The metabolism of apolipoprotein B in subjects with hypertriglyceridemia and polydisperse LDL

Waldo R. Fisher, Loren A. Zech, Promode Bardalaye, Germaine Warmke, and Mones Berman

University of Florida, Departments of Medicine and Biochemistry, and Laboratory of Theoretical Biology, NCI, NIH

Abstract This research concerns the metabolism of apolipoprotein B (apoB) in hypertriglyceridemic subjects with polydisperse or heterogeneous LDL. Five subjects maintained under constant dietary control had blood samples fractionated into very low density lipoprotein (VLDL), S_f 20, S_f 10, and S_f 4 LDL, and plasma free leucine, and in three subjects VLDL was further fractionated by size. Apo B was isolated, and the masses of the plasma apo B pools were measured for these lipoproteins. Following injection of [^{3}H]leucine as a metabolic tracer the specific activity of apo B in these lipoproteins and of plasma leucine were measured over 7 or 14 days. The kinetic data were examined using multicompartmental analysis and interpreted in terms of our previous model of apo B metabolism (1975. *Federation Proc.* **24:** 2263.). Newly synthesized apo B, secreted as large VLDL, is metabolized by a delipidation chain yielding intermediate density lipoprotein (IDL), consisting of small VLDL and S_f 20 LDL, and eventually forms small, S_f 4 LDL. LDL is metabolized in a steplike process from S_f 20 to S_f 10 and S_f 4 LDL. A second major biosynthetic input of apo B enters directly into IDL and ¼ to ⅔ of newly synthesized apo B enters plasma by this route. Total apo B synthesis in these subjects is 5- to 10-fold greater than reported for normals. The rate of transport of VLDL apo B to IDL is slower than normal with a residence time which is increased about twofold; however, the VLDL apo B pool is enlarged 5- to 10-fold, and thus the quantity of apo B entering and leaving this pool per hour is much greater than in the normal. Two major pathways for apo B catabolism occur. Between 1/3 and 2/3 of apo B is metabolized through LDL, disappearing from plasma as S_f 4 LDL. The remainder of apo B disappears from plasma IDL directly. The four major findings in this kinetic study of apo B metabolism in hypertriglyceridemic subjects with polydisperse LDL are: *1*) The marked increase in apo B synthesis; *2*) the biosynthetic input of much of this apo B directly into IDL; *3*) the large catabolic pathway of apo B which leaves IDL, and *4*) the stepwise metabolism of LDL by which S_f 20, S_f 10, and S_f 4 LDL are generated.—**Fisher, W. R., L. A. Zech, P. Bardalaye, G. Warmke, and M. Berman.** The metabolism of apolipoprotein B in subjects with hypertriglyceridemia and polydisperse LDL. *J. Lipid Res.* 1980. **21:** 760–774.

Supplementary key words tritiated leucine metabolism · multicompartmental kinetic analysis · intermediate density lipoprotein · very low density lipoprotein

LDL consists of a spectrum of macromolecules found within the density range of 1.006–1.063 g/ml. In most normotriglyceridemic individuals, the predominant species of LDL exist within a narrowly delineated region of this density range, and such monodisperse LDL, isolated from an individual, is homogeneous with respect to molecular weight (1, 2). By contrast, LDL isolated from most hypertriglyceridemic subjects is heterogeneous with respect to density as well as molecular weight, and the occurrence of polydisperse LDL may be readily demonstrated upon either analytical ultracentrifugation of the total LDL fraction or upon equilibrium ultracentrifugation within a density gradient (3).

In polydisperse LDL, lipoproteins are predominantly distributed within three regions of the LDL class (4). S_f 20 LDL is a large, relatively lipid-enriched macromolecule with a molecular weight of about 4.9×10^6 and is isolated in the density interval of 1.006–1.020 g/ml. S_f 10 LDL has a molecular weight approximating 3.2×10^6 and is isolated between d 1.020 and 1.040 g/ml. S_f 4 LDL is generally the predominant species of LDL with a small molecular weight averaging 2.5×10^6 and recoverable in the 1.040–1.063 g/ml density fraction. Interestingly, S_f 4 LDL is a considerably smaller and more dense lipoprotein than are the lipoproteins found in most individuals with monodisperse LDL (2, 4).

A study of the metabolic relationship of these lipoproteins has been undertaken using tritiated leucine as an endogenous metabolic tracer, and an account of our observations with one subject was reported (5). Most studies of lipoprotein metabolism in human subjects have been performed with the use of radioiodine as an exogenous label that is chemically bonded to

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; apo B, apolipoprotein B; S.A., specific radioactivity.

an isolated lipoprotein and then reinjected into the subject (6, 7). By contrast, when using an endogenous label, it is possible to study the biosynthesis of the apoprotein directly and also to avoid possible denaturation artifacts produced during the isolation, iodination, and reinjection of the labeled lipoprotein. The recent use of selenomethionine as an endogenous tracer of lipoprotein metabolism has been reported by Eaton and Kipnis (8). In the present report, we present the kinetic data on apolipoprotein B metabolism in four additional subjects with polydisperse LDL.[1]

SUBJECT DATA

Subject 1 was a 41-year-old white female with an 11-year history of insulin-dependent diabetes and of hyperlipemia for 2 years. The patient's diabetes had been complicated by a retinal hemorrhage and intermittent symptoms of neuropathy including a Bell's palsy. During the year preceding this study she had two hospital admissions for acute pancreatitis, during which times her triglycerides rose to about 11,000 mg/dl. On these occasions, lipoprotein electrophoresis revealed a marked increase in pre-β- lipoprotein with some chylomicrons present. Subsequently the hyperlipemia responded to diet, clofibrate, and nicotinic acid; however, at the time of the present study she had been off these drugs for 1 month. Physical examination revealed no xanthoma, xanthelasma, nor arcus cornea, and was positive for hepatomegaly. There were no bruits. She weighed 67 kg. Laboratory data revealed a normal blood count. Urinalysis showed neither ketones nor proteinuria. The plasma albumin was 3.9 g/dl. Admission triglycerides and cholesterol were 4690 and 580 mg/dl, respectively, with a Type IV pattern on lipoprotein electrophoresis. The fasting level of glucose was 210 mg/dl on admission and remained at this level throughout the study.

Seven members of the patient's family had been studied; four were hypertriglyceridemic with normal cholesterols and three were diabetic. Five were also found to have LDL polydispersion. The patient was considered to have familial hyperpre-β-lipoproteinemia.

Subject 2 was a 47-year-old white male with elevated plasma triglycerides for 12 years. The triglyceride concentrations ranged between 5955 mg/dl, at which time lipoprotein electrophoresis showed a Type V pattern, and many values in the 300–400 mg/dl range, when Type IV patterns were recorded. Aside from hypertriglyceridemia he was in good health, without diabetes, without clinically evident arteriovascular disease, and with no metabolic disorders predisposing to hyperlipemia. At the time of the study he had received no medications for 1 month.

Physical examination was normal and specifically without xanthomata, xanthelasma, arcus cornea, or arterial bruits. His weight was 66 kg. Laboratory data revealed normal hematologic and urinary analyses and chemistries which were abnormal only for triglycerides (386 mg/dl) and cholesterol (188 mg/dl) with a Type IV lipoprotein electrophoretic pattern. A 3-hour glucose tolerance test yielded, respectively, values for plasma glucose and insulin as follows: fasting, 80 mg/dl, 5.5 mu/ml; 30 min, 105 and 12.5; 60 min, 170 and 41.0; 120 min, 108 and 35.5; and 180 min, 78 and 19.0.

Twenty-four individuals in this family had been studied and previously reported (3, Family #2). Six were hypertriglyceridemic, each with Type IV patterns, but only two subjects had diabetes. Eight subjects had polydisperse LDL, twelve monodisperse LDL, and four did not have LDL dispersion determined. The patient was considered to have familial hyperpre-β-lipoproteinemia.

Subject 3 was a 55-year-old white female, with insulin-dependent diabetes for 13 years and with known hyperlipemia for 4 years. One year previously, triglycerides of 1210 mg/dl and cholesterol of 396 mg/dl were measured, at which time xanthomas and hepatomegaly with fatty infiltration were noted. The patient had a myocardial infarction two years previously with subsequent coronary artery by-pass surgery. She also had extensive atherosclerotic disease demonstrable on angiography of the aorta, iliac, and femoral arteries. There was no hypertension, but hyperuricemia had been recorded. She was not receiving hyperlipemic medications.

On physical examination, she weighed 66 kg. Clusters of approximately 3 mm diameter, tuberoeruptive xanthomas were present over both elbows. There was no arcus cornea. Arterial pulses were generally diminished and femoral bruits were present. The liver measured 17.5 cm but with no splenomegaly.

Laboratory evidence showed a normal blood count and a urinalysis positive for only a trace of protein with a serum albumin of 3.2 g/dl. Blood chemistries were normal except for a fasting glucose, triglyceride, and cholesterol concentrations of 188, 1810, and 349 mg/dl, respectively. Lipoprotein electrophoresis revealed a marked increase in pre-β-lipoprotein without chylomicrons.

The family was not available for study. One brother was diabetic and died at age 63, while a second brother had a heart attack at 50 years of age. Her other sib-

[1] These studies were presented at the American Heart Association meetings in Dallas in 1978.

