Studies on the metabolism of apolipoprotein B in hypertriglyceridemic subjects using simultaneous administration of tritiated leucine and radioiodinated very low density lipoprotein

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Abstract To study the metabolic pathways of apolipoprotein B (apoB), a series of studies were carried out in which both radioiodinated very low density lipoproteins (VLDL) and tritiated leucine were simultaneously injected into three hypertriglyceridemic subjects. The appearance and disappearance of tritium activity in VLDL apoB, intermediate density lipoprotein (IDL) apoB, and low density lipoprotein (LDL) apoB were followed as was the disappearance of iodine activity from VLDL and the appearance and disapperance of iodine activity in IDL apoB and LDL apoB. It was found that a delipidation chain could describe the kinetics of both endogenously and exogenously labeled VLDL. A slow component of VLDL was necessary to fit the VLDL ^{131}I-labeled apoB data and was consistent with the observed VLDL [^{3}H]apoB kinetics. In addition, the estimated rate of conversion of VLDL apoB to LDL exceeded that which appeared to pass through the measured IDL pools, suggesting that a fraction of the IDL was not directly observed. It was also found that a higher percentage of VLDL ^{131}I-labeled apoB was converted to LDL apoB than was VLDL [^{3}H]apoB. To evaluate possible causes of this apparent anomaly, simultaneous examination of all kinetic data was performed. This exercise resulted in the resolution of removal pathways from multiple compartments in the VLDL delipidation chain and the conversion of slowly metabolized VLDL to IDL and LDL. The wide spectrum of this loss pathway indicates that previous estimates of VLDL apoB production rate using the radioiodinated methodology probably represent lower bounds for the true physiologic variable. It is important to note that these direct losses were apparent only when the combination of endogenous and exogenous labeling was used. —**Beltz, W. F., Y. A. Kesäniemi, N. H. Miller, W. R. Fisher, S. M. Grundy, and L. A. Zech.** Studies on the metabolism of apolipoprotein B is hypertriglyceridemic subjects using simultaneous administration of tritiated leucine and radioiodinated very low density lipoprotein. *J. Lipid Res.* 1990. **31:** 361–374.

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The kinetics of very low density lipoprotein (VLDL) apolipoprotein B (apoB) have been studied using a number of tracer methods. The primary goals of these studies have included the determination of accurate production rates for VLDL apoB, the quantification of the conversion of VLDL apoB to intermediate density lipoproteins (IDL) and low density lipoproteins (LDL), and the identification of the points of entry of apoB into the plasma lipoprotein system. Methods of exogenously radioiodinating and reinjecting VLDL have been used extensively by many investigators (reviewed in reference 1). Exogenous labeling has the advantage that label may be introduced directly into the system under study and the observed kinetics are determined solely by removal pathways. One possible disadvantage is that modification of the particles during separation and labeling may lead to nonphysiological kinetics. Also, heterogeneous systems are labeled according to the masses in the different pools. Calculation of absolute transport rates then requires some assumption concerning the relative sizes of these pools. The usual assumption is that all plasma pools are labeled with equal

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TG, triglyceride; TMU, tetramethylurea; FCR, fractional catabolic rate.

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efficiency and therefore have equal specific activities immediately following labeling.

To avoid these potential labeling problems, other researchers have injected radioactive amino acids to endogenously label apolipoproteins. Phair et al. (2) and Fisher et al. (3), using [^{3}H]leucine, and Eaton, Allen, and Schade (4), using [^{75}Se]selenomethionine, have used this approach. The endogenous labeling technique has the advantage that tracer is introduced to the system under study via the true input pathways and may therefore better reflect apoprotein production. The labeling also leaves the proteins biologically functional. The method suffers from the disadvantage that the kinetics of the labeled particles are not always easily interpretable since they are a convolution of both input and removal kinetics.

Thus, exogenous and endogenous labeling each have theoretical advantages and disadvantages for the study of apoprotein kinetics. To date, no direct comparison of results obtained using these distinctly different methodologies for studying apoB metabolism has been made. Although Le et al. (5) gave simultaneous injections of ^{125}I-labeled VLDL and [^{3}H]leucine into monkeys, they did not report a quantitative comparison of the two isotopes. We therefore performed a study in which iodinated VLDL was injected along with tritiated leucine. We could then directly compare results obtained for the two methods as well as combine the data to resolve any apparent discrepancies between them.

METHODS

Three hypertriglyceridemic subjects were admitted to the Special Diagnostic and Treatment Unit of the Veterans Administration Medical Center, San Diego, California. The age, sex, body habitus, and plasma lipid concentrations at the times of the studies are presented in **Table 1**.

During hospitalization, the subjects were each fed an isocaloric, weight-maintenance diet. The basic composition and pattern of this diet have been described previously (6). Except as noted below, three liquid meals were given each day. Subject 1 also received a fourth meal of solid food. Fat comprised 40% of the calories and was mostly in the form of lard. The ratio of polyunsaturated to saturated fats was 0.2. For 36 h before injection and 48 h after injection, nonfat liquid meals were given every 3 h in equally divided amounts. Throughout the study, calories were divided approximately equally among the feedings. Leucine intake was constant during the study at 4.18 mg/kcal. Cholesterol intake was less than 200 mg/day. Vitamin and mineral supplements were given daily. Each subject was weighed daily and caloric intake was adjusted to maintain total body weight at a constant level. These investigations were approved by the appropriate review boards and all subjects gave informed consent for the study.

TABLE 1. Clinical data

	Subject		
	1	2	3
Age (yr)	60	58	60
Sex	F	M	M
Clinical diagnosis	N	N	CHD
Weight (kg)	65	76	75
(% Ideal)	120	122	120
Ideal body weight (kg)	54	62	62
Plasma volume (dl)	25.3	29.2	29.2
	mg/dl		
Total cholesterol	306 ± 13	276 ± 16	251 ± 5
VLDL cholesterol	175 ± 10	153 ± 7	115 ± 4
LDL cholesterol	105 ± 3	97 ± 7	109 ± 6
HDL cholesterol	25 ± 1	24 ± 3	25 ± 1
Total triglyceride	690 ± 31	745 ± 90	419 ± 15
VLDL triglyceride	610 ± 25	694 ± 86	348 ± 17
LDL triglyceride	53 ± 7	31 ± 4	48 ± 5
HDL triglyceride	26 ± 1	19 ± 3	21 ± 1

Lipid concentrations are means ± standard errors of three determinations for subject 1, five determinations for subject 2, and five determinations for subject 3. N, normal; CHD, coronary heart disease.

Preparation of iodinated VLDL

After each subject had been on the metabolic diet for 7 to 14 days, plasmapheresis was carried out by collecting blood (500 ml) into sterile, pyrogen-free bags containing 500 mg of disodium EDTA. After centrifugation at 5500 *g* and 20°C for 5 min, plasma was separated from cells and the cells were returned to the patient. VLDL for iodination were isolated according to Lindgren et al. (7) at a density of 1.006 g/ml in an SW-27 swinging bucket rotor in an L2-65 B ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA) at 23,000 rpm and 10°C for 22 h. VLDL were aspirated from the top by Pasteur pipet, mixed with sterile NaCl solution (d 1.006 g/ml) and subjected to further ultracentrifugation for 22 h in a 40.3 rotor at 39,000 rpm and 10°C. The concentrated VLDL were dialyzed against 0.15 M NaCl containing 0.01% EDTA (pH 7.4).

Whole VLDL were labeled with ^{131}I by the iodine monochloride method of McFarlane (8) as modified by Bilheimer, Eisenberg, and Levy (9). The molar ratio of iodine to protein was less than one. The efficiency of radiolabeling averaged 13%. The extent of lipid labeling was determined by chloroform–methanol 2:1 (v/v) extraction (10). The organic phase was evaporated to dryness and radioactivity in the residual lipid was determined. Radioactivity in the lipid moiety was 24 to 33%. The amount of unbound iodine after dialysis was determined by thin-layer chromatography on Gelman ITLC-SG sheets (Gelman Sciences, Inc., Ann Arbor, MI) using

20% trichloroacetic acid containing 0.125% sodium metabisulfate. The amount of ^{131}I not bound to the lipoprotein averaged less than 1%.

After dialysis, the labeled VLDL preparation was subjected to centrifugation at 20,000 *g* and 4°C for 30 min to remove any precipitated material. The supernate was removed, passed through a 0.22-μm filter (Millipore Corp, Bedford, MA), and diluted to a concentration of 20 μCi/ml using a sterile solution of 5% human albumin. The solution was then passed again through a 0.22-μm filter and aliquots were tested for sterility and pyrogenicity.

