Comparison of deuterated leucine, valine, and lysine in the measurement of human apolipoprotein A-I and B-100 kinetics

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Abstract The production rates of apolipoprotein(apo)B-100 in very low density lipoprotein and in low density lipoprotein and apolipoprotein A-I in high density lipoprotein were determined using a primed-constant infusion of [5,5,5,-$^{2}H_3$]leucine, [4,4,4,-$^{2}H_3$]valine, and [6,6-$^{2}H_2$,1,2-$^{13}C_2$]lysine. The three stable isotope-labeled amino acids were administered simultaneously to determine whether absolute production rates calculated using a stochastic model were independent of the tracer species utilized. Three normolipidemic adult males were studied in the constantly fed state over a 15-h period. The absolute production rates of very low density lipoprotein apoB-100 were 11.4 ± 5.8 (leucine), 11.2 ± 6.8 (valine), and 11.1 ± 5.4 (lysine) mg per kg per day (mean ± SDM). The absolute production rates for low density lipoprotein apoB-100 were 8.0 ± 4.7 (leucine), 7.5 ± 3.8 (valine), and 7.5 ± 4.2 (lysine) mg per kg per day. The absolute production rates for high density lipoprotein apoA-I were 9.7 ± 0.2 (leucine), 9.4 ± 1.7 (valine, and 9.1 ± 1.3 (lysine) mg per kg per day. There were no statistically significant differences in absolute synthetic rates of the three apolipoproteins when the plateau isotopic enrichment values of very low density lipoprotein apoB-100 were used to define the isotopic enrichment of the intracellular precursor pool. Our data indicate that deuterated leucine, valine, or lysine provided similar results when used for the determination of apoA-I and apoB-100 absolute production rates within plasma lipoproteins as part of a primed-constant infusion protocol.—**Lichtenstein, A. H., J. S. Cohn, D. L. Hachey, J. S. Millar, J. M. Ordovas, and E. J. Schaefer.** Comparison of deuterated leucine, valine, and lysine in the measurement of human apolipoprotein A-I and B-100 kinetics. *J. Lipid Res.* 1990. **31:** 1693-1701.

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The use of isotopically labeled amino acids to characterize in vivo apolipoprotein synthesis has gained increasing popularity in recent years (1-5). Labeled amino acids offer several advantages over in vitro radiolabeled lipoproteins and apolipoproteins. Alterations in lipoproteins or apolipoproteins created during isolation prior to in vitro labeling or during the in vitro labeling procedure are eliminated (6, 7). Rates of production are measured directly rather than inferred from rates of catabolism. Shorter duration studies (hours versus days) minimize effects due to the recycling of the apolipoproteins and discomfort to the subjects. Very low density lipoprotein (VLDL) apoB-100 kinetics may be assessed without altering the fat and/or calorie content of the diet. Specifically with respect to stable isotope-labeled amino acids, their relative safety compared to radiolabeled amino acids affords the opportunity to study the same person under a variety of different conditions. Additionally, a wider range of subjects with respect to age and physiological state can be studied. Since all newly synthesized proteins are labeled after administration of the labeled amino acid, production rates of a number of proteins can be studied simultaneously. A potential disadvantage of the method is the requirement for sophisticated and expensive gas-liquid chromatography-mass spectrometric (GLC-MS) analysis. However, such equipment is becoming more available to the biomedical community.

As issue concerning this methodology is to establish the validity of using specific isotope-labeled amino acids to obtain kinetic parameters. This study examines this issue by comparing the fractional production rates (FPR) and absolute production rates (APR) obtained for VLDL

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; FPR, fractional production rate; APR, absolute production rate; TG, triglyceride; C, cholesterol; GLC-MS, gas-liquid chromatography-mass spectrometry.

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apolipoprotein (apo)B-100, low density lipoprotein (LDL) apoB-100, and high density lipoprotein (HDL) apoA-I using three different deuterated amino acids; leucine, valine, and lysine. Our hypothesis was that all amino acids are handled identically with respect to their incorporation into newly synthesized apolipoproteins. Our data indicated that a bolus followed by a constant infusion of either deuterated leucine, valine, or lysine yielded similar results with regard to VLDL apoB-100, LDL apoB-100, and HDL apoA-I absolute production rates.

