Catabolism of Very Low Density Lipoprotein B Apoprotein in Man

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ABSTRACT The turnover and the catabolic fate of the B apoprotein of very low density lipoprotein (VLDL-B) was studied in 15 normal and hyperlipidemic subjects using reinjected autologous VLDL labeled with radioiodine. The specific radioactivity-time curve of the B apoprotein in total VLDL $(S_f 20-400)$ was multiexponential but conformed to a two-pool model during the first 48 h of catabolism. The flux was highest in several hypertriglyceridemic subjects. The mass of pool A exceeded the intravascular content of VLDL-B by 30% on average, indicating extravascular metabolism of VLDL. The two-pool model might reflect the input of several populations of particles or heterogeneity of catabolic processes or pools. The flux of B apoprotein was also measured in several subclasses of VLDL, in smaller intermediate density lipoproteins, and in low density lipoproteins (LDL). In three subjects the flux was similar in $S_f 60-400$ and in $S_f 12-60$ lipoproteins, suggesting that VLDL was catabolized at least to a particle in the density range S_f 12-60. Subsequent catabolism appeared to proceed by two pathways: in normotriglyceridemic subjects, B apoprotein flux in the $S_f 20-400$ and in $S_f 12-20$ lipoproteins was similar, whereas in hypertriglyceridemic subjects flux through S_f 12-20 accounted for only part of the VLDL-B flux.

The flux of low density lipoprotein B apoprotein (LDL-B), which is believed to be derived from VLDL catabolism, was calculated from the area between the specific activity time curves of VLDL-B and LDL-B. In subjects with normal plasma triglyceride concentration, LDL-B flux was from 91% to 113% of that of VLDL-B; but in three hypertriglyceridemic subjects showing high rates of VLDL-B transport, LDL-B flux was only one-third that of VLDL-B. This suggests that

when VLDL-B flux is high, VLDL is substantially catabolized by a route other than through LDL and possibly leaves the circulation as a particle in the S_f 20–60 density range.

INTRODUCTION

The metabolism of plasma lipids is closely linked to that of the apoproteins. The influx of lipids from the liver or gut requires the secretion of the B apoprotein (1). Newly synthesized triglyceride (2), and in all probability cholesterol (3), enter plasma within very low density lipoprotein (VLDL)1 particles that, in their nascent form, may contain only the single B apoprotein (4-6). Within the plasma, VLDL acquire additional proteins, the C apoproteins (7) that are related functionally to the catabolism of VLDL. As the triglyceride is removed through the action of a lipolytic system that is regulated by the C apoproteins (8, 9), additional changes take place both to the surface and the core constituents of the lipoprotein. These include the progressive removal of the C apoproteins and enrichment with esterified cholesterol that lead to the formation of smaller cholesterol-rich lipoproteins (10, 11).

Low density lipoproteins (LDL) are believed to originate in this way. Recent studies in man by Eisenberg et al. (10) and by Sigurdsson et al. (12) have shown that possibly most of LDL B apoprotein (LDL-B) is derived from the catabolism of VLDL. Less clear is the extent to which VLDL are converted to LDL and whether VLDL B apoprotein (VLDL-B) can be cleared from the circulation through alternative pathways. During the initial stages of VLDL breakdown, lipoproteins of in-

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¹ Abbreviations used in this paper: IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LDL-B, B apoprotein of low density lipoprotein; TMU, tetramethyl urea; VLDL, very low density lipoprotein; VLDL-B, B apoprotein of very low density lipoprotein.

termediate density and size are formed (13), most of which are removed from the circulation without prior conversion to LDL in the rat (14). Excessive accumulation of a subclass of intermediate lipoproteins may reflect the failure of VLDL catabolism to proceed beyond a specific point, and it has been postulated that Type 3 hyperlipoproteinemia, which is characterized by the accumulation of a cholesterol-rich intermediate density lipoprotein, develops in this way (15).

The present study has investigated the catabolism of VLDL, or more specifically, that of its B apoprotein, in patients with and without increased concentrations of VLDL. The kinetics of VLDL removal and the transfer of B apoprotein radioactivity into intermediate lipoproteins and LDL have provided estimates of VLDL turnover in subjects with different degrees of hyper-lipoproteinemia and the extent of VLDL conversion to smaller lipoproteins. In several subjects, two subclasses of VLDL have been labeled with separate isotopes and injected together to define the probable na-

ture of the intermediate or "remnant" lipoprotein. Finally, a comparison of VLDL turnover and LDL turnover (calculated from the rate of labeling of LDL from radiolabeled VLDL) has suggested different pathways of VLDL catabolism that appear to bypass LDL.

METHODS

Materials

¹²⁵I-Na and ¹³¹I-Na, carrier free for protein iodination, were obtained from the Radiochemical Centre, Amersham, England. Urea (Univar, Ajax Co, Australia) was deionized by passing through Rexyn I-300 columns immediately before use in gel filtration or preparation of polyacrylamide gels. 1,1,3,3 tetramethyl urea (TMU) from Sigma Chemical Co., St. Louis, Mo., was redistilled twice before use and stored in dark glass bottles under N₂ at 4°C.

