Effects of 1,2-cyclohexanedione modification on the metabolism of very low density lipoprotein apolipoprotein B: potential role of receptors in intermediate density lipoprotein catabolism[1]

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Abstract The conversion of very low density (VLDL) to low density lipoproteins (LDL) is a two-step process. The first step is mediated by lipoprotein lipase, but the mechanism responsible for the second is obscure. In this study we examined the possible involvement of receptors at this stage. Apolipoprotein B (apoB)-containing lipoproteins were separated into three fractions, VLDL (S_f 100–400), an intermediate fraction IDL (S_f 12–100), and LDL (S_f 0–12). Autologous ^{125}I-labeled VLDL and ^{131}I-labeled 1,2-cyclohexanedione-modified VLDL were injected into the plasma of four normal subjects and the rate of transfer of apoB radioactivity was followed through IDL to LDL. Modification did not affect VLDL to IDL conversion. Thereafter, however, the catabolism of modified apoB in IDL was retarded and its appearance in LDL was delayed. Hence, functional arginine residues (and by implication, receptors) are required in this process. Confirmation of this was obtained by injecting ^{125}I-labeled IDL and ^{131}I-labeled cyclohexanedione-treated IDL into two additional subjects. Again, IDL metabolism was delayed by approximately 50% as a result of the modification. These data are consistent with the view that receptors are involved in the metabolism of intermediate density lipoprotein. — **Packard, C. J., D. E. Boag, R. Clegg, D. Bedford, and J. Shepherd.** Effects of 1,2-cyclohexanedione modification on the metabolism of very low density lipoprotein apolipoprotein B: potential role of receptors in intermediate density lipoprotein catabolism. *J. Lipid Res.* 1985. **26:** 1058–1067.

Supplementary key words low density lipoprotein

Apolipoprotein B (apoB),[1] the major protein component of very low density, intermediate density, and low density lipoproteins (VLDL, IDL, and LDL) is unique among the apolipoproteins in that it remains associated with its parent particle throughout its lifetime in the plasma. This property has led to its use as a tracer of the metabolic interrelationships that exist between these species. Early investigations (1) indicated that it is secreted by the liver in triglyceride-rich particles that undergo continuous remodelling in the plasma via the agency of lipoprotein lipase until small cholesteryl ester-enriched LDL are formed. Ultimately, removal of LDL from the system is under the regulatory control of high affinity receptors on cell membranes (2).

Recent studies have extended this simple concept to reveal metabolic complexities that reflect the structural heterogeneity in the VLDL–IDL–LDL spectrum. Streja, Kallai, and Steiner (3) examined the secretion of triglyceride into VLDL and showed that it appeared at multiple sites across the VLDL (S_f 20–400) flotation interval; and Fisher et al. (4) have confirmed that this phenomenon also applies to apolipoprotein B. It follows then that smaller VLDL particles may derive from direct secretion or from lipolysis of larger particles. In a recent publication (5) we have examined the possibility of the existence of multiple catabolic channels within VLDL. Large (S_f 100–400) particles were labeled with ^{125}I and the metabolic fate of its apoB was determined in normal and hyperlipemic individuals. It transpired that whereas these large particles were delipidated rapidly and quantitatively to remnants of S_f 12–100, subsequent catabolism was slow and did not result in the formation of LDL. Rather, the latter arose from small (S_f 20–60) VLDL particles secreted directly into the plasma. Bezafibrate, which is known to activate lipoprotein lipase, accelerated the clearance of the large triglyceride-rich particle but had

Abbreviations: S_f, negative sedimentation coefficient at d 1.063 kg · l^{-1} and 26°C; VLDL, very low density lipoproteins, S_f 100–400; IDL, intermediate density lipoproteins, S_f 12–100; LDL, low density lipoproteins, S_f 0–12; apoB, apolipoprotein B; apoE, apolipoprotein E; apoC, apolipoprotein C; CHD, 1,2-cyclohexanedione; TMU, 1,1,3,3-tetramethylurea.

[1] Part of this study was presented at the 14th Meeting of the Federation of European Biochemical Societies in Edinburgh, March 29–April 3, 1981, and has appeared in abstract form (*Biochem. Soc. Trans.* 1981. **9:** 211).

no effect on the remnants (6). So it appears that two distinct catabolic processes are involved.

In this report we address the mechanism involved in the catabolism of remnants of large triglyceride-rich lipoproteins. Our stratagem was to trace labeled S_f 100–400 VLDL isolated by cumulative flotation ultracentrifugation and examine the influence of arginine modification on its plasma clearance. The rationale to this approach is based upon our earlier observations (7) that 1,2-cyclohexanedione modification of the arginine residues of LDL retarded its catabolism by interfering with its interaction with high affinity cell membrane receptors. It is known that VLDL can interact with the same receptor but the physiological significance of this is not yet clear. Our findings in the present study indicate that arginine residues play a role in remnant lipoprotein clearance, and we infer from this that receptors are involved in the process.

