Production of apolipoprotein B-67 in apolipoprotein B-67/B-100 heterozygotes: technical problems associated with leucine contamination in stable isotope studies

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Abstract In vivo kinetics of apoB were performed in three apoB-67/apoB-100 heterozygotes using a primed-constant infusion of (5,5,5-$^2H_3$)-leucine. Mean plasma VLDL and LDL apoB-67 concentrations were 0.05 ± 0.01 mg/dl and 0.62 ± 0.17 mg/dl, respectively, which were 0.2% and 2.8% of total plasma apoB concentrations. When cation exchange chromatography was used to separate plasma amino acids from fragments of polyacrylamide gels, the tracer/tracee ratio at plateau for VLDL apoB-67 was less than 50% of that observed when centrifugation was used. Mean fractional catabolic rate for LDL apoB-67 was 2-fold higher when the lower plateau was used in multicompartmental analysis compared to the higher plateau (0.70 ± 0.21 versus 0.37 ± 0.06 pools per day, P = 0.06). The lower plateau resulted from introduction of unlabeled leucine during cation exchange chromatography; therefore, all samples were processed with centrifugation. Mean fractional catabolic rates for VLDL and LDL apoB-67 were not significantly different from VLDL and LDL apoB-100 (VLDL: 9.7 ± 3.4 versus 18.1 ± 8.6 pools per day, respectively, P = 0.19; LDL: 0.37 ± 0.06 versus 0.34 ± 0.11 pools per day, respectively, P = 0.66). Mean secretion rate of VLDL apoB-67 was 5.6% of VLDL apoB-100 (0.20 ± 0.04 versus 3.6 ± 1.3 mg/kg/day, P = 0.01). Fifty-three % of apoB-67 was directly removed from VLDL compared to only 3.5% of apoB-100 (P = 0.003), thus accounting for the lower proportion of apoB-67 in LDL (3.3 ± 1.8%) as compared to VLDL (11.2 ± 2.5%). Mean LDL production rate for apoB-67 was 2.6% of LDL apoB-100 (0.09 ± 0.02 versus 3.50 ± 1.39 mg/kg/day, P = 0.05). Thus, decreased secretion of apoB-67 is responsible for low levels of apoB-67.—**Welty, F. K., A. H. Lichtenstein, P. H. R. Barrett, G. G. Dolnikowski, J. M. Ordovas, and E. J. Schaefer.** Production of apolipoprotein B-67 in apolipoprotein B-67/B-100 heterozygotes: technical problems associated with leucine contamination in stable isotope studies. *J. Lipid Res.* 1997. **38:** 1535–1543.

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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. The analysis of data from stable isotope studies requires the isolation of the protein and derivatization of the labeled amino acid in preparation for determination of the ratio of labeled to unlabeled amino acid (tracer/tracee ratio) by gas chromatography mass spectrometry.

Apolipoprotein (apo) B, the main protein of low density lipoprotein (LDL), normally exists in two isoforms in plasma, apoB-100 and apoB-48 (1). Synthesized by the liver, apoB-100 is secreted in the form of very low density lipoprotein (VLDL) which is metabolized in plasma to form LDL. In the human, apoB-48 is synthesized in the intestine in response to dietary fat and secreted in the form of chylomicrons (1). In stable isotope studies of the metabolism of apoB, the apoB isoforms are separated using SDS gradient gel electrophoresis (2–4). Cation exchange chromatography has been used to separate plasma amino acids from fragments of polyacrylamide gels (2–9).

Familial hypobetalipoproteinemia is an autosomal codominant disorder characterized by plasma con-

Abbreviations: apo, apolipoprotein; FCR, fractional catabolic rate; FSD, fractional standard deviation; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LDL-C, low density lipoprotein cholesterol; SDS, sodium dodecyl sulfate; VLDL, very low density lipoprotein.

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centrations of apolipoprotein B and LDL cholesterol (LDL-C) that are about one-third of normal (10). Some cases of hypobetalipoproteinemia have been shown to be due to a truncated form of apoB-100 (10). ApoB-67 is due to deletion of an adenine at cDNA 9327 (11). Affected family members have LDL-C levels one-third of normal. Plasma levels of apoB-67 in the apoB-67/B-100 heterozygotes range from 1 to 4% of total apoB levels (12). In this study, we investigated the mechanism for the low plasma levels of apoB-67 by using a primed-constant infusion of deuterated leucine and performing multicompartmental modeling to determine apolipoprotein kinetic parameters. When cation exchange chromatography was used to separate plasma amino acids from fragments of polyacrylamide gels, the tracer/tracee ratio at plateau for VLDL apoB-67 was less than 50% of that observed when centrifugation was used. This difference was due to the introduction of unlabeled leucine during cation exchange chromatography; therefore, all samples were processed with centrifugation. Decreased secretion of apoB-67 was the primary reason for the low levels of apoB-67 in apoB-67 heterozygous subjects.

