Metabolism of Apolipoproteins B-48 and B-100 of Triglyceride-rich Lipoproteins in Patients with Familial Dysbetalipoproteinemia

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Abstract

The metabolism of apolipoproteins B-48 and B-100 (apo B-48 and B-100) in large triglyceride-rich lipoproteins was studied in three adults with familial dysbetalipoproteinemia (F. dys.) and compared to that of normolipidemic subjects. One Caucasian F. dys. subject was apparently homozygous for the common form of apo E-2, (Arg_{158} → Cys), whereas the two Black subjects were homozygous for a different apo E-2 mutant (Arg_{145} → Cys), which displays much less defective binding to cells than apo E-2 (Arg_{158} → Cys).

The lipoproteins were labeled with ^{125}I and injected intravenously into fasted recipients. The results indicate that (*a*) the terminal catabolism of triglyceride-rich lipoproteins of intestinal and hepatic origin is markedly impaired in apo E2/2 homozygotes with alleles Arg_{158} → Cys and Arg_{145} → Cys; (*b*) despite long residence times, apo B-48 of chylomicrons and apo B-100 of large very low density lipoproteins are not converted appreciably to intermediate or low density lipoproteins in apo E2/2 homozygotes.

Introduction

Familial dysbetalipoproteinemia (F. dys.)[1] is a monogenic disorder characterized by accumulation of remnants of triglyceride-rich lipoproteins of both intestinal and hepatic origin in blood plasma (1, 2). Affected homozygotes who are hyperlipidemic (type III hyperlipoproteinemia) exhibit characteristic xanthomatosis and have an increased risk of premature atherosclerotic disease (3). In contrast to normal very low density lipoproteins (VLDL) which have prebeta mobility, the remnant particles in these patients have beta electrophoretic mobility and are enriched with cholesteryl esters (β-VLDL). Remnant particles are normally removed rapidly into the liver by receptor-mediated endocytosis (4). Apolipoprotein (apo) E is critical to this process (4). F. dys. is associated with homozygosity for a particular isoform of this protein (apo E-2), which is taken up poorly by the liver (5) and binds poorly to lipoprotein receptors (6). Rall and his associates have shown that the isoforms of apo E differ by amino acid substitutions in the primary structure of the protein (7). Homozygosity for the form of apo E in which cysteine replaces arginine at residue 158 in the amino acid sequence, designated E-2 (Arg_{158} → Cys), has been found most commonly in patients with F. dys. and is associated with grossly defective binding to lipoprotein receptors. The defective binding of apo E-2-containing lipoproteins to cellular receptors is thought to be responsible for the accumulation of β-VLDL, but because homozygosity for apo E-2 isoforms is often observed in the absence of hyperlipoproteinemia (8), additional genetic or environmental determinants are evidently required for the appearance of hyperlipoproteinemia. Two additional E-2 mutants, E-2 (Arg_{145} → Cys) and E-2 (Lys_{146} → Gln), bind to LDL receptors on human fibroblast receptors with about one-half of the normal affinity but are associated with classical F. dys. (9, 10).

From measurements of the rate of clearance of retinyl esters contained in chylomicrons, it has been concluded that chylomicron remnants are cleared poorly from the blood of subjects with F. dys. (11). Retinyl esters, like the other core lipid constituents of chylomicrons, can be transferred to other lipoproteins by an exchange protein, making them an uncertain marker of particle clearance (12). Apo B remains with newly secreted triglyceride (TG)-rich lipoprotein particles until they are cleared from the blood (4). We have used a recently described method (13) to study the metabolism of two species of apo B (apo B-48 and apo B-100) in TG-rich lipoproteins in F. dys. subjects possessing two different forms of apo E-2. These two apo Bs are thought to be synthesized primarily in the intestine and liver, respectively, and therefore reflect directly the metabolism of TG-rich lipoproteins originating in these organs (14). We prepared radioiodinated TG-rich lipoproteins containing apo B-48 and apo B-100 from a subject with familial lipoprotein lipase deficiency, a condition in which these lipoproteins are not normally degraded to remnant particles. These lipoproteins were injected intravenously and the rate of removal of these two forms of apo B from the blood and the extent of their appearance in lipoproteins of higher density were determined. In our earlier studies using this approach, we showed that both forms of apo B are rapidly removed from the blood of normolipidemic subjects; neither was converted to apo B of low density lipoproteins (LDL) (13).