METHODS

Subjects

Three healthy normal volunteers in their mid-twenties underwent a medical history, physical examination, and had clinical chemical analyses performed prior to enrollment in the study. The subjects had no evidence of any chronic illness including hepatic, renal, thyroid, or cardiac dysfunction, nor family history of these disorders. They did not smoke, nor were taking medications known to affect plasma lipid levels (anion exchange resins, fibric acid derivatives, niacin, hydroxymethylglutaryl CoA reductase inhibitors, probucol, hormonal preparations, beta-adrenergic blocking agents, or thiazide diuretics). Percent of ideal body weight was determined from the Metropolitan Life tables (8). The experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University.

Experimental protocol

Subjects resided in the Metabolic Research Unit of the USDA Human Nutrition Research Center on Aging for a 3-day period to acclimatize them to the surroundings. During this time they consumed a normal weight-maintaining diet. Baseline plasma lipid values were determined after a 12-h overnight fast on the second day of the study period, and on the third day the subjects underwent a primed-constant infusion experiment in which three deuterated amino acids [5,5,5-$^{2}H_3$]L-leucine, [4,4,4,-$^{2}H_3$]-L-valine, and [6,6,-$^{2}H_2$;1,2-$^{13}C_2$]L-lysine were infused over a 15-h period. The protocol for the primed-constant infusion experiment has been described in detail previously (5). Briefly, at 6 AM on the day of the infusion, the subjects received the first of 20 meals, representing 4/23rd of their estimated caloric requirement (40 calories/kg body weight per day); subsequent hourly meals represented 1/23rd of their estimated caloric requirement. The meals were composed of natural foods and contained (as percent of total calories) 45% carbohydrate, 15% protein, and 40% fat (17% saturated, 17% monounsaturated, and 6% polyunsaturated fatty acids) and 180 mg cholesterol/1000 calories. Five hours later, with two intravenous lines in place, one for the infusate and one for blood sampling, subjects were given a bolus injection (10 μmol/kg) and then a constant infusion (10 μmol/kg body weight per h) of the three deuterated amino acids dissolved in saline. The infusion was terminated 15 h later at 2 AM.

Isolation of lipoproteins

Blood was collected in tubes containing EDTA (0.1%) and lipoproteins were isolated from plasma by sequential ultracentrifugation as described previously (6). VLDL and chylomicrons were isolated by a single ultracentrifugational spin (39,000 rpm, 18 h, 4°C) and will be collectively referred to as VLDL. Plasma and VLDL were assayed for total cholesterol and triglyceride (TG) with an Abbott Diagnostics ABA-200 bichromatic analyzer using enzymatic reagents (9). High density lipoprotein cholesterol (HDL-C) was measured as described by Warnick, Benderson, and Albers (10). Lipid assays were standardized through the Centers for Disease Control's Lipid Standardization Program.

Quantitation of apolipoproteins

ApoB was assayed with a noncompetitive, enzyme-linked immunosorbent assay using immunopurified polyclonal antibodies (11). ApoA-I was assayed with the same system, using apoA-I polyclonal antibodies. Coefficients of variation for both assays were less than 5% within runs and less than 10% between runs. Concentrations of apoB in plasma and VLDL were measured directly. Due to losses incurred during the isolation procedure for LDL, apoB was calculated as the difference between plasma and VLDL plus intermediate density lipoprotein (IDL) apoB. The concentration of HDL apoA-I was taken to be the plasma concentration of apoA-I (assuming that >95% of plasma apoA-I is on HDL) (5). In order to estimate the plasma pool size of VLDL apoB-100, VLDL apoB concentration measurements were taken to represent the concentration of VLDL apoB-100. We have previously shown that in the fed state, less than 3% of total VLDL apoB is apoB-48 (12).

ApoE phenotypes were determined by minigel isoelectric focusing as previously described (13). Lipoprotein [a] (Lp[a]) was undetectable when plasma was screened using 2–16% nondenaturing polyacrylamide gradient gel electrophoresis (14). This elimated the possibility that LDL apoB-100 was significantly contaminated with Lp[a] associated apoB-100.

