Endogenously labeled low density lipoprotein triglyceride and apoprotein B kinetics

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Abstract The kinetics of endogenously labeled low density lipoprotein (LDL) triglycerides (TG) and apoprotein B (apoB) have been studied in four normal and in four hyperlipemic subjects using double tracers. Analysis of the data suggests that most LDL triglycerides turn over about 10 times faster than apoB (0.003/min vs. 0.0003/min) and that about 10% of the LDL particles contain most of the TG found with LDL. It is not possible to determine from the analysis whether each new LDL particle arrives with the excess TG or whether only a subpopulation of particles contains most of the TG. The kinetic analysis further suggests that triglyceride-rich LDL particles do not exchange with an extraplasma compartment as most LDL particles do, and thus, they behave more like very low density lipoprotein particles. A compartmental model accounting for both the LDL-TG and LDL-apoB kinetics is proposed.

Supplementary key words fractional turnover rate · multicompartmental analysis · intermediate density lipoprotein · very low density lipoprotein

In previous work, the metabolism of low density lipoproteins (LDL) in human subjects has been studied mostly by injecting exogenously ^{131}I- or ^{125}I-labeled autologous lipoproteins (1–5). Kinetics of endogenously labeled LDL have also been studied by the incorporation of amino acids, such as leucine (6) and selenomethionine (7), into the apoproteins. Little has been done on LDL triglyceride kinetics (LDL-TG). Quarfordt et al. (8) observed a precursor–product relationship between very low density lipoprotein (VLDL) and LDL triglycerides but did not analyze the kinetics.

The present study involves the injection of endogenously labeled VLDL-, IDL-, and LDL-TG into a number of normal and hyperlipemic subjects. The labeled material was obtained from donors to whom ^{14}C- or ^{3}H-labeled palmitate was administered. In some subjects endogenously labeled apoB derived from ^{14}C-labeled leucine given to the same donors was also followed simultaneously with the triglyceride label.

MATERIAL AND METHODS

Material

L-[1-^{14}C]Leucine (56–60 mCi/mmol), [1-^{14}C]palmitic acid (57.9–59.0 mCi/mmol), and [9,10(n)-^{3}H]palmitic acid (500 mCi/mmol) were purchased from the Radiochemical Center, Amersham, England.

Only decerebrated patients were used as donors, and they received large doses of ^{14}C- and ^{3}H-labeled leucine and/or palmitic acid. The donors were carefully selected among young, well-nourished subjects with accidental cerebral damage (EEG altered) but free of infection, shock, or any metabolic alterations. In particular, their respiratory, cardiovascular, and renal functions were followed and considered normal. All blood and urine checkup examinations gave values within normal limits.

Preparation of biosynthetically doubly labeled lipoproteins from a donor

A single dose of 5 mCi of [1-^{14}C]palmitate or [9,10-^{3}H]palmitate, complexed to albumin, and 5 mCi of L-[1-^{14}C]leucine were injected intravenously into a human donor who had been fasting for 12 hr. From 90 to 390 min later, 500–600 ml of blood was collected aseptically from the brachial artery of the opposite arm into 600-ml sterile plastic bags containing EDTA solutions (0.1% final concentration). The bags were centrifuged at 5,000 rpm for 20 min at 20°C in a Sorvall type RC-3 automatic refrigerated centrifuge. The plasma was separated carefully and the red blood cells in saline were reinfused into the donor within 45 min of their withdrawal. Plasma was centrifuged in 38.5-ml polyallomer tubes using the Beckman model L2-65B preparative ultracentrifuge (rotor 60 Ti). The

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; apoB, apoprotein B; TLC, thin-layer chromatography.