TABLE 1. Clinical parameters

	Subject			
	#1	#2	#3	#4
Diet				
Total calories	1314	1343	1500	1400
Carbohydrate (g/day)	220	227	239	204
Fat (g/day)	15	19	22	22
Polyunsaturated/ saturated ratio	0.40	0.45	0.95	1.1
Protein (g/day)	69	66	95	90
Leucine (g/day)	5.5	5.6	6.5	6.0
Weight (kg)				
Admission	67	66	66	87
Injection of [^{3}H]leucine	68	66	66	87
Discharge	68	65	65	86
Triglycerides (mg/dl)				
Admission	4690	386	1810	678
Injection of [^{3}H]leucine	3270	255	1200	340
Discharge	2190	204	615	268
Glucose (mg/dl)				
Admission	210	80	188	124
Injection of [^{3}H]leucine				116
Discharge	221		147	100

lings, parents, and grandparents were not known to have heart disease nor diabetes. She had no children. The diagnosis was hyperpre-β-lipoproteinemia with unknown familial involvement.

Subject 4 was a 56-year-old white female with diabetes of 4 years duration, initially treated with insulin and then by diet alone. Her hypertriglyceridemia was diagnosed 1 year previously, and her triglycerides ranged from 498 to 3680 mg/dl with corresponding cholesterols of 280 and 754 mg/dl, respectively. Four years previously she had the onset of angina and an EKG showed a previous anteroseptal infarction; subsequently she experienced episodic transient blindness of her right eye. The patient had a 10-year history of hypertension controlled medically and intermittent attacks of gout. Throughout this study she was receiving 1500 mg of clofibrate daily. On physical examination she weighed 87 kg. No xanthelasma, xanthomas, nor arcus cornea were present, and the remainder of the examination was normal with adequate pulse and no bruits.

Laboratory evaluation revealed a normal blood count and urinalysis. Blood chemistries were normal with the exception of concentrations of uric acid of 8.4 mg/dl, fasting glucose of 125 mg/dl, and triglycerides and cholesterol of 678 and 323 mg/dl, respectively. Lipoprotein electrophoresis revealed an increase in pre-β-lipoprotein and was interpreted as a Type 2B pattern. The family history revealed an absence of premature vascular disease, and longevity with no known diabetes nor hyperlipemia. None of the family were available for study. The patient was considered to have sporadic hypertriglyceridemia which could not be further classified.

Our initial subject, F.T. was a 53-year-old white male with a 15 year history of diabetes and known hyperlipemia for 5 years. An account of his medical condition and his experimental protocol while on the Clinical Research Center has been published (5). An investigation of the occurrence of hyperlipemia in three generations of the subject's family has also been reported (3, Family #1), and the findings are consistent with a diagnosis of familial combined hyperlipoproteinemia. Generally the subject presented with a Type IV phenotype that converted to a Type V pattern on one occasion when his triglycerides rose to values of about 7000 mg/dl. The polydisperse character of his LDL has been previously described (4).

CLINICAL PROTOCOL

Subjects were admitted to the Clinical Research Center for one week of metabolic stabilization prior to injection with [^{3}H]leucine. During this time each patient was maintained on a constant diet. Triglyceride, cholesterol, glucose, and other clinical chemistry determinations were performed to assess the subjects' metabolic status, plasma volume was measured, and plasma was drawn for physical characterization and measurement of plasma pool size of VLDL and LDL.

On the 6th hospital day the diet was shifted to four equal feedings given at 7 AM, 1 PM, 6 PM, and 11 PM. The following morning 4 hours after breakfast, [^{3}H]L-leucine was injected intravenously and blood-sampling commenced thereafter as indicated in the Results section. In the first of these four subjects, 1.3 mCi of [^{3}H]leucine was injected; however, it was found that smaller doses could be used, and the last two subjects received 0.20 mCi each. On the 9th day the diet was readjusted to three meals per day, and the subject was discharged on the 14th day. With two subjects, an additional blood sample was obtained a week after discharge. During the initial week of hospitalization, the total calories were adjusted to keep the subjects' weight constant. The composition of the diet was determined by the requirement for a fat intake of about 20 g daily, in order to minimize chylomicron contamination of the VLDL, and of a leucine intake of 5–6 g daily. **Table 1** summarizes these clinical parameters for the four new subjects.

METHODS

Processing of blood samples for specific activity determination

Measurement of plasma leucine specific activity. Serum separated from 5 ml of blood was made 3% in sulfosali-

cylic acid and centrifuged. The deproteinized serum was applied to a Beckman 120C amino acid analyzer in two aliquots; in one analysis, the leucine in the sample was quantitated, and in the second, the leucine was recovered by diverting the column effluent prior to entering the reaction coil. The recovered solution of leucine was lyophilized, solubilized in a triton scintillation solution (9), and the radioactivity was measured, thus permitting S.A. calculation.

Fractionation of lipoproteins. Blood samples of 50 ml were collected on ice in the presence of 500 units of heparin, 5 mg of merthiolate, 5 mg of EDTA and 10 mg of sodium azide. The plasma was dialyzed against two exchanges of 0.01 M L-leucine in 0.1 M KBr followed by one exchange against 0.1 M KBr in order to remove free [^{3}H]leucine. Lipoprotein fractionation was performed ultracentrifugally by differential density flotation at solution densities of 1.006, 1.02, 1.04 and 1.06 g/ml with recovery of VLDL, S_f 20, S_f 10, and S_f 4 LDL respectively as previously described (4).[2] Recovered LDL fractions were dialyzed against 0.05 M NH_4HCO_3 then extracted with diethylether–ethanol 3:1 (v/v); the protein pellet was dissolved in 3% SDS by warming in the presence of dilute NaOH. Aliquots were removed for protein determination and for scintillation counting.

VLDL was further fractionated by either gel filtration chromatography on Sepharose 2B (5 × 80 cm column) as described by Quarfordt et al. (10) and Sata, Havel, and Jones (11), or by sequential flotation in the swinging bucket rotor according to Lindgren et al. (12). Recovered VLDL fractions were delipidated with tetramethyl urea (13), and the insoluble apo B was washed twice with water, extracted with diethylether–ethanol 3:1 (v/v) and dissolved in 0.5 ml of 3% SDS. Aliquots of this solution were withdrawn for measurement of protein content and for radioactivity measurement. The precipitated protein, when solubilized in 3% SDS and electrophoresed on acrylamide gels, showed a predominant high molecular weight protein band.

The VLDL from subjects #1 and #2 were fractionated on the Sepharose 2B column and the elution pattern appeared similar to those previously reported (11). Five fractions were separated from the VLDL of subject 1, these being numbered sequentially as they eluted from the column with fraction 1 constituting large-sized particles. For subject 2, the amount of VLDL present was much less, and the VLDL recovered from the Sepharose column was combined into two fractions, fraction 1 comprising VLDL eluting ahead of the peak and fraction 2, VLDL eluting after the peak.

The VLDL from subject 3 was separated into three fractions ultracentrifugally. The fast floating VLDL fraction contained so little protein that it could not be analyzed. The next two fractions, however, contained ample protein and are designated as fractions 2 and 3. The serum from subject 4 contained too little VLDL to render fractionation possible, and her VLDL was studied as a single fraction.

The quantity and radioactivity of apo B were measured on aliquots of protein solubilized in 3% SDS. Protein was measured by the method of Lowry et al. (14). For scintillation counting, protein was maintained in solution by the addition of Soluene 350 (Packard) and counted using a Permablend #3 scintillator solution (Packard). Counting was performed with a Packard Tricarb scintillation spectrophotometer using an external standard, and correction to dpm was accomplished using the Wang Liquid Scintillation Data Program.

Characterization of plasma lipoproteins

In all subjects VLDL, S_f 4, 10, and 20 LDL were subjected to acrylamide gel electrophoresis in SDS or urea (15, 16). The patterns revealed the presence of apo B near the top of the gels in all samples. In the three LDL fractions this protein comprised 95% or more of the Coomassie blue-staining material, although in S_f 20 LDL some C-apoproteins were present. In VLDL the lower molecular weight staining proteins were reduced to trace proportions after purification of apo B by the tetramethyl urea procedure.

S_f 4, 10, and 20 LDL were each subjected to analytical ultracentrifugation in order to measure their flotation rates, thus confirming their assignment to these LDL classes (4). **Fig. 1** displays photographs taken during the analysis of the LDL from subject 1 to demonstrate the apparent homogeneity of the lipoproteins. These LDL were also banded by equilibrium density gradient ultracentrifugation (4), and the banding patterns for S_f 4, 10 and 20 LDL from the same subject are in **Fig. 2.**

VLDL were characterized by several criteria following fractionation. For subjects 1 and 2, the elution pattern from the Sepharose 2B column was similar to that reported by Sata et al. (11) in their studies of the size fractionation of VLDL on agarose columns. Analytical ultracentrifugation of VLDL from subject 1 revealed the early eluting fractions to have fast flotation rates which decreased progressively in the fractions eluted sequentially from the column, consistent with the fractionation of VLDL on the basis of particle size. Lipoprotein paper electrophoresis was used to monitor the column fractions in a manner similar to

[2] VLDL was removed in three successive centrifugations, in each case overlaying with KBr solution of d 1.006 g/ml, initially at 20,000 rpm for 6 hr, then twice at 40,000 rpm for 20 hr.