Subjects

15 subjects, 12 men and 3 women, were studied in the metabolic ward. Four were normolipidemic and the remainder were hyperlipidemic, as shown in Table I. Three suffered

 TABLE I

 Clinical and Lipid Status of Subjects Studied

Subject	Sex	Age	Weight	Cholesterol	Triglyceride	Lipoprotein phenotype*	Clinical status	Familial hyperlipidemia	Response to drug therapy‡
		yr	kg	mg/100 ml					
1	М	21	56	110	62	Normal	Normal		_
2	М	51	56	202	110	Normal	Normal		
3	М	54	72	230	120	Normal	Coronary disease		_
4	F	67	70	351	145	2a	Coronary disease	+(2b)§	Clofibrate+
5	М	25	69	295	150	2a	Hyperlipidemia	+(2a,2a,2b)	Clofibrate, cholestyramine+
6	F	51	55	409	209	2b	Hyperlipidemia	+(2a,2a)	Clofibrate, cholestyramine+
7	М	58	73	294	252	2b	Coronary disease	+(2b)	Clofibrate+
8	М	40	85	281	324	2b	Hyperlipidemia	+(2b,4)	Clofibrate+
9	М	69	61	266	323	4	Hyperlipidemia	Not studied	Diet alone+
10	F	62	82	213	405	4	Hyperlipidemia	Not studied	Diet alone+
11	М	44	81	365	765	4	Hyperlipidemia	+(4)	Clofibrate, nicotinic acid+
12	М	36	90	707	4,050	5	Hyperlipidemia	+(4,4)	Clofibrate+
13	М	51	58	176	77	Normal	Normal		
14	М	34	67	265	320	4	Hyperlipidemia	_	Clofibrate –
15	М	38	68	341	426	2b	Hyperlipidemia	Not studied	Clofibrate, nicotinic acid–

* World Health Organization classification (46).

‡ + denotes satisfactory lowering of plasma lipids with the treatment shown.

\$ Lipoprotein phenotype found in affected first-degree relatives; in some cases more than one affected relative was found.

from coronary heart disease and two showed abnormal glucose tolerance, though not glycosuria (3 and 12). None was receiving medication.

The presence of hyperlipidemia in other members of the family was sought in 8 of the 11 hyperlipidemic subjects, though the number of first-degree relatives available for study varied between one and four. Some subjects were deliberately studied because of the known occurrence of hyperlipidemia in other members of the family. Familial hyperlipidemia was not established in only one of the eight subjects (subject 14, who had no children and only one surviving parent who had normal plasma lipid levels). In the others, the affected relatives most commonly, but not invariably, showed a similar lipoprotein phenotype.

The response to drug treatment is also shown in Table I. As shown in Table I, some of the patients were overweight, especially those who were hypertriglyceridemic.

Preparation of radioiodinated lipoproteins

Lipoproteins were separated from 40-50 ml of plasma obtained after subjects had fasted overnight. EDTA was added to 1 mM final concentration. Chylomicrons ($S_f > 400$), if present, were removed by centrifuging plasma at 20,000 g for 30 min. VLDL (Sf 20-400) were isolated by ultracentrifugation (16) at d 1.006 g/ml for 16 h in a 40 Beckman rotor (Beckman Instruments, Inc., Fullerton, Calif.) and were washed once by flotation through an equal volume of 0.15 M NaCl, pH 7.4, containing 1 mM EDTA (0.15 M NaCl buffer). In other studies, VLDL was defined as the S_f 60-400 fraction and isolated according to Gustafson et al. (17). Lipoproteins of S_f 12-60 and $S_f 12-20$ were isolated after removing the $S_f > 60$ and $S_f > 20$ lipoproteins, respectively, by centrifugation at d 1.019 g/ml for 18 h and have been referred to as intermediate density lipoproteins (IDL). These fractions were also purified by recentrifugation at the appropriate densities. Low density lipoproteins $(S_f 0-12)$ were isolated at d 1.063 g/ml according to Havel et al. (16) and recentrifuged once; LDL refers to the d 1.019–1,063 fraction throughout.

Lipoproteins obtained by increasing the solvent density with KBr were dialyzed against 0.15 M NaCl buffer, and the protein concentrations were determined by the method of Lowry et al. (18). Any turbidity was removed by clearing with diethyl ether. 2-ml aliquots of lipoproteins containing 2-3 mg of apolipoprotein per ml were filtered through 0.45-µm Millex filters (Millipore Corp., Bedford, Mass.) and dialyzed against 0.4 M glycine:NaOH buffer, pH 10.0. Lipoproteins were iodinated with either ¹²⁵I or ¹³¹I (see Results) according to a modification of McFarlane (19), as described previously (20). All labeled preparations contained less than one atom of iodine per mole of protein (13). 89-95% of the ¹²⁵I was attached to the protein, with most of the remainder in the lipid moiety and <1% remaining unbound. Both the B and C apoproteins of VLDL were labeled by this procedure; after separation of the insoluble and soluble apoprotein with TMU, as described by Kane (21), 38-41% of the protein-bound ¹²⁵I was found associated with the B apoprotein and the remainder with the soluble proteins of VLDL S_f 20-400. The physical properties of iodinated VLDL did not appear to have been altered by iodination; 95% of the radioactivity was recovered in the d< 1.006 g/ml fraction after centrifugation of labeled VLDL and the immunoelectrophoretic properties were identical with those of unlabeled VLDL.

Radioiodinated lipoproteins were sterilized by filtration through 0.22- μ m filters.