MATERIALS AND METHODS

Subjects

Six normolipemic and two hypertriglyceridemic subjects volunteered for the study. Their ages, body weights, and plasma lipid and lipoprotein profiles are documented in **Table 1.** None showed evidence of hepatic, renal, endocrine, or hematologic dysfunction, and throughout the study all drug therapy was proscribed with the exception of KI administered at a dose of 60 mg thrice daily for 3 days before and 1 month after administration of radioactivity in order to block its thyroidal sequestration. According to the Fredrickson classification system (8), the two hypertriglyceridemic subjects had Type V hyperlipoproteinemia. There was no obvious underlying cause for their disease and, in particular, alcohol abuse was excluded. The project protocol conformed to the requirements of the Ethical Committee of Glasgow Royal Infirmary.

Experimental protocol

Lipoprotein isolation. In this study we decided to separate the apolipoprotein B-containing lipoproteins into three density classes, using a cumulative flotation ultracentrifugation procedure modified from that described by Lindgren, Jensen, and Hatch (9). Two-milliliter aliquots of plasma were raised to a density of 1.182 $kg \cdot l^{-1}$ by the addition of solid NaCl (0.345 g) and layered over a 0.5-ml cushion of 1.182 $kg \cdot l^{-1}$ NaBr solution in a Beckman SW40 cellulose nitrate tube (Beckman Instruments Co., Palo Alto, CA). Above this was constructed the discontinuous gradient described by Lindgren et al. (9) for LDL subfraction separation. The preparation was centrifuged at 23°C for 66 min at 37,000 rpm, decelerated without applying the brake, and the top 1.0 ml, containing VLDL of S_f 100–400, was removed carefully with a Pasteur pipette. This volume was replaced with 1.0 ml of 1.0588 $kg \cdot l^{-1}$ solution and centrifugation was continued at 23°C for a further 17 hr and 36 min at 27,000 rpm. IDL, S_f 12–100, accumulated at the gradient surface during this time and was removed in 0.5 ml. Finally, centrifugation for 21 hr and 12 min at 28,000 rpm and 23°C permitted the isolation of LDL (S_f 0–12) in the top 1.0 ml of the tube. Chylomicrons, if present, were removed in a preliminary centrifugation at 23°C for 30 min at 10,000 rpm in a Beckman 40.3 rotor. For the normal subjects whose VLDL metabolism was investigated, it was necessary to prepare an initial concentrate of total VLDL ($d < 1.006$ $kg \cdot l^{-1}$, S_f 20–400) by the standard procedure of Havel, Eder, and Bragdon (10) in which 200 ml of plasma from a fasting subject was ultracentrifuged for 18 hr at 23°C and 40,000 rpm in a Beckman Ti 60 rotor. The crude preparation was then treated as described above for plasma to isolate VLDL of S_f 100–400. This preliminary step was unnecessary when preparing the material from the hypertriglyceridemic subjects.

We chose the above density intervals on the basis of previously published work which suggests that lipoproteins of S_f 12–100 form a metabolically distinct class (1, 11, 12). In contrast to the less dense VLDL of S_f 100–400, their clearance rate does not correlate with plasma lipoprotein lipase activity (13), nor does their catabolism accelerate following intravenous administration of heparin (12). Rather, they accumulate, at least initially, in

TABLE 1. Plasma lipids and lipoproteins in normal and hypertriglyceridemic subjects

Subject	Age	Body Weight	Plasma Triglyceride	Plasma Cholesterol	VLDL Cholesterol	LDL Cholesterol	HDL Cholesterol
	yr	*kg*			*mmol · l⁻¹*		
N_1 (M)	27	68	2.25	5.5	0.9	3.55	1.10
N_2 (M)	23	76.4	1.20	6.60	0.20	4.10	2.20
N_3 (F)	48	50	1.90	5.55	0.70	3.70	1.20
N_4 (M)	36	62	1.95	5.80	0.75	3.25	2.10
N_5 (M)	28	68	0.86	4.65	0.30	2.67	1.68
N_6 (M)	20	87	1.05	5.04	0.27	3.11	1.67
HTG_1 (M)	48	81.4	23.6	10.15	7.60	1.85	0.73
HTG_2 (M)	56	87.6	52.9	17.3	16.0	0.60	0.66

response to the polyanion-induced rapid clearance of VLDL.

Radiolabeling and chemical modification of lipoproteins. VLDL (S_f 100–400) or IDL (S_f 12–100) was concentrated where necessary by pressure filtration (Amicon XM100A filters, Amicon Corp., Bedford, MA) to a protein content of 1–5 $mg \cdot ml^{-1}$. The material was then radiolabeled in two aliquots with ^{125}I and ^{131}I according to the procedure of Bilheimer, Eisenberg, and Levy (14). Labeling efficiencies for VLDL and IDL were typically between 5–10% and 10–20%, respectively. The amount of ICl in the reaction mixture was adjusted to incorporate no more than 1 mole of iodine per 300,000 daltons of protein. Lipid labeling was less than 15% for VLDL and 8% for IDL. Of the remaining radioactivity, about 40% and 70%, respectively, was in 1,1,3,3-tetramethylurea (TMU)-precipitable protein (15). Following the labeling procedure the lipoprotein was desalted by gel filtration through a Pharmacia PD 10 column (Pharmacia, London, UK) using 0.15 M NaCl–0.01% Na_2EDTA, pH 7.0, as eluant. One of the labeled lipoproteins (usually ^{131}I) was then treated with 1,2-cyclohexanedione (16). One milliliter, containing 1–5 mg of protein, was incubated at 35°C for 2 hr with 2.0 ml of 0.15 M 1,2-cyclohexanedione in 0.2 M borate buffer, pH 8.1. Thereafter the lipoprotein was again desalted by gel filtration through a PD 10 column and exhaustively dialyzed at 4°C for 18 hr using the same buffer as described above. This procedure modified more than 55% of the arginine residues on the apolipoprotein B moiety of VLDL and has been shown by Gianturco et al. (17) to block binding of the lipoprotein to the high affinity LDL receptor on fibroblasts.

