Enhanced clearance from plasma of low density lipoproteins containing a truncated apolipoprotein, apoB-89

Klaus G. Parhofer, Alan Daugherty,* Makoto Kinoshita,[1] and Gustav Schonfeld

Division of Atherosclerosis and Lipid Research and Cardiovascular Division,* Department of Internal Medicine, Washington University School of Medicine, 4566 Scott Avenue, Box 8046, St. Louis, MO 63110

Abstract Previously we have reported on a kindred with hypobetalipoproteinemia in which three sisters were found to be compound heterozygotes for two newly described truncated forms of apoB, apoB-40 and apoB-89. ApoB-89-containing low density lipoproteins (LDL) bound with increased affinity to cultured normal human fibroblasts and were internalized and degraded at increased rates, suggesting that the low plasma concentrations of apoB-89-LDL of the patients could be due to enhanced rates of clearance through LDL-receptors. To examine this hypothesis, apoB-89-LDL was isolated from the three study subjects and apoB-100-LDL from two control subjects. LDL was conjugated to the radioiodinated residualizing label, dilactitol tyramine (*I-DLT, containing either ^{125}I or ^{131}I). *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL were simultaneously injected into ear veins of rabbits. The clearance from plasma and hepatic accumulations of both radiolabeled LDL fractions were followed over 24 h. Fractional catabolic rates (FCR) of apoB-89-LDL were $0.105 \pm 0.012\ h^{-1}$ compared to $0.054 \pm 0.007\ h^{-1}$ for apoB-100-LDL. In agreement with the enhanced clearance from plasma, 1.72 to 1.87 times more *I-DLT-apoB-89-LDL than *I-DLT-apoB-100-LDL accumulated in the livers 24 h after injection. There was no significant difference in splenic accumulation, suggesting that LDL-receptors rather than scavenger receptors mediated the enhanced clearance of apoB-89-LDL. To assess further the importance of LDL-receptors, *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL were reductively methylated to inhibit their interactions with LDL-receptors. Reductive methylation resulted in a marked decrease in FCRs for both LDL preparations (apoB-89-LDL: $0.028\ h^{-1}$ vs. $0.105\ h^{-1}$ unmodified; apoB-100-LDL: $0.023\ h^{-1}$ vs $0.054\ h^{-1}$ unmodified) and almost eliminated the difference in the FCR between apoB-89-LDL and apoB-100-LDL. The injection of *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL into a WHHL rabbit yielded similar results (apoB-89-LDL: $0.043\ h^{-1}$; apoB-100-LDL: $0.032\ h^{-1}$). These data suggest that deletion of 11% of the carboxyterminal of apoB-100 resulted in enhanced plasma clearance of apoB-89-LDL by liver primarily mediated by LDL-receptors. This may contribute to their low concentrations in plasma.—**Parhofer, K. G., A. Daugherty, M. Kinoshita, and G. Schonfeld.** Enhanced clearance from plasma of low density lipoproteins containing a truncated apolipoprotein, apoB-89. *J. Lipid Res.* 1990. **31:** 2001–2007.

Supplementary key words LDL receptor • fractional catabolic rate • dilactitol tyramine • WHHL rabbits • hypobetalipoproteinemia

The mechanisms responsible for the reduced concentrations or absence of apolipoprotein B (apoB)-containing lipoproteins observed in plasmas of hypobetalipoproteinemic subjects have not been defined. Potential mechanisms include a diminished ability to synthesize and/or secrete apoB from intestinal and hepatic tissue. In addition, this state may be a consequence of enhanced clearance of apoB-containing lipoproteins from plasma (1–4).

We have reported recently on a kindred with hypobetalipoproteinemia (5). In this kindred three compound heterozygous sisters were identified with fasting plasma concentrations of low density lipoprotein (LDL)-cholesterol and apoB that were < 5 percentile for their sex, age, and race. The apoB in the plasmas of the three sisters was not the normal apoB-100. Instead, low concentrations of two truncated forms of apoB were present, which were designated as apoB-40 and apoB-89 based on the percentile system (5). Heterozygotes for apoB-100/apoB-89 and apoB-100/apoB-40 also were found in this kindred. The genetic defects responsible for both apoB-89 and apoB-40 variants appeared to be the presence of premature stop codons (6). LDL isolated by ultracentrifugation at a density of 1.019 - 1.063 g/ml from these sisters contained ~ 95% apoB-89, < 5% apoB-40, and no detectable apoE. The high density lipoprotein (HDL) fraction (1.063–1.21 g/ml) contained most of the small amounts of apoB-40 present in plasma in addition to the usual apoproteins associated with this fraction (apoproteins A-I, A-II, E, and the Cs) (5). ApoB-89-containing LDL were smaller than most normal LDL and were bound and processed

Abbreviations: apoB, apolipoprotein B; LDL, low density lipoprotein; HDL, high density lipoprotein; DLT, dilactitol tyramine; GGE, gradient gel electrophoresis; FCR, fractional catabolic rate; NZW, New Zealand White; WHHL, Watanabe Heritable Hyperlipidemic; *I-DLT, DLT labeled with either ^{125}I or ^{131}I.

