Metabolism of Human Intermediate and Very Low Density Lipoprotein Subfractions from Normal and Dysbetalipoproteinemic Plasma

In Vivo Studies in the Rat

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Subfractions of radiolabeled d < 1.019 g/ml lipoproteins were isolated by nonequilibrium density gradient ultracentrifugation (DGU) from normal and dysbetalipoproteinemic human plasma and were injected into rats. Size and density (d) of lipoprotein products formed over 8 hours were assessed by gradient gel electrophoresis and equilibrium DGU, respectively. Subfractions containing a subspecies of very low density lipoproteins (VLDL) of particle diameter > 350 Å were cleared rapidly from the plasma and formed only small amounts of low density lipoproteins (LDL). Fractions containing VLDL subspecies of smaller diameter (300 to 350 Å) were cleared much more slowly, and formed greater amounts of a discrete LDL product with the characteristics of human LDL-II (peak particle diameter 255 to 265 Å, d = 1.030 to 1.040 g/ml). A similar LDL product was formed from subfractions containing intermediate density lipoproteins (IDL). Cholesterol-enriched subspecies within the smaller, denser portion of the IDL spectrum, however, yielded two additional products. One had size and density characteristic of the major human LDL-I subclass reported previously (265 to 275 Å, d = 1.025 to 1.030 g/ml), while the other was yet larger (275 to 285 Å) and overlapped normal IDL in size and density. In dysbetalipoproteinemic plasma, the metabolic precursors of the largest product were shifted from the IDL to the small VLDL (β-VLDL) particle distribution. Since β-VLDL are known to predispose to accelerated atherosclerosis in dysbetalipoproteinemia, it may be that metabolically homologous cholesterol-enriched IDL subspecies in other subjects have similar atherogenic properties.

Low density lipoproteins (LDL) are believed to originate from the sequential catabolism of very low density lipoprotein (VLDL) precursors. As VLDL triglyceride is hydrolyzed by lipoprotein lipase, smaller, cholesterol-enriched intermediate density lipoproteins (IDL) are formed and are subsequently converted to LDL by mechanisms that are poorly understood. In normal humans, nearly all of the protein moiety of plasma LDL derives from VLDL through IDL intermediates, although in patients with familial hypercholesterolemia and in some primate species independent production of LDL has been reported. A portion of the apolipoprotein (apo) B contained in VLDL, moreover, is removed from the circulation before conversion to LDL. This is the fate of 10% to 60% of the VLDL apo B in normal human subjects and a still greater proportion of the VLDL apo B in hypertriglyceridemic subjects and in some animal species. This direct catabolism of VLDL or VLDL remnants is thought to occur in the liver.

Human VLDL and LDL are known to represent heterogeneous populations of particles with respect to size, physical properties, and chemical composition. The techniques of density gradient ultracentrifugation (DGU) and gradient gel electrophoresis (GGE), moreover, have revealed multiple discrete subspecies within the conventional LDL density range (1.019 to 1.063 g/ml). Recent studies of human d < 1.019 g/ml lipoproteins in our laboratory have also demonstrated multiple subspecies in the IDL (1.006 < d < 1.019 g/ml) as well as the VLDL (d < 1.006 g/ml) density ranges. The existence of this heterogeneity raises the question whether subfractions of VLDL and IDL differ in their capacity to form LDL and, further, whether they give rise to the same or different LDL products. In the studies reported here, we have investigated these questions using a new electrophoretic "blotting" technique which exploits the resolving power of GGE and permits accurate measurement of size transformations of radiolabeled lipoprotein subspecies as they are metabolized in vivo. Series of radiolabeled d < 1.019 g/ml lipoprotein subfractions of sequentially smaller particle diameter were isolated by nonequilibrium DGU from the plasma of normolipidemic subjects and from a patient with dysbetalipoproteinemia.
The clearance and metabolic transformations of these subfractions were then studied in the rat, a species in which the presence of an active lipolytic pathway and the absence of significant cholesteryl ester/triglyceride exchange activity makes it possible to identify direct precursor-product relationships in an in vivo model. Despite potential interspecies differences in lipoprotein metabolism, our findings in rats support the view that specific precursors are capable of generating different LDL products.

