Human Apolipoprotein (Apo) B-48 and ApoB-100 Kinetics With Stable Isotopes

Francine K. Welty, Alice H. Lichtenstein, P. Hugh R. Barrett, Gregory G. Dolnikowski, Ernst J. Schaefer

Abstract—The kinetics of apolipoprotein (apo) B-100 and apoB-48 within triglyceride-rich lipoproteins (TRLs) and of apoB-100 within IDL and LDL were examined with a primed-constant infusion of (5,5,5-$^2H_3$) leucine in the fed state (hourly feeding) in 19 subjects after consumption of an average American diet (36% fat). Lipoproteins were isolated by ultracentrifugation and apolipoproteins by SDS gels, and isotope enrichment was assessed by gas chromatography/mass spectrometry. Kinetic parameters were calculated by multicompartmental modeling of the data with SAAM II. The pool sizes (PS) of TRL apoB-48, VLDL apoB-100, and LDL apoB-100 were 17±10, 273±167, and 3325±1146 mg, respectively. There was a trend toward a faster fractional catabolic rate (FCR) for VLDL apoB-100 than for TRL apoB-48 (6.73±3.48 versus 5.02±2.07 pools/d, respectively, $P=0.06$). The mean FCRs for IDL and LDL apoB-100 were 10.07±7.28 and 0.27±0.08 pools/d, respectively. The mean production rate (PR) of TRL apoB-48 was 6.5% of VLDL apoB-100 (1.3±0.90 versus 20.06±6.53 mg · kg^{-1} · d^{-1}, $P<0.0001$). TRL apoB-48 PS was correlated with apoB-48 PR ($r=0.780$, $P<0.0001$) but not FCR ($r=-0.1810$, $P=0.458$). VLDL apoB-100 PS was correlated with both PR ($r=0.713$, $P=0.0006$) and FCR ($r=-0.692$, $P=0.001$) of VLDL apoB-100 and by apoB-48 PR ($r=0.728$, $P=0.0004$). LDL apoB-100 PS was correlated with FCR ($r=-0.549$, $P=0.015$). These data indicate that (1) the FCRs of TRL apoB-48 and VLDL apoB-100 are similar in the fed state, (2) TRL apoB-48 PS is correlated with TRL apoB-48 PR, (3) VLDL apoB-100 PS is correlated with both PR and FCR of VLDL apoB-100 and PR of TRL apoB-48, and (4) LDL apoB-100 PS is correlated with LDL FCR. **(*Arterioscler Thromb Vasc Biol*. 1999;19:2966-2974.)**

Key Words: apolipoprotein B ■ metabolism ■ stable isotopes ■ LDL cholesterol ■ lipoproteins

The mechanisms regulating the synthesis and secretion of apolipoprotein (apo) B-100 and apoB-48 are incompletely understood but are of importance because elevated levels of apoB, the main protein in LDL, are associated with an increased risk of coronary heart disease.[1] ApoB exists in 2 forms in plasma, apoB-100 and apoB-48,[2] both of which are products of the same structural gene on chromosome 2.[3] ApoB-100 is synthesized by the liver and secreted within VLDLs, which are metabolized in plasma to form LDL. ApoB-100 contains the LDL receptor–binding domain; therefore, VLDL remnants (IDL) and LDL are removed from the circulation by binding to hepatic LDL receptors.[2]

Synthesized in the intestine in response to dietary fat, apoB-48 is produced as a result of a premature stop codon at the apoB-100 codon 2153 by tissue-specific mRNA processing and secreted within chylomicrons.[4] Both chylomicrons and VLDL are the major triglyceride carriers in plasma, and the triglycerides therein are hydrolyzed by lipoprotein lipase to form chylomicron remnants and VLDL remnants, respectively. ApoB-48 does not contain an LDL receptor–binding domain; therefore, the chylomicron remnants are most likely taken up by the liver by receptors that recognize apo E.[5,6]

Increasing evidence suggests that chylomicron and VLDL remnants are atherogenic; however, chylomicron remnant clearance has not been examined extensively in humans because of the difficulty in studying apoB-48 kinetics. Furthermore, the interrelationships between intestinal apoB-48 production and apoB-100 secretion from the liver have not been studied in humans. Using different approaches, both Nestel[7] and Grundy and Mok[8] showed that the triglyceride component of chylomicrons has a half-life of 5 to 8 minutes in healthy subjects and a longer half-life of 26 minutes in hypertriglyceridemic subjects with coronary heart disease. Although a substantial mass of the triglyceride is rapidly hydrolyzed by lipoprotein lipase in chylomicrons and VLDL, the nontriglyceride core components (cholesteryl ester) and apoB-48 remain with the lipoprotein particle until receptor-mediated uptake occurs. Previous kinetic studies of triglyceride-rich lipoproteins (TRLs) using radiolabeled particles have suggested that catabolism of both apoB-48 and apoB-100 within TRL is very rapid in normal humans, ie, 15 to 30 minutes.[9,10]