MATERIALS AND METHODS

Three subjects of the apoB-67 kindred were studied (5). They have been described previously as have the details of the experimental protocol (5). Lipid levels were determined as previously described (2–5, 13). The VLDL (d $<$ 1.006 g/ml), IDL (d 1.006–1.019 g/ml) and LDL (d 1.019–1.063 g/ml) fractions were isolated from fresh plasma by ultracentrifugation (14). ApoB was assayed in plasma and lipoprotein fractions with a noncompetitive, enzyme-linked immunosorbent assay using immunopurified polyclonal antibodies (15). The coefficient of variation for the apoB assay was less than 5% within runs and less than 10% between runs (15).

Quantitation and isolation of the apolipoproteins

ApoB-67 and apoB-100 were isolated from lipoproteins by preparative sodium dodecyl sulfate (SDS) polyacrylamide gradient gel electrophoresis using a Tris-glycine buffer system as previously described (2–5, 13, 16). Based on the assumption that both forms of apoB have the same chromogenicity as assumed by others for apoB-48 and other truncations (2–8, 17), apoB concentration within individual apoB species was assessed by scanning each gel with laser densitometry as previously described (2–5, 13, 17). We scanned VLDL, IDL, and LDL fractions from each time point and averaged all ten to calculate ratios and to estimate concentrations of apoB-67 and apoB-100.

Isotopic enrichment determinations

ApoB-67 and apoB-100 bands were excised from polyacrylamide gels. Plasma (0.3 ml) and the excised apoB-67 and apoB-100 bands were hydrolyzed in 12 N HCl at 100°C for 24 h. The free amino acids were initially isolated using Dowex AG-50W-X8 100–200 mesh cation exchange chromatography and subsequently with centrifugation at 2,000 *g* for 5 min. The amino acids were converted to the n-propyl ester, N-heptafluorobutyramide derivatives prior to analysis on a Hewlett-Packard 5890/5988A gas chromatograph/mass spectrometer.

Isotope enrichment and tracer/tracee ratio (as percents) were calculated from the observed ion current ratios using the method of Cobelli et al. (18) as described previously (5).

Kinetic analysis

The kinetics of apoB-67 in VLDL, IDL, and LDL fractions were described by a multicompartmental model previously used to describe the kinetics of apoB-100 in apoB-67 subjects and six normolipidemic subjects (5). This model is a modification of a multicompartmental model previously used to describe the kinetics of apoB in subjects heterozygous for apoB-75 (6, 7) apoB-89 (7, 8), apoB-54.8, apoB-52 and apoB-31 (7, 9), in normolipidemic subjects who had plasma cholesterol levels between the 5th and 50th percentile of age matched controls (19) and subjects with cholesteryl ester transfer protein deficiency (20). Each apoB-67 subject studied is heterozygous for apoB-67/apoB-100; therefore, this permitted a comparison of metabolic parameters for apoB-67- and apoB-100-containing particles. As noted in the studies of apoB-31, apoB-52, and apoB-54 (7, 9), apoB-75 (6, 7) and apoB-89 (7, 8), differences in metabolic parameters between apoB-67 and apoB-100 are largely independent of the model chosen because parameters for the truncation are compared to those of apoB-100 obtained in the same subject, at the same time, and under the same experimental conditions.

The SAAM II program (21) was used to fit the model to the observed tracer data using a weighted least squares approach to find the best fit. The model has been described in detail previously (5). A difference from the previous model is that a direct loss from compartment 3 (designated as k(0,3)) rather than the delipidation chain was required to fit the observed tracer/tracee ratios of VLDL-apoB-67 (5).

Because of low IDL apoB-67 concentrations, tracer/tracee ratios could not be determined in this fraction. This situation is similar to that observed in previous ki-

netic studies of apoB truncations (6, 8). Therefore, it was assumed that, as has been with IDL apoB-75 (6) and apoB-89 (8), all IDL apoB-67 was converted to the LDL fraction in the apoB-67 subjects. Similar to the previous studies, the fractional catabolic rate of the IDL compartment was free to adjust to provide the delay necessary to fit the LDL data (6, 8).