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first run was done at a density adjusted to 1.006 with 0.9% NaCl solution at 42,000 rpm for 16 hr at 10°C in order to separate VLDL from the other lipoproteins. Second, third, and fourth runs were done with densities adjusted to d 1.019 g/ml and 1.063 g/ml with NaCl–KBr solutions of known densities or to d 1.21 g/ml with solid KBr, at 42,000 rpm and 10°C for 21 hr to separate successively IDL, LDL, and HDL. The lower phase of the final centrifugation was considered free of major lipoproteins. Each lipoprotein fraction initially isolated was recentrifuged in 13-ml polyallomer tubes (rotor 65 or 50 Ti) in solutions of precisely regulated densities: VLDL, $d < 1.006$ g/ml; IDL, $1.006 < d < 1.019$ g/ml; and LDL, $1.019 < d < 1.063$ g/ml. After ultracentrifugation, the tubes were sliced at 1.2 cm from the top, verifying the location of each fraction at the meniscus of the tube and of the clear zone underneath.

An aliquot (0.1 ml) of each labeled lipoprotein preparation was mixed with 3 ml of the recipient's unlabeled serum, obtained at time 0 before injection of the tracer, and ultracentrifuged as described above. This procedure gave the rough percentage of contamination of the fraction by other lipoproteins.

Each purified, concentrated lipoprotein solution was sterilized by Millipore filtration (pore size 0.45 μm). Merthiolate was added in the solution to a final 1/10,000 concentration. The solution kept at 4°C was shown to be free of bacteriological contamination. The maximal time of storage before use was 10 days.

Experimental procedures in recipient subjects

All recipient subjects were maintained for 2 weeks prior to the tests on a standard diet containing 45% of the total calories as carbohydrates, 40% as fat, and 15% as protein. The subjects fasted overnight (14 hr) prior to injection of the tracer. A Cournand needle was placed in the brachial artery of one arm. Subjects received 2–12 μCi of L[1-^{14}C]leucine and/or [9,10-^{3}H]palmitate-labeled lipoprotein. The dose in a volume of approximately 10 ml was injected within 20 sec into one of the antecubital veins of the opposite arm. Blood was collected in tubes containing EDTA solution (0.1% final concentration) as anticoagulant and was placed immediately in the refrigerator until analytical procedures could be initiated. Sampling was done before the injection and at regular time intervals up to 540 min. A normal meal was given at this time and duplicate final samples were taken at 1440 min after a repeated overnight fast.

Ultracentrifugations and lipid separations

Plasma was separated from cells at 5°C in a refrigerated International PR-2 centrifuge at 2,500 rpm for 20 min. Four lipoprotein subfractions were isolated by differential ultracentrifugation at 10°C in 13-ml polyallomer tubes as described above, but without recentrifugation. Each subfraction was extracted with Dole's solution (9) in the following proportions: lipoprotein–Dole's solution–isooctane–water 2:10:6:4 (by vol.) or with chloroform–methanol 2:1. The isooctane or chloroform phase was recovered and dried under nitrogen at 40°C. The residue dissolved in chloroform was applied to a silicic acid column (Mallinckrodt 100 mesh) in order to separate neutral lipids from phospholipids (10). Neutral lipids were eluted with 30 ml of chloroform and phospholipids with 30 ml of methanol. The methanol phospholipid fraction was submitted again to silicic acid chromatography. The chloroform phase was evaporated to dryness and the residue was dissolved in 10 ml of isooctane. The separation procedure of Borgstrom (10) was applied to this phase. FFA and triglycerides were evaporated and separately counted after addition of 15 ml of PPO liquid scintillation solution. Aliquots of neutral lipids were subjected to analytical TLC at regular intervals in order to determine the relative amount of radioactivity incorporated in cholesteryl esters compared to that of triglycerides and diglycerides. The recovery of radioactivity after TLC was found to be over 90%.

Delipidation of lipoproteins

Determination of radioactivity. One or two ml of each lipoprotein with 50–100 μl of plasma (unlabeled lipoprotein load) was delipidated by a chloroform–methanol extraction according to Scanu and Edelstein (11) but at 4°C. The precipitate, after washing with ether, was dried under nitrogen and redissolved in 1 ml of a 5% solution of sodium dodecylsulfate (SDS) in 0.5 N NaOH by overnight incubation at 37°C. All the solution was transferred quantitatively with 11 ml of Instagel (New England Nuclear Corp., Boston, MA) to counting vials containing 100 μl of 6 N HCl.