Injection procedure and sample collection

Five days after plasmapheresis, subjects were simultaneously injected with 30 to 50 μCi of ^{131}I-labeled VLDL and 340 μCi of [^{3}H]leucine (Amersham Corp., Arlington Heights, IL). Blood samples (10 ml) were collected at 0.25, 0.5, 0.75, 1, 2, 3, 5, 9, 12, 18, 24, 30, 36, and 48 h after injection, and daily thereafter until 14 days after injection.

Separation of lipoproteins

Four ml of plasma was overlaid with 2 ml of NaCl (d 1.006 g/ml) and centrifuged in a 40.3 rotor at 39000 rpm and 15°C for 20 h. VLDL ($d < 1.006$ g/ml) were isolated by tube slicing. The infranate was adjusted to a density of 1.019 g/ml by the addition of NaCl and NaBr and subjected again to ultracentrifugation in a 40.3 rotor at 39000 rpm and 15°C for 20 h. IDL ($1.006 < d < 1.019$ g/ml) were isolated by tube slicing and dialyzed to d 1.006 g/ml in 0.15 M NaCl containing 0.01% EDTA (pH 7.4). The infranate was adjusted to a density of 1.063 g/ml by the addition of NaCl and NaBr and subjected to ultracentrifugation in a 40.3 rotor at 39000 rpm and 15°C for 20 h. LDL ($1.019 < d < 1.063$ g/ml) was isolated by tube slicing and dialyzed to d 1.006 g/ml.

Plasma lipid and lipoprotein determinations

Plasma lipid and apoB concentrations were estimated three to five times during the first 48-h period using procedures detailed elsewhere (11). Total plasma cholesterol and triglycerides (TG) were determined with a Technicon AutoAnalyzer II (Technicon Instruments Corp., Tarrytown, NY). Concentrations of cholesterol and TG in VLDL, IDL, LDL, and HDL were determined as described in the Lipid Research Clinics Manual of Laboratory Operations (12). Percentage recoveries of lipoprotein lipids were estimated by comparison to the total plasma concentrations. Lipoprotein cholesterol and TG concentrations were corrected for losses by using the same percentage recovery for each lipoprotein fraction. ApoB concentrations were determined by precipitation with 9 M tetramethylurea (TMU), resolubilization in 1 M NaOH, and measurement of protein (13). Ratios of apoB to TG and apoB to cholesterol were calculated for each sample. Concentrations of VLDL and IDL apoB were corrected for losses by multiplying the VLDL and IDL apo BTG ratios by the corrected VLDL and IDL TG concentrations, respectively. The concentration of LDL apoB was corrected for losses by multiplying the apoB cholesterol ratio by the corrected LDL cholesterol concentration. Mean concentrations of VLDL apoB, IDL apoB, and LDL apoB used in the analyses were those obtained during the first 48 h of the study.

Specific activity determinations

Specific activities of ^{131}I-labeled apoB in VLDL, IDL, and LDL were determined according to the method of Le et al. (14) One ml of isolated lipoprotein was delipidated using acetone and isopropanol and 9 M TMU was added. After standing overnight, 2 ml of water was added, the samples were centrifuged and the supernate was recovered. The apoB pellet was washed with 9 M TMU, washed with water, and evaporated to dryness. The pellet was then added to 1 M NaOH (0.5 ml for VLDL, 0.25 ml for IDL and 1.0 ml for LDL) and sonicated at 37°C for 1 to 3 days to resolubilize the protein. ^{131}I in an aliquot of the resolubilized protein was counted in a Packard 5820 scintillation spectrometer (Packard Instrument Co., Downer's Grove, IL). The concentration of the resolubilized apoB was determined by the method of Lowry et al. (13). VLDL and IDL ^{131}I-labeled apoB specific activities were determined for only the first 48 h of the turnover study. LDL ^{131}I-apoB specific activity was followed for the full 14 days.

An aliquot of each lipoprotein fraction was used for determination of [^{3}H]apoB specific activity as previously described (15). Briefly, the VLDL, IDL, or LDL fraction was dialyzed against 0.05 M sodium phosphate, 0.15 M NaCl (pH 7.2), extracted with diethylether–ethanol, and electrophoresed in a preparative 4.5% polyacrylamide tube gel using 0.05 M imidazole, 0.1% SDS, pH 7 buffer. The apoB band was excised and transferred to a separate gel tube, overlaid with an acrylamide plug, and the bottom of the tube was sealed with dialysis membrane to form a sample collection chamber. The apoB was then electroeluted and recovered. The amount of protein was quantified by a fluorescamine assay and the tritium activity was measured using a scintillation counter (Beckman Instruments, Inc., Palo Alto, CA). For subjects 2 and 3, the specific activity of plasma leucine was determined as previously described (3). All tritium specific activities were followed for 14 days after injection.

For the two methods for measuring apoB specific activities, it was necessary that mass measurements for apoB be identical by the Lowry procedure (13) and the fluorescamine assay (15). In both cases, bovine serum albumin (BSA) was used as the standard and all masses

were related to BSA assuming a chromatogenecity factor of 1.0. Previous studies in one author's (W.R.F.) laboratory have compared the relative chromatogenecity of apoB, using amino acid analysis, with BSA as determined by Lowry and fluorescamine methods. Both give identical chromatogenecities for apoB relative to BSA, and therefore, specific activities for apoB measured by the two methods will be identical.

Kinetic analysis

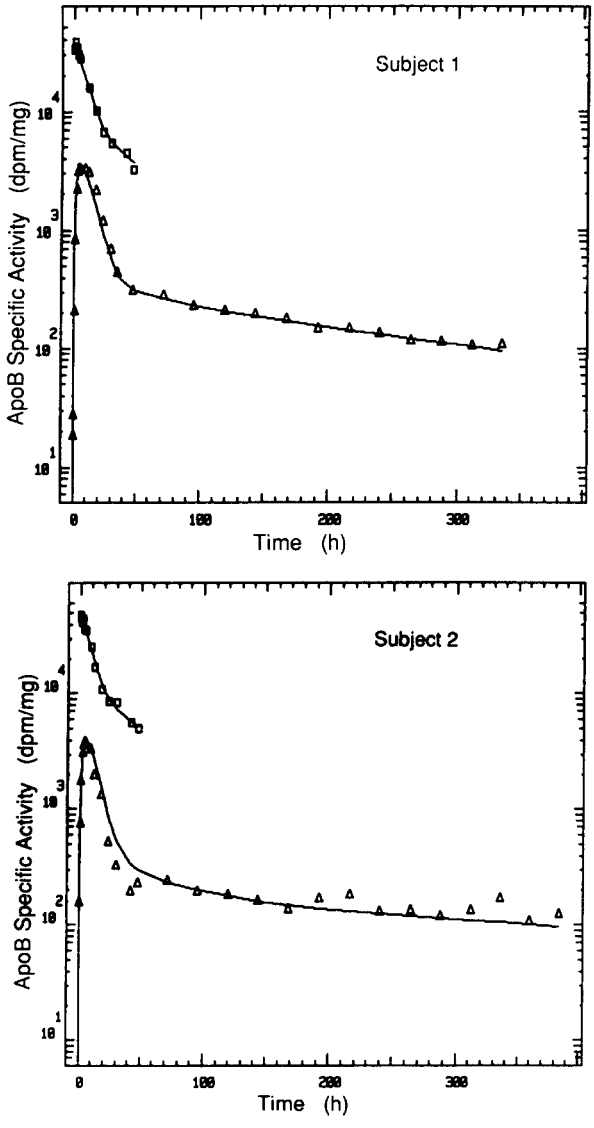
The specific activity data for apoB in VLDL, IDL, and LDL were analyzed using multicompartmental analysis. All computations were performed on a VAX 11/780 computer (Digital Equipment Corp., Maynard, MA) using the SAAM/CONSAM computer programs (16, 17). Kinetic analysis was performed in two steps. First, results obtained using the two methods of VLDL labeling were directly compared by separately analyzing the [^{3}H]apoB data and ^{131}I-labeled apoB data using slightly modified versions of published models. Second, all kinetic data were analyzed simultaneously using a single model. The latter method maximized the information obtained from the available data.

RESULTS

Independent analyses

Kinetic data for VLDL, IDL, and LDL are shown as open symbols in **Fig. 1, Fig. 2,** and **Fig. 3**, respectively. Each figure shows specific activity data for both isotopes and for all subjects.

