol	.001	−68.33	213.19	.749	.001	−68.33	213.19	.749				
LDL cholesterol	.006	79.78	119.61	.507	.006	79.78	119.61	.507				
VLDL apoB	.294	10.55	1.36	.000	.222	9.18	1.67	.000	.216	8.69	2.88	.005

was lower in men versus women (8.34 ± 0.5 *v* 12.59 ± 1.5 pools/d, $P = .010$) and in carriers of the apoE4 genotype compared with noncarriers (5.73 ± 0.42 *v* 8.72 9 ± 0.67, $P = .012$). The FCR also decreased with increasing body weight ($P = .038$) but was not significantly related to age, BMI, or prandial status. The FCR of apoB was significantly and inversely related to plasma cholesterol ($P = .004$), triglyceride ($P < .001$), LDL cholesterol ($P < .001$), and VLDL apoB ($P = .003$), as well as positively related to plasma HDL cholesterol ($P < .001$) (Table 5). In multiple regression analysis including age, sex, weight, and prandial status, prandial status was the only variable that was a significant predictor of VLDL apoB secretion ($P = .006$); this remained significant after adjusting for differences in the sample size and type of apoB assay and modeling methods. In the fasted state, male sex, weight, and apoE4 genotype were inversely related to the VLDL apoB FCR and the associations between the FCR and plasma lipid and VLDL apoB remained significant. The effect of sex on the FCR remained significant after adjusting for age and weight. In a subset analysis, the effect of sex disappeared with the inclusion of apoE genotype. In the fed state, no statistically significant associations were found between the fractional catabolism of VLDL apoB and any of the variables studied, although a trend for a significant association was found with VLDL apoB ($P = .072$) (Table 5).

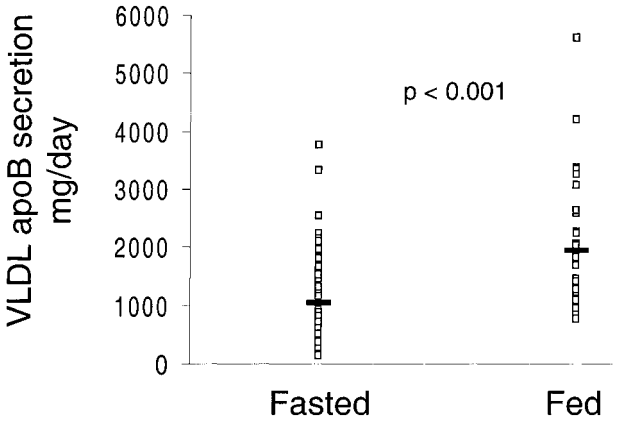

Fig 1. Hepatic secretion of VLDL apoB in healthy normolipidemic individuals studied in the fasted and fed states using stable isotopically labeled amino acids.

DISCUSSION

This pooled analysis of a large number of stable-isotope studies in healthy subjects shows that constitutional, nutritional, and genetic factors are significant determinants of the transport of apoB in plasma. Age, sex, weight, apoE genotype, and particularly prandial status were the significant determinants of the kinetics of apoB in a group of subjects with a wide variation in the hepatic secretion and catabolism of this apolipoprotein. As anticipated, plasma apoB pool size was dependent on both hepatic secretion and catabolism of apoB. Our principal findings were independent of the assay used for apoB and the method of kinetic analysis.

The determinants of apoB kinetics have not been previously examined in a meta-analysis. However, a few individual studies recruited a sufficient number of patients to demonstrate significant correlates of apoB secretion.[22,27] In a previous report included in this analysis, we showed that VLDL apoB secretion was primarily determined by the waist to hip ratio, a measure of visceral fat.[27] In this group of subjects, plasma triglycerides were inversely correlated with the fractional catabolism of VLDL apoB, consistent with our present findings and those of radioisotopic studies.[31] Millar et al[22] reported that the hepatic output of VLDL apoB increased with age during constant feeding of a standardized diet, and we have extended this observation in a larger number of subjects by demonstrating that the effect of age is also present in the fed state. We acknowledge that exogenous labeling of lipoproteins with radioisotopes has been used in other studies,[31] but in our view, the experimental protocols involved are less desirable than endogenous labeling with stable isotopes,[7] and inclusion of these more limited data would have further biased the present analysis.