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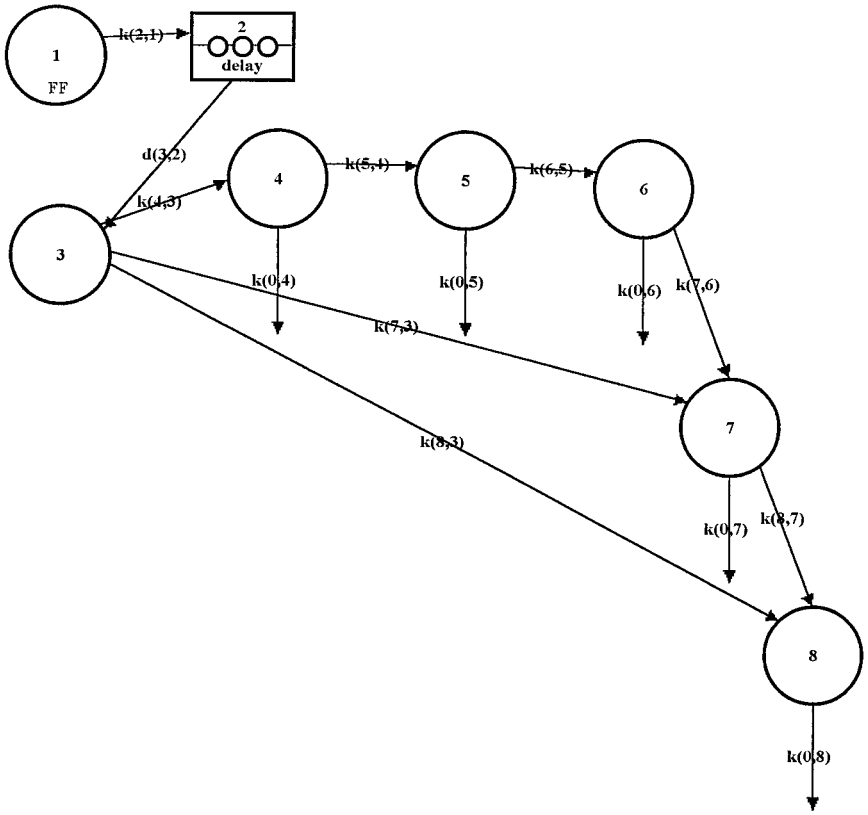

Figure 1. Multicompartmental model for apoB-100 metabolism. Compartment 1 is the plasma amino acid forcing function. Compartment 2 is an intracellular delay compartment representing the synthesis of apoB and assembly of lipoproteins in the liver. Compartments 3, 4, 5, and 6 represent plasma VLDL; compartment 7, IDL; and compartment 8, LDL.

Kinetic studies using stable isotopes of amino acids allow for the direct evaluation of endogenously synthesized protein and therefore have an advantage over radioactive studies in which a protein is isolated, labeled, and reinjected. Using stable isotopes and a monoexponential analysis, Lichtenstein et al[11] studied 8 subjects to determine whether an intestinal apolipoprotein such as apoB-48 would have a similar level of enrichment at plateau compared with a hepatically derived protein such as VLDL apoB-100. They did not study IDL and LDL kinetics. They observed that the tracer-tracee ratio at plateau for TRL apoB-48 was ≈50% of that for VLDL apoB-100 and concluded that maximal enrichment at plateau differed between the liver and intestine. Using a different methodology to prepare apoB-48 and apoB-100 samples for mass spectrometry,[12] we observed that the plateau for TRL apoB-48 approached that of VLDL apoB-100 in the present study. We then investigated the kinetics of TRL apoB-48 and apoB-100 within VLDL-, IDL-, and LDL-containing lipoproteins in 19 humans by using a primed-constant infusion of deuterated leucine with the subject in the constantly fed state and performing

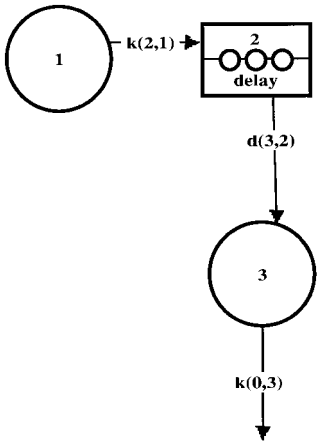

Figure 2. Multicompartmental model for determination of kinetic parameters for apoB-48. Compartment 1 represents the amino acid forcing function. Compartment 2 is an intracellular delay compartment representing the synthesis of apoB-48 and assembly of lipoproteins in the intestine. Compartment 3 accounts for the kinetics of the TRL containing apoB-48. See Methods for details.

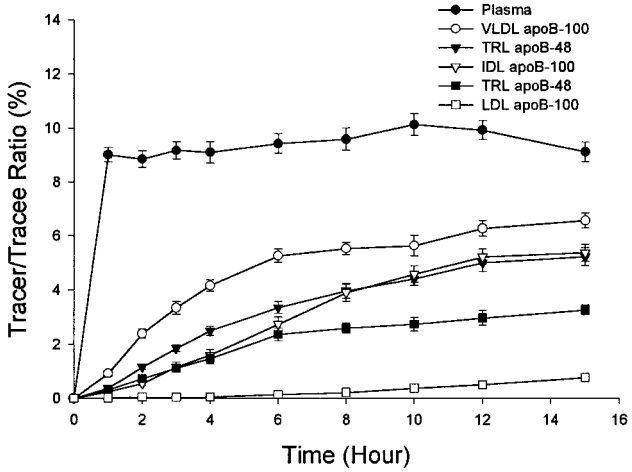

Figure 3. ApoB leucine tracer/tracee ratios for plasma (●), VLDL apoB-100 (○), TRL apoB-48 processed with the new methodology (▼), IDL apoB-100 (▽), TRL apoB-48 processed with the old methodology (■), and LDL apoB-100 (□). Results are shown as mean±SEM for all 19 subjects.

TABLE 1. Baseline Characteristics of the Subjects

Subject	Age, y	Weight, kg	BMI, kg/m^2	TC, mg/dL	LDL Cholesterol, mg/dL	HDL Cholesterol, mg/dL	TG, mg/dL
Female (n=8)							
Mean±SD	67.9±4.9	66.6±13.2	26.1±4.0	223.4±39.6	153.4±36.5	57.0±7.3	94.8±31.6
Range	58–71	50.9–88.1	22.6–30.8	185–283	111–209	41–64	53–146
Male (n=11)							
Mean±SD	57.1±12.2	77.4±9.1	25.3±1.8	204.1±32.6	136.5±26.4	43.5±8.5	115.0±36.8
Range	41–74	62.3–91.8	21.6–28.1	159–277	94–191	29–60	76–182
P	0.03	0.05	0.54	0.26	0.26	0.002	0.23

multicompartmen-tal modeling to determine apoB kinetic parameters and correlations between apoB-48 and apoB-100 kinetics.