**Methods**

**Isolation and Iodination of Lipoproteins**

Blood was collected from normolipidemic and hyperlipidemic adult male volunteers, after a 12 to 14-hour fast, into tubes containing disodium ethylene diaminetetraacetate (EDTA) at a final concentration of 1.5 mg/ml. The tubes were immediately placed on ice and plasma was separated at 4°C by low-speed centrifugation. The plasma total cholesterol of the three normal donors ranged from 179 to 213 mg/dl and the triglycerides, from 59 to 164 mg/dl. The patient with dysbetalipoproteinemia had a ratio of VLDL cholesterol to plasma triglycerides of >0.30, 3-VLDL demonstrable on agarose electrophoresis of the d < 1.006 g/ml lipoproteins, and the apo E2/2 phenotype by isoelectric focusing. A cysteine residue at position 158 of the amino acid sequence of his apo E had previously been demonstrated. At the time of the study, this patient was taking no lipid-altering drug, and his plasma cholesterol ranged from 268 to 289 mg/dl and his triglycerides, from 123 to 279 mg/dl.

The d < 1.019 g/ml lipoproteins were isolated from 30 to 120 ml of plasma by preparative ultracentrifugation under standard conditions. Where necessary, lipoprotein solutions were concentrated by dry dialysis using Aquacide I-A (Calbiochem, La Jolla, California) or by ultrafiltration using Amicon (Danvers, Massachusetts) XM50 filters. Lipoproteins (5 to 8 mg protein) were iodinated using the iodine monochloride method. One to three atoms of iodine were substituted for each mole of protein, assuming a molecular weight of 350,000 for apo VLDL. Free iodine was removed by gel chromatography with Sephadex G25, followed by sequential dialysis against 0.1 M potassium iodide in normal saline (pH 7.4), normal saline containing 0.01% EDTA (pH 7.4), and normal saline alone (pH 7.4), with seven changes over a period of 18 hours. Approximately 95% of the label remained bound to protein after delipidation.

**Separation of VLDL and IDL Subfractions**

The iodinated d < 1.019 g/ml lipoproteins (0.5 to 1.0 mg/ml protein, specific activity 28 to 62 μCi/mg protein) were subjected to nonequilibrium density gradient ultracentrifugation (DGU) as described in detail elsewhere. Briefly, the density of the lipoprotein solution was adjusted to 1.21 g/ml and a 4.5 ml sample was placed in a 9/16 x 3 ½-inch Ultraclear tube (Beckman Instruments, Mountain View, California) and was sequentially overlaid with 3.0, 3.0, and 1.5 ml, respectively, of solutions of densities 1.020, 1.010, and 1.000 g/ml. The tubes were centrifuged for 6 hours in a Beckman SW41 rotor at 40,000 rpm at 17°C. The contents of the tube were withdrawn by pipetting four consecutive 1.0 ml fractions followed by four 0.5 ml fractions, designated 1 through 8 in order from the top of the tube. Frequently, the first and second 0.5 ml portions of fraction 1 were pipetted separately and designated 1a and 1b, respectively.

**In Vivo Studies**

Male Sprague-Dawley albino rats (Simonson Laboratories, Gilroy, California) weighing between 280 and 350 grams were used for all studies. The rats had free access to water and standard Purina lab chow.

Iodinated VLDL or IDL subfractions (50 to 100 μg of protein) from individual donors were injected into an exposed jugular vein with the animal under light methoxyflurane anesthesia. Samples were dialyzed against normal saline and were diluted before infusion so that each rat received the same quantity of 125I-apo B in an identical volume (ranging from 0.5 to 1.2 ml). Blood samples of 0.3 to 0.5 ml were withdrawn from the opposite jugular vein for analysis. At 8 hours, the rats were exsanguinated from the inferior vena cava or abdominal aorta. Blood samples were anticoagulated with EDTA at a final concentration of 1.5 mg/ml of blood. Tubes were immediately placed on ice and plasma was separated by low-speed centrifugation at 4°C.