Methods

Subjects. Three subjects with F. dys. and one normal control subject were injected intravenously with TG-rich lipoproteins from a patient with lipoprotein lipase deficiency. Their clinical characteristics are summarized in Table I. The three F. dys. subjects (R.O., W.McC., and H.S.) had tubero-eruptive xanthomas, β-VLDL, and a homozygous apo E2/

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1. *Abbreviations used in this paper:* apo, apolipoprotein; F. dys., familial dysbetalipoproteinemia; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; TG, triglyceride; VLDL, very low density lipoproteins.

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Table I. Characteristics of Recipients of TG-rich Lipoproteins

Subjects	Sex	Age	Height	Weight	Apo E Genotype	Lipid levels (mg/dl)									
						Plasma		VLDL		IDL		LDL		HDL	
						C*	TG*	C	TG	C	TG	C	TG	C	TG
		yr	*cm*	*kg*											
Normolipidemic															
R.M.	M	24	181	80.0	3/3	176	90	15	48	—	—	98‡	20‡	54	18
F. Dys.															
R.O.	M	41	179	96.2	2/2($Arg_{145} \rightarrow$ Cys)	188	128	63	86	17	7	43	10	43	14
W.McC.	M	55	185	99.9	2/2($Arg_{145} \rightarrow$ Cys)	298	397	192	330	8	5	32	16	40	27
H.S.	F	79	162	61.5	2/2($Arg_{158} \rightarrow$ Cys)§	442	395	250	315	32	13	65	23	31	14

Lipoprotein fractions were separated as described in reference 27. Total cholesterol and triglycerides were measured as described in reference 19. * C = total cholesterol. ‡ Includes IDL. § Based upon mobility of apo E in SDS PAGE (15).

2 phenotype. They had been prescribed diets restricted in fat (~30% cal) and cholesterol (~300 mg daily). Only W.McC. took a hypolipidemic drug, clofibrate, which was discontinued 1 week before this study. Total plasma cholesterol and triglyceride levels were normal in R.O. and elevated in W.McC. and H.S. W.McC. is known to have an apo E-2 variant with only moderately deficient receptor binding properties, apo E-2 ($Arg_{145} \rightarrow$ Cys) (9). Partial sequence analysis demonstrated that R.O. is also homozygous for apo E-2 ($Arg_{145} \rightarrow$ Cys). H.S. has the common apo E-2 mutant, E-2 ($Arg_{158} \rightarrow$ Cys), based upon the electrophoretic mobility of her apo E in sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) as described by Utermann et al. (15) (Fig. 1).

The subjects were admitted to the Metabolic Ward 1 d before the study and were given potassium iodide (1,000 mg/d) for 5 d to block the uptake of ^{125}I by the thyroid. The protocol was approved by the Committee on Human Research of the University of California, San Francisco. Written informed consent was obtained from each subject. Three additional normolipidemic subjects were studied separately in an identical manner. They received TG-rich lipoproteins of similar composition from the same LPL-deficient donor. Their clinical characteristics are described elsewhere (13). Data on the early removal of apo B-100 and apo B-48 from their blood have not been described and are presented here for comparison with the fourth normolipidemic subject and those with F. dys.

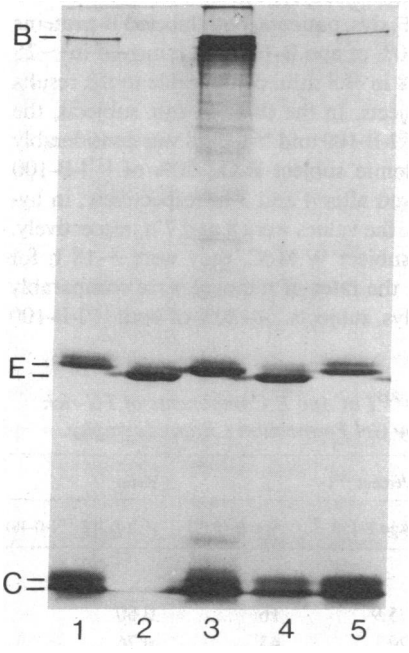

Figure 1. SDS polyacrylamide gel electrophoretograms of apo VLDL (lanes *1, 3, 4, 5*) and apo E (lane *2*) prepared and run as described by Utermann and associates (15). Lanes *1* and *5*, H.S., lane *2*, W.McC., lane *3*, A.G., lane *4*, R.O. W.McC. and R.O. have apo E ($Arg_{145} \rightarrow$ Cys) and A.G. has apo E ($Arg_{158} \rightarrow$ Cys) that binds very poorly to LDL receptors (6, 7). Note that the mobility of apo E from H.S. is the same as that of A.G. The minor apo E components of higher apparent molecular weight are sialylated forms (15).