[1]Present address: First Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo Bunkyo, Tokyo, Japan.

by normal fibroblasts in culture with greater affinity than apoB-100-containing LDL isolated either from normal subjects or from patients with hypobetalipoproteinemia born into other, unrelated kindreds, whose LDL contained only apoB-100 (5). Based on these results, we hypothesized that the low plasma concentrations of apoB-89-LDL were due to apoB-89-containing LDL being cleared more rapidly than apoB-100-containing LDL (5, 6). To test the hypothesis, LDL were isolated from all three compound heterozygous sisters (apoB-89 and apoB-40) and two control subjects (apoB-100). Metabolism of these LDL preparations was determined in vivo following conjugation to the residualizing protein label, dilactitol tyramine, radiolabeled with either ^{125}I or ^{131}I (*I-DLT). LDL preparations were simultaneously injected into marginal ear veins of New Zealand White (NZW) rabbits. Clearance from plasma of *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL and extent of accumulation within hepatic tissue and the spleen were determined. To establish whether the different clearance rate of apoB-89-LDL is mediated via LDL-receptors, experiments were performed using reductively methylated LDL particles that are known to interact poorly, if at all, with LDL-receptors (7). Furthermore, the clearance rates of apoB-89-LDL and apoB-100-LDL were studied in a Watanabe Heritable Hyperlipidemic (WHHL) rabbit, an animal totally deficient in functional LDL-receptors (8).

MATERIALS AND METHODS

Materials

NZW rabbits (1.68–2.49 kg body weight) were obtained from Boswell Rabbit Farm (Pacific, MO) and maintained on standard laboratory diet (Ralston Purina, St. Louis, MO). The WHHL rabbit (3.42 kg body weight) was derived from a stock supplied by Dr. J. L. Goldstein, (Dallas, TX). $Na^{125}I$ and Na ^{131}I (carrier free) were obtained from Amersham Radiochemical Center, (Arlington Heights, IL).

Preparation of lipoproteins

Blood from fasted patients (subjects, Nos. 1-3 in Fig. 1 of reference 5) and two normolipidemic control donors were drawn into tubes containing EDTA (1.0 mg/ml). After separating plasma from blood cells by centrifugation, D-Phe-L-Phe-L-Arg chloromethylketone (20 μM; PPACK) and D-Phe-L-Pro-L-Arg chloromethylketone (20 μM; Pierce Chemical Co., Rockford, IL) were added promptly to prevent cleavage of apoB by kallikrein or thrombin, respectively (9). Control LDL (apoB-100-LDL) and patients' LDL (apoB-89-LDL) were isolated from plasma by sequential ultracentrifugation between the densities of 1.019 and 1.063 g/ml. LDL preparations were dialyzed at 4°C against phosphate-buffered saline (pH 7.4). The sizes of donor lipoproteins were evaluated by subjecting the plasmas of the donors to nondenaturing gradient gel electrophoresis (GGE) in 2–20% (w/v) gels, followed by transferring the protein to nitrocellulose and immunoblotting (10) with an anti-apoB monoclonal antibody directed against an NH_2 terminal epitope of apoB-100 (5, 11).

Radioiodination of lipoproteins

ApoB-89-LDL and apoB-100-LDL were radiolabeled with ^{131}I-DLT or ^{125}I-DLT, respectively, by reductive amination as described previously (12, 13). To evaluate isotope-specific effects, apoB-89-LDL was radiolabeled with ^{125}I-DLT and apoB-100-LDL with ^{131}I-DLT in two animals receiving LDL from subject #1. After conjugation, the radiolabeled LDL particles were separated from free *I-DLT by gel filtration using QUIK-SEP (Isolab; Akron, Ohio) and dialyzed overnight against EDTA/saline. The specific radioactivities of *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL were between 58 cpm/ng and 505 cpm/ng protein.

Reductive methylation of lipoproteins

*I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL were modified by reductive methylation of lysine residues as described previously (7). Briefly, *I-DLT-apoB-100-LDL and *I-DLT-apoB-89-LDL (in EDTA/saline) were diluted with borate buffer (0.3 M, pH 9.0) to 1.5 the original volume. Sodium borohydride (1 mg total) was added with stirring at 4°C followed by seven aliquots of 1 μl of 37% w/v aqueous formaldehyde over 36 min (at time 0, and thereafter every 6 min). After the last addition of formaldehyde the reaction was stopped by chromatography on Sephadex G50. Reductively methylated *I-DLT-LDL were dialyzed against EDTA/saline at 4°C overnight.

Metabolism of lipoproteins in vivo

Mixtures of LDL preparations (84–149 μg protein) labeled with either ^{125}I-DLT or ^{131}I-DLT were injected simultaneously via a marginal ear vein of NZW rabbits or a WHHL rabbit. The following kinetic studies were performed: 1) ^{131}I-DLT-apoB-89-LDL and ^{125}I-DLT-apoB-100-LDL in eight NZW rabbits (four rabbits for subject #1, four rabbits for subject #2, two rabbits for subject #3); 2) ^{125}I-DLT-apoB-89-LDL and ^{131}I-DLT-apoB-100-LDL in two NZW rabbits (subject #1); 3) ^{131}I-DLT-apoB-100-LDL and methylated ^{125}I-DLT-apoB-100-LDL in two NZW rabbits; 4) methylated-^{131}I-DLT-apoB-89-LDL and methylated-^{125}I-DLT-apoB-100-LDL in two NZW rabbits; and 5) ^{131}I-DLT-apoB-89-LDL and ^{125}I-DLT-apoB-100-LDL in one WHHL rabbit.