**Determination of Apo B Radioactivity in Plasma and Lipoprotein Fractions**

The isopropanol precipitation method of Egusa et al. was employed to quantify 125I-apo B radioactivity. In brief, plasma or lipoprotein samples in a volume of 1 ml were mixed with equal volumes of isopropanol and were left overnight at 4°C. The precipitate was isolated by low-speed centrifugation and was washed once with 50% (vol/vol) isopropanol/saline. The pellet and supernatant radioactivity were counted in a Packard gamma scintillation counter, and the percent 125I-apo B was calculated as the ratio of precipitable 125I to total 125I x 100. Control experiments demonstrated that identical results were obtained whether the 125I-labelled VLDL or LDL were precipitated in ultracentrifugally separated lipoprotein fractions as originally described by Egusa et al. or in mixtures with plasma. When isopropanol precipitation was performed on lipoprotein fractions containing less than 100 μg/ml of protein, 50 μl of unlabelled normal human plasma or normal human LDL were added to increase the size of the pellet and minimize nonspecific losses. The percent 125I-apo B remaining in rat plasma was calculated relative to the 125I-apo B present in a sample drawn between 60 and 90 seconds after injection of the tracer. While this method may underestimate the decay of very rapidly cleared lipoproteins, errors associated with the calculation of rat plasma volumes are avoided.

**Polyacrylamide Gradient Gel Electrophoresis**

Electrophoresis of plasma samples or lipoprotein fractions was performed on 2% to 16% polyacrylamide gradient gels (Pharmacia PAA 2/16, Piscataway, New Jersey) for 24 hours at 125 V in Tris (0.09 M)/boric acid (0.08 M) buffer.
M)/Na₂EDTA (0.003 M) buffer (pH 8.3) as described elsewhere. Gels were stained for proteins in a solution containing 0.1% Coomassie brilliant blue R-250, 50% ethanol, and 9% acetic acid (vol/vol), or for lipid with oil red O or Sudan black in 60% ethanol at 55°C. Gels were scanned at 555 nm for Coomassie blue staining or 530 nm for oil red O staining, using a Transidyne RFT densitometer or at 363 nm using an LKB Instruments Ultrascan Laser Densi-

ometer. Migration distances for each absorbance peak were determined and the molecular diameters corresponding to each peak were calculated from a calibration curve as described elsewhere.

Equilibrium Density Gradient Ultracentrifugation

The products of lipoprotein catabolism in the rat were analyzed by equilibrium DGU, as previously described. In brief, the d = 1.019 to 1.063 g/ml LDL fraction was isolated by preparative ultracentrifugation and dialyzed to d = 1.0400 g/ml. The LDL (2 ml) were layered above a NaBr solution of d = 1.0540 g/ml (2.5 ml) in a cellulose nitrate tube. This was overlayered with 2.5 ml of a NaBr solution of d = 1.0275 g/ml. Tubes were centrifuged to equilibrium at 40,000 rpm in a Beckman SW 41 rotor at 22°C. The tube contents were then withdrawn by sequential pipetting the top 0.5 ml, six 1-ml fractions, and the bottom 0.5 ml. A background salt tube was included for determination of the salt densities of the pipetted fractions.