Preparation, labeling, and analysis of TG-rich lipoproteins. We performed all procedures under sterile conditions as described (13). 4 h after the lipoprotein lipase-deficient donor, J.C. (phenotype E4/2), received a meal of 100 g olive oil dispersed in milk, ~300 ml of blood was obtained by venipuncture, mixed with 1 mg EDTA/ml and centrifuged at 1,000 *g* for 20 min at room temperature to separate formed elements. The postprandial plasma TG concentration of the donor was 1,300 mg/dl and total plasma cholesterol concentration was 239 mg/dl. 3 ml of plasma were overlayered with 0.15 M NaCl/1 mM EDTA, pH 7.4, in each of 54 polyallomer centrifuge tubes, and centrifuged in 40.3 rotors of Beckman ultracentrifuges (Beckman Instruments, Inc., Palo Alto, CA) at 25°C for 30 min at 30,000 rpm. The TG-rich particles were recovered in the top 0.75 ml. To facilitate layering with the salt solution, the density of the isolated material was increased by addition of 0.50 volume of ^2H_2O containing 1 mM EDTA. Centrifugation was then repeated under the same conditions. The TG-rich particles were labeled with ^{125}I by the iodine monochloride method (16). Unreacted iodine was removed by dialysis against 0.15 M NaCl/1 mM EDTA, pH 7.4 and Rexyn I-300 (Fisher Scientific Corp., Springfield, NJ) for 2 h at 4°C and then for 24 h in the absence of Rexyn with several changes of dialysis liquid. In previous experiments (13) we incubated the labeled TG-rich lipoproteins with the recipient's plasma and reisolated them before injection in order to exchange labeled proteins other than apo B with their counterparts in high density lipoproteins. We omitted this step in the current study because the reduction of ^{125}I in proteins other than apo B (mainly C apoproteins) was small. Apo E of the recipients also exchanged with that of the donor during these incubations, as shown by alteration of the isoform pattern of apo E. For example, incubation with plasma of M.B. (phenotype E3/3) increased the E3 component of the TG-rich lipoproteins of donor J.C. (phenotype E4/2). Tests were performed for the presence of endotoxins (Pyrogent, Mallinckrodt, St. Louis, MO) and portions were taken for culture of bacteria. We injected the labeled TG-rich lipoproteins (~50 μCi) about 60 h after the donor was bled. The total amount of TG injected into each subject was 12.3 mg and the amount of protein injected was 0.6 mg. Fractions of the injected TG-rich lipoproteins, differing in size or density, were separated by gel permeation chromatog-

raphy (17) and by density gradient ultracentrifugation (18). To determine the composition of the TG-rich lipoproteins and its fractions, TG (19), free and esterified cholesterol (20), phospholipids (21), and protein (22) were estimated. The TG-rich lipoproteins were further characterized by analytical isoelectric focusing polyacrylamide gel electrophoresis (pH 3.5–7.0) of the component proteins (23). We separated molecular species of apo B in polyacrylamide gels with a gradient from 2.5 to 27% as described (24) with some modifications (13). The percentage of total ^{125}I in apo B-100 and B-48 of the injected TG-rich lipoproteins was 7.2 and 4.5%, respectively. The calculated labeling of total apo B was 0.4 mol ^{125}I/mol, assuming a molecular mass of 400,000 D. Lipid labeling was 24.7% and 98.8% of the label was precipitated by 10% trichloroacetic acid (TCA). Electron microscopy of the TG-rich lipoproteins and its fractions was performed on samples negatively stained with potassium phosphotungstate (25). Particle diameters were measured on the photographic prints at a magnification of 180,000 (26).

