Measurement of human apolipoprotein B-48 and B-100 kinetics in triglyceride-rich lipoproteins using [5,5,5-$^2H_3$]leucine[1]

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Abstract A primed-constant infusion of deuterated leucine was used in humans to determine the maximal level of enrichment at plateau of apolipoprotein (apo)B-48 and apoB-100 which are synthesized in the intestine and liver, respectively, and to compare the kinetics of these two proteins under identical conditions. Eight normal subjects (four post-menopausal females and four males) over the age of 40 were studied in the constantly fed state over a 20-h period by providing small hourly feedings of identical composition. [5,5,5-$^2H_3$]Leucine (10 μmol/kg body weight followed by 10 μmol/kg body weight per hour) was infused over 15 h intravenously. The enrichment of deuterated leucine in apoB-48 and apoB-100 triglyceride-rich lipoproteins isolated by ultracentrifugation (d<1.006 g/ml) was determined during the entire infusion period. The plateau level of enrichment in triglyceride-rich lipoprotein apoB-48 was 3.96 ± 1.41 tracer/tracee ratio (%) which was 39.7% of the plasma leucine enrichment level. The plateau level of enrichment in triglyceride-rich lipoprotein apoB-100 was 7.23 ± 1.17 tracer/tracee ratio (%) which was 72.5% of the plasma leucine enrichment level. Mean fractional secretion rates of triglyceride-rich lipoprotein apoB-48 and apoB-100 were 4.39 ± 2.00 and 5.39 ± 1.98 pools per day, respectively, with estimated residence times of 5.47 and 4.45 hours, respectively. The data indicate that in the fed state there is about a twofold difference in the plateau enrichment of an intestinally derived protein, as compared to one of hepatic origin, most likely attributable to differences in the enrichment of the intracellular leucine in the two organs. Our data indicate that in the fed state apoB-48 and apoB-100 within triglyceride-rich lipoproteins are catabolized at similar fractional rates, and that the major pool size differences between these two proteins are due to differences in the absolute secretion rate. Moreover, the data are consistent with the view that one cannot use the triglyceride-rich lipoprotein apoB-100 plateau as an indicator of precursor pool enrichment in protein kinetic studies via endogenous labeling with labeled amino acids to assess the kinetics of apolipoproteins partially or totally synthesized by the intestine, such as apoB-48 or apoA-I.—**Lichtenstein, A. H., D. L. Hachey, J. S. Millar, J. L. Jenner, L. Booth, J. Ordovas, and E. J. Schaefer.** Measurement of human apolipoprotein B-48 and B-100 kinetics in triglyceride-rich lipoproteins using [5,5,5-$^2H_3$]leucine. *J. Lipid Res.* 1992. **33:** 907–914.

Supplementary key words: kinetics • deuterated leucine • apolipoprotein B-48 • apolipoprotein B-100

Plasma lipid concentrations are a function of lipoprotein secretion and catabolism. However, the methodology used to characterize lipoprotein metabolism is complicated by the dynamic nature of the system. Much of the current understanding of lipoprotein metabolism comes from studies that use decay curves generated from the radio-iodination of apolipoproteins within lipoprotein particles (1, 2). Concerns have arisen over possible alterations in the lipoprotein particles created during isolation and radio-iodination (3–5). An alternate approach for the study of lipoprotein metabolism has been the use of an endogenous metabolic tracer in order to follow apolipoprotein or lipoprotein fractional secretion in vivo. Using a single dose of labeled amino acid, multicompartmental analysis has been used to assess the kinetics of apolipoproteins (5–10). Subsequent work adopted a primed-constant infusion of labeled amino acid to directly address the issue of endogenous synthesis of apolipoproteins (11–16). The use of labeled amino acids has the advantage of allowing for the simultaneous measurement of the kinetics of multiple apolipoproteins (7, 13, 14). Additionally, the use of stable isotope-labeled amino acids, in contrast to radio-labeled amino acids, allows for multiple measures of apolipoprotein metabolism in the same individual.

With respect to the methodological aspects of the work, we have previously examined the use of different deuterated amino acids, leucine, valine, and lysine, in the measurement of human apolipoprotein kinetics and observed

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Abbreviations: TRL, triglyceride-rich lipoproteins; FSR, fractional secretion rate; TC, total cholesterol; TG, triglyceride; ASR, absolute secretion rate; GC-MS, gas chromatography-mass spectrometry.

