Conversion of Very Low Density Lipoprotein to Low Density Lipoprotein

A METABOLIC STUDY OF APOLIPOPROTEIN B KINETICS IN HUMAN SUBJECTS

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ABSTRACT The interrelationship between apolipoprotein B in very low density lipoprotein (VLDL-B) and in low density lipoprotein (LDL-B) was studied in seven normal and hyperlipidemic men and women, with purified radioiodinated VLDL. The time-course of the appearance of radioactivity in LDL was followed. As the specific activity curves intersected at the maximal height of the LDL-B curve, it was inferred that all or most LDL-B peptide is derived from VLDL-B peptide.

This transfer was further quantitated in seven normotriglyceridemic subjects by simultaneous i.v. injection of purified ^{131}I-VLDL and ^{125}I-LDL. By a deconvolution method, a quantitative description of the rate of entry of ^{131}I-VLDL-B into ^{131}I-LDL-B was derived by analysis of ^{131}I-LDL-B and ^{125}I-LDL-B radioactivity in plasma. The results indicate that approximately 90% of VLDL-B mass is converted into LDL-B in subjects with normal serum triglyceride concentrations.

The synthetic rates of VLDL-B and LDL-B peptide were simultaneously measured in six normal subjects, and two patients with heterozygous familial hypercholesterolemia (type IIa). The turnover rates for VLDL-B and LDL-B peptide were similar in these subjects. The findings in the three parts of this study were consistent with the view that most if not all VLDL-B is converted into LDL-B peptide, and most if not all LDL-B is derived from VLDL-B peptide in normotriglyceridemic subjects.

This work was presented in part to the Medical Research Society, London, in December 1974 and published in abstract form.

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Received for publication 24 February 1975 and in revised form 20 August 1975.

INTRODUCTION

The lipoproteins of plasma comprise a broad spectrum of molecules varying in size and in lipid and protein content. These lipoproteins have been classified, chiefly operationally, into four major classes, but there is growing evidence that these lipoproteins form a dynamic system within which exchange and mass transfer of lipid and protein content occur. The origin of these lipoproteins has been a matter of extensive research and it has been shown that very low density lipoprotein VLDL)[1] is secreted by the liver and small intestine (2) in nascent form (3). Similarly it has been shown that high density lipoprotein is secreted by the liver and small intestine (2, 4) in nascent form.

On the other hand, the origin of low density lipoprotein (LDL) is less clear. Graham et al. (5) showed that heparin injection into rabbits and humans induced a fall in plasma lipoproteins of S_f 20-400 and a concomitant rise in the level of lipoproteins of S_f 12-20. Similar changes were found in vitro after incubation of VLDL with postheparin plasma (6). Gitlin et al. (7) injected radioiodinated VLDL of S_f 10-100 into human subjects and observed that a significant proportion of label appeared in LDL. Similarly, LDL triglyceride appears to be derived from VLDL in rabbits (8) and humans (9).

The protein moiety in VLDL (apo VLDL) consists of several different peptides including apolipoprotein B, which constitutes about 50% of apo VLDL (10). This B peptide in VLDL is identical to the major peptide of LDL. Bilheimer et al. (11) have shown in

[1] *Abbreviations used in this paper:* FCR, fractional catabolic rate; LDL, low density lipoproteins; U/P ratio, urinary radioactivity/plasma radioactivity; VLDL, very low density lipoproteins.

vivo using radioiodinated VLDL that a proportion of the radiolabeled B peptide of VLDL appears in the LDL fraction, first into the *d* 1.006-1.019 range and later into the *d* 1.019-1.063 range; specific activity data were not obtained in this study.

In the present investigation the interrelationship between the B peptide (apo B) in VLDL and LDL was quantitatively studied to elucidate what proportion of B peptide in LDL is derived from VLDL and what proportion of VLDL mass is converted into LDL.

For this purpose radioiodinated VLDL was reinjected into human subjects and specific activity curves for VLDL-B and LDL-B peptide were obtained (study A). By simultaneous injection of VLDL and LDL, labeled with different iodine isotopes, the transfer of VLDL-B peptide into LDL-B peptide was quantitated (study B) and the turnover rates of VLDL-B and LDL-B peptides were compared (study C).

METHODS

Subjects

Study A. Four men and three women were studied. Three subjects had normal blood lipid concentrations. Three subjects had primary endogenous hypertriglyceridemia, known underlying causes being excluded; two had prebeta-hyperlipoproteinemia (type IV), and one also had chylomicronemia (type V). One subject with heterozygous familial hypercholesterolemia (type IIa) was studied. The diagnosis was based on an elevated plasma LDL cholesterol concentration, the presence of tendon xanthomata, and an affected first-degree relative. Normal levels for cholesterol and triglyceride were defined as below 95th percentile as found in a London population (12).

Study B. Seven subjects were studied, including five normals and two patients with heterozygous familial hypercholesterolemia; the latter had elevated LDL-cholesterol levels, tendinous xanthomata, and affected first-degree relatives. Subject D. L. also participated in study A.

Study C. Eight subjects were studied, including six normals and two patients with heterozygous familial hypercholesterolemia, five of whom were also included in study B. The diagnosis of familial hypercholesterolemia was based on elevated LDL-cholesterol levels, tendinous xanthomata, and affected first-degree relatives.

Clinical data for all the studies are summarized in Table I. Fully informed consent was obtained from all participants.

Preparation of radiolabeled lipoproteins

Blood was drawn from the subjects after a 14-h fast with 0.1% EDTA as an anticoagulant. VLDL was isolated at plasma density ($d = 1.006$) by ultracentrifugation for 16 h at 105,000 *g* (13). The VLDL was then twice washed and concentrated by further ultracentrifugations at plasma density ($d = 1.006$) for 16 h each at 105,000 *g*.