Plasma amino acid concentrations

Amino acid concentrations were determined by isotope dilution mass spectrometry. Known amounts of [UL-$^{2}H_8$]valine (39.60 nmol), [UL-$^{2}H_{10}$]leucine (36.31 nmol), and [$^{2}H_8$]lysine (21.62 nmol) were added to 100 μl of plasma. The amino acids were isolated by cation exchange

chromatography using a Dowex AGx50W-X8 column. The free amino acids were converted to the n-propyl ester and n-heptafluorobutyramide derivatives. The isotopic enrichment of the free amino acid was determined by negative chemical ionization GLC–MS, as previously described (15). Molar ratios of the individual isotopomers, corrected for overlap of natural abundance amino acids in the sample with the tracer amino acid, were obtained by solving a set of simultaneous linear equations which contained terms for the unlabeled amino acids, the deuterated amino acids administered to the subjects during the infusion, and for the deuterated internal standards (16, 17). The plasma amino acid concentrations were obtained using equation 1:

$$[\text{Amino Acid}]_x = \frac{MR_x * \text{Internal Standard (nmol)}}{\text{Volume of Plasma (ml)}} \quad \textit{Eq. 1)}$$

where MR_x is the molar ratio of isotopomer X to the internal standard.

Isolation of apolipoproteins

ApoB-100 was isolated from VLDL and LDL, and apoA-I from HDL by preparative SDS polyacrylamide gradient gel electrophoresis (4–22.5%) using a Tris-glycine buffer system as previously described (12, 18). Lipoprotein samples were loaded by volume. Apolipoproteins were identified by comparing migration distances with those of known molecular weight standards.

Isotopic enrichment determinations

Apolipoprotein bands were excised from polyacrylamide gels, dried and hydrolyzed in 12 N HCl at 100°C for 24 h. The hydrolyzates were dried and the free amino acids were isolated using 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 GLC–MS. Samples were analyzed by methane negative chemical ionization GLC–MS using a Hewlett-Packard 5988A instrument. Chromatographic separations were performed using a 30 m × 0.32 mm DX 4 capillary column (J & W Scientific, Inc., Rancho Cordova, CA). The flow rate was 2.3 ml/min and the temperature was programmed from 80 to 250°C at 10°C/min. The ion source pressure was 0.6 torr methane and the source temperature was 200°C. Isotope ratios were determined in triplicate using the [M], [M + 1], and [M + 3] or [M + 4] isotope satellite peaks surrounding the base peak, [M-HF]. The isotopic enrichment of amino acids and protein samples was determined as delta % excess (d%e), calculated as follows:

$$d\%e = 100(IR_t - IR_o) \quad \textit{Eq. 2)}$$

where IR_t is the isotopic ratio of sample at time 't' and IR_o is the isotopic ratio of the baseline sample at zero time which averaged 0.147 ± 0.028 (leucine), 0.124 ± 0.017 (valine), and 0.033 ± 0.002 (lysine).

Plasma amino acids were isolated and derivatized using the same procedures as described above for quantitation of their levels, but omitting the addition of deuterated internal standards. Apolipoprotein production was measured as the rate of incorporation of deuterated amino acid into circulating apolipoproteins. This parameter, by definition, reflects the rate of apolipoprotein assembly into lipoproteins within the liver and secretion of these lipoproteins into the circulation. The fractional production rate (FPR) for each apolipoprotein (expressed as pools/day) was calculated as follows:

$$FPR = \frac{\text{rate of increase of amino acid enrichment(d\%e/h)}}{\text{enrichment of VLDL apoB-100 at plateau (d\%e)}} \times 24 \quad \textit{Eq. 3)}$$

where enrichment of VLDL apoB-100 at plateau was taken as the average of VLDL apoB-100 enrichment at the last two time-points. A linear rate for increase in apolipoprotein enrichment was calculated using the method of least squares with 6–9 time-points for the LDL and HDL and 4–6 time-points for the VLDL. Only those points that fell on the linear portion of the curve were used to calculate the slope. Absolute production rates (APR) for each apolipoprotein (expressed as mg/kg body weight per day) were calculated as follows:

$$APR = \frac{\text{FPR (pools/day)} * \text{apolipoprotein pool size}}{\text{body weight (kg)}} \times 24 \quad \textit{Eq. 4)}$$

where apolipoprotein pool size equals apolipoprotein concentration times plasma volume (0.045 l/kg body weight) (19).

Statistical analysis

Analysis of variance was used to assess the significance of differences between production rates among the three amino acids.