Preparation of lipoproteins and isolation of apo B-100 and B-48. After injection of the TG-rich lipoproteins, the recipients were given meals containing <5 g fat (total) for the next 24 h. Blood was collected at intervals into EDTA (1 mg/ml) and chilled on ice. Preparation of the lipoprotein fractions was begun the same day. We separated lipoproteins sequentially by ultracentrifugation of 6 ml plasma in 40.3 rotors of Beckman ultracentrifuges at nonprotein solvent densities of 1.006, 1.019, and 1.063 g/ml (27). Densities were adjusted to 1.019 g/ml with ^2H_2O in 0.15 M NaCl and with KBr to 1.063 g/ml. Top fractions were collected in a volume of 1.5 ml. Radioactivity was measured in all fractions in a gamma spectrometer; recovery of total ^{125}I in the serial samples taken after injection was 91–99% for R.M., 90–103% for R.O., 88–103% for W.McC., and 86–99% for H.S. Correction was made for KBr quenching in the fractions $1.019 < d < 1.063$ g/ml and $d > 1.063$ g/ml.

Apo B-100 and apo B-48 were isolated from the $d < 1.006$ g/ml fractions by electrophoresis in polyacrylamide gradient gels (13). In this fraction, 91.2–97.7% of ^{125}I was precipitated by 10% TCA. 50 to 200 μl (100–300 μg of protein) were mixed with SDS and sucrose to give final concentrations of each of 3%, heated for 30 s in boiling water, and applied to the gels. Electrophoresis was carried out in the presence of reducing agents (1% mercaptoacetic acid and 1% mercaptopropanediol) for 16 h at 2 mA. (These reducing agents were added to prevent disulfide bridging between apo B species. The mercaptoacetic acid purges the gel of persulfate and the mercaptopropanediol remains at the top of the gel where aggregation is likely to occur.) After staining and destaining (13), the gels were sliced into six segments and radioactivity was measured in each segment, including narrow segments of 3 mm that contained apo B-100 and B-48. Electrophoresis was performed in multiple runs when necessary to obtain sufficient protein for measurement of ^{125}I.

The amount of total ^{125}I-apo B was determined in the lipoprotein fractions $1.006 < d < 1.019$ g/ml [intermediate density lipoproteins (IDL)] and $1.019 < d < 1.063$ g/ml (LDL) from the difference between ^{125}I in the total sample and ^{125}I in the isopropanol-soluble fraction (28).

Results

Characterization of the labeled TG-rich lipoproteins. The labeled lipoprotein preparation was free of endotoxin and was sterile. Its composition and that of the three fractions separated by density gradient ultracentrifugation are shown in Table II. The content of all components except triglycerides was directly related to density. The amount of ^{125}I-B-100 as compared with ^{125}I-B-48 was the lowest in the less dense fractions and fivefold higher in the fraction of highest density. The same patterns were found in five fractions separated by gel permeation chromatography (Table III). In these fractions the ^{125}I-B-100:^{125}I-B-48 ratio increased from 0.60 to 5.76 with decreasing particle diameter. Particles with diameters greater than 800 Å were separated most effectively by ultracentrifugation; those of smaller diameter by gel chromatography. From these data, it can be estimated that the median diameter of particles containing apo B-48 was about 700 Å and that of particles containing apo B-100 was about 500 Å.

Table II. Properties of Fractions of Injected Labeled TG-rich Lipoproteins Separated by Density Gradient Ultracentrifugation

	Fraction*		
	1	2	3
Composition (percent mass)			
Cholesteryl esters	3.0	7.4	10.5
TG	87.7	79.1	66.4
Cholesterol	1.8	3.1	4.5
Phospholipids	5.8	8.1	13.3
Protein	1.7	2.4	5.3
Mean diameter (Å)	1,724	868	608
Percent ^{125}I			
Apo B-100	1.3	13	85
Apo B-48	5.1	41	54
Ratio, ^{125}I-B-100:^{125}I-B-48	0.43	0.54	2.64

* Fractions of progressively increasing density were recovered from the top of the gradient after centrifugation at 35,000 rpm for 1.5 (1), 3.5 (2), and 10 h (3) (18).

