

# Relationships between LDL density and kinetic heterogeneity in subjects with normolipidemia and familial combined hyperlipidemia using density gradient ultracentrifugation

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**Abstract** The metabolism of heterogeneous subpopulations of low density lipoprotein (LDL) apoB-100 was examined in three normolipidemic and two familial combined hyperlipidemic subjects. Autologous radioiodinated plasma LDL ( $1.019 < d < 1.063$  g/ml) were injected into each subject and the disappearance and appearance of radiolabeled lipoproteins into various LDL subpopulations were examined using density gradient ultracentrifugation. Eleven to 13 fractions ( $\sim 320$   $\mu$ l each) were collected within LDL defined uniquely in each subject. In all subjects, the disappearance of radiolabeled LDL from plasma was biexponential. However, changes with time in the distribution of radiolabeled LDL among the various LDL density subpopulations revealed complex metabolic behavior that differed among the subjects. ■ When the relationships between density and kinetic characteristics were examined in more detail by following the disappearance of individual fractions defining LDL in each subject, the data suggested that: 1) the kinetic behavior of the LDL fractions was more complex than suggested by the disappearance of radiolabeled LDL from plasma; 2) certain fractions within specific density ranges were kinetically similar; 3) distinct differences in the disappearance curves among the fractions occurred within narrow density ranges; and 4) precursor-product relationships were seen among specific LDL density fractions and varied from subject to subject. These studies underscore the complexities of plasma LDL apoB-100 metabolism. More detailed characterizations of the kinetic behavior of various LDL subpopulations should help in our understanding of the origin(s) and potential physiological consequences of different LDL subpopulations. —Marzetta, C. A., D. M. Foster, and J. D. Brunzell. Relationships between LDL density and kinetic heterogeneity in subjects with normolipidemia and familial combined hyperlipidemia using density gradient ultracentrifugation. *J. Lipid Res.* 1989. 30: 1307–1317.

**Supplementary key words** apoB • low density lipoprotein fractions

Plasma LDL consist of populations of particles that differ in size, density, apoprotein and lipid composition among, as well as within, individuals (1–5). The characteristics of plasma LDL appear to be influenced by

diet, intravascular environment, and genotype (6–13). Although the physical properties of LDL have been described for many years, the origin(s) and physiological consequences of different LDL subpopulations are unknown. Recently, metabolic variability of different isolated LDL subfractions has been observed in normal and hyperlipidemic subjects (14–17); however, the mechanisms responsible for this kinetic heterogeneity are poorly understood but thought to be due to the presence of different populations of LDL, each having their own distinct metabolic fates.

Traditionally, the kinetic behavior of plasma LDL has been characterized by following its disappearance from plasma. While much information has been derived from studies of this kind, the distribution and kinetic behavior of various LDL subfractions can only be inferred from compartmental analyses of the disappearance curves of radiolabeled LDL from whole plasma.

In the current studies, autologous radiolabeled LDL were injected into three normolipidemic subjects and two subjects with familial combined hyperlipidemia (FCHL). These studies were designed to examine the relationships between the physical and kinetic heterogeneity of LDL by following the distribution of the radiolabeled lipoproteins among the various LDL density subpopulations within each individual using density gradient ultracentrifugation.

Abbreviations: DGUC, density gradient ultracentrifugation; EDTA, ethylenediaminetetraacetic acid; FCHL, familial combined hyperlipidemia; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase; LTP, lipid transfer protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gradient gel electrophoresis; VLDL, very low density lipoprotein; TG, triglyceride; FCR, fractional catabolic rate.

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## MATERIAL AND METHODS

### Subjects

Three normolipidemic volunteers and two subjects with FCHL were studied as outpatients of the Clinical Research Center at the University Hospital of the University of Washington. All normal subjects were free of any lipid disorders and were not receiving medication known to alter lipid metabolism. Subject 4 with FCHL is a member of family 41 published previously (18). This subject has been predominantly hypercholesterolemic but has multiple first and second degree relatives with hypertriglyceridemia (18). Subject 5 (FCHL) was usually hypertriglyceridemic but has had LDL cholesterol levels above the 99th percentile with normal triglyceride levels. This subject has first degree relatives with elevated cholesterol concentrations and others with elevated triglyceride levels. Both subjects 4 and 5 have had elevated apoB levels (>90th percentile) as have members of their respective families. Subject 5 was taking ethinyl estradiol (0.05 mg, qd). The subjects carefully maintained and consumed their own typical diet throughout the study. Potassium iodide (500 mg) was given orally 3 times a day beginning 3 days before the injection of radiolabeled lipoproteins and throughout the study. The study protocol was approved by the Human Subjects Review Committee of the University of Washington and informed consent was obtained from all subjects.

### Preparation of radiolabeled lipoproteins

A blood sample was taken from each subject after an overnight fast and placed immediately into tubes on ice containing 1 mg/ml EDTA, 1 mg/ml NaN<sub>3</sub>, and 10 U/ml aprotinin (final concentrations). Plasma was isolated by centrifugation at 2600 *g* for 30 min at 4°C. Plasma LDL (1.019 < *d* < 1.063 g/ml) were isolated by sequential ultracentrifugation by first raising the density of whole plasma to 1.019 g/ml with solid KBr, overlaying with 9 ml of a *d* 1.019 g/ml solution and then ultracentrifuging the samples in a 60 Ti rotor at 50,000 rpm for 20 h at 10°C. The *d* < 1.019 g/ml lipoproteins were then isolated by tube slicing, raised to *d* 1.063 g/ml with solid KBr, overlaid with at least 8 ml of a *d* 1.063 g/ml solution, and subjected to further ultracentrifugation in a 60 Ti rotor at 50,000 rpm for 24 h at 10°C. Isolated lipoproteins were concentrated to >3 mg protein/ml in dialysis tubing using carboxymethyl cellulose (Sigma, St. Louis, MO) and 10 mg of lipoprotein protein was iodinated with <sup>125</sup>I or <sup>131</sup>I (New England Nuclear, Boston, MA) by the method of McFarlane (19) as modified by Bilheimer, Eisenberg, and Levy (20). Less than 2% of the LDL radioactivity was free iodine (TCA-soluble). Approximately 85.3 ± 11% of the TCA-precipitable radioactivity was isolated in apoB-100 as determined by isopropanol precipitation (21). An

average of 2.2 ± 0.6% of the total radioactivity was lipid-extractable. The radiolabeled lipoproteins were sterilized using a 0.2-μm filter and checked for pyrogenicity in a rabbit before injection.