Methods

Subjects

Nineteen subjects, 8 women and 11 men, underwent a medical history and physical examination. They had no evidence of any chronic illness, including endocrine, hepatic, renal, thyroid, or cardiac dysfunction. They did not smoke and were not taking any medications known to affect lipid levels. All female subjects were postmenopausal. The experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University.

Experimental Protocol for In Vivo Stable Isotope Kinetics

To determine the kinetics of TRL apoB-48 and VLDL, IDL, and LDL apoB-100, the subjects underwent a primed-constant infusion of deuterated leucine while they were in the fed state as previously described.[11–15] Starting at 6 AM, the subjects received 20 identical small hourly meals, each equivalent to 1/20th of their daily food intake, with 15% of calories as protein, 49% carbohydrate, 36% fat (15% saturated, 15% monounsaturated, 6% polyunsaturated), and 180 mg cholesterol/1000 kcal. At 11 AM, with 2 intravenous lines in place, 1 for the infusate and 1 for blood sampling, (5,5,5-$^2H_3$)-L-leucine (10 μmol/kg body wt) was injected as a bolus IV over 1 minute and then by continuous infusion (10 μmol · kg body wt^{-1} · h^{-1}) over a 15-hour period. Blood samples (20 mL) were collected at hours 0, 1, 2, 3, 4, 6, 8, 10, 12, and 15.

Plasma Lipid and Lipoprotein Characterization

Blood was collected in sterile tubes containing EDTA (0.1% final concentration). Plasma was separated from red cells in a refrigerated centrifuge at 3000 rpm for 30 minutes at 4°C. Plasma and lipoprotein fractions were assayed for total cholesterol and triglyceride with an Abbott Spectrum analyzer with enzymatic reagents.[16,17] HDL cholesterol was measured as previously described.[18] Lipid assays were standardized through the Centers for Disease Control Lipid Standardization Program.

The VLDL (d<1.006 g/mL), IDL (d=1.006 to 1.019 g/mL), and LDL (d=1.019 to 1.063 g/mL) fractions were isolated from fresh plasma by ultracentrifugation.[19] ApoB was assayed in plasma and lipoprotein fractions with a noncompetitive ELISA using immunopurified polyclonal antibodies.[20] The coefficient of variation for the apoB assay was <5% within runs and <10% between runs.[20]

Quantification and Isolation of the Apolipoproteins

ApoB-48 and apoB-100 were isolated from lipoproteins by preparative SDS-PAGE using a Tris-glycine buffer system as previously described.[21,22] Based on the assumption that both apoB-100 and apoB-48 have the same chromogenicity, apoB concentrations within individual apoB species were assessed by scanning each gel with laser densitometry as previously described.[11–15,23] We scanned VLDL fractions from each time point and averaged all 10 to calculate ratios and to estimate concentrations of apoB-48 and apoB-100 using the total apoB concentration as determined by ELISA.

Isotopic Enrichment Determinations

ApoB-48 and apoB-100 bands were excised from the polyacrylamide gels. Plasma (0.3 mL) and the excised apoB-48 and apoB-100 bands were hydrolyzed in 12N HCl at 100°C for 24 hours.[11–15] The free amino acids were isolated from plasma by Dowex AG-50W-X8 100- to 200-mesh cation exchange chromatography as previously described.[11–15] The free amino acids for apoB-48 and apoB-100 were initially isolated with the Dowex columns and subsequently with centrifugation at 2000*g* for 5 minutes as previously described for apoB-67.[12] The amino acids were converted to the n-propyl ester *N*-heptafluorobutyramide derivatives before analysis on a Hewlett-Packard 5890/5988A gas chromatograph/mass spectrometer.

Isotope enrichment (%) and tracer/tracee ratio (%) were calculated from the observed ion current ratios by the method of Cobelli et al.[24] Data in this format are analogous to specific radioactivity in radiotracer experiments. The isotopic enrichment of leucine in the apolipoproteins was expressed as tracer/tracee ratio (%).[24]

Kinetic Analysis

The kinetics of apoB-100 in the VLDL, IDL, and LDL fractions were described by a multicompartmental model (Figure 1) as previously

TABLE 2. Nonfasting Apolipoprotein Concentrations (mg/dL) During Kinetic Studies

Subject	Total Plasma ApoB	ApoB-48	VLDL ApoB-100	IDL ApoB-100	LDL ApoB-100
Female					
Mean±SD	113.6±38.6	0.5±0.4	8.1±4.9	2.5±.8	102.6±39.3
Range	83.5–194.1	0.32–1.41	2.90–18.76	0.93–3.77	76.13–186.4
Male					
Mean±SD	110.3±19.5	0.5±0.3	8.5±5.4	1.7±1.1	99.6±21.8
Range	78.2–143.1	0.31–1.19	2.07–18.59	0.61–4.23	72.5–135.9
P value	0.81	0.90	0.88	0.10	0.84