[1]Presented in part at the 63rd sessions of the American Heart Association, November 14, 1990, Dallas Texas.

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that each of these amino acids gave similar results (14). In addition, we confirmed the reproducibility of this methodology within individuals and the comparability of data obtained using this methodology relative to that derived from radiodecay curves (13). In calculating kinetic parameters for these studies, the plateau at enrichment for triglyceride-rich lipoprotein (TRL) apolipoprotein (apo)B-100 has been assumed to be a valid marker of a precursor pool enrichment (11, 13, 14). Theoretically, the level at which the enrichment of any protein plateaus would give an estimate of the precursor pool of enrichment of the protein under study, a term necessary for estimating the fractional secretion rate (FSR).

Experimental evidence indicates that this maximal level of enrichment may be unique to the organ in which the protein in question is synthesized and may not be constant throughout the body (12, 14, 17–19). A concern would then be that the kinetics of apolipoproteins such as apoA-I, which is synthesized in both the liver and the intestine, may not be accurately assessed. We therefore addressed the question as to whether an intestinal apolipoprotein such as apoB-48 would have a similar level of enrichment at plateau as compared to a hepatically derived protein such as apoB-100. The purpose of the current study was to assess TRL apoB-48 and apoB-100 kinetics as well as their enrichments at plateau in the constantly fed state following a primed-constant infusion of deuterated leucine. To our knowledge these issues have not been previously examined.

METHODS

Experimental protocol

Eight volunteers (four post-menopausal females and four males) over the age of 40 underwent a complete medical history and physical examination. The subjects had no evidence of any chronic illness including endocrine, hepatic, renal, thyroid, or cardiac dysfunction. They did not smoke, and were not taking medications known to affect plasma lipid levels (cholestyramine, colestipol, niacin, clofibrate, gemfibrozil, probucol, lovastatin, beta blockers, thiazide diuretics, diphenylhydantoin, *cis*-retinoic acid, ascorbic acid, estrogens, progestins, anabolic steroids, hydrocortisone, fish oil capsules, or thyroxine). The experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University. During the 6 weeks prior to the kinetic studies, the subjects consumed an isocaloric diet (15% of calories as protein, 49% carbohydrate, 36% fat; 15% saturated, 15% monounsaturated, 6% polyunsaturated, 180 mg cholesterol/1000 kcal) that was provided to them. Plasma fatty acid patterns were used to monitor adherence to the diets and no significant deviation from the mean values was seen in any of the study subjects. Additionally, body weight was constant throughout the study period, suggesting that the subjects were consuming the diet as provided. Plasma lipid concentrations were monitored during weeks 4, 5, and 6. There were no statistically significant differences among these values, therefore the subjects were assumed to be in a steady state.

In order to determine the kinetics of TRL apoB-48 and apoB-100, the subjects underwent a primed-constant infusion of deuterated leucine while they were in the fed state as previously described (13, 14). Briefly, starting at 6 AM, the subjects received 20 identical small hourly meals, each equivalent to 1/20th of their daily food intake. At 11AM, with two intravenous lines in place, one for the infusate and one for blood sampling, 10 μmol/kg body weight [5,5,5-$^2H_3$]-L-leucine was injected as a bolus intravenously over 1 min and 10 μmol/kg body weight per h was infused over a 15-h period. Blood samples (20 ml) were collected at hours 0, 1, 2, 3, 4, 6, 8, 10, 12, and 15. An additional 30 ml of blood was collected at 0 hour.

Plasma lipids and lipoprotein determinations

Blood was collected in tubes containing EDTA (0.1% final concentration). Other than during the infusion, blood samples were obtained after a 14-h overnight fast. Plasma was separated from red cells in a refrigerated centrifuge at 3,000 rpm for 30 min at 4°C. TRL were isolated after a single ultracentrifugational spin (39,000 rpm, 18 h, 4°C, d 1.006 g/ml). Plasma and TRL were assayed for total cholesterol (TC) and triglyceride (TG) with an Abbott Diagnostics ABA-200 bichromatic analyzer using enzymatic reagents (20, 21). High density lipoprotein (HDL) cholesterol was measured as previously described (22). Lipid assays were standardized through the Centers for Disease Control's Lipid Standardization Program.

Quantitation and isolation of the apolipoproteins

ApoB was assayed in plasma and TRL with a noncompetitive, enzyme-linked immunosorbent assay (ELISA) using immunopurified polyclonal antibodies (23). The coefficient of variation for the apoB assay was less than 5% within runs and less than 10% between runs.