### Design of the studies

Each subject was injected with 50 μCi of radiolabeled LDL at 8 AM after an overnight fast. Blood samples were obtained after 10 min, 3, 6, 9, 24, 34, 48, and 58 h, and then daily (8 AM after an overnight fast) for up to 9 days after the injection of radiolabeled lipoproteins. Plasma was isolated from each blood sample as described previously. Two 500-μl aliquots were taken to determine total radioactivity in each whole plasma sample. Radioactivity was quantitated using a Packard 5160 gamma counter (Laguna Hills, CA) and corrections were made for background, quench, and decay.

### Lipoprotein separation and characterization

The density distribution of the radiolabeled lipoproteins was determined using density gradient ultracentrifugation (DGUC). Discontinuous salt gradients were set up by underlayering the following solutions: 5.2 ml of *d* 1.006 g/ml, 4 ml of *d* 1.063 g/ml, and 2.6 ml (including 2.2 ml of each plasma sample at *d* 1.21 g/ml) of *d* 1.21 g/ml for subjects 1 and 5; or 3.7 ml of *d* 1.006 g/ml, 5.7 ml of *d* 1.063 g/ml, and 2.3 ml of each whole plasma sample (at 1.21 g/ml) for subjects 2, 3, and 4. The second salt gradient was developed to improve the separation within the LDL density range (Marzetta, C. A., unpublished observations). For subject 5, VLDL (*d* < 1.006 g/ml) was first isolated from whole plasma by ultracentrifugation in a 40.3 rotor at 40,000 rpm for 18 h at 10°C and the *d* > 1.006 g/ml fraction was then subjected to DGUC as described. All density solutions were made by adding solid KBr to 0.9% NaCl containing 0.01% EDTA and 0.01% NaN<sub>3</sub>. For each DGUC run, a tube containing the same density solutions but without the lipoprotein sample was prepared at the same time. All samples were subjected to ultracentrifugation in an SW-41 rotor at 41,000 rpm for 24 h at 15°C. After ultracentrifugation, each sample was drained and 38 to 43 fractions were collected as described previously (22). The refractive index (ABBE-3L Refractometer; Bausch and Lomb, Rochester, NY) was measured on alternate fractions of the blank sample at 15°C and densities were determined using the relationship between standard solutions of known densities and their refractive indexes. The distribution of radioactivity among the lipoprotein subfractions was determined by counting each fraction. The reproducibility of the salt gradients established using DGUC from two fractions (8 and 22) from each sample from a representative study were 1.0203 ± 0.0006 and 1.0548 ± 0.0026, respectively (mean ± SD; *n* = 11 each; coefficient of varia-

tion < 1.0%). Recovery of radioactivity after DGUC from two representative studies was  $94.5 \pm 7.7\%$  (mean  $\pm$  SD;  $n = 22$ ). The percentage of the total LDL radioactivity that was isolated within apoB-100 (21) for each subject after the injection of the iodinated particles was  $94.8 \pm 1.9\%$ ; therefore all the radioactivity within LDL was considered to be associated with apoB-100.

### Chemical analyses

Total plasma and lipoprotein cholesterol and triglyceride concentrations were measured according to the methods described by Warnick (23). ApoB measurements were made using the RIA methods of Albers, Cabana, and Hazzard (24). For the lipoprotein fractions, the radioactivity in each sample was less than 0.5% of the total radioactivity used in each apoB assay.

## RESULTS

Total plasma apoB concentrations were elevated in the FCHL subjects (>90th percentile) (24) and normal in the control subjects (Table 1). Total plasma TG and LDL cholesterol concentrations in the normal subjects were below the 80th percentile while subject 4 had LDL cholesterol concentrations above the 95th percentile and subject 5 had total plasma TG concentrations above the 80th percentile (25) at the initiation of the study.

A representative elution profile of whole plasma separated by DGUC from each subject in the study is shown in Fig. 1. In these gradients, VLDL and IDL are isolated at the top of the gradient (left portion of the elution profiles), LDL is distributed throughout the middle of the gradient (major peak), and HDL and the plasma proteins are isolated at the bottom of the gradient (right side of the elution profiles). The increase in relative absorbance within the HDL region of the gradient is due primarily to plasma proteins. This method separates the

major classes of lipoproteins as well as helps to resolve density heterogeneity within LDL (26). Variability in the density of the LDL peaks can be seen among the subjects, ranging from 1.033 g/ml (first peak of subject 3) to 1.0475 g/ml (subject 4). In addition, LDL density heterogeneity within an individual can be seen in subjects 2, 3, 4, and 5.