Turnover studies

To minimize the input of VLDL apoprotein from the gut. the subjects received a diet containing less than 5% fat during the first 2 days of the studies. The carbohydrate intake was not increased to replace the fat totally to prevent an increase in hepatic VLDL production. In practice, this led to an acceptably constant concentration of VLDL-B in that the fluctuations in concentration appeared to be random. The standard deviations of the VLDL-B concentrations for the samples collected during the first 48 h in each study have been calculated and expressed as a percentage of the mean concentration: they varied from as low as 5% to as high as 23%, being mostly of the order of 8-15%. A considerable part of the variability would derive from inconstant losses during ultracentrifugation, which was confirmed from simultaneous measurements of cholesterol. Alternative dietary approaches such as virtual fasting or a eucaloric low fat diet would have led, respectively, to a reduction or to an increase in VLDL levels. With the hypocaloric diet there was no suggestion of either a progressive decrease or increase in apoprotein levels.

For the next 2 days of the turnover studies, the fat intake was less than 25% of total calories. Before and during the study, the weight and plasma lipid levels of all subjects remained steady, the weight change not exceeding 0.4 kg in any subject over 3 days. The variability in the lipid levels in whole plasma was considerably less than in the VLDL-B concentration. Potassium iodide (180 mg) was administered before, during, and after the study to inhibit thyroid uptake of ¹²⁵I. The nature of the investigations was explained, and informed consent was obtained.

Approximately 3–5 mg of apoprotein and 20–70 μ Ci of autologous sterile ¹²⁵I-labeled lipoprotein was injected into each subject in the morning after an overnight fast. Double-labeled experiments were performed in some subjects (see Results). In these studies, ¹³¹I-labeled lipoprotein containing 10–20 μ Ci in 3–5 mg of apoprotein was injected at the same time as ¹²⁵I-labeled lipoprotein. During the first 24 h, samples of blood (20 ml) were withdrawn at regular intervals through an in-dwelling cannula kept patent by infusing 0.9% NaCl solution and by venipuncture thereafter for 3–14 days, depending on the study.

Determination of B apoprotein specific radioactivity

Lipoprotein separation. Plasma samples (10 ml) were centrifuged at 4°C in the 40 rotor of a Beckman L3-50 ultracentrifuge (Beckman Instruments, Inc.). VLDL or VLDL subfractions were separated as described above. Lipoproteins of $S_r 12-20$ or $S_r 12-60$ were obtained by adjusting the density of the infranate to 1.019 g/ml after removal of the lower density VLDL fraction. LDL ($S_r 0-12$) was isolated at d 1.063 g/ml and high density lipoprotein at d 1.21 g/ml by sequential ultracentrifugation according to Havel et al. (16). All lipoproteins were washed once at the appropriate density and dialyzed against 0.15 M NaCl buffer, and aliquots were removed to determine total radioactivity and protein concentrations of each fraction.

Separation of the B apoprotein from VLDL and LDL. Isolation of B apoprotein was achieved by one of two methods. In several studies we used the technique described by Kane (21) which depends on the insolubility of the B apoprotein in TMU. The procedure was modified to obtain a direct estimate of the B apoprotein specific radioactivity. This was achieved by washing the lipid-protein pellicle once with 4.2 M TMU and twice with H_2O , followed by extraction with chloroform, methanol, and diethyl ether (see below). The insoluble B apoprotein precipitate was then dried under N_2 and dissolved in 0.1 M NaOH. Aliquots were removed for protein determination and radioassay.

Another procedure for separating B from soluble apoproteins was developed for some of these studies (subjects 2 and 7-15) and did show some advantages over the gel filtration and TMU methods and has been described elsewhere (22). The method depends on the insolubility of B apoprotein in a low ionic strength buffer (5 mM NH4HCO3). An aliquot of VLDL or LDL (containing from 0.5 to 2.0 mg of apoprotein) was dialyzed against 5 mM NH4HCO3, pH 8.0, and lyophilized. The samples were delipidated, using a modification of the chloroform, methanol, diethyl ether system described by Windmueller et al. (5). The protein precipitate was washed with methanol plus diethyl ether and then with diethyl ether alone, after which it was dried, 0.5 ml of 5 mM NH₄HCO₃ added, and the sample mixed and left overnight at room temperature. After the insoluble protein was sedimented, the supernate was removed and the pellet was washed once in 0.5 ml mM NH₄HCO₃ and centrifuged. After drying, the protein was dissolved in 0.1 M NaOH and assayed for radioactivity and protein content (18). Purified B apoprotein gave practically identical standard curves as bovine serum albumin, which was subsequently used to standardize protein assays. Similar observations have been reported by Kane et al. (23).

To validate the method described above, aliquots of some samples were also processed with TMU to compare the specific radioactivities of the B apoprotein by both methods. That this new method described above led to the isolation of B apoprotein apparently free of soluble apoprotein was established as follows. First, the presence of soluble proteins in the insoluble pellet was checked by electrophoresis on polyacrylamide gels (21), which showed only the presence of B apoprotein. Second, the amino acid composition of the NH4HCO3-insoluble protein of VLDL was compared with the insoluble protein of LDL and the protein in the first half of the first peak obtained by gel filtration (Sephadex G-200 superfine, Pharmacia Fine Chemicals Inc., Piscataway, N. J.) of apoprotein which contains only B apoprotein. Amino acid analyses were performed by the method of Spackman et al. (24) on a Beckman 120B amino acid analyzer. The amino acid compositions of the NH₄HCO₃-insoluble apoprotein of S_f 20-400, 12-20, and 0-12 lipoproteins were found to be virtually identical (22) and similar to the B apoprotein data reported by others (23). Third, the B apoprotein specific radioactivity of VLDL samples obtained during turnover studies was determined by both the TMU method and the procedure described above and found to be identical.