ApoB-48 and apoB-100 were isolated from TRL by preparative SDS polyacrylamide gradient gel electrophoresis (4–22.5%) using a Tris-glycine buffer system as previously described (24, 25). Apolipoproteins were identified by comparing migration distances with those of known molecular weight standards. The gels were scanned in two dimensions and the staining intensity was quantitated. Staining intensity was assumed to be similar for the two proteins (26) and the relative amount of each protein was estimated from the total apoB values in the TRL fraction obtained by ELISA.

Isotopic enrichment determinations

ApoB-48 and B-100 bands were excised from polyacrylamide gels and hydrolyzed in 12 N HCl at 100°C for

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24 h. The free amino acids were isolated using a Dowex AG-50W-X8 100–200 mesh cation exchange resin (Bio-Rad Labs., Richmond, CA). The purified hydrolyzates were converted to the n-propyl ester, N-heptafluorobutyramide derivatives prior to analysis by GC-MS as has been previously described (14). The isotopic enrichment of leucine and proteins in the samples was expressed as tracer/tracee ratio (%).

Hypothesis generation

In order to analyze the data generated from these experiments, a number of assumptions were made. We made the assumption that the study subjects were in a steady state, achieved in part by the design of the protocol which entailed the consumption of small frequent meals of constant composition. Inherent in the interpretation of the data was the assumption that for every organ synthesizing the protein of interest the relative level of enrichment of leucine with deuterated leucine within plasma, the intracellular space, and the tRNA was constant throughout the study period. During the primed-constant infusion period, we assumed that there was a constant incorporation of deuterated leucine into the proteins of interest and constant secretion of these proteins into the plasma pool with negligible recycling of deuterated leucine.

Implicit in our analysis was the assumption that the maximal level of enrichment observed for TRL apoB-100 and TRL apoB-48 represents the theoretical maximal enrichment of any protein synthesized by the liver and the intestine, respectively. Also assumed was that the absolute secretion rates (ASR) can be accurately calculated from the FSR and that treating TRL apoB-100 and TRL apoB-48 as a single pool (based on our sample) provides a reasonable assessment of all the components within each of these pools.

Calculation of kinetic parameters

The TRL apoB-48 and apoB-100 data were analyzed by fitting data points to a monoexponential curve (RS/1, BBN Research System, Cambridge MA). The data were fit to the function $A(t) = A_p(1-e^{-kt})$ where A(t) is the tracer/tracee ratio at time t, A_p is the tracer/tracee ratio (%) of the plateau of the curve representing the tracer/tracee ratio (%) of the precursor pool, and k is the FSR.

RESULTS

The characteristics of the study subjects are shown in **Table 1.** The mean age of the subjects was 64 ± 6 years. On average they were 11 ± 9% above ideal body weight. Their mean plasma cholesterol during the last 3 weeks of the diet period was 205 ± 30 mg/dl with an unremarkable cholesterol distribution among the lipoprotein fractions. Mean plasma triglyceride values were 112 ± 32 mg/dl. The maximum body weight change during the 6 weeks prior to the kinetic studies was < ± 1 kg.

Plasma and TRL lipid and apolipoprotein values during the 15-h infusion period are shown in **Fig. 1.** All parameters were stable by the start of the infusion period (5 h post-feeding) and did not change significantly throughout the infusion period. Lipid and apolipoprotein values during the 15-h time period are shown in **Table 2.** Plasma cholesterol averaged 194 ± 36 mg/dl and TRL cholesterol averaged 15 ± 10 mg/dl. Plasma apoB averaged 104 ± 24 mg/dl and TRL apoB averaged 14 ± 7 mg/dl.