Blood was drawn into EDTA-containing tubes at the indicated times over 24 h and plasma was separated by centrifugation. Radioactivity was determined in 150-μl aliquots of whole plasma and in 10% trichloroacetic acid (TCA)-precipitated fractions. For determination of the accumulation of radioactivity in liver, animals were anesthetized with sodium pentobarbital 24 h after injection. The abdominal cavity was opened, a cannula was placed in the

portal vein, and EDTA/saline was introduced into the portal vein at a rate of 10 ml/min while blood was withdrawn from the inferior vena cava. After perfusing the liver with 50 ml of EDTA/saline, livers were removed, weighed, and samples were taken for determination of radioactivity. In most animals, the spleens were also removed, weighed, and samples were counted. Lipoprotein accumulation in tissue was expressed as the percentage of injected dose of radiolabeled lipoproteins that accumulated in the organs 24 h after injection. Clearance curves were constructed from the TCA-precipitable fractions of plasma radioactivity. In all radioactivity determinations, the values for ^{125}I radioactivity were corrected for spillover from ^{131}I, and for the radionuclide decay of ^{131}I. Fractional catabolic rates (FCR) for the radiolabeled LDL were computed based on the two-pool model using the RStrip program (MicroMath Scientific Software, Salt Lake City, UT), run on a DOS-based computer. The model selection criterion (a program-specific quality control) showed that all clearance curves could be described best by a two-exponential equation.

As the LDL fraction of the subject also contained small amounts of apoB-40 (<5%) there was some concern that any differences in the clearance rate could be due to a rapidly turning over subfraction of LDL (containing apoB-40). Therefore, the localization of the radioactivity was assessed in the labeled LDL preparations by SDS gradient polyacrylamide gel (3–10% w/v) electrophoresis (14) followed by autoradiography.

To assess the density distributions of injected radiolabeled LDL in plasma during 24 h of observation, aliquots of plasma (of two rabbits) were subjected to density gradient ultracentrifugation in a SW 40 Ti rotor (Beckmann Instruments, Palo Alto, CA) as described by Terpstra, Woodward, and Sanchez-Muniz (15) at 40,000 rpm at 20 °C for 22 h. Fractions (0.33–0.40 ml) were collected, and radioactivities and densities of fractions were determined. In addition, plasmas of rabbits were subjected to electrophoresis in 2–20% (w/v) nondenaturing gradient polyacrylamide gels (GGE) followed by drying of the gel and autoradiography.

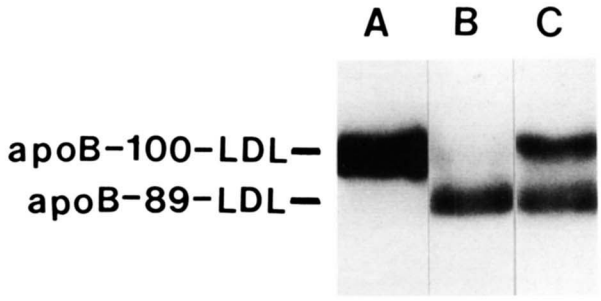

Fig. 1. Particle diameters of LDL. Plasmas from a control subject (homozygous for apoB-100) (lane A), subject #1 (heterozygous for apoB-89 and apoB-40) (lane B), and her brother (heterozygous for apoB-100 and apoB-89) (lane C) (nos. 1 and 8, respectively, of Fig. 1 in reference 5) were separated by 2–20% (w/v) GGE. After electrophoresis, proteins were transferred to nitrocellulose paper by the method of Lefevre, Goudey-Lefevre, and Roheim (10), and the nitrocellulose paper was incubated with the monoclonal anti-apoB, C1.4, that recognizes amino acids near the N-terminal of apoB-100 (11). The reactive proteins were detected by incubating the nitrocellulose paper with ^{125}I-labeled goat anti-mouse lgG.

