Metabolism of apoB and apoC lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects

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Abstract The kinetics of apolipoproteins B and C were studied in 14 normal and hyperlipoproteinemic subjects after injection of exogenously ^{125}I-labeled very low density lipoprotein (VLDL) particles. Plasma radioactivities of apoB and apoC were determined over a period of 4 days in VLDL ($d < 1.006$) and total radioactivity in intermediate (IDL) ($1.006 < d < 1.019$), low (LDL) ($1.019 < d < 1.063$), and high (HDL) ($1.063 < d < 1.21$) density lipoproteins. The data were analyzed by the use of a model, developed mostly from these data, with the following results. The VLDL particle undergoes a series of incremental density changes, most likely due to a number of delipidation steps, during which apoB stays with the particle until the density reaches the IDL range. There is, however, a loss of apoC associated with these delipidation steps. In our normal subjects, all IDL apoB eventually becomes LDL. In our hyperlipemic subjects some of the apoB on IDL is also degraded directly. The apoC lost by VLDL and IDL recycles to HDL, and most of it is picked up again by newly synthesized VLDL. There is a slowdown of the stepwise delipidation process in all hyperlipemic individuals studied. Three additional features became apparent in the type III subjects. First, there is a significant increase (a factor of 2 compared to normal) in the apoB synthesis rate by way of VLDL; second, there is an induced direct apoB synthesis pathway by way of IDL (and/or LDL); third, a bypass of the regular stepwise VLDL delipidation pathway is induced by which VLDL particles lose apoC but none of their apoB, thereby forming a new particle with metabolic properties similar to LDL, but with a density still in the VLDL density range. Two type III patients treated with nicotinic acid and clofibrate showed a sharp decrease in their VLDL apoB synthesis rates. This was somewhat compensated by an increased IDL apoB synthesis rate. A type I patient on a medium chain triglyceride diet also showed a number of metabolic changes, including reduced VLDL apoB synthesis and the induction of considerable IDL and/or LDL apoB synthesis.

Supplementary key words mathematical models · apolipoproteins · VLDL · LDL · IDL · HDL · compartmental models · type III hyperlipoproteinemia

Very low density lipoprotein (VLDL) is a complex particle, composed primarily of protein, triglyceride, cholesterol, and phospholipid. Its synthesis and metabolism have been poorly understood though it has been the subject of much recent study. Its primary sites of synthesis appear to be the liver and the intestine. (See reviews by Fredrickson and Levy (1); Havel (2); Eisenberg and Levy (3).) Much quantitative data concerning VLDL metabolism has been derived from triglyceride studies using isotopically labeled fatty acids or glycerol (4–7). Several metabolic studies utilizing various apoprotein labeling techniques have shown that VLDL is at least partially metabolized to low density lipoprotein (LDL) (8–17).

This report describes the results of metabolic studies using ^{125}I-labeled VLDL (^{125}I-VLDL) in 14 human subjects, both normal and hyperlipemic (**Table 1**), and it reflects mostly the kinetics of the apoproteins apoB and apoC. After injection, the kinetics of labeled apoproteins were followed in the various lipoprotein subfractions VLDL, IDL (intermediate density lipoprotein), LDL (low density lipoprotein) and HDL (high density lipoprotein), yielding a more integrated picture of metabolic pathways. Based on these data a unified mathematical model was developed and is presented in detail elsewhere (16). Analysis of all the studies in terms of the proposed model yield parameter values for the normal and abnormal subjects. The physiological and clinical signifi-

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; MCT, medium chain length triglyceride; ^{125}I-VLDL, ^{125}I-labeled VLDL.

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TABLE 1. Patients' clinical data

Patient	Age	Sex	Weight	Height	Obesity Index[a]	Chol.	TG	Diet	Medication[b]	Comment
			kg	*cm*		*mg/dl*	*mg/dl*			
Normal										
J.Pr.	23	M	58.7	161	1.01	174	111	800 mg chol normal P/S	None	
C.Br.	21	M	78.4	197	0.91	90	169	Hi CHO 5 g fat	None	Participated in paired VLDL–LDL study
S.Sa.	19	M	68.1	183	0.92	165	110	Low chol normal P/S	None	
W.Du.	21	M	62.1	176	0.90	107	69	Hi CHO 5 g fat	None	
Type I										
P.Pr.	23	M	68.1	181	0.93	235	477	Hi CHO 5 mg fat	Tetracycline 2 g/day	
L.Wy.	29	M	60.6	168	0.97	222	236	Hi CHO 5 g fat	None	
J.Kn.	21	M	60.9	164	1.02	101	347	Hi CHO 60 g MCT 5 g fat	MCT diet	
Type II										
H.Mo.[c]	41	M	77.4	178	1.10	219	446	Hi CHO 5 g fat	None	
Type III										
M.Da.	60	F	51.2	148	1.06	181	154	Low chol normal P/S	Nicotinic acid 2 g/day	
T.Pl.(1)	53	F	96.2	162	1.77	224	339	Low chol normal P/S	Dessicated thyroid	Euthyroid (clinically)
(2)	53	F	94.6	162	1.74	239	340	Low chol normal P/S	Dessicated thyroid	Euthyroid (clinically)
(3)	53	F	87.4	162	1.60	152	152	Low chol normal P/S	Atromid 2 g/day Dessicated thyroid	Euthyroid (clinically)
J.Tu.	45	M	93.3	181	1.29	252	393	Low chol normal P/S	Atromid 2 g/day	
B.Ra.	51	F	60.3	161	1.11	315	423	Low chol normal P/S	Hydralazine Reserpine	
S.Ja.	46	M	82.6	175	1.21	246	382	Low chol normal P/S	None	Participated in paired VLDL–LDL study
Type IV										
E.We.(1)	60	M	76.0	169	1.20	211	728	Low chol normal P/S	None	
(2)	60	M	76.0	169	1.20	192	576	Low chol normal P/S	None	

[a] Based on Metropolitan Life Insurance Tables, 1968.
[b] All patients received supersaturated potassium iodide, beginning 3 days prior to the study, until the study was completed.
[c] This patient had a type IV pattern during this study while on a high CHO low fat diet. Prior to this he had a typical type II pattern with cholesterol levels in the 300 mg/dl range and TG levels in the 150 mg/dl range.
Abbreviations: CHO, carbohydrate; Chol, cholesterol; P/S, polyunsaturated/saturated.

cance of these results and their implications to the general understanding of lipoprotein metabolism are examined. Preliminary observations from these studies, without detailed kinetic modeling, were published earlier by Bilheimer, Eisenberg, and Levy (11) and Eisenberg, et al. (13).

The nomenclature employed to describe the lipoprotein moieties and the model is presented in **Table 2**.

TABLE 2. Nomenclature

VLDL	Very low density lipoprotein (d < 1.006 g/ml).
IDL	Intermediate density lipoprotein (1.006 < d < 1.019 g/ml).
LDL	Low density lipoprotein (1.019 < d < 1.063 g/ml).
HDL	High density lipoprotein (1.063 < d < 1.21 g/ml).
Q	Any class of lipoprotein particles: VLDL, IDL, LDL, HDL, LP.
Q-B	Suffix B represents apoproteins apoB on the particle Q.
Q-C	Suffix C represents apoproteins apoC on the particle Q.
Q-P	Suffix P represents total apoproteins on particle Q.
Q-TG	Suffix TG represents total triglyceride on particle Q.
Q-ARP	Suffix ARP represents arginine-rich peptides on particle Q.
α_2-VLDL	VLDL having α_2-mobility on electrophoresis. Assumed to correspond to compartments 7–16 in the model.
β-VLDL	VLDL having β-mobility. Assumed to correspond to compartment 21 in the model.
X-VLDL	Unidentified VLDL. Represented by compartment 5 in the model.
*	A labeled moiety, e.g., VLDL-B* is the labeled apoB on VLDL particles, whereas VLDL-B is the unlabeled counterpart. IDL* is the total label on IDL.
C(I)	Compartment I in the model.
$L_{m,n}$	Fraction of material in C(n) transported to C(m) per day.
$L_{0,n}$	Fraction of material lost irreversibly from C(n) per day.
U_i	Rate of entry of new material (mg/day) into compartment i from the outside.
$R_{m,n}$	Flux of material in compartment n transported to compartment m in mg per day.
M_i	Steady state mass of material in compartment i.

Superscript on symbols $L_{m,n}$, $R_{m,n}$, M_i and U_i designates moiety of material to which it applies.

METHODS

Patients

Fourteen subjects participated in lipoprotein turnover studies while housed on a metabolic ward at the NIH Clinical Center (Table 1). There were four normal volunteers, three patients with familial lipoprotein lipase deficiency (familial type I hyperlipoproteinemia), one with familial hypercholesterolemia (type II hyperlipoproteinemia), five with familial type III hyperlipoproteinemia, and one with endogenous hypertriglyceridemia (type IV hyperlipoproteinemia). Hyperlipoproteinemia is classified according to the system of Fredrickson and Levy (1). One patient (T.Pl.) participated in three separate studies while another (E.W.) took part in two. Two patients (T.Pl. and E.We.) were paired for their studies, each receiving radiolabeled VLDL from the other during one of their paired studies. Three of the normal subjects and one subject with type III underwent separate studies of ^{125}I-labeled LDL (^{125}I-LDL) turnover 4–6 weeks after the ^{125}I-VLDL injection, while on the same metabolic diet and at a stable weight. All subjects were tested for and found free of renal, hepatic, or hematologic abnormalities. One patient (T.Pl.) had treated hypothyroidism (on 3 grains of dessicated thyroid (Armour) per day). The remainder of the patients were euthyroid and all subjects had normal fasting blood sugar levels. Five subjects had atherosclerotic cardiovascular disease but all were in stable medical condition during their studies. L.Wy. suffered from abdominal pain of unknown etiology during one day of a 10-day study but was otherwise stable. The remaining subjects were in stable health throughout the study period.