It is assumed that each subject remains in steady state with respect to apoB metabolism during the course of the study (2–5). Under this condition, the fractional synthetic rate is equivalent to the fractional catabolic rate. ApoB secretion rates were determined by the formula: secretion rate (mg/kg/day) = [FCR (pools/day) × apoB concentration (mg/dL) × plasma volume (1)]/body weight (kg). Plasma volume was estimated as 4.5% of body weight.

Statistical analysis

Data were analyzed using the SigmaStat program and presented as mean ± standard deviation (SD) and % fractional standard deviation (FSD). Unpaired *t* tests were performed. *P* values ≤ 0.05 were considered to be significant.

RESULTS

Characteristics of the subjects

There was no significant difference in age or body mass index between the apoB-67 subjects and control subjects (5). In the apoB-67 subjects, total cholesterol levels were 50% of those of the control subjects, LDL-C levels were 19% of control, HDL-C levels were 143% of control, and triglyceride levels were 36% of control (5). The non-fasting plasma apoB concentrations in the VLDL, IDL, and LDL lipoprotein fractions (**Table 1**) represent means of measures at all 10 time points during the study period. The mean plasma VLDL and LDL apoB-67 concentrations were 0.05 ± 0.01 mg/dl and 0.62 ± 0.17 mg/dl which were 0.2% and 2.8% of the total plasma apoB concentrations, respectively. The percentage of apoB-67 was higher in the VLDL fraction (11.2%) compared to the LDL fraction (3.3%) (Table 1).

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 (2–5). Plasma leucine tracer/tracee ratios ranged from 5 to 7% for the subjects and remained constant during the course of the infusion (**Fig. 1**). A representative 4–12% SDS gel of the VLDL fractions is shown in **Fig. 2.** We initially used the excised band from one SDS gel for apoB-67 at each time point to determine enrichment; however, the enrichment was undetectable, probably due to extremely low plasma levels of apoB-67, a situation similar to that reported previously for the apoB-40 truncation (8), for IDL and LDL in the apoB-54.8 truncation (7), and for IDL in the apoB-75 (6) and apoB-89 truncations (7, 8). When four apoB-67 bands from four SDS gels were combined for each time point, the plateau for VLDL apoB-67 (closed triangles, Fig. 1) was approximately 50% lower than the plateau for VLDL apoB-100 (closed circles, Fig. 1). When centrifugation was used to separate SDS gel fragments from amino acids rather than cation exchange columns, the plateau for VLDL apoB-67 (open squares, Fig. 1) was similar to that for VLDL apoB-100 (closed circles, Fig. 1). When the distilled water used to wash the columns was derivatized and analyzed on the mass spectrometer, unlabeled leucine was detected. The leucine was thought to result from bacterial contamination of the distilled water. A very small amount of leucine resulting from bacterial breakdown could dilute deuterated leucine, especially with proteins present in low concentrations. This leucine may not be removed by the bacterial filter in the still containing the distilled water. Therefore, centrifugation was performed to remove

TABLE 1. Non-fasting plasma apolipoprotein concentrations during kinetic studies

Subject	Plasma ApoB	VLDL ApoB-100	VLDL ApoB-67	VLDL B-67/B-100 Ratio	IDL ApoB-100	IDL ApoB-67	LDL ApoB-100	LDL ApoB-67	LDL B-67/B-100 Ratio
	mg/dl	*mg/dl*		*%*	*mg/dl*		*mg/dl*		*%*
1	14.9 ±0.30	0.40 ±0.09	0.04 ±0.02	10.0	0.16 ±0.02	0.008 10.001	13.6 ±0.39	0.70 ±0.27	5.1
2	26.7 ±1.22	0.35 ±0.09	0.05 ±0.01	14.0	0.22 ±0.05	0.011 ±0.002	25.6 ±1.15	0.42 ±0.29	1.6
3	23.7 ±1.34	0.63 ±0.14	0.06 ±0.01	9.5	0.19 ±0.02	0.009 ±0.001	22.1 ±1.22	0.74 ±0.19	3.3
Mean ± SD	21.8 ±6.1	0.46 ±0.15	0.05 ±0.01	11.2 ±2.5	0.19 ±0.03	0.009 ±0.001	20.4 ±6.2	0.62 ±0.17	3.3 ±1.8