Turnover study protocol. Preparation of the radiolabeled and modified lipoproteins took 48 hr. Following sterilization (0.45 μm filters, Amicon Corp., Bedford, MA) the native and cyclohexanedione-treated preparations were injected, via an intravenous catheter, into the bloodstream of the donor, separated by a bolus of sterile 0.15 M NaCl at 8.00 AM. Blood samples were collected thereafter at frequent intervals over the next 24 hr and then daily in the fasting state for 14 days. On the first day of the VLDL studies, the fat intake of each volunteer was restricted to less than 5.0 g to minimize chylomicron production. The other dietary components were maintained at the level that the subject normally consumed. These nutritional procedures were under the control of a dietitian.

Plasma aliquots taken at each time point were subjected to the cumulative ultracentrifugation procedure described above to isolate VLDL (S_f 100–400), IDL (S_f 12–100), and LDL (S_f 0–12). All samples were stored until the end of the 2-week turnover and then treated (5) with TMU at 37°C as described by Kane et al. (15) in order to prepare apolipoprotein B. In the normal subjects whose VLDL concentration was low, an approximately equivalent amount of unlabeled carrier VLDL was added to the isolated labeled VLDL fractions prior to TMU precipitation. Radioactivity and protein measurements were made on the apoB samples from each lipoprotein fraction as described elsewhere (18). The pool size of apolipoprotein B in IDL and LDL was calculated as the mean TMU-precipitable protein recovered in each fraction obtained from all fasting blood samples. The total apoB radioactivity in each fraction was expressed as a percentage of the initial (i.e., 10 min) plasma sample. The plasma volume was calculated as 4% of the subjects' body weight which gave a value comparable to that obtained by isotope dilution over the first 10 min.

Apolipoprotein B kinetic analysis. The apolipoprotein B decay curves from VLDL→IDL→LDL were analyzed by a computer-based multicompartmental modeling program.[2] The algorithm used incorporated commercially available subroutines (Numerical Algorithms Group, Cambridge, UK) into a Fortran program that permitted data input, model specification, and output of the final parameters. The input to the program included time, apolipoprotein B radioactivity as a fraction of that present in the initial sample, and a weighting factor associated with each observed datum, calculated as described by Berman and Weiss (19). The model shown in **Fig. 1** is the simplest that gave an acceptable fit to the observed data and is described in detail elsewhere (5, 6). It includes two VLDL pools (1 and 2) within the vascular compartment. In normal subjects, VLDL apoB was cleared monoexponentially and therefore the tracer here was confined to pool 1. Pool 2, however, was required to explain the biexponential decay of VLDL apoB in the Type V subjects. IDL apoB exhibited biexponential decay, whether injected directly or as its precursor in VLDL. Hence, it required two compartments, again located within the vascular space. LDL apoB kinetics were analyzed according to the conventional two-pool model, catabolism occurring from the intravascular compartment. The capillary transfer rates between the intra- and extravascular pools were limited to retain 72% of the LDL apoB within the

[2]Multicompartmental analysis was performed using a Fortran program similar to SAAM (19) and NONLIN (31). Three commercially available subroutines (Numerical Algorithms Group, Cambridge, UK) were used to provide: *a*) integration of first order differential equations (DO2BBF); *b*) minimization of the sum of squared differences between observed and calculated values (EO4JAF); and *c*) matrix inversion (FO1ADF).

The algorithm proceeded as follows. The program input parameters were *i*) time of sampling; *ii*) observed apoB radioactivities expressed as a fraction of the total apoB radioactivity present in the 10-min sample; *iii*) a weight for each datum calculated from its estimated error (19); and *iv*) initial estimates for the rate parameters described in the models (Figs. 1 and 4). An iterative procedure adjusted the rate constants to minimize the sum of squares between observed and calculated data. The final solution was accepted when it gave a consistent and unique fit to the data (32).