Subjects were admitted to the ward at least 2 weeks prior to study for a period of stabilization and dietary adjustment. Calories were adjusted to maintain body weight within ±0.5 kg. Diets for the type III and type IV subjects contained 40% of the calories as fat, 40% as carbohydrate, and 20% as protein. Cholesterol intake was limited to 300 mg/day and the polyunsaturated/saturated fatty acid ratio was normal (~0.2). Attempts were made to study normal subjects as well as the one type II on this diet but, in two normal subjects and the type II subject, the plasma VLDL levels were too low to permit isolation of sufficient material for iodination. To circumvent this problem subjects were studied while consuming an isocaloric high carbohydrate–5 g fat diet (7 g of carbohydrate/kg body weight per day) which elevated the plasma VLDL to levels adequate for conducting the study.

Two of the three subjects with familial lipase deficiency were maintained on the high carbohydrate–5 g fat diet to minimize exogenous hypertriglyceridemia. In a third subject with type I (J.Kn.), 60–100 g/day of medium chain length triglyceride (MCT) was substituted isocalorically for carbohydrate on an otherwise fat-free diet.

On the first day of the experiment the patients were on a low-fat (<10 g/day), isocaloric diet. This diet was instituted in order to minimize chylomicron production since plasma samples were collected throughout the day. On subsequent days, plasma samples were taken after an overnight fast and the patients were placed on their specified diets for the remainder of the day.

All patients were permitted ad libitum activity and none was confined to bed.

Isolation of VLDL

Four days prior to study, subjects fasted for 14 hr were plasmapheresed using EDTA anticoagulant (0.5 g/500 ml of blood) to obtain two units of plasma (250–280 ml per unit). Initial isolation of VLDL was performed by ultracentrifugation at 4°C in a Beckman L2-65B ultracentrifuge using a 60 Ti rotor at

speeds of 60,000 rpm for 12 hr (1.84×10^8 average *g*-min). At the end of the run, VLDL was isolated by the tube slicing technique, resuspended in sterile saline (0.15 M) and subjected to a second ultracentrifugation in a 60 Ti rotor at speeds of 60,000 rpm for 8 hr (1.23×10^8 average *g*-min). The isolated VLDL was again suspended in sterile saline (0.15 M) and subjected to a final wash–concentration spin using a Beckman 65 rotor at speeds of 65,000 rpm for 6 hr (9.79×10^7 average *g*-min). The concentrated VLDL was then resuspended in small amounts of sterile 0.15 M saline by gentle stirring on a magnetic stirrer at 4°C. Each VLDL preparation was found free of contamination by other plasma proteins as judged by immunoelectrophoresis in 1% agarose gel using specific antisera prepared against whole human serum, human albumin, human HDL, and human LDL (18). Small aliquots of VLDL were taken for determination of protein, cholesterol, and triglyceride (vida infra).

Radioiodination

VLDL protein concentration was measured by modification of the method of Lowry, et al. (19) using bovine serum albumin as a standard. Turbidity was removed by addition of diethyl ether to the fractions and standards prior to recording absorbance. Radioiodination of VLDL was performed by the method of MacFarlane (20) as modified by Bilheimer, et al. (11). In this procedure, usually 10 or more mg of VLDL protein in a total volume not exceeding 2 ml was mixed with one ml of 1 M glycine buffer, pH 10.0. One to 3 mCi of carrier-free $Na^{125}I$ (New England Nuclear, Boston, MA) was added, followed by rapid injection of ICl solution. (0.9 M ICl stock containing 2.4 mg/ml diluted 1:50 with 2 M NaCl.) The quantity of ICl added was calculated to yield a stoichiometry of one atom of I per mole of VLDL protein, assuming a VLDL protein molecular weight of 300,000 and an iodination efficiency of 15%. Unbound iodide was removed by dialysis in casein (Union Carbide, Chicago, IL), at 4°C against a total of 12 l (one l changed every 20 min.) of 0.15 M NaCl, 0.01% EDTA, pH 7.4. Ninety-nine percent of the radioactivity remaining after dialysis was attached to the lipoprotein as determined by descending paper chromatography in methanol–water 85:15 followed by scanning on a Packard Model 7201 radiochromatogram scanner (21).

Duplicate aliquots of the labeled VLDL were subjected to delipidation by the method of Folch, Lees, and Sloane Stanley (22) and the chloroform phase was assayed for radioactivity. After correction for quenching, lipid-bound iodide was found to vary between 7.7 and 26% (mean 17%). The immunoreactivity and paper electrophoretic behavior of ^{125}I-VLDL were identical to those of native VLDL (18).

Preparation of ^{125}I-VLDL for in vivo use

Following addition of carrier human albumin, sterilization by Millipore filtration (0.45 μm) (Millipore Corp., Bedford, MA), and pyrogen testing (23), the VLDL was ready for injection. Total time from plasmapheresis to injection was 96 hr.

In the three normal subjects and one type III who participated in paired ^{125}I-LDL–^{125}I-VLDL studies, the LDL was isolated, tested for purity, labeled, and prepared exactly as described previously (24).

Study protocol

Fasting, supine, normal subjects and patients were administered 25–50 μCi of ^{125}I-VLDL (0.6–5.6 mg of VLDL apoprotein) intravenously from a calibrated syringe via a continuous saline infusion. Subsequently, 40 ml of blood were collected in evacuated glass tubes containing EDTA at 10 min, 3 hr, 6 hr, 9 hr, 12 hr, 24 hr, and then daily thereafter for 4 days. From the 5th day to the end of the study (10–14 days), only 10 ml of blood were taken each morning. Plasma was immediately separated at 4°C by centrifugation in an IEC refrigerated centrifuge (1500 rpm for 20 min) and portions were removed for counting, cholesterol and triglyceride determination (AAII), and separation of lipoproteins by ultracentrifugation (25). Twenty-four-hour urine collections were made using glass jars containing 5 ml of a preservative designed to minimize volatilization of ^{125}I or its adsorption to glass.[7] Supersaturated potassium iodide (1 g/day) was administered in three divided doses daily beginning 3 days prior to the study and extending throughout the study period. Informed consent was obtained from each patient and normal volunteer.

Sample analysis

A 2-ml aliquot of plasma was removed for measurement of radioactivity content using a Packard Model 3375 gamma spectrometer.

Five-ml portions of urine were also analyzed for radioactivity and the total daily urine volume was recorded. Three aliquots of plasma (5 ml) from each time point through 4 days of study (6 days in a few subjects) were adjusted, respectively, to d 1.006, 1.019,

[7] Preservative solution consisted of 25 g of KI, 44 g of $NaHSO_3$, and 80 g of NaOH in 100 ml of water. Stock solution was diluted 1:100 before use (26).

and 1.063 g/ml by the method of Havel, Eder, and Bragdon (27) as modified by Furman, Howard, and Nocia (28). A fourth aliquot of 1 ml was diluted with 4 ml of the subjects' original plasma remaining from the initial plasmapheresis and adjusted to d 1.21 g/ml. The samples were centrifuged in a Beckman Model L2-65B ultracentrifuge using a 40.3 rotor at speeds of 40,000 rpm for 24 hr (1.65×10^8 average *g*-min). Tubes were sliced in the clear zones between the top and bottom fractions. These fractions were subsequently transferred quantitatively to 5-ml volumetric flasks and brought to volume. Samples were assayed for radioactivity before and after addition of a $Na^{125}I$ internal standard that was needed to correct for absorption of the relatively low-energy gamma emission of ^{125}I in the salt solutions of higher concentration. Radioactivity in each of the four lipoprotein density regions was then determined by calculating the additional quantity of radioactivity floated up at each subsequently higher density. Recovery ranged between 88 and 100% (and was usually over 95%).

Whole plasmas as well as aliquots from the d 1.006 top and bottom fractions were taken for determination of cholesterol (29) and triglyceride (30). An HDL cholesterol determination was performed on the plasma and the d 1.006 bottom fraction (18, 31).

The VLDL isolated at each time point as outlined above was delipidated and solubilized as previously described (32–34). Within the error of the method, solubilization was believed to be complete. The solubilized VLDL apoprotein was then subjected to polyacrylamide gel electrophoresis (34). Peptide zones were detected by Coomassie blue staining, sliced from the gel, and assayed for radioactivity (11, 13).

Material isolated in zone III included what is now referred to as arginine-rich peptides (ARP). Labeled apoB was defined as radioactivity associated with zone II and labeled apoC that associated with zones VI and VII (11). Protein loads varied between 75 and 300 μg, the size of the load varying with the degree of radioactivity present. About 95% of the radioactivity recovered was associated with protein bands.

For IDL, LDL, and HDL only total radioactivities were measured. For LDL all the radioactivity was assumed to be in apoB; for HDL it was assumed to be in apoC. For IDL the radioactivity was assumed to be in both apoB and apoC and the model was able to quantify each.

Data analysis

Apoprotein concentrations were calculated from measured cholesterol and triglyceride concentration values and from measured or published composition data on each class of particles. These data are presented in **Table 3.** Radioactivity in plasma is expressed as percent injected dose per liter of plasma. 'Plasma volume' was calculated by isotopic dilution using the 10-min total plasma radioactivity value.