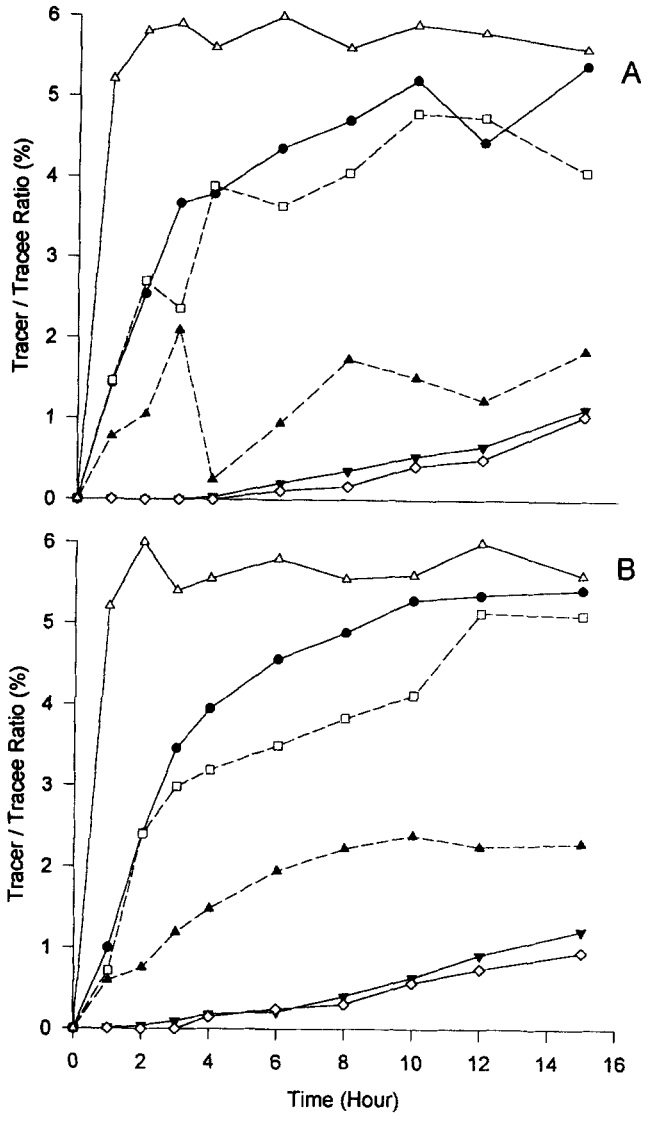

Fig. 1. ApoB leucine tracer/tracee ratios (%) for plasma (open triangles); VLDL apoB-100 processed without columns (solid circles with solid line); VLDL apoB-67 processed without cation exchange columns (open squares with dashed line); VLDL apoB-67 processed with cation exchange columns (solid triangles with dashed line); LDL apoB-100 processed without columns (inverted solid triangles); and LDL apoB-67 processed without columns (open diamonds) in two apoB-67/B-100 heterozygous subjects. A, subject 1; B, subject 2.

SDS gel fragments from all samples obtained from SDS gels rather than cation exchange chromatography.

The tracer/tracee data with and without cation exchange chromatography were subjected to multicompartmental modeling to determine whether the differences at plateau affected the fractional catabolic rates. The VLDL and LDL apoB leucine tracer/tracee ratios and model predicted values obtained without the cation exchange columns are shown for the apoB-67 subjects in **Fig. 3.** There is good agreement between the model-derived fits and the observed tracer data.

Table 2 compares the apoB-67 VLDL and LDL fractional catabolic rates calculated using the tracer/tracee ratios obtained both by using the cation exchange columns (designated as "column") and without the cation exchange columns (designated "no column"). When the lower tracer/tracee ratio at plateau generated using column chromatography was used in multicompartmental analysis, the fractional catabolic rate for LDL apoB-67 was about 2-fold higher compared to the higher plateau generated using centrifugation rather than column chromatography (0.70 ± 0.21 versus 0.37 ± 0.06 pools per day, $P = 0.06$) and was 2.5-fold higher for VLDL apo-B-67 for subject 1. We think the higher plateau and consequent lower fractional catabolic rate reflect the physiological situation as there is no contamination with unlabeled leucine in the preparation of these samples.

Table 3 compares the fractional catabolic rates for apoB-67 and apoB-100 in each subject when the samples were processed with centrifugation. The apoB-100 values have been previously reported for the apoB-67 subjects (5). The mean fractional catabolic rate for VLDL apoB-67 was not significantly different from VLDL apoB-100 (9.7 ± 3.4 versus 18.1 ± 8.6 pools per day, respectively, $P = 0.19$). The mean fractional catabolic rate for LDL apoB-67 was not significantly different from LDL apoB-100 (0.37 ± 0.06 versus 0.34 ± 0.11 pools per day, respectively, $P = 0.66$).