RESULTS

The characteristics of the study subjects are shown in **Table 1**. All had normal body weights and fasting plasma lipids. Two subjects had an apoE4/E3 phenotype and the third had an apoE3/E3 phenotype. The primed-constant infusion was well tolerated by the subjects. The subjects were restricted to bed rest during the infusion period but

TABLE 1. Characteristics of the subjects

Variable	LD	JM	JJ	Mean ± SDM
Age (years)	24	25	27	25 ± 2
Height (cm)	174	177	165	172 ± 5
Weight (kg)	62	73	71	69 ± 6
Ideal Body Weight (%)	84	98	109	97 ± 12
Cholesterol (mg/dl)	143	144	157	148 ± 8
Triglyceride (mg/dl)	33	61	70	54 ± 20
HDL-cholesterol (mg/dl)	37	45	37	39 ± 5
LDL-cholesterol (mg/dl)	99	87	106	97 ± 10
ApoE phenotype	E4/E3	E3/E3	E4/E3	

were encouraged to exercise their limbs frequently. Total plasma cholesterol remained stable during the 15-h experimental period (**Fig. 1A**). Plasma TG varied somewhat during the infusion period, increasing slightly at the beginning and then normalizing during the later three-fourths of the study period. There was virtually no fluctuation in the plasma apoB concentration during the 15-h time period.

Compositional data for VLDL are shown in Fig. 1B. VLDL cholesterol and apoB remained relatively constant during the infusion period. The variation in VLDL-TG values mirrored that seen in plasma TG. Similarly, LDL cholesterol and apoB concentrations did not vary during the infusion period (Fig. 1C). The data for HDL-C showed a slight downward trend during the infusion period; however, HDL apoA-I was constant throughout the study period (Fig. 1D).

Shown in **Fig. 2** are the plasma amino acid concentrations during the primed-constant infusion period. Mean values over the 15-h infusion period were 194 ± 1 (leucine), 316 ± 2 (valine), and 231 ± 22 μmol/l (lysine). An initially large meal followed by small hourly feedings resulted in relatively constant plasma amino acid concentrations. These values were 33% to 61% higher than mean normal fasting levels reported by Waterlow, Garlick, and Milwood (20), and were similar to mean fed levels reported by Cohn et al. (5).

The isotopic enrichment of plasma leucine, valine, and lysine during the infusion period is shown in **Fig. 3**. Plasma enrichment (d%e) of leucine rose rapidly after the initial bolus injection of isotope, reached a maximal level of enrichment within the first sampling period (1 h), and remained relatively constant for the 15 h duration of the studies. Valine and lysine reached 77% and 84%, respectively, of maximum enrichment by 1 h. There was a slow rate of increase in plasma enrichment for the next 3–4 h after which maximum enrichment was observed.