The [^{3}H]apoB data were first analyzed according to the model shown in **Fig. 4A**. This model is essentially that proposed by Fisher et al. (3). The originally proposed mo-



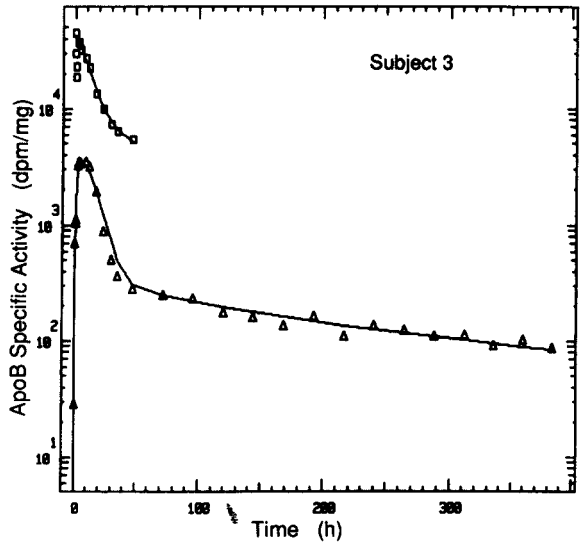


Fig. 1. VLDL apoB turnover curves after the injection of tritiated leucine and radioiodinated VLDL. Open triangles are observed values of VLDL [^{3}H]apoB specific activity. Open squares are observed values of VLDL ^{131}I-labeled apoB specific activity. Solid lines are computer-generated fits to the data.

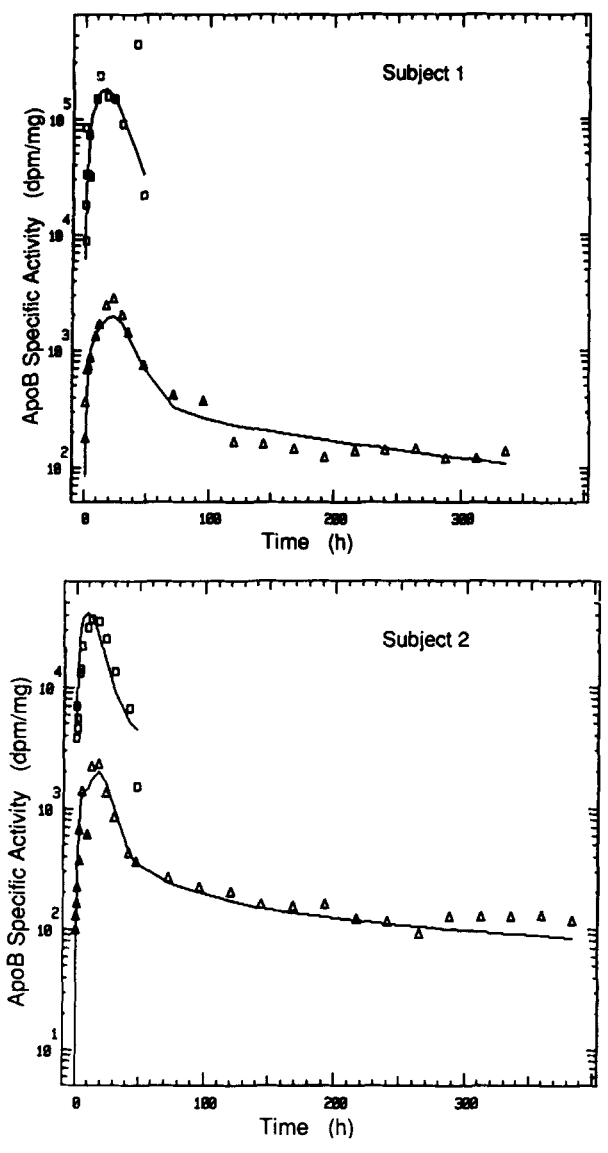
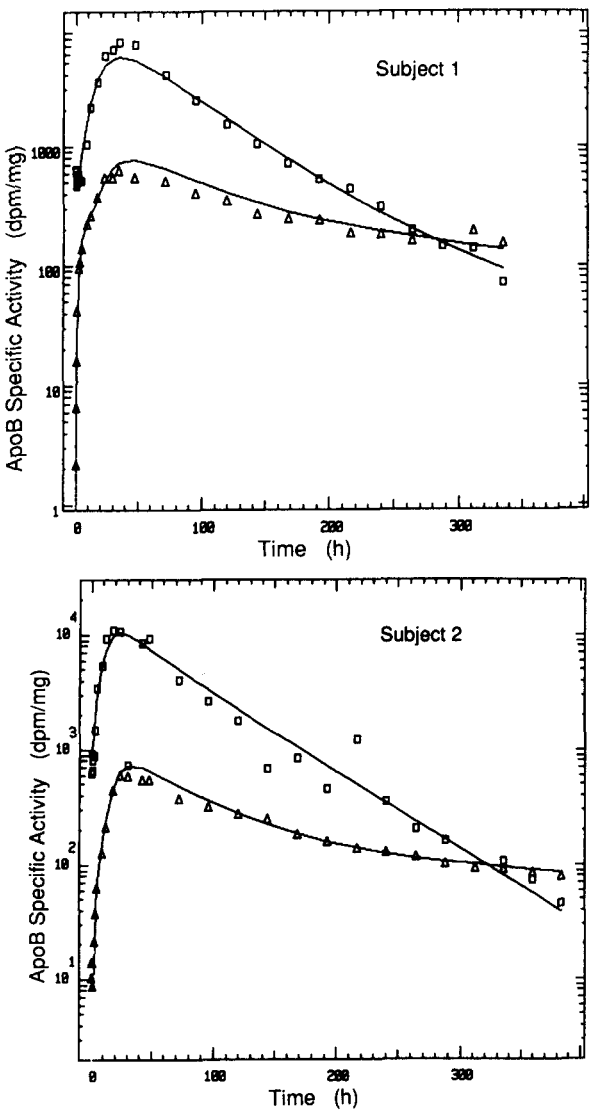


Fig. 2. IDL apoB turnover curves after the injection of tritiated leucine and radioiodinated VLDL. Open triangles are observed values of IDL [^{3}H]apoB specific activity. Open squares are observed values of IDL ^{131}I-labeled apoB specific activity. Solid lines are computer-generated fits to the data.

del has been modified as described below to conform to the present experimental design. In the previous study (3), IDL and LDL were subfractionated. In the present study, subfractionation was not performed and a single pool of plasma IDL and a single pool of plasma LDL were found to be adequate to fit the available data. In the model used, [^{3}H]leucine is introduced into a precursor subsystem. This system is represented by the single compartment Lu and the amount of tritium in Lu available for incorporation into apoB at any time was set by an explicit equation. In subjects 2 and 3, this forcing function was a sum of three exponential terms obtained by fitting their respective plasma free leucine activity curves. It was assumed that plasma leucine turnover in subject 1 was similar to that observed in the other two subjects and a leucine-forcing function for subject 1 was obtained by fitting the composite data of subjects 2 and 3. Labeled leucine can directly enter VLDL, IDL, or LDL apoB plasma pools via the precursor pools Vp, Ip, and Lp, respectively. The latter pools represent delays in the appearance of apoB in plasma. VLDL apoB passes through a delipidation chain of multiple subcompartments (V1 through V4) and ultimately can have either of two fates: direct removal from the circulation or conversion to IDL. IDL apoB (compartment I) can either be converted to LDL or removed directly. LDL apoB consists of intravascular and extravascular pools (compartments L and Lx, respectively), with irreversible removal occurring only from intravascular pool. The results obtained for the leucine data using this model are presented in **Table 2**.

The ^{131}I-labeled apoB data were first analyzed using the model shown in Fig. 4B. This model is basically that used by Beltz et al. (18). In the model used, VLDL apoB consists of a delipidation cascade with a variable number of



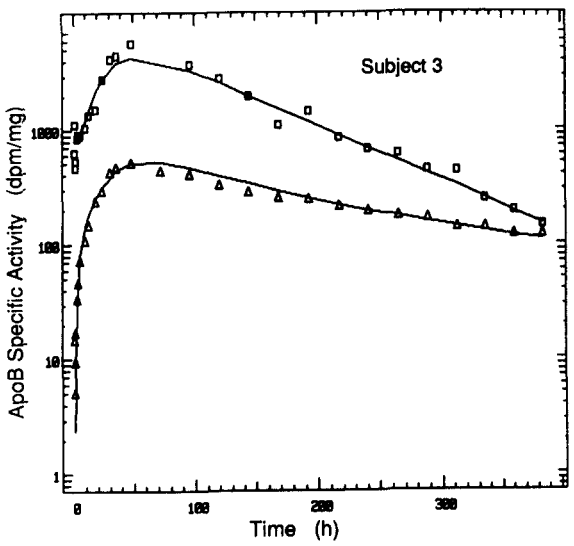


Fig. 3. LDL apoB turnover curves after the injection of tritiated leucine and radioiodinated VLDL. Open triangles are observed values of LDL [^{3}H]apoB specific activity. Open squares are observed values of LDL ^{131}I-labeled apoB specific activity. Solid lines are computer-generated fits to the data.

compartments (V1 through Vn) and a slowly catabolized pool (Vs). IDL apoB consists of a single pool (compartment I). IDL apoB can be either converted to LDL or removed directly. In the present study, as in the previous study (18), if all VLDL to LDL transport was assumed to pass through IDL, the estimated IDL apoB concentration exceeded what was actually measured. A fraction of IDL compartment I may therefore be outside the measured circulating IDL pool. LDL apoB is a two-compartment subsystem consisting of intravascular (L) and extravascular (Lx) pools. The model allows for irreversible removal of apoB from the last compartment of the VLDL chain and direct input of IDL and LDL. The results for the iodine data using this model are presented in Table 2.