The radioactivities in the various lipoprotein subfractions (up to at least 10 days) were determined by differential centrifugation of the plasma at four salt densities, and the results were expressed as percent injected dose per liter of plasma. The percent of injected radioactivity in individual VLDL apoproteins was obtained by multiplying the relative fraction of the counts found in a given peptide separated on disc gel electrophoresis by the percent of the injected dose in VLDL per liter of plasma at the same time.[8]

All resultant data were analyzed on a Univac 1108 computer using the SAAM computer program (version 26) of Berman and Weiss (35, 36). The development of a multicompartmental model to account for the tracer and tracee (nonlabeled) kinetic data is discussed elsewhere (16). The nomenclature employed to describe the derived model is presented in Table 2. The specific pathways and lipoprotein moieties defined are underlined when first presented in the subsequent sections.

Typical sets of data are shown in Figs. 1–3. **Figs. 1** and **2** show apoB and apoC data respectively for a normal and a type III subject. Such data were initially used separately in deriving the apoB and apoC models. **Fig. 3** shows a typical set of data (apoB and apoC) on a single subject, which were fitted simultaneously to the apoB and apoC models, satisfying common constraints. Urinary excretions of labeled iodide, released as a result of lipoprotein degradation, were included in the combined data fitting. These data served as an additional independent constraint on the model by requiring that all the label be accounted for. The average variation of the data about the model predicted values ranged from 5 to 10% for the various studies.

RESULTS

Analysis of the kinetics of apoproteins is complicated not only by the heterogeneity of the protein moieties but also by their disparate metabolic behavior. Whereas apoB appears to stay with the VLDL particle during its catabolism, apoC shuttles between VLDL and HDL. Due to these complexities it is necessary to analyze the apoB and apoC moieties individually as well as jointly in order to integrate the results

[8] This overestimates the radioactivity of each protein moiety by a factor P/(P − Y) where Y (Col. (1), Table 4) is the nonprotein (lipid, etc.) and P is the total radioactivity in plasma (Table 4). An appropriate correction for this was made in the modeling process.

TABLE 3. Plasma LP concentration data

		LDL			VLDL							HDL	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Patient	Space of Distr.	LDL-CH	LDL-P to LDL-CH Ratio	LDL-B	VLDL-CH	VLDL-P to VLDL-CH Ratio	VLDL-B to VLDL-P Ratio	VLDL-B	Ratio	VLDL-C	VLDL-ARP	HDL-CH	HDL-C
	ml	*mg/dl*		*mg/dl*	*mg/dl*			*mg/dl*		*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
Normals													
J.Pr.	3200	108	0.48	52	19	0.63	0.41*	4.9	0.79	5.6	1.5	45	9.8
C.Br.	3480	54	0.50*	27	29	0.65*	0.41*	7.7	0.79	8.8	2.3	42	9.1
S.Sa.	3200	98	0.41	40	14	1.09	0.41*	6.2	0.79	7.1	1.9	41	8.9
W.Du.	3420	68	0.45	31	10	0.75	0.41*	3.1	0.79	3.5	0.9	20	4.4
Type I													
P.Pr.	3120	86	0.50*	43	114	0.37	0.41*	17.2	0.79	19.7	5.2	29	6.3
L.Wy.	2780	111	0.50*	56	96	0.58	0.41*	22.8	0.79	26.0	6.9	15	3.3
J.Kn.	2610	92	0.50*	46	27	0.51	0.41*	5.6	0.79	6.4	1.7	10	2.2
Type II													
H.Mo.	3210	143	0.50*	72	62	0.78	0.41*	19.8	0.79	22.5	6.0	27	5.9
Type III													
M.Da.	2630	66	0.50*	33	34	0.46	0.48	7.5	0.79	6.4	1.7	77	16.7
T.Pl.(1)	2700	82	0.50*	41	117	0.65*	0.64	48.7	0.61	16.7	10.7	36	7.8
(2)	2700	78	0.50*	39	123	0.55	0.66	44.6	0.61	14.3	9.0	36	7.8
(3)	2700	65	0.50*	33	44	0.56	0.52	12.6	0.79	9.3	2.5	44	9.6
J.Tu.	3420	69	0.50*	35	143	0.65*	0.59	54.8	0.61	23.2	14.9	33	7.2
B.Ra.	2390	152	0.50*	76	132	0.63	0.56	46.6	0.61	22.3	14.3	36	7.8
S.Ja.	3360	68	0.51	35	128	0.41	0.59	31.0	0.61	13.2	8.4	30	6.5
Type IV													
E.We.(1)	2980	51	0.50*	26	118	0.67	0.41*	32.4	0.79	36.8	9.8	33	7.2
(2)	3030	65	0.50*	33	96	0.65*	0.41*	25.6	0.79	29.0	7.7	31	6.7

Col. (1). Space of distribution (ml). Calculated from total radioactivity concentration in plasma at 10 min. Col. (2). Measured LDL-cholesterol. Col. (3). Measured ratios except starred (*) values which are estimates based on literature values (1). Col. (4). LDL-B calculated as the product (Col. 2) × (Col. 3). Col. (5). Measured VLDL cholesterol. Col. (6). Measured VLDL-P/VLDL-CH ratio except starred (*) values which are estimates based on literature values (1). Col. (7). Measured VLDL-B/VLDL-P ratio, except starred (*) values which are estimates based on literature values (3). Col. (8). VLDL-B calculated from product (Col. 5) × (Col. 6) × (Col. 7). Col. (9). Estimated (1) ratio of VLDL-C/(VLDL-C + VLDL-ARP) based on literature values (37). All patients except untreated Type IIIs were assumed to have normal ratios of (VLDL-C)/(VLDL-C + VLDL-ARP). Col. (10). VLDL-C calculated from product (Col. 5) × (Col. 6) × (1-Col. 7) × (Col. 9). Col. (11). VLDL-ARP calculated from product (Col. 5) × (Col. 6) × (1 − Col. 7.) × (1 − Col. 9). Col. (12). Measured HDL-cholesterol. Col. (13). HDL-C protein concentration calculated from (Col. 10) × 0.075 × 2.90. This assumes an HDL-C/HDL-P ratio of 0.075 (Based on C. Blum, 12 studies in 6 patients (unpublished data)) and an HDL-P/HDL-CH ratio of 2.90.

into a composite picture of the carrier particles: VLDL, IDL, LDL, and HDL.

The apolipoprotein data were analyzed using the apoB and apoC models developed in a previous report (16) and are presented in **Figs. 4** and **5.** The models propose that apoB first enters the plasma (apoB synthesis) with newly synthesized VLDL (compartment 7). The metabolism of VLDL-apoB then proceeds by either one of two pathways: the α_2-pathway or the β-pathway. The α_2-pathway involves a series of delipidation steps (compartments 7, 11, 12, and 16) resulting in the formation of progressively less lipidated α_2-VLDL. Subsequently the particles are further delipidated to form IDL ($1.006 < d < 1.019$ g/ml, compartment 13), and then LDL ($1.019 < d < 1.063$ g/ml, compartment 1). The β-pathway leads to the formation of β-VLDL (compartment 21). β-VLDL particles disappear from the circulation without being converted to either IDL or LDL, and have a catabolic rate constant similar to that for LDL.

IDL particles are metabolized by two pathways: conversion to LDL or direct degradation.

X-VLDL* denotes part of the injected ^{125}I-labeled VLDL that behaves differently than α_2- and β-VLDL and is required in the analysis to account for all the VLDL label. It reflects, in part, lipid label but also some VLDL that has been altered or denaturated during preparation (compartment 5). It is of interest to note that, in the study of S.Ja., special care was taken to minimize physical agitation of VLDL during its isolation and preparation for radiolabeling, and a relatively small amount of X-VLDL* was found.

It is difficult to determine from our data whether newly synthesized apoC first enters plasma with the HDL or with the VLDL particle. In some patients the data suggest the first mode and, in others, the second.

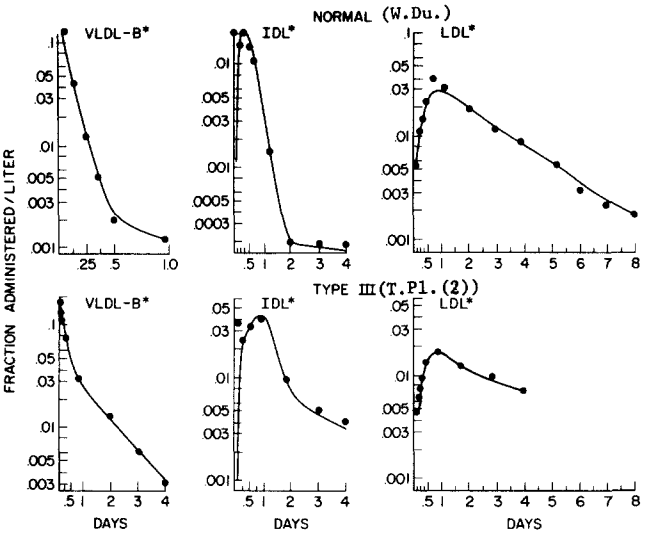

Fig. 1. Two sets of typical apoB tracer data: one for a normal and one for the type III patient. The dots are the observed values and the solid lines are the model predictions.

Once with HDL, apoC seems to be distributed to only newly synthesized VLDL particles. It recycles back to HDL as VLDL is delipidated (HDL-C recycling path) and is eventually degraded through the HDL particle. X-VLDL-C seems at first to disappear from the system completely, but much of it reappears with newly synthesized VLDL (X-VLDL-C recycling pathway) after a 3–9 hr delay.