Table 4 shows the secretion rates for apoB-67 compared to apoB-100. The mean secretion rate of VLDL apoB-67 was 5.6% of VLDL apoB-100 (0.20 ± 0.04 versus 3.6 ± 1.3 mg/kg/day, $P = 0.01$). The mean IDL production rate for apoB-67 was 4.3% of apoB-100 (0.05 ± 0.04 versus 1.17 ± 0.49 mg/kg/day, $P = 0.017$). The mean LDL production rate for apoB-67 was 2.6% of LDL apoB-100 (0.09 ± 0.02 versus 3.50 ± 1.39 mg/kg/day, $P = 0.05$).

Table 5 shows the metabolic channeling of apoB-100. Fifty-three % of apoB-67 was directly removed from VLDL compared to only 3.5% of apoB-100 ($P = 0.003$); thus, less apoB-67 reached the LDL fraction as compared to apoB-100. This increased direct removal of apoB-67 from VLDL is reflected in the fact that the percent of apoB-67 mass relative to apoB-100 mass was lower in the LDL fraction (3.3%) compared to the VLDL fraction (11.2%) (Table 1).

Mean VLDL apoB-67 pool size was 10.0% of apoB-100 (1.58 versus 15.75 mg, $P = 0.033$) due to a 94.4% lower mean secretion rate (5.6% of apoB-100). Mean LDL apoB-67 pool size was 3.2% of apoB-100 (21.2 versus 665.3 mg, $P = 0.020$) due to a 97.4% lower mean production rate (2.6% of LDL apoB-100). Thus, de-

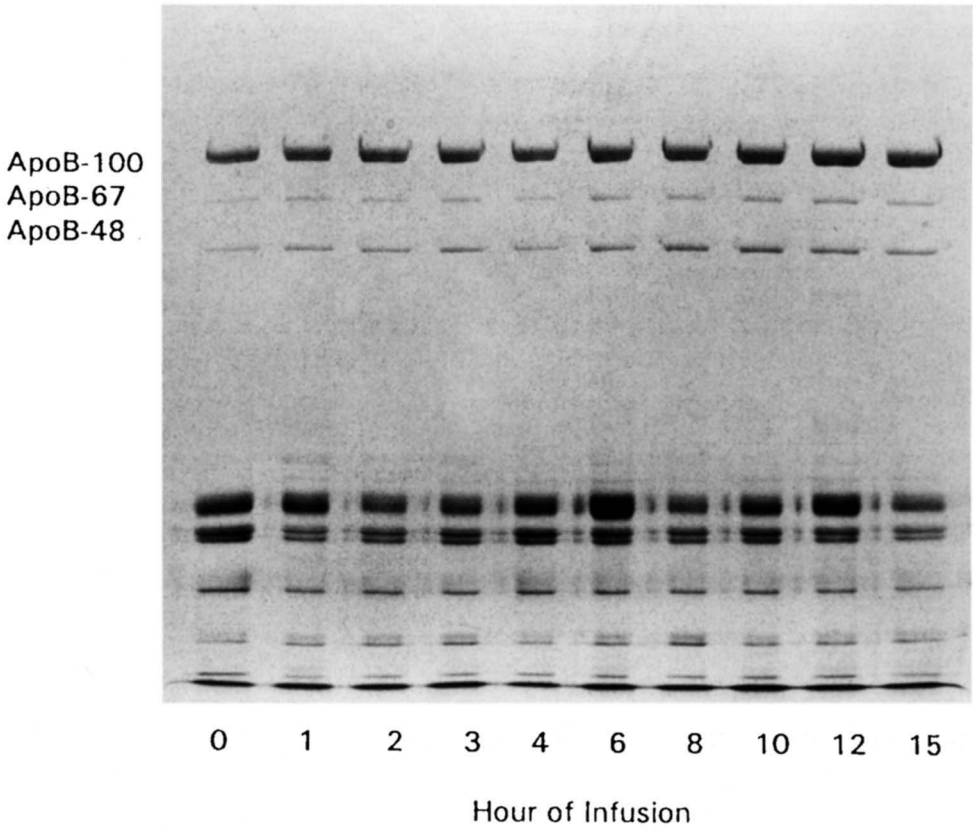

Fig. 2. 4–12% gradient SDS polyacrylamide gel electrophoresis of VLDL fractions at each time point during the infusion of deuterated leucine.

creased secretion of apoB-67 is responsible for the low levels of apoB-67 in apoB-67 subjects.