Determination of total plasma VLDL-B and LDL-B concentrations

Lipoproteins were isolated from duplicate 5-ml plasma samples of each subject. After removal of VLDL by ultracentrifugation, LDL were precipitated with heparin and $MnCl_2$, as described by Burstein and Samaille (25). Distribution of cholesterol and triglyceride among VLDL, LDL, and high density lipoproteins (supernate obtained after LDL precipitation) was determined using a Technicon AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (Methods N24A and N78, respectively). The VLDL-B was isolated as described above (insolubility in 5 mM NH₄HCO₃); B apoprotein in VLDL and LDL were quantified by the Lowry procedure (18).

Kinetic analyses

VLDL turnover. As shown in Figs. 1 and 5, the kinetics of VLDL-B in the $S_f 20-400$ and $S_f 60-400$ species appeared to conform more closely to a two-pool than to a one-pool model in all studies except in subject 1, who had a very low concentration of VLDL. The curves were resolved visually by curve-peeling.

The two-pool model proposed by Gurpide et al. (26) which describes the relationship between two pools, A and B, allows for independent entry and exit from both pools. As will be discussed later, the catabolism of VLDL-B may occur in a variety of tissues, as well as in the generally accepted manner by conversion to LDL within the plasma; since it seemed likely that catabolism might occur in both pools, we selected a model that incorporated efflux of material from pool B as well as from pool A. This does not imply that pool A is limited to the plasma and pool B to extravascular sites; in fact, pool A was found to extend beyond plasma. The flux of material through pool A, representing the influx of VLDL B apoprotein, is given by the equation:

$$PR_{A} = \frac{R_{A} \cdot \alpha \cdot \beta}{\beta \cdot C_{A} + \alpha \cdot C_{B}},$$

where $PR_A =$ the flux through pool A (other than material recycled through pool B) and equals input into pool A or output from both pools; $R_A =$ dose of radioactivity reinjected into pool A; α and β are the rate constants of the exponentials describing the biexponential specific radioactivity-time curve of VLDL-B (Fig. 1); and C_A and C_B are the specific radioactivities of VLDL apoprotein on the y axis which represent the intercepts of the two rate constants, α and β . It is worth noting that this equation does not require knowledge of pool sizes.



FIGURE 1 The specific activity-time curve of $^{125}\mbox{I-labeled}$ B apoprotein in Sf 20–400 VLDL. The curve has been resolved into two functions.

However, the mass of VLDL-B in pool A (M_A) can be derived from the equation:

$$M_{A} = \frac{R_{A}}{C_{A} + C_{B}}$$

The mass in pool B cannot be calculated. The model is shown in Fig. 2.

Although loss of label might well occur in tissues that can degrade apoprotein B and that may make up part of pool B, it seems highly probable that input into the plasma occurs only from the liver and gut. The VLDL in plasma, liver, and gut are likely to belong to the rapidly turning over pool A, since nascent VLDL in those organs are the precursors of plasma VLDL.

In two studies (subject 1, $S_f 20-400$; and subject 14, $S_f 12-60$) the pattern of removal of the lipoproteins appeared to be monoexponential until nearly 90% of the radioactivity had been removed from plasma. The turnover rate (k) was then calculated by the equation:

$$k = \frac{0.693}{t_{1/2}} \tag{27},$$

where $t_{1/2}$ = the half life of removal. The flux, in milligrams per day, was calculated by multiplying the turnover rate per day (k) by the intravascular pool B apoprotein in the respective lipoprotein fraction. Apparently, monoexponential decay may occur with small pools and rapid turnover rates or when the amount of injected radioactivity is small. However, whenever it was possible to calculate flux also by two-pool analysis, the two calculations differed by less than 25% (lower values being obtained with the two-pool analysis).

The approximate flux of apoprotein B in the IDL after the injection of the radiolabeled VLDL was estimated by measuring the areas under the specific activity-time curves:

$$Flux (mg/day) = \frac{Dose of injected radioactivity}{Area under specific activity-time curve (27)}.$$

If all the labeled precursor (VLDL) is converted to the product (IDL), then the dose will be common to both and the flux through IDL can be calculated. If, however, only part of the precursor is metabolized to the product, then the flux of the latter will be overestimated.

This method was also used as a comparison for values obtained by two-pool analysis of VLDL $S_f 20-400$ turnover, since the equations are similar. The agreement between the two methods of analysis averaged 95%.