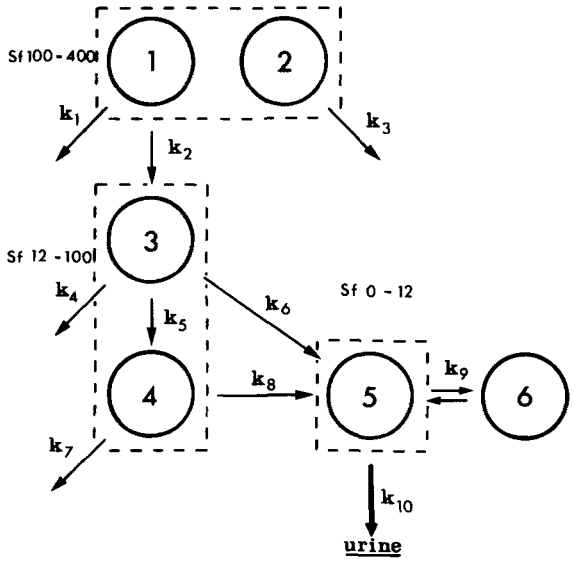

Fig. 1. Metabolic model used in the analysis of apolipoprotein B kinetics from VLDL to LDL. The fractional clearance rate of S_f 12–100 apoB was calculated from the formula:

$$FCR = \frac{\frac{k_4}{k_5}(k_7 + k_8) + \frac{k_6}{k_5}(k_7 + k_8) + k_7 + k_8}{1 + \frac{(k_7 + k_8)}{k_5}}$$

1) Residence time $(\tau_{IDL}) = \frac{1}{k_4 + k_5 + k_6} + \frac{k_5}{(k_4 + k_5 + k_6)} \times \frac{1}{k_7 + k_8}$

2) $FCR = 1/\tau$.

plasma, in accord with data obtained from prior studies of LDL kinetics (7, 20). In preliminary models, VLDL apoB was allowed to feed the intravascular LDL pool directly, but this was not a requirement in fitting the data of either the normal or the hypertriglyceridemic subjects and was abandoned. Fractional clearance rates for total VLDL, IDL, and LDL apolipoprotein B were determined from the relationships shown in Fig. 1.

RESULTS

The apolipoprotein B moiety of VLDL (S_f 100–400) in the plasma of normolipemic subjects transfers rapidly into the IDL (S_f 12–100) density range. Examination of the decay profile for a typical individual (**Fig. 2**) shows that within 6 hr the radioactivity present in VLDL apoB is lost monoexponentially, reaching peak values in IDL within 2–3 hr of injection. The kinetic parameter for the conversion of VLDL to IDL (i.e., rate constant k_2, Fig. 1) was of the same order in all four normal subjects. However, one (subject N_2) exhibited significant direct catabolism of VLDL apoB (as indicated by the high value of k_1, **Table 2**). We cannot exclude the possibility that denaturation during VLDL tracer preparation was responsible for this finding. Clearance from the intermediate density fraction proceeds slowly and in a biphasic fashion. IDL transfer into LDL (S_f 0–12) however is incomplete. Fifteen hours after injection, IDL apoB radioactivity had fallen to about 20% of the injected dose from a peak value of 60%. Over the same time period, LDL apoB radioactivity had reached a plateau of only 6% of the initially injected apoB radioactivity. This plateau was maintained for approximately 80 hr and thereafter the die-away curve for the apolipoprotein was characteristically slow. When the decay curves were analyzed by the model shown in Fig. 1, the rate constants in Table 2 were obtained. These were used to calculate the fractional clearance rates presented in **Table 3.** The analysis indicated that VLDL apoB had a plasma residence time of 1.1 hr. As would be expected from inspection of the decay curves, the conversion of VLDL apoB to IDL was virtually quantitative in three of the four normal subjects (N_1, N_3, and N_4, Table 2). The plasma fractional clearance of this intermediate (1.29 ± 0.23 pools $\cdot d^{-1}$) was much slower than that of VLDL apoB and the majority of IDL apoB radioactivity was removed directly from the plasma and did not reappear in LDL. The fractional catabolism of apoB in LDL (0.34 ± 0.07 pools $\cdot d^{-1}$) was very similar to the values obtained previously in our laboratory by direct measurement (7).

Cyclohexanedione treatment of VLDL prior to its injection into the volunteers substantially altered the metabolic behavior of its apolipoprotein B moiety. While the plasma residence time of VLDL apoB remained unchanged at 1.1 hr and its appearance in IDL reached a maximum within the same time period as did the native material, its subsequent clearance was delayed. The decay curve of IDL apoB radioactivity retained its biexponential appearance but catabolism of both components was substantially retarded relative to the unmodified lipoprotein (compare rate constants k_4, k_6, k_7, and k_8 for native and modified apoB, Table 2). In fact, the overall fractional clearance rate of cyclohexanedione-modified apoB from the IDL density range was only half normal, suggesting that functional arginine residues on apoVLDL must be required in the subsequent catabolism of IDL. Examination of the rise curve of radioactivity in LDL apoB supports this proposal. The appearance of radioiodide in this fraction from modified VLDL was retarded and its peak value was delayed by about 60 hr (Fig. 2). Finally, the clearance rate of modified apoB from the LDL density range was slower than normal, in keeping with the well-established observation that cyclohexanedione modification inhibits LDL catabolism.