Gel filtration. Pooled lipoprotein preparations containing 5–10 mg of protein were dialyzed, concentrated, lyophilized, delipidated, and resolubilized as described elsewhere (12).

Gel chromatography of apoproteins

Gel filtration of solubilized apoprotein was carried out using Sephadex G-200 as described elsewhere (12). This chromatography generated five individual 280 nm absorption peaks designated I, II, III, IV, and V in their order of elution from the column. Peaks were pooled, dialyzed for 48 hr against 5 mmol/l NH_4HCO_3, pH 8.0, and concentrated to 5.0 ml by ultrafiltration (UM-2 Diaflo membrane, Amicon UF

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TABLE 1. Clinical data of donors and recipient patients

Donor	Clinical State	Age	Sex	Weight	Chol.	TG	PL	Glucose	Donor	Time of Plasma Collection after Injection
		yr		*kg*		*mg/dl*				*min*
1. V.B.M.		38	F		175	138	172	130		120(PA-^{14}C)[a]
2. M.L.		28	M		250	151	242			90(PA-^{3}H)
3. M.L.		28	M							390(PA-^{14}C)
4. R.H.		44	M		190	177	213			210(PA-^{3}H, LE-^{14}C)
5. J.L.		39	M		210	161	205			115(PA-^{3}H, LE-^{14}C)
6. D.G.		42	M		191	103	250			125(PA-^{3}H, LE-^{14}C)
Patient										**Labeled Material Injected**
1. J.U.	Normal	20	M	85	177	70	204	84	2	VLDL(PA)
2. C.M.	Normal	40	M	65	258	126	280	93	1	LDL(PA)
3. D.D.	Normal	19	M	75	178	81	275	93	1	IDL(PA)
4. J.B.	Normal	25	M	65	159	34	263	97	4	LDL(PA-LE)
5. R.D.	Type IIa[b]	39	M	76	354	85	310	92	5	VLDL(PA-LE)
6. D.V.	Type IIa	33	F	68	400	154	279	86	6	LDL(PA-LE)
7. B.E.	Type IV	36	M	87	185	312	229	84	1	VLDL(PA)
8. R.B.	Type IV	64	M	78	214	360	280	97	5	LDL(PA-LE)

[a] PA, palmitate; LE, leucine.
[b] According to classification of Frederickson et al. (16).

cell, model 52). Aliquots of 0.1–0.5 ml were taken for protein determination (13). To 4 ml were added 2 drops of 1 N HCl and 10 ml of Instagel in counting vials and the radioactivity was measured. The remaining solution was lyophilized, resolubilized in Tris-HCl buffer containing 100 mmol/l SDS, diluted with the same buffer containing 2 mmol/l SDS, and subjected to polyacrylamide gel electrophoresis after addition of half the volume of a 50% sucrose solution. The final protein concentration was about 100 μg/100 μl.

Radioactivity measurements

Each sample was counted for a time sufficient to achieve a standard deviation of less than 2% (unless otherwise indicated). At 1440 min two samples were taken to yield the desired precision. Radioactivity was determined in Packard scintillation vials using a Mark II Nuclear Chicago automated liquid scintillation spectrometer equipped with an automated external standard.

General chemical methods

Protein concentrations of the lipoprotein solutions were determined by a modification (13) of the technique of Lowry et al. (14) using bovine serum albumin standard (Sigma, St. Louis, MO). Lipoprotein electrophoresis, serum cholesterol, free fatty acids, phospholipids, triglycerides, and blood glucose were determined by methods already described (15).

Patients, donors, and studies

The recipient and donor populations and the clinical data related to them are given in **Table 1.** The experimental details related to the time of collection of plasmas from the donors and the labels used are also given in this table. Typical kinetic curves for LDL are shown in Figs. 1, 3, and 4.

Informed written consent was obtained from all recipients of endogenously labeled material. For all donors, approval was obtained from a Board of the Neurosurgery Department and, when available, from the immediate families.