Initial labeling

The initial amounts of label present on the various lipoprotein species as required by the model and that best approximate the observations are given in **Table 4.** The fraction of the injected activity of ^{125}I-labeled apoprotein in VLDL-B* ranged 28–52%. For VLDL-C* the range was 13–27%. In addition, in all studies, initial labeling was required for HDL-C* and ranged 3–18%. Small amounts of label (~3%) were initially present in IDL and LDL, and were neglected in the present analysis.

In the 10-min sample of VLDL about 5% (Table 4) of the total injected radioactivity was found in the polyacrylamide gel zone III (13), presumably equivalent to apoA or the arginine-rich peptide of Havel and Kane (37). The low levels of radioactivity in this apoprotein precluded effective analysis of its metabolism. Lipid labeling accounted for about 17% of the injected ^{125}I (range 7–26%). In this analysis we chose to focus on the metabolism of the labeled apoB and apoC. The labeling of lipid was taken into account in the model (through compartment 5), but, since it declined rapidly after injection of VLDL, its effect became negligible after the early phases of the protein kinetics.

The wide variation in initial labeling values observed in these studies must be due, in part, to variations between individuals, but probably also to differences in dietary conditions and the modalities of drug treatment.

ApoB and apoC metabolism in normal subjects

VLDL-B* metabolism in all subjects followed a precursor–product relationship from VLDL to IDL and from IDL to LDL. In normal subjects the fraction of apoB diverted to β-VLDL ($L_{21,7}/L_{12,11}$, **Table 5**) was very small, 2% or less. The average residence time of apoB in the α_2-delipidation chain was 0.25 days, implying a turnover rate of 4.0/day. The rate constant of apoB turnover in IDL ($L_{13,13}$, Table 5) was fairly rapid, 4.8/day, and its turnover in LDL ($L_{3,1}$, Table 5) was about one-tenth as rapid, 0.46/day. No direct degradation of IDL-B ($L_{3,13}$, Table 5) was observed in the normal subjects. ApoB synthesis (U_7^B, **Table 6**) averaged 556 mg/day (range, 443–667 mg/day).

In order to determine how much newly synthesized apoC may appear first with VLDL (U_7^C) or with HDL (U_9^C) particles, the plasma concentrations of apoC in both VLDL and HDL need be known. These values are given in Table 3. If one uses these values, however, negative values are derived in some patients for either U_9^C or U_7^C. This implies an inconsistency in either the model or the data. Because the assumptions involved

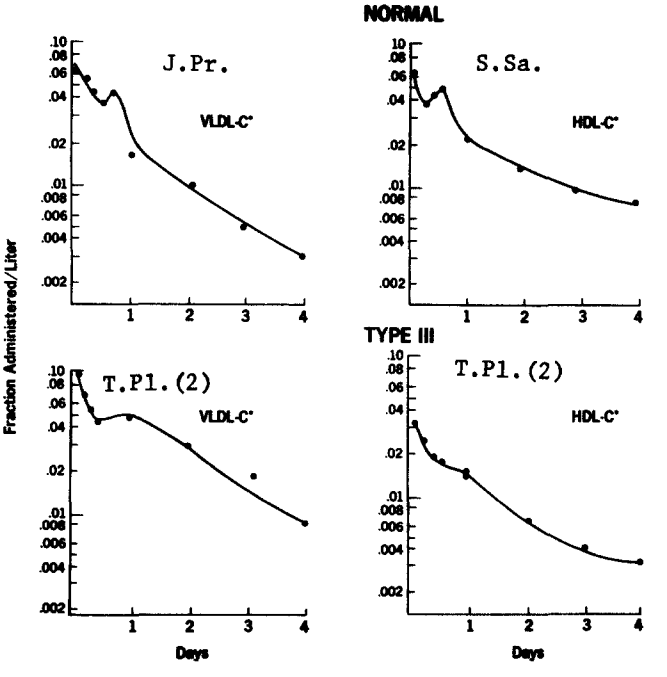

Fig. 2. Two sets of typical apoC tracer data: one for a normal and one for the type III patient. The dots are the observed values and the solid lines are the model predictions.

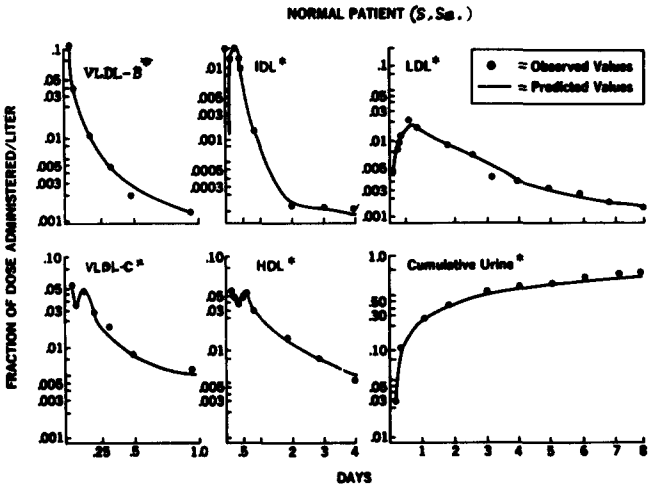

Fig. 3. Complete set of apoB and apoC data on a normal subject. All these data were fitted simultaneously to the apoB and apoC models.

in the calculation of HDL apoprotein levels in plasma involve values from the literature (1, 3, 37) rather than actual measured values in these patients, we chose to satisfy only the VLDL apoC concentration data. Under these conditions the model is compatible with either a U_7^C or a U_9^C, but then a discrepancy arises between the model calculated and 'observed' HDL apoC masses. This is shown in Table 6 as the ratio M_9^Cobs/M_9^Ccalc both for $U_7^C = 0$ and for $U_9^C = 0$, since we cannot choose a preferred site of first entry of newly synthesized apoC from the results.

As VLDL moves through progressive stages of delipidation, apoC is transferred to HDL. By the time VLDL becomes IDL, most of the apoC is lost. HDL apoC is transferred to newly synthesized VLDL with a rate of about 500–1300 mg/day ($R_{7,9}^C$, **Table 7**). ApoC associated with X-VLDL after being lost from the plasma reappears with newly secreted VLDL after a delay of about 3–9 hr. ApoC synthesis in normal subjects ranges between 250 and 660 mg/day if entry into plasma is with the HDL particle (U_9^C, Table 7) and 175–440 mg/day if entry into plasma is with VLDL (U_7^C, Table 7). There is no obvious correlation between apoB and apoC synthesis rates.

Thus, over the first 24 hr the kinetics of labeled VLDL particles reflect mostly changes due to the metabolism of apoB. After that, only apoC is seen and the kinetics of VLDL parallel those of HDL.

ApoB and apoC metabolism in hyperlipoproteinemic patients (other than type III)

Six studies were conducted in five patients. Three patients had documented lipoprotein lipase deficiency (J.Pr., L.Wy., and J.Kn. (38)); one had endogenous hypertriglyceridemia with normal lipase activity (E.We. (38)); and one had familial type II hyperlipoproteinemia (H.Mo.). ApoB synthesis (U_7^B, Table 7) was

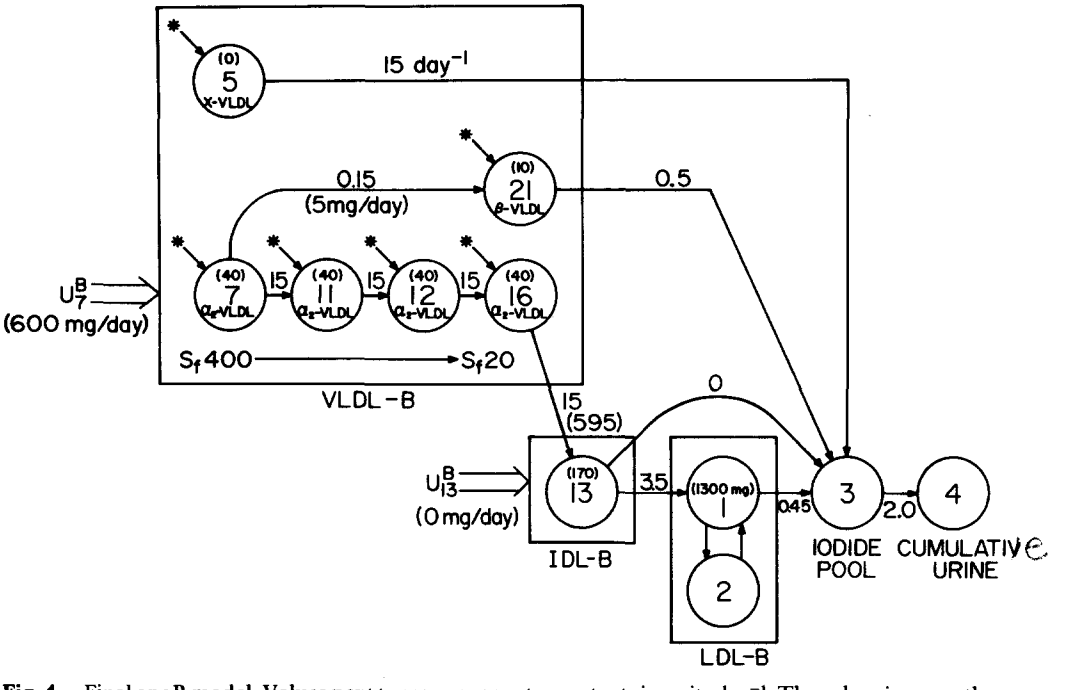

Fig. 4. Final apoB model. Values next to arrows are rate constants in units day^{-1}. The values in parentheses are calculated or measured steady state transports of apoB lipoproteins in mg/day. Value in parentheses inside a circle is the calculated steady state amounts of apoB in that compartment. The values are approximations for a normal individual.