Removal of apo B-100 and apo B-48 in TG-rich lipoproteins from blood plasma. The patterns of removal of ^{125}I-B-100 and ^{125}I-B-48 from the $d < 1.006$-g/ml fraction are shown in Fig. 2. Previous results in three normolipidemic subjects (13) who received labeled TG-rich lipoproteins from the same donor are included. In normolipidemic subject R.M., who received the same preparation as the F. dys. patients, both labeled B-proteins were removed rapidly: 50% of apo B-100 was removed in ~25 min and 50% of apo B-48 in ~8 min, comparable to the results in the other normal subjects. In the three F. dys. subjects, the rate of removal of both ^{125}I-B-100 and ^{125}I-B-48 was considerably retarded. In normolipidemic subject R.O., 50% of ^{125}I-B-100 and ^{125}I-B-48 was removed after 4 and 3 h, respectively; in hyperlipidemic subject H.S. the values were 8 and 7 h, respectively, and in hyperlipidemic subject W.McC. they were ~18 h for both proteins. After 4 h the rates of removal were comparably retarded in all three F. dys. subjects. 50–80% of both ^{125}I-B-100

Table III. Distribution of ^{125}I in Apo B Components of TG-rich Lipoproteins Separated by Gel Permeation Chromatography

Fraction*	Mean diameter	Percent ^{125}I		Ratio
		Apo B-100	Apo B-48	(^{125}I-B-100:^{125}I-B-48)
	Å			
1	915	5.9	16	0.60
2	666	20	43	0.76
3	563	27	22	2.09
4	470	33	15	3.61
5	465	14	4	5.76

* Fraction 1 eluted in the void volume of the column of 2% agarose gel; fractions 2–5 represent succeeding fractions that contain particles of progressively smaller diameter (17).

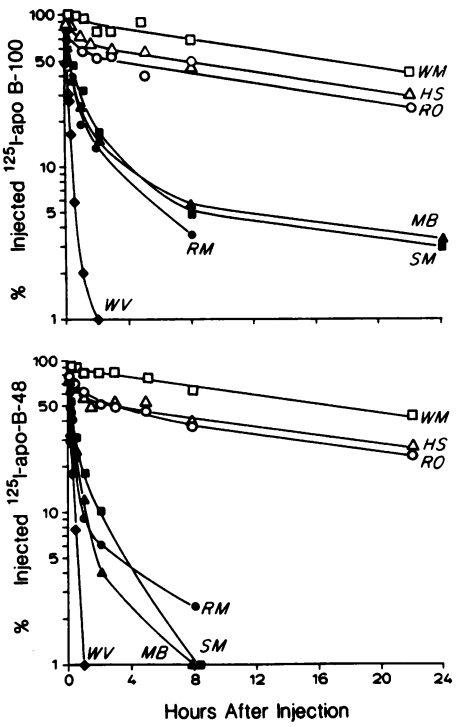

Figure 2. Removal of ^{125}I-apo B-100 and ^{125}I-apo B-48 from TG-rich lipoproteins of blood plasma of patients with F.dys. (R.O., H.S., and W.McC.) and in normal subjects W.V. (E 3/3), M.B. (E 3/3), S.M. (E 3/2), and R.M. (E-3/3). The same labeled lipoprotein preparation was given to R.M. and the three F. dys. subjects. The other normal subjects were studied previously (13). The results are expressed as percentage of the initial value, which was estimated by dividing the counts per minute of the injected ^{125}I-B-100 and ^{125}I-B-48 by the plasma volume, taken as 40 ml/kg body wt (for H.S., the plasma volume was estimated from the dilution of the injected ^{125}I in the blood sample taken 2 min after injection). For subjects of the current study, values for ^{125}I in the 2-min samples were 1,866 to 5,056 cpm/ml for B-100 and 1,214 to 3,036 cpm/ml for apo B-48. All cpm values were at least six times background counts except for apo B-100 at 8 h and apo B-48 at 1, 2, and 8 h in subject R.M.