The observation that the VLDL apoB to IDL apoB conversion was not affected by 1,2-cyclohexanedione treatment, whereas subsequent clearance of the apolipoprotein was, led us to examine directly the transit of radioactivity from native and modified IDL to LDL. **Fig. 3** describes

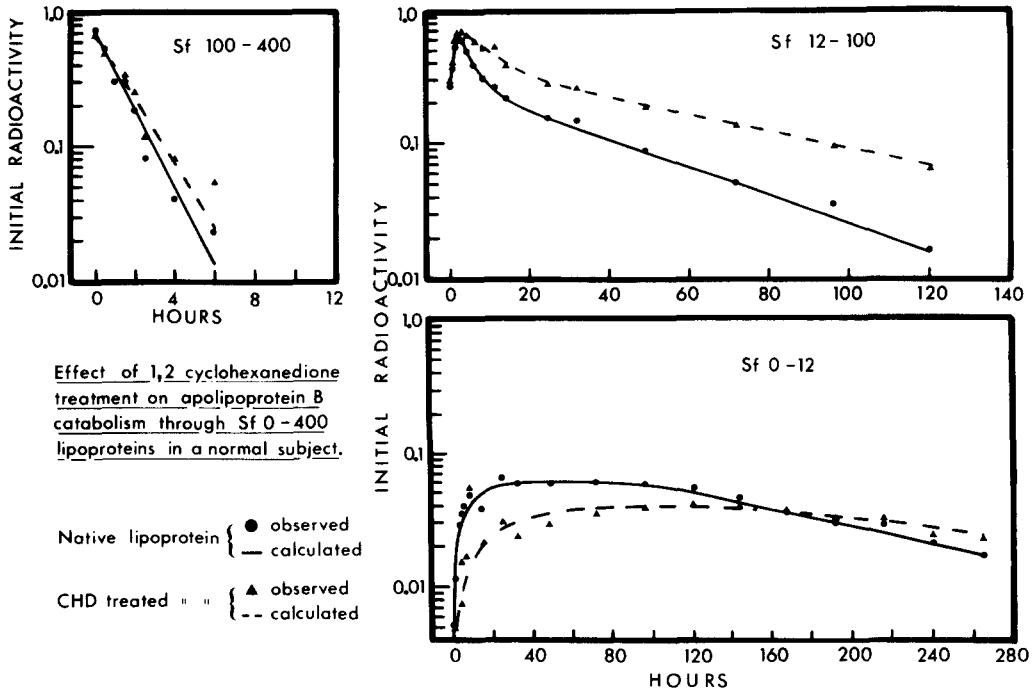

Fig. 2. Transit of native and 1,2-cyclohexanedione-modified apoB from VLDL (S_f 100–400) through IDL (S_f 12–100) to LDL (S_f 0–12) in subject N_3.

the results obtained from a normal subject. As before, IDL catabolism was biexponential and again both phases of the decay curve were slowed by cyclohexanedione treatment. The fractional clearance rates obtained for the native and cyclohexanedione-treated IDL in the two subjects were 1.99, 3.12 and 0.88, 1.18 pools · d^{-1}, respectively. However, conversion of IDL to LDL apoB differed in extent from that seen following injection of VLDL. This is apparent from the LDL apoB radioactivity profiles in Figs. 2 and 3. In Fig. 3, 44% of the apoB radioactivity in native IDL was lost within 15 hr and seemed to be virtually quantitatively transferred into the LDL density range where peak radioactivity values of 38% of the injected dose were attained. It should be noted that the rise in LDL radioactivity is associated with the more rapid initial clearance of IDL; the slower terminal IDL apoB exponential function apparent by 20 hr following injection seemed to contribute little to LDL apoB whose plasma radioactivity was itself falling at this time. The clearance curves of apoB from the modified intermediate density lipoprotein followed a similar pattern, but the appearance of radioactivity in LDL apoB was slower, the

TABLE 2. Kinetic parameters of apoB metabolism in native and arginine-modified very low density, intermediate density, and low density lipoprotein

	Rate Constants (hr^{-1})								
Subject	k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_{10}
N_1 native	0.002	0.47	0.0	0.095	0.055	0.0	0.0	0.026	0.017
N_1 CHD-treated	0.0	0.58	0.0	0.034	0.067	0.0	0.0064	0.011	0.011
N_2 native	1.15	0.94	0.0	0.21	0.12	0.0	0.014	0.009	0.012
N_2 CHD-treated	0.50	1.4	0.0	0.11	0.084	0.0	0.012	0.002	0.008
N_3 native	0.006	0.68	0.0	0.22	0.077	0.017	0.014	0.009	0.011
N_3 CHD-treated	0.090	0.49	0.0	0.080	0.045	0.004	0.011	0.003	0.006
N_4 native	0.0	0.40	0.0	0.12	0.026	0.004	0.0014	0.01	0.017
N_4 CHD-treated	0.04	0.32	0.0	0.063	0.011	0.0013	0.0013	0.006	0.011
HTG_1 native	0.039	0.011	0.05	0.079	0.17	0.047	0.022	0.0	0.014
HTG_1 CHD-treated	0.032	0.019	0.0026	0.007	0.024	0.004	0.0086	0.006	0.009
HTG_2 native	0.007	0.10	0.027	0.041	0.010	0.026	0.008	0.004	0.041
HTG_2 CHD-treated	0.022	0.027	0.015	0.0	0.012	0.009	0.015	0.0	0.010

The rate constants above are defined in Fig. 1.