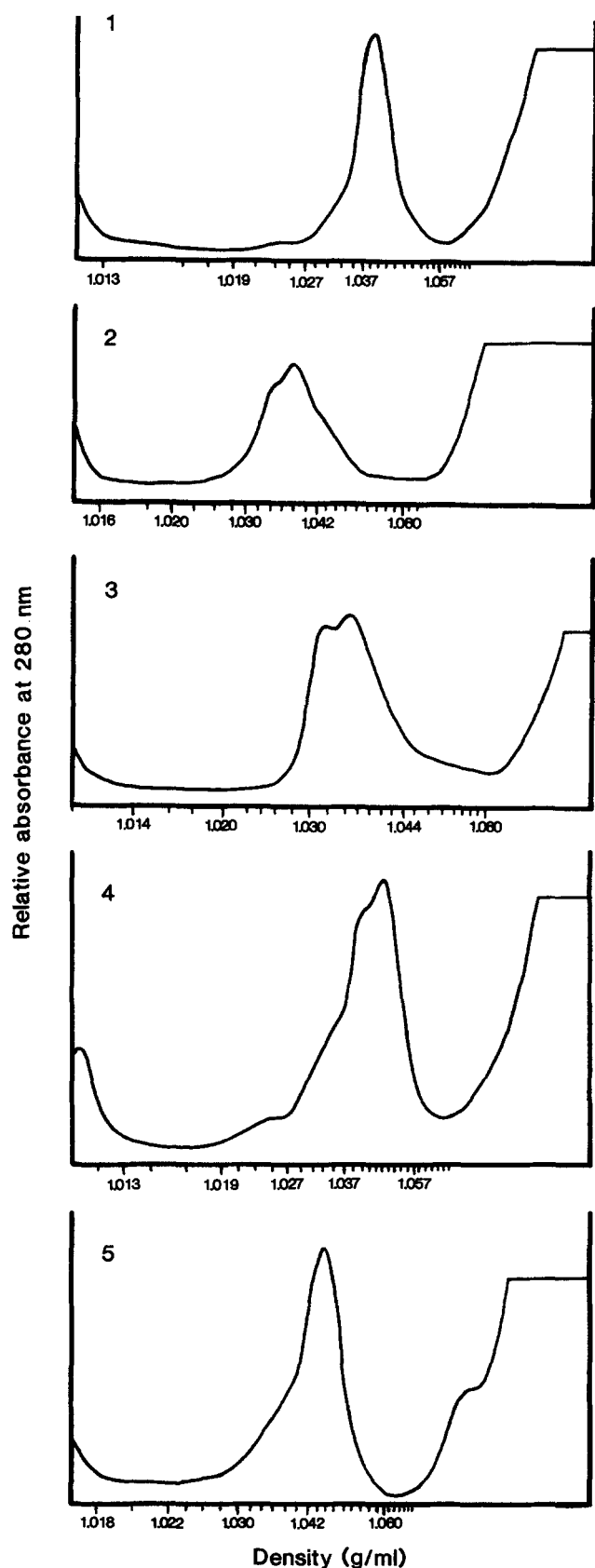
The disappearance curves of radiolabeled LDL apoB-100 from plasma are shown for subjects 3 and 5 in Fig. 2. In both subjects, the radiolabeled LDL disappeared from plasma biexponentially. Each datum point on these disappearance curves, however, represents the sum of the radioactivity distributed among the various LDL density subpopulations. To examine this in more detail, the distribution of the radiolabeled LDL among the various LDL subfractions at each time point was characterized using DGUC. The relationship between plasma LDL protein mass (relative absorbance at 280 nm) and the distribution of the iodinated LDL at various time points is illustrated for subjects 3 and 5 in Fig. 3 and Fig. 4, respectively. In subject 3, the distribution of the radiolabeled LDL resembled the heterogeneous distribution of the plasma LDL protein mass 1 day after the injection of the  $^{131}\text{I}$ -labeled LDL (Fig. 3, 1 day). With time, however, the peak density of the radiolabeled LDL was isolated in a slightly more buoyant region of the gradient than the more buoyant LDL subpopulation (Fig. 3, days 3, 4, and 6). The peak of the LDL apoB-100 radioactivity remained in this buoyant density range throughout the remainder of the study (7 days).

In subject 5, the distribution of the radiolabeled LDL was similar to the distribution of the plasma LDL mass 1 day after the injection of  $^{125}\text{I}$ -labeled LDL (Fig. 4, day 1). In contrast to subject 3, however, with time the peak density of the radiolabeled LDL was isolated in a denser region of the gradient than the peak density of the major LDL subpopulation (Fig. 4, days 3, 5, and 7). In subject 2, the distribution of radiolabeled LDL with time resembled that shown for subject 5; in subjects 1 and 4, the

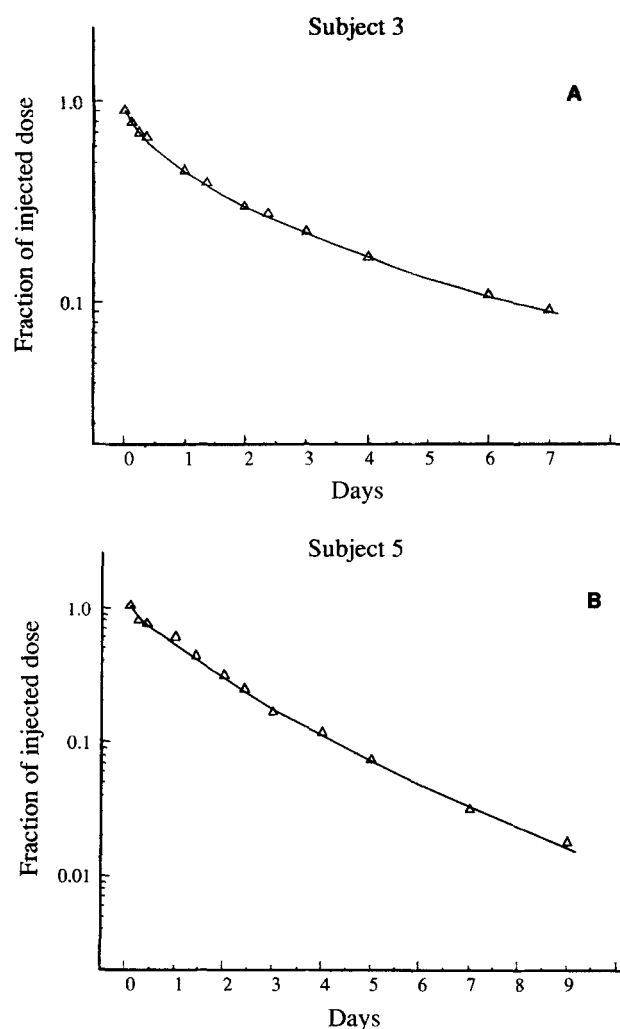
TABLE 1. Clinical characteristics of the subjects

Subjects	Gender	Age	Weight	Height	Plasma			Cholesterol		
					CHOL	TG	ApoB	VLDL	LDL	HDL
		yr	kg	cm	mg/dl			mg/dl		
NL										
1	M	67	80	164	233	69	129	11	175	47
2	F	30	70	179	136	42	68	7	70	59
3	M	29	78	178	166	65	85	13	96	57
FCHL										
4	M	67	88	185	278	113	144	19	211	48
5	F	58	58	166	263	188	141	22	153	88

Abbreviations: CHOL, cholesterol; TG, triglyceride; ApoB, apoprotein B; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; NL, normolipidemic; FCHL, familial combined hyperlipidemic.