For all three subjects, the residence time for VLDL apoB estimated from the model in Fig. 4B using the ^{131}I data was greater than that estimated from the model in Fig. 4A using the ^{3}H data. The transport rates for VLDL apoB were therefore determined to be lower with the iodine label than with the tritium. With the tritium data it was not possible to estimate the size of the slowly catabolized VLDL pool (compartment Vs, Fig. 4B) because its contribution to the tail of the VLDL specific activity curve could not be distinguished from recycling of tritium via the leucine subsystem. Based on the iodine data, the size of the slowly catabolized pool ranged from 19 to 27% of the total VLDL apoB pool.

One of the striking differences between the two analyses was in the fraction of VLDL apoB converted to LDL. For the ^{131}I data, 50 to 76% of VLDL apoB reached LDL, while for the [^{3}H]leucine data, only 20 to 32% of VLDL apoB appeared to be transformed to LDL apoB. Based upon the [^{3}H]leucine data, the fraction of VLDL apoB converted to IDL apoB ranged from 21 to 55%. In two

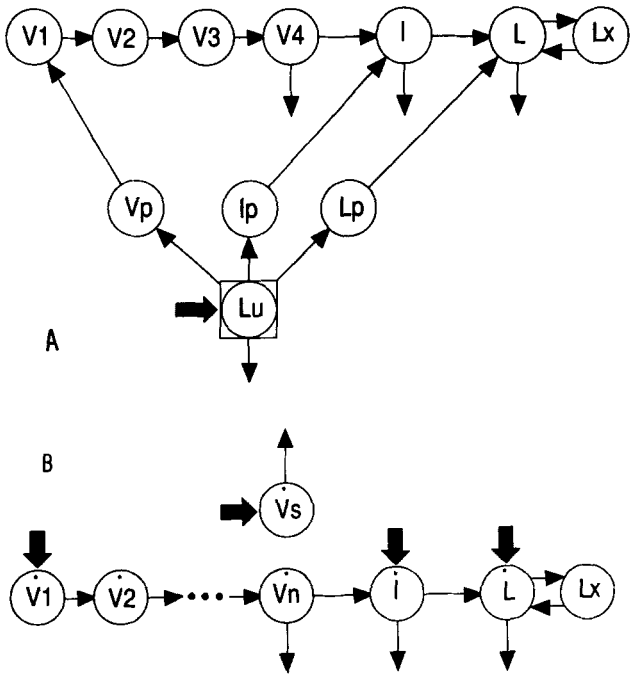


Fig. 4. A. Model used in independent analysis of tritiated apoB specific activity data. B. Model used in independent analysis of radioiodinated apoB specific activity data. Large arrows represent sources of unlabeled apoB. Other arrows indicate transfer of unlabeled apoB and apoB tracers between compartments. The compartments are interpreted as follows: Lu, plasma leucine; Vp, delay pool for leucine incorporation into VLDL; Ip, precursor pool for apoB production into IDL; Lp, delay pool for apoB production into LDL; V1, V2, ..., Vn or V4, VLDL delipidation chain; Vs, slowly catabolized VLDL; I, rapidly catabolized IDL; L, plasma LDL; Lx, extravascular LDL exchange pool. Measured VLDL apoB radioactivity is the sum of activities in the delipidation chain and Vs. Measured IDL apoB tritium radioactivity is the activity in compartment I. Measured IDL apoB iodine radioactivity is only a fraction of the activity in compartment I. The remainder of the iodine activity in compartment I is considered to be "sequestered" IDL pool that is not measured when sampling plasma IDL. Measured LDL apoB radioactivity is the activity in compartment L. The box around compartment Lu indicates that a forcing function was used to drive the cellular uptake of radiolabeled plasma leucine. This forcing function was set equal to the observed plasma leucine specific activity curve. Compartments marked with stars are sites of entry of ^{131}I into the system. Small amounts of ^{131}I were observed in the IDL and LDL apoB pools at t = 0.

subjects this fraction was higher than that for conversion of VLDL apoB to LDL. All VLDL to LDL tritium conversion could be accounted for by passage through the IDL pool. VLDL to IDL conversion could not be confidently estimated for the iodine data, not because of any theoretical problem, but because of uncertainty in the IDL ^{131}I-labeled apoB specific activity determinations due to a low recovered mass of IDL apoB.

For IDL apoB, the residence time estimated from the model in Fig. 4B using the iodine data was greater than that estimated from the model in Fig. 4A using the leucine data. Consequently, the IDL apoB fractional catabolic rate (FCR) and transport rates were lower when estimated using iodine than when using leucine. The leucine data provided evidence for direct input of IDL independent of the VLDL chain. This input could not be detected with the ^{131}I data. The fraction of IDL apoB converted to LDL apoB also could not be calculated from iodine specific activities.

For LDL apoB, the residence time estimated from the model in Fig. 4B using the iodine data was considerably lower than that estimated from the model in Fig. 4A using the leucine data. Hence, transport rates for LDL apoB were estimated to be higher using ^{131}I than those using leucine. For subject 1, direct input of LDL apoB appeared to be higher with [^{3}H]leucine while it was lower in subject 3 with this isotope. There was no difference between the two isotopes for direct LDL input in subject 2.

Further model development

To resolve the differences found when the two labels were analyzed independently, all kinetic data were combined for simultaneous analysis. This allowed the explicit testing of various hypotheses that might account for these differences. To resolve the apparent discrepancy in the fraction of VLDL converted to LDL, it was necessary to add direct removal pathways along the delipidation chain as well as have a significant fraction of the slowly catabolized VLDL be converted to LDL. Since leucine predominantly enters the chain as large VLDL (compartment V1), the removal of a fraction at each step leads to a higher fraction of total input directly removed for leucine than for iodine, which enters the VLDL system at all points along the chain.

The pool of slowly catabolized VLDL (compartment Vs) that was necessary to fit the iodine data was found to be larger than what was compatible with the leucine data. To resolve this discrepancy, it was necessary to postulate unequal initial specific activities between the VLDL chain and the slow VLDL compartment. It was also necessary to have some of the slowly catabolized VLDL converted to LDL. This conversion was hypothesized to pass through an intermediate pool of slow IDL, though the kinetics of this pool were poorly defined.

Incorporating these changes resulted in the model shown in **Fig. 5**. In this model, as with that in Fig. 4A, [^{3}H]leucine is introduced into a precursor compartment that is seen by the rest of the system as a forcing function. ApoB may initially appear in any lipoprotein fraction, that entering VLDL doing so through the first compartment of a delipidation chain. VLDL apoB is directly removed from all compartments of the chain. A portion of VLDL from the end of the chain can be transformed into slowly catabolized VLDL (compartment Vs) which is then converted to IDL and LDL or removed directly. VLDL apoB at the end of the chain that is not irreversibly removed is converted to IDL. IDL apoB consists of two pools. As with the model of Fig. 4B, part of the total IDL subsystem appeared to be sequestered and not measured in circulating IDL. LDL apoB exists in intravascular and

TABLE 2. ApoB concentrations and transport rates: independent analysis of either leucine data (^{3}H) or iodine data (^{131}I)