The isotopic enrichment levels of plasma leucine and TRL apoB-48 and apoB-100 are shown in **Table 3** and **Table 4**, respectively. Representative data are plotted in **Fig. 2.** By 1 h, plasma leucine enrichment appeared to reach maximal levels and remained nearly constant throughout the study period [averaged 9.97 ± 1.71 tracer/tracee ratio (%)]. The enrichment of TRL apoB-48 increased gradually during the initial part of the infusion period and then appeared to approach a plateau during the subsequent infusion period. The level of enrichment at the plateau averaged 3.96 ± 1.41 tracer/tracee ratio (%) and was 39.7% of the plasma level. Plasma enrichment of

TABLE 1. Characteristics of the subjects

Subject	Age	Sex	IBW	ApoE Phenotype	TC	TRL-C	LDL-C	HDL-C	TG
	yr		*%*		*mg/dl*				
1	69	F	101	3/3	238 ± 9	8 ± 6	172 ± 6	59 ± 1	84 ± 13
2	68	F	128	4/3	254 ± 5	9 ± 3	183 ± 4	61 ± 1	96 ± 12
3	64	F	116	3/3	191 ± 9	17 ± 7	119 ± 3	55 ± 3	89 ± 17
4	60	M	110	3/3	211 ± 5	15 ± 5	148 ± 5	48 ± 2	79 ± 7
5	73	M	114	3/3	204 ± 8	22 ± 12	133 ± 6	49 ± 1	125 ± 28
6	53	M	112	3/3	159 ± 5	36 ± 4	94 ± 3	29 ± 2	174 ± 32
7	58	F	102	3/3	185 ± 6	16 ± 8	128 ± 9	41 ± 4	121 ± 14
8	65	M	104	3/2	195 ± 2	28 ± 4	128 ± 3	39 ± 2	128 ± 5
Mean[a]	64 ± 6		111 ± 9		205 ± 30	19 ± 9	138 ± 29	48 ± 11	112 ± 32

[a]Mean ± SD. Lipid values for each individual subject represent the mean of determinations made during the last 3 weeks of the study period.

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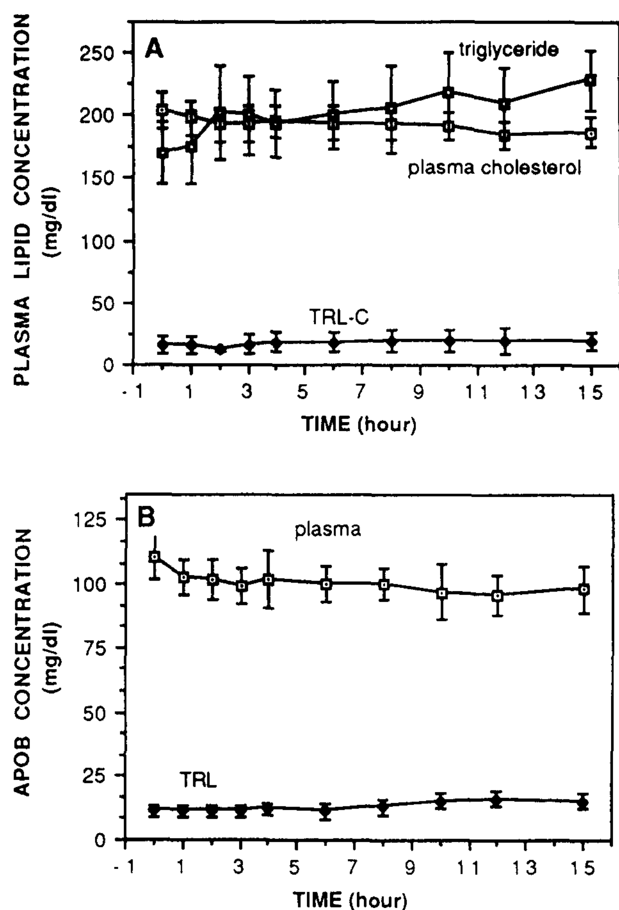

Fig. 1. Concentrations of plasma lipids and apoB during the primed-constant infusion experiment in the fed state. (A) Plasma cholesterol (open squares), plasma triglyceride (solid squares) and triglyceride-rich lipoprotein (TRL) cholesterol (solid diamonds). (B) Plasma apoB (open squares) and TRL apoB (solid diamonds).

TRL apoB-100 rose more sharply than TRL apoB-48 and again approached a plateau towards the end of the infusion period. The level of enrichment at the plateau averaged 7.23 ± 1.17 tracer/tracee ratio (%) and was 72.5% of plasma level or almost double that of TRL apoB-48. The effect of gender on apolipoprotein kinetics in this sample was not statistically significant, therefore kinetic data was presented for the entire group.