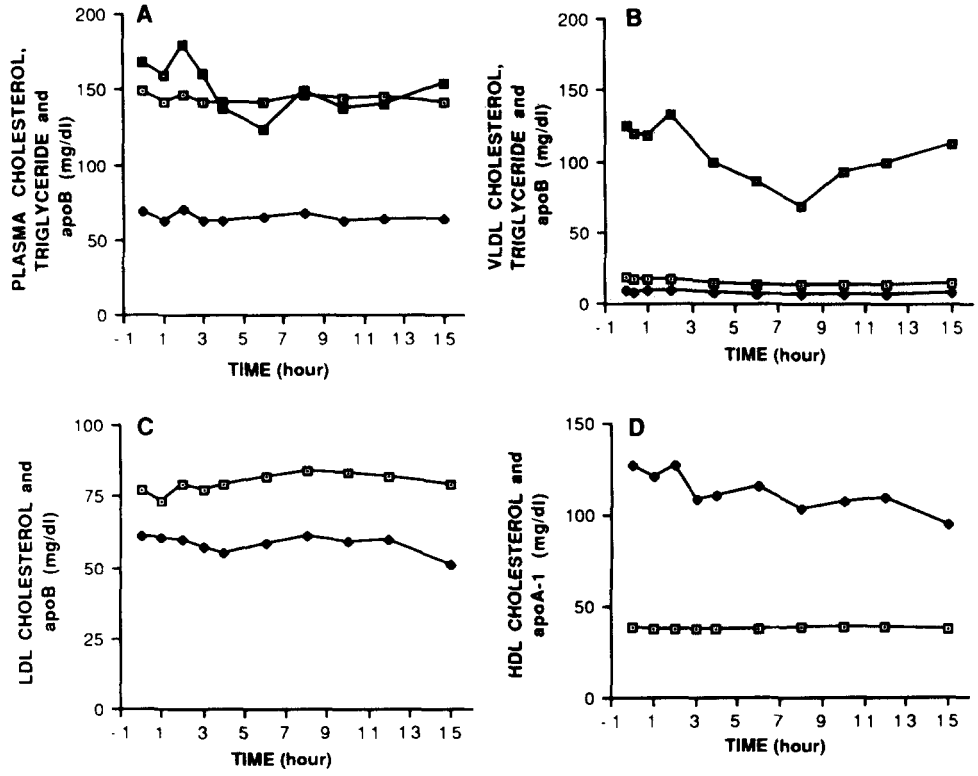

Fig. 1. Concentrations of cholesterol (open squares), triglycerides (solid squares), and apolipoproteins (solid diamonds) in plasma (A), VLDL (B), LDL (C), and HDL (D) of subjects during the constant infusion experiments.

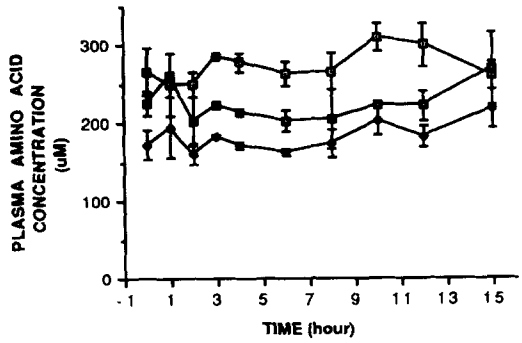

Fig. 2. Plasma amino acid concentrations of valine (open squares), leucine (solid diamonds), and lysine (solid squares) during the constant infusion experiments.

Shown in **Fig. 4A** is the enrichment of VLDL-apoB-100 with the three deuterated amino acids. There was a period of rapid rise in the incorporation of the amino acids into VLDL apoB-100 and then, towards the end of the infusion period, a plateau. This plateau enrichment was taken to represent the enrichment of the precursor amino acid pool from which VLDL apoB-100 was derived, since in an isotopic steady state, the enrichment of a product should reach the enrichment of its precursor. Any protein derived from this amino acid pool will eventually reach this same level of enrichment. The time taken to reach this level represents the time it takes for the protein pool in question to turnover one complete time. A perfect plateau enrichment was not reached, possibly due to recycling of labeled amino acid. There were no differences in the shape of the curve for leucine, valine, or lysine. The differences observed in the relative enrichment of VLDL-apoB-100 within the different amino acids reflected relative differences in the plasma enrichment (Fig. 3) and dilution of the tracer by the intracellular free amino acid pool.

The enrichment of LDL-apoB-100 and HDL-apoA-I with leucine, valine, and lysine is shown in Figs. 4B and 4C, respectively. After a short lag period there was a

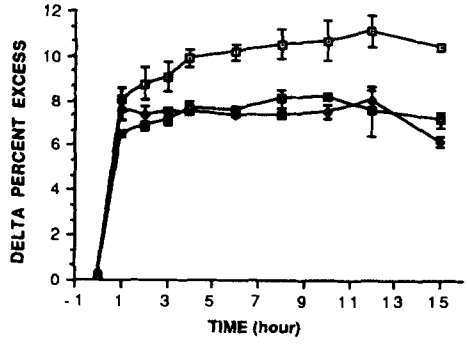

Fig. 3. Enrichment of plasma with deuterated leucine (solid diamonds), valine (open squares), and lysine (solid squares) during the constant infusion experiments.