	Subject 1		Subject 2		Subject 3	
	^{3}H	^{131}I	^{3}H	^{131}I	^{3}H	^{131}I
Total apoB transport (mg/h)	162 ± 11	113 ± 15	197 ± 7	131 ± 17	81 ± 2	59 ± 5
VLDL apoB						
Concentration (mg/dl)	90 ± 4		78 ± 10		39 ± 2	
Residence time (d)	0.67 ± 0.02	0.85 ± 0.10	0.49 ± 0.02	0.72 ± 0.05	0.64 ± 0.02	0.99 ± 0.09
FCR (d^{-1})	1.49 ± 0.05	1.18 ± 0.14	2.04 ± 0.07	1.39 ± 0.10	1.56 ± 0.05	1.01 ± 0.11
Transport (mg/kg/d)	52.2 ± 1.9	41.5 ± 4.8	60.7 ± 2.1	41.5 ± 3.7	23.6 ± 0.7	15.2 ± 1.9
Fraction in slow pool		0.21 ± 0.11		0.27 ± 0.09		0.19 ± 0.14
Fraction to IDL	0.24 ± 0.11		0.21 ± 0.04		0.55 ± 0.10	
Fraction to LDL via IDL	0.20 ± 0.07	0.76 ± 0.12	0.21 ± 0.02	0.50 ± 0.11	0.32 ± 0.03	0.66 ± 0.18
Fraction catabolized	0.77 ± 0.11	0.24 ± 0.12	0.79 ± 0.04	0.50 ± 0.11	0.45 ± 0.10	0.34 ± 0.18
IDL apoB						
Concentration (mg/dl)	9.2 ± 2.6		6.3 ± 1.8		6.9 ± 0.8	
Residence time (d)	0.27 ± 0.13	0.38 ± 0.09	0.19 ± 0.03	0.35 ± 0.03	0.19 ± 0.03	0.52 ± 0.10
FCR (d^{-1})	3.70 ± 1.40	2.63 ± 0.59	5.26 ± 0.83	2.85 ± 0.26	5.26 ± 0.78	1.92 ± 0.38
Transport (mg/kg/d)	13.5 ± 5.8	9.6 ± 2.2	14.1 ± 2.2	7.6 ± 0.7	14.2 ± 2.1	5.2 ± 1.0
Fraction from VLDL	0.91 ± 0.04		0.91 ± 0.02		0.91 ± 0.01	
Fraction direct input	0.09 ± 0.04		0.09 ± 0.02		0.09 ± 0.01	
Fraction to LDL	0.86 ± 0.40		1.00 ± 0.02		0.59 ± 0.01	
LDL apoB						
Concentration (mg/dl)	131 ± 8		92 ± 4		95 ± 3	
Residence time (d)	2.85 ± 0.31	1.62 ± 0.25	2.24 ± 0.13	1.70 ± 0.35	3.64 ± 0.14	2.70 ± 0.63
FCR (d^{-1})	0.35 ± 0.04	0.62 ± 0.10	0.45 ± 0.03	0.59 ± 0.12	0.27 ± 0.01	0.37 ± 0.09
Transport (mg/kg/d)	17.9 ± 1.9	31.4 ± 4.9	14.5 ± 0.9	20.8 ± 4.4	9.5 ± 0.4	13.7 ± 3.7
Fraction from VLDL via IDL	0.59 ± 0.19	0.99 ± 0.14	0.89 ± 0.03	1.00	0.81 ± 0.04	0.74 ± 0.08
Fraction from direct IDL input	0.06 ± 0.03		0.09 ± 0.02		0.08 ± 0.01	
Fraction direct input	0.35 ± 0.19	0.01 ± 0.14	0.03 ± 0.03	0.00	0.12 ± 0.04	0.26 ± 0.08

ApoB concentrations are means and standard errors of three determinations for subject 1, five determinations for subject 2, and five determinations for subject 3. All other errors are estimate standard deviations as calculated by SAAM (16).

extravascular pools (compartments L and Lx, respectively).

The data for each subject were analyzed using the model shown in Fig. 5. These results are presented in **Table 3, Table 4,** and **Table 5**. Transport rates of VLDL apoB were similar to those obtained with the tritium data alone. The fraction of VLDL apoB transport converted to LDL was less than that obtained from the tritium data alone but more than that estimated from the iodine data. The fraction of VLDL in the slowly catabolized pool was less than that estimated with the iodine data alone. Transport rates for LDL apoB were similar to those obtained with the iodine kinetics alone.

DISCUSSION

The purpose of this study was to compare endogenous and exogenous labeling for the study of apoB kinetics in VLDL, IDL, and LDL. Both labeling techniques have their theoretical advantages and disadvantages. If the two approaches had given identical results for the three lipoprotein fractions, then ease of methodology would be the deciding factor in selecting a technique to use. In fact, the two gave somewhat different results, and this finding requires consideration of the relative merits of each. In the discussion to follow, the two approaches will be compared for each of the lipoprotein fractions, and an attempt will be made to integrate the data to obtain a more complete picture of the metabolism of apoB.

VLDL apoB kinetics

Two major differences were noted for kinetics of VLDL apoB between the two isotopic approaches. First, residence times for VLDL apoB estimated with [^{3}H]leucine labeling were less than with iodination. Second, the fraction of VLDL apoB converted to LDL was greater for ^{131}I. Both of these differences may be related to common factors that may be biological, methodological, or both.

One of the major concerns with exogenous labeling is that isolation and labeling procedures may alter the structure and composition of the native lipoprotein. If so, labeled tracer may not accurately follow the tracee. For example, ultracentrifugation may cause the loss of soluble apolipoproteins, thus depleting labeled VLDL of apolipoprotein E (apoE) and altering its kinetics by reducing its ability to bind to cell-surface receptors. An abnormally large fraction of labeled VLDL may then be converted to LDL. Further, the possibility cannot be excluded that a subfraction of VLDL that is converted to LDL may be ra-

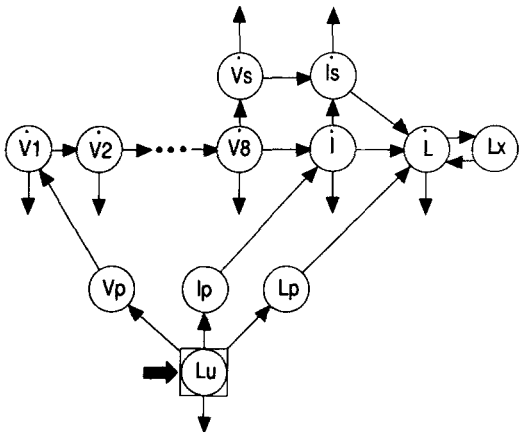


Fig. 5. Compartmental model used in simultaneous analysis of all tracer kinetic data. Large arrow into Lu represents the sole source of unlabeled apoB. Other arrows indicate transfer of unlabeled apoB and apoB tracers between compartments. The compartments are interpreted as follows: Lu, plasma leucine; Vp, precursor pool for apoB production into VLDL; Ip, precursor pool for apoB production into IDL; Lp, precursor pool for apoB production into LDL; V1, V2, ..., V8, VLDL delipidation chain; Vs, slowly catabolized VLDL; I, rapidly catabolized IDL; Is, IDL pool by which slow VLDL (Vs) is converted to LDL; L, plasma LDL; Lx, extravascular LDL exchange pool. Measured VLDL apoB radioactivity is the sum of activities in compartments V1 to V8 and Vs. Measured IDL apoB radioactivity is the sum of the activity in compartments Is and a fraction of the radioactivity in compartment I. The remainder of the activity in compartment I is considered to be a "sequestered" IDL pool that is not measured when sampling plasma IDL. Measured LDL apoB radioactivity is the activity in compartment L. The box around compartment Lu indicates a forcing function used to drive the cellular uptake of radiolabeled plasma leucine. This forcing function was set equal to the observed plasma leucine specific activity curve. Compartments marked with stars are sites of entry of ^{131}I into the system. Small amounts of ^{131}I were observed in the IDL and LDL apoB pools at t = 0.

dioiodinated with greater efficiency than other VLDL subfractions. For either example, direct removal of ^{131}I-labeled VLDL apoB would be lower than that of endogenously labeled VLDL apoB. Thus, exogenous labeling could produce artifacts in the tracer that would lead to a misrepresentation of the true kinetics.

Since radioiodination theoretically labels apoB in proportion to its steady-state mass in each compartment, whereas endogenous labeling essentially traces input pathways, exogenous labeling will predominantly be in particles that have accumulated near the end of the delipidation chain, because the major mass of VLDL apoB resides in smaller VLDL particles (19). Remnant-like particles may be unusually prone to conversion to LDL, compared to larger VLDL, many of which are cleared directly from the circulation (20). Over-representation of smaller VLDL particles in labeling might be partially overcome by endogenous labeling.

With the model used for iodine data (Fig. 4B), all VLDL apoB was assumed to leave the circulation from the end of the delipidation chain, whether via direct removal or conversion to IDL. One of the features of this model is that it allows the number of subcompartments in the chain to vary to give the best fit to the data. However, data from tritiated leucine suggested that VLDL apoB left the delipidation chain after passing through fewer compartments than required to fit the iodine data. This difference may have been due in part to the the fact that the leucine label predominantly enters the delipidation chain, whereas iodinated VLDL consists of particles from all compartments of the delipidation chain.

The over-representation of small VLDL by labeling with ^{131}I undoubtedly includes slowly catabolized VLDL (compartment Vs). According to the ^{131}I-labeled apoB data, this fraction comprised about 20% of total VLDL apoB. This percentage in our hypertriglyceridemic subjects was higher than reported previously for normotriglyceridemic subjects (11). Slowly catabolized VLDL cannot be readily discerned after endogenous labeling because the tail of the VLDL tritium activity curve is derived in part from slow components of leucine incorporation. This slow incorporation is due to recycling of labeled leucine through protein pools of diverse residence times. Nonetheless, an exponential component of the same rate as the tail of the VLDL ^{131}I-labeled apoB activity curve could be discerned in the VLDL ^{3}H activity curves of two of the subjects. This suggests that the tail of the ^{131}I curve reflects a metabolically significant component of VLDL and is not merely an artifact of iodination. It is possible that apoB in slowly catabolized VLDL was initially labeled with a greater specific activity than apoB in the VLDL chain. The much greater conversion of VLDL apoB to LDL apoB with ^{131}I compared with that from endogenous labeling is consistent with this concept. This could have been due to greater exposure of apoB on the surface of smaller VLDL particles than on larger particles, either naturally or artificially after ultracentrifugation.