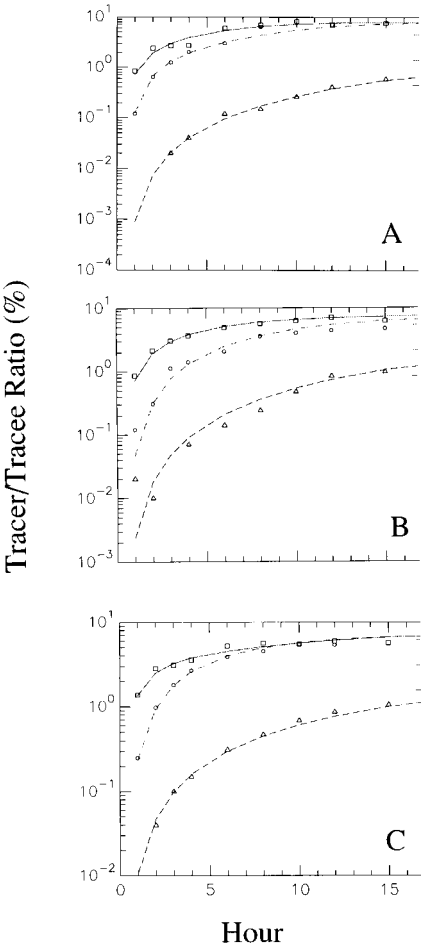

Figure 4. ApoB-100 leucine tracer/tracee ratios (percent) for VLDL apoB-100 (□), IDL apoB-100 (○), and LDL apoB-100 (△) versus time after a primed-constant infusion of [5,5,5-$^2H_3$]leucine over 15 hours in 3 study subjects: A, subject 1; B, subject 2; and C, subject 3. Observed values are given as symbols and model-predicted values as lines. No data points were excluded from the fitting process.

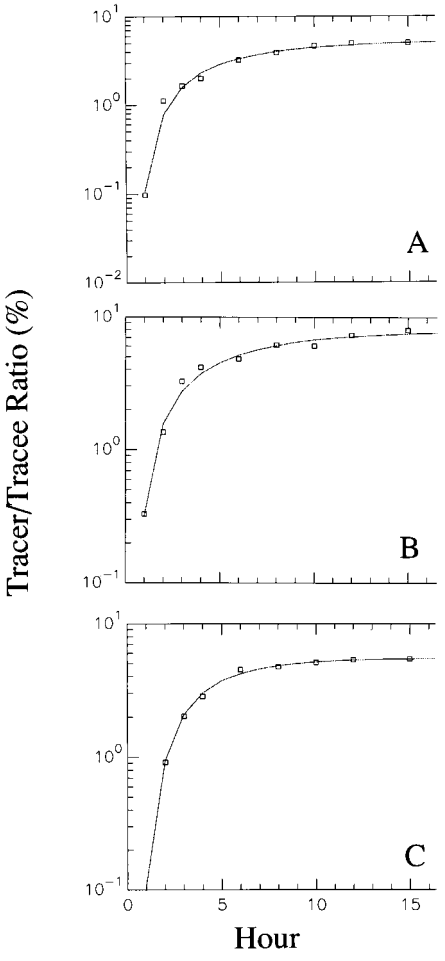

Figure 5. ApoB-48 leucine tracer/tracee ratios (percent) versus time after a primed-constant infusion of [5,5,5-$^2H_3$]leucine over 15 hours in 3 study subjects: A, subject 1; B, subject 2; and C, subject 3. Observed values are given as symbols and model-predicted values as lines. No data points were excluded from the fitting process.

described.[15] The SAAM II program was used to fit the model to the observed tracer data by a weighted-least-squares approach to find the best fit as previously described.[15,25] Compartment 1 is the plasma amino acid forcing function. Compartment 2 is an intracellular delay compartment representing the synthesis of apoB in the liver. Compartments 3, 4, 5, and 6 represent plasma VLDL; compartment 7, IDL; and compartment 8, LDL. The details of the model have been described previously.[15]

The kinetics of apoB-48 in the VLDL fraction were described by the multicompartmental model shown in Figure 2. The model consists of a precursor compartment (compartment 1), which is the plasma leucine pool. Compartment 2 is an intracellular delay compartment accounting for the synthesis of apoB-48 and the assembly of lipoproteins. Compartment 3 accounts for the kinetics of the TRL containing apoB-48. The fractional catabolic rate (FCR) of TRL apoB-48 corresponds to the rate of irreversible loss from compartment 3, k (0,3). The SAAM II program was used to fit the model to the observed tracer data by use of a weighted-least-squares approach to find the best fit.[25]

It is assumed that plasma leucine (compartment 1) is the source of the leucine that is incorporated into apoB and that all apoB enters the plasma via compartment 3. Therefore, transport rates into compartment 3 correspond to total apoB-100 and apoB-48 production in both models. Figure 3 shows the mean tracer-tracee ratio for plasma, VLDL apoB-100, TRL apoB-48, IDL apoB-100, and LDL apoB-100 for the 19 subjects at each time point. The mean tracer-tracee ratio for plasma leucine at plateau is ≈30% higher than that for VLDL apoB-100 at its plateau. Fisher et al[26] showed that the labeling of intracellular and extracellular leucine is not always similar; therefore, plasma leucine may not accurately reflect the precursor pool and may not be a suitable forcing function, especially for slowly-turning-over lipoprotein particles. We assumed a constant enrichment of the precursor pool and used the VLDL apoB-100 plateau for liver-derived VLDL apoB-100 as previously described[27] and the TRL apoB-48 plateau for intestinally derived apoB-48. Calculation of the plateau values was based on the solution of an exponential model assuming a single compartment. Thus, the VLDL apoB-100 plateau and TRL apoB-48 plateau tracer-tracee ratio data were used as the forcing functions to drive the appearance of tracer into apoB-100 and apoB-48, respectively.