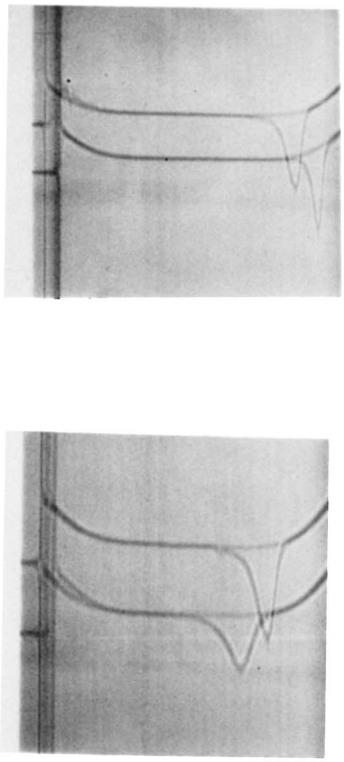

Fig. 1. Analytical ultracentrifugation of isolated LDL fractions in KBr density 1.20 g/ml at 25°C and 42,040 rpm. Top: S_f 10 LDL upper (wedged) cell and S_f 4 LDL lower cell. Bottom: S_f 10 LDL upper cell and S_f 20 lower cell.

Sata et al. (17). The patterns revealed that the larger, early eluting VLDL remained at the origin, suggesting a very low apoprotein content. Subsequent column fractions contained increasing amounts of pre-β-migrating lipoproteins, and a final fraction in which the

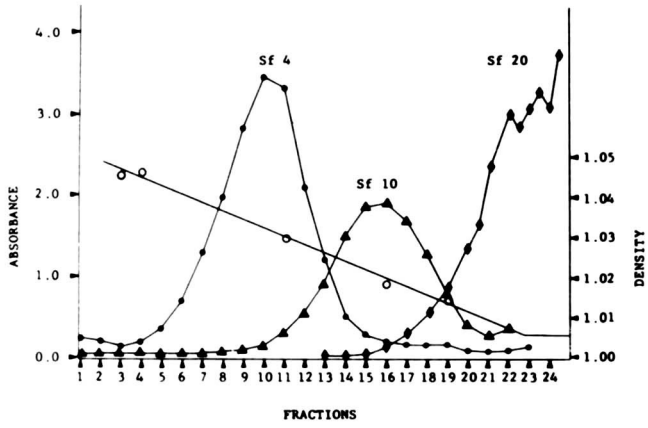

Fig. 2. Equilibrium gradient ultracentrifugation of LDL from Subject 1 in sucrose density gradient in SW 25 rotor at 25,000 rpm for 48 hr at 5°C. The banding profiles of the separately banded S_f 20, S_f 10, and S_f 4 LDL are plotted as a composite. Absorbance is measured at 280 nm. ○, density gradient.

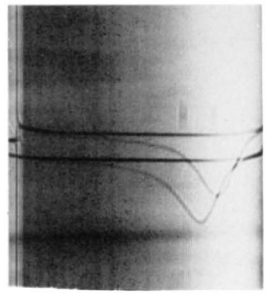

Fig. 3. Analytical ultracentrifugation of VLDL fractions 2 and 3 from Subject 3 in 1.061 g/ml KBr at 42,040 rpm and 25°C. Upper (wedged) cell, fraction 3. Lower cell, fraction 2.

mobility decreased toward that of a β-lipoprotein, suggesting a physical similarity to LDL.

Analytical ultracentrifugation was used to characterize the VLDL fractions isolated from subject 3. While three fractions were isolated by the method of sequential ultracentrifugal flotation, as described by Lindgren et al. (12), the first fraction contained so little protein that it could not be analyzed. Flotation patterns of fractions 2 and 3 are shown in **Fig. 3** and had S_f values of 81 and 60. These VLDL fractions were also examined by transmission electron microscopy using osmium tetroxide fixation in a 0.1 M sodium cacodylate buffer and sodium phosphotungstate as a negative stain.[3] The particles were spherical, and the average diameters measured approximately 490Å, 400Å, and 350Å for fractions 1, 2, and 3, respectively. Although this subject had plasma triglycerides of 1810 mg/dl, most was transported in relatively dense VLDL particles having low flotation rates and smaller diameters.

Measurement of metabolic pools

Plasma volume was measured by the Evans blue dye method as modified for use in hyperlipemic subjects (18). To determine plasma pools the concentrations of each component were multiplied by the plasma volume. Plasma leucine was measured on sulfosalicylic acid filtrates of plasma as described previously.

Plasma VLDL apo B concentration was measured by addition of a tracer of radioiodinated VLDL to a sample of plasma from which VLDL was then isolated. After delipidation with diethylether–ethanol 3:1 (v/v), the mass of VLDL apoprotein recovered was measured (14) and the proportion of radioiodinated VLDL recovered was used to calculate plasma VLDL apoprotein concentration. VLDL apo B content was meas-

[3] Directions for this procedure were provided by Dr. Trudy Forte of the Donner Laboratory, and the study was performed by Dr. Sylvia Coleman of the Gainesville Veterans Administration Hospital.

ured by the tetramethyl urea method of Kane et al. (13), from which plasma VLDL apo B could be calculated.

Plasma total LDL apoprotein concentration was similarly measured following the addition of radioiodinated LDL to plasma. The concentrations of S_f 20, S_f 10, and S_f 4 LDL were determined after flotational ultracentrifugation of plasma and recovery of the components from a known quantity of total LDL. Since the LDL apoprotein in each of these species was previously shown to be predominantly apo B (4), an observation reconfirmed with the LDL fractions of each subject by SDS acrylamide gel electrophoresis, the recovered protein in each fraction was considered to consist solely of apo B for purposes of this study.

RESULTS

Analyses

Table 2 shows the measured plasma masses for apo B in various lipoprotein pools from the four subjects. The total VLDL apo B mass for normal individuals is about 182 mg and for LDL is about 1236 mg (7). The ratio, mass of S_f 10 LDL/mass of total LDL, serves to estimate the degree of polydispersity, and these values contrast to those of normal subjects with monodisperse LDL in whom the ratio approximates one. The mass of the plasma leucine pool is also tabulated.

Fig. 4 presents the data on the S.A. of apo B isolated from VLDL and from S_f 20, 10, and 4 LDL for the four subjects. Each value is the average of duplicate determinations. VLDL was fractionated by Sepharose 2 B chromatography in two subjects, 1 and 2, and by ultracentrifugational flotation in subject 3. By both procedures VLDL was fractionated according to size with large, triglyceride-enriched fractions being recovered initially. The fractions were numbered in the order in which they were recovered. For subject 1, fraction 2 was lost; however, this fraction comprises only 2% of the total VLDL apo B mass.

Fig. 4A shows that for subject 1, all VLDL fractions rose rapidly, peaked at about the same time, but did not decay uniformly. S_f 20 LDL also had an initial rapid rise to a shoulder but then ascended to its maximum after VLDL and decayed more slowly. S_f 10 and S_f 4 LDL rose and decayed at progressively slower rates. For Subject 2, Fig. 4B, a similar pattern was observed, though because of the smaller amount of VLDL present, VLDL from the Sepharose column was only combined into two fractions.

The ultracentrifugal fractionation of VLDL from subject 3 yielded too little protein in the initial fraction to permit measurement of its S.A. VLDL-2 peaked and decayed ahead of VLDL-3 followed by S_f 20, 10, and 4 LDL in progression, Fig. 4C.

VLDL from subject 4 were not fractionated due to the small amount of apo B present; however, one again sees the sequential labeling of the LDL lipoproteins, Fig. 4D. The fact that this subject was receiving clofibrate during the study undoubtedly altered her VLDL triglyceride metabolism. However, the LDL remained polydisperse when the subject was on and off the clofibrate, and the kinetic features of her apo B metabolism were similar to the other subjects.

The plasma leucine S.A. curves for all subjects were very similar and hence only the data for subject 1 is presented in **Fig. 5A.**

TABLE 2. Plasma pool sizes and concentrations

	Subject				
	1	2	3	4	Normal
Plasma volume (ml)	2900	2520	2140	2500	
VLDL protein conc. (mg/ml)	1.98	0.75	2.58	0.92	
VLDL apo B conc. (mg/ml)	0.66	0.26	0.90	0.32	0.055[a]
LDL apo B conc. (mg/ml)	0.42	1.09	0.76	3.38	0.38[a]
Pools					
VLDL apo B (mg)	1924	644	1930	810	182[a]
Total LDL apo B (mg)	1218	2754	1626	8441	1236[a]
S_f 20 LDL apo B (mg)	319	449	341	1301	
S_f 10 LDL apo B (mg)	406	980	488	2079	
S_f 4 LDL apo B (mg)	493	1325	797	5061	
Mass of $\frac{S_f\ 10\ LDL}{Total\ LDL}$	0.33	0.36	0.30	0.25	
Plasma leucine conc. (μmol/ml)	0.130	0.106	0.183	0.151	0.15–0.23[b]
Plasma leucine pool (μmol)	377	267	392	377	

[a] Berman et al. (7).
[b] Wurtman et al. (19).