IDL apoB kinetics

One limitation of ^{131}I labeling was that we could not accurately estimate the fraction of VLDL converted to IDL from ^{131}I data alone. This was due to an apparent overestimation of the IDL ^{131}I-labeled apoB specific activity and a consequent overestimation of the absolute amount of radioactive iodine in IDL when specific activity was multiplied by a reliable IDL apoB pool size. Consequently, with iodine data alone, we could not accurately estimate amounts of VLDL actually converted to IDL or amounts of IDL converted to LDL. For the independent analysis of the iodine data, we therefore assumed that all IDL apoB was derived from VLDL. In our previous study (18), the data suggested that all of the mass of VLDL apoB converted to LDL apoB did not pass through the circulating IDL apoB pool. We thus postulated the existence of a pool of IDL apoB that was not isolated in the density 1.006–1.019 g/ml fraction. Such a pool could be se-

TABLE 3. ApoB concentrations and transport rates: simultaneous analysis of all leucine and iodine data

	Subject 1	Subject 2	Subject 3
Total apoB transport (mg/h)	155 ± 11	194 ± 9	93 ± 5
VLDL apoB			
Concentration (mg/dl)	90	78	39
Residence time (d)	0.76 ± 0.07	0.51 ± 0.03	0.58 ± 0.04
FCR (d^{-1})	1.32 ± 0.12	1.95 ± 0.09	1.72 ± 0.12
Transport (mg/kg/d)	46.3 ± 4.4	58.5 ± 2.8	26.2 ± 1.8
Fraction in slow pool	0.21 ± 0.01	0.17 ± 0.01	0.27 ± 0.01
Fraction to IDL	0.66 ± 0.10	0.60 ± 0.08	0.41 ± 0.08
Fraction to LDL via IDL	0.44 ± 0.04	0.27 ± 0.02	0.21 ± 0.01
Fraction catabolized	0.34 ± 0.10	0.40 ± 0.08	0.59 ± 0.08
IDL apoB			
Concentration (mg/dl)	21.0 ± 0.9	23.8 ± 0.6	12.8 ± 0.2
Residence time (d)	0.21 ± 0.02	0.25 ± 0.02	0.40 ± 0.05
FCR (d^{-1})	4.7 ± 0.4	4.0 ± 0.2	2.5 ± 0.3
Transport (mg/kg/d)	38.4 ± 3.7	36.9 ± 2.6	12.4 ± 1.5
Fraction in slow pool	0.07 ± 0.02	0.22 ± 0.09	0.69 ± 0.02
Fraction from VLDL	0.79 ± 0.05	0.94 ± 0.02	0.88 ± 0.01
Fraction directly synthesized	0.21 ± 0.05	0.06 ± 0.02	0.12 ± 0.01
Fraction to LDL	0.66 ± 0.06	0.45 ± 0.04	0.52 ± 0.08
LDL apoB			
Concentration (mg/dl)	139.0 ± 2.0	82.2 ± 1.4	97.5 ± 2.6
Residence time (d)	1.9 ± 0.05	1.8 ± 0.1	4.5 ± 0.2
FCR (d^{-1})	0.52 ± 0.01	0.55 ± 0.03	0.22 ± 0.01
Transport (mg/kg/d)	28.2 ± 0.9	17.5 ± 0.9	8.5 ± 0.4
Fraction from VLDL via IDL	0.73 ± 0.03	0.90 ± 0.02	0.66 ± 0.02
Fraction from direct IDL input	0.17 ± 0.05	0.05 ± 0.02	0.10 ± 0.01
Fraction directly synthesized	0.10 ± 0.05	0.05 ± 0.01	0.24 ± 0.03

For each subject, VLDL apoB concentration was set to its observed value and a production rate into compartment Lu was calculated (Fig. 5). Observed apoB concentrations for IDL and LDL were included in the modeling as weighted data. All errors are estimate standard deviations as calculated by SAAM (16).

TABLE 4. Kinetic parameters (h^{-1}): simultaneous analysis of both leucine and iodine data

	Subject 1	Subject 2	Subject 3
L(Vp,Lu)	0.026 ± 0.020	0.044 ± 0.002	0.015 ± 0.010
L(Ip,Lu)	0.0045 ± 0.0014	0.0016 ± 0.0008	0.0009 ± 0.0001
L(Lp,Lu)	0.0016 ± 0.0009	0.0007 ± 0.0001	0.0011 ± 0.0002
L(0,Lu)	9.97 ± 0.02	9.95 ± 0.01	9.98 ± 0.01
L(V1,Vp)	0.23 ± 0.03	0.61 ± 0.07	0.67 ± 0.07
L(I,Ip)	0.055 ± 0.018	0.003 ± 0.005	0.41 ± 0.05
L(L,Lp)	0.053 ± 0.029	0.065 ± 0.106	0.12 ± 0.02
L(0,V1)	0.024 ± 0.009	0.040 ± 0.007	0.058 ± 0.009
L(V2,V1)	0.38 ± 0.01	0.51 ± 0.01	0.42 ± 0.01
L(Vs,V8)	0.073 ± 0.012	0.050 ± 0.005	0.11 ± 0.02
L(0,Vs)	0	0	0
L(Is,Vs)	0.027 ± 0.001	0.024 ± 0.016	0.024 ± 0.008
L(0,I)	0.072 ± 0.018	0.098 ± 0.009	0.089 ± 0.035
L(Is,I)	0	0.026 ± 0.015	0.041 ± 0.009
L(L,I)	0.11 ± 0.01	0.074 ± 0.003	0.14 ± 0.01
L(0,Is)	0	0.074 ± 0.105	0.032 ± 0.010
L(L,Is)	0.34 ± 0.07	0.080 ± 0.021	0.014 ± 0.003
L(0,L)	0.022 ± 0.001	0.023 ± 0.001	0.009 ± 0.001
L(Lx,L)	0.0032 ± 0.0010	0.0030 ± 0.0007	0
L(L,Lx)	0.015 ± 0.003	0.012 ± 0.002	

L(I,J) is the fraction of material in compartment J which is transferred to compartment I per hour. Compartment names refer to the model shown in Fig. 5. Fractional rate constants for each compartment in the VLDL chain were assumed to be equal: L(V2,V1) = L(V3,V2) = ... = L(I,V8) and L(0,V1) = L(0,V2) = ... = L(0,V8). All errors are estimate standard deviations as calculated by SAAM (16).

TABLE 5. ApoB transport rates (mg/h): simultaneous analysis of *both leucine and iodine data*

	Subject 1	Subject 2	Subject 3
R(V1,Vp)	125.4 ± 11.9	185.2 ± 8.8	81.8 ± 5.5
R(I,Ip)	21.6 ± 6.8	6.5 ± 3.2	4.7 ± 0.5
R(L,Lp)	7.7 ± 4.2	2.9 ± 0.6	6.4 ± 0.9
R(0,V1)	7.4 ± 3.3	13.4 ± 2.3	9.9 ± 2.0
R(V2,V1)	117.9 ± 8.7	171.8 ± 6.6	71.9 ± 3.6
R(Vs,V8)	13.3 ± 1.3	9.8 ± 0.8	7.3 ± 0.7
R(I,V8)	69.1 ± 5.9	100.4 ± 5.5	26.7 ± 2.8
R(0,V8)	4.4 ± 1.3	7.8 ± 1.3	3.7 ± 0.3
R(0,Vs)	0	0	0
R(Is,Vs)	13.3 ± 1.3	9.8 ± 8.0	7.3 ± 2.5
R(0,I)	35.4 ± 9.3	52.8 ± 7.0	10.2 ± 3.7
R(Is,I)	0	14.9 ± 11.0	4.7 ± 0.7
R(L,I)	55.3 ± 4.1	40.2 ± 6.2	16.5 ± 0.9
R(0,Is)	0	11.4 ± 7.0	8.2 ± 2.9
R(L,Is)	13.3 ± 1.3	12.4 ± 7.5	3.7 ± 0.9
R(0,L)	76.4 ± 2.4	55.5 ± 2.9	26.6 ± 2.3
R(Lx,L)	11.2 ± 3.6	7.2 ± 1.8	0
R(L,Lx)	11.2 ± 3.6	7.2 ± 1.8	0

R(I,J) is the rate of transfer of apoB from compartment J to compartment I in mg/h. Compartment names refer to the model shown in Fig. 5. All errors are estimate standard deviations as calculated by SAAM (16).

questered in the liver in the space of Disse and there be transformed to LDL without appearing in the measured IDL apoB pool (18). Alternatively, IDL apoB might be incompletely isolated for analysis (21) A similar phenomenon was noted in the present study, as reflected by the finding that the observed concentration of IDL apoB was lower than what would be predicted if all VLDL converted to LDL apoB had passed through the circulating IDL. This finding does not rule out the possibility of direct input of IDL apoB nor of direct removal.