It was assumed that each subject remains in steady state with respect to apoB-48 and apoB-100 metabolism during the study as previously shown.[11–15] Under this condition, the FCR is equivalent to the fractional synthetic rate. ApoB production rates (PRs) were determined by the formula PR ($mg \cdot kg^{-1} \cdot d^{-1}$)=[FCR (pools/d)× apoB concentration (mg/dL)×plasma volume (L)]/body wt (kg).[13,14] Plasma volume was estimated as 4.5% of body weight.

Statistical Analysis

Data were analyzed with the SysStat program and presented as mean±SD. Unpaired t tests were performed. Spearman correlation coefficients were determined. Probability values ≤0.05 were considered to be significant.

Results

Characteristics of the Subjects

Table 1 shows the characteristics of the subjects. The men were significantly younger and had significantly lower levels of HDL cholesterol than the women. The nonfasting plasma apoB-100 concentrations in the VLDL, IDL, and LDL lipoprotein fractions and apoB-48 in chylomicrons (Table 2) represent means of measures at all 10 time points during the study period.

In Vivo Kinetics of ApoB

During the kinetic studies, plasma apoB and lipid concentrations did not change significantly throughout the infusion period, indicating steady-state conditions as previously shown.[11–15]

When cation-exchange columns were used to separate SDS gel fragments from amino acids in apoB-48 samples, the plateau for TRL apoB-48 was ≈50% lower than the plateau for VLDL apoB-100 (Figure 3). Lichtenstein et al[11] reported similar results in 8 subjects. When centrifugation was used to separate SDS gel fragments from amino acids rather than cation exchange columns, the plateau for TRL apoB-48 (Figure 3) approached the plateau for VLDL apoB-100. We previously reported similar findings for apoB-67, a protein also present in low concentrations, and showed that the differences in tracer-tracee ratio significantly affected the FCRs for apoB-67.[12] Therefore, centrifugation was performed to remove SDS gel fragments from all samples obtained from SDS gels rather than cation exchange chromatography as previously described for apoB-67.[12]

Representative VLDL, IDL, and LDL apoB-100 leucine tracer/tracee ratios and model-predicted values are shown in Figure 4. Representative apoB-48 leucine tracer-tracee ratios and model-predicted values are shown in Figure 5.

Table 3 shows the pool sizes (PS), FCRs, and PRs for apoB-48 and apoB-100 grouped by sex. There were no sex differences. The remainder of the results are given for the entire group. The PS of TRL apoB-48, VLDL apoB-100, and LDL apoB-100 were 17±10, 273±167, and 3325±1146 mg, respectively; therefore, the mean TRL apoB-48 PS was only 6.2% of that of VLDL apoB-100. There was a trend toward a faster FCR for VLDL apoB-100 than for TRL apoB-48 (6.73±3.48 versus 5.02±2.07 pools/d, respectively, $P=0.06$). The mean FCRs for IDL and LDL apoB-100 were 10.07±7.28 and 0.27±0.08 pools/d, respectively. The mean PR of TRL apoB-48 was 6.5% of VLDL apoB-100 (1.3±0.90 versus 20.06±6.53 mg · kg^{-1} · d^{-1}, $P<0.0001$). The mean PRs of IDL and LDL apoB-100 were 7.6±3.9 and 11.3±2.3 mg · kg^{-1} · d^{-1}, respectively.

TRL apoB-48 PS was correlated with apoB-48 PR ($r=0.780$, $P<0.0001$) but not FCR ($r=-0.1810$, $P=0.458$). VLDL apoB-100 PS was correlated with both PR ($r=0.713$, $P=0.0006$) and FCR ($r=-0.692$, $P=0.001$) of VLDL apoB-100 and with apoB-48 PR ($r=0.728$, $P=0.0004$). LDL apoB-100 PS was correlated with FCR ($r=-0.549$, $P=0.015$). TRL apoB-48 PR and PS were correlated inversely with VLDL apoB-100 FCR ($r=-0.597$, $P=0.007$ and $r=-0.721$, $P=0.0005$, respectively) and directly with VLDL apoB-100 PR ($r=0.507$, $P=0.027$ and $r=0.534$, $P=0.018$). ApoB-48 PS was directly correlated with VLDL apoB-100 PS ($r=0.837$, $P<0.0001$).

Discussion

The present study provides information on the interrelationships between TRL apoB-48 and VLDL, IDL, and LDL apoB-100 kinetics in 19 human subjects on an average American diet by multicompartmental modeling. Cell culture studies have shown that the regulation of apoB-containing lipoprotein secretion occurs mainly posttranslationally, because de novo synthesized apoB molecules are targeted for either secretion or intracellular degradation.[28–30] The availability of lipid appears to play a major role in determining the amount of apoB that is secreted from the liver. Oleic acid significantly stimulates apoB secretion from cultured HepG2 cells[29] by facilitating the translocation of newly synthesized apoB away from proteases[31] that appear to be associated with the endoplasmic reticulum[29] and thereby protecting newly synthesized apoB from intracellular degradation.

On the basis of these in vitro results, we hypothesized that humans who have higher rates of apoB-48 production would have more fat delivered to their liver in the form of chylomicron remnants and therefore have higher rates of production of VLDL apoB-100 from the liver. The positive correlation between apoB-48 PS and VLDL apoB-100 PR observed in the present study suggests that when more lipid and/or cholesteryl ester is delivered to the liver in the form of chylomicron remnants, the secretion of VLDL apoB-100 from the liver is increased. Thus, our results provide support for our hypothesis.