LDL turnover. This was calculated in two ways. The specific radioactivity-time curve of LDL-B was obtained whenever labeled VLDL-B was injected. Since most of these studies did not extend beyond 4 days, LDL turnover was calculated as follows. The specific radioactivity-time curves described by VLDL-B and LDL-B were plotted on arithmetic paper. The two curves intersected at various times between 18 and 36 h after injecting the labeled VLDL, generally at or



FIGURE 2 Postulated two-pool model for VLDL metabolism.

near the peak specific radioactivity of LDL. This is presumptive evidence for LDL being derived from VLDL, though it does not require that all VLDL is converted to LDL (27). Recent data in man suggest that LDL-B is solely derived from VLDL-B (12). Since this is believed to occur during the intravascular catabolism of VLDL, the area between the respective specific radioactivity-time curves should reflect the catabolic rate (k) of LDL. We have calculated this value of k for LDL in 12 subjects from the first 18 h of each experiment from the equation: k (per day) = Rate of rise in LDL specific radioactivity/Area between VLDL and LDL specific radioactivity curves (28) (the rate of rise was derived from the differences between the 18- and 2-h LDL specific radioactivities). The turnover of flux (in milligrams per day) was calculated by multiplying k by the intravascular pool of LDL-B.

In three subjects, the values for LDL turnover, as measured by the above technique, were compared with the more conventional two-pool analysis obtained over 14 days after the injection of labeled LDL. This was carried out by simultaneously injecting LDL labeled with ¹²⁵I and VLDL with ¹³¹I. The analysis was performed as described by others (29). Agreement between the two methods was, for the three subjects, 82, 76, and 90%, lower values being obtained with the area between the specific activity-time curves method.

RESULTS

VLDL-B ($S_f 20-400$) turnover. This was carried out in 12 subjects, 7 of whom showed varying degrees of hypertriglyceridemia and hence increased amounts of $S_f 20-400$ lipoproteins (Tables I and II).

In every study, the specific radioactivity-time curve resolved visually by curve-peeling was multiexponential over at least 48 h (Fig. 1). The first five points on the curve encompassing the first 12 h almost described a monoexponential function in most subjects; however, when the 48-h curve was resolved into two exponential functions by the curve-peeling technique, the first and faster exponential of the two-pool model conformed closely to a monoexponential function (Fig. 1). However, in the normolipidemic subject (subject 1), in whom the concentration of VLDL-B was only 1.7 mg/ 100 ml, the specific activity-time curve appeared monoexponential until 95% of the radioactivity had left the $S_f 20-400$ fraction. In this single subject it seemed more appropriate to calculate the flux from a single-pool analysis and it was found to be 359 mg/day. VLDL-B flux was calculated with the two-pool model in the other 11 subjects and gave values between 637 and 3,060 mg/ day (Table II).

The mass of pool A was also estimated by compartmental analysis and was found to be on average 30% larger than the intravascular content of VLDL-B (Table II).

In the subjects with normal or moderately raised levels of triglyceride, flux and pool size appeared to be related, suggesting that expansion of the pool was a function of increased input (Fig. 3). The three subjects with the highest triglyceride levels showed an increase in pool size that was out of proportion to the increase

 TABLE II

 Very Low Density B Apoprotein Turnover Data Derived from Two-Pool Analysis after

 Injecting Labeled S120-400 VLDL

		Apoprotein concentration	Apoprotein turnover	Rate constants		B apoprotein mass	
Subject	Lipoprotein phenotype			α	β	Pool A	Intravascular*
		mg/100 ml	mg/day	day^{-1}		mg	
1	Ν	1.7	359‡	_			44
2	Ν	5.2	637	22.18	1.06	181	131
3	Ν	6.0	680	5.54	1.51	235	194
4	2a	6.8	1,076	4.75	0.89	330	214
5	2a	10.8	1,092	12.91	1.03	554	334
6	2b	11.4	1,133	11.09	0.55	306	282
7	2b	12.7	1,238	4.27	0.91	641	415
8	2b	12.7	1,671	4.63	0.41	654	485
9	4	13.0	3,345	8.52	0.67	732	355
10	4	25.5	2,323	4.15	0.77	1,061	942
11	4	35.6	1,928	2.78	0.53	1,544	1,326
12	5	53.0	3,060	3.07	0.94	2,611	2,147
Mean±1 SD		16.2 ± 14.9	$1,545 \pm 952$	7.6 ± 5.8	0.84 ± 0.31	804 ± 719	620 ± 620

* B apoprotein concentration × plasma volume (4.5% body weight).

‡ Calculated by one-pool analysis (see text). All other values obtained by two-pool analysis.

in flux. On the other hand, one normolipidemic subject showed a disproportionately high flux of S_f 60–400 lipoproteins (subject 13).

in the other two studies, the crossover was at peak S_f 12–20 specific radioactivity.

VLDL-B kinetics in VLDL subfractions. The relationship between the metabolism of the $S_f 20-400$ and the $S_f 12-20$ lipoprotein fractions was examined in the studies with labeled $S_f 20-400$. In seven of nine studies, the $S_f 12-20$ specific radioactivity curve intersected the $S_f 20-400$ curve before the specific radioactivity of the product had reached its peak (Fig. 4); 12–20 specific radioactivity. The proportion of S_f 20–400 lipoprotein that might be catabolized to the smaller S_f 12–20 lipoprotein class

be catabolized to the smaller $S_f 12-20$ lipoprotein class was measured directly in subjects 4 and 10. The removal of the $S_f 12-20$ was multiexponential in both





FIGURE 3 Relationship between intravascular content and the turnover of VLDL-B ($S_f 20-400$).

FIGURE 4 Relationship between specific activity curves for B apoprotein in $S_f 20-400$ VLDL (injected fraction) and $S_f 12-20$ (IDL) and $S_f 0-12$ (LDL) lipoproteins. Subject 11.

subjects, with a calculated flux which was only 20% of that of its precursor $S_f 20-400$ fraction in the hypertriglyceridemic subject 10 but very similar to that of the precursor in the hypercholesterolemic but normotriglyceridemic subject 4. This suggested that though most if not all of the $S_f 12-20$ fraction was derived from the $S_f 20-400$ fraction, not all of the $S_f 12-20$ fraction when $S_f 20-400$ flux is high.