TABLE 4. Initial labeling

Patient	(1) Lipid	(2) VLDL-ARP*	(3) VLDL-B*[a]	(4) VLDL-C*[a]	(5) HDL-C*	(6) Fraction of VLDL-B* in β-VLDL	(7) Fraction of VLDL-B* (and VLDL-C*) in X-VLDL
	%	%	%	%	%		
Normal							
J.Pr.	25.6	8.7	33.9	16.0	16.0	0.035	0.17
C.Br.	12.2	3.0	41.4	26.8	9.8	0.032	0.56
S.Sa.	16.7	2.8	30.4	13.6	18.0	0.120	0.28
W.Du.	24.1	2.0	28.4	12.7	12.0	0.025	0.41
Type I							
P.Pr.	21.7	4.3	40.2	16.4	4.7	0.025	0.31
L.Wy.	9.1	5.3	43.5	24.5	6.1	0.046	0.31
J.Kn.	6.9	2.9	52.2	24.1	7.8	0.008	0.61
Type II							
H.Mo.	10.6	4.1	31.3	25.5	7.0	0.032	0.17
Type III							
M.Da.	24.8	2.1	34.6	15.5	4.5	0.101	0.09
T.Pl. (1)	19.1	3.6	36.4	17.9	10.3	0.055	0.58
(2)	18.5	3.6	39.1	20.2	8.2	0.263	0.21
(3)	18.7	2.6	38.6	14.4	13.5	0.114	0.41
J.Tu.	16.7	5.8	41.1	14.9	8.5	0.182	0.28
B.Ra.	14.4	3.5	37.2	15.4	12.0	0.218	0.11
S.Ja.	8.1	8.5	39.7	26.3	3.0	0.259	0.10
Type IV							
E.We. (1)	19.1	4.6	35.0	23.4	6.0	0.046	0.16
(2)	18.5	5.4	38.4	19.3	7.0	0.232	0.30

[a] Labeled VLDL-B* and VLDL-C* include compartments 7 through 16 plus compartments 5 (minus lipid label) and 21. The lipid label is assumed to reside wholly in compartment 5 and to contaminate apoB and apoC in proportion to the corresponding protein labeling. The differences between the amount injected (100%) and the amounts shown in this table are due to initial activities in IDL, LDL, protein of density >1.21, and polyacrylamide gel residues.

Col. (1). Determined experimentally from 10-min plasma sample. Col (2). Determined experimentally from 10-min plasma sample as label in zone 3. Col. (3). Derived by the model. Col. (4). Derived by the model. Col. (5). Derived by the model assuming that all HDL label is in apoC. Col. (6). Derived by the model assuming that the initial labeling of β-VLDL-B and α_2-VLDL-B was proportional to their relative steady state masses as calculated by the model. Col. (7). Derived by the model.

within the range of normal in four studies and slightly elevated in H.Mo. In J.Kn., however, fed a MCT diet, direct synthesis of IDL-B or LDL-B was required. There are no apparent differences in apoC synthesis for the various populations studied.

In all these patients, the fraction of apoB in newly synthesized VLDL that passes through the β-VLDL pathway is minimal. The residence time of apoB in the α_2-VLDL delipidation pathway is, however, prolonged considerably and varies between 0.73 and 1.08 days (Table 5), compared to about 0.25 days for normal subjects. The turnover rate of IDL-B is decreased ($L_{13,13}$, Table 5), and the turnover rate of LDL ($L_{3,1}$) is comparable to that found in the normal subjects, except in the type II patient in whom it is decreased. In contrast to the normal, there is some direct loss (11–48%) of IDL without conversion to LDL ($L_{3,13}$).

ApoC kinetic parameters are generally similar to those observed in normal subjects. The rate constant for apoC degradation, however, is somewhat increased (~20%). Since we have no information on ARP/apoC mass ratios for these patients and a ratio equal to that for normal subjects was assumed, we cannot say much about the steady state masses and transports.

ApoB and apoC metabolism in type III hyperlipoproteinemia

VLDL metabolism in our type III patients is unique. A considerable fraction of the injected ^{125}I-labeled apoB is associated with β-VLDL (Table 4). The amount of apoC associated with β-VLDL was neglected. The fraction of newly-secreted VLDL-B that passes through the β-pathway ($L_{21,7}/L_{12,11}$, Table 5) in untreated patients ranged from 10 to 29% whereas this pathway was negligible in all other subjects. The apoC associated with particles that entered the β-VLDL pathway was diverted to HDL. In the untreated patients there was a 2 to 3-fold increase in total apoB synthesis ($U^B_7 + U^B_{13}$, Table 7). In addition, considerable direct IDL-B (or LDL-B) synthesis (U^B_{13}, Table

7) was required in all type III patients. ApoC synthesis (U_7^C or U_9^C, Table 7) was normal, except in the type III subject on nicotinic acid in whom it was elevated.

When the blood lipids were effectively lowered by treatment with clofibrate (T.Pl. (3) or with nicotinic acid (M.Da.)), the fraction of newly synthesized VLDL-B diverted via the β-VLDL pathway was greatly reduced. The drugs did not lower IDL-B synthesis (U_{13}^B); in fact, it seems even to have been raised. Thus, the model predicts some increase in the IDL-B and LDL-B masses.

As in other hypertriglyceridemic patients, the residence time of VLDL particles in the α_2-VLDL delipidation pathway in type III patients (Table 5) is prolonged by a factor of 3–4. The turnover rate of IDL-B is comparably decreased; however, a larger fraction of IDL-B (28–62%) is degraded directly ($R_{3,13}^B$) (Table 7). The turnover rates of β-VLDL ($L_{3,21}$) are comparable to those of LDL ($L_{3,1}$) which are not significantly different from normal.

DISCUSSION

The present report includes the results of 17 studies in which the fate of VLDL, labeled predominantly in its apoB and apoC protein moieties with ^{125}I, was determined in a diverse population of individuals. The mathematical model developed to explain the data is presented elsewhere (16). The model satisfies the data for all individuals studied, including four normal subjects and ten patients with four different patterns of hyperlipoproteinemia (types I, IIa, III, and IV), untreated and treated. Hence, the universal feature of the model is self-evident. We like to emphasize that the number of individuals within each population is too small to consider the derived values as necessarily typical of their population. Some of the changes observed may be due to other uncontrolled variables (e.g., obesity). Nevertheless several interesting patterns emerge with respect to the classified abnormalities and drug action.

The model represents an integration of all available data into a consistent framework. Some of its elements correspond directly to observed physiological entities; others, however, were introduced in order to satisfy the features of the data and are thus hypotheses to be further tested experimentally.

Several studies were designed specifically to answer certain questions about the model (supplementary studies in four patients using labeled LDL to determine the LDL subsystem (16) and crossover experi-

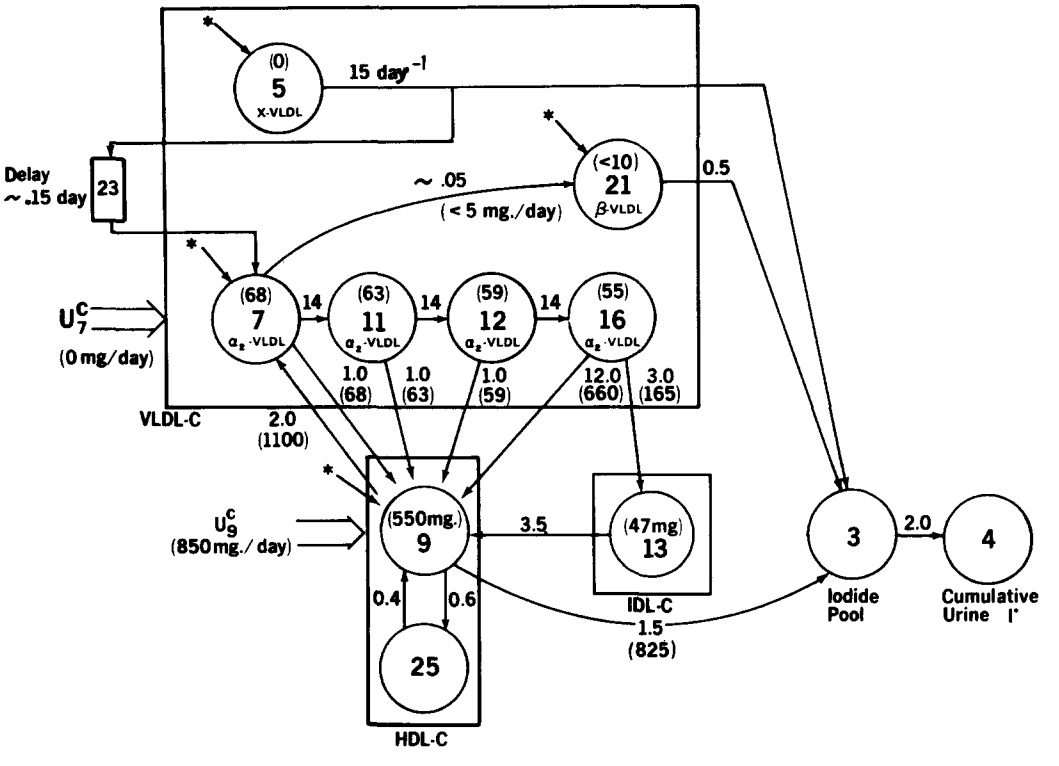

Fig. 5. Final apoC model. Values next to arrows are rate constants in units day^{-1}. The values in parentheses are calculated or measured steady state transports of apoC lipoproteins in mg/day. Value in parentheses inside a circle is the calculated steady state amount of apoC in that compartment. The values are approximations for a normal individual.