Table 6 shows the fractional rate constants of individual lipoprotein pools. These are the rate constants for each individual compartment in each subject; they correspond to the arrows in the model (5). They are used to derive the fractional catabolic rates which are listed for each individual subject in Table 3. The fractional standard deviations provide a measure of the error for each rate constant.

DISCUSSION

In the present study, the tracer/tracee ratio at plateau for VLDL apoB-67 was 50% lower when cation exchange columns were used to remove SDS gel fragments compared to centrifugation. This resulted from the introduction of unlabeled leucine during the washing of cation exchange columns with distilled water. The low protein concentration of apoB-67 may render it more susceptible to dilution from an exogenous source of unlabeled leucine as compared to apoB-100, which is present at a much higher protein concentration. Multicompartmental modeling with the lower tracer/tracee ratios resulted in a 2-fold increase in the fractional catabolic rate for LDL apoB-67 compared to the higher plateau. Therefore, the differences in plateau had an effect on the fractional catabolic rates.

Differences in the tracer/tracee ratio at plateau have been previously noted for apoB-100 compared to apoB-54.8, which is also present at a very low concentration. In a metabolic study in six human subjects heterozygous for an apoB-54.8 truncation, the plateau values for VLDL apoB-54.8 were lower than VLDL apoB-100 in all six subjects (7). The fractional catabolic rates for VLDL apoB-54.8 were significantly faster compared to VLDL apoB-100 (7). The authors suggested that the different plateau values could result from dilution of samples (from an unknown source) but that this would not affect the fractional catabolic and secretion rates as the time when the plateau is reached would not change (7). The results in the present study suggest that differences in the plateau may affect the fractional catabolic rate.

The kinetic analysis in the present study demonstrates that apoB-67 levels are low in the apoB-67 subjects due to decreased secretion of apoB-67 containing

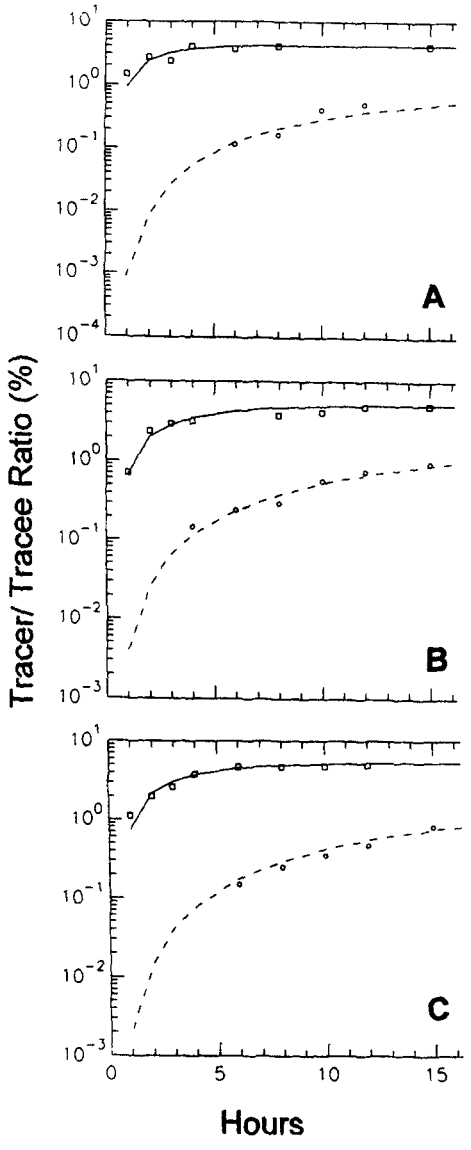

Fig. 3. ApoB-67 leucine tracer/tracee ratios (%) for VLDL apoB-67 (squares with solid lines) and LDL apoB-67 (circles with dotted lines) processed without cation exchange columns versus time after a primed constant infusion of (5,5,5-$^2H_3$)-leucine over 15 h in three apoB-67/apoB-100 heterozygous subjects: A, subject 1; B, subject 2; C, subject 3. Observed values are given as symbols and model predicted values as lines.