Both chylomicrons and VLDL increase after a fat-rich meal. Cohn et al[13,22] showed that the apoB-48 PS is 10-fold higher and VLDL apoB-100 PS is 22% higher in the fed state than in the fasted state. The data in the present study indicating that TRL apoB-48 PS is correlated with PR and not FCR suggest that the increase in chylomicrons is due to an increase in PR. In contrast, VLDL apoB-100 PS is correlated with both production and catabolism of VLDL apoB-100. In addition, the inverse correlation between TRL apoB-48 PS

TABLE 3. PS, FCR, and PR

	TRL B-48			VLDL B-100			IDL B-100		
	PS, mg	FCR, pools/d	PR, mg · kg^{-1} · d^{-1}	PS, mg	FCR, pools/d	PR, mg · kg^{-1} · d^{-1}	PS, mg	FCR, pools/d	PR, mg · kg^{-1} · d^{-1}
Female									
Mean±SD	15.0±9.6F	5.1±2.7	1.2±0.8	237.8±127.5	7.1±2.4	22.0±5.8	77.4±37.6	7.4±2.3	8.3±3.5
Male									
Mean±SD	18.8±10.5	5.0±1.5	1.4±0.9	298.3±193.1	6.4±4.2	18.7±6.9	60.1±35.6	12.0±9.0	7.0±4.3
P	0.41	0.94	0.55	0.45	0.70	0.29	0.32	0.18	0.48

TABLE 4. Kinetic Studies of ApoB-48

Author	Subjects, n	Tracer	Conclusions
Schaefer et al,[37] 1978	1	^{125}I chylomicrons	Labeled pleural fluid chylomicrons used; rapid clearance of chylomicron apoB (<1% remaining by 6 hours) and very little conversion to other lipoproteins
Nestel et al,[9] 1983	6 HTG 2 Normal	^{125}I-TRL	Donor was HTG; apoB-100 cleared more rapidly than apoB-48 in 4 HTG; in 2 HTG and 2 normals, rates were similar
Stalenhoef et al,[10] 1984	2 HTG	^{125}I-TRL	Donor was HTG; in 2 normals, most of apoB-48 cleared within 15 minutes and most of apoB-100 within 30 minutes, whereas in HTG, clearance of both proteins markedly delayed
Stalenhoef et al,[38] 1986	3 DBL 1 Normal	^{125}I-TRL	Donor was DBL; impaired TRL apoB-100 and apoB-48 clearance in the DBL subjects
Schaefer et al,[39] 1986	1 ApoE-deficient 2 Normal	^{125}I-TRL	Donor was apoE-deficient; impaired TRL apoB-100 and apoB-48 clearance in apoE-deficient subject
Lichtenstein et al,[11]* 1992	8 Subjects	Endogenous stable isotope labeling	Similar TRL apoB-100 and apoB-48 FCRs; monoexponential analysis
Present study*	19 Subjects	Endogenous stable isotope labeling	Trend toward faster FCR for VLDL apoB-100 than TRL apoB-48 (6.73±3.48 vs 5.02±2.07 pools/d, $P=0.06$) as assessed by multicompartmental modeling; subjects studied in constantly fed state

*All studies performed in the fasting state except for those with *. HTG denotes hypertriglyceridemic; DBL, dysbetalipoproteinemic.

and PR and VLDL apoB-100 FCR suggests that an increase in the apoB-48 PS slows down the catabolism of VLDL apoB-100, a finding suggesting that TRL apoB-48 and VLDL apoB-100 may be competing for the same catabolic pathway. Thus, the increase in VLDL apoB-100 PS after a fat-rich meal is due to increased production and decreased clearance of VLDL apoB-100. These results are supported by previous observations with fat feeding in humans. Bjorkegren et al[32] infused a chylomicron-like triglyceride emulsion intravenously for 60 minutes to healthy young men. Using simultaneous stable isotope studies, they observed that concomitant with a 3-fold increase in plasma triglycerides, large VLDL increased and small VLDL decreased. They concluded that the formation of chylomicrons and their remnants during feeding inhibited lipolysis of large VLDL to small VLDL and accounted for the increase in VLDL after meals. In another study, after an oral fat load, Karpe et al[33] observed an increase in both small and large chylomicron remnants in control subjects and normotriglyceridemic patients and an increase in only large chylomicron remnants in hypertriglyceridemic patients. They also concluded that chylomicrons compete with VLDL for removal of triglycerides by lipoprotein lipase.

In the present study, we observed that LDL apoB-100 PS was correlated with FCR but not PR. This finding indicates an important role for the LDL receptor in regulating LDL levels in humans. It is important to note that the correlations we observed between apoB-48 and apoB-100 kinetics provide insight into possible mechanisms that can now be examined by studying subjects under various metabolic and dietary conditions.

Our second hypothesis was that the FCRs of apoB-48 and apoB-100 would be similar, a finding Lichtenstein et al[11] observed in a monoexponential analysis. However, we observed a trend toward faster catabolism for VLDL apoB-100 than for TRL apoB-48 ($P=0.06$). ApoB-48 kinetics have been difficult to perform because of the low protein concentration of apoB-48, especially in the fasting state, and difficulty in measuring the concentration of apoB-48.[22,34] Some investigators have used retinyl esters as a marker of chylomicron remnant clearance. However, it has been noted that retinyl ester can exchange between lipoproteins and therefore may not be an ideal marker of chylomicron remnants.[35,36] ApoB-48 appears to be a better marker for chylomicrons and their remnants in plasma than retinyl palmitate because there is no exchange of apoB-48 between lipoproteins, and the liver secretes only apoB-100 in humans.[2]