TABLE 5. Model rate

Patient	'Chain' Rate $L_{12,11}$	VLDL-B FCR[2]	VLDL[b] Particle 'Lifespan' τ	$L_{5,5}$	Fraction X-VLDL-C Recycled	τ_{23}	$L^{B}_{21,7}$	$\frac{L^{B}_{21,7}}{L_{12,11}}$	$L_{3,21}$[a]	$L^{C}_{9,7}$
	day^{-1}	*day*$^{-1}$	*days*	*day*$^{-1}$		*day*	*day*$^{-1}$		*day*$^{-1}$	*day*$^{-1}$
Normals										
J.Pr.	25.0 ±1.1	6.25	0.16 ±0.01	8.0 ±0.2	1.00 ±0.22	0.25 ±0.05	0.13 ±0.01	0.005 ±0.001	0.40	2.10 ±3.00
C.Br.	11.0	2.75	0.36	4.5	1.00	0.38	0.22	0.020	0.60	2.20
S.Sa.	17.0	4.25	0.24	15.0	1.00	0.13	0.30	0.020	0.41	4.60
W.Du.	16.0	4.00	0.25	10.0	0.42	0.25	0.13	0.008	0.42	3.30
Type I										
P.Pr.	4.5	1.12	0.89	4.0	0.50	0.50	0.045	0.010	0.35	0.27
L.Wy.	3.7 ±0.1	0.92	1.08 ±0.04	3.0 ±0.5	0.33 ±0.20	0.38 ±0.16	0.085 ±0.005	0.023 ±0.001	0.35	0.46 ±0.20
J.Kn.	5.5	1.37	0.73	5.5	0.20	0.13	0.022	0.004	0.82	0.57
Type II										
H.Mo.	5.0 ±0.4	1.25	0.80 ±0.06	4.0 ±1.2	0.25 ±0.35	0.13 ±0.23	0.050 ±0.004	0.010 ±0.001	0.28	1.60 ±0.60
Type III										
M.Da.	8.0	2.00	0.50	7.5	1.00	0.38	0.040	0.050	0.80	2.00
T.Pl. (1)	4.0	1.00	1.00	4.5	0.33	0.13	1.16	0.290	0.57	2.20
(2)	4.8	1.2	0.84	5.0	0.70	0.50	1.38	0.290	0.68	2.30
(3)	6.5	1.62	0.62	6.5	1.00	0.13	0.65	0.100	0.70	2.30
J.Tu.	2.8 ±0.2	0.70	1.43 ±0.06	3.0 ±0.5	1.00 ±0.30	0.13 ±0.05	0.70 ±0.10	0.250 ±0.027	0.50	2.10 ±0.40
B.Ra.	4.4	1.10	0.92	8.0	0.25	0.13	0.44	0.100	0.35	1.10
S.Ja.	4.5	1.12	0.89	2.6	0.50	0.13	0.50	0.110	0.30	1.40
Type IV										
E.We. (1)	4.0 ±0.13	1.10	1.00	3.0 ±0.2	0.51 ±0.10	0.13 ±0.06	0.12 ±0.01	0.030 ±0.002	0.40	0.44 ±0.50
(2)	4.8	1.20	0.84	6.0	0.83	0.13	0.10	0.020	0.40	0.57

[a] $L_{3,21}$ set equal to $L_{3,1}$ for all patients except type III.
[b] τ is inverse of commonly used Fractional Catabolic Rate (FCR).
[c] 20% uncertainty imposed a priori based on either literature values or separate LDL experiments.

ments between type III and type IV patients to determine the nature of the type III defect). These were pooled with the original studies to yield single sets of parameter values. The role of the special experiments in defining particular features of the model are discussed in the modeling paper (16). In view of the diverse data and information that went into the generation and the testing of the model, it is difficult to associate the confidence in the determination of any one parameter value with a particular segment of the data. The information is usually spread over the entire spectrum of the experimental observations and the imposed constraints. Thus, for example, even though apoB and apoC were not isolated in IDL, the model was able to predict these from the VLDL, LDL, HDL, and urinary excretion data.

Standard deviations for the parameter values for typical subjects are given with caution. First, nonlinear regression analysis uses linear regression approximations and the estimation of errors based on linear ex-

constants and delays

$L^c_{7,9}$	$L^c_{9,11}$	$\frac{L^c_{9,11}}{L_{11,11}}$	$\frac{L^c_{13,16}}{L_{16,16}}$	$L_{13,13}$	Dir. IDL-B Degrad. $\frac{L_{3,13}}{L_{13,13}}$	$L^c_{2,11}$	$L^c_{1,2}$	$L_{3,1}$	$L_{25,9}$	$L_{9,25}$	$L_{3,9}$
day^{-1}	day^{-1}			day^{-1}		day^{-1}	day^{-1}	day^{-1}	day^{-1}	day^{-1}	day^{-1}
3.5 ±0.9	2.0 ±3.0	0.08 ±0.13	0.10 ±0.04	7.2 ±0.5	0.0 ±0.07	0.25 ±0.04	0.70 ±0.09	0.40 ±0.06	0.60 ±0.10	0.40 ±0.13	1.5 ±0.10
3.0	2.0	0.18	0.10	3.0	0.0	0.25	0.50	0.60	0.60	0.40	1.5
2.0	4.3	0.25	0.20	2.9	0.0	0.12	0.24	0.41	0.60	0.40	1.5
3.5	3.2	0.20	0.20	6.0	0.0	0.15	0.23	0.42	0.60	0.30	1.5
2.5	0.23	0.05	0.10	1.3	0.20	0.25	0.50	0.35	0.60	0.20	2.0
3.5 ±0.8	0.37 ±0.17	0.10 ±0.05	0.20	0.9 ±0.9	0.20 ±0.08	0.25 ±0.05	0.50 ±0.10	0.35 ±0.06	0.60 ±0.37	0.10 ±0.11	2.2 ±0.4
3.5	0.55	0.10	0.10	3.0	0.48	0.10	0.43	0.82	0.30	0.10	2.2
5.0 ±1.5	1.50 ±0.60	0.30 ±0.12	0.20 ±0.09	1.6 ±0.35	0.20 ±0.10	0.10 ±0.02	0.20 ±0.04	0.28 ±0.07	0.80 ±0.50	0.40 ±0.38	2.0 ±0.4
2.5	1.60	0.20	0.45	1.5	0.35	0.25	0.50	0.81	0.80	0.20	2.2
3.5	1.00	0.25	0.80	1.9	0.45	0.25	0.50	0.50	0.60	0.30	1.7
4.5	0.95	0.20	0.25	1.5	0.46	0.25	0.50	0.60	0.60	0.30	2.5
2.0	1.60	0.25	1.00	2.2	0.53	0.25	0.50	0.60	0.80	0.40	2.2
4.5 ±2.1	1.40 ±0.40	0.50 ±0.24	0.15 ±0.36	1.0 ±0.13	0.60 ±0.07	0.25 ±0.04	0.50 ±0.08	0.55 ±0.14	0.60 ±0.24	0.20 ±0.14	1.8 ±0.3
3.5	0.65	0.15	0.40	1.5	0.40	0.67	0.66	0.55	0.60	0.20	2.0
2.0	0.90	0.20	0.40	2.5	0.28	0.22	0.29	0.65	0.80	0.20	1.8
7.5 ±2.3	0.32 ±0.50	0.08 ±0.13	0.10 ±0.03	2.6 ±0.18	0.11 ±0.07	0.50 ±0.05	0.50 ±0.10	0.40 ±0.08	0.60 ±0.54	0.30 ±0.36	2.0 ±0.3
7.5	0.48	0.10	0.10	2.6	0.36	0.50	0.50	0.40	0.60	0.30	2.7

trapolation can be quite erroneous, especially for complex systems. Second, the proposed model does not fit all the data perfectly and there are some systematic deviations, so that even linear regression error estimates are somewhat questionable. Furthermore, the necessity for some parameters is sometimes based on only a single datum having an unusual displacement from the rest and the confidence for such a datum comes from repetition of the pattern in many of the subjects. Statistically, however, such a datum may not carry much weight. In view of these various factors, estimates of uncertainties for individual studies are not necessarily reliable. We chose, therefore, to give only one set of uncertainties for a typical individual in each of the populations studied. These were derived using standard nonlinear regression techniques (35). The variation of parameter values between subjects and patterns of changes between "populations" should serve as additional qualitative indicators for the confidence in the derived values.