TABLE 2. ApoB-67 fractional catabolic rates (FCR) calculated from samples prepared with column chromatography (column) and with centrifugation (no column)

	VLDL FCR		LDL FCR	
Subject	Column	No Column	Column	No Column
1	34.0	13.6	0.56	0.24
2	8.4	7.63	0.94	0.45
3	8.4	7.78	0.59	0.32
Mean ± SD	16.9 ± 14.8	9.7 ± 3.4	0.70 ± 0.21	0.37 ± 0.06
P value	0.19		0.06	

VLDL, IDL and LDL lipoprotein particles. Two possibilities for the lower levels of apoB-67 compared to apoB-100 in apoB-67 heterozygotes include low levels of mRNA or decreased mRNA translation rates, both of which may lead to low levels of protein. Many of the mutations causing truncated apoBs are nonsense mutations. Nonsense mutations have been shown to result in low mRNA levels and diminished protein synthesis rates in several other genes (22–24). In nonsense and frameshift mutations in the B-globin gene, the presence of early translation termination codons correlates with a decrease in the steady-state level of mRNA (25). Premature termination of translation is presumed to destabilize the mRNA released by polysomes; however, a premature stop codon could also induce intranuclear instability of the transcript and impair its transport to the cytoplasm (26). As the apoB-67 mutation is a frameshift mutation, it is possible that similar mechanisms could account for low levels of truncated apoB proteins.

Liver biopsies in humans with heterozygous hypobetalipoproteinemia would be required to determine whether mRNA levels are low; however, it is extremely difficult to justify this invasive procedure in a healthy subject. Therefore, the mechanism for the reduced amount of plasma truncated apoB protein in heterozygous hypobetalipoproteinemia was investigated by examining the amount of apoB mRNA in liver in transgenic mice. Using gene-targeting techniques, transgenic mice heterozygous and homozygous for the apoB-70 truncation were produced (27). The levels of LDL apoB-70 were 9% of the amount of apoB-100 in these mice, similar to that observed in the human. The amount of mRNA for the truncated apoB protein was very low compared to the mRNA for apoB-100 (27). Therefore, it is possible that the mRNA for the truncated apoB protein is also low in human subjects heterozygous for apoB truncations.

Post-transcriptional events could also cause low levels of truncated apoB proteins. Studies of cultured hepatoma cells demonstrate that apoB secretion is under post-transcriptional control and that most of the apoB is degraded before secretion (28–33). There is evidence that apoB is produced intracellularly at a constant rate but that the amount secreted depends on the amount of lipid available to prevent intracellular degradation (28–33). Addition of oleate to culture medium resulted in increased secretion of apoB-containing lipoprotein particles as a result of decreased degradation of apoB prior to secretion (30–32). Therefore, it is possible that decreased availability of hepatic lipid, whether triglyceride, cholesteryl ester, or phospholipid, may result in enhanced intracellular apoB-67 degradation prior to secretion in the apoB-67 subjects. Alternatively, due to its

TABLE 3. Fractional catabolic rates (FCR) for apoB-67 compared to apoB-100

	VLDL FCR		IDL FCR		LDL FCR	
Subject	ApoB-67	ApoB-100[a]	ApoB-67	ApoB-100[a]	ApoB-67	ApoB-100[a]
1	13.6	11.8	22.2	8.5	0.24	0.31
2	7.63	27.9	2.0	14.3	0.45	0.38
3	7.78	14.7	13.47	17.2	0.32	0.42
Mean ± SD	9.7 ± 3.4	18.1 ± 8.6	12.6 ± 10.1	13.3 ± 4.4	0.37 ± 0.06	0.34 ± 0.11
P value	0.190		0.91		0.66	

[a]Reference 5 for kinetic analysis.

TABLE 4. Secretion rates for apoB-67 compared to apoB-100

	VLDL		IDL		LDL	
Subject	ApoB-67	ApoB-100[a]	ApoB-67	ApoB-100[a]	ApoB-67	ApoB-100[a]
	mg/kg/day		*mg/kg/day*		*mg/kg/day*	
1	0.24	2.1	0.08	0.6	0.08	1.9
2	0.16	4.4	0.01	1.4	0.08	4.4
3	0.19	4.2	0.06	1.5	0.11	4.2
Mean ± SD	0.20 ± 0.04	3.6 ± 1.3	0.05 ± 0.04	1.17 ± 0.49	0.09 ± 0.02	3.50 ± 1.39
P value	0.010		0.017		0.05	

[a]Reference 5 for kinetic analysis.