The associations of apoB transport reported here are consistent with other observations. Premenopausal women have lower plasma cholesterol and triglycerides than men,[32] and this may be due to the stimulatory effect of estrogen on hepatic LDL and remnant receptors.[33] This is consistent with the lower fractional catabolism of apoB noted in men in both the fasting and fed states. Estrogen replacement therapy in postmenopausal women

Table 5. Univariate Associations of the FCR of VLDL ApoB for All Stable-Isotope Studies Combined and Studies Divided Into Fasted and Fed States

	All Studies (N = 142)				Fasted Studies (n = 107)				Fed Studies (n = 35)			
Variable	R^2	Regression Coefficient	SE	*P*	R^2	Regression Coefficient	SE	*P*	R^2	Regression Coefficient	SE	*P*
Age	.006	−0.04	0.04	.374	.016	0.06	0.05	.184	.003	−0.03	0.11	.791
Sex	.028	−2.69	1.32	.043	.112	−5.07	1.36	.000	.062	4.66	3.16	.150
Weight	.029	−0.09	0.05	.038	.066	−0.13	0.05	.006	.004	0.05	0.12	.707
BMI	.005	−0.18	0.22	.422	.009	−0.20	0.22	.350	.005	−0.23	0.73	.754
Prandial status	.002	0.77	1.29	.552								
ApoE	.088	−2.98	1.15	.012	.137	−4.08	1.43	.006	.000	0.03	1.13	.978
Cholesterol	.067	−1.92	0.66	.004	.076	−2.11	0.69	.003	.103	0.66	0.80	.437
Triglyceride	.251	−5.23	0.83	.000	.295	−7.35	1.08	.000	.081	−0.62	0.85	.496
HDL cholesterol	.217	9.78	1.86	.000	.217	9.78	1.86	.000				
LDL cholesterol	.164	−4.22	1.10	.000	.164	−4.22	1.10	.000				
VLDL apoB	.061	−0.04	0.01	.003	.076	−0.05	0.02	.004	.095	−0.04	0.02	.072

increases the production of both $VLDL_1$ and $VLDL_2$ apoB,[12] but we show that this may not apply under physiologic conditions. Plasma lipid and lipoprotein levels increase with age,[34] which may be a consequence of a reduction in growth hormone levels[35] and insulin sensitivity,[36] both of which may induce hepatic oversecretion of apoB and a reduction in its fractional catabolism. The inverse association between body weight and the FCR of VLDL apoB may also be due to the effects of increasing insulin resistance on the clearance of apoB.[37] As shown by others,[38,39] feeding increases the hepatic output of VLDL apoB, which appears to be mainly due to oversecretion of the $VLDL_1$ subfraction,[40] and the effect of insulin resistance[20] and growth hormone deficiency[34] on this process may explain the positive correlation we report with age in the prandial state. The fractional catabolism of VLDL apoB may also decrease postprandially due to a reduction in the apoC-II content of VLDL[40] or to the competition of remnant lipoproteins for a common removal pathway, but these phenomena are not consistent with our findings. However, the effect of feeding on the hepatic production of apoB is compatible with the regulatory role of lipid substrates in the synthesis and secretion of apoB.[6,41] The fact that we did not find an effect of feeding on the fractional clearance of apoB in the present study might have been due to the selection of normolipidemic subjects and the use of multiple dietary feeds of low fat composition. Our findings with respect to apoE genotype agree with those of Demant et al,[42] who found that subjects with the E4 allele have decreased direct catabolism of all apoB-100–containing lipoproteins, consistent with an affect of increased lipid supply to the liver to downregulate hepatic LDL receptors.[43] We have previously reported that the E4 allele is associated with increased hepatic production of apoB,[44] but this finding was restricted to patients with visceral obesity and insulin resistance.