Chylomicrons ($S_f > 400$) were removed before VLDL separation from plasma from patient B. W. (type V), by ultracentrifugation, 20,000 *g* for 30 min twice (14).

Low density lipoproteins (LDL_1 and LDL_2) were isolated in the density range 1.006–1.063 and recentrifuged at a background density of 1.063 (13) for 20 h at 105,000 *g*.

The apoproteins of VLDL (apo B and apo C chiefly) were radioiodinated with ^{131}I by the iodine monochloride (ICl) method of MacFarlane (15), as modified for iodination of apolipoproteins by Langer et al. (16). 1 M glycine: NaOH buffer at pH 10.0 and initial concentration of VLDL protein in the range of 5–15 mg/ml were used. If the molecular weight is 300,000 for the whole apo VLDL complex, the amount of ICl added was such that less than one atom of iodine was introduced per molecule of VLDL.

LDL was radioiodinated with ^{125}I in the same way, after dialysis against 0.15 M NaCl solution containing 1 mM

TABLE I
Clinical Data: Studies A, B, and C

Subjects	Study	Sex	Age	Weight	Height	Plasma triglycerides	Plasma cholesterol	Lipoprotein pattern
			yr	*kg*	*cm*	*mg/100 ml*	*mg/100 ml*	*WHO type (42)*
C. L.	A	F	61	50	166	135	255	N
J. K.	A	F	55	100	173	378	228	IV
O. C.	A	F	35	61	165	84	380	IIa
W. B.	A	M	59	87	169	184	182	N
L. C.	A	M	63	80	168	393	219	IV
B. W.	A	M	47	85	179	606	196	V
D. L.	A + B + C	M	30	67	168	168	201	N
H. J.	B + C	M	74	91	183	189	145	N
R. J.	B + C	M	38	82	171	180	190	N
M. K.	B	M	37	78	180	125	218	N
L. B.	B + C	F	55	69	153	100	234	N
M. B.	B + C	F	51	58	156	127	594	IIa
M. A.	B	F	63	58	156	111	376	IIa
E. H.	C	F	49	71	179	154	224	N
D. B.	C	M	38	82	178	71	169	N
A. S.	C	M	37	81	173	95	335	IIa

EDTA at pH 7.4. The molecular weight of apo LDL was assumed to be 100,000.

Unbound iodine was removed from the iodinated sample with a column of Sephadex G 10, suspended in sterile 0.15 M NaCl. The eluate was dialyzed against 0.15 M NaCl, 1 mM EDTA, pH 7.4. After dialysis the radioiodinated preparations contained less than 2% unbound iodine, as determined by precipitation with 15% trichloroacetic acid (TCA) in the presence of carrier albumin.

As determined by ethanol/ether extraction (3:1 vol/vol) (Scanu and Edelstein [17]), 8–22% of radiolabeling in VLDL and less than 5% in LDL was in the lipid moiety. We confirmed that by this procedure 98% of the lipid moiety is extracted. The fraction of apo VLDL soluble in 3:1 ethanol/ether was precipitated by adding sufficient ether to bring the ethanol to ether ratio of the mixture to 3:5 as these authors described.

Both the B and C peptide components of VLDL became labeled during the iodination procedure and 43–57% of radiolabeling in apo-VLDL was bound to B-peptide, as determined by Sephadex G 150 chromatography (18) (see study B). More than 95% of ^{125}I radioactivity in the LDL dose (d 1.006–1.063) was in the density range 1.019–1.063 (LDL_2) as determined by ultracentrifugal fractionation (13), presumably reflecting the relative protein concentrations in the subjects studied.

The iodinated lipoproteins were sterilized by Millipore filtration (pore size 0.45 μM) (Millipore Corp., Bedford, Mass.).

A maximum of 96 h elapsed between the taking of the initial blood sample and reinjection of the labeled VLDL and LDL. The iodinated lipoproteins had the same mobility as native lipoproteins on cellulose acetate electrophoresis. The preparations were found to be free of albumin as determined by double immunodiffusion with antisera reacting against human serum albumin. After final dialysis, sterile human albumin was added to the iodinated preparation to minimize self-radiation.

Radioactivity was determined with a double-channel gamma spectrometer.

Study protocol

The sterile radiolabeled ^{131}I-VLDL (total radioactivity 10–30 μCi in approximately 1–5 mg of apoprotein) was reinjected i.v. on the morning after a 14-h fast. In studies B and C, radiolabeled ^{125}I-LDL (total radioactivity 20–30 μCi in approximately 2–8 mg of apoprotein) was simultaneously injected i.v. For 12–30 h after the injection, blood samples (12 ml) were withdrawn at 2–3-h intervals, through an indwelling cannula. In study C further blood samples were taken daily for 2 wk.

During the first 24 h after the injection, the subjects received a diet containing 300 g of carbohydrate, 60 g of protein and less than 5 g of fat; this was to minimize change in caloric balance and to avoid intestinal VLDL synthesis. One patient (H. J.) received this diet for 48 h. Subsequently they received their normal free diet. Body weight remained steady (±0.5 kg) during the 2 wk of study. The subjects received 180 mg of potassium iodide daily, by mouth, in divided doses for 4 days before and throughout the study, to inhibit uptake of radioiodine by the thyroid.

A further study was performed in two subjects with elevated serum triglyceride levels (part of study C). Autologous ^{125}I-VLDL, in the presence of carrier albumin, (total dose 30 μCi in 5 mg of apoprotein) was infused i.v. at a constant rate after a priming dose. This infusion was continued overnight for 16 h; it was then stopped and a bolus i.v. injection of radiolabeled ^{131}I-VLDL (15 μCi) was given and the plasma disappearance curves of ^{125}I-VLDL-B and ^{131}I-VLDL-B were compared.