Parameters of TRL apoB-48 and apoB-100 kinetics are shown in Tables 3 and 4, respectively. The rate of enrichment of each protein was calculated by fitting the data to a monoexponential curve (representative data shown in Fig. 2). Mean apolipoprotein concentrations (Table 2) during the 15-h infusion period were used to estimate pool size. The mean FSR of TRL apoB-48 was 4.39 ± 2.00 and apoB-100 was 5.39 ± 1.98 pools/day. The reciprocal of FSR or fractional catabolic rate is residence time. The mean residence time for apoB-48 was 0.23 days (5.47 h) and apoB-100 was 0.19 days (4.45 h). The pool size of TRL apoB-48 was about 5.3% that of TRL apoB-100. This difference resulted in a significantly higher ASR of TRL apoB-100 than TRL apoB-48 under these study conditions.

DISCUSSION

The current studies suggest that with the methodology described, the FSR for apoB-48 and apoB-100 are similar within the TRL fraction in the constantly fed state when the subjects consumed the specified diets. Additionally, the current data indicate that the plateau of enrichment in terms of the isotopic ratio of deuterated leucine to normal leucine (Fig. 2) is consistently greater for TRL apoB-100 than for TRL apoB-48. The magnitude of this difference is approximately twofold which we interpret to indicate different intracellular levels of enrichment of the leucyl tRNA precursor in the liver and intestine. While there is still some controversy as to whether all of apoB-100 is synthesized in the liver or whether a small fraction is derived from the intestine (27–29), there is a general consensus that all or almost all of the apoB-48 in the plasma is derived from the intestine (27, 30–32).

Possibly of greatest significance in these studies is the finding that the residence times of apoB-48 and apoB-100 within TRL in plasma are similar in humans in the fed

TABLE 2. Non-fasting plasma lipid and apolipoprotein concentrations during the kinetic studies

Subject	TC	TG	TRL-C	ApoB	TRL-ApoB
			mg/dl		
1	227 ± 6	127 ± 22	5 ± 2	97 ± 12	9 ± 5
2	267 ± 11	136 ± 9	6 ± 1	153 ± 12	8 ± 1
3	167 ± 6	151 ± 21	12 ± 2	79 ± 8	6 ± 1
4	195 ± 7	173 ± 25	9 ± 1	96 ± 8	25 ± 8
5	193 ± 5	269 ± 36	27 ± 3	110 ± 10	12 ± 1
6	152 ± 5	355 ± 42	32 ± 8	86 ± 12	16 ± 3
7	176 ± 7	153 ± 42	10 ± 3	101 ± 12	23 ± 5
8	175 ± 8	223 ± 53	22 ± 1	111 ± 8	12 ± 2
Mean[a]	194 ± 36	198 ± 79	15 ± 10	104 ± 24	14 ± 7

[a]Mean ± SD. Values for each individual subject represent the mean of all determinations (10) made during the 15-h infusion period.

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TABLE 3. Parameters of TRL apoB-48 kinetics

Subject	Plateau (Tracer/Tracee)	Plasma Volume	ApoB-48	FSR	ASR[a]
	%	*l*	*mg/l*	*pools/day*	*mg/kg/day*
1	2.37	2.46	0.5	6.48	1.46
2	4.96	3.44	0.6	2.64	0.71
3	3.11	2.83	0.4	6.82	1.23
4	5.00	3.33	1.5	3.17	2.15
5	2.16	3.85	0.5	6.53	1.47
6	4.43	3.99	0.8	3.41	1.23
7	6.19	2.31	1.1	1.63	0.81
8	3.49	3.43	0.5	4.44	1.00
Mean[b]	3.96 ± 1.41	3.21 ± 0.62	0.7 ± 0.4	4.39 ± 2.00	1.26 ± 0.46

[a]ASR (mg/kg/day) = $\frac{\text{FSR (pools/day)} \times \text{apolipoprotein pool size (mg)}}{\text{body weight (kg)}}$

Pool size = plasma apolipoprotein concentration (mg/dl) × plasma volume (0.045 l/kg body weight).
[b]Mean ± SD.

state (on the order of 5.0 h). As apoB-48 does not contain the recognition site for the LDL receptor (in contrast to apoB-100) (33), our data would suggest that the fractional catabolism of these two proteins is possibly regulated by a putative apoE receptor within the liver (34,35). We have previously reported that the catabolism of these proteins within TRL was markedly impaired in familial apoE deficiency, and this also is the case in dysbetalipoproteinemia associated with apoE2 homozygosity (due to a cysteine for arginine substitution at residue 158) (36,37). These issues as well as the effects of dietary variation on the kinetics of these two proteins within TRL using this methodology remain to be explored.