**Fig. 1.** Density gradient elution profiles of whole plasma separated by density gradient ultracentrifugation from each study subject. The solid line represents relative protein (absorbance at 280 nm).



**Fig. 2.** Disappearance of LDL  $^{125}\text{I}$ -labeled apoB-100 from plasma for subject 3 (A) and subject 5 (B). The symbols represent the observed data and the lines represent the best fit drawn by hand.

radiolabeled LDL remained similar to the plasma LDL protein mass (data not shown).

To examine the relationship between density and kinetic heterogeneity, the radioactivity in each fraction within plasma LDL defined for each subject was plotted against time. For clarity, only 3 or 4 fractions were plotted in each graph for subject 3 (**Fig. 5**) and subject 5 (**Fig. 6**). In subject 3, fractions 12, 13, and 14 ( $1.024 < d < 1.031$  g/ml) disappeared with an initial rapid clearance followed by a slower turning over portion (**Fig. 5, A**). However, fractions 15 and 16 ( $1.031 < d < 1.0355$  g/ml) disappeared less rapidly in the earlier portion of the disappearance curves and then more rapidly than fractions 12, 13, and 14 after 1 day (**Fig. 5, panels A and B**). In contrast, fractions 17–19 ( $1.0355 < d < 1.044$  g/ml) showed a slight delay in the clearance of radioactivity in the initial portion of the curves with the terminal disappearance of radioactivity

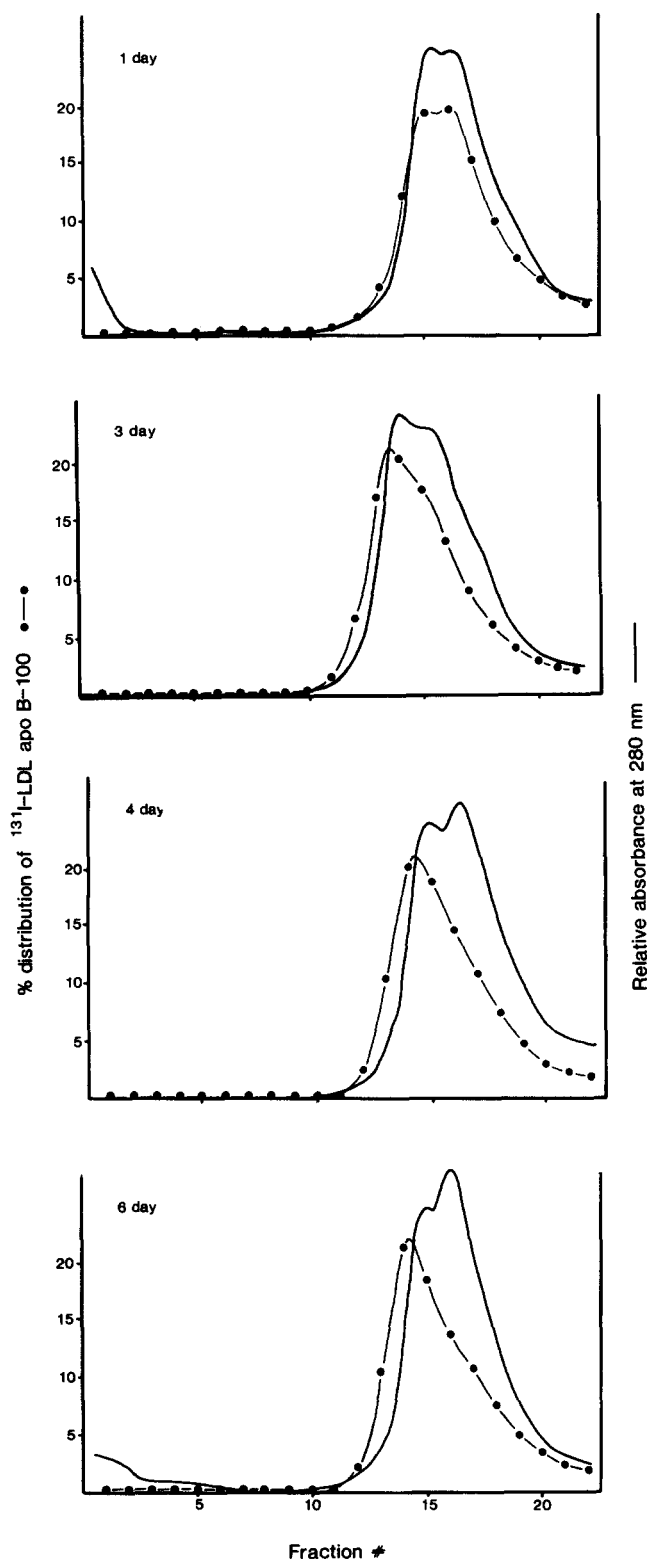


Fig. 3. Changes in the distribution of protein mass (—) and LDL apoB-100 radioactivity (●—●) 1, 3, 4, and 6 days after the injection of the radiolabeled lipoproteins in subject 3.