Sample analysis

Determination of ^{131}I-VLDL-B specific activity curve (Study A). The plasma was immediately separated at 4°C. VLDL was isolated by ultracentrifugation for 16 h at 105,000 g (13) and removed by tube slicing.

VLDL was delipidated by ethanol/ether and ether extraction (17). To obtain purified B-peptide, the apoprotein precipitate was first washed with 0.2 M Tris HCl buffer, pH 8.2, to remove Tris-soluble peptides; the remaining insoluble material was then dissolved in 0.2 M Tris-HCl, pH 8.2, containing 0.1 M sodium decyl sulphate. This solution was subjected to Sephadex G 150 chromatography (column size 60 × 0.8 cm) (18).

The fraction that corresponded to the maximum of the peak eluting at the void volume was collected for determination of VLDL-B peptide specific activity. The amino acid composition of this fraction was determined and compared to that of the corresponding peak obtained from Sephadex G 150 chromatography of delipidated and resolubilized LDL_2 (d 1.019–1.063). A JEOL JLC 6AH amino acid analyzer (JEOL Analytic Instruments, Cranford, N. J.) was used. Compositions were expressed as moles of amino acid relative to aspartic acid = 100 mol. An average difference of 3.6% was found between the amino acid composition of VLDL-B and LDL-B, purified as described above. No residues showed more than 10% difference. The mean amino acid composition differed by less than 5% from that published by Levy et al. for an LDL fraction of density 1.019–1.063 (19). Thus the VLDL-B peptide obtained as above was considered to be virtually identical in amino acid composition with LDL-B peptide.

Protein concentrations of fractions containing VLDL-B peptide were determined by precipitation in 15% TCA followed by Amidoblack binding (20). The method was sensitive down to protein concentrations of 5 μg/ml and was not affected by the presence of Tris or sodium decyl sulphate.

Determination of ^{131}I-LDL-B specific activity curve. LDL was isolated from the infranate of the same plasma samples from which the VLDL was isolated. This was accomplished by centrifugation for 20 h at 105,000 g at a background density of 1.063 g/ml. The LDL fractions were carefully overlayered with a solution of density 1.063 g/ml and washed by recentrifugation under the same conditions.

The ^{131}I radioactivity in the washed LDL fractions and in the VLDL-B peptide fractions was determined at the same time. Radioactivity associated with the lipid and with the tetramethyl urea-soluble apoproteins (including C peptide) in LDL fractions was determined. This was subtracted from the total radioactivity. Lipid-associated radioactivity was assayed by ethanol-ether extraction (17). Radioactivity associated with soluble apoproteins in LDL was assayed in the aqueous phase after tetramethyl urea precipitation (21); this was found to be less than 5% of the total radioactivity except in the samples taken during the first 3–4 h. The protein concentration of the LDL fractions was determined by TCA precipitation and Amidoblack binding, as described for VLDL-B peptide.

In calculating the specific activity, the radioactivity (minus the lipid and soluble apoprotein contributions) was divided by the protein concentration. The specific activities

so obtained were compared with those of material isolated by Sephadex G 150 chromatography of delipidated and resolubilized portions of LDL from the same samples: the values corresponded to within ±6%.

Cholesterol concentrations were determined by the Technicon N 24a method (Technicon Instruments Corp., Tarrytown, N. Y.). Triglyceride levels were measured by the Technicon N 78 method, the fluorimetric procedure being modified (22). These levels remained within ±8% of the initial fasting values.

Sample analysis (study B). A fraction of the ^{131}I-VLDL dose was delipidated (17) and fractionated with Sephadex G 150 (18). The fraction of the dose injected as ^{131}I-VLDL-B was thus obtained.

The recovery of radioactivity from the column was about 80%. The recovery rate was the same for B and C peptides (as demonstrated by reapplication of the eluted fractions on to new columns); the error introduced by the incomplete recovery was therefore considered minimal, and not selective for either peptide.

VLDL and LDL fractions from the plasma samples were isolated by ultracentrifugation (13). Total ^{131}I and ^{125}I radioactivity in the LDL fractions were determined simultaneously along with appropriate standards. The quenching of ^{125}I due to high salt concentration was corrected for, with Na^{125}I as internal standard. The LDL fractions were delipidated (ethanol/ether 3:1 vol/vol) (17) and the radioactivity in the lipid moiety was subtracted from the total counts. The radioactivity bound to soluble apoproteins in the LDL fractions was determined as described in study A and subtracted. The ^{131}I and ^{125}I radioactivity in LDL-B peptide were thus obtained and were expressed as percentages of the dose of ^{131}I-VLDL-B and ^{125}I-LDL-B.

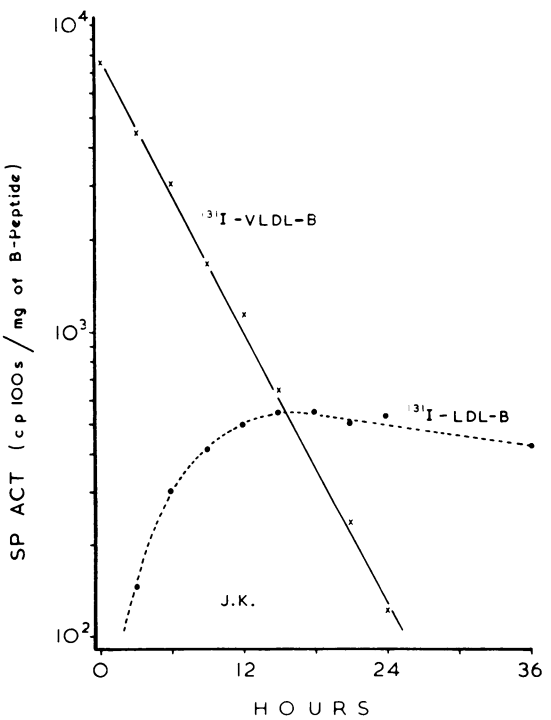

FIGURE 1 Study A. Specific activity-time curves for ^{131}I-VLDL-B and ^{131}I-LDL-B in plasma of a representative patient (J. K.).