MODEL DEVELOPMENT AND RESULTS

Analysis of the kinetic curves (**Fig. 1**) resulting from simultaneous injections of LDL labeled in the apoB and TG moieties show a more rapid initial disappearance of labeled TG. Since both moieties share a common particle, this means that LDL loses most of its TG independently of the apoB. Some TG must also be lost simultaneously with apoB when the entire particle is degraded.

The LDL model shown in **Fig. 2** is proposed to explain the observed kinetics. Imbedded in this model are the notions that the kinetics of an LDL particle are tightly coupled to its apoB, and that the latter can be represented by a two-compartment model (3, 5). In this model, compartment 1 (*C1*)[3] represents newly

[3] We shall denote compartment 1 in the model as *C1*. A rate constant is given as $L_{i,j}$ and represents the fraction of compartment j transported to compartment i per unit time (minutes). $L_{o,j}$ is the fraction of compartment j lost to the outside per unit time. The superscripts *TG* and *B* on the $L_{i,j}$ identify the respective moieties when they are numerically distinguishable.

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entered LDL particles that are triglyceride-rich. Most of the TG in *C1* is lost by way of $L_{0,1}(L_{0,1}^{TG} \gg L_{2,1}^{TG})$. Once TG passes on to *C2* it follows the kinetics of apoB which is represented by its conventional model (*C2* in exchange with *C3* and a degradation path $L_{0,2}$). *C4* represents IDL as a precursor for LDL.

The model for LDL-TG also applies to the apoB moiety. ApoB stays in the *C1* state until its TG complement is depleted to a level at which it enters *C2* with the remaining TG. Contrary to TG, most of the apoB goes via $L_{2,1}(L_{2,1}^{B} \gg L_{0,1}^{B})$.

The LDL-apoB studies of Langer, Strober, and Levy (3) show biphasic LDL-apoB kinetics: a rapid phase, lasting about 1–2 days, followed by a slow one, lasting 4–6 days. Our LDL kinetics were followed for only one day and therefore reflect only the early LDL decay phase which effectively equals the sum of the rate constants $L_{0,2} + L_{3,2}$. We cannot determine from our studies the individual values of the rate constants $L_{0,2}$, $L_{3,2}$, and $L_{2,3}$. We therefore assigned the literature values to $L_{0,2}$ and $L_{2,3}$ (Langer et al. (3); Sigurdsson, Nicoll, and Lewis (5)) and fitted the data by adjusting $L_{0,2}$, $L_{2,1}$ and $L_{3,2}$. It was further assumed that apoB in *C1* was degraded with equal probability as in *C2* ($L_{0,1}^{B} = L_{0,2}$). An effort to add to *C1* an exchange compartment equivalent to *C3* was inconsistent with the data. This is due to the fact that initially the injected LDL-apoB decayed slowly but, after a time lag comparable to the residence time for its TG, the disappearance rate increased (see Fig. 1). Activities derived from labeled IDL as a precursor were too small to account for the initial slow LDL-apoB decay.

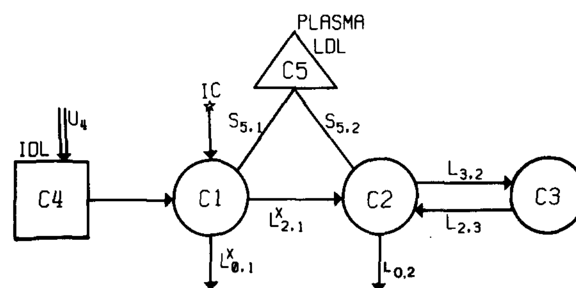

Fig. 2. The 3-compartment LDL model. The circles are the LDL compartments, the square is the IDL precursor, and the triangle represents the observed data. *C5* is a summer that simulates a *measured* quantity representing the sum of *C1* and *C2*, and $S_{5,1}$ and $S_{5,2}$ are the summing coefficients and are equal to unity: $q_5(t) = S_{5,1}q_1(t) + S_{5,2}q_2(t)$. $L_{i,j}$ are rate constants and represent the fraction of compartment *j* transported to compartment *i* per unit time. U_4 is the rate of input of material necessary to satisfy the steady state LDL data. Without superscripts, the rate constants have the same values for TG as for apoB. A superscript indicates a distinction between the two.