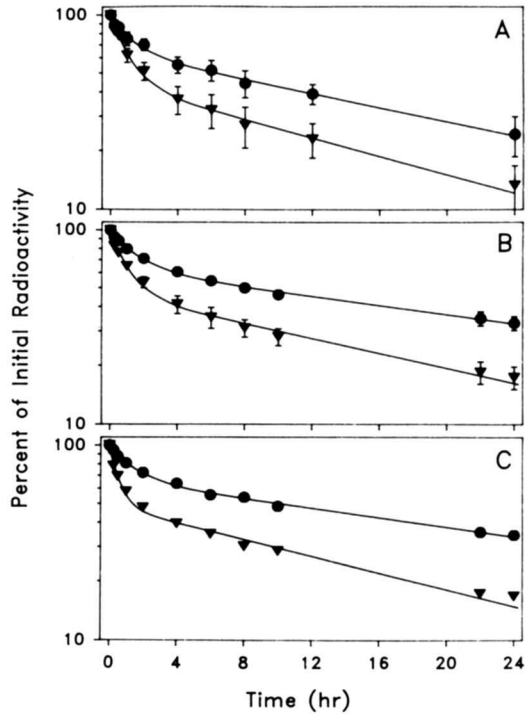

Fig. 2. Clearance curves from plasma of *I-DLT-apoB-89-LDL (triangles) and *I-DLT-apoB-100-LDL (circles) in NZW rabbits. A) subject #1, B) subject #2, C) subject #3. Points are means ± SEM from four animals for subject #1 (A) and subject #2 (B), and are means of two animals for subject #3 (C). Solid lines are predicted values from the two-exponential equation used to fit the data and to calculate FCRs. Concentration of radioactivity in plasma 1 min after injection was used as the 100% value.

RESULTS

Particle diameters of donor apoB-100-LDL and apoB-89-LDL were analyzed from unfractionated plasmas of a control subject, the compound heterozygous patient, and her apoB-100/apoB-89 heterozygous brother, by 2–20% w/v GGE (**Fig. 1**). LDL were subsequently visualized by immunoblotting. The apoB-100-LDL of the control subject and of the apoB-100/apoB-89 heterozygote were larger than apoB-89-LDL. This result confirms previous findings obtained by gel permeation chromatography (5).

Clearance curves of *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL from plasma were obtained in a total of ten NZW rabbits each of which was injected with both radiolabeled LDL preparations (**Fig. 2**). Fig. 2A–2C shows the clearance kinetics for the different subjects (#1–#3 respectively). Indicated concentrations of radioactivity represent mean values ± SEM for each time point (four animals for subject #1 and #2, two animals for subject #3). In all ten rabbits, *I-DLT-apoB-89-LDL was cleared more rapidly from plasma than *I-DLT-apoB-100-LDL. The FCRs of the

TABLE 1. Fractional catabolic rates of native and reductively methylated *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL in NZW and WHHL rabbits

Injected Lipoprotein	Recipient Rabbit	Subject	n	FCR (h^{-1}) ApoB-89-LDL	FCR (h^{-1}) ApoB-100-LDL	Ratio FCR-apoB-89-LDL / FCR-apoB-100-LDL
LDL	NZW	#1	4	0.126 ± 0.030	0.069 ± 0.017	1.83
LDL	NZW	#2	4	0.087 ± 0.007	0.045 ± 0.004	1.93
LDL	NZW	#3	2	0.099	0.048	2.06
Reductively methylated LDL	NZW	#1	2	0.028	0.023	1.23
LDL	WHHL	#1	1	0.043	0.032	1.34

FCRs were calculated on the basis of a two-compartment model. FCR values represent means ± SEM. When n < 4 no SEM was calculated.

LDL from the different study subjects are listed in **Table 1**. In every animal studied the ratio of FCRs of apoB-89-LDL and of apoB-100-LDL was very consistent (1.19 ± 0.08). Accumulations in the hepatic tissue of apoB-89-LDL and apoB-100-LDL over 24 h also were different, with apoB-89-LDL accumulation being 1.72–1.87 times that of apoB-100-LDL (**Table 2**). Only a small amount of the label (≤ 2%) could be found in the spleen after 24 h, with somewhat greater accumulation of apoB-100 particles than apoB-89 particles (Table 2). TCA precipitabilities of *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL were both 99.0% before injection, and between 91% and 98% for all rabbits in the plasma samples taken at 24 h.

On density gradient ultracentrifugation, flotation rates of the radioactivity peaks of both ^{125}I-DLT-apoB-89-LDL and ^{131}I-DLT-apoB-100-LDL were similar after injection and both remained in the LDL density range, but with increasing time the former moved to a slightly denser fraction, while ^{131}I-DLT-apoB-100-LDL moved to a slightly less dense fraction (**Fig. 3**). On GGE, LDL particles remained intact (**Fig. 4**).

Before injection the radiolabeled LDL fractions were subjected to SDS polyacrylamide gel electrophoresis and autoradiography to assess whether any apoB-40 was radiolabeled in the LDL of the subject (**Fig. 5**). More than 99% of the label in the LDL fraction was in the study subjects' apoB-89 and in the control subjects' apoB-100.

To study the effect of chemical modification of radiolabeled LDL particles on their clearance rate from plasma and their accumulation in tissues, reductively methylated *I-DLT-apoB-100-LDL and native *I-DLT-apoB-100-LDL were compared. Reductive methylation decreased the FCR of apoB-100-LDL from 0.080 h^{-1} to 0.032 h^{-1}. Correspondingly, the accumulation of methylated apoB-100-LDL in the liver was approximately 50% of the accumulation of unmodified apoB-100-LDL (20.1% and 11.1%, respectively). Next, the plasma kinetics and hepatic accumulation of reductively methylated-*I-DLT-apoB-89-LDL were compared to reductively methylated-*I-DLT-apoB-100-LDL in two NZW rabbits (**Fig. 6A**). Both reductively methylated LDL preparations were cleared considerably slower than native LDL. In contrast to the native LDL, the plasma clearance kinetics of modified apoB-89-LDL and modified apoB-100-LDL were very similar (Table 1). Also, both methylated preparations accumulated in the liver to the same extent (Table 2). A similar result was achieved when *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL were injected into a WHHL rabbit (Fig. 6B).