Sample analysis (study C). VLDL-B peptide was isolated and specific activity/time curves obtained as described in study A. Total VLDL-B peptide concentration in plasma was determined by tetramethylurea precipitation on respun VLDL fractions as described by Kane (21). Protein estimations were carried out by the method of Lowry et al. (23). The turbidity caused by high lipid content was cleared by extracting the solution with diethyl ether immediately before optical density reading. Because of difference in chromogenicity in the Lowry reaction between the albumin standard and the soluble apolipoproteins, a correction factor of 0.77 (24) was applied in the calculation of B peptide concentrations. ^{125}I-LDL radioactivity was counted in 5-ml portions of the daily plasma samples along with appropriate standards.

Total LDL-B peptide concentration in plasma was determined by the method of Lowry et al. (23) on respun LDL fractions. The same correction factor (0.77) was used as for estimation of VLDL-B peptide.

Calculations

Study A. The theoretical basis of this study was the analysis of specific activity/time curves of precursor and product by Zilversmit et al. (25). The points of intersection of the curves for VLDL-B peptide and LDL-B peptide were observed (Fig. 1). Zilversmit has shown that if a product receives its A atoms only from one immediate precursor (which has been labeled) the values of the specific activity curve for the product will lie below the values of the curve for the precursor until the specific activity curve for the product reaches its maximum. At the maximum the curves will intersect, the product curve thereafter lying above the curve for the immediate precursor. But if the product receives its A atoms from any other source in addition to the labeled precursor, the curve for the product will remain below the curve for the precursor at least for some time after the curve for the product has reached its maximum; depending on the parameters of the system the curves may or may not eventually cross (25).

Intersection of specific activity curves is thus compatible with a precursor-product relationship but does not prove it. For example, if A is the precursor of B, but C is in rapid equilibrium with A, then the specific activity curve for C would also cross that for B and yet C would not be a precursor (26). However, in the case of VLDL-B to LDL-B peptide, this situation does not appear likely.

Study B. A quantitative description of the rate of entry of ^{131}I-VLDL-B into ^{131}I-LDL-B was derived as a transfer function $[I(t)]$ from the changes after a simultaneous i.v. injection of a unit of ^{131}I-VLDL-B and ^{125}I-LDL-B. The mathematical procedure for this derivation is known as deconvolution or inverse convolution. With an integral equation, Volterra's equation, an unknown function can be derived when the other two functions are known (27):

$$R(t) = \int_0^t I(T) \cdot W(t - T) \cdot dT$$

$I(t)$ (the unknown input function) is the transfer function of ^{131}I-VLDL-B into ^{131}I-LDL-B; $R(t)$, the known response function to the input function $I(t)$ equals ^{131}I-LDL-B curve (which reflects both the appearance of ^{131}I into the LDL-B pool and the disappearance from this pool); $W(t)$ (the known weighting function) equals ^{125}I-LDL-B curve; T is the age variable of integration (Fig. 2).

By inserting the values of the functions $R(t)$ and $W(t)$ obtained from appropriate curves (see Methods) we obtained $I(t)$, whose integral gave the fraction of dose injected as ^{131}I-VLDL-B which had been converted into ^{131}I-LDL-B.

This technique is based on the assumption that the fractional removal rate is the same for ^{131}I-LDL-B and ^{125}I-LDL-B. The validity of this assumption is supported by the parallelism of the disappearance curves of these isotopes in LDL (Fig. 2).

The mean conversion time ($\bar{t}$), i.e. the mean time required for VLDL-B molecule to become an LDL-B molecule, is given by (28):

$$\bar{t} = \frac{\int_0^\infty tI(t)dt}{\int_0^\infty I(t)dt}$$

Computation of data was performed on a digital computer, with the discrete formulation of the equation described by Stephenson (29) with a program similar to that of Hart and Spencer (30).

Alternatively the fraction of VLDL-B converted into LDL-B may be calculated if the ^{125}I-LDL-B curve is regarded conceptually as reflecting 100% conversion into LDL (as it was injected in this form). The ratio of the isotope doses remaining,

$$\frac{^{131}\text{I-LDL-B } \%\text{ dose (of } ^{131}\text{I-VLDL-B)/liter plasma}}{^{125}\text{I-LDL-B, } \%\text{ dose/liter plasma}},$$

when the curves have become parallel, therefore gives the fraction of radiolabeled VLDL-B dose converted into LDL. Allowance was made for the fact that the injected ^{125}I-LDL-B had been in the circulation longer than ^{131}I-LDL-B, the latter was being synthesized from ^{131}I-VLDL-B. The ratio was calculated by taking the value of ^{125}I-LDL-B at t_1 but taking the corresponding ^{131}I-LDL-B value at time $t_1 + \bar{t}$ (the mean conversion time for the particular subject).

Study C. When the specific activity-time curve for ^{131}I-VLDL-B after a bolus injection is plotted semilogarithmically, the curve appears to be monoexponential for at least 3–4 half-lives (Fig. 3), during which time most of labeled B peptide enters LDL (study B). By the time that the second exponential appears, at the most 10% of the injected dose remains in VLDL-B peptide. Therefore the fractional catabolic rate (FCR) (the fraction of the intravascular VLDL-B peptide pool catabolized per hour) may be calculated from the slope of the first exponential according to the formula (31): $FCR = 0.693/t_{\frac{1}{2}}$. The slope of the disappearance curve was determined by the method of least squares.