Several studies have used radioactivity to study the kinetics of apoB-100 and apoB-48 within TRL simultaneously. These studies are summarized in Table 4. Using labeled pleural chylomicrons, Schaefer et al[37] showed that <1% of apoB labeled within TRL remained at 6 hours in the fasting state with virtually no transfer to LDL. Nestel et al[9] labeled TRL from hypertriglyceridemic subjects with ^{125}I and reinjected it into 4 hypertriglyceridemic subjects. ApoB-100 was cleared more rapidly than apoB-48, whereas the rates were similar when reinjected into 2 hypertriglyceridemic and 2 normal subjects.[9] When ^{125}I-labeled TRL from a hypertriglyceridemic subject was reinjected into 2 normal subjects, Stalenhoef et al[10] observed rapid clearance of most of the apoB-48 in 15 minutes and most of the apoB-100 within 30 minutes, whereas in their hypertriglyceridemic subject, clearance of both proteins was markedly delayed. In a second study, Stalenhoef et al[38] used ^{125}I-TRL from a dysbetalipoproteinemic subject; clearance of both TRL apoB-100 and apoB-48 was impaired. When Schaefer et al[39] reinjected ^{125}I-TRL from an apoE-deficient subject, delayed clearance of both the apoB-48 and apoB-100 occurred in the apoE-deficient subject compared with rapid clearance in the normal subjects.

TABLE 3. Continued

LDL B-100		
PS, mg	FCR, pools/d	PR, mg · kg^{-1} · d^{-1}
3205±1741	0.29±0.09	12.4±2.1
3413±470	0.26±0.07	10.8±2.5
0.71	0.33	0.16

TABLE 5. Rate Constants of Individual Lipoprotein Pools and Delay for ApoB-100

Subject	*k*(2,1)	d(3,2)	*k(4,3)**	*k*(0,4)†	*k*(7,3)	*k*(8,3)	*k*(8,7)	*k*(0,8)
1	0.54±0.06	0.2	0.26±0.03	0.12±0.03	0.03±0.006	0.01±0.004	0.38±0.08	0.012±0.001
%FSD	11.0	...	10.3	22.6	0.5	40.2	17.9	11.4
2	0.57±0.07	0.2	0.50±0.16	0.46±0.14	0.13±0.02	0.03±0.002	0.33±0.08	0.010±0.001
%FSD	12.0	...	31.7	31.9	19.0	12.8	24.6	10.5
3	0.44±0.036	0.5	0.21±0.07	0.21±0.06	0.07±0.01	0.36±0.04	0.28±0.05	0.014±0.0008
%FSD	3.8	...	35.1	29.1	15.1	11.2	18.7	5.5
4	0.85±0.09	0.92±0.04	0.43±0.06	0.18±0.06	0.08±0.01	0.16±0.03	0.23±0.04	0.015±0.001
%FSD	10.3	4.8	13.7	31.8	18.5	15.5	16.8	8.0
5	0.58±0.06	0.82±0.05	0.67±0.09	0.30±0.09	0.27±0.04	0	0.42±0.05	0.016±0.0009
%FSD	11.1	5.7	13.9	29.2	15.0	...	11.0	5.64
6	0.44±0.02	0.27±0.06	0.57±0.05	0	0.21±0.03	0.16±0.05	0.43±0.05	0.007±0.0003
%FSD	4.8	23.4	9.6	...	15.2	32.2	11.8	5.7
7	0.36±0.02	0.30±0.11	0.04±0.004	0.09±0.05	0.18±0.03	0.42±0.07	0.16±0.02	0.016±0.001
%FSD	5.1	35.0	10.0	63.5	16.9	16.8	12.5	8.4
8	0.77±0.09	0.5	0	0	0.10±0.02	0.04±0.008	0.23±0.02	0.008±0.0004
%FSD	12.1	...	...	...	15.2	19.1	7.8	5.5
9	0.73±0.08	0.40±0.05	0.22±0.06	0.21±0.06	0.12±0.02	0	1.18±0.14	0.009±0.0005
%FSD	10.7	13.9	25.0	29.6	14.5	...	11.6	5.9
10	0.85±0.10	0.5	0.52±0.05	0.13±0.04	0.16±0.03	0	0.68±0.08	0.017±0.001
%FSD	11.2	...	9.2	35.8	16.0	...	11.0	5.8
11	1.16	0.50	0.12±0.005	4.39	0.02±0.002	0.02±0.002	0.16	0.011±0.0003
%FSD	...	...	4.2	...	10.2	8.1	...	2.5
12	0.40±0.025	0.50	0.36±0.12	0.13±0.05	0.21±0.05	0.62±0.12	0.26±0.06	0.011±0.0006
%FSD	6.2	...	33.3	41.0	22.5	20.1	23.7	5.8
13	0.37±0.03	0.73±0.04	0.08±0.02	0.14±0.06	0.09±0.01	0.25±0.03	0.13±0.01	0.008±0.0008
%FSD	7.4	6.1	25.0	41.9	14.9	13.3	10.7	10.8
14	0.52±0.06	0.62	0.44±0.02	0.08±0.03	0.02±0.004	0.03±0.005	0.59±0.06	0.009±0.0004
%FSD	10.9	...	5.2	34.4	16.4	19.2	11.1	7.2
15	0.48±0.04	0.77±0.04	0.08±0.04	0.18±0.07	0.12±0.02	0.07±0.01	0.15±0.02	0.008±0.0008
%FSD	9.3	5.2	53.3	40.5	14.2	19.1	14.4	10.6
16	0.45±0.02	1.11±0.05	1.07±0.16	0	0.44±0.09	1.37±0.23	0.85±0.11	0.011±0.0006
%FSD	4.1	4.9	15.4	...	20.1	16.6	12.9	5.3
17	0.40±0.04	0.50	0.43±0.05	0.06±0.04	0.06±0.01	0.32±0.06	0.98±0.22	0.010±0.0008
%FSD	9.6	...	12.7	74.1	23.6	18.3	22.3	7.5
18	0.42±0.04	0.50	0.36±0.10	0.21±0.06	0.08±0.02	0.23±0.04	0.38±0.12	0.009±0.0007
%FSD	8.5	...	28.6	30.4	19.1	15.7	30.3	8.0
19	0.96±0.10	0.50	0.23±0.02	0.17±0.03	0.01±0.002	0.06±0.01	0.16±0.02	0.015±0.001
%FSD	10.0	...	6.8	16.7	16.2	16.8	15.6	8.8

FSD indicates fractional standard deviation.