TABLE 5. Metabolic channeling of apoB-67 and apoB-100

			Conversion from VLDL to LDL					
	VLDL Directly Removed		Via Shunt[a]		Via IDL		Via Delipidation Cascade	
Subject	B-67	B-100	B-67	B-100	B-67	B-100	B-67	B-100
	%		%		%		%	
1	66.2	10.5	0	60.0	33.8	17.5	0	22.5
2	47.8	0	45.9	67.0	6.2	15.7	0	17.3
3	45.1	0	30.5	67.6	30.5	3.7	0	28.7
Mean	53.0	3.5	25.5	64.9	23.5	12.3	0	22.8
± SD	±11.5	±6.1	±23.4	±4.2	±15.1	±7.5		±5.7
P value	0.003		0.040		0.313			

[a]The shunt pathway refers to the rapid conversion of VLDL apoB (compartment 3) directly to LDL apoB (compartment 8) depicted in the model (5).

TABLE 6. Fractional rate constants of individual lipoprotein pools and delay

Subject	k(2,1)	k(0,3)	k(7,3)	k(8,3)	k(8,7)	k(0,8)
1	0.008	0.37	0.19	0	0.92	0.011
	±0.001	±0.07	±0.02		±0.09	±0.0006
%FSD	13.9	17.5	11.4	—	9.4	5.3
2	0.004	0.15	0.02	0.14	0.08	0.019
	±0.0005	±0.04	±0.005	±0.03	±0.02	±0.003
%FSD	12.0	25.9	22.0	18.3	24.8	18.1
3	0.007	0.14	0.10	0.08	0.56	0.013
	±0.0009	±0.03	±0.02	±0.02		±0.001
%FSD	12.0	21.1	13.9	25.7	—	9.6

FSD indicates fractional standard deviation.

shorter length compared to apoB-100, apoB-67 may not bind enough lipid to prevent intracellular degradation before secretion. The conformation of apoB-67 may be altered due to improper folding of the truncated protein such that it is targeted for degradation. Thus, a larger portion of a newly synthesized truncated apoB may be degraded intracellularly before secretion than apoB-100.

Experiments in rat hepatoma McA-RH7777 cells transfected with constructs containing apoB-60, 72, 80, 88, and 94 are secreted as efficiently from cells as apoB-100 in nontransfected cells (34). The intracellular retention time, secretion efficiency, and extent of intracellular degradation of apoB-72 and apoB-80 were simi-

lar to apoB-100 in nontransfected cells (34). These data suggest that low levels of truncated apoBs in subjects with hypobetalipoproteinemia may not be due to a secretory problem. However, because expression of the apoB mutants in the transfected cells is constitutively driven by the cytomegalovirus promoter, the authors cautioned that these in vitro results may not reflect the in vivo situation in hypobetalipoproteinemia.

In the present study, there was increased direct removal of apoB-67 from the VLDL fraction with subsequent decreased conversion of the VLDL apoB-67 to LDL apoB-67. Similar results have been observed for other truncations (6, 8). This may be due to an increased ratio of apoE/apoB-100 in the VLDL apoB-67-containing particles compared to VLDL apoB-100-containing particles such that the increased direct removal is mediated by apoE. Alternatively, the conformation of the apoB-67 VLDL particles may be altered such that more apoE binding sites are exposed compared to apoB-100-containing VLDL particles.

Metabolic studies in human subjects with the apoB-31, apoB-54.8, apoB-75, and apoB-89 mutations have shown decreased secretion rates of the truncated protein compared to apoB-100 (7). The secretion rates increased in a linear fashion as the length of the truncation increased and were 13%, 37%, 64%, and 92% of that of apoB-100, respectively (7). In the present study, the mean secretion rate for VLDL apoB-67 was 5.6% of apoB-100 and for LDL apoB-67, 2.6% of apoB-100. When expressed on a molar basis as a percent of apoB-100 secretion, our results are lower than predicted by the other study. Our fractional catabolic rates are about the same as those for the apoB-75 and 89 truncations; however, the concentration of apoB-67 is much lower than that of apoB-75 and apoB-89. As secretion rate is calculated from fractional catabolic rate and pool size, our secretion rates will, accordingly, be lower than those observed in the other study.

In summary, we have shown that the introduction of unlabeled leucine during cation exchange chromatography to isolate free amino acids in stable isotope studies of truncated apoBs results in lower tracer/tracee ratios that significantly alter the calculated fractional catabolic rates. This study demonstrates a solution to the difficulty in performing kinetic studies with proteins present at low concentrations. In addition, we have shown that decreased secretion rates of apoB-67 are responsible for the low levels of apoB-67 in apoB-67/B-100 heterozygotes. In a prior study we have shown that the secretion of apoB-100 is also low in these subjects compared to apoB-100 in control subjects. Future studies will be required to elucidate the cellular mechanism underlying this decreased secretion of apoB. As apoB plays a major role in atherogenesis, this mechanism is of great interest and may have therapeutic potential.

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