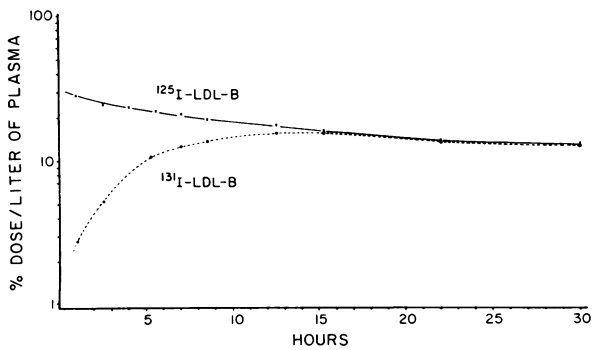

FIGURE 2 Study B. ^{125}I-LDL-B disappearance curve (upper curve) and ^{131}I-LDL-B curve (lower curve, reflecting appearance of ^{131}I into LDL-B from VLDL-B and disappearance from LDL pool) in plasma from one subject (D. L.).

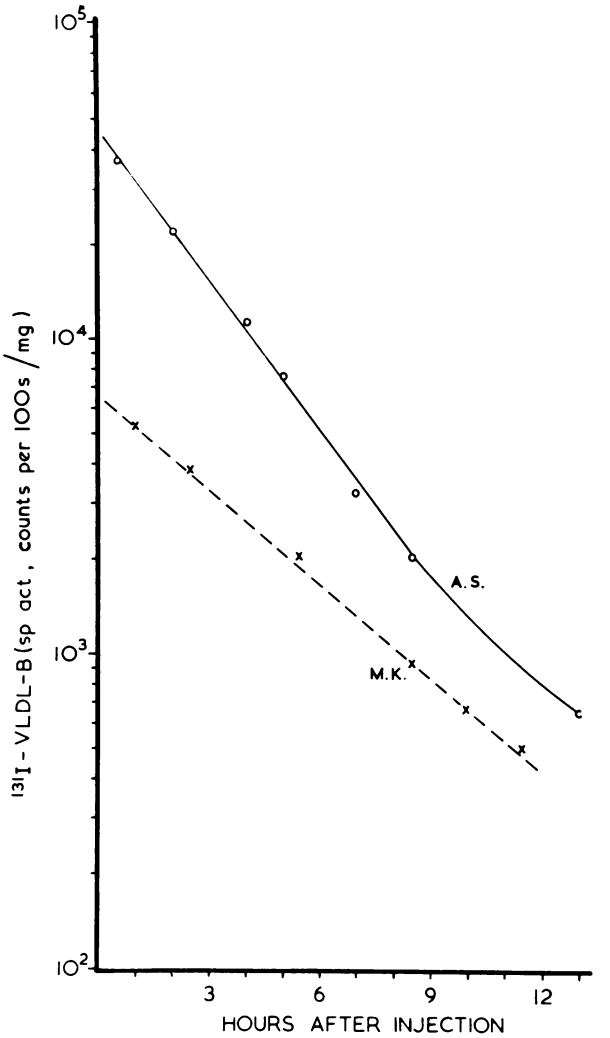

FIGURE 3 Study C. Two representative plasma ^{131}I-VLDL-B specific activity-time curves (M. K., A. S.).

The possible existence of more than one quantitatively significant fraction differing in half-life is unlikely, as the disappearance curve follows closely a monoexponential curve. The later exponentials might reflect some minor fractions within VLDL with considerably longer half-lives; but as this reflects the catabolism of only a small fraction (less than 10%) of VLDL-B mass, it would have a negligible effect on the FCR as calculated from the first exponential.

There is no evidence that B peptide recycles from LDL to VLDL. Injected radiolabeled ^{125}I-LDL remains in the LDL fraction and is not found to any extent in the VLDL fraction (16). However, as the LDL-B pool turns over relatively slowly compared to the rapid turnover of VLDL-

B, a minor recycling cannot be ruled out; this might contribute to the long half-life of later exponentials of the ^{131}I-VLDL-B curve.

The plasma volume (in liters) was assumed as 4.5% of body weight (32) and the total intravascular VLDL-B pool was then calculated as the product of VLDL-B concentration and the plasma volume. The steady state synthetic rate of VLDL-B (or absolute catabolic rate) was calculated as follows: synthetic rate = FCR × intravascular VLDL-B peptide.

The synthetic rate was expressed as milligrams of VLDL-B peptide synthesized per 24 h, normalized for body weight (milligrams per kilogram per 24 h).

The fraction of the intravascular LDL-B peptide pool catabolized per day (FCR) was calculated by analysis of plasma decay curve of ^{125}I-LDL-B injected i.v. by methods described by Matthews (33). The LDL-B peptide concentration in plasma was determined by the method of Lowry et al. (23) on recentrifuged LDL fractions. Correction for incomplete recovery of lipoproteins was based on measurements of cholesterol concentration before and after recentrifugation. The same correction factor (0.77) was used as for estimation of VLDL-B peptide. The total intravascular pool of LDL-B peptide was calculated from the product of LDL-B peptide concentration in plasma and the plasma volume (by the same volume as for VLDL-B turnover). This assumed volume was within ±5% of the volume obtained by isotope dilution method by measuring radioactivity in samples taken 10 min after injection.

The steady state synthetic rate of LDL-B peptide was then calculated in the same way as that of VLDL-B peptide: synthetic rate = FCR × intravascular LDL-B peptide, (milligrams/kilogram per day).