Results

Characterization of Radioiodinated VLDL and IDL Subfractions

Nonequilibrium DGU of d < 1.019 g/ml lipoprotein fractions provided a series of fractions of progressively smaller mean particle diameter. The chemical and physical properties of the VLDL and IDL subspecies contained in these fractions from normolipidemic and dysbetalipoproteinemic subjects have been previously described in detail. Representative gradient gel electrophoretograms of nonequilibrium DGU fractions 1a through 8 from one of the normolipidemic subjects (total cholesterol 179 mg/dl, triglyceride 134 mg/dl) whose plasma was used in subsequent experiments are shown in Figure 1A. Fraction 1a included nearly all of the VLDL of particle diameter >350 Å. A smaller subspecies of VLDL was also found in small amounts in fraction 1, and extended as a broad distribution of particles through fraction 4. The particles of IDL density contained in fractions 5 to 7, as previously described, were seen to include two subspecies of overlapping buoyant density. The predominant IDL species in this subject displayed a peak particle diameter extending from 291 Å in fraction 5 to 283 Å in fraction 7. Lipoprotein subspecies in corresponding fractions from all of the normal donors were within 4 Å in size of those shown for the subject in Figure 1A, and variation in particle diameters for each subtraction from individual subjects at differing sampling times during the study were no greater than 5 Å. Lipoproteins of similar size were also found in the corresponding nonequilibrium DGU fractions from the plasma of the patient with dysbetalipoproteinemia (total cholesterol 274 mg/dl, triglycerides 279 mg/dl) (Figure 1B) used in subsequent experiments, with differences from the normal pattern as previously described. Agarose electrophoresis showed the presence of α₂-VLDL only in fraction 1a, while β-VLDL was found in fractions 1a through 4 (data not shown).

The d < 1.019 g/ml lipoproteins from these two subjects were radioiodinated, and the distribution of apo B-associated (isopropanol-precipitable) radioactivity among the nonequilibrium DGU subfractions was determined (Table 1). Compared with the normal subject, the patient with dysbetalipoproteinemia showed a greater proportion of counts in the fractions containing small VLDL and IDL. The
Figure 1. Densitometric scans of 2% to 16% gradient gels of subfractions obtained by nonequilibrium density gradient ultracentrifugation of the d < 1.019 g/ml lipoproteins from a normal male subject (A) and a male subject with dysbetalipoproteinemia (B). The gels were stained for protein with Coomassie blue R-250. Particle diameters of the major peaks (in Å) were determined from calibration curves as described in Methods.

Table 1. Distribution of Apo B-Associated Radioactivity among VLDL and IDL Subfractions

<table>
<thead>
<tr>
<th>SW41 fraction</th>
<th>Percent isopropanol precipitability</th>
<th>Distribution of apo B in d&lt;1.019 g/ml fractions*</th>
<th>Percent isopropanol precipitability</th>
<th>Distribution of apo B in d&lt;1.019 g/ml fraction*</th>
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<tr>
<td>1a</td>
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<td>9.3</td>
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<td>1b</td>
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<tr>
<td>8</td>
<td>92</td>
<td>4.2</td>
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*Values represent percent of total isopropanol precipitable counts in the d < 1.019 g/ml fraction.

percentage of radioactivity precipitable by isopropanol progressively increased in fractions 1a through 7, as shown in Table 1. Measurement of the distribution of radioactivity among apolipoproteins by densitometric scanning of SDS gradient gel blot autoradiograms (data not shown) revealed similar proportions of ¹²⁵I-apo B and non-B apolipoprotein as found by isopropanol precipitation. The percent isopropanol-precipitable radioactivity in VLDL fraction 1a for all of the preparations used ranged from 50% to 62% for normals and 51% to 66% for type 3 dysbetalipoproteinemia.

VLDL and IDL subfractions contained somewhat greater amounts of apo E than normals, but in both cases ¹²⁵I-apo E represented only a minor component (less than 2%) of the total labelled apolipoprotein, in agreement with previous reports of dissociation of apo E from lipoproteins during ultracentrifugation as well as inefficient labelling of this apolipoprotein. Nonequilibrium DGU was shown to result in substantial losses of apo E from the d < 1.019 subfractions, with greater than 90% of the recoverable apo E (as measured by radioimmunossay in the laboratory of John Albers) found in the lipoprotein-free fraction at the bottom of the tube. A band corresponding to apo B-48 was sometimes seen in fraction 1, with negligible amounts in subsequent fractions. In the patient with dysbetalipoproteinemia, the amount of ¹²⁵I-associated with this band was minimal when plasma triglycerides were maintained below 150 mg/dl by dietary treatment.