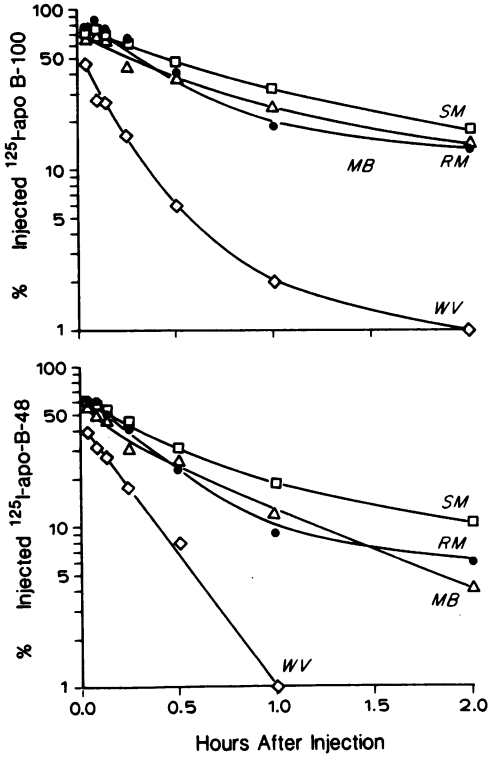

Figure 3. Removal of ^{125}I-apo B-100 and ^{125}I-apo B-48 from TG-rich lipoproteins of blood plasma of the four normolipidemic subjects during the first 2 h after injection. Data are the same as those shown in Fig. 2 and are depicted on an expanded time scale. An appreciable fraction of ^{125}I was removed from apo B-100 and apo B-48 within 2 min, in some cases followed by plateau before further removal occurred (see text).

and ^{125}I-B-48 remained in the plasma lipoproteins of $d < 1.006$ g/ml after 3 h and 25–50% after 22 h. Removal in the two subjects with the apo E-2 ($Arg_{145} \rightarrow$ Cys) mutation did not differ systematically from removal in the subject with the apo E-2 ($Arg_{158} \rightarrow$ Cys) mutation.

Fig. 3 shows the patterns of removal of ^{125}I-B-100 and ^{125}I-B-48 in the $d < 1.006$-g/ml fraction of the four normolipidemic subjects during the first 2 h after injection. It can be seen that a substantial fraction of both proteins had been removed by 2 min; for apo B-100 this was followed by a plateau for several minutes in three of the four before both proteins were removed further. A briefer plateau was evident for apo B-48 in two subjects. At 2 min 68–77% of the injected ^{125}I-B-100 and 60% of ^{125}I-B-48 was found in three subjects; in subject W.V., who had a very low serum TG level (29 mg/dl) and a very rapid removal, the values were 50 and 40%, respectively.

Conversion of ^{125}I-B-100 and ^{125}I-B-48 from the lipoprotein fraction of $d < 1.006$ g/ml to lipoproteins of higher density. The amounts of total ^{125}I-apo B in the IDL ($1.006 < d < 1.019$ g/ml) and LDL ($1.019 < d < 1.063$ g/ml) fractions are given in Table IV. The values found 2 min after injection were generally comparable to those observed when a portion of the injected TG-rich lipoproteins was mixed with the recipients' plasma in vitro to give a concentration comparable to that occurring after injection in vivo (data not shown). 1 h after injection in subject R.M., when most of the injected ^{125}I apo B had been removed from the VLDL fraction, an amount of ^{125}I equivalent to 3% of the injected ^{125}I-B-100 remained in IDL and 5% in LDL. These values can be considered upper bounds for appearance in these lipoprotein fractions, provided that rates of removal are appreciably slower than rates of formation from the injected TG-rich lipoproteins. In F. dys. subjects R.O. and W.McC., increases in ^{125}I-apo B were found in IDL after 15–90 min (4–10% in excess of the 2-min value). Little or no increase in ^{125}I-apo B was found in LDL of the three F. dys. subjects.

Discussion

The secretion of distinct B apoproteins by the human liver and intestine (14) provides a means to study the metabolism of chylomicrons and VLDL separately and to determine the fate of their remnants (13). Apo B does not appear to transfer among lipoprotein particles and can therefore serve as a marker for the particles that it contains until they leave the blood. Milne and associates have shown by immunoaffinity chromatography that apo B-100 and apo B-48 in the VLDL fraction of plasma from subjects with familial dysbetalipoproteinemia reside on distinct particles, as would be expected if they are synthesized in liver

Table IV. Total ^{125}I-Apo B in IDL (1.006 < d < 1.019 g/ml) and LDL (1.019 < d < 1.063 g/ml) after Intravenous Injection of Labeled TG-rich Lipoproteins