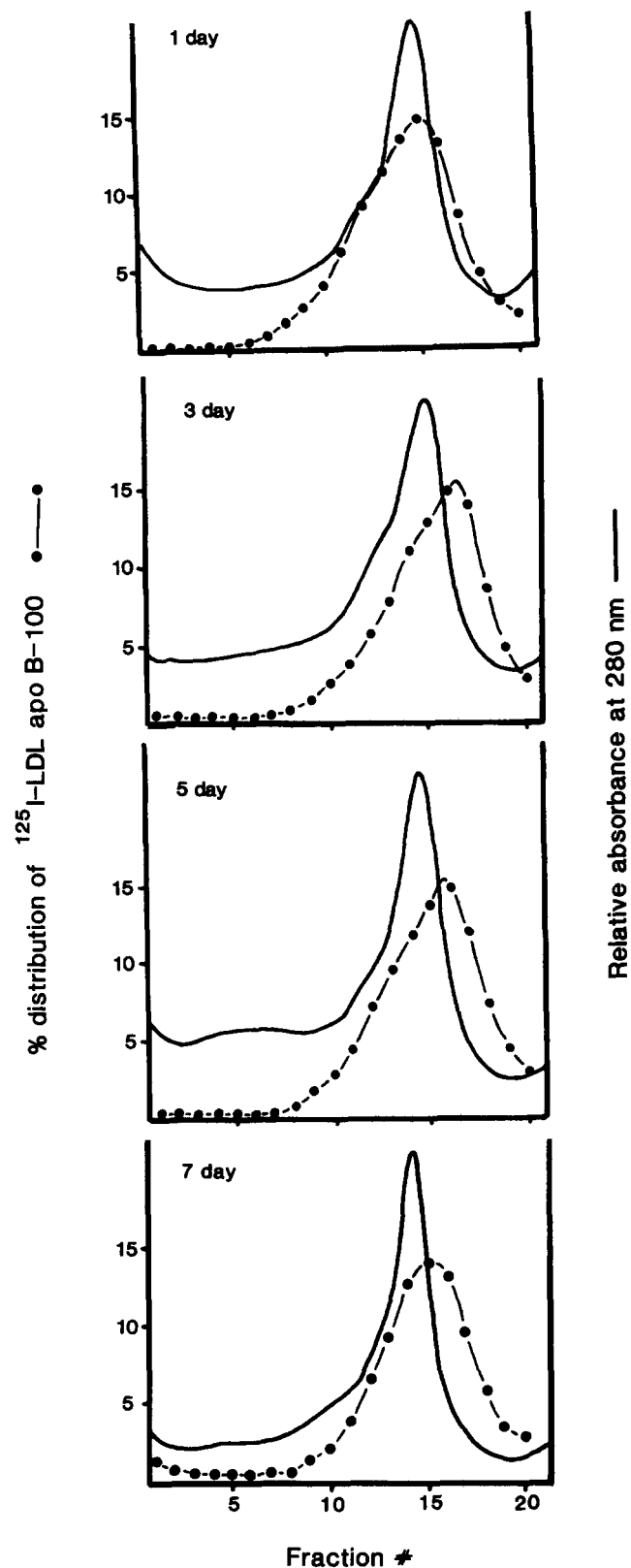
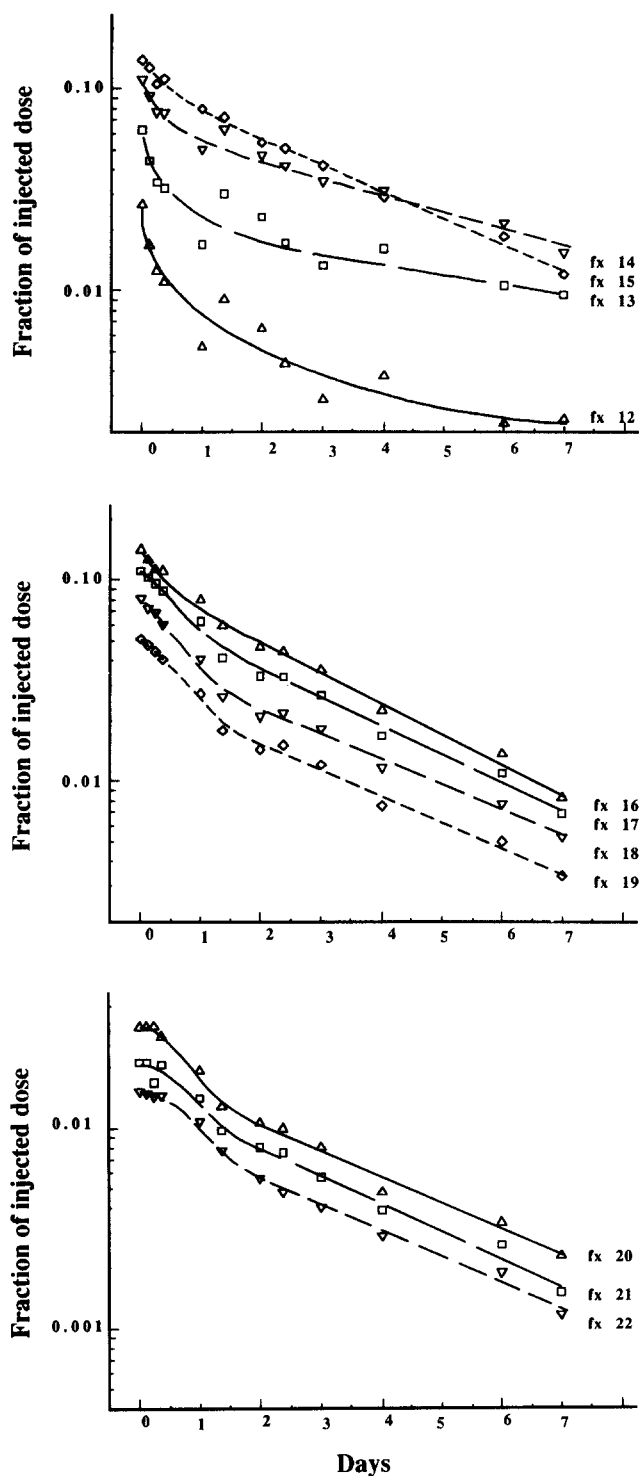
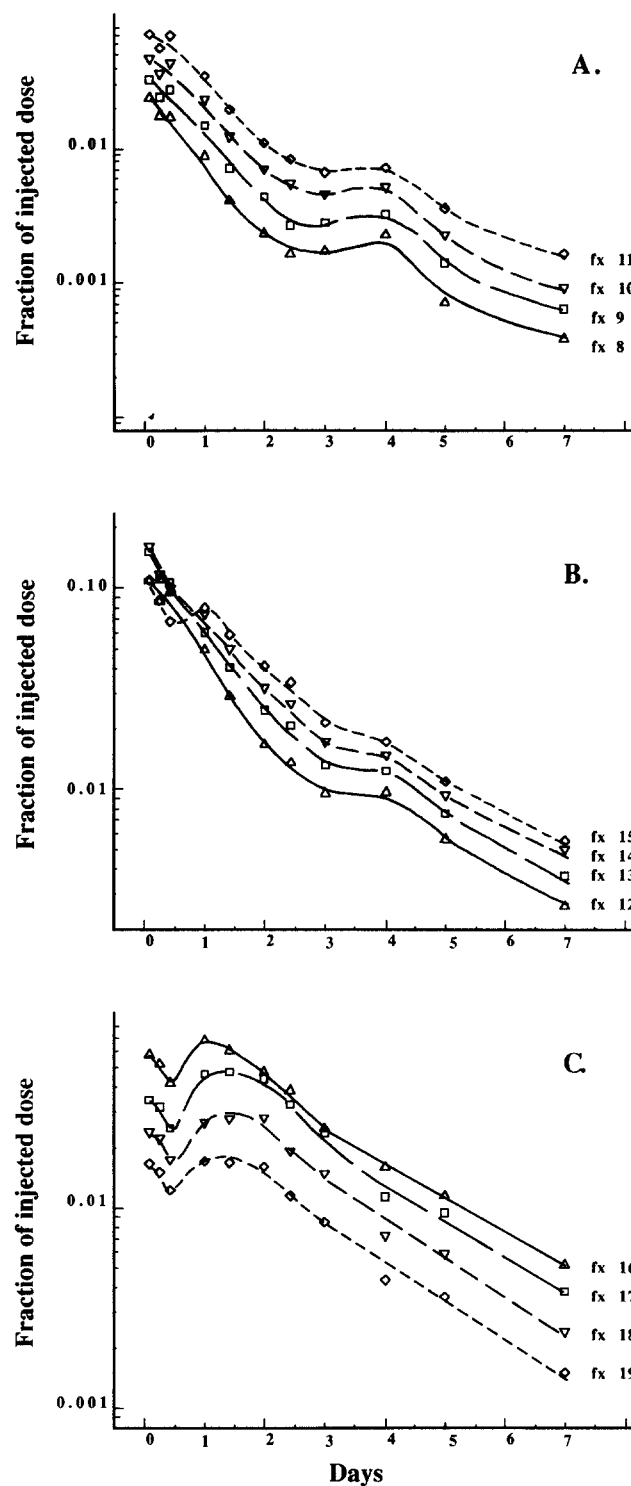