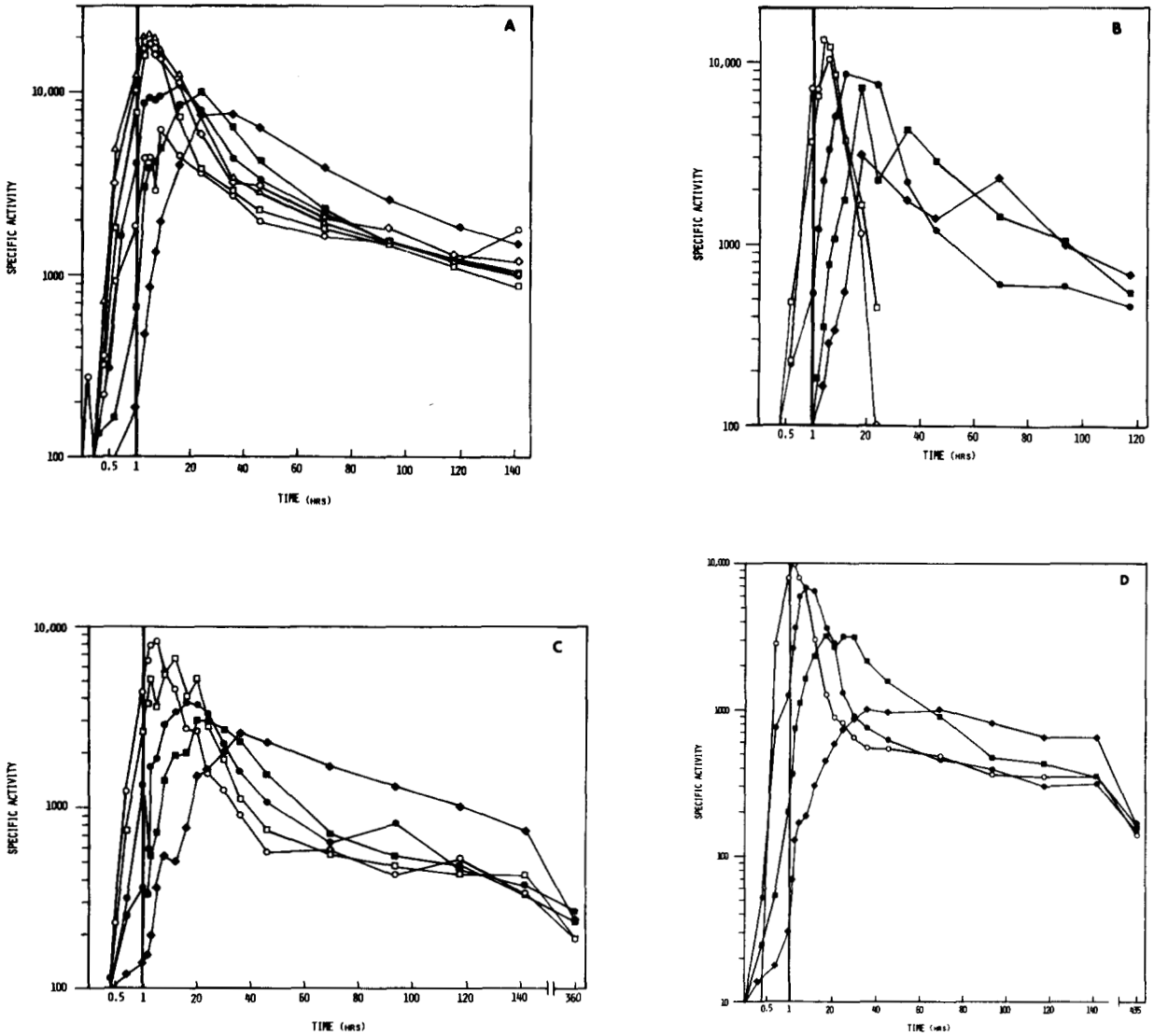

Fig. 4. Specific activities of apo B, dpm/mg protein, in VLDL and LDL for four subjects. Closed symbols, LDL fractions (●S_f 20, ■ S_f 10, and ◆ S_f 4 LDL). Open symbols, VLDL fractions as specified: A) Subject 1, ○ VLDL-1, □ VLDL-3, ◇ VLDL-4, and △ VLDL-5; B) Subject 2, ○ VLDL-1 and □ VLDL-2; C) Subject 3, ○ VLDL-2 and □ VLDL-3; and D) Subject 4, ○ total VLDL.

Model development and results of kinetic analysis

The experimental data generated in this study were subjected to multicompartmental analysis with the aid of the SAAM computer program (20). A description of the development of the model of apo B metabolism in our initial subject, F.T., has been published (5). Subsequently, the apo B model was further developed by Phair et al. (21) and Berman et al. (7). The data from our four new subjects, in combination with the previous models, have enabled us to refine this model further. The revised model is shown in **Fig. 6.**

Plasma leucine, compartment 1, is the precursor for apo B and also exchanges with all other body proteins, compartment 2. Direct measurements of labeled free leucine in plasma, Fig. 5A, permitted us to demonstrate the rapid equilibration of plasma leucine with the intracellular precursor of apo B and to define the rate constants for this subsystem. As previously modeled, apo B synthesis is depicted by a delay in series with a rapidly turning over compartment 3.

The broad peak of the VLDL apo B specific activity curve from our initial subject was accommodated by modeling VLDL as a delipidation chain in which VLDL was depicted as "a class of particles formed from one another by progressive delipidation", with IDL being formed as the end product (5). VLDL from three subjects has now been fractionated according to size, and the S.A. of apo B in these fractions again fits a model which represents VLDL by a delipidation chain

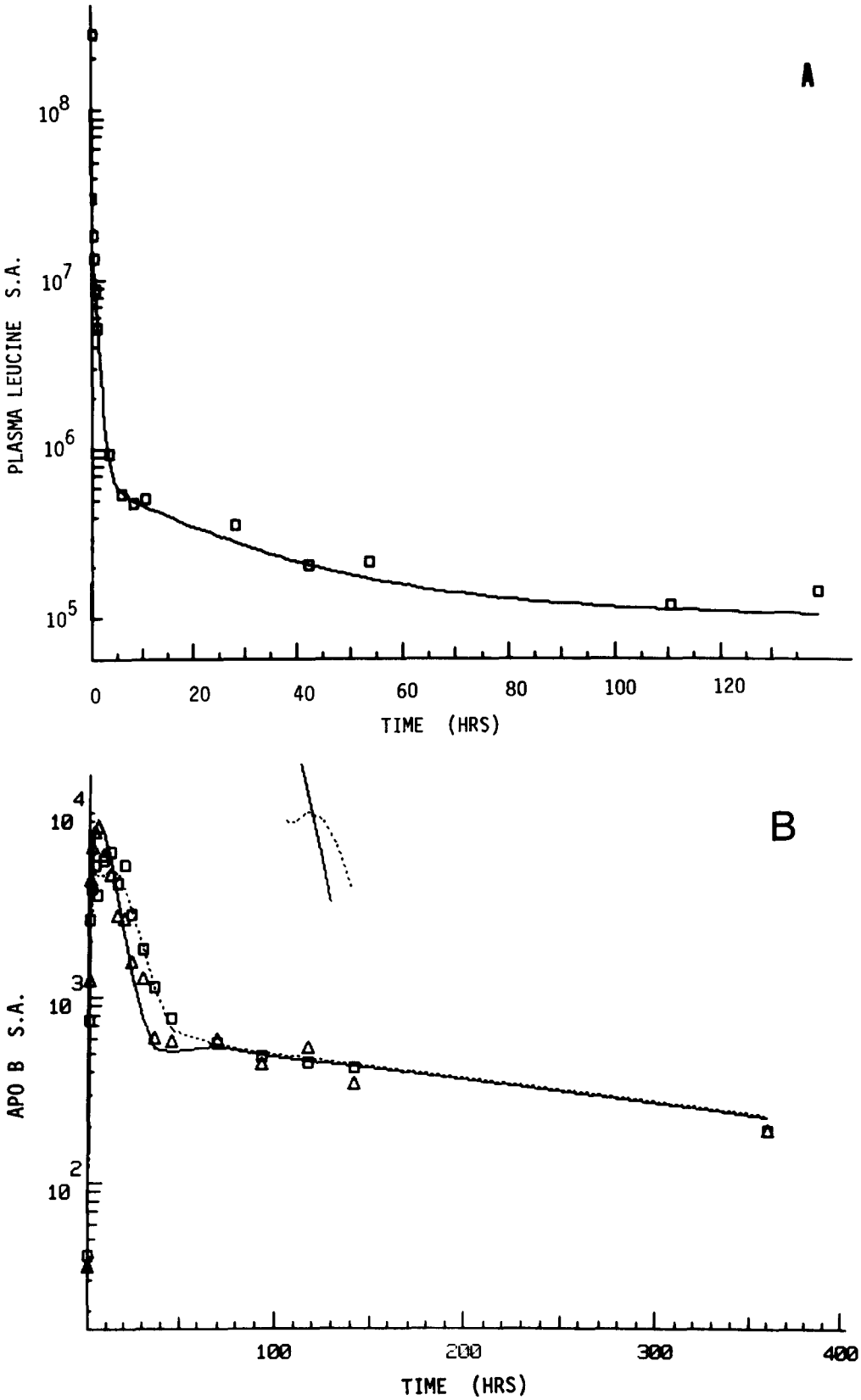

Fig. 5. A: specific activity of plasma leucine for Subject 1, dpm/mg. □, Experimental data and line is theoretical response from model for plasma leucine, compartment 1 of model, Fig. 6. B: Experimental data (△ and □) and theoretical response using model, (– – –) and (· · ·), for apo B specific activity of large VLDL-2 and small VLDL-3, respectively, from Subject 3. Inset shows an expansion of the model-generated curves for large and small VLDL apo B at the cross-over which occurs at about 15 hr and shows that large VLDL decays before small VLDL in a manner consistent with a precursor to its product.