	Percentage remaining in blood plasma*							
	Normolipidemic		F. dys.					
	R.M.		R.O.		W.McC.		H.S.	
Minutes after injection	IDL	LDL	IDL	LDL	IDL	LDL	IDL	LDL
2	4.9	6.2	4.4	11.3	—	4.5	6.2	5.3
15	4.3	7.4	14.4	5.3	4.3	7.9	2.6	4.7
30	3.7	6.4	9.3	8.5	4.3	10.0	3.2	6.5
60	3.2	5.2	11.2	12.3	5.1	10.5	8.3	7.4
90	—	—	—	—	6.9	—	9.8	8.0
120	—	4.8	9.2	9.6	—	10.5	7.1	7.1
180	—	—	10.9	6.2	9.1	8.6	5.8	7.9
300	—	—	6.9	9.8	6.0	12.3	6.7	7.4

* Results are expressed as percentage of ^{125}I-B-100 injected (see legend to Fig. 2). Only values that exceeded background by at least 100 cpm are recorded (background was ~70 cpm).

and intestine, respectively (29). The difference in particle size distribution of labeled apo B-48 and apo B-100 (Tables II and III) suggests that this is the case for subjects with lipoprotein lipase deficiency as well.

In the normolipidemic, E3/3 subject of the current study, apo B-48 in chylomicrons was removed in a few minutes, and the removal of apo B-100 derived from the large VLDL was only slightly slower. These results are similar to those obtained earlier in normal persons (13); we suggested that the very short residence times for apo B-100 were explained by the nature of the particles injected, i.e., their large size and the fact that they have not been acted upon by lipoprotein lipase. In most studies in which the entire lipoprotein fraction of $d < 1.006$ g/ml has been labeled (for example, ref. 30), the apo B is contained predominantly in particles that are considerably smaller and contain not only nascent particles but also a variable number of remnants. Furthermore, in this as well as in our earlier study, very little labeled apo B-48 or apo B-100 was found in lipoproteins of $d > 1.006$ g/ml of normal persons, indicating that few large nascent VLDL are converted to LDL. Consistent with our findings is a recent report by Packard and associates (31). These investigators injected VLDL (S_f 100–400) obtained from subjects with endogenous hypertriglyceridemia into normolipidemic subjects. The residence times that they observed for total apo B approach the values found by us, and only a small fraction (~10%) of the apo B associated with these particles was found in LDL. These investigators concluded that a subfraction of VLDL, which is secreted from the liver as small particles, is mainly converted to LDL.

We found that only 70–80% of the apo B-100 and about 60% of apo B-48 was present in the VLDL fraction of plasma of normal subjects 2 min after injection, followed usually by a plateau during the new few minutes (Fig. 3). Only about 10% of the injected ^{125}I-apo B was recovered in other lipoprotein fractions at this time. We found close to 100% of apo B-100 in the VLDL fraction from 2 to 15 min after injection into two subjects with lipoprotein lipase deficiency (13). Therefore, mixing of the lipoprotein particles in the plasma compartment appears to be essentially complete 2 min after injection. We postulate that the early removal reflects partition of the injected TG-rich lipoproteins onto lipoprotein lipase sites on the capillary endothelium ("margination") and that, following partial lipolysis, the particles are released back into the plasma compartment. In hyperlipemic subjects, such as those with F. dys., the injected particles presumably compete with a larger number of endogenous particles, so that fewer marginate initially. The greater apparent margination of chylomicrons as compared with VLDL can be explained by the higher affinity of the former for the enzyme.

In sharp contrast to their rapid disappearance in the normolipidemic subjects was the slow rate of removal of both apo B-48 and apo B-100 in those with F. dys. This observation, particularly in R.O., who had normal total plasma lipid levels, is consistent with a profound defect in the hepatic uptake of chylomicron and VLDL remnants in F. dys. The amount of normal apo E contained in the injected TG-rich lipoproteins was negligible and presumably exchanged rapidly with that of the recipients. The initial hydrolysis of TG-rich lipoproteins and consequent formation of remnants is presumably unimpaired in F. dys., because lipoprotein lipase is normal (32, 33). This conclusion is supported by recent studies of Packard and associates (31), who injected autologous large TG-rich lipoproteins (S_f 100–400) into three hyperlipidemic F. dys. subjects. They did not separate species of apo B, but found that most of the total apo B in these lipoprotein fractions was cleared rapidly from S_f 100–400 lipoproteins and appeared in VLDL of lower S_f rates (mainly S_f 20–60), from which it was cleared slowly. In addition, a fraction of the injected apo B, which accounted for 15–39% of the total, was cleared slowly from S_f 100–400 lipoproteins. Because a slowly catabolized fraction of apo B was not evident when S_f 100–400 lipoproteins from a subject with endogenous hypertriglyceridemia were injected into an F. dys. recipient, they suggested that the slowly catabolized fraction represented chylomicron remnants present in the S_f 100–400 lipoproteins of the F. dys. subjects. Our results, which show a profound and comparable defect in the removal of apo B-48 and apo B-100 from the VLDL of F. dys. subjects, are consistent with this interpretation.