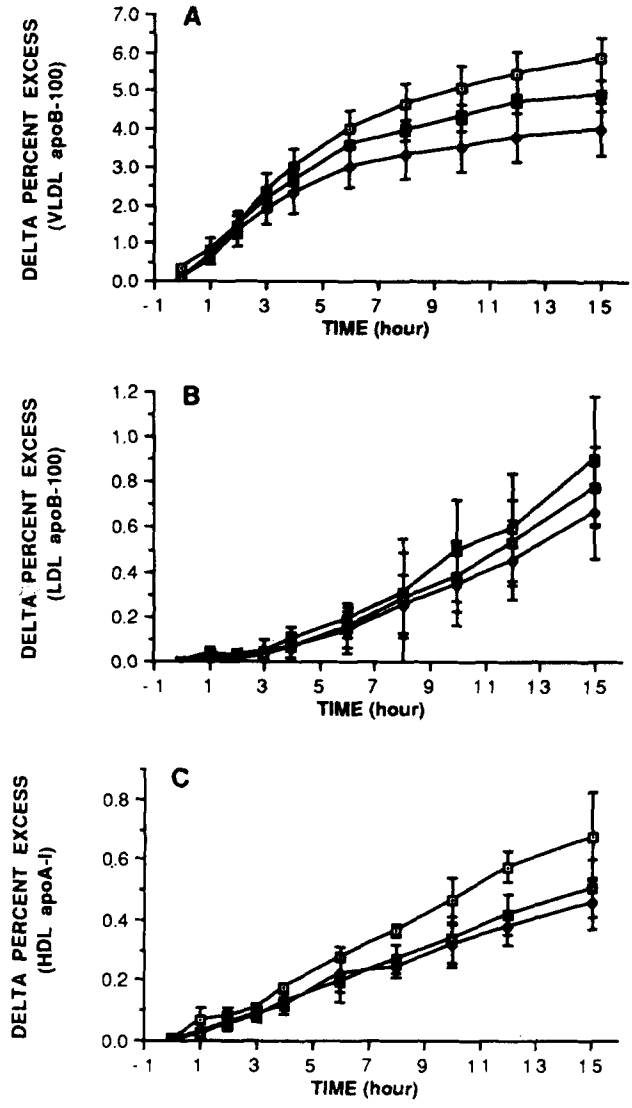

Fig. 4. Enrichment of VLDL (A), LDL (B), and HDL (C) with deuterated leucine (solid diamonds), valine, (open squares), and lysine (solid squares) during the constant infusion experiments in the fed state.

linear rise in the incorporation of the deuterated amino acids into LDL-apoB-100. This lag period was probably attributable to the known metabolic conversion of VLDL-apoB-100 to LDL-apoB-100. The absence of a lag period for the synthesis of HDL likely reflects a more direct route of synthesis. Once enrichment of the apolipoproteins was achieved, this rate was relatively constant throughout the 15-h study period, indicating that only a fraction of the total pool of LDL-apoB and HDL-apoA-I was produced during the 15-h infusion period. Again, the relative levels of enrichment of the three amino acids reflected that of the plasma enrichment pool, and were therefore consistent among the three amino acids for all the proteins studied.

Parameters of VLDL-apoB-100 production are shown in **Table 2**. The rate of enrichment (d%e) was calculated

TABLE 2. Parameters of VLDL-apoB-100 production as measured with leucine, valine, and lysine

Variable	Leucine	Valine	Lysine
Rate of enrichment (d%e/h)	0.54 ± 0.16[a]	0.72 ± 0.27	0.63 ± 0.11
Fractional production rate (pools/day)	3.20 ± 0.51	3.06 ± 0.88	3.14 ± 0.47
Pool size (mg)	258 ± 168	258 ± 168	258 ± 126
Absolute production (mg/kg/day)	11.43 ± 5.82	11.22 ± 6.82	11.05 ± 5.37

[a]Mean ± SDM.

TABLE 4. Parameters of HDL-apoA-I production as measured with leucine, valine, and lysine

Variable	Leucine	Valine	Lysine
Rate of enrichment (d%e/h)	0.03 ± 0.01[a]	0.05 ± 0.01	0.040 ± 0.01
Fractional production rate (pools/day)	0.19 ± 0.04	0.21 ± 0.02	0.18 ± 0.03
Pool size (mg)	3534 ± 517	3534 ± 517	3534 ± 517
Absolute production (mg/kg/day)	9.66 ± 0.16	9.40 ± 1.65	9.06 ± 1.27

[a]Mean ± SDM.

from the linear portion of the data shown in Fig. 3A. The enrichment at plateau of VLDL-apoB-100 was used to calculate the FPR for all apolipoproteins. These values were (d%e) 3.98 (leucine), 5.63 (valine), and 4.80 (lysine). Mean apolipoprotein concentrations over the 15-h infusion period (Fig. 1) were used to estimate pool size. The FPR of the three amino acids for VLDL-apoB-100 averaged 3.13 ± 0.07 pools/day and were virtually identical for all the amino acids studied. The APR of VLDL-apoB-100 are also shown in Table 2 and averaged 11.23 ± 0.19 mg/kg body weight per day with greatest mean percent differences among the three amino acids being 3.3%. The data for LDL-apoB-100 and HDL-apoA-I are shown in **Table 3** and **Table 4**. The FPR and APR for the apolipoproteins as measured by leucine, valine, and lysine are again similar. The mean FPR for LDL-apoB-100 was 0.29 ± 0.01 and HDL-apoA-I was 0.19 ± 0.01 pools/ day. The mean APR of LDL-apoB-100 was 7.68 ± 0.28 and for HDL-apoA-I was 9.37 ± 0.30 mg/kg body weight per day. For the three amino acids studied the greatest mean differences among them were 6.0% for LDL-apoB and 6.2% for HDL-apoA-I. These data indicate that the method of utilizing deuterated amino acids to measure the rate of production of apolipoproteins into plasma is independent of the specific amino acid labeled, at least for the three amino acids that we studied.