An apparent advantage of the endogenous labeling methodology is that it could better define routes of entrance and exit of IDL apoB. Our current data indicated that about 10% of IDL apoB entered the IDL fraction independently of the VLDL delipidation chain. It was also revealed that a portion of IDL was removed directly from the circulation without conversion to LDL. The size of a sequestered IDL pool could not be estimated from the leucine data because of the limitations on the analysis of kinetics of LDL apoB (see below). Still, unless the ^{131}I label in VLDL apoB was grossly maldistributed, it seems unlikely that all VLDL apoB converted to LDL apoB passed through the measurable, circulating pool of IDL. As with ^{131}I, tritium-labeled VLDL apoB that could not be detected in the measured IDL pool in its conversion to LDL nevertheless experienced a delay before its appearance in LDL. The duration of this delay was similar to the residence time of apoB in IDL, suggesting that the process by which sequestered IDL was converted to LDL was similar to that for circulating IDL.

LDL apoB kinetics

Since LDL apoB was not labeled exogenously, it was necessary to model both the input and clearance of label in the LDL subsystem simultaneously. Consequently, removal rates of LDL were somewhat less well defined than if the kinetics of exogenously labeled LDL had been available. In the analysis using ^{131}I alone, it was assumed that slowly catabolized VLDL is not converted to LDL (Fig. 4B). If some of this pool is transformed to LDL, it would raise the tail of the LDL activity curve and the estimated FCR for LDL would be an underestimation of the true value.

Another limitation of the LDL apoB kinetic analysis for the subjects of this study was that they probably had multidisperse LDL, which is characteristic of patients with hypertriglyceridemia (22). Fisher et al. (3), Teng et al. (23), and Vega and Grundy (24) have shown that multidisperse LDL is kinetically heterogenous. In the current study, a single intravascular pool of LDL was used because LDL was neither subfractionated nor exogenously labeled, and a single pool of plasma LDL therefore was adequate to fit the available data. Analysis of LDL kinetics with this two-pool model is undoubtedly an oversimplified approach and may not give an entirely accurate estimate of true LDL apoB transport. Recent studies by Vega and Grundy (24) have shown that transport rates of LDL apoB in patients with multidisperse LDL may be in error by as much as 15% when the two-pool analysis is used. The same limitation exists for analysis of the tritium data. Fisher et al. (3) have demonstrated kinetic heterogeneity of LDL subfractions in patients with multidisperse LDL when apoB metabolism is studied with [^{3}H]leucine as a precursor. Kinetic heterogeneity of LDL likewise has been demonstrated when LDL is radioiodinated and its plasma decay curve has been carefully analyzed simultaneously with the appearance of radioiodine in the urine (25, 26).

Tritiated leucine has two disadvantages as a precursor in the analysis of LDL kinetics. First, it primarily labels VLDL apoB and delays in the conversion of VLDL to LDL complicate the kinetic analysis for LDL, as mentioned previously for iodinated VLDL apoB. Second, there is a slow component in the appearance of ^{3}H in VLDL apoB and this adds further complexity to the LDL activity curve. Both of these factors contribute to the tail of the LDL curve and their individual and summed contributions cannot be differentiated with confidence. On the other hand, the endogenous tracer is valuable for detecting direct input of LDL apoB, which is evident as an appearance of tritium in LDL apoB that is too rapid to be accounted for by conversion from VLDL.

Integrated analysis and further model development

One approach to the study of apoB kinetics is to combine endogenous and exogenous labeling. The data from

both isotopes can then be combined for further analysis and hypothesis testing. Such was carried out in the current study. For example, to account for the discrepancies in the fraction of VLDL apoB converted to LDL, direct removal pathways were introduced along the delipidated chain. Since the leucine-labeled VLDL is assumed to enter the delipidated chain primarily at the first VLDL compartment, removal of a fraction from several pools along the chain leads to a higher percentage of label estimated to be removed directly than for ^{131}I, which is introduced into all pools in the chain. Although some apoB likely enters each compartment in the VLDL chain, the distribution of this input could not be determined from the measurement of whole VLDL specific activities, and it was assumed that all VLDL input was into the first chain compartment. Theoretical calculations (W. F. Beltz, unpublished observations) indicate that estimates of VLDL apoB transport rates are not sensitive to this assumption and that the estimated chain length is an average of the chain lengths appropriate for the various inputs.

Previously, Beltz et al. (18) proposed that newly secreted VLDL enters into a compartment prior to the first compartment of the VLDL delipidation chain. This compartment could not be identified with iodine labeling because of its small pool size and rapid turnover rate. Furthermore, it was postulated that a significant fraction of apoB was removed directly from the circulation before entering identifiable plasma compartments. Evidence for the existence of such a compartment has been obtained from studies in both humans (19, 27) and laboratory animals (20), and the hypothesis has been used in subsequent interpretation of kinetic data (28, 29). The current data with endogenous labeling provide additional support for the concept that greater quantities of apoB enter and exit initial pools of the delipidation chain than can be identified with iodine labeling. Thus, exogenous labeling underestimates the true production rate of apoB-containing lipoproteins to a greater extent than does endogenous labeling.

To determine possible sources for the slowly catabolized VLDL compartment, various hypotheses were tested to see whether any could reconcile the differences obtained for the two labels. These included direct production of slowly catabolized VLDL and inputs from various combinations of chain compartments. The largest discrepancy with the observed data was found with slowly catabolized VLDL being synthesized directly and the smallest with slow VLDL arising from the last compartment of the chain. In the latter case, the slowly catabolized pool of VLDL apoB estimated from ^{131}I was still larger than that consistent with the leucine data. This discrepancy could only be resolved by postulating that the initial specific activities in the chain pools and in the slowly catabolized pool were different, implying that apoB in the latter pool was labeled more efficiently than apoB in the delipidation chain. An alternate possibility is that during isolation and iodination, some VLDL was artificially converted to slowly catabolized material. This might occur if apoE were removed from a portion of VLDL particles during ultracentrifugation. A third possibility must be considered in view of the fact that the method by which VLDL was initially obtained for iodination was not identical to that by which it was isolated for specific activity determination. If these two procedures yielded different populations of lipoproteins particles, some of the VLDL would appear to be preferentially labeled. All of these possibilities illustrate ways in which iodination of isolated VLDL could introduce artifacts in the study of VLDL apoB kinetics.

Previous isotope-kinetic studies have suggested that LDL apoB is secreted directly into plasma (30–32). This observation has suggested to some workers that the liver synthesizes LDL or lipoproteins that closely resemble LDL. Studies in cholesterol-fed animals (33–37), but not in normal animals (38–41), are consistent with direct LDL synthesis. To avoid the necessity for implicating direct LDL synthesis, Beltz et al. (18) proposed that newly secreted VLDL can be transformed rapidly into LDL without passing through the VLDL delipidation chain pathway. In the current study, endogenous labeling likewise revealed some "direct" input of LDL apoB. This input once more bypassed the VLDL chain even though endogenous labeling identified apoB much earlier in the VLDL chain than exogenous labeling. Whether the precursor pools for direct LDL input reside exclusively in intracellular synthesis pools or partly within newly secreted lipoproteins could not be determined.

By combining data for the two isotopes, it was possible to use the model shown in Fig. 5, which not only combined features of the two models in Fig. 4, but contained features that could not be resolved using either isotope alone. It should be noted that this model structure could only be determined using the combination of endogenous and exogenous labeling.

In future studies it will be valuable to compare endogenous labeling of LDL using [^{3}H]leucine and exogenously labeled LDL. If only one iodine isotope is to be used, this technique may give a better overall picture of apoB metabolism than the present one because it would better define LDL kinetics and the conversion of VLDL apoB into kinetically distinct LDL pools such as those suggested by Goebel, Garnick, and Berman (25) and Foster et al. (26). VLDL kinetics would still be estimable from the leucine data. Even more information could be obtained using simultaneous injections of tritiated leucine, radioiodinated VLDL, and radioiodinated LDL.