Fig. 4. Changes in the distribution of protein mass (—) and LDL apoB-100 radioactivity (●—●) 1, 3, 5, and 7 days after the injection of the radiolabeled lipoproteins in subject 5.





**Fig. 5.** The disappearance of LDL apoB-100 radioactivity with time in each fraction isolated within the LDL density range after separation by density gradient ultracentrifugation in subject 3. Fraction numbers coincide with those given in Fig. 3 and are numbered from buoyant to dense (protein peak of buoyant LDL ~ fraction 15, dense LDL ~ fraction 16). The symbols represent the observed data and the lines represent the best fit drawn by hand. Each symbol represents a different LDL fraction (fx) as labeled.



**Fig. 6.** The disappearance of LDL apoB-100 radioactivity with time in each fraction isolated within the LDL density range after separation by density gradient ultracentrifugation in subject 5. Fraction numbers coincide with those given in Fig. 4 and are numbered from buoyant to dense (protein peak ~ fraction 14). The symbols represent the observed data and the lines represent the best fit drawn by hand. Each symbol represents different LDL fraction (fx) as labeled. Note: 3 cycle y-axis for A, 2 cycle y-axis for B and C.

from these fractions similar to fractions 15 and 16 (Fig. 5, panel B). With increasing density (fractions 20–22) a more prominent shoulder or delay was seen in the early portion of the disappearance curves followed by the clearance of the remaining radioactivity at rates similar to that seen in fractions 15–19.

In subject 5, the more buoyant LDL fractions appeared to have an initial delay in clearance followed by a more rapid disappearance with an increase in all fractions at day 4 and somewhat slower removal from day 4 through day 7 (Fig. 6, panel A). Fractions 8 through 12 ( $1.025 < d < 1.038$  g/ml) were remarkably similar in their kinetic behavior; however, fractions 13 and 14 ( $1.038 < d < 1.044$  g/ml) do not appear to have the same shoulder or initial delay as that seen in the more buoyant fractions although the characteristics of the disappearance curves 1 day after the injection of the radiolabeled lipoproteins resembled those seen in fractions 8 through 12 (Fig. 6, panel B). Remarkably different kinetic behavior was seen in the more dense fractions (15–19;  $1.044 < d < 1.068$  g/ml). In these fractions, the radiolabeled particles disappeared rapidly for the first 10 h after the injection of the iodinated LDL, and then distinct increases in radioactivity were seen by 24 h, characteristic of a precursor-product relationship (Fig. 6, panel C). In addition, no increase in radioactivity was seen at day 4 in fractions 16 through 19.

Finally, although total plasma cholesterol concentrations remained relatively stable in each subject throughout the course of these studies, the distribution of LDL mass among various density subpopulations varied with time in several subjects. Fig. 7A illustrates shifts in the distribution of LDL protein mass in normolipidemic subject 3. At the beginning of the study (0 min) the more dense subpopulation ( $LDL_2$ ) appeared to be the major population of particles. One day after the injection of the radiolabeled lipoproteins, however, the distribution between  $LDL_1$  (buoyant) and  $LDL_2$  appeared to be about the same. However, the next day (2.5 days) the predominant population appeared to be  $LDL_1$  with evidence of a third, more dense, subfraction. A day and a half later (4 days), the distribution between the two major subfractions was more similar with a distinct shift to a more predominant  $LDL_2$  subfraction appearing once again at day 6.

To quantitate these apparent changes between  $LDL_1$  and  $LDL_2$  in this subject,  $LDL_2/LDL_1$  apoB and cholesterol ratios were calculated for each time point throughout the study (Fig. 7B). As expected, the distribution of cholesterol and apoB mass between the two major density subfractions varied greatly from day to day. Similar, but less extreme changes were seen in normal subject 2 and FCHL subjects 4 and 5 with only very little change in the distribution of LDL in subject 1 (data not shown).