TABLE 6. Model

Patient	VLDL					IDL		
	M^{C}_{chain}[a] α_2-VLDL-C	M^{B}_{chain} α_2-VLDL-B	M^{B}_{21} β-VLDL-B	Total VLDL-Bcalc. / Total VLDL-Bobs.	U^{B}_{7}[c] VLDL-B Synth.	U^{B}_{13}[c] IDL-B Synth.	M^{B}_{13} IDL-B	M^{C}_{13} IDL-C
	mg/dl	*mg/dl*	*mg/dl*		*mg/day*	*mg/day*	*mg/dl*	*mg/dl*
Normal								
J.Pr.	5.6	3.3	0.1	0.71	667.	0.	2.88	0.43
C.Br.	8.8	6.9	0.5	0.82	575.	0.	5.41	0.58
S.Sa.	7.1	3.9	0.8	0.75	538.	0.	5.68	0.83
W.Da.	3.5	3.2	0.2	1.09	443.	0.	2.14	0.32
Type I								
P.Pr.	19.7	16.7	0.6	1.00	593.	0.	15.1	1.58
L.Wy.	26.0	26.8	1.9	1.23	691.	0.	27.6	4.62
J.Kn.	6.4	5.5[(2)]	0.1	1.00	199.	1700.	24.2	0.25
Type II								
H.Mo.	22.5	19.9	0.8	1.05	808.	0.	15.9	1.94
Type III								
M.Da.	6.4	6.7[(2)]	0.8	1.00	368.	731.	27.4	2.68
T.Pl. (1)	16.7	29.5[(2)]	19.2	1.00	1020.	284.	19.9	3.58
(2)	14.3	29.7[(2)]	15.0	1.00	1230.	215.	29.8	1.99
(3)	9.3	10.4[(2)]	2.4	1.00	502.	672.	19.0	4.26
J.Tu.	23.2	40.6[(2)]	14.2	1.00	1220.	650.	47.4	0.85
B.Ra.	22.3	35.5	11.0	1.00	1020.	741.	46.4	4.99
S.Ja.	13.2	21.9[(2)]	9.0	1.00	1000.	243.	12.5	1.64
Type IV								
E.We. (1)	36.8	11.5	1.1	0.40	352.	0.	4.41	1.25
(2)	39.0	17.0	0.6	0.69	628.	0.	7.82	1.13

[a] These values were taken from Col. (10) of Table 3 and were fixed in the model.
[b] The sum $M_{chain} + M_{21}$ was constrained to equal values given in Col. (8), Table 3.
[c] Steady state uncertainties are difficult to estimate. Uncertainties in U^{B}_{7} are directly related to uncertainties in VLDL-B lifespan (τ in Table 5) and the VLDL-B mass and is probably less than 20%. Error estimate for U^{B}_{13} is somewhat higher, but probably less than 30%.

TABLE 7. State

Patient	U^{B}_{7} VLDL-B Synth.	$R^{B}_{11,7}$ α_2-VLDL-B Path	$R^{B}_{21,7}$ β-VLDL-B Path	U^{B}_{13} IDL-B Synth.	$R^{B}_{3,13}$ IDL-B Degrad.	$R^{B}_{1,13}$ IDL-B→LDL-B Path	$U^{B}_{7} + U^{B}_{13}$ Total ApoB Synth.
	← APO-B →						
Normal							
J.Pr.	667	663	3	0	0	663	667
C.Br.	575	564	11	0	0	564	575
S.Sg.	538	527	11	0	0	527	538
W.Du.	443	439	4	0	0	439	443
Type I							
P.Pr.	593	587	6	0	117	470	593
L.Wy.	691	675	16	0	135	540	691
J.Kn.	199	198	1	1700	909	985	1900
Type II							
H.Mo.	808	800	8	0	160	640	808
Type III							
M.Da.	368	350	18	731	379	703	1100
T.Pl. (1)	1020	723	295	284	453	554	1300
(2)	1230	951	276	215	536	629	1450
(3)	502	456	46	672	598	530	1170
J.Tu.	1220	973	243	650	973	649	1870
B.Ra.	1020	923	92	741	666	999	1760
S.Ja.	1000	902	99	243	321	825	1240
Type IV							
E.We. (1)	352	342	10	0	38	304	352
(2)	628	616	13	0	222	394	628

steady state masses

LDL		HDL						
		For $U_9^C = 0$			For $U_7^C = 0$			
M_1[a] LDL-B	$\frac{M_2}{M_1}$ (ApoB)	U_7^C	M_9	$\frac{M_9 calc.}{M_9 obs.}$	U_9^C	M_9	$\frac{M_9 calc.}{M_9 obs.}$	$\frac{M_{25}}{M_9}$
mg/dl	*Ratio*	*mg/day*	*mg/dl*	*Ratio*	*mg/day*	*mg/dl*	*Ratio*	*Ratio*
51.8	0.36	349	7.29	0.74	499	10.4	1.04	1.50
27.0	0.50	355	6.81	0.74	532	10.1	1.11	1.50
40.2	0.50	440	13.83	1.55	660	20.7	2.32	1.50
30.6	0.65	175	3.42	0.79	250	4.9	1.12	2.00
43.0	0.50	305	4.90	0.77	548	8.8	1.39	3.00
56.7	0.50	262	4.39	1.31	433	7.2	2.13	6.00
46.0	0.23	96	1.67	0.77	156	2.7	1.25	3.00
71.1	0.50	383	5.98	1.02	536	8.4	1.42	2.00
32.7	0.50	175	3.03	1.82	329	5.7	0.34	4.00
41.0	0.50	226	4.93	0.63	336	7.3	0.94	2.00
38.9	0.50	253	3.75	0.48	393	5.8	0.74	2.00
32.8	0.50	212	3.58	0.38	446	7.5	0.79	2.00
34.5	0.50	417	7.26	0.95	583	9.5	1.32	3.00
76.0	1.00	226	4.73	0.06	355	7.4	0.95	3.00
34.7	0.76	314	4.77	0.73	596	9.0	1.38	4.00
25.5	1.00	247	4.17	0.58	314	8.9	0.73	2.00
32.5	1.00	307	3.76	0.56	417	5.1	0.75	2.00

transports (mg/day)

For $U_7^C = 0$		For $U_9^C = 0$				
U_9^C HDL-C *Synth.*	$R_{7,9}^C$ HDL-C→VLDL-C *Path*	U_7^C VLDL-C *Synth.*	$R_{7,9}^C$ HDL-C→VLDL-C *Path*	$R_{11,7}^C$ α_2-VLDL-C *Path*	$R_{13,16}^C$ VLDL-C→IDL-C *Path*	$R_{9,7}^C$ VLDL-C→HDL-C *Path*
← APO-C →						
499	1169	349	814	1161	98	107
532	1067	355	711	909	61	221
660	1319	440	885	1059	119	382
250	585	175	410	517	66	134
548	687	305	382	706	64	45
433	680	262	418	698	113	96
156	249	96	153	241	20	28
536	1343	383	956	995	98	442
329	374	175	200	367	106	115
336	689	226	466	402	182	472
393	708	253	455	487	78	299
446	405	212	194	450	253	209
584	1458	417	1043	594	22	891
355	622	226	396	622	179	182
596	662	314	349	586	150	227
314	2354	247	932	1140	96	137
417	1161	307	853	1098	89	150

Examination of the distribution of ^{125}I label in the various protein bands showed that most of it was associated with apoB and apoC. Little label was contained in a band now identified retrospectively as arginine-rich protein. Since the proposed model depends only on the tracer kinetics, it is felt that it is a good representation of the apoB and apoC subsystems. The steady state mass and flux calculations, however, are dependent—in addition to the model—on plasma VLDL, LDL, and HDL apoB and apoC concentrations. A correction based on literature estimates (37) was introduced in the calculated apoC masses to allow for the contribution from arginine-rich peptides. Only in type III patients was this correction significant. No correction was necessary for the apoB masses.

The proposed model is compatible with previously reported observations on the routes of formation and disappearance of VLDL. Independent routes of synthesis of apoB and apoC are indicated in the studies cited by Hamilton (39) on the lipoprotein lipase activator content of nascent VLDL and in the report by Windmueller, Herbert, and Levy (40) on the incorporation of labeled amino acids into apoproteins of newly synthesized VLDL in liver and intestine. A precursor–product relationship for both triglycerides (7, 41) and apoB (13) between VLDL and subfractions of higher density and lower S_f has also been demonstrated. Direct degradation of partially delipidated triglyceride-rich lipoproteins (including IDL particles), though not previously demonstrated in man, has been observed in other animals for both chylomicrons (42, 43) and VLDL (44–46).

In all subjects, except for type III patients, essentially all VLDL was metabolized via the α_2 chain-like pathway. This presumably involved delipidation through the activity of lipoprotein lipase. Such a stepwise delipidation scheme implies that VLDL particles lose some triglycerides at a delipidation site and reappear in the plasma as VLDL particles of higher density. This process is repeated a number of times until the particle reaches the IDL density range. This kinetic scheme was first proposed by Phair, et al. (15, 47) for a type IV patient. More recently Higgins and Fielding (48) have presented more direct experimental evidence in support of such a mechanism.

In the type III patients some VLDL is metabolized via the β-pathway. This pathway leads to the formation of what most likely is β-VLDL (compartment 21), which is degraded slowly. Because the turnover rate of compartment 21 is very similar to that of LDL-B, we think of β-VLDL as a triglyceride-rich, LDL-like particle. Because of limited resolution in our data, the β-pathway transition is shown as a single event.

The β-VLDL particles predicted from the kinetics seem to correspond to the "floating beta-lipoprotein" particles previously isolated in type III patients by Quarfordt, Levy, and Fredrickson (7), where a precursor–product relationship between α_2-VLDL-TG and β-VLDL-TG was also shown. Labeled arginine-rich peptides with type III VLDL (37) could not be modeled in our studies, since ARP was poorly labeled.

Our data are inadequate to evaluate the amount of apoC that accompanies apoB along the β-VLDL path. From Havel and Kane studies (37) perhaps about half of the apoC on the particles may remain with β-VLDL.