TABLE 3. Apoprotein B kinetics in VLDL, IDL, and LDL from normal subjects

Subject	Lipoprotein Class	Fractional Clearance Rate (pools · d^{-1})		
		Native	CHD-treated	Difference (native minus CHD-treated)
N_1	VLDL	11.4	14.0	0
N_2	VLDL	50.00	47.0	3.0
N_3	VLDL	6.4	14.0	2.4
N_4	VLDL	9.6	8.6	1.0
Mean ± SD		21.9 ± 16.4	20.9 ± 15.2	1.6 ± 1.2
N_1	IDL	1.15	0.55	0.60
N_2	IDL	1.25	0.70	0.55
N_3	IDL	1.67	0.70	0.96
N_4	IDL	1.08	0.58	0.50
Mean ± SD		1.29 ± 0.23	0.63 ± 0.07	0.65 ± 0.18
N_1	LDL	0.4	0.26	0.14
N_2	LDL	0.28	0.20	0.08
N_3	LDL	0.26	0.14	0.12
N_4	LDL	0.40	0.26	0.14
Mean ± SD		0.34 ± 0.07	0.22 ± 0.05	0.12 ± 0.02

peak was later, and subsequent catabolism was retarded.

The model adopted for the kinetic analysis of these decay curves (**Fig. 4**) is a subset of that shown in Fig. 1. IDL apoB was considered to occupy two pools within the plasma compartment, neither of which penetrated the extravascular space. This is consistent with the models adopted by others (21, 22). Catabolism into a single intravascular pool of LDL could occur from either IDL compartment, and LDL was free to exchange with the extravascular space. It was considered to be catabolized only in the plasma compartment. Unlike VLDL apoB, which in the normal subjects had a monoexponential plasma decay and therefore could be considered as a single pool of tracer, the radioactivity injected as IDL apoB had to be distributed between the two IDL compartments. This was achieved, assuming that each was labeled to the same specific activity, by determining the Y axis intercepts of the two exponential functions and distributing the radioactivity accordingly. The rate constants derived for IDL apoB using this model (**Table 4**) confirmed our impressions obtained by visual inspection of the curves. IDL was converted quantitatively to LDL. In addition, not only was direct catabolism (k_1, k_4, Table 4) delayed, but the rate of conversion of IDL to LDL (k_3, k_5, Table 4) was also slower.

Having established the pattern in normal subjects, we proceeded to examine the transit of native and modified apolipoprotein B through VLDL to LDL in two individuals in whom the conversion process was severely compromised. The first clear difference from normal (**Fig. 5**, Table 2) was that VLDL apoB catabolism was slow and biexponential. Moreover, if modified, the VLDL was catabolized at only half of the rate of the unmodified tracer. This derived primarily from a delay in the disappearance of the second exponential, so that direct catabolism of VLDL apoB was substantially compromised by cyclohexanedione treatment. The transit of radioactivity through IDL to LDL was more difficult to interpret than in the normal situation. However, both IDL and LDL catabolism were obviously delayed by modification, and kinetic analysis again suggested that the catabolism of IDL requires functional arginine residues on the lipoprotein apoprotein (Table 2).

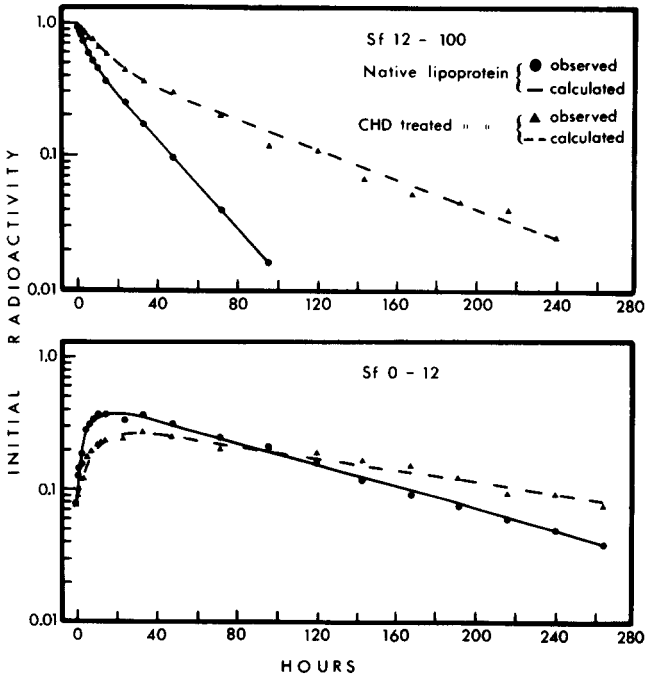

Fig. 3. Effects of 1,2-cyclohexanedione treatment on the conversion of IDL apoB to LDL apoB in subject N_6.

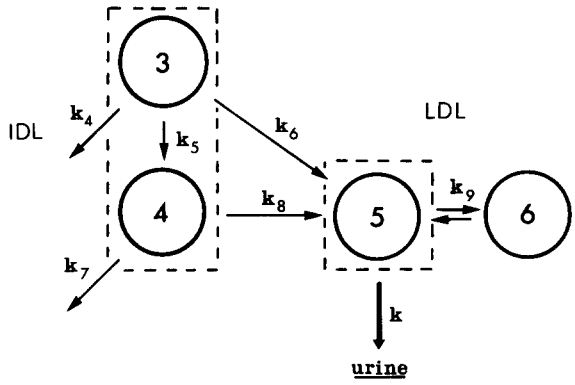

Fig. 4. Metabolic model used in the kinetic analysis of IDL apoB→LDL apoB conversion.