Our results suggest that in the fasting state, plasma cholesterol and triglyceride levels are determined principally by the catabolic rate of apoB and not by its hepatic secretion. However, in states of high lipid substrate supply to the liver, hepatic secretion may assume a greater role than the clearance rate.[15,45] We could not adequately explore the association between the kinetics of VLDL apoB and plasma lipids in the fed state, owing to insufficient availability of data. The inverse association between the fractional catabolism of VLDL apoB and the plasma LDL and non-HDL cholesterol concentration suggests that the clearance of VLDL remnants and both LDL and intermediate-density lipoproteins may share common removal pathways,[46] involving the LDL supergene family of receptors. An alternative explanation is that the delayed fractional catabolism of VLDL results in increased absolute delivery of lipid substrates to the liver and increased direct input of both IDL and LDL.[41,47] However, our study cannot distinguish between these two possibilities. Our results are compatible with the notion that newly secreted VLDL can be taken up directly by the liver before entering the delipidation cascade and apoB may be secreted directly in plasma as cholesterol-rich, triglyceride-depleted particles.[47]

The wide variation in kinetic data from stable-isotope studies in normolipidemic subjects has also been found in radiokinetic studies.[30] The dispersion in the data may reflect not only the variation in plasma apoB transport but also errors in measurement of the apoB pool size, which is dependent on body size, and plasma apoB concentration. Our subjects were all of desirable body weight, and we also adjusted for variations in weight in multiple regression analyses. Despite the wide range in plasma apoB levels in this normal group, we did not find systematic differences among different studies. A significant proportion of the between-subject variation in the present study might have been due to assay variation among laboratories,[48] and this would have increased the standard errors of the regression coefficient in correlational analyses. However, in a previous report, we have found that immunochemical and standard Lowry assays for VLDL apoB are closely correlated.[49] Since the sample size of the studies varied, we also adjusted for this in regression analyses. However, the unexplained variation in apoB kinetics could also be due to a number of unmeasured factors. These include the habitual background diet,[50] alcohol intake,[51] level of physical exercise,[52] and genes regulating intrahepatic processing of apoB and lipid substrate supply to the liver, such as microsomal triglyceride transfer protein and apoB signal peptide.[44,53] The kinetic studies selected for this analysis involved rigorous clinical protocols such that we consider that,

with the exception of prandial status, the subjects were studied under fairly uniform conditions.

The use of different mathematical techniques among studies may also account for the variation in the reporting of kinetic data.[23,54] Linear regression analysis tends to overestimate rate constants compared with monoexponential and multicompartmental analyses, both of which generally produce comparable results.[23] We did not find any systematic differences in the present study between these 2 forms of data analysis. Neither were there differences among studies with respect to the use of different stable isotopically labeled amino acids, consistent with another report.[55]

In conclusion, this meta-analysis of healthy normolipidemic subjects has highlighted some constitutional, physiologic, and genetic factors that may influence apoB kinetics. It is likely that these factors will also influence the expression of apoB transport in genetic hyperlipidemias,[56] with a dominant effect of the fed state. The clinical importance of the analyses presented relates to elucidating the factors that contribute to high flux rates of apoB in apparently healthy normolipidemic subjects that may signify an increased risk of atherosclerotic vascular disease.[57] The impact of these variables on other aspects of lipoprotein metabolism including chylomicron remnant and apoA-I kinetics merits further examination, although at present, data from tracer studies are too limited to permit meta-analysis.

ACKNOWLEDGMENT

We are grateful to Fiona Riches for assisting with data collection, and to Professor F. Sacks and Dr M. de Sain for supplying individual data from the studies by Walsh and Sacks[25] and de Sain et al,[17] respectively.

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