DISCUSSION

Although no formal surveys have been performed, it is probable that most patients with hypobetalipoproteinemia have normal sized apoB-100 molecules. However, a number of molecular defects of apoB have also been identified in hypobetalipoproteinemia. The variant proteins range in size from apoB-27 to apoB-89 (5, 6, 16–20). All are carboxyl terminal deletions of apoB-100 due to deletions of single bases or larger pieces of genomic DNA.

TABLE 2. Accumulation of native and reductively methylated *I-DLT-apoB-89-LDL and *I-DLT-apoB-100-LDL in liver and spleen of NZW rabbits

Studied Tissue	Injected Lipoprotein	Subject	n	Accumulation (% of injected dose) ApoB-89-LDL	Accumulation (% of injected dose) ApoB-100-LDL	Ratio ApoB-89-LDL / ApoB-100-LDL
Liver	LDL	#1	4	36.5 ± 13.8	21.1 ± 7.5	1.73
Liver	LDL	#2	4	15.9 ± 1.5	8.5 ± 1.1	1.87
Liver	LDL	#3	2	13.1	7.6	1.72
Liver	Reductively methylated LDL	#1	2	10.2	10.3	0.99
Spleen	LDL	#1	2	1.51	1.77	0.85
Spleen	LDL	#3	2	0.70	0.97	0.72
Spleen	Reductively methylated LDL	#1	2	1.92	1.99	0.96

Values represent the precentage of the injected dose of *I-DLT-apoB-89-LDL or *I-DLT-apoB-100-LDL that accumulated in livers and spleens of rabbits 24 h after injection. Values represent means ± SEM. When n < 4 no SEM was calculated.

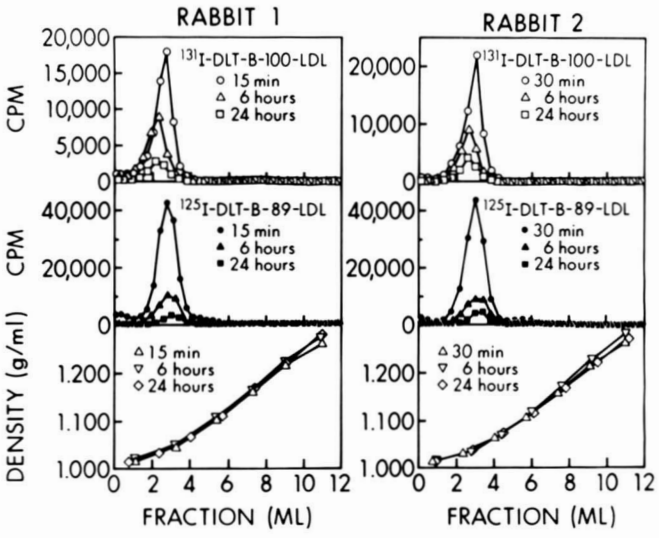

Fig. 3. Distribution of radioactivity in plasma fractions separated by density gradient ultracentrifugation. The top two panels represent the radioactivity of *I-DLT-apoB-100-LDL and the middle panels represent the *I-DLT-apoB-89-LDL. Plasma samples were collected at 15 min, 6 h, and 24 h from rabbit 1 (left) and at 30 min, 6 h, and 24 h from rabbit 2 (right). Bottom panels represent the density curves of three samples.

The mechanisms responsible for the reduced plasma LDL concentrations are unknown for most hypobetalipoproteinemia patients. ApoBs that are shorter than apoB-48 are not likely to be cleared by LDL-receptors because apoB-48 contains the amino terminal ~ 2200 amino acids, which do not include the LDL-receptor recognition domain (21). Thus, chylomicron remnants that contain apoB-48 and apoE are not cleared via LDL-receptors but rather via chylomicron remnant receptor (22) or the LDL-receptor-like protein (23) on hepatocytes that recognize apoE. Since LDL isolated from compound heterozygous hypobetalipoproteinemia patients with apoB-40 and apoB-89 contain very little, if any, apoE (5), one would expect their LDL to be cleared slowly and to accumulate in plasma. The fact that they do not suggests that production and/or secretion rates are very low.

ApoB-89 contains the LDL-receptor recognition region and the present data demonstrate that apoB-89-LDL is cleared more rapidly from plasma than apoB-100-LDL and that the enhanced clearance was due to increased hepatic accumulation, mediated primarily by LDL-receptors (Fig. 2, Tables 1 and 2). As the radioactivity in the spleen is minimal it is probable that trapped label in the liver was present in hepatocytes rather than in reticuloendothelial cells.