DISCUSSION

Available evidence supports the view that within the S_f 12–400 flotation interval there is continuous secretion of nascent lipoproteins that undergo progressive delipidation and remodelling until they are removed from the system. Early investigations (23) suggested that LDL was the point of exit for the majority of the secreted apoB. However, more detailed recent studies (4–6) have shown that there are multiple exit points; apoB secreted in large triglyceride-rich VLDL need never reach LDL since it can form a remnant within the S_f 20–400 range that can be removed directly from the circulation. In fact, VLDL of $d < 1.006$ kg/l comprises a mixture of newly secreted and lipolyzed VLDL remnants. By selecting a tracer of large (S_f 100–400) VLDL we hoped to be able to follow sequentially the processes of lipolysis and remnant removal.

In order to assess the potential involvement of receptors in VLDL catabolism, we chose to modify the lipoprotein in a way that is known to block LDL receptor binding in vitro. Mahley et al. (16) have shown that this could be achieved by derivatization of either arginyl or lysyl residues on the lipoprotein apoprotein. The relative merits of each modification have been examined in a number of earlier publications (7, 24). Ideally, irreversible chemical treatment of lysyl residues using reductive methylation or the newly described 2-hydroxyacetaldehyde procedure (25) would have been preferable. However, both caused VLDL to be cleared rapidly from the plasma. Consequently, we chose 1,2-cyclohexanedione to modify the VLDL apoprotein arginyl residues. We have found this agent to be satisfactory for the quantitation of LDL receptor-mediated catabolism in man (7). In fact, CHD-modified LDL is handled identically to the 2-hydroxyacetaldehyde-treated particle (25). Therefore, we were confident in its use in this situation, although we emphasize at this stage that the results should be interpreted qualitatively rather than quantitatively since we do not have prima facie evidence that the CHD-modified lipoprotein is truly representative of physiological tracees.

In the four normal subjects examined here, 1, 2-cyclohexanedione treatment did not affect the clearance rates of VLDL of S_f 100–400. Since catabolism of triglyceride-rich particles from this particular density range is dependent on the activity of lipoprotein lipase (13), it can be inferred that lipase-directed triglyceride hydrolysis was not altered in vivo by cyclohexanedione treatment of the substrate. Moreover, in unpublished in vitro studies, we have failed to demonstrate a difference in the clearance rates of ^{125}I-labeled native and ^{131}I-labeled cyclohexanedione-treated VLDL apoB from the $d < 1.006$ kg · l^{-1} density fraction when incubated with dialyzed $d > 1.21$ kg · l^{-1} postheparin plasma. These findings are not at variance with the observation that cyclohexanedione-treated apoC-II fails to activate lipoprotein lipase (26) since the rapidity with which this protein transfers between lipoprotein particles in vivo (14, 27) will ensure its virtual instantaneous acquisition by the injected, modified VLDL; and we have found that cyclohexanedione modification of VLDL does not significantly affect exchange of apoC between particles. A similar argument also holds for VLDL apolipoprotein E (apoE) which may have an involvement in receptor binding (14, 28, 29), although, in vitro, modified apolipoprotein E appears to exchange less freely (unpublished data). Thus, it is likely that the high molecular weight apolipoprotein B, which is conserved in

TABLE 4. Kinetic parameters of apoB metabolism in IDL and LDL from two normal subjects

Subject	Plasma Concentration (mg · dl^{-1})		Rate Constants (hr^{-1})					
	IDL	LDL	k_4	k_5	k_6	k_7	k_8	k_{10}
N_5 native	5.5	35.5	0.039	0.01	0.11	0.014	0.0	0.015
N_5 CHD-treated			0.01	0.016	0.047	0.0	0.007	0.011
N_6 native	8.5	39.2	0.002	0.0	0.153	0.016	0.021	0.015
N_6 CHD-treated			0.0	0.0075	0.072	0.011	0.003	0.009

See Fig. 4 for a definition of the above rate constants.