The metabolic homogeneity of radiolabeled ^{125}I-LDL was assessed from the daily urinary radioactivity excretion from day 2 to day 8 of each study, by the metabolic clearance method of Berson and Yalow (34). With this method the clearance of ^{125}I-LDL was calculated for each day of study from the ratio of total urinary radioactivity excreted per day to the mean plasma radioactivity during the interval (U/P ratio).

RESULTS

Study A. Fig. 1 shows the specific activity-time curves for ^{131}I-VLDL-B and ^{131}I-LDL-B for a representative subject (J. K.). The curves in the other six subjects showed a very similar pattern, i.e., they appeared to intersect at the maximum of the product curve, ^{131}I-LDL-B.

TABLE II
Metabolic Parameters (Study A)

Subject	VLDL triglyceride	LDL cholesterol	Time of intersection	^{131}I-VLDL-B half-life
	mg/100 ml	*mg/100 ml*	*h*	*h*
J. K.	316	139	16.0	4.1
C. L.	71	150	8.7	2.7
O. C.	60	345	6.5	1.8
W. B.	135	110	8.5	1.9
D. L.	130	124	13.8	4.2
L. C.	351	104	19.2	4.8
B. W.	385	83	14.8	5.7

TABLE III
Study B

Subject	VLDL triglycerides	Fraction of injected ^{131}I-VLDL-B dose converted into LDL-B		Mean conversion time
		a*	b‡	c
	mg/100 ml			*h*
D. L.	130	0.86	0.89	6.7
H. J.	140	0.89	0.89	7.6
R. J.	140	0.87	0.93	10.4
M. K.	74	0.81	0.82	6.7
L. B.§	52	>0.80	>0.80	3.7
M. B.	75	0.99	0.98	7.0
M. A.	65	1.01	1.05	6.1
Mean		0.89	0.91	6.9

* Calculated by integration of $I(t)$.
‡ Calculated from the ratio of ^{131}I-LDL-B against ^{125}I-LDL-B once the curves became parallel, corrected for mean conversion time (see section on calculation, study B).
§ Minimal value: the curves were still converging at 12 h.

Table II shows the time course of this intersection, which was directly related to the VLDL-triglyceride concentration of these subjects ($r = +0.86$, $P < 0.02$) and to the half-life of ^{131}I-VLDL-B in plasma ($r = +0.87$, $P < 0.02$). It did not bear any relationship to the LDL-cholesterol concentration.

Study B. Table III shows in column *a* the fraction of dose injected as ^{131}I-VLDL-B that was converted into ^{131}I-LDL-B, calculated by integration of function $I(t)$; the fraction appearing in column *b* is the ratio of ^{131}I-LDL-B to ^{125}I-LDL-B after the curves had become parallel (corrected for the mean conversion time). Both methods gave similar results.

Column *c* shows the mean conversion time of VLDL-B into LDL-B. The mean conversion time was proportional to the VLDL-triglyceride concentration ($r = +0.76$, $P < 0.05$).

Samples were obtained from one subject (H. J.) for 48 h after the injection. Subfractions of density ranges 1.006-1.019 and 1.019-1.063 were obtained by further ultracentrifugation (13) and analyzed separately. By the same methods of calculation it was found that 0.84 (method *a*) and 0.86 (method *b*) of the dose injected as ^{131}I-VLDL-B was recovered in the LDL fraction 1.019-1.063. The mean conversion time into LDL fraction 1.019-1.063 was 15.8 h, compared to 7.6 h into LDL fraction 1.006-1.063.

Study C. VLDL-triglyceride and VLDL-B concentrations remained within ±10% of the initial fasting value during the time of VLDL-B turnover study (12-24 h). Fasting LDL-cholesterol levels remained within ±8% of the initial fasting value and the cholesterol to protein ratio remained constant during the 2 wk of study. Body weight remained steady (±0.5 kg) during the 2 wk of study.

TABLE IV
Metabolic Parameters (Study C)

Subject	VLDL-B parameters: Plasma concn.	$t_{\frac{1}{2}}$	FCR	Synthetic rate		VLDL triglyceride concn.	LDL-B parameters: Plasma concn.	FCR: From plasma curve	FCR: U/P ratio	Synthetic rate		LDL cholesterol concn.
	mg/100 ml	*h*	*fraction i.v. pool/h*	*mg/day*	*mg/kg/day*	*mg/100 ml*	*mg/100 ml*	*fraction i.v. pool/day*		*mg/day*	*mg/kg/day*	*mg/100 ml*
L. B.	4.7	2.5	0.277	972	14.1	52	75.0	0.33	0.30	770	11.2	169
E. H.	3.75	2.9	0.239	694	9.7	102	86.6	0.26	0.23	725	10.1	133
H. J.	11.3	5.2	0.133	1,417	15.5	140	53.5	0.36	0.30	801	8.75	106
D. L.	11.5	4.2	0.165	1,317	19.7	133	67.1	0.43	0.35	866	12.9	124
D. B.	4.6	2.0	0.346	1,412	17.2	51	61.0	0.49	0.51	1,103	13.5	113
R. J.	12.1	6.4	0.108	1,156	14.1	140	60.0	0.51	0.51	1,132	13.8	100
M. B.	6.5	3.3	0.210	864	14.9	75	244.0	0.13	0.12	834	14.24	519
A. S.	2.64	1.8	0.385	878	11.0	57	153.0	0.16	0.17	881	11.0	256

The turnover rate of VLDL-B peptide ranged from 9.7 to 19.7 mg/kg/day, with a mean value of 14.53± 3.18 mg/kg/day (SD). The LDL-B peptide turnover ranged from 8.75 to 14.24 mg/kg/day, with a mean of 11.94±1.96 mg/kg/day (Table IV).