TABLE 3. Parameters of LDL-apoB-100 production as measured with leucine, valine, and lysine

Variable	Leucine	Valine	Lysine
Rate of Enrichment (d%e/h)	0.05 ± 0.01[a]	0.07 ± 0.02	0.06 ± 0.02
Fractional production rate (pools/day)	0.30 ± 0.08	0.30 ± 0.06	0.28 ± 0.08
Pool size (mg)	1734 ± 807	1734 ± 807	1734 ± 807
Absolute production rate (mg/kg/day)	8.00 ± 4.72	7.52 ± 3.80	7.52 ± 4.22

[a]Mean ± SDM.

The use of deuterated leucine to determine APR was reproducible when the method was assessed in the same three individuals on two separate occasions differing by an interval of approximately 1 year (5) (**Table 5**). The protocols followed were identical except that in the first study only deuterated leucine was infused in contrast to the present study in which deuterated leucine, valine, and lysine were infused. The maximum mean percent differences between the two measures were VLDL-apoB-100, 3%; LDL-apoB-100, 10%; and HDL-apoA-I, 4%.

DISCUSSION

We have used three different stable isotope-labeled amino acids to measure apolipoprotein production rates with a primed-constant infusion technique. No significant difference was observed between kinetic parameters obtained with the three tracers. VLDL- and LDL-apoB-100, and HDL-apoA-I production rates were essentially identical when measured with deuterated leucine, valine, or lysine. Furthermore, kinetic parameters obtained with deuterated leucine in the present study were similar to those obtained for the same subjects in our previous study (5). Moreover, these values are similar to those previously reported by investigators utilizing radioiodinated lipoproteins or apolipoproteins (21, 22). These data suggest that deuterated leucine is a viable tracer for measuring apolipoprotein production rates with the present method, consistent with previous observations utilizing radioactive leucine (1).

TABLE 5. Comparison of absolute production rates of VLDL-apoB-100, LDL-apoB-100, and HDL-apoA-I as measured with deuterated leucine on two separate occasions in the same individuals

	Subject							
	LD		JM		JJ		Mean ± SEM	
Apolipoprotein	I	II	I	II	I	II	I	II
	mg/kg body weight/day							
VLDL-apoB-100	7.3	6.3	7.3	8.1	16.8	16.1	10.5 ± 3.2	10.2 ± 3.0
LDL-apoB-100	4.4	2.7	7.5	7.5	14.3	13.2	8.7 ± 2.9	7.8 ± 3.0
HDL-apoA-I	12.1	9.6	8.0	9.6	10.1	9.8	10.1 ± 1.2	9.7 ± 0.1

I, data from reference 5; II, data from current study.

There were several reasons why deuterated leucine was originally selected for our in vivo studies: *1*) leucine is an essential amino acid and is not synthesized to any extent in humans; the enrichment of plasma leucine pools in the liver is therefore not affected by endogenously synthesized amino acid; *2*) unlike most other amino acids, leucine is metabolized to a large extent in muscle, reducing the possibility of label recycling in the liver; *3*) leucine is converted to its keto acid (alphaketoisocaproate) and is not converted to other amino acids, resulting in less effect of the injectate on other amino acid pools in the liver; and *4*) deuterated leucine is commercially available and is relatively affordable in the quantities required for this technique.