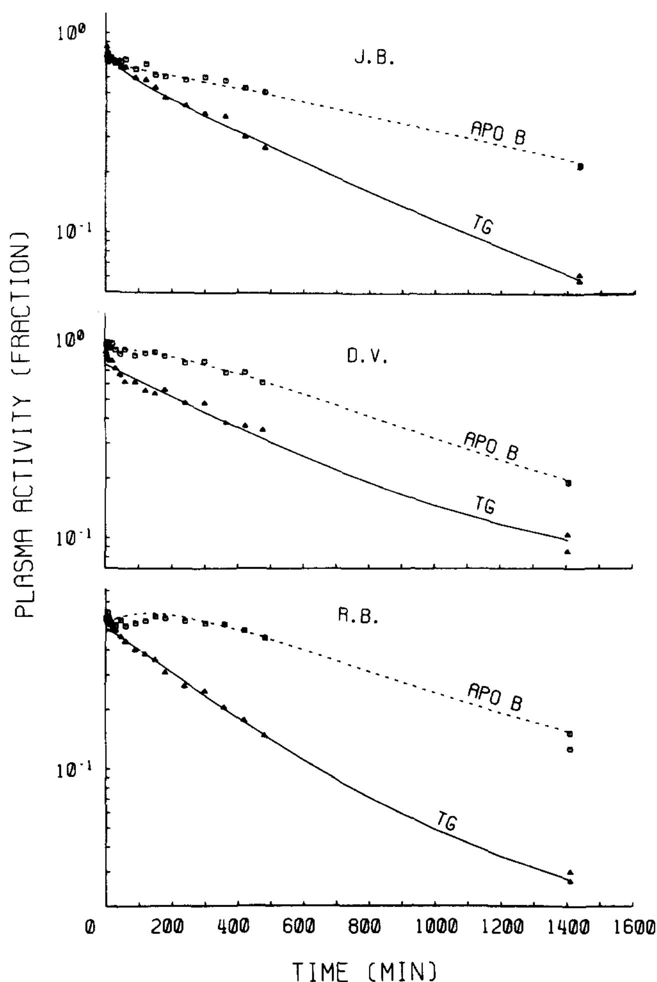

Fig. 1. Plasma LDL disappearance curves in three patients to whom LDL labeled in the apoB and TG moieties was administered simultaneously. The squares and triangles are the data for the apoB and TG moieties, respectively, and the dotted and solid lines are the model predicted curves.

In fitting the data, it was necessary that, initially, the injected label be in compartment *C1*. Efforts to bypass *C1* and put material directly into *C2* were unsuccessful. The most striking argument against this are the LDL-apoB data (Fig. 1) after an injection of labeled LDL-apoB. The initial flatness of curve disappears when the injection is into *C2*.

The above assumptions have negligible effect in estimating the TG fractional catabolic rate, $L_{0,1}^{TG}$.

For the studies where labeled VLDL or IDL were injected (TG and/or apoB moieties), the IDL curve was used as the precursor for LDL. These fits are shown in **Fig. 3.**

It was observed that in most studies there was an initial, rapidly decaying component (10–30 min) in the IDL and LDL curves for both TG and apoB. This is readily seen in Fig. 3. For a number of cases this component does not follow a simple equilibration pattern (see dip in IDL curve of Fig. 3). Since the decay constant of the rapid component was about the same for IDL and LDL (also for VLDL), it was postulated

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that some unknown moiety was responsible for it, and it was neglected in the present analysis. The rapid component in IDL did not serve as a precursor for LDL.

For each individual study the parameters ($L_{i,j}$) of the model were adjusted to fit the data using a least-squares criterion. All modeling and data fitting were done with the SAAM27 digital computer program (17). Typical fits of LDL data using the model are shown in Figs. 1 and 3. The results of the kinetic analyses for the proposed model are given in **Table 2.**

As can be seen, not all parameter values in the model can be determined with high precision. The most reliable values are obtained from the doubly labeled (apoB and TG) experiments (J.B., R.D., D.V., R.B.), especially those in which labeled LDL was administered. The patterns of behavior that are suggested by the model, however, are consistent for all studies.