The half-lives of the plasma disappearance curves for ^{131}I-VLDL-B (after an i.v. bolus injection) and ^{125}I-VLDL-B were compared. The latter was determined after stopping a constant infusion continued for 16 h, by which time a plateau for the specific activity for ^{125}I-VLDL-B had been reached. The half-lives were 9.3 h and 8.6 h for ^{131}I and ^{125}I, respectively, in one patient, and 4.1 h and 3.9 h in the other (Fig. 4).

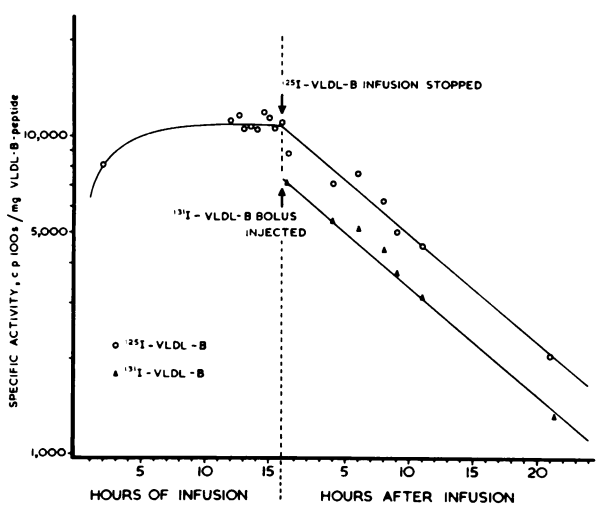

FIGURE 4 Study C. The upper curve shows plasma ^{125}I-VLDL-B specific activity-time curve during and after a constant infusion of ^{125}I-VLDL ($t_{\frac{1}{2}}$ = 9.3 h). The lower curve shows plasma ^{131}I-VLDL-B specific activity-time curve after a bolus injection of ^{131}I-VLDL ($t_{\frac{1}{2}}$ = 8.6 h).

The turnover rate of VLDL-B peptide, as determined by the formula: [Rate of infusion of ^{125}I-VLDL-B (in dpm/h)/Specific activity of plasma ^{125}I-VLDL-B at equilibrium (in dpm/mg B peptide)], was within ±5% of the turnover rate obtained by a bolus injection of ^{131}I-VLDL-B.

As study B indicated that most of the VLDL-B is converted into LDL-B peptide, the mean conversion time ($\bar{t}$) (study B) should be similar to the mean turnover time of VLDL-B peptide in the circulation. VLDL-B turnover rate can be calculated from: VLDL-B turnover (in mg)=[24 h/$\bar{t}$ (in h)]× i.v. pool size of VLDL-B (in mg).

Table V shows a comparison of VLDL-B turnover data in five subjects included in both study B and C; in column *a* the turnover rate is derived from the mean conversion time (study B) and in column *b* from specific activity curves (study C). The two methods gave

TABLE V
VLDL-B Peptide Turnover

Subject	VLDL-B peptide turnover, a*	b‡
	mg/24 h	
D. L.	1,236	1,317
H. J.	1,465	1,417
R. J.	1,031	1,156
L. B.	906	972
M. B.	589	864
Mean	1,045	1,145

* Calculated from mean conversion time (study B).
‡ Calculated from VLDL-B peptide specific activity curves (study C).

similar turnover rates and the difference was statistically not significant ($P > 0.05$).

The FCR for LDL-B peptide, calculated from the plasma curve, was slightly higher than that derived from the mean U/P ratio. The U/P ratio was observed for 7 days; it remained constant, after the initial delay in excretion due to a retention of ^{125}I within the iodide pool (16), indicating that no significant amount of rapidly degradable protein had been present.

No direct test of the metabolic integrity of radioiodinated VLDL is available, since the protein moiety is heterogeneous. Biologic screening is difficult due to the rapid turnover of VLDL and its prompt conversion to LDL. But as the rate of removal of radiolabeled ^{131}I-LDL-B (conceptually corresponding to endogenously labeled LDL) was the same as for ^{125}I-LDL-B (Fig. 2) and as the U/P ratio for ^{125}I-LDL remained constant, this would indirectly suggest a metabolic homogeneity, at least for that part of radiolabeled VLDL-B converted into LDL-B.

DISCUSSION

Studies of specific activity curves for apo B in VLDL and LDL (study A). The intersection of the specific activity-time curves of VLDL-B and LDL-B at the maximum of the curve for LDL-B suggests that most if not all LDL-B peptide (under the experimental conditions) is derived from VLDL-B peptide. This occurred in normal subjects, patients with endogenous hypertriglyceridemia and in one patient with heterozygous familial hypercholesterolemia. The same conclusion has been suggested by previous studies (7, 11, 35), but not on the basis of specific activity-time curves.

In the present study the density range 1.006-1.063 was used in the isolation of LDL to include all B peptide within LDL. Lee and Alaupovic (10) have shown that the apoprotein of density range 1.006-1.019 contains about 30-40% C peptide. The total apoprotein concentration of density range 1.006-1.019 in the subjects studied was only a small fraction of the total apoprotein concentration of the whole LDL density range of 1.006-1.063; the small amount of C peptide included should therefore introduce only a minor error into the measurement of specific activity for ^{125}I-LDL-B. This is also supported by the finding that the method used gave specific activity values closely similar (±6%) to those obtained by fractionation of apo-LDL on Sephadex G 150 columns. Ultraviolet monitoring of the eluates indicated that less than 10% of the protein in lipoproteins of density 1.006-1.063 was apo C.