Additional estimates along these lines were made from six of the preceding studies with the labeled S_f 20-400 lipoproteins in which the data allowed accurate measurements of the areas under the specific radioactivity-time curves of the Sf 20-400 and the Sf 12-20 fractions. The area under the S_f 12-20 curve was similar to that under the Sf 20-400 curve in subjects 5, 6, and 7 (ratios of the areas under S_f 20-400 and S_f 12-20 were 1:1.4, 1:1.4, and 1:1.1, respectively). However, in the three subjects with Type 4 lipoprotein phenotype (subjects 9, 10, and 11), the corresponding ratios were 1:1.9, 1:3.1, and 1:1.9. The areas under the S_f 12-20 curves were therefore greater than those under the S_f 20-400 curves, especially in the hypertriglyceridemic subjects; since flux is inversely related to the area under the curve, S_f 12-20 flux was clearly less than that of $S_f 20-400$ in subjects 9, 10, and 11. If some of the label injected with the S_f 20-400 lipoproteins was removed by a process other than through the S_f 12-20 fraction, then the estimated turnover of the latter fraction would be even less (as suggested by the direct measurement in subject 10). It therefore seems likely that in hypertriglyceridemic subjects a substantial fraction of VLDL-B catabolism may leave the circulation before being degraded to a lipoprotein of the S_f 12-20 size, and the possibility was considered that this may lie within the range S_f 12-60. It has recently been suggested on the basis of in vivo and in vitro studies that the appropriate density of the VLDL remnant might be in the range of $S_f 20-60$ (11, 30). Accordingly, double-labeled experiments were carried out in two subjects, one with normal plasma lipids (subject 13) and one with moderately severe Type 4 hyperlipoproteinemia (subject 14). Fig. 5 shows one such experiment in which S_f 60-400 lipoproteins, labeled with ¹²⁵I, and S_f 12-60 lipoproteins, labeled with ¹³¹I, were injected together. Also shown is the S_f 12-60 curve derived from the catabolism of ¹²⁵I S_f 60-400. The S_f 60-400 specific radioactivity-time curves could be resolved into two exponentials. In subject 13 the $S_f 12-60$ curve could also be resolved into two exponentials, but in subject 14, the S_f 12-60 curve appeared monoexponential and flux was calculated accordingly. The flux of B apoprotein in the two lipoprotein fractions, measured from the two labels, were of a similar order (Table III), which, taken together with the precise precursorproduct characteristics of their respective specific radioactivity curves (determined from the $S_f 60-400$ labeling, Fig. 5), suggests that the major VLDL remnant has a density of around $S_f 12-60$. In a third subject (subject 15), only $S_f 60-400$ lipoproteins were injected and the flux of the $S_f 12-60$ fraction was calculated by the areas under the curves method: fluxes were again similar.

Conversion of VLDL to LDL. The specific radioactivity of the B apoprotein in LDL (d 1.019–1.063) was also measured in most subjects, primarily to define the nature of the VLDL to LDL relationship. In addition, the fractional rate of labelling of LDL protein was calculated from the rate of rise in LDL specific radioactivity and the rate of fall in VLDL specific radioativity during the first 18 h. As described in Methods, this model requires that LDL-B is derived solely from the corresponding protein in VLDL. In all subjects, the LDL specific radioactivity curve at or very near to the peak specific radioactivity of LDL (Fig. 4). This is consistent with VLDL-B being the source of LDL protein.

The calculation of LDL flux by this method was compared in three subjects (subjects 5, 6, and 8) with the



FIGURE 5 Relationship between specific activity curves of B apoprotein in $S_f 60-400$ VLDL (injected fraction labeled with ¹²⁵I) and $S_f 12-60$ lipoprotein (labeled with ¹²⁵I from $S_f 60-400$ lipoproteins.) Also shown is the $S_f 12-60$ fraction labeled with ¹³¹I.

Subject	Lipoprotein phenotype	Lipoprotein fraction studied	_	Rate constants			
			B apoprotein concentration	k*	α‡	βţ	Turhover
			mg/100 ml		day-1		mg/day
4	2a	$S_{f} 20 - 400 (^{125}I)$	6.8		4.75	0.89	1,076
		$S_f 12-20 (^{131}I)$	8.3		18.48	2.30	1,104
10	4	$S_f 20 - 400 (^{125}I)$	25.5		4.15	0.77	2,322
		$S_{f} 12 - 20 (^{131}I)$	3.6		4.16	0.66	462
13	Ν	$S_{f} 60 - 400 (^{125}I)$	5.6		9.79	2.21	2,061
		$S_f 12-60 (^{131}I)$	7.3		8.33	0.62	1,968
14	4	$S_{f} 60 - 400 (^{125}I)$	6.6		2.64	0.40	605
		$S_{f} 12-60 (^{131}I)$	13.0	1.39			544
15	2b	$S_{f} 60 - 400 (^{125}I)$	11.1		1.96	0.92	488
		$S_{f} 12-60$	22.0				462"

 TABLE III

 B Apoprotein Metabolism in Subclasses of Very Low Density Lipoproteins and

 Smaller Intermediate Lipoproteins

* One-pool analysis: apparently monoexponential removal rate.