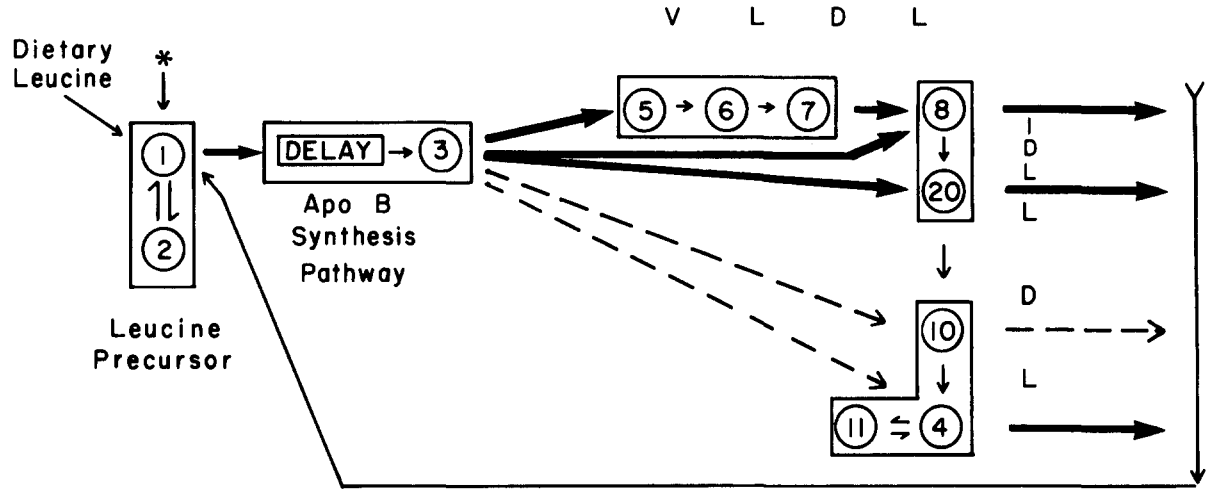

Fig. 6. Modified model of apo B metabolism in hypertriglyceridemic subjects with polydisperse LDL. Compartment 1 is plasma leucine which feeds the cellular apo B synthesis pathways and also is in equilibrium with other body protein, Compartment 2. It is into this space that the [^{3}H]leucine tracer (*) is introduced. For explanation of model, see text.

that is entered at compartment 5, but also required an input near the bottom of the chain.

In our previous model, additional pathways for the direct synthesis of apo B in LDL were recognized as necessary because of the early rise in S.A. of LDL. The magnitude of these pathways, by which apo B appears directly in plasma LDL bypassing the VLDL delipidation chain, was not fully appreciated, however, since the masses of the various plasma apolipoprotein pools had not been measured in subject F.T. In the present study the direct synthesis of apo B entering small VLDL (compartment 8), S_f 20, S_f 10, and S_f 4 LDL has been calculated and is reported in **Table 3A.**

The biosynthetic input of apo B, along pathways which enter plasma below compartment 5, can be appreciated from inspection of the kinetic curves. **Figs. 7A** and **7B** show the total VLDL and S_f 20 LDL apo B curves from subject 1. It can be seen that the S.A. rises rapidly in both fractions, suggesting kinetically similar rates of synthesis from a common precursor. VLDL apo B, however, peaks at 5 hr and then decays, while S_f 20 LDL rises to a shoulder which coincides with the VLDL peak and then slowly ascends to its maximum at 16 hr. Measurements of apo B transport show that this latter ascent results from the metabolic conversion of apo B from VLDL to S_f 20 LDL by way of the delipidation pathway. Figs. 7C and 7D show the kinetic curves for S_f 10 and 4 LDL, and again one sees the initial rapid synthesis of apo B in S_f 10 LDL: however the direct synthesis of apo B from a common precursor constitutes a minor input into this pool and, after the initial rapid rise, the S.A. ascends more slowly to peak at 24 hr, demonstrating that S_f 10 LDL is

TABLE 3. Kinetic values of apo B transport

A Biosynthesis of Apo B (mg/hr)

	Subject 1	2	3	4	Normal[a]
Total apo B synthesis	201	78	146	350	23
Entry into VLDL					
Compartment (5)	69	52	92	219	
Entry into IDL					
Compartment (8)	113	12	50	0	
Compartment (20)	12	8	2	108	
Entry into LDL					
Compartment (10)	6	3	1	13	
Compartment (4)	0	3	1	10	

B Catabolism of Apo B (mg/hr)

	Subject 1	2	3	4	Normal[a]
Total apo B catabolism	200	78	146	351	23
Disappearance from IDL					
Compartment (8)	97	24	88	86	
Compartment (20)	59	0	7	147	
Disappearance from LDL					23
Compartment (10)	0	8	4	17	
Compartment (4)	44	46	47	101	

C Residence Time of Major Plasma Apo B Pools (hr)[a]

	Subject 1	2	3	4	Normal[b]
VLDL	10.0	10.1	14.0	3.3	6.0
S_f 20 LDL	5.2	5.2	5.2	1.9	5.0
S_f 10 LDL	9.1	17.0	11.0	20.0	52.0
S_f 4 LDL	11.0	33.0	17.0	50.0	

[a] Residence time or particle lifespan is the inverse of the fractional catabolic rate for the particle.
[b] Berman et al. (7).

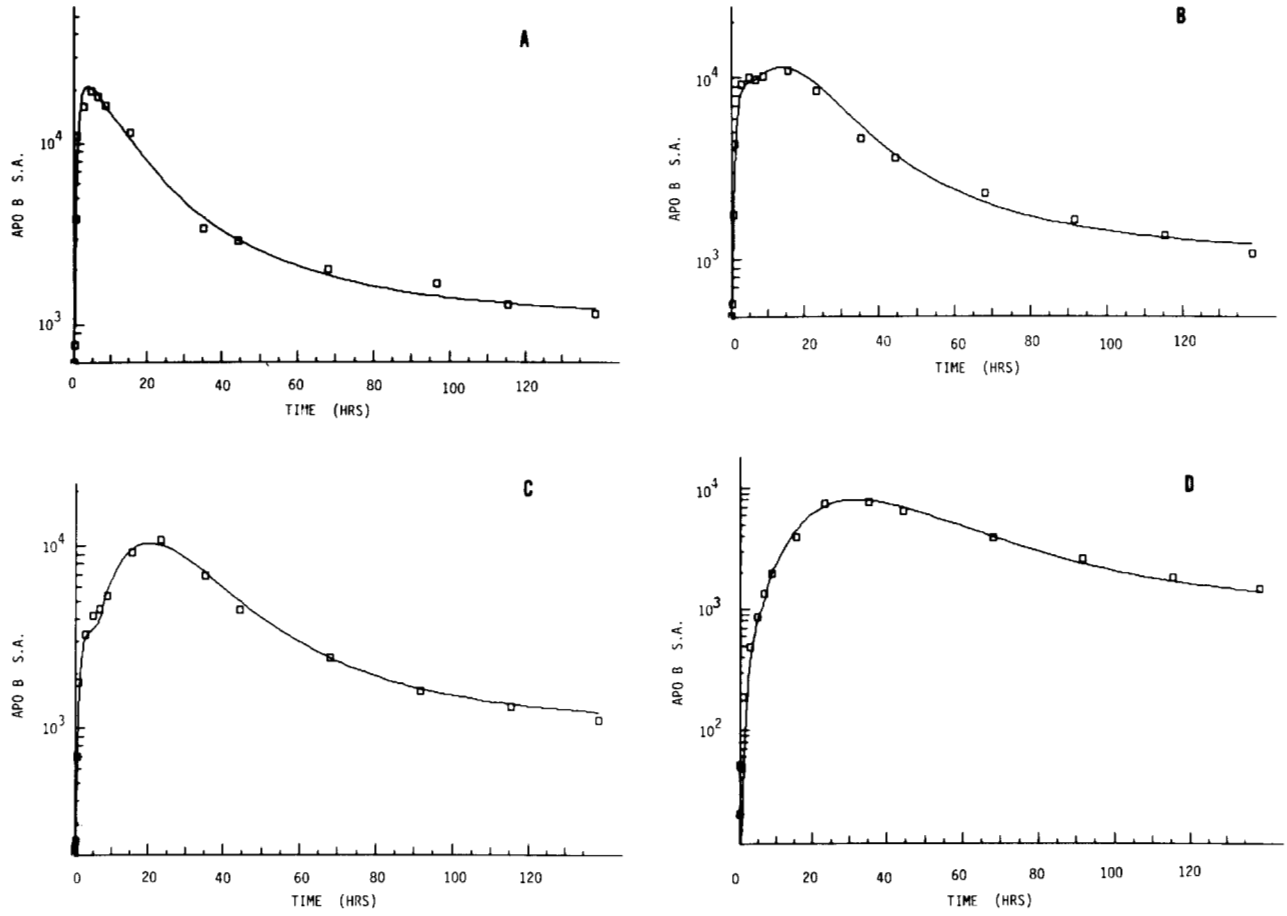

Fig. 7. Experimental specific activity data points, dpm/mg protein, and theoretical response from the model for apo B in various lipoprotein fractions of Subject 1. A), Total VLDL apo B; B), S_f 20 LDL; C), S_f 10 LDL; and D), S_f 4 LDL.

primarily the product of S_f 20 LDL catabolism. Table 3A tabulates the amount of direct synthesis of apo B into each lipoprotein fraction.