Bilheimer et al. (11) have shown that after an intravenous injection of radioiodinated apo-VLDL into humans, radioactivity is transferred first into LDL of density 1.006-1.019 and later into *d* 1.019-1.063. In the present study no attempt was made to separate these fractions because of difficulty in obtaining reliable specific activity curves within the density range 1.006-1.019 due to the low protein concentration. The intersection of the VLDL and LDL curves at the apparent maximum specific activity of the latter suggests that B peptide within the whole LDL pool is derived exclusively or almost exclusively from VLDL. That all B peptide within the LDL subclasses is derived from one source makes it likely, as the work of Bilheimer et al. (11) has indicated, that all LDL-B peptide in the density range 1.019-1.063 is derived from LDL-B peptide of lipoproteins in the density range 1.006-1.019.

The mechanism and site of this transfer of VLDL-B into LDL-B is not clear. It is accompanied by transfer of triglycerides, first into smaller VLDL molecules and then into LDL. This has been thought to be accomplished by the enzyme lipoprotein lipase situated in the capillary endothelial wall, which cleaves off most of the triglyceride. There is some evidence that other lipases may be involved (36). However, the remodeling of VLDL to LDL clearly involves extensive changes in composition as not only is most of triglyceride lost but also most of apolipoprotein C, shown to exchange with high density lipoprotein (11).

The cross-over of the specific activity curves of VLDL-B (precursor) and LDL-B peptide (product) does not indicate how much of VLDL-B is converted into LDL-B peptide. It could still occur if only a part of VDL-B mass was converted into LDL-B. To answer this question, study B was performed.

Quantitation of transfer of VLDL-B into LDL-B peptide (study B). The mathematical technique used for the quantitation of this interconversion has been termed impulse analysis (noncompartmental analysis). The underlying theory represents a development of indicator dilution theory (e.g. the Stewart-Hamilton method for cardiac output) (28, 37). It has proved informative, for example, in determining the percentage absorption of calcium when one calcium is isotope given orally and another intravenously (38); Stephenson (29) used similar techniques for analysis of fatty acid transport. The findings based on this model were in good agreement with those obtained from the ratio method. In the latter, ^{131}I-VLDL-B and ^{125}I-LDL-B were injected and the ^{131}I and ^{125}I present in LDL-B were compared once the disappearance curves had become parallel. The results from both these methods indicate that in the patients studied, 90% of VLDL-B mass on average is converted into LDL in normotriglyceridemic subjects.

This is in contrast with the findings in rats: less than 10% of VLDL-B appears in LDL and most is removed by the liver (39, 40). This could explain the low concentration of LDL in rats compared with humans.

Most of radiolabeled VLDL-B peptide was recovered in the LDL fraction 1.019-1.063 in the subject in whom this was quantitated. This may be different in hyperlipidemic subjects; Hall et al. (41) have recently reported that a part of the intermediate density fraction in hyperlipidemics appears to bypass the density fraction 1.019-1.063. The present study has also demonstrated that the conversion time from VLDL into LDL of *d* 1.019-1.063 is considerably longer than when *d* 1.006-1.063 is used because of the time taken to pass through the intermediate density range 1.006-1.019.

Studies of turnover rates of VLDL-B and LDL-B peptide (study C). Studies A and B thus suggested that most VLDL-B peptide is converted into LDL-B peptide and that most or all LDL-B peptide is derived from VLDL-B peptide. In study C, synthetic rates for this peptide were compared in these two lipoprotein classes.

Of the eight subjects studied, four showed closely similar synthetic rates for VLDL-B and LDL-B (less than 5% difference). In the other subjects VLDL-B turnover was higher than that of LDL-B; this conceivably reflects a true biological heterogeneity, but it is possible that the discrepancy results from purely analytical factors. It is evident from Kane's data (21) that the tetramethylurea precipitate may include a trace of protein other than apo B. Resultant overestimation of VLDL-B pool size would lead to a magnified error in estimating synthetic rate of VLDL-B peptide, as the pool is turned over several times per day. It is to be noted that VLDL-B turnover was measured on a very low fat diet when no chylomicrons were circulating. One could therefore not exclude that chylomicrons might (under other nutritional circumstances) contribute to the production of LDL-B. The observation that LDL-B turnover, measured during 2 wk of normal free diet, was still similar to the VLDL-B turnover would make this possibility unlikely.

For the estimation of LDL-B turnover, the density range 1.006-1.063 g/ml was used to obtain the total turnover rate of LDL-B peptide. Lee and Alaupovic (10) have shown that the apoprotein of *d* range 1.006-1.019 contains 30-40% apo C. But, as discussed previously, the total apoprotein concentration of *d* 1.006-1.019 is only a small part of the total apoprotein concentration of *d* 1.006-1.063 (at least in the normoglyceridemic subjects of study C). The use of *d* 1.006-1.063 should therefore only introduce a minor overestimation of LDL-B pool size.

The parallelism of the disappearance curves in plasma after a bolus injection of ^{131}I-VLDL-B and after a constant infusion of ^{125}I-VLDL-B suggests that no significant mixing with extravascular pools occurred. The slopes were monoexponential from the first observation (at 10 min) and for at least 3-4 half-lives, during which time most of ^{131}I-VLDL-B is recovered in LDL (study B). This suggests that the disappearance for ^{131}I-VLDL-B in plasma after a bolus injection is mostly determined by the rate of conversion of VLDL into LDL and is not to any extent affected by mixing with extravascular pools. By contrast, about one third of LDL-B peptide is in extravascular compartments (16).

ACKNOWLEDGMENTS

We are grateful to Mrs. S. Grundy and Mr. A. February for skillful technical assistance. We thank Dr. J. Reeve for constructing a computer program and helpful criticism on the manucript. We thank Dr. P. Byfield for the amino acid analyses.

This study was supported by a grant from the Medical Research Council.

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