Although the β-VLDL pathway has only been found to be necessary in the type III patients, its existence in other abnormalities cannot be excluded, since we have studied only very few patients. In a type IV patient previously studied by Hammond, et al. (15, 47, 49) using leucine as an endogenous label, a diversion of VLDL to an LDL subfraction identified as S_f 17 also had to be introduced into the model. A similar observation was also made by Hazzard and Bierman (50). It is therefore possible that a branching pathway similar to that for β-VLDL may exist as a more universal mechanism of VLDL metabolism and that the particles so generated span a wide density spectrum.

The results of the analysis of the data support the idea that in normal man essentially all LDL particles are derived from VLDL and all VLDL goes to LDL. This conclusion was also reached recently by Sigurdsson, Nicoll, and Lewis (17). This is clearly different in our type III subjects in whom less than half of the synthesized VLDL-B is degraded through LDL, with the remainder being metabolized through the β-VLDL pathway and by direct IDL degradation ($L_{3,13}$, Table 5).

It is most interesting that, in all the type III subjects and in one type I (J.Kn., MCT diet), a VLDL-independent pathway for apoB synthesis was necessary to satisfy both the VLDL and LDL apoB plasma concentrations. It is not possible to determine from our data whether this pathway is via IDL or LDL because IDL apoB plasma levels were not measured. In view of the fact that the analytic ultracentrifuge patterns show large concentrations of lipoprotein particles in the IDL range for type III patients, we chose the IDL route. It is also interesting that the patients requiring direct IDL-B synthesis also require direct IDL-B degradation. It is tempting to speculate that this is more than a coincidence. Direct IDL synthesis could also be interpreted as the synthesis of VLDL particles having low lipid content and thus bordering the IDL density range.

The turnover rate of IDL-B ($L_{13,13}$, Table 5) follows a pattern similar to that of VLDL-B ($L_{12,11}$). In our

hyperlipoproteinemia patients it is down by a factor of about 3 compared to normal subjects, as is the case for VLDL. This strongly suggests that at least some IDL particles are VLDL-like and represent the tail end of the VLDL density spectrum.

There is also a high correlation between the turnover rate of X-VLDL ($L_{5,5}$) and the 'chain' rate constant ($L_{12,11}$, Table 5). This is not too surprising since much of compartment 5 radioactivity is due to lipid and therefore reflects directly the delipidation process. The fraction of X-VLDL-C that recycles to α_2-VLDL-C varies from 20 to 100% and there seems to be no particular pattern to it. The apoC recycling may be part of the more general pattern of apoC recycling associated with delipidation as seen in all studies. Why lipid recycles is not clear, unless it accompanies apoC during the VLDL–HDL exchanges.

As pointed out earlier, we could not determine the site where newly synthesized apoC first enters the system. In our type III and type IV subjects, newly synthesized apoC entry with HDL ($U_9^C \neq 0$, $U_7^C = 0$) gives a better agreement with HDL apoC masses (M_9^Ccalc/M_9^Cobs, Table 6) than first entry with VLDL ($U_7^C \neq 0$, $U_9^C = 0$), and in the other patients it is the other way around. Hamilton (39) has presented evidence to support HDL as a major first entry site for apoC. In a more recent paper by Noel and Rubinstein (51) VLDL rather than HDL was suggested as the first entry site, although a simulation using our model and their data led us to the opposite conclusion. In view of the large transport of apoC from HDL to newly synthesized VLDL ($R_{7,9}^C$, Table 7), HDL as the first entry site is more appealing. This, however, requires that there be significant compositional changes under perturbed or abnormal conditions. Better compositional data should help to resolve this.

No significant changes were seen in the rate of synthesis of apoC for the various patient populations, regardless of first entry site. Nor was there any significant change in any of the HDL-C kinetic parameters, except for the type I and IV patients. In these, there is a reduction in $L_{9,7}^C$, the rate constants for the transport of apoC from VLDL to HDL per delipidation step. The reason for this is not clear.

Several features of VLDL metabolism were common to all hypertriglyceridemic patients, regardless of the nature of the disorder. These include an increased residence time of particles in the VLDL and IDL density ranges, which, in our model, is interpreted as a reduced delipidation rate constant, and some direct degradation of IDL particles, bypassing the LDL route. A reduced delipidation rate constant was expected in the three type I patients, all of whom had a deficiency of the extrahepatic (protamine-inhibited) lipoprotein lipase (38). A reduced delipidation rate constant, however, was also found in other patients with documented normal levels of heparin releaseable extra hepatic lipoprotein lipase. This suggests that the apparent delipidation rate constant in situ is concentration dependent, consistent with observations of Quarfordt, et al. (52) and Havel et al. (53) on a decreased removal rate of VLDL triglyceride fatty acids in hypertriglyceridemic patients, and a saturable delipidation process as suggested by Reaven, et al. (4) and Brunzell, et al. (54).

Because of the limited number of observations and lack of suitable control studies, we cannot comment here on the effects of dietary perturbations on lipoprotein metabolism.

In two of the three type I patients (P.Pr., L.Wy.) the parameter values show the general features of hyperlipoproteinemia already mentioned. In addition, there is a reduced exchange of apoC between HDL and VLDL. No other features were striking. In the third patient (J.Kn.) there are a number of striking changes: an apoB synthesis pathway via IDL, reduced apoB synthesis via VLDL, and decreased HDL-C synthesis. This patient was on a medium chain triglyceride diet and it is tempting to suggest a cause–effect relationship. Obviously more studies are required to establish this.

In the type II patient (H.Mo.) a reduced turnover rate of LDL-B was observed. This is consistent with previously reported findings for type II patients (24).

Seven studies were carried out in five patients with type III hyperlipoproteinemia. In these studies, apoB synthesis was 2–3 times higher than in all other subjects, much of it (about 30–60%) directly through IDL (U_7^B, U_{13}^B, Table 7). The high levels of plasma VLDL are accounted for not only by the slowdown of the delipidation process but also by the increased synthesis rate and the large, slowly turning over β-VLDL compartment. There is also considerable direct degradation of IDL, which bypasses the LDL route. It is because of these various bypasses in the degradation of the α_2-VLDL and IDL particles that LDL-B concentrations in type III patients remain relatively normal. Thus it seems that a major defect in our type III patients is an overproduction of apoB, probably resulting in the generation of U_{13}^B and $L_{21,7}^B$. This explanation is at variance with a previously proposed explanation for the type III disorder (55) in which a defect of IDL catabolism was suggested because of the observed high levels of IDL. In addition to direct IDL apoB synthesis, IDL levels may also appear high as a result of β-VLDL contamination of the IDL moiety in the original experi-

mental separations. β-VLDL has previously been demonstrated to have a higher average density (lower mean S_f rate) than α_2-VLDL (7) and appears to clearly overlap the density range of IDL as defined in our studies.

Aside from the general pattern for hyperlipoproteinemia, no particular pattern of changes was noticed for the type IV patient (E.We.-(1) and -(2)).

Several studies represent perturbations. M.Da., a type III patient, was given nicotinic acid. Although we do not have a control study on this patient, the derived kinetic parameter values are between our type III patients and normal population values, consistent with her positive clinical response. The 'chain' rate constant is higher compared to the other type III patients, and VLDL apoB synthesis is lower (U_7^B, Table 7); however, IDL apoB synthesis U_{13}^B (Table 7), if anything, is higher. Consistent with a reduction in VLDL apoB synthesis, there is also a reduced β-VLDL ($R_{21,7}$) pathway.

Two other type III patients (T.Pl.-(3), J.Tu.) were given clofibrate (Atromid). T.Pl.-(3) had a positive clinical response and shows a kinetic response pattern similar to that of M.Da. on nicotinic acid. T.Pl. also had two other studies without clofibrate and hence is her own control. J.Tu. showed no clinical response to clofibrate and his kinetic parameters are not significantly different from the other type III patients.

There is an increase in HDL-C synthesis in M.Da. but not in T.Pl.-(3). In view of the uncertainty about the relative amounts of apoC in HDL a lower HDL-C plasma concentration level could be present in response to a lower synthesis rate.

It could be argued that the changes in our type III patients were due to obesity since these patients were the most obese. We do not think so, although our populations are too small to reject this hypothesis statistically. There is much circumstantial evidence to argue against it. For example, our most obese patient, T.Pl., who served as his own control, when put on Atromid reverted toward a more normal VLDL pattern. Patients B.Ra. and S.Ja. were not strikingly obese, yet displayed a striking type III pattern. M.Da., who had an obesity index of 1.06, still displayed some of the type III symptoms ($U_{13} = 731$, $U_7 + U_{13} = 1099$). All our patients predict β-VLDL-B masses comparable with previously reported floating β in type III patients. A more definitive answer must eventually come from new experiments.

The detailed analysis of the data obtained in these studies was possible through the rigorous modeling carried out with the aid of the SAAM program. The complexities involved in simultaneously studying the metabolism of at least two kinetically different labeled proteins in several different lipoprotein density fractions precluded the usual manual techniques for data analyses (56, 57). The formal approach allowed us to develop and quantify a composite model of apoprotein metabolism. The combination of the fitting of all the data and the emergence of patterns of changes in the kinetic parameters for the different populations and perturbations tend to strengthen our confidence in the model as well as in its predictions. It must be appreciated, however, that a good model, in addition to fitting the data and having known physiological mechanisms imbedded in it, may also contain speculative predictions for which more direct experimental evidence will be required. Further experiments to test the predictions of the model and to refine it are thus essential. In its present form, however, the model already provides new insights and a quantitative tool for examining the metabolic derangements found in several types of hyperlipoproteinemia and the effects of diet and drugs on normal and abnormal lipoprotein metabolism.

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