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REFERENCES

1. Kesäniemi, Y. A., G. L. Vega, and S. M. Grundy. 1982. Kinetics of apolipoprotein B in normal and hyperlipidemic man: review of current data. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 181–205.
2. Phair, R. D., M. G. Hammond, J. A. Bowden, M. Fried, W. R. Fisher, and M. Berman. 1975. A preliminary model for human lipoprotein metabolism in hyperlipoproteinemia. *Fed. Proc.* **34:** 2263–2270.
3. Fisher, W. R., L. A. Zech, P. Bardalaye, G. Warmke, and M. Berman. 1980. The metabolism of apolipoprotein B in subjects with hypertriglyceridemia and polydisperse LDL. *J. Lipid Res.* **21:** 760–774.
4. Eaton, R. P., R. C. Allen, and D. S. Schade. 1983. Overproduction of a kinetic subclass of VLDL-apoB, and direct catabolism of VLDL-apoB in human endogenous hypertriglyceridemia: an analytical model solution of tracer data. *J. Lipid Res.* **24:** 1291–1303.
5. Le, N-A., I. J. Goldberg, H. N. Ginsberg, and W. V. Brown. 1983. Direct production of low density lipoprotein in the monkey: comparison of endogenous versus exogenous tracers. *Circulation, Suppl. III.* **68:** 119.
6. Grundy, S. M. 1975. Effects of polyunsaturated fats on lipid metabolism in patients with hypertriglyceridemia. *J. Clin. Invest.* **55:** 269–282.
7. Lindgren, F. T., L. C. Jensen, R. D. Wills, and N. K. Freeman. 1969. Flotation rates, molecular weights and hydrated densities of low density lipoproteins. *Lipids.* **4:** 337–344.
8. McFarlane, A. S. 1958. Efficient trace-labelling of proteins with iodine. *Nature (London).* **182:** 53–57.
9. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoproteins proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
10. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
11. Kesäniemi, Y. A., W. F. Beltz, and S. M. Grundy. 1985. Comparisons of metabolism of apolipoprotein B in normal subjects, obese patients, and patients with coronary heart disease. *J. Clin. Invest.* **76:** 586–595.
12. Manual of Laboratory Operations, Lipid Research Clinics Program. Lipid and Lipoprotein Analysis. 1974. DHEW Publication No. (NIH) 75-628. U. S. Government Printing Office, Washington, DC.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
14. Le, N-A., J. S. Melish, B. C. Roach, H. Ginsberg, and W. V. Brown. 1978. Direct measurement of apoprotein B specific activity in ^{125}I-labeled lipoproteins. *J. Lipid Res.* **19:** 578–584.
15. Kilgore, L. L., J. L. Rogers, B. W. Patterson, N. H. Miller, and W. R. Fisher. 1985. The determination of specific radioactivity of proteins eluted intact from polyacrylamide gels, utilizing a fluorescamine assay. *Anal. Biochem.* **145:** 113–119.
16. Berman, M., and M. F. Weiss. 1978. SAAM Manual. DHEW Publ. No. (NIH) 78-180. U. S. Govt. Printing Office, Washington, DC.
17. Berman, M., W. F. Beltz, P. C. Greif, R. Chabay, and R. C. Boston. 1983. CONSAM User's Guide. PHS Publ. No. 1983-421-132:3279. U. S. Govt. Printing Office, Washinton, DC.
18. Beltz, W. F., Y. A. Kesäneimi, B. V. Howard, and S. M. Grundy. 1985. Development of an integrated model for analysis of the kinetics of apolipoprotein B in plasma lipoproteins VLDL, IDL and LDL. *J. Clin. Invest.* **76:** 575–585.
19. Packard, C. J., A. Munro, A. R. Lorimer, A. M. Gotto, and J. Shepherd. 1984. Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2178–2192.
20. Yamada, N., D. M. Shames, K. Takahashi, and R. J. Havel. 1988. Metabolism of apolipoprotein B-100 in large very low density lipoproteins in blood plasma. Kinetics studies in normal and Watanabe heritable hyperlipidemic rabbits. *J. Clin. Invest.* **82:** 2106–2113.
21. Fisher, W. R. 1970. The characterization and occurrence of an S_f 20 serum lipoprotein. *J. Biol. Chem.* **245:** 877–884.
22. Hammond, M. G., M. C. Mengel, G. L. Warmke, and W. R. Fisher. 1977. Macromolecular dispersion of human plasma low-density lipoproteins in hypertriglyceridemia. *Metabolism.* **26:** 1231–1242.
23. Teng, B., A. D. Sniderman, A. K. Soutar, and G. R. Thompson. 1986. Metabolic basis of hyperbetalipoproteinemia. Turnover of apolipoprotein B in low density lipoprotein and its precursors and subfractions compared with normal and familial hypercholesterolemia. *J. Clin. Invest.* **77:** 663–672.
24. Vega, G. L., and S. M. Grundy. 1986. Kinetic heterogeneity of low density lipoproteins in primary hypertriglyceridemia. *Arteriosclerosis.* **6:** 395–406.
25. Goebel, R., M. Garnick, and M. Berman. 1976. A new model for low density apoprotein kinetics: evidence for two labeled moieties. *Circulation.* **54:** II–4.
26. Foster, D. M., A. Chait, J. J. Albers, R. A. Failor, C. Harris, and J. D. Brunzell. 1986. Evidence for kinetic heterogeneity among human low density lipoproteins. *Metabolism.* **35:** 685–696.
27. Stalenhoef, A. F. H., M. J. Malloy, J. P. Kane, and R. J. Havel. 1984. Metabolism of apolipoprotein B-48 and B-100 of triglyceride-rich lipoproteins in normal and lipoprotein lipase-deficient humans. *Proc. Natl. Acad. Sci. USA.* **81:** 1839–1843.
28. Vega, G. L., and S. M. Grundy. 1988. Lovastatin therapy in nephrotic hyperlipidemia: effects on lipoprotein metabolism. *Kidney Int.* **33:** 1160–1168.
29. Vega, G. L., C. East, and S. M. Grundy. 1988. Lovastatin therapy in familial dysbetalipoproteinemia: effects on kinetics of apolipoprotein B. *Atherosclerosis.* **70:** 131–143.
30. Janus, E. D., A. Nicoll, R. Wootton, P. R. Turner, P. J. Magill, and B. Lewis. 1980. Quantitative studies of very low density lipoprotein: conversion to low density lipoprotein in normal controls and primary hyperlipidemic states and the role of direct secretion of low density lipoprotein in heterozygous familial hypercholesterolemia. *Eur. J. Clin. Invest.* **10:** 149–159.
31. Janus, E. D., A. M. Nicoll, P. R. Turner, P. Magill, and B. Lewis. 1980. Kinetic bases of the primary hyperlipoproteinemias: studies of apolipoprotein B turnover in genetically defined subjects. *Eur. J. Clin. Invest.* **10:** 161–172.
32. Bilheimer, D. W., Y. Watanabe, and T. Kita. 1982. Imparied receptor-mediated catabolism of low density lipoprotein in the WHHL rabbit, an animal model of familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **79:** 3305–3309.
33. Illingworth, D. R. 1975. Metabolism of lipoproteins in nonhuman primates. Studies on the origin of low density lipoprotein apoprotein in the plasma of the squirrel monkey. *Biochim. Biophys. Acta.* **388:** 38–51.

34. Nakaya, N., B. H. Chung, and O. D. Taunton. 1977. Synthesis of plasma lipoproteins by the isolated perfused liver from the fasted and fed pig. *J. Biol. Chem.* **252:** 5258–5261.
35. Nakaya, N., B. H. Chung, J. R. Patsch, and O. D. Taunton. 1977. Synthesis and release of low density lipoproteins by the isolated perfused pig liver. *J. Biol. Chem.* **252:** 7530–7533.
36. Noel, S. P., L. Wong, P. J. Dolphin, L. Dory, and D. Rubenstein. 1979. Secretion of cholesterol-rich lipoproteins by perfused livers of hypercholesterolemic rats. *J. Clin. Invest.* **64:** 674–683.
37. Johnson, F. L., R. W. St. Clair, and L. L. Rudel. 1983. Studies on the production of low density lipoproteins by perfused livers from nonhuman primates. Effect of dietary cholesterol. *J. Clin. Invest.* **72:** 221–236.
38. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perused rat liver. *J. Clin. Invest.* **58:** 667–680.
39. Guo, L. S. S., R. L. Hamilton, R. Ostwald, and R. J. Havel. 1982. Secretion of nascent lipoproteins and apolipoproteins by perfused livers of normal and cholesterol-fed guinea pigs. *J. Lipid Res.* **23:** 543–555.
40. Hornick, C. A., T. Kita, R. L. Hamilton, J. P. Kane, and R. J. Havel. 1983. Secretion of lipoproteins from the liver of normal and Watanabe heritable hyperlipidemic rabbits. *Proc. Natl. Acad. Sci. USA.* **80:** 6096–6100.
41. Marsh, J. B. 1976. Apoproteins of the lipoproteins in a nonrecirculating perfusate of rat liver. *J. Lipid Res.* **17:** 85–90.