Two approaches were used to test whether LDL-receptors mediated the enhanced clearance of apoB-89-LDL: *1)* injection of reductively methylated LDL; and *2)* injection of LDL into a WHHL rabbit. The former abolishes the ability of LDL to be recognized by LDL-receptors (7, 24, 25) while in the latter, no functional LDL receptors are present (8). An experiment was performed to confirm that reductive methylation decreased the clearance of apoB-100-LDL from plasma as reported by others (7, 24, 25). This process resulted in a marked decrease of FCR and liver uptake. The observed reduction of FCR of 60% is within the range reported by other investigators (24, 25). The delayed clearance from plasma of methylated apoB-89-LDL to nearly the same extent as methylated apoB-100-LDL suggests that the more rapid clearance of apo-B-89-LDL is mainly mediated by LDL-receptors. Similarly, in the WHHL rabbit, the decreased FCRs of both unmodified LDL preparations suggest that LDL receptors are important, while the absence of selective uptake by the spleen further suggested that scavenger receptors are not important in mediating the different metabolic behavior.

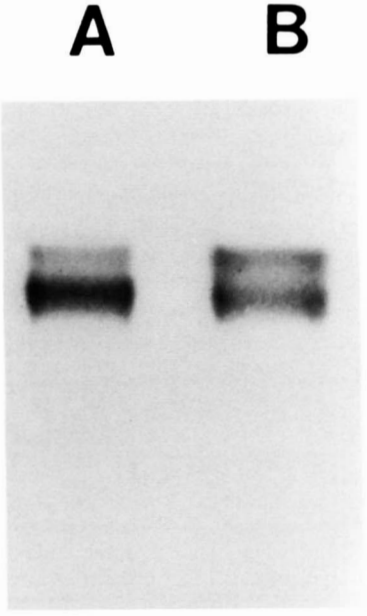

Fig. 4. Electrophoresis of injected radiolabeled lipoproteins. Plasma samples were drawn 6 h (lane A) and 24 h (lane B) after injection, and analyzed by 2-20% (w/v) GGE. After electrophoresis, this gel was fixed, dried, and autoradiographed to detect radiolabeled lipoproteins.

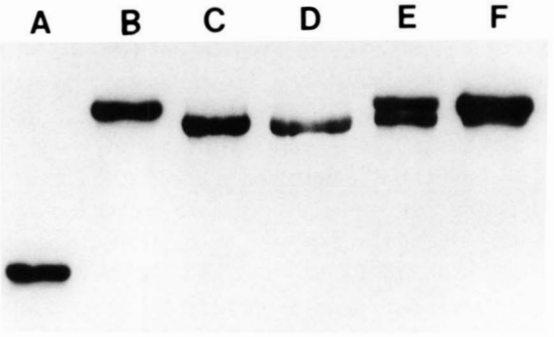

Fig. 5. Polyacrylamide gel electrophoresis (3-10%, w/v) followed by autoradiography of the injected material. Lane A: labeled myosin (M_r 200 kDa); lane B; *I-DLT-apoB-100; lanes C and D; *I-DLT-apoB-89 (subjects #2 and #3, respectively); lanes E and F: injected mixtures (*I-DLT-apoB-100 and *I-DLT-apoB-89 from control subject and subject #2 and subject #3, respectively). Only tracer amounts of radiolabeled apoB-40 were detectable.

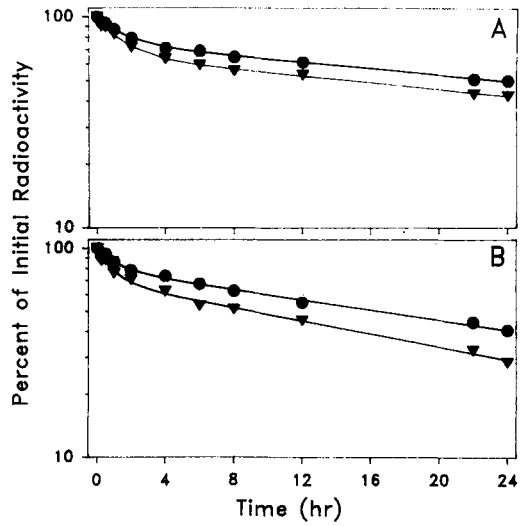

Fig. 6. A) Clearance curves from plasma of reductively methylated- *I-DLT-apoB-89-LDL (triangles) and reductively methylated- *I-DLT-apoB-100-LDL (circles) in two NZW rabbits. B) Clearance curves from plasma of *I-DLT-apoB-89-LDL (triangles) and *I-DLT-apoB-100-LDL (circles) in a WHHL rabbit. Concentration of radioactivity in plasma 1 min after injection was used as the 100% value. Solid lines are predicted values from the two-exponential equation used to fit the data and to calculate FCRs.

The primary difference in the metabolic behavior of apoB-89-LDL and apoB-100-LDL occurred within the first 4 to 8 h. Interpretation of these data could have been confounded by the presence of apoB-40 in the LDL fraction. This, however, is unlikely to have any significant effects, since as discussed earlier, apoB-40 lacks the receptor binding region and an increase in FCR would be expected rather than the observed decrease. Even more important, only a tracer amount of radioactivity could be detected in apoB-40 following the conjugation to *I-DLT (Fig. 5). Another possible confounding factor in the enhanced clearance from plasma of *I-DLT-apoB-89-LDL could have been the presence of denatured protein rapidly degraded by the reticuloendothelial system. However, denatured protein did not contribute significantly to the clearance as judged by the minimal accumulation of radioactivity within the spleen. Also, reductive methylation would not have retarded the clearance from plasma of denatured protein. Thus, the observed differences in the metabolic behavior of apoB-89-LDL were not due to contamination by rapidly turning over radiolabeled apoB-40-LDL or the presence of denatured material.

Further indication that apoB-89 is responsible for the enhanced clearance of apoB-89-LDL comes from another study. Krul et al. (5) reported that the monoclonal anti-apoB antibody B1B3 (an antibody that does not recognize apoB-40) inhibited the binding of ^{125}I-apoB-89-LDL to fibroblast receptors, whereas an anti-apoE antibody did not. This suggests that apoB-89 and not apoB-40 or apoE is responsible for the binding of these LDL particles.

Control LDL were obtained from two different donors, thus excluding the possibility that an unusual control LDL was responsible for the different metabolic behavior of apoB-89-LDL.

These studies were performed under nonsteady-state conditions since human LDL was injected into rabbits. Calculations of FCR, therefore, may not provide data directly applicable to situations where homologous lipoproteins and subjects are used. Nevertheless, it is possible to compare the relative behavior of two tracers and it is clear that apoB-89-containing LDL particles were cleared considerably faster than apoB-100-LDL.

Both apoB-100-LDL and apoB-89-LDL remained intact as LDL particles in rabbit plasma over 24 h, but they did undergo small changes in flotation rates (Fig. 3). The metabolic events responsible for these small shifts are not known, but it is unlikely that these shifts contributed to the differences between the metabolic behavior of the two LDL, for two reasons. First, the plasma clearance curves of the two LDL began to diverge within 15–30 min after injection, before any changes in physical properties were noted, and remained divergent for the 24 h. No changes were seen in the flotation properties of apoB-89-LDL even 6 h after injection (Fig. 4) by which time 50–80% of the injected dose had left the plasma (Fig. 2A). Second, in cultured fibroblast studies conducted in lipoprotein-deficient serum where the changes in LDL probably did not occur, apoB-89-LDL was bound (5) and degraded (Krul, E. S., unpublished data) more avidly than apoB-100-LDL. Thus, it appears that the conformations present on apoB-89 at the time of injection of the radiolabeled LDL were responsible for its accelerated clearance from plasma.

In summary, we conclude that the enhanced clearance from plasma of apoB-89-LDL compared to apoB-100-LDL may be a contributing factor to the low plasma concentration of this lipoprotein in these hypobetalipoproteinemia subjects.

We thank Dr. Suzanne R. Thorpe (Department of Chemistry, University of South Carolina) for supplying dilactitol tyramine. We also acknowledge the technical assistance of Debra L. Rateri. Supported in part by Grants HL36822, HL15308, HL42460, and HL17646 (SCOR in Coronary and Vascular Diseases).

Manuscript received 16 January 1990, in revised form 18 April 1990, and in re-revised form 12 July 1990.

REFERENCES

1. Bouma, M. E., I. Beucler, L. P. Aggerbeck, R. Infante, and J. Schmitz. 1986. Hypobetalipoproteinemia with accumulation of an apoprotein B-like protein in intestinal cells. *J. Clin. Invest.* **78:** 398–410.
2. Dullaart, R. P. F., B. Speelberg, H-J. Schuurman, R. W. Milne, L. M. Havekes, Y. L. Marcel, H. J. Geuze, M. M. Huishof, and D. W. Erkelens. 1986. Epitopes of apolipoprotein

B-100 and B-48 in both liver and intestine. Expression and evidence for local synthesis in recessive abetalipoproteinemia. *J. Clin. Invest.* **78:** 1397-1404.
3. Malloy, M. J., J. P. Kane, D. A. Hardman, R. L. Hamilton, and K. B. Dalal. 1981. Normotriglyceridemic abetalipoproteinemia — absence of the B-100 apolipoprotein. *J. Clin. Invest.* **67:** 1441-1450.
4. Talmud, P. J., J. K. Lloyd, D. P. R. Muller, D. R. Collins, J. Scott, and S. Humphries. 1988. Genetic evidence from two families that the apolipoprotein B gene is not involved in abetalipoproteinemia. *J. Clin. Invest.* **82:** 1803-1806.
5. Krul, E. S., M. Kinoshita, P. Talmud, S. E. Humphries, S. Turner, A. C. Goldberg, K. Cook, E. Boerwinkle, and G. Schonfeld. 1989. Two distinct truncated apolipoprotein B species in a kindred with hypobetalipoproteinemia. *Arteriosclerosis.* **9:** 856-868.
6. Talmud, P., L. King-Underwood, E. S. Krul, G. Schonfeld, and S. Humphries. 1989. The molecular basis of truncated forms of apolipoprotein B in a kindred with compound heterozygous hypobetalipoproteinemia. *J. Lipid Res.* **30:** 1773-1779.
7. Weisgraber, K. H., T. L. Innerarity, and R. W. Mahley. 1978. Role of lysine residues of plasma lipoproteins in high affinity binding to cell surface receptors on human fibroblasts. *J. Biol. Chem.* **253:** 9053-9062.
8. Yamamoto, T., R. W. Bishop, M. S. Brown, J. L. Goldstein, and D. W. Russell. 1986. Deletion in cysteine-rich region of LDL-receptor impedes transport to cell surface in WHHL rabbit. *Science.* **232:** 1230-1238.
9. Cardin, A. D., K. R. Witt, J. Chao, H. S. Margolius, V. H. Donaldson, and R. L. Jackson. 1984. Degradation of apolipoprotein B-100 of human plasma low density lipoproteins by tissue and plasma kallikreins. *J. Biol. Chem.* **259:** 8522-8528.
10. Lefevre, M., J. C. Goudey-Lefevre, and P. S. Roheim. 1987. Gradient gel electrophoresis-immunoblot analysis (GGCI): a sensitive method for apolipoprotein profile determinations. *J. Lipid Res.* **28:** 1495-1507.
11. Krul, E. S., Y. Kleinman, M. Kinoshita, B. Pfleger, K. Oida, A. Law, J. Scott, R. Pease, and G. Schonfeld. 1988. Regional specificities of monoclonal anti-human apolipoprotein B antibodies. *J. Lipid Res.* **29:** 937-947.
12. Daugherty, A., S. R. Thorpe, L. G. Lange, B. E. Sobel, and G. Schonfeld. 1985. Loci of catabolism of β-very low density lipoproteins in vivo delineated with a residualizing label, ^{125}I-dilactitol tyramine. *J. Biol. Chem.* **260:** 14564-14570.
13. Strobel, J. L., J. W. Baynes, and S. R. Thorpe. 1985. ^{125}I-Glycoconjugate labels for identifying sites of protein catabolism in vivo: effect of structure and chemistry of coupling to protein on label entrapment in cells after protein degradation. *Arch. Biochem. Biophys.* **240:** 635-645.
14. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227:** 680-685.
15. Terpstra, A. H. M., C. J. H. Woodward, and F. J. Sanchez-Muniz. 1981. Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation: visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum. *Anal. Biochem.* **111:** 149-157.
16. Collins, D. R., T. J. Knot, R. J. Pease, L. M. Powell, S. C. Wallis, S. Robertson, C. R. Pullinger, R. W. Milne, Y. L. Maral, S. E. Humphries, P. J. Talmud, J. K. Lloyd, N. E. Miller, D. Muller, and J. Scott. 1988. Truncated variants of apolipoprotein B cause hypobetalipoproteinaemia. *Nucleic Acids Res.* **16:** 8361-8378.
17. Schonfeld, G. 1990. The inherited disorders of lipid transport. *Endocrinol. Metab. Clin. North Am.* **19:** 229-257
18. Young, S. G., S. T. Northey, and B. J. McCarthy. 1988. Low plasma cholesterol levels caused by a short deletion in the apolipoprotein B gene. *Science.* **241:** 591-593.
19. Young, S. G., S. T. Hubl, D. A. Chappell, R. S. Smith, F. Claiborne, S. M. Snyder and J. F. Terdiman. 1989. Familial hypobetalipoproteinemia associated with a mutant species of apolipoprotein B (B-46). *N. Engl. J. Med.* **320:** 1604-1610.
20. Huang, L-S., M. E. Ripps, S. H. Korman, R. J. Deckelbaum, and J. L. Breslow. 1989. Hypobetalipoproteinemia due to an apolipoprotein B gene exon 21 deletion derived by alu-alu recombination. *J. Biol. Chem.* **262:** 1394-1400.
21. Knott, T. J., S.C. Rall, Jr., T. L. Innerarity, S. F. Jacobson, M.S. Urdea, B. Levy-Wilson, L. M. Powell, R. J. Pease, R. Eddy, H. Naki, M. Byers, L. M. Priestley, E. Robertson, L. D. Rail, C. Betscholtz, T. B. Shows, R. W. Mahley, and J. Scott. 1985. Human apolipoprotein B: structure of carboxylterminal domains, sites of gene expression, and chromosomal localizations. *Science.* **230:** 37-43.
22. Mahley, R. W., D. Y. Hui, T. L. Innerarity, and K. H. Weisgraber. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man. *J. Clin. Invest.* **68:** 1197-1206.
23. Kowal, R. C., J. Herz, J. L. Goldstein, V. Esser, and M. S. Brown. 1989. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. USA.* **86:** 5810-5814.
24. Carew, T. E., R. C. Pittman, and D. Steinberg. 1982. Tissue sites of degradation of nature and reductively methylated [^{14}C]sucrose-labeled low density lipoprotein in rats. *J. Biol. Chem.* **257:** 8001-8008.
25. Malmendier, C. L., C. Delcroix, and J. F. Lontie. 1989. Kinetics of a heterogeneous population of particles in low density lipoprotein apolipoprotein B. *Atherosclerosis.* **80:** 91-100.