**k*(0,4)=*k*(0,5)=*k*(0,6)=*k*(0,7).

†*k*(4,3)=*k*(5,4)=*k*(6,5)=*k*(7,6).

In these early studies,[9,10,38,39] the half-life of VLDL apoB-100 and apoB-48 was much shorter than the longer half-lives observed in the present study and many other recent studies using either radioactivity or endogenous labeling techniques.[11–14,27,40,41] Differences in half-lives between the present study and the previous studies may be due to differences in the design of the studies. In the previous studies, either TRL isolated from hypertriglyceridemic subjects or pleural chylomicrons were used; thus, the particles were abnormal. The TRL was then radioactively labeled and reinjected into both abnormal and normal subjects who were fasting. The isolation, labeling, and concentration of the lipoproteins for radioactive studies may alter the protein and thus affect its FCR. In addition, the populations of particles studied with exogenous labeling may differ from those studied with endogenous labeling. In exogenous labeling studies, lipoproteins are removed at 1 point in time and concentrated; therefore, the population of particles that are labeled are representative of the distribution at that time. As a result, a large proportion of these lipoproteins may consist of remnant-like particles rather than nascent particles, and thus, the kinetics of the nascent particles may not be apparent. In

TABLE 6. Rate Constants of Individual Lipoprotein Pools and Delay for ApoB-48

Subject	*k*(2,1)	d(3,2)	*k*(0,3)
1	0.06±0.002	0.50	0.19±0.009
%FSD	3.0	...	4.7
2	0.04±0.003	0.61±0.11	0.35±0.03
%FSD	17.9	17.9	8.5
3	0.02±0.001	1.1±0.11	0.25±0.019
%FSD	5.8	9.8	7.4
4	0.03±0.003	0.50	0.13±0.02
%FSD	7.6	...	19.6
5	0.009±0.001	0.50	0.39±0.08
%FSD	15.8	...	21.1
6	0.02±0.002	0.93±0.08	0.21±0.04
%FSD	11.8	8.5	19.6
7	0.002±0.0008	0.62	0.05±0.009
%FSD	18.4	...	18.4
8	0.02±0.0006	0.50	0.10±0.005
%FSD	2.4	...	5.7
9	0.02±0.002	1.04±0.07	0.15±0.03
%FSD	10.6	6.8	21.7
10	0.03±0.005	1.79±0.20	0.22±0.06
%FSD	18.1	11.2	27.1
11	0.08±0.007	0.50	0.21±0.04
%FSD	9.1	...	17.2
12	0.02±0.002	0.50	0.33±0.05
%FSD	11.3	...	15.6
13	0.01±0.002	0.50	0.12±0.05
%FSD	21.1	...	39.8
14	0.04±0.005	1.27±0.08	0.21±0.04
%FSD	11.8	6.1	19.8
15	0.06±0.01	0.96±0.26	0.24±0.06
%FSD	19.2	27.3	26.7
16	0.009±0.001	0.83±0.08	0.25±0.05
%FSD	11.8	9.4	18.9
17	0.02±0.004	1.54±0.22	0.26±0.08
%FSD	20.8	14.2	29.8
18	0.02±0.003	0.98±0.08	19±0.04
%FSD	12.6	8.0	21.4
19	0.03±0.003	0.50	0.12±0.03
%FSD	10.3	...	27.2

TABLE 7. ApoB PS

		ApoB-100		
Subject	ApoB-48	VLDL	IDL	LDL
1	32.4	430.5	51.9	1753.4
2	11.1	210.0	60.3	2117.0
3	9.8	153.0	26.2	2380.1
4	26.8	419.8	149.3	3846.4
5	8.7	164.3	82.1	2070.7
6	12.2	181.2	82.3	6673.0
7	1.9	76.1	60.6	2087.7
8	20.1	267.5	106.3	4712.7
9	17.7	425.1	31.6	3305.2
10	17.1	408.8	85.6	2448.3
11	39.4	613.4	76.7	2489.4
12	12.2	124.7	51.8	3648.2
13	11.2	128.8	61.0	3816.2
14	31.2	315.5	46.4	3544.2
15	25.5	257.7	133.7	4153.8
16	3.7	57.9	26.3	3805.2
17	10.2	187.2	19.7	3404.4
18	13.6	156.5	28.9	2995.0
19	25.4	605.3	99.4	2935.4

Values are in milligrams.

contrast, endogenous labeling ensures that all particles are labeled proportionally, and thus, the kinetics of both nascent and remnant particles should be apparent.

In summary, we have shown that apoB-48 PS is only 6.2% of that of VLDL apoB-100 and is correlated with PR, not FCR; VLDL apoB-100 PS is correlated with both PR and FCR of VLDL apoB-100 and with PR of apoB-48; and LDL PS is correlated with FCR. In addition, there is a trend toward faster catabolism for VLDL apoB-100 than for TRL apoB-48. The analytic and modeling approach in the present study and the improvement in the methodology for preparation of apoB-48 samples for mass spectrometry should permit the use of stable isotopes to elucidate key features of both apoB-48 and apoB-100 metabolism in normal and pathological states and to examine factors influencing dietary responsiveness. Determination of these factors will have important implications for cardiac risk reduction in the primary and secondary prevention of coronary heart disease.

Appendix

For Appendix, please see Tables 5, 6, and 7.

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