These findings contrast with the conclusions drawn in some previous studies using methodology involving radioiodination, which indicated that in the fasting state the fractional catabolic rate of exogenously introduced apoB-48 within TRL is faster than that of apoB-100 (36–38). Other studies also using the radio labeled methodology of exogenous labeling came to conclusions similar to ours, albeit in hypertriglyceridemic subjects, that the FSR for apoB-48 and apoB-100 are similar (39, 40). The methodology in the previously work used non-autologous lipoproteins isolated from hyperlipidemic subjects radiolabeled, and reinjected into normal individuals. The composition of the introduced lipoproteins was most probably broad in spectrum ranging from newly secreted lipid-laden particles to extensively delipidated particles which would vary widely in their metabolic rates. Moreover, these subjects were not studied in the fed state. It is difficult at this point to conclude that either method provides data more indicative of the true physiological state than the other. We do feel that given the limitations of the current methodology the assessment of de novo synthesized protein is an appropriate method for assessing apoB-48 kinetics.

A limitation of using the present protocol is that small frequent doses of fat are introduced into the intestine which may result in a relatively small, dense triglyceride pool of apoB-48-containing lipoproteins being formed.

TABLE 4. Parameters of TRL apoB-100 kinetics

Subject	Plateau (Tracer/Tracee)	Plasma Volume	ApoB-100	FSR	ASR[a]
	%	*l*	*mg/l*	*pools/day*	*mg/kg/day*
1	6.07	2.46	8.8	8.50	33.56
2	9.39	3.44	7.6	4.94	16.85
3	7.02	2.83	5.7	6.77	17.36
4	7.87	3.33	23.3	3.96	41.58
5	5.70	3.85	11.1	7.61	37.96
6	7.25	3.99	15.1	3.38	22.94
7	6.62	2.31	21.6	3.41	33.10
8	7.92	3.43	11.8	4.54	24.13
Mean[b]	7.23 ± 1.17	3.21 ± 0.62	13.1 ± 6.4	5.39 ± 1.98	28.44 ± 9.39

[a]ASR (mg/kg/day) = $\frac{\text{FSR (pools/day)} \times \text{apolipoprotein pool size (mg)}}{\text{body weight (kg)}}$

Pool size = plasma apolipoprotein concentration (mg/dl) × plasma volume (0.045 l/kg body weight).
[b]Mean ± SD.

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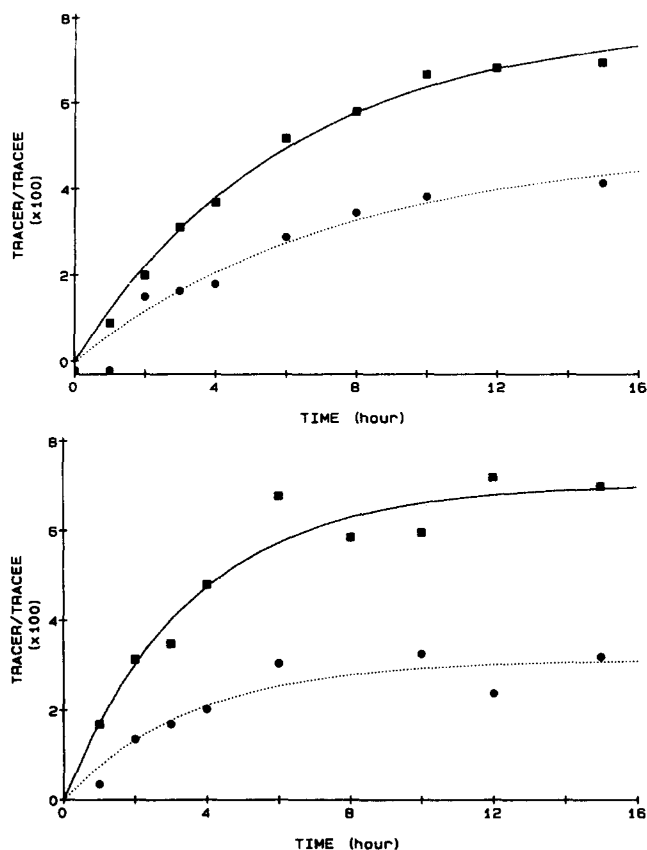

Fig. 2. Representative enrichment data of apoB-48 (circles) and apoB-100 (squares) with [5,5,5-$^2H_3$]-L-leucine during the primed-constant infusion in the fed state. Upper panel, subject #4, lower panel, subject #3.