DISCUSSION

It is evident that LDL-TG disappears from plasma with a rate constant ($L_{0,1}{}^{TG}$) about 5–10 times faster than LDL-apoB ($L_{0,1}{}^{B}$) and probably represents the final TG delipidation stages of the particle.

The extent of LDL-TG loss before the apoB is degraded is also surprising. The calculated TG masses $M_1{}^{TG}$ and $M_5{}^{TG}$ (Table 2) indicate that most of the LDL triglycerides are on the relatively new LDL particles (*C1*), which constitute only about 10% of all particles. Such a distribution of TG on LDL should generate a bimodal LDL density spectrum. This, however, has not been observed[4]. Several explanations may be given for this. For example, TG loss may be accompanied by a protein loss (e.g., apoC) or it may exchange with very slowly turning over nonplasma TG pools or with plasma cholesteryl ester, as has previously been suggested (8). The latter would be consistent with the finding that in LCAT deficiency LDL is rich in TG and poor in cholesteryl ester (8).

The two-stage LDL model proposed to account for the difference in the disappearance of LDL-apoB and LDL-TG is supported not only by the LDL-TG curves but also by the relatively slow initial decay of LDL-apoB. This slow initial decay also implies that TG-rich LDL particles do not diffuse out of the plasma compartment to exchange with an extraplasma compartment. The significance of this is not clear. One could speculate that not until most TG (and apoC) is removed can LDL particles exchange with an extraplasma compartment. The TG-rich LDL particles may also have a rate of apoB degradation different from normals, but our data are inadequate to determine this. In line with this it may be of interest to determine whether there is a correlation between LDL-TG and LDL-apoC kinetics.

[4] A population of floating LDL particles has been observed recently by Dr. Scott Grundy, using an electron microscope. Personal communication.

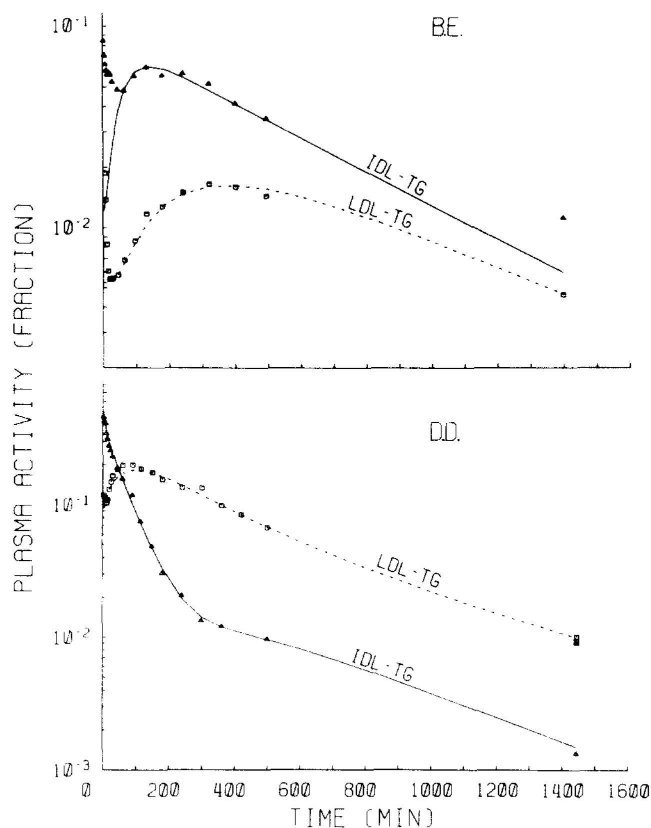

Fig. 3. Plasma LDL-TG and IDL-TG disappearance curves in patients B.E. and D.D. Patient B.E. was given labeled VLDL-TG whereas patient D.D. was given labeled IDL-TG. The squares and triangles are the LDL-TG and IDL-TG data and the dotted and solid lines are the model generated curves, respectively.