‡ Two-pool analysis: biexponential removal rate.

§ Injected lipoprotein.

"Calculated by area under curve method (see text).

conventional technique of a two-pool analysis of LDL protein specific radioactivity over a period of 14 days after i.v. administration of ¹³¹I-labeled LDL. The present method gave values that were 24%, 18%, and 10% less than by two-pool analysis. However, the two-pool method may overestimate flux when compared with the urine:plasma ratio technique (31). On the other hand, the present method may underestimate LDL flux, since

the calculation utilizes only the mass of intravascular LDL protein.

This much more rapid estimate of LDL-B flux was therefore compared in all subjects with the turnover of VLDL S_f 20–400 lipoproteins (Table IV). The LDL flux varied from 5.5 to 18.7 mg/kg per day, which is in line with findings reported by others with the two-pool model (29). There was little difference in LDL flux

 TABLE IV

 Comparison of Turnover of B Apoprotein in Very Low Density and Low Density Lipoprotein Fractions

		LDL-B data					
Subject	Lipoprotein phenotype	Concentration	Turnover rate	Turnover		VLDL-B turnover	LDL/VLDL turnover
		mg/100 ml	k/day	mg/day	mg/day per kg	mg/day	%
1	Ν	47	0.33	405	5.5	359	113
2	Ν	80	0.29	579	10.3	637	91
3	Ν	82	0.24	652	9.1	680	96
4	2a	150	0.24	1,125	16.0	1,076	105
5	2a	135	0.29	1,177	17.3	1,092	107
6	2b	183	0.16	702	12.8	1,133	62
7	$2\mathbf{b}$	131	0.23	990	13.7	1,238	80
8	2b	112	0.36	1,542	18.1	1,671	92
9	4	97	0.43	1,144	18.7	3,345	34
10	4	77	0.27	772	9.4	2,323	33
11	4	102	0.37	1,368	17.0	1,928	70
12	5	78	0.34	1,074	11.9	3,060	35
Mean±1 SD	Mean±1 SD		0.30 ± 0.07	961 ± 341	13.3 ± 4.2	$1,545 \pm 952$	76.5 ± 30

between the four subjects with hypertriglyceridemia alone and the others. However, whereas LDL flux was substantially less than VLDL flux in at least three of the four purely hypertriglyceridemic subjects, the two measurements were much closer in the other subjects. This suggests that at higher concentrations of VLDL a considerable fraction of VLDL-B turnover is catabolized by a route other than through LDL. In the one subject with severe xanthomatous hypercholesterolemia (subject 6), LDL flux was 12.8 mg/kg per day by this technique.

DISCUSSION

VLDL-B turnover. In nearly every study, the specific radioactivity-time curve during the first 48 h conformed to a two-pool model. Sigurdsson et al. (12) had also noted the curvilinear nature of the log-linear plot of the specific radioactivity-time curve, but concluded that the initial 24-h segment was consistent with a monoexponential rate of removal. The data from some of our studies, and in particular in the normolipidemic subjects, could also be plotted visually in an apparently monoexponential manner but usually not beyond 12 h. By extending the duration of the studies, the multiexponential nature of VLDL catabolism was more clearly defined.

The two-pool characteristics of VLDL-B flux could have arisen artifactually if the specific radioactivity values were distorted by contaminating labeled proteins with turnover kinetics that differed from those of the B apoprotein. This possibility has been dealt with above. The absence of soluble proteins with polyacrylamide-gel electrophoresis of ammonium bicarbonate or TMU-insoluble proteins, the similarity of the amino acid composition of the insoluble proteins derived from VLDL and LDL, and the similarity in the turnover rates derived from analysis of NH₄HCO₃ and TMU-insoluble protein strongly suggest that contamination of the VLDL-B with other proteins was minimal (see Methods).

A two-pool model is consistent with some of the known metabolic characteristics of VLDL. First, more than one population of particles may be secreted into the plasma. It is known that carbohydrate-induced hypertriglyceridemia leads to the secretion of VLDL that are larger than those found in subjects eating normally (32), and measurements of VLDL particle size show that hyperlipidemic subjects may have a much higher proportion of large VLDL than do fasting normolipidemic individuals (23).

However, it has not yet been shown that in any single individual VLDL particles of different size are secreted into the plasma at the one time.

The two-pool model might also reflect heterogeneity of catabolic processes. It is clear from experiments in

which the protein or the lipid moieties of VLDL have been labeled that smaller species of lipoproteins are progressively formed as triglyceride and surface components of VLDL are removed (10, 11). Barter and Nestel considered the possibility of independently metabolized pools of VLDL triglyceride that would lead to differing turnover rates for different subclasses of VLDL (33). Instead, the findings suggested a stepwise transformation of larger to smaller lipoproteins with LDL representing the final step. However, the catabolic fate of the B apoprotein moiety of VLDL may diverge from that of triglyceride during the formation of VLDL remnants which contain all the B apoprotein of the parent VLDL but very much less triglyceride (23, 34). Experiments in the rat show that catabolism of VLDL-B may indeed proceed by more than one route (14): Less than 10% of labeled B apoprotein in VLDL was reported to have been recovered in LDL, whereas the uptake of label in the liver was substantial (14). The rate and extent of uptake of the B apoprotein in rat liver resembles that of the cholesteryl esters (35) which remain as the major core component of the partially catabolized VLDL. This provides strong evidence that at least in the rat, some remnant particles are removed by the liver while others are destined for conversion to LDL.