Kinetic studies with multicompartmental modeling techniques in F. dys. have suggested that apo B is directly synthesized in the IDL density range and that secretion of VLDL apo B is increased (34) or normal (35). Although direct synthesis of apo B in IDL cannot be excluded as a factor contributing to the accumulation of remnants, these studies did not take into account the structural and metabolic heterogeneity of the precursors of IDL (2, 36). Other kinetic studies in which the whole VLDL fraction was radiolabeled and injected without differentiating lipoproteins of different sources also suggested that catabolism of VLDL is impaired in F. dys. (37, 38). That a portion of the accumulated remnant particles in F. dys. originated as chylomicrons was suggested earlier from compositional analysis of fractions of triglyceride-rich lipoproteins (39) before the presence of the intestinal apo B-48 in the β-VLDL fraction was known (2, 36). An abnormality of chylomicron metabolism has been further supported by the retention in VLDL of retinyl esters which were given with an oral fat load as a marker for particles of dietary origin (11). In an extension of this observation, it was recently reported that the catabolism of chylomicrons labeled with retinyl esters is markedly retarded in normolipidemic E2/2 homozygotes (40).

The elucidation of the structure of apo E and its role in the uptake of TG-rich lipoproteins by the liver have been of major importance to our understanding of the metabolic defects in F. dys. (5–7). Apo E exhibits genetically determined polymorphism (8) produced by multiple alleles occurring at a single locus (41). These alleles are for the structural genes of apo E and specify the apo E proteins that differ by a single amino acid (7, 9, 10). As compared with normal apo E, the forms of the protein that are associated with F. dys. have reduced affinity for cellular receptors (6) and are removed slowly into perfused rat livers (5) and from the blood of subjects with F. dys. (42). Three variant apo E-2's are now known (9, 10). In addition to the common form of apo E-2 in which cysteine replaces arginine at residue 158, designated as apo E-2 ($Arg_{158} \rightarrow Cys$), cysteine-arginine interchanges have been found at residue 145 in three subjects, apo E-2 ($Arg_{145} \rightarrow Cys$) (9). All of these subjects are Black; the two who are homozygotes took part in the current study. A new structural variant recently has been reported in one subject with F. dys. with a glutamine for lysine interchange at residue 146 (10). The binding of these two additional apo E-2 mutants to LDL (apo B-100/E) receptors in vitro was found to be much less defective than that of apo E-2 ($Arg_{158} \rightarrow Cys$). Nevertheless, the severity of the defect in remnant clearance was comparable in all three of our F. dys. subjects, two with $Arg_{145} \rightarrow Cys$ and one with $Arg_{158} \rightarrow Cys$. The reason for the apparent discrepancy between the equilibrium binding affinity and metabolic behavior of the two apo E-2 variants is unclear. Our results raise the possibility that endocytosis of remnants in vivo, which occurs under nonequilibrium conditions, may not be reflected by the affinity for the receptor at equilibrium. Alternatively, the conformation of the apo E in isolated lipoproteins may differ from its conformation in lipoproteins in the blood.

As in our normolipidemic subjects, apo B-48 of chylomicrons and apo B-100 of large VLDL were not converted appreciably to LDL in our F. dys. patients, despite long residence times of these proteins. Reduced conversion of remnant particles to LDL may explain the low LDL concentrations in F. dys. (37, 38). Apo E may have a role in this conversion (4). It has recently been demonstrated that apo E-2 can impede the conversion of hepatic β-VLDL to LDL-like particles by lipoprotein lipase or hepatic lipase in vitro (43). Low LDL levels in F. dys. could also reflect increased LDL clearance due to upregulation of the LDL receptors (44) as a result of the impaired uptake of lipoprotein remnants, but the eventual site of uptake of lipoproteins that contain apo B-48 and B-100 in this disorder remains to be determined.

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