If the proposed model is correct then a factor to be considered in connection with endogenous LDL labeling is the time of collection of donor blood. At early times, most of the LDL activity should be in *C1*, whereas at late times, most of it should be in *C2*. In all present studies donor LDL was isolated about 120 min after injection and this is compatible with most of the initial label being in compartment 1, since it takes several hours before VLDL is fully converted to LDL (18).

We know of no studies that permit us to compare endogenously to exogenously labeled LDL-TG kinetics. As for LDL-apoB, however, it seems that our calculated values for total plasma turnover, $L_{2,2}$ ($= L_{0,2} + L_{3,2}$) (~0.0011/min), are quite comparable to those obtained by Eaton et al. (7) for endogenously labeled LDL using [^{75}Se]selenomethionine but are somewhat higher than values obtained for exog-

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TABLE 2. Model parameter values

Subject	Rate constants (1) $L_{1,1}$	(2) $L_{0,1}^{TG}/L_{1,1}$	(3) $L_{0,1}^{B} = L_{0,2}$	(4) $L_{2,2}$	Plasma LDL-TG Conc. (5) M_1^{TG}	(6) M_5^{TG}	Plasma LDL-apoB Conc. (7) M_1^{B}	(8) M_5^{B}	(9) FCR LDL-TG	(10) LDL-TG Transport
	min^{-1}			min^{-1}	mg/ml	mg/ml	mg/ml	mg/ml	min^{-1}	mg/ml/min
Normal										
J.B.	0.0020 ± 0.0007	0.90 ± 0.14	0.0003	0.0013 ± 0.0003	0.17 ± 0.12	0.292	0.078 ± 0.028	0.52	0.00120	0.00035 ± 0.00012
J.U.	0.0101 ± 0.0012	0.95 ± 0.02	0.0003	0.0013 ± 0.0005	0.13 ± 0.03	0.375			0.00347	0.0013 ± 0.0002
D.D.	0.0040 ± 0.0027	0.98 ± 0.42	0.0003	0.0008 ± 0.0240	0.46 ± 2.15	0.589			0.00322	0.0019 ± 0.0070
C.M.	0.0035 ± 0.0023	0.92 ± 0.30	0.0003	0.0008 ± 0.0053	0.41 ± 0.88	0.800			0.00175	0.0014 ± 0.0021
Type IIa										
R.D.	0.0023 ± 0.0012	0.96 ± 0.14	0.0002	0.0005 ± 0.0002	0.49 ± 0.62	0.720	0.156 ± 0.085	1.79	0.00153	0.0011 ± 0.0008
D.V.	0.0022 ± 0.0040	0.95 ± 2.10	0.0002	0.0025 ± 0.0041	1.31 ± 19.44	2.025	0.214 ± 0.381	2.35	0.00144	0.0029 ± 0.0400
Type IV										
B.E.	0.0043 ± 0.0086	0.97 ± 1.20	0.0003	0.0008 ± 0.0600	0.39 ± 5.03	0.525			0.00318	0.0017 ± 0.0019
R.B.	0.00320 ± 0.00020	0.98 ± 0.14	0.0003	0.0018 ± 0.0001	0.77 ± 0.90	0.825	0.068 ± 0.004	0.72	0.00279	0.0023 ± 0.0029

(1) $L_{1,1}$ is total fractional turnover rate of TG-rich LDL (*C1*) and is the same for TG and apoB moieties ($=L_{0,1} + L_{2,1}$).
(2) Fraction of *C1* triglycerides lost from LDL.
(3) $L_{0,1}^{B}$ is irreversible apoB loss from *C1*. $L_{0,2}$ is irreversible loss from *C2* for both TG and apoB. Values taken from the literature (3).
(4) $L_{2,2}$ is total fractional turnover rate of *C2* ($=L_{3,2} + L_{0,2}$).
(5), (6), (7), (8), (10) Since the LDL apoB and TG share the same particle, their spaces of distribution are also the same. Hence, all steady state masses and transports are expressed in concentration units (mg/ml, mg/ml/min). To obtain total plasma masses and transports these quantities must be multiplied by plasma volume.
(6), (8) M_5^x is the total plasma concentration of moiety x ($M_5^x = M_1^x + M_2^x$).
(9) FCR is fractional catabolic rate, defined as fraction of total plasma LDL-TG lost irreversibly per day.