In subject 3, a pathway for the direct synthesis of apo B can be shown to feed compartment 8 or small VLDL, i.e., fraction 3 of VLDL from this subject. Fig. 5B compares the kinetic curves for large VLDL (fraction 2) and small VLDL (fraction 3) and again one sees the rapid rise in S.A. of apo B in each compartment. Large VLDL peaks around 3 hr and then decays; however, small VLDL shows a double peak with maxima at 4 and 12 hr and then decays after large VLDL, as shown in the inset to Fig. 5B. The delay in the decay of small VLDL, such that it decays behind large VLDL, is interpreted as resulting from the conversion of large to small VLDL via the delipidation chain. Thus one sees both direct and indirect synthesis of small VLDL apo B. In this subject very little direct synthesis of S_f 20 LDL apo B is evident, most of this lipoprotein being derived from small VLDL, compartment 8, as shown in Table 3A.

In our first model (5), VLDL apo B was considered to be entirely converted to LDL, and the S.A. data indicated that the LDL subspecies were formed in a unidirectional metabolic sequence: S_f 20 → S_f 10 → S_f 4 LDL, with disappearance of apo B from the plasma S_f 4 LDL compartment.[4] This precursor to product relationship is still evident for each of our current subjects, and the kinetic data are accommodated by assuming unidirectional flow of apo B. A major catabolic pathway for apo B from either small VLDL or S_f 20 LDL, compartments 8 and 20, however, became obvious when the masses of the plasma pools of these lipoproteins were measured in the four new subjects. The mass of apo B leaving these compartments was considerably greater than that entering the next compartment in the plasma LDL metabolic chain. Table 3B tabulates the magnitude of the disappearance pathways by which apo B is removed from plasma

[4] In our preliminary subject, F.T., the largest molecular weight LDL subspecies was S_f 17 LDL, whereas the four subjects in the present study have S_f 20 LDL as their predominant high molecular weight subspecies of LDL.

at varying stages in its metabolism, and it is seen that in three subjects over 2/3 of apo-B is removed from plasma without being metabolized through LDL.

It has been previously recognized the plasma LDL exchanges with the extravascular space (5), and compartment 11 accommodates this exchange. **Table 4** provides a complete list of the model rate constants and transport parameters for each of the subjects.

DISCUSSION

In this investigation apo B metabolism has been studied in five hypertriglyceridemic subjects: two with familial hyperpre-β-lipoproteinemia, one with familial combined lipoproteinemia presenting with a Type IV phenotype, and two without a pedigree. Although the experimental data and the kinetic analyses have improved as the study progressed, in all five subjects the metabolism of apo B is very similar and no qualitatively unique differences appear among the subjects.

The metabolic tracer chosen for this kinetic analysis was tritiated leucine injected intravenously, for it has been shown that extracellular leucine is a good marker for cellular protein synthesis and protein turnover studies, providing the recycling of leucine is monitored and accounted for (22, 23). Measurements of plasma leucine S.A. throughout these studies permitted us to account for recycled leucine in the kinetic analysis. The reproducibility of the experimental data obtained using tritiated leucine as a metabolic tracer was established with the first subject, in which the initial study was repeated after an interval of 3 weeks. The S.A. data obtained for VLDL and the three LDL lipoproteins were very similar on both occasions (5).

By using tritiated leucine as an endogenous tracer, it was possible to study apo B synthesis directly, which constituted a major advantage of the use of this tracer in contrast to radioiodination of circulating lipoproteins followed by their reinfusion. In the analysis of the kinetic data from our initial subject, VLDL apo B metabolism was depicted as a delipidation chain with VLDL apo B entering plasma as large, triglyceride-enriched VLDL particles and then proceeding through a delipidation cascade (5). Such a delipidation chain has also been found applicable in the analysis of VLDL

TABLE 4. Model parameters

	Subject				
	1	2	3	4	Units
R(5,3)	69 ± 0.06[a]	52 ± 0.06	92 ± 0.06	219 ± 0.08	mgB/hr
R(8,3)	113 ± 0.07	12 ± 0.19	50 ± 0.07	0	mgB/hr
R(20,3)	12 ± 0.10	8 ± 0.11	2 ± 0.08	108 ± 0.08	mgB/hr
R(10,3)	6 ± 0.09	3 ± 0.10	1 ± 0.07	13 ± 0.09	mgB/hr
R(4,3)	0	3 ± 0.10	1 ± 0.04	10 ± 0.08	mgB/hr
R(6,5)	69 ± 0.06	52 ± 0.06	92 ± 0.06	219 ± 0.08	mgB/hr
R(7,6)	69 ± 0.06	52 ± 0.06	92 ± 0.06	219 ± 0.08	mgB/hr
R(8,7)	0	52 ± 0.06	84 ± 0.07	219 ± 0.08	mgB/hr
R(0,8)	97 ± 0.11	24 ± 0.19	88 ± 0.05	86 ± 0.35	mgB/hr
R(20,8)	16 ± 0.30	39 ± 0.06	37 ± 0.06	13.4 ± 0.18	mgB/hr
R(20,7)	31 ± 0.21	0	0	0	mgB/hr
R(0,20)	59 ± 0.10	0	7 ± 0.01	147 ± 0.19	mgB/hr
R(10,20)	0	48 ± 0.05	31 ± 0.07	95 ± 0.07	mgB/hr
R(10,7)	38 ± 0.16	0	0	0	mgB/hr
R(0,10)	0	8 ± 0.03	4 ± 0.01	17 ± 0.57	mgB/hr
R(4,10)	44 ± 0.05	43 ± 0.06	38 ± 0.05	91 ± 0.09	mgB/hr
R(0,4)	44 ± 0.05	46 ± 0.05	47 ± 0.03	101 ± 0.08	mgB/hr
Delay	0.25 ± 0.01	0.50 ± 0.01	0.40 ± 0.01	0.35 ± 0.01	hr
L(6,5)	0.20 ± 0.04	0.46 ± 0.05	0.25 ± 0.03	1.17 ± 0.08	hr^{-1}
L(7,6)	0.20 ± 0.04	0.46 ± 0.05	0.25 ± 0.03	1.17 ± 0.08	hr^{-1}
L(8,7)	0	0.46 ± 0.05	0.23 ± 0.03	1.17 ± 0.08	hr^{-1}
L(0,8)	0.12 ± 0.15	0.15 ± 0.15	0.11 ± 0.13	0.46 ± 0.35	hr^{-1}
L(20,8)	0.02 ± 0.29	0.25 ± 0.09	0.04 ± 0.09	0.71 ± 0.18	hr^{-1}
L(20,7)	0.09 ± 0.19	0	0	0	hr^{-1}
L(0,20)	0.19 ± 0.15	0.15 ± 0.15	0.11 ± 0.13	0.46 ± 0.35	hr^{-1}
L(10,20)	0	0.04 ± 0.06	0.08 ± 0.05	0.07 ± 0.07	hr^{-1}
L(10,7)	0.11 ± 0.17	0	0	0	hr^{-1}
L(0,10)	0	0.01 ± 0.60	0.01 ± 0.60	0.01 ± 0.58	hr^{-1}
L(4,10)	0.11 ± 0.06	0.05 ± 0.06	0.08 ± 0.05	0.04 ± 0.08	hr^{-1}
L(0,4)	0.09 ± 0.05	0.03 ± 0.07	0.06 ± 0.03	0.02 ± 0.08	hr^{-1}

[a] ± Fractional standard deviation or coefficient of variation.

Nomenclature: R(m,n), flux of material in compartment n transported to compartment m in mg/hr; L(m,n), fraction of material in compartment n transported to compartment m per hr; L(o,n), fraction of material in compartment n lost irreversibly per hr.

apo B metabolism in studies from other laboratories using radioiodinated apo B (7, 21), and also for VLDL triglyceride as studied with a glycerol tracer (24). Thus these different metabolic tracers yield similar data.

In three of our subjects we have fractionated VLDL according to size. By fractionating VLDL as well as LDL, it is possible to delineate those sites where apo B makes its initial appearance in plasma. The kinetic data from subjects 1, 2, and 3 all show a simultaneous, early rise in the S.A. of each of the VLDL apo B fractions, and initial, rapid labeling of S_f 20 LDL is also seen in all the subjects, Figs. 4, 5B, and 7. The data imply direct synthesis of apo B containing lipoproteins of various sizes.