The use of deuterated leucine as a tracer requires several assumptions. It must be assumed that this tracer is handled in an identical way to endogenous unlabeled leucine and is not selectively incorporated into proteins or stored within specific amino acid pools in the liver. It is assumed that the rate of equilibration of this tracer with intracellular pools is relatively rapid to allow for an accurate estimation of apolipoprotein production. In this and the previous study (5), we observed that the enrichment of a hepatic protein, VLDL-apoB-100, at what we assumed to be at or near the plateau value, did not reach plasma levels of enrichment. Similar observations have been made by Cryer et al. (3) and Matsushima et al. (4) who investigated synthesis of VLDL-apoB-100 using [^{15}N]glycine. Influx of unlabeled amino acids from the portal vein in the fed state and intracellular protein turnover serve to dilute the intracellular pool of deuterated leucyl-tRNA, hence would result in a lower level of enrichment intracellularly. Interestingly, for tissues that derived their blood supply from the peripheral circulation, such as cardiac muscle and lung, this discrepancy has not been reported, at least during the initial stages of the infusion (23–25). The production rates reported for HDL-apoA-I were calculated under the assumption that the levels of liver and intestinal enrichment are similar. This assumption requires further validation.

Support for the assumption that deuterated leucine could be used to accurately estimate apolipoprotein kinetics can be derived from comparisons between APR generated from work using labeled amino acids and catabolic rates using radiolabeled lipoproteins or apolipoproteins. Reported values for the catabolic rate of VLDL-apoB-100 range from 9.1 to 15.5 mg/kg per day (21), LDL-apoB-100 range from 7.6 to 14.4 mg/kg per day (21) and HDL-apoA-I range from 8.7 to 15.8 mg/kg per day (22). The data presented in this paper and previously (5) compare favorably, given the variability in the characteristics of the subjects and the protocols they were on. We have now attempted to further validate the methodology of using stable isotope labeled amino acids to describe apolipoprotein kinetics by infusing three deuterated amino acids, leucine, valine, and lysine, into subjects and comparing lipoprotein kinetics simultaneously derived from all three. Our data are consistent with the concept that the use of leucine, valine, or lysine yields similar results when their individual isotopic enrichments of VLDL-apoB-100, LDL-apoB-100, and HDL-apoA-I were used to calculate FPR or APR. No evidence that there was differential handling of any of the amino acids was observed with respect to incorporation into newly synthesized apolipoproteins. This was found for valine, a branched chain amino acid similar in physical and metabolic properties to leucine, and for lysine, a basic amino acid that is potentially a major substrate for intermediary metabolism and differs in physical characteristics when compared to leucine.

In the present study, we have compared rates of apolipoprotein production with the three deuterated amino acids in the fed state, although in our previous study we carried out experiments in both the fed and fasted state (5). Due to the labor-intensive nature of these stable isotope experiments, we have not made a comparison of the different amino acids in the fasted state, though we would presume that a similar result would be obtained. We chose to do the present comparison in the fed state, because we believe that this state is more physiologically relevant and because this metabolic state may be of particular importance in the pathogenesis of atherosclerosis.

The present technique has been derived from a method described by Cryer et al. (3) in which VLDL-apoB (apoB-100 plus apoB-48) kinetics were assessed with a bolus and

then a constant infusion of [^{15}N]glycine. The enrichment of urinary hippurate at plateau was used to measure the enrichment of the glycine precursor pool. This approach is potentially useful in the situation where VLDL-apoB-100 enrichment does not reach a plateau within the time course of the experiment (e.g., patients with hypertriglyceridemia). Subsequent work by this group utilizing isolated nonrecirculating perfused rat livers demonstrated that the enrichment of perfusate hippurate with [^{15}N]glycine was 30% higher than that of glycyl-tRNA and hence when used as a measure of maximal enrichment of VLDL-apoB-100, overestimated the FPR (4). They estimated that this discrepancy in humans was closer to 20% and suggested incorporating a correction factor into the FPR calculations to make the plateau enrichment of hippurate a more accurate measure of glycine precursor enrichment. These findings reinforce the essential nature of choosing a rapidly turning over protein from which to estimate maximal enrichment for the calculation of FPR, one that is made in the same compartment of the cell as the protein in question. Alternatively, the relative difference in the potential enrichment of the protein in question must be estimated from the tissue secretory product and this value must be incorporated into the calculations. At this point we feel that deuterated leucine enrichment of VLDL-apoB-100 at plateau is a reasonable means of assessing the enrichment of the precursor amino acid pool.

We have further validated the methodology of using deuterated leucine to estimate apolipoprotein kinetics by running duplicate determinations in the same subjects about 1 year apart. The results were highly reproducible. Our combined results are consistent with the concepts that deuterated leucine, valine, and lysine yield similar results when used to determine lipoprotein kinetics, that the method is reproducible, and that the results obtained with this methodology are similar to those previously obtained with radioiodinated kinetic studies.

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