$$FCR = \frac{L_{0,1}M_1 + L_{0,2}(M_5 - M_1)}{M_5}.$$

(10) LDL-TG transport is the triglyceride transport through the LDL subsystem and equals FCR*M_5.

enously labeled LDL-apoB (~0.0005/min, ref. 3). The numbers of studies we have, however, are too small to make statistically significant comparisons. Since the LDL studies were carried out over a period of one day only, we could not estimate a fractional catabolic rate (FCR = $L_{0,2}$) for LDL-apoB. In fact, a literature value was assumed for it in our modeling.

In patients in whom labeled LDL was injected, some label was also found in IDL particles but it had relatively little effect on the kinetics of LDL. When labeled VLDL was injected, IDL was the dominant precursor for LDL. In two of the patients, it was found that the slow component in IDL made too large a contribution to LDL and a good LDL fit could be obtained only by reducing the tail of the IDL curve (see Fig. 3*a*). This suggests that the slow decaying component of IDL contains a moiety different from that in the rapid component. Further analysis of this, by investigating more fully the kinetics of IDL, is necessary.

The fraction of apoB mass in the TG-rich plasma LDL (M_1^B/M_5^B) closely reflects the fraction of the LDL particles in that moiety and averages about 10%. For TG this ratio averages about 60%, although this value is poorly determined. The ratios M_1^{TG}/M_1^B and M_2^{TG}/M_2^B are measures of average TG/apoB ratios for the different particles.

Although the model proposed seems reasonable, we cannot exclude other explanations for the data. One possibility is that only about 10% of the particles measured in the LDL range carry extra triglycerides and the rest do not. Such an explanation, however, would require that each TG-rich particle carry about ten times as much TG as the measured average. It is not likely that such a particle would be found in the LDL range. In any case such a model would not significantly change the major conclusions drawn earlier.

The individual studies here fall into several populations with respect to their lipid metabolism. We do not see any striking differences as to LDL-TG kinetic parameters between the populations. The number of

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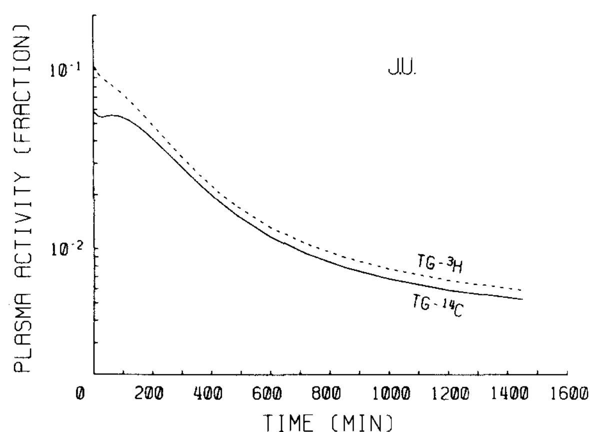

Fig. 4. Plasma LDL disappearance curves for ^{14}C- and ^{3}H-labeled TG in same patient. Data are not shown but the fit was very good for both curves.

individuals involved, however, is too small to conclude whether this is real or a random occurrence.

Note added in proof. Although not shown here, it is of interest to mention that most of the fatty acid label on LDL triglycerides was recovered in the plasma free fatty acids. This supports the notion that hydrolysis rather than exchange processes is the major route of LDL triglyceride loss.

A comment about the precision of the results. As can be seen from Table 2, quite a few of the values are poorly defined. In each column, however, there are some values that do have reasonable precision to indicate roughly where the values and estimates for their upper limits lie. The best data arise from the double experiments in which both the protein and triglyceride moieties were labeled. The double study in J.U. (**Fig. 4**) is also helpful. It is unfortunate that greater precision cannot be derived from the present studies; longer range studies are necessary to get more information in the future.

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