Plasma Decay of ¹²⁵I-Apo B

Radiiodinated nonequilibrium DGU fractions isolated from the d < 1.019 g/ml lipoproteins from individual normolipidemic donors were infused into rats in separate experiments, and their catabolism was followed over an 8-hour period. The total amount of protein injected ranged from 50 to 100 μg per rat. At intervals from 1 minute to 8 hours after infusion, residual ¹²⁵I-apo B was measured and lipoprotein products were analyzed by GGE. The behavior of corresponding subfractions from the three normal donors was similar. Figure 2A illustrates plasma decay curves of ¹²⁵I-apo B for fractions 1a, 3, 5, and 7, averaged for these three experiments. Fraction 1a, containing the larger VLDL species, was cleared much more rapidly than the other fractions and showed a biphasic exponential decay curve. Greater than 50% of the ¹²⁵I-apo B present at 1 minute was cleared within 30 minutes. Fraction 3 containing small VLDL and fraction 5 containing predominantly the larger...
IDL species also displayed an early phase of more rapid clearance, quantitatively much less pronounced than for fraction 1a. The decay curve for fraction 7, which was enriched in the smaller, denser IDL species, was monoeXponential. The rates of decay for all of the fractions after the first 2 hours were similar and roughly paralleled that of radiolabelled human LDL (d = 1.030 to 1.040 g/ml) infused into rats under similar conditions (Figure 2B).

**Analysis of Residual Lipoprotein Species in Plasma**

Lipoprotein species in the plasma samples from the above studies were characterized by 2% to 16% GGE. Gradient gels were blotted onto nitrocellulose paper, as described in Methods, and the lipoprotein bands were visualized by autoradiography. The findings for the three subjects were similar. Densitometric scans of representative gel-blot autoradiograms for fractions 1a, 3, 5, and 7 from one of the subjects (cholesterol 251 mg/dl, triglycerides 164 mg/dl) are shown in Figure 3. The residual radioactive tracer in the rat injected with fraction 1a moved rapidly into the IDL size range, with a peak particle diameter of 302 Å at 30 minutes and 285 Å at 1 hour. By 2 hours, the major band was reduced in size to 280 Å and a discrete shoulder was evident with a particle diameter of 261 Å. At 4 hours, most of the radioactivity was confined to the peak at 260 Å. The amount of this species appeared to gradually decrease over the following 4 hours, but its particle diameter changed little (257 Å at 8 hours). The major LDL sub-species found in the plasma of the donor subject had a peak particle diameter of 259 Å.

The small VLDL species contained in fractions 2 through 4 formed a similar LDL-sized particle, as shown for fraction 3 in Figure 3. The starting material in fraction 3 consisted of a sharp band with particle diameter 322 Å, compared to the VLDL of peak particle diameter 372 Å in fraction 1a. One hour after infusion, the bulk of the material appeared as a band in the size range of IDL (287 Å peak particle diameter) with a shoulder at 266 Å. Over the following hours, the IDL-sized material diminished and eventually disappeared, while the relative magnitude of an LDL peak of final diameter 258 Å increased. The amount of label appearing in the LDL peak was much greater for fractions 2 through 4 compared with fraction 1a (Figure 2).

Fraction 5 contained primarily the larger IDL species, with a peak particle diameter of 297 Å. At 1 hour after infusion, the bulk of the lipoprotein remaining in the plasma was found in a peak at 287 Å, with a shoulder at 264 Å. Over the ensuing hours, the relative intensity of the smallest band again increased; by 6 to 8 hours, however, there remained two closely overlapping bands at 280 Å and 268 Å in addition to the more prominent peak at 258 Å. For fraction 7 containing a larger proportion of smaller, denser IDL a similar pattern was seen. Three overlapping peaks were present at 6 to 8 hours; however, in this case the largest lipoprotein sub-species, with particle diameter measuring 284 Å, was the predominant species.

To further assess the density distribution of the 1.019 to 1.063 g/ml 125I-labelled lipoprotein products at 8 hours, they were