The magnitude of the apo B secretory pathways which bypass VLDL is appreciable, Table 3A. Observations suggesting more than one biosynthetic pathway for VLDL have been made by Streja, Kallai, and Steiner (25) in their study of VLDL triglyceride metabolism in hypertriglyceridemic humans, and in greater detail, by Zech et al. (24). Berman et al. have reported a major pathway for direct apo B synthesis into IDL in Type I and Type III subjects in studies using a ^{125}I-VLDL-apo B tracer (7). Whereas the direct synthesis of S_f 10 and S_f 4 LDL apo B constitute minor pathways in our subjects with hypertriglyceridemia, this is in apparent contrast to subjects with familial hypercholesterolemia in whom the direct synthesis of LDL apo B may become a major pathway (26).

Animals studies indicate that apo B may be synthesized by either the liver or the gut, and in this whole patient study it is impossible to determine the site of synthesis of the lipoproteins. The possibility that some of the apo B biosynthesis observed in these subjects occurs as a consequence of chylomicron production must also be considered. During the study the subjects were on a diet containing approximately 20g of fat which was dispersed in feedings throughout the day. In order to transport this lipid as chylomicrons, a maximum of no more than 17 mg/hr of chylomicron apoprotein would be required, of which only a portion is apo B. Thus, for these subjects, chylomicron synthesis does not provide a major input of nascent apo B.[5]

The metabolism of apo B in these subjects is represented as unidirectional, and the cascade feature is evident from the kinetic curves. For VLDL the kinetic curves from subject 3, Fig. 5B, provide visual evidence for the conversion of large VLDL to small VLDL. This type of observation complements the quantitative data on the mass of apo B transported through VLDL over a given time, which provides the primary justification of the delipidation pathway. The sequential nature of apo B metabolism extending from VLDL to IDL and LDL is demonstrated in the kinetic curves of all subjects, for example, subject 1, Fig. 7.

Since the size of VLDL particles reflects primarily their triglyceride content, and in these studies VLDL was fractionated by size, the model in Fig. 6 implies that apo B may be secreted with variable amounts of associated lipid and thus suggests a looser coupling between apo B and triglyceride secretion. The requirement of apo B for triglyceride secretion, however, is demonstrated by the failure to secrete significant triglyceride in the disease abetalipoproteinemia.

In nonlipemic subjects, most, if not all, of VLDL apo B is believed to be converted to LDL and then disappears from plasma (7, 27). In the study of our initial subject, we assumed this would be the case and we constrained our kinetic analysis to fit this assumption. The error in this assumption became obvious during the study of these last four subjects when the mass of apo B in each of the metabolic pools was measured, and it became evident that, for three of the four subjects, the major portion of apo B disappeared from plasma in the form of either small VLDL or S_f 20 LDL, Table 3B. Similar observations have been reported in hypertriglyceridemic subjects by Reardon, Fidge, and Nestel (28), who demonstrated a major catabolic pathway for small VLDL particles, and by Berman et al. (7), who showed a similar pathway in Types II, III, and IV subjects, where apo B departs from plasma IDL.

In our model, Fig. 6, we have designated small VLDL and S_f 20 LDL, compartments 8 and 20, as IDL. A class of lipoproteins in man isolatable within the density range of 1.006–1.019 g/ml was initially observed at the Donner laboratory (29, 30), confirmed by Shore and Shore (31), and physically characterized by Hammond and Fisher (4) and Fisher (32). Eisenberg and Rachmilewitz (33) demonstrated the accumulation of similar lipoproteins in the rat after the action of lipoprotein lipase. The hydrated density of S_f 20 LDL is 1.004 g/ml, and hence it is not surprising that recent studies in man on the fractionation of lower density lipoproteins by zonal ultracentrifugation demonstrate that IDL may also be isolated within the traditional VLDL fraction (density $<$ 1.006 g/ml) (34).

Firm evidence for IDL as an intermediary in apo B metabolism was provided by the ^{125}I-VLDL-apo B studies in man by Eisenberg et al. (35) and the [^{3}H]-leucine study from our laboratories (5). IDL is a heterogeneous fraction containing VLDL remnants and S_f 20 LDL. It has been considered a metabolic intermediate positioned between VLDL and IDL, the

[5] In subject 1, the first "VLDL" fraction eluted from the agarose column was triglyceride-rich and contained very little apo B. As seen in Fig. 4A this fraction had quite different kinetic properties from the other VLDL fractions, and we wonder if it might not be a chylomicron fraction.

end product of the action of the plasma lipases. The present findings support the placement of IDL at a complex metabolic crossroads fed both by apo B from the delipidation chain and from de novo synthesis. Metabolically, IDL is both the precursor for LDL and is also directly removed from plasma. Physically, we consider IDL to consist of the small lipoproteins of VLDL, that comprise compartment 8, and the fraction of large LDL, S_f 20 LDL.

The metabolic fate of IDL which disappears from compartments 8 and 20 is unknown in man. Recently, Gianturco et al. (34) have reported that VLDL and IDL isolated from hypertriglyceridemic subjects will suppress cholesterol biosynthesis in fibroblasts, presumably secondarily to binding to the LDL receptor on the fibroblast plasma membrane, while VLDL isolated from normolipemic subjects fails to do so. This set of observations is consistent with the apparent differences in the metabolism of normal VLDL and hypertriglyceridemic VLDL. In three of our hypertriglyceridemic subjects, apo B, present as small VLDL or S_f 20 LDL, disappears from plasma at a rate of approximately 100 mg/hr or greater, Table 3B. Interestingly, the fractional rate of irreversible loss for these pathways, L (0,8) and L (0,20), averages 0.17 hr^{-1}, as compared to an average fractional rate for apo B disappearance from S_f 4 LDL, L(0,4), of 0.05 hr^{-1}, indicating a more efficient removal of IDL than of LDL, Table 4.

Even with this major direct catabolic pathway for apo B from IDL, the quantity of apo B transported through S_f 10 to S_f 4 LDL remains large. The loss of apo B from plasma S_f 10 LDL is minimal, as this lipoprotein is almost totally converted to S_f 4 LDL before being removed from the plasma space. The mechanism of the conversion of S_f 20 to S_f 10 to S_f 4 LDL remains unknown. The change in composition of these lipoproteins indicates that there is no appreciable loss of protein during this metabolic sequence (4); rather, a mixture of lipids comprising cholesterol, triglyceride, and phospholipid are removed in a discontinuous process in which S_f 20 is converted to S_f 10 and then to S_f 4 LDL, as observed by the kinetic data from all five of the subjects studied.

In this study of apo B metabolism in hypertriglyceridemic subjects with polydisperse LDL, the kinetic data were derived by the use of an endogenous tritiated leucine tracer. Comparative data from nonlipemic control subjects are not available as yet. In order to explore differences between these subjects and normals, published data on subjects studied by reinjection of radioiodinated VLDL are used. In particular, the data from Berman et al. (7) are compared since the kinetic analyses in that study and in the present one are essentially the same. Tables 2–4 contain a tabulation of the major kinetic data for our four new subjects and comparable data for normal subjects derived from the study by Berman et al. (7). There are a number of striking differences. These human subjects have markedly increased VLDL apo B masses, and the total apo B synthesis is likewise strikingly increased as compared to the normal. With the exception of subject 4, the quantity of apo B entering the VLDL delipidation chain, compartment 5, is only moderately increased, and the major increase in apo B synthesis in subjects 1 and 3 enters IDL. Since this pathway has not been previously quantitated in either Type IIB or Type IV subjects, no comparisons can be made with the literature; however, this appears a major pathway for apo B synthesis. Despite the large mass of apo B in VLDL, Table 2, the residence time of this apoprotein within VLDL is only moderately increased compared to normal values, Table 3C. This increase in residence time presumably arises from the large mass of apo B to be transported through VLDL per unit time.

In contrast to normal subjects, a large fraction of newly synthesized apo B entering IDL is removed from plasma without passing through LDL. Even so, the quantity of apo B which is transported through LDL is increased compared to the normal, Table 3B, though only the Type IIB subject, #4, has a markedly enlarged LDL mass, Table 2. The residence times of apo B in S_f 10 and S_f 4 LDL, Table 3C, reflect both the transport of apo B through LDL as well as the magnitude of the LDL apo B mass. Thus, one sees a strikingly increased apo B synthesis in these subjects with only a moderately increased apo B transit through LDL, for in three subjects the major portion of apo B which is synthesized is removed from plasma as IDL. An increase in apo B synthesis in hypertriglyceridemic subjects has also been emphasized by Sigurdsson, Nicoll, and Lewis (36).

It is interesting that in kinetic studies of endogenous VLDL triglyceride synthesis, using a fatty acid tracer, subjects with hypertriglyceridemia rarely show more than a 4-fold increase in triglyceride transport (37), in striking contrast to the 4- to 10-fold apparent increase in apo B synthesis observed here. The fact that in these subjects much of apo B enters IDL, and thus transports less triglyceride, may well provide an explanation for this finding and focuses the question of whether the primary metabolic defect in these hypertriglyceridemic subjects with polydisperse LDL is the overproduction of apo B. If so, then perhaps the availability of triglyceride precursors determines the extent to which apo B is secreted as VLDL, IDL, or LDL.