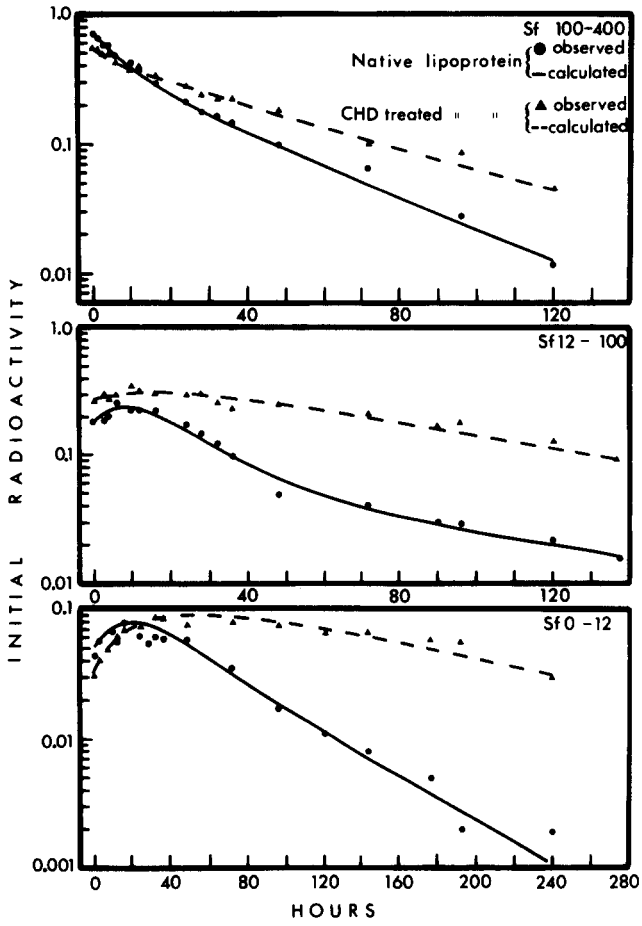

Fig. 5. Native and 1,2-cyclohexanedione-treated apolipoprotein B transit from VLDL (S_f 100–400) through IDL (S_f 12–100) to LDL (S_f 0–12) in Type V hyperlipoproteinemic subject HTG_1.

the particle during its catabolism, is primarily responsible for the changes induced by arginine modification. These changes first become apparent when the particle leaves the IDL S_f 12–100 density range. At this point the metabolism of the native and modified lipoproteins becomes divergent and the phenomenon continues through into LDL. Tables 2 and 3 show that cyclohexanedione treatment reduces the fractional clearance rate of IDL apoB to half its normal value. This phenomenon has been observed earlier in studies of LDL metabolism and has generally been interpreted as signifying a role for receptors in the catabolism of this lipoprotein. Although there are other potential explanations for our findings with regard to IDL, it is reasonable to assume that receptors may also be involved here. One potential aspect of this involvement may be in the direct removal of IDL from the plasma, presumably via the LDL receptor in both liver and extrahepatic tissues. However, this proposal does not completely explain our findings since the modification procedure also delays the appearance of radioactivity in LDL apoB. That is, modification must also slow the conversion process, suggesting that arginine residues and, by implication, receptors have an involvement here as well. This thesis was examined directly by following the pathways of catabolism of native and modified IDL to LDL in two normal subjects. It was immediately apparent that the modified IDL was cleared more slowly, resulting in a delay in the appearance of radiolabeled modified apoB in LDL. Following their transit to LDL, the terminal clearances of native and modified apoB occurred at rates consistent with values already obtained in LDL turnover studies (7). This implies that the arginine residues that are modified in VLDL apoB are those same residues that have a functional role in binding LDL to the receptor. It therefore follows that the charged domains responsible for the LDL/receptor interaction are exposed both on the surface of VLDL and IDL.

Another interesting point emerges from examination of the quantitative conversion of IDL apoB to LDL. Following injection of VLDL, its apolipoprotein B complement was rapidly and quantitatively transferred to IDL so that by 3 hr the latter contained the majority of the plasma apoB radioactivity (Fig. 2). However, only 21 ± 9% of the latter subsequently appeared in LDL. This contrasts with the situation that followed the direct injection of radiolabeled IDL (Fig. 3). In this instance there was virtually a precursor-product relationship between apoB in IDL and LDL (Tables 2 and 3). It would therefore seem that the IDL derived from VLDL is not metabolically identical to the S_f 12–100 fraction that was isolated from the plasma, radiolabeled, and reinjected. The nature of this apparent metabolic channelling is presented elsewhere (5, 6).

The catabolism of apoB in the Type V subjects differed from normal. Here, VLDL modification did affect the clearance of the B apolipoprotein from the S_f 100–400 density range. Although k_1 and k_2 were not markedly changed (see Fig. 1 and Table 2), clearance of the slowly metabolized VLDL apoB species (represented by k_3) was substantially retarded by cyclohexanedione treatment. Elsewhere (18) we have argued that this material in Type V subjects may represent apoB resident in partially degraded chylomicrons. Since, according to the argument presented above, modified apoC and apoE in VLDL should have been replaced, through exchange with native apoproteins, one can infer that apoB may play a role in the receptor-mediated uptake of these slowly degraded particles. IDL and LDL apoB catabolism in the Type V individuals was also retarded by cyclohexanedione treatment, in accord with the data obtained from the normal volunteers.

Reardon, Sakai, and Steiner (13) have shown that whereas the clearance rates of S_f 100–400 lipoproteins correlate significantly with plasma lipase activity, this is not true for intermediate density (S_f 12–60) particles whose catabolism shows no dependency on the activities

of either lipoprotein lipase or hepatic lipase. This is consonant with the recent finding from this laboratory that the clofibrate analogue bezafibrate, while activating VLDL apoB catabolism, has no effect on IDL apoB (6). Another regulatory mechanism may be involved in this process. The present study suggests that receptors may play important, perhaps regulatory, roles both in the direct catabolism of IDL and in its conversion to LDL. Whether they correspond either to the chylomicron remnant receptor or to the LDL receptor is not clear, although the accumulation of IDL in the plasma of familial hypercholesterolemic heterozygotes (30) makes the latter a more likely candidate.

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