These particles, in turn, may be a poorer substrate for lipoprotein lipase than the larger less dense particles formed during a more conventional feeding regime. All of the subjects studied had a clear and sustained post-absorptive triglyceride increase.

This work has allowed us to explore the feasibility of using the incorporation of stable isotope-labeled amino acids to describe the kinetics of proteins of both intestinal and hepatic origin. These data indicate that an intestinally derived protein (i.e., apoB-48) reaches a different enrichment plateau than that observed for a liver-derived protein, specifically TRL apoB-100. It should be stressed that our studies were carried out in the constantly fed state and that different results might have been obtained in the fasting state or after consumption of a diet of different composition. It is our view, however, that apolipoprotein kinetic studies should be carried out in the constantly fed state because this condition is more physiologic than is the fasting state.

The difference in the plateau enrichment of the two proteins may be due to the influx of unlabeled dietary leucine into intestinal cells, in contrast to the liver which receives leucine from the portal vein and the hepatic artery. Since the tracer is introduced into the vascular compartment, the hepatocytes receive a more highly enriched supply of free amino acids for protein synthesis than the intestinal lumenal cells which receive amino acids from the mesenteric artery and gut. This would be reflected in different levels of enrichment of the intracellular leucyl tRNA precursor available for de novo protein synthesis. The potential value of this hypothesis as a contributory factor is supported by evidence suggesting that tissues that derive their blood supply solely from the peripheral circulation, the vascular compartment that would be most highly enriched in deuterated leucine, such as heart muscle and lung, synthesize protein with levels of enrichment which closely approximate that of the arterial blood supply (30-32). While it would have been of interest to carry out similar studies in the fasting state to more clearly address the question of differential maximal levels of enrichment in the different organs, normal subjects have only small or undetectable amounts of TRL apoB-48 in the fasted state which makes such studies unfeasible. It is of interest to note that in a previous study it was reported that plasma enrichment of deuterated leucine in the fed state was 83% of that in the fasting state and similarly, the enrichment of VLDL apoB-100 was 79% of that in the fasting state (13).

An alternate explanation and/or contributing factor for the lower level of enrichment of TRL apoB-48 relative to TRL apoB-100 is the existence of a preformed, therefore unlabeled, pool of intestinal apoB. This pool has previously been identified to be present in the intestine and is releasable during fat absorption (41, 42). The impact of this factor might be expected to be minimized in the current situation due to the experimental design, that is, that the kinetic studies were started 5 h after feeding began. Therefore, part or all of the preformed pool would have been exhausted prior to the kinetic measures. The comparison of the relative enrichment patterns of apoB-48 and apoB-100 in normal and hypertriglyceridemic subjects might provide some indication of the relative significance of this issue.

For investigators not in the lipoprotein field, our data indicate that TRL apoB-100 can be used as a marker of the precursor pool for hepatically derived proteins, and that TRL apoB-48 in the fed state can be used as a marker of the precursor pool for intestinally derived proteins. The utility of these proteins in this regard is due to their fairly rapid turnover rates. Enrichment for these proteins approaches a plateau within the 15 h of the primed-constant infusions carried out under these experimental conditions. To our knowledge this issue has not been previously addressed.

Our studies also raise an additional issue as to how to handle an analysis of the kinetics of such proteins as apoA-I, which are derived from both the liver and the intestine. In the future such studies will have to estimate the relative amounts of this protein that are derived from each

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organ to obtain estimates of enrichment for the precursor pool. This represents a clear limitation of this methodology. It will also be difficult to interpret the data on apolipoproteins that can rapidly exchange between lipoprotein particles. Nevertheless, we feel that the stable isotope methodology allows us to examine apolipoprotein kinetics in ways that were not previously possible.

The authors would like to thank the staff of the Metabolic Research Unit for the expert care provided to the study subjects. This research was supported by grant HL 39326 from the National Institutes of Health and contract 53-3KO6-5-10 from the United States Department of Agriculture Research Service at Tufts University. This work is, in part, a publication of the USDA/ARS Childrens Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX, and has been funded with federal funds from the U.S. Department of Agriculture, Agricultural Research Service under Cooperative Agreement number 58-7MN1-6-100. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Manuscript received 31 May 1991 and in revised form 31 January 1992.

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