The lesser increases in apo B synthesis in hyper-

triglyceridemic subjects observed when using reinfused, radioiodinated VLDL as a tracer appear to constitute a discrepant finding among experimental studies. While this could reflect differences in subjects, the method of exogenous labeling with radioiodinated VLDL permits only an indirect estimate of apo B synthesis that is subject to errors that arise if the tracer fails to equilibrate with all of the pools into which newly synthesized apo B is secreted. In studies using ^{125}I-VLDL apo B, biosynthetic input into IDL has generally not been observed in hypertriglyceridemia, and the large input through this pathway in our subjects primarily accounts for the increased magnitude of apo B synthesis which we observe.

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Experimental observations which could constitute additional metabolic mechanisms by which IDL may be directly removed from plasma have recently been reported from several laboratories and assume particular interest in view of the large magnitude of IDL-apo B cleared directly from plasma in these hypertriglyceridemic subjects (38, 39).

REFERENCES

1. Fisher, W. R., M. G. Hammond, and G. L. Warmke. 1972. Measurements of the molecular weight variability of plasma low density lipoproteins among normals and subjects with hyper-β-lipoproteinemia. Demonstration of macromolecular heterogeneity. *Biochemistry.* **11:** 519.
2. Fisher, W. R., M. G. Hammond, M. C. Mengel, and G. L. Warmke. 1975. A genetic determinant of the phenotypic variance of the molecular weight of low density lipoprotein. *Proc. Nat. Acad. Sci. USA.* **72:** 2347–2351.
3. Hammond, M. G., M. C. Mengel, G. L. Warmke, and W. R Fisher. 1977. Macromolecular dispersion of human plasma low-density lipoproteins in hyperlipoproteinemia. *Metabolism* **26:** 1231–1242.
4. Hammond, M. G., and W. R. Fisher. 1971. The characterization of a discrete series of low density lipoproteins in the disease, hyper-pre-β-lipoproteinemia. *J. Biol. Chem.* **246:** 5454–5465.
5. Phair, R. D., M. G. Hammond, J. A. Bowden, M. Fried, W. R. Fisher, and M. Berman. 1975. A preliminary model for human lipoprotein metabolism in hyperlipoproteinemia. *Federation Proc.* **34:** 2263–2270.
6. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
7. Berman, M., M. Hall III, R. I. Levy, S. Eisenberg, D. W. Bilheimer, R. D. Phair, and R. H. Goebel. 1978. Metabolism of apo B and apo C lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **19:** 38–56.
8. Eaton, R. P., and D. M. Kipnis. 1972. Incorporation of SE^{75} selenomethionine into a protein component of plasma very-low-density lipoprotein in man. *Diabetes.* **21:** 744–753.
9. Boyce, R. P., and J. W. Farley. 1968. Production of single-strand breaks in covalent circular λ phage DNA in superinfected lysogens by monoalkylating agents and the joining of broken DNA strands. *Virology.* **35:** 601–609.
10. Quarfordt, S. H., A. Nathans, M. Dowdee, and H. L. Hilderman. 1972. Heterogeneity of human very low density lipoproteins by gel filtration chromatography. *J. Lipid Res.* **13:** 435–444.
11. Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13:** 757–768.
12. Lindgren, F. T., A. V. Nichols, F. T. Upham, and R. D. Wills. 1962. Subfractionation of the S_f 20–10^5 lipoproteins in a swinging bucket rotor. *J. Phys. Chem.* **66:** 2007–2011.
13. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
14. Lowry, O. N., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951 Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
15. Kobylka, D., A. Khettry, B. C. Shin, and K. L. Carraway. 1972. Proteins and glycoproteins of the erythrocyte membrane. *Arch. Biochem. Biophys.* **148:** 475–487.
16. Herbert, P. N., R. S. Shulman, R. I. Levy, and D. S. Fredrickson. 1973. Fractionation of the C-apoproteins from human plasma very low density lipoproteins. *J. Biol. Chem.* **14:** 4941–4946.
17. Sata, R., D. L. Estrich, P. D. S. Wood, and L. W. Kinsell. 1970. Evaluation of gel chromatography for plasma lipoprotein fractionation. *J. Lipid Res.* **11:** 331–340.
18. Chinard, R. P., and H. A. Eder. 1948. The determination of the concentration of the dye T-1824 in normal and lipemic plasmas. *J. Exp. Med.* **87:** 473–487.
19. Wurtman, R. J., C. M. Rose, C. Chou, and F. F. Larin. 1968. Daily rhythms in the concentrations of various amino acids in human plasma. *New Engl. J. Med.* **279:** 171–176.
20. Berman, M., and M. F. Weiss. 1967. SAAM Manual. U.S. Public Health Service Publication No. 1703, Washington, DC. U.S. Government Printing Office.
21. Phair, R. D., M. Hall, D. W. Bilheimer, R. I. Levy, R. Goebel, and M. Berman. 1976. Modeling lipoprotein metabolism in man. Proc. Summer Simulation Conference. Simulation Councils, Inc., La Jolla, CA. 486–492.
22. Poole, B. 1971. The kinetics of disappearance of labeled leucine from the free leucine pool of rat liver and its

effect on the apparent turnover of catalase and other hepatic proteins. *J. Biol. Chem.* **246:** 6587–6591.
23. Glass, R. D., and D. Doyle. 1972. On the measurement of protein turnover in animal cells. *J. Biol. Chem.* **247:** 5234–5242.
24. Zech, L. A., S. M. Grundy, D. Steinberg, and M. Berman. 1979. Kinetic model for production and metabolism of very low density lipoprotein triglycerides. *J. Clin. Invest.* **63:** 1262–1273.
25. Streja, D., M. A. Kallai, and G. Steiner. 1977. The metabolic heterogeneity of human very low density lipoprotein triglyceride. *Metabolism.* **26:** 1333–1344.
26. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1978. Metabolism of apolipoprotein B-containing lipoproteins in familial hypercholesterolaemia. *Atherosclerosis.* **32:** 315–325.
27. Sigurdsson, G., A. Nicoll, and B. Lewis. 1975. Conversion of very low density lipoprotein to low density lipoprotein. *J. Clin. Invest.* **56:** 1481–1490.
28. Reardon, M. F., N. H. Fidge, and P. J. Nestel. 1978. Catabolism of very low density lipoprotein in man. *J. Clin. Invest.* **61:** 850–860.
29. Lindgren, F. T., H. A. Elliott, and J. W. Gofman. 1951. The ultracentrifugal characterization and isolation of human blood lipids and lipoproteins, with application to the study of atherolsclerosis. *J. Phys. Colloid Chem.* **55:** 80–93.
30. Nichols, A. V., E. H. Strisower, F. T. Lindgren, G. L. Adamson, and E. L. Coggiola. 1968. Analysis of the changes in ultracentrifugal lipoprotein profiles following heparin and ethyl-p-chlorophenoxyisoputyrate administration. *Clin. Chim. Acta* **20:** 122–283.
31. Shore, B., and V. Shore. 1962. Some physical and chemical properties of the lipoproteins produced by lipolysis of human serum S_f 20–400 lipoproteins by postheparin plasma. *J. Atherosclerosis.* **2:** 104–114.
32. Fisher, W. R. 1969. The characterization and occurrence of an S_f 20 serum lipoprotein. *J. Biol. Chem.* **245:** 877–884.
33. Eisenberg, S., and D. Rachmilewitz. 1975. The interaction of rat plasma very low density lipoprotein with lipoprotein lipase-rich (postheparin) plasma. *J. Lipid Res.* **16:** 341–351.
34. Gianturco, S. H., A. M. Gotto, Jr., R. L. Jackson, J. R. Patsch, H. D. Sybers, O. D. Taunton, D. L. Yeshurun, and L. C. Smith. 1978. Control of 3-hydroxy-3-methylglutaryl-CoA reductase activity in cultured human fibroblasts by very low density lipoproteins of subjects with hypertriglyceridemia. *J. Clin. Invest.* **61:** 320–328.
35. Eisenberg, S., D. W. Bilheimer, R. I. Levy, and F. T. Lindgren. 1973. On the metabolic conversion of human plasma very low density lipoprotein to low density lipoprotein. *Biochim. Biophys. Acta* **326:** 361–377.
36. Sigurdsson, G., A. Nicoll, and B. Lewis. 1976. Metabolism of very low density lipoproteins in hyperlipidaemia: studies of apolipoprotein B kinetics in man. *Europ. J. Clin. Invest.* **6:** 167–177.
37. Grundy, S. M., H. Y. I. Mok, L. Zech, D. Steinberg, and M. Berman. 1979. Transport of very low density lipoprotein triglycerides in varying degrees of obesity and hypertriglyceridemia. *J. Clin. Invest.* **63:** 1274–1283.
38. Goldstein, J. L., Y. K. Ho, M. S. Brown, T. L. Innerarity, and R. W. Mahley. 1980. Cholesterol ester accumulation in macrophages resulting from receptor-mediated uptake and degradation of hypercholesterolemic canine B-very low density lipoproteins. *J. Biol. Chem.* **255:** 1839–1848.
39. Chao, Y., E. E. Windler, G. C. Chen, and R. J. Havel. 1979. Hepatic catabolism of rat and human lipoproteins in rats treated with 17α-ethinyl estradiol. *J. Biol. Chem.* **254:** 11360–11366.