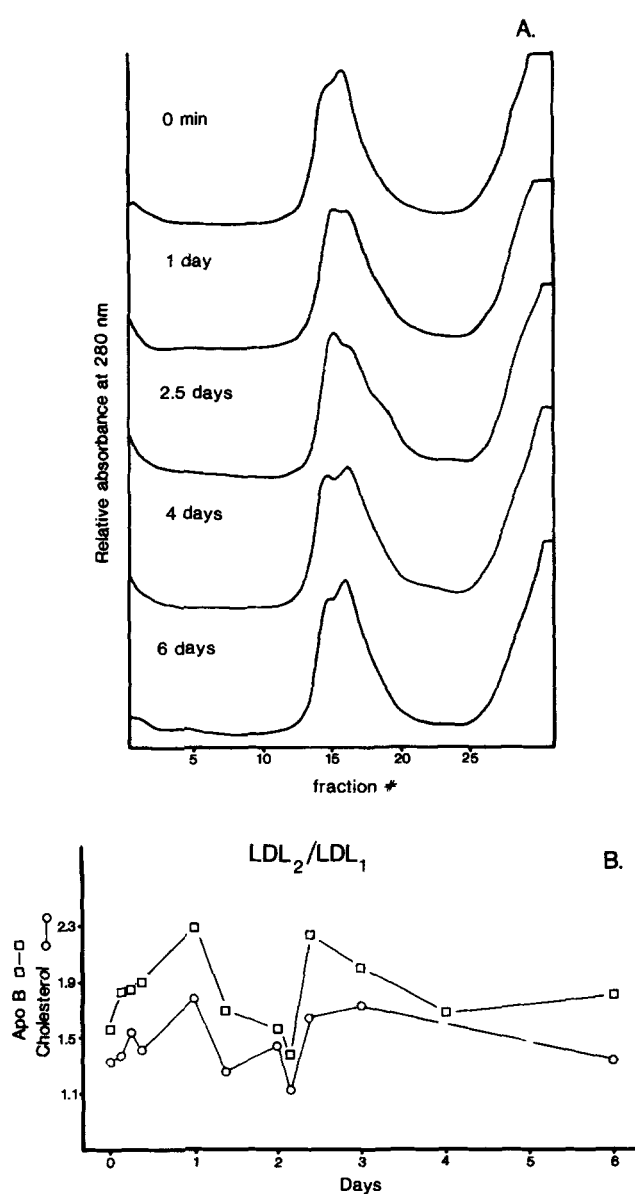


Fig. 7. A) Changes in the density gradient ultracentrifugation profiles with time in normolipidemic subject 3. Times given are minutes (min) and days after the injection of radiolabeled lipoproteins. B) Changes in the ratio of  $LDL_2/LDL_1$  apoB ( $\square$ ) and cholesterol ( $\circ$ ) mass with time after the injection of radiolabeled lipoproteins. The cholesterol ratio for day 4 was lost; therefore, the line connecting the day 3 and day 6 points has been dashed.

## DISCUSSION

Physical heterogeneity of plasma LDL has been recognized for many years (1–5). Although the density heterogeneity described among the subjects in these studies is not a new observation, it is nonetheless remarkable how

much variability in peak density, as well as in the distribution of protein mass among various density subpopulations within an individual, was seen among the five study subjects (Fig. 1). Although the average size and density of plasma LDL have been shown to be related to plasma triglyceride concentrations (8, 10), factors that influence LDL heterogeneity within an individual are poorly understood. The observations made in the current studies suggest that the identification and classification of LDL heterogeneity within any given individual are not readily predictable and require further analyses.

Traditionally, the kinetic behavior of radiolabeled LDL has been described by following its disappearance from plasma and estimating the fractional catabolic rate (FCR) of the entire LDL class. However, FCRs represent a weighted average of the kinetic behavior of all the LDL subpopulations within any individual and, therefore, much of the potential information on the metabolic pathways among LDL subpopulations is obscured within a plasma LDL disappearance curve. Although much information has been derived from such studies, the associations between density and kinetic characteristics cannot be determined from these analyses. To examine the relationships between density and kinetic heterogeneity in more detail, we have separated plasma lipoproteins using density gradient ultracentrifugation and examined the kinetic behavior of radiolabeled plasma LDL among the various LDL subpopulations within each individual.

In all subjects, the disappearance of radiolabeled LDL from plasma was biexponential. However, when examined in more detail using DGUC, the distribution of the radiolabeled LDL among the various plasma density subfractions varied with time and differed among the subjects. No striking differences were seen between the normal subjects compared to those with FCHL. However, the disappearance curves of individual fractions defining LDL in each subject suggested that: 1) the kinetic behavior of the LDL fractions was more complex than suggested by the whole plasma decay curves; 2) certain fractions within specific density ranges were kinetically similar; 3) distinct differences in the disappearance curves among the fractions occurred within narrow density ranges; and 4) precursor-product relationships were seen among specific LDL density fractions and varied from subject to subject.

Many models have been proposed to describe the disappearance of LDL from plasma (27–30) but, although many include more than one plasma pool (suggesting at least two kinetically distinct kinds of LDL), how the metabolic pools relate to the physical properties of the plasma LDL is unknown. In the current studies, we examined the distribution of the radioactivity among the LDL subpopulations with time using DGUC. When the disappearance curves of individual fractions defining LDL for

each subject were examined, several additional kinds of information were suggested by the data.

First, certain fractions within a given density range were kinetically similar. This included similarities within specific fractions in the disappearance, as well as precursor-product relationships. These data suggest that LDL within specific density ranges appear to share some common characteristics that rendered them kinetically comparable. Furthermore, the kinetic behavior of certain LDL fractions changed within a relatively narrow density range (Figs. 5 and 6). Although the lipid and protein compositions could not be examined in each fraction across the LDL for these subjects, other studies have suggested that continual compositional changes are seen across the density ranges defining LDL (3, 22). These studies have shown decreases in the proportion of cholesteryl ester, increases in the proportion of protein, and only slight changes in the proportion of free cholesterol, phospholipid, and triglyceride with increasing density. In both of these studies though, the density ranges used for compositional analyses were broader than those used in the studies presented here and, therefore, subtle differences in compositions could have been masked. In other studies, however, complete lipid compositions were determined for individual fractions across LDL separated by DGUC in three normolipidemic subjects (including subject 2 approximately 1 year after the lipoprotein turnover study) and again, no striking or abrupt compositional changes were seen (Marzetta, C. A., and J. A. Albers, unpublished observations).