It is therefore possible that VLDL may not only be catabolized by more than one process, but that the turnover rate of protein through successive metabolic steps becomes altered. Eisenberg et al. (10) have reported a faster rate of transfer of labeled B apoprotein from VLDL to S_f 12–20 lipoproteins during the initial stages of heparin-induced VLDL catabolism than during the subsequent transfer of label to LDL. Such a heterogeneity of catabolic steps might be expected to lead to multicompartmental kinetics for VLDL-B turnover. The removal of VLDL-B in the rat also appears to be multiexponential (13). This may reflect the very rapid rate of removal of VLDL apoprotein from the plasma coupled to the much slower rate of uptake and catabolism of remnants in the liver (14, 36).

A third contributing factor to multiexponential kinetics might occur from the distribution of VLDL-B in more than one pool, with each pool having different turnover characteristics. While it is highly probable that the catabolism of VLDL takes place largely within the plasma, lipoproteins resembling VLDL have been identified in human lymph (37). Catabolism of VLDL remnants has been described in several tissues (14, 36, 38), and VLDL-B can be degraded in a variety of cultured cells² (39). The presence of VLDL-like par-

² In human lymphocytes recently removed from the body, the uptake and degradation of VLDL-B is almost as great as that of LDL-B. Vijayagopal, P. and P. J. Nestel. *Artery*. In press.

ticles in the intestine (40) represents another possible pool of VLDL that might exchange with the intravascular pool.

The multiexponential nature of the specific activity curves might therefore reflect several factors: heterogeneity of turnover rates among several populations of VLDL particles, alternative catabolic processes, and catabolism within several tissue or lipoprotein pools.

VLDL remnant. The present findings, though limited, suggest that the major remnant of VLDL catabolism is in the S_f 12-60 range. This is based on the similar flux of B apoprotein through the $S_f 60-400$ and the S_f 12–60 lipoprotein fractions (subjects 13, 14, and 15) and the smaller flux of B apoprotein in the $S_f 12-20$ compared with the S_f 20-400 lipoproteins in the hypertriglyceridemic subjects (Table III). This is consistent with two studies in the rat: Mjøs et al. have shown that the size of VLDL remnants is distributed mainly in the 300 to 400-Å range (11), and Eisenberg and Rachmilewitz have reported the remnant to have a mean S_f value of 30 and a diameter of 296 Å (30). In humans, VLDL-B is rapidly transferred from the S_f 100-400 to the S_f 20–60 fraction when heparin is injected (10). On the other hand, with constant prolonged infusions of heparin, smaller lipoproteins in the S_f 12–20 range are also rapidly formed (41), and in vitro NaCl-suppressible postheparin lipolytic activity can transform VLDL to LDL (22).

Our findings suggest that most of VLDL may be degraded to $S_f 20-60$ lipoproteins but that in some subjects only a proportion of this is further catabolized to $S_f 12-20$, the remainder leaving the circulation without conversion to $S_f 12-20$ and then to $S_f 0-12$ lipoproteins. Compartmental studies reported by Hall et al. also suggest that the $S_f 12-20$ fraction, which had until recently been considered to be the major VLDL remnant, may be partly "bypassed" (42). Conversion to LDL is clearly one fate of the VLDL remnant, and an estimate of this was obtained from our comparison of VLDL-B turnover and LDL turnover.

LDL flux. The studies of Sigurdsson et al. suggest that in man LDL is solely derived from VLDL (12). However, both in the monkey (43) and in the rat³ some LDL may be formed independently of VLDL catabolism and this may yet be demonstrated in man under certain circumstances.

The values obtained by the present technique were in reasonable agreement in three subjects with the turnover calculated from reinjected labeled LDL and were in the range reported for LDL turnover by others (29). Of special interest was the similarity between VLDL B- and LDL-B flux (as well as S_f 12–20 B apoprotein) in the five subjects without hypertriglyceridemia, suggesting total conversion of VLDL particles to LDL. However, as suggested by the data of Sigurdsson et al. (44), alternate paths may be available for VLDL catabolism in hypertriglyceridemic subjects. In three of our four hypertriglyceridemic subjects, as little as onethird of VLDL-B flux appeared in LDL. Since the flux of LDL-B was similar in those with and without hypertriglyceridemia (Table IV), whereas that of VLDL-B was much higher in the hypertriglyceridemic subjects, it is possible that LDL turnover cannot be readily increased to accommodate a higher flux of VLDL-B. A proportion of the latter is then catabolized elsewhere, possibly in the liver as occurs in the rat. Sigurdsson et al. have also reported normal values for LDL flux in hypertriglyceridemic subjects, many of whom presumably showed increased VLDL turnover (45).

The present studies in which the protein of VLDL has been labeled also suggest that expansion of VLDL pools may be associated with increased input of VLDL-B. However, since several distinct metabolic processes contribute to the catabolism of VLDL, it is likely that turnover and pool size will not always conform to the relationship shown in Fig. 3.

The most relevant factor in the present context is the influence of overweight which is known to stimulate VLDL secretion. It is therefore important to note that some of the hypertriglyceridemic subjects who showed increased VLDL-B turnover were also overweight.

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