The presence of non-apoB apoproteins could also influence the metabolic behavior of different LDL subpopulations. Gibson et al. (4) and Lee and Alaupovic (5) have shown the presence of variable amounts of apoE, apoA-I, and apoCs in particles isolated within plasma LDL. In two subjects in the current study (#2 and #3), we determined the percentage of the total protein (Lowry method) that was apoB (RIA) in buoyant and dense LDL subpopulations. In subject 2,  $93.1 \pm 4.6\%$  and  $92.1 \pm 7.2\%$  (mean  $\pm$  SD;  $n = 9$ ) of the total protein was apoB in the buoyant ( $1.026 < d < 1.0345$  g/ml) and dense ( $1.0345 < d < 1.055$  g/ml) LDL, respectively. In subject 3,  $89.7 \pm 5.0\%$  and  $97.8 \pm 7.3\%$  (mean  $\pm$  SD;  $n = 11$ ) of the total protein was in apoB in the buoyant ( $1.029 < d < 1.033$  g/ml) and dense ( $1.033 < d < 1.0525$  g/ml) LDL subpopulations, respectively. Perhaps, in subject 3, changes in apoproteins that occurred within the narrow density range could help account for the differences in the disappearance curves between fractions 14 and 15 (Fig. 5), but specific information regarding this possibility remains to be determined.

Second, it was important to try to correlate the metabolic differences in the radiolabeled LDL with the density heterogeneity unique to each subject. In each subject, the changes in the disappearance curves did not necessarily



correspond to the density heterogeneity seen in the DGUC elution profiles. For example, in subject 5, the disappearance curves for fractions 8 through 12 were similar, fractions 13 and 14 were similar (but different from fractions 8–12 and 15–19), and fractions 15 through 19 were similar but distinct from fractions 8–14. When compared to the radioactivity and protein elution profiles shown in Fig. 4, these fractions corresponded to the heterogeneity seen in the radioactivity profiles more closely than the protein profiles. That is, the distinct differences in the apparent precursor-product relationships that begin in fraction 15 do not correspond to the plasma LDL protein heterogeneity seen in this subject, whereas, in general, fractions 15–19 correspond to the major portion of the main peak of radioactivity throughout the study and fractions 13 and 14 correspond more closely to the shoulder seen in the buoyant portion of the radioactivity profiles (Fig. 4).

Little information is known about factors that may contribute to the complexities of the LDL radioactivity and protein profiles seen in these studies. However, variations in potential interactions between specific LDL subpopulations and various intravascular enzymes (lipid transfer protein, lecithin:cholesterol acyltransferase, lipoprotein lipase, and hepatic lipase) could influence the metabolic behavior of these lipoproteins. Studies by Deckelbaum et al. (31, 32) have shown that the physical and compositional characteristics of plasma LDL can be modified by lipid transfer protein and lipoprotein lipase. More recently, Chait et al. (33) demonstrated that compositional changes in plasma LDL induced by lipid transfer protein resulted in changes in the cellular metabolism of these *in vitro*-modified LDL. In addition, hepatic lipase has been associated with the physical and compositional properties of plasma LDL (34). However, little is known about potential differences in the substrate specificity of these intravascular enzymes to various subpopulations of LDL.

An additional hypothesis for the differences in the kinetic behavior of various LDL subpopulations could relate to the source of the LDL. A number of studies have suggested that LDL may be derived from a variety of different sources such as plasma VLDL, plasma IDL, and the direct production of LDL. Direct LDL production includes the direct production of LDL-like particles from the liver (35, 36) and/or the rapid conversion of nascent precursors to LDL (27, 37, 38). Hypothetically, for example, LDL derived directly from the liver may contain apoB-100 only (LDL<sub>B</sub>), whereas LDL derived from VLDL may still have apoE associated with them (LDL<sub>B,E</sub>). Since apoE has been shown to influence the metabolism of plasma LDL (39), in this scenario, LDL<sub>B</sub> derived directly from the liver might be expected to have different kinetic characteristics from LDL<sub>B,E</sub> derived from plasma VLDL. In the current studies plasma LDL isolated, iodinated, and reinjected into the study subjects would have

included particles derived from a variety of sources, each potentially having its own metabolic fate.

Lastly, although basic steady-state requirements of relatively constant lipoprotein pool sizes were observed for each subject throughout the time course of these studies, the distribution of apoB-100 and cholesterol mass among LDL density subfractions within an individual was shown to change considerably with time in some subjects. Although it is unclear how frequently our apparent steady-state assumptions may be violated in subjects with heterogeneous LDL, nonlinear compartmental analyses may be employed to help analyze and quantitate the kinetic behavior of LDL subpopulations in these subjects in more detail.

Changes in the characteristics and composition of LDL have been shown to be influenced by dietary cholesterol, fat, and certain lipid-lowering drugs (6, 9, 40). However, how these factors may influence the distribution of LDL mass among various subpopulations has not been examined in detail. For the current studies, each subject was advised to maintain his usual diet and dietary habits throughout the study with the exceptions of no alcoholic consumption throughout the study and no food consumption after 8 PM. For subject 3, daily dietary records were kept and although this subject maintained a relatively stringent low fat diet, subtle variations in the diet may have influenced the distribution of mass among the various LDL density subfractions. Why different LDL subfractions are formed, what the various factors are that influence their metabolic behavior, and what their physiological consequences are, especially as they relate to the development of atherosclerosis, remain unclear.

In conclusion, the present studies underscore the complexities of plasma LDL apoB-100 metabolism. The kinetic behavior of the LDL fractions suggested that metabolically similar subpopulations are seen among density fractions uniquely defining LDL in each subject. Furthermore, the distinct differences in the disappearance curves among the fractions occurred within narrow density ranges and varied from subject to subject. These data are consistent with previous observations that suggest plasma LDL are made up of a variety of particles having their own potentially different metabolic fates. Kinetic analyses of these complicated data will require the testing of many models and hypotheses, but further insights into LDL metabolism should be derived from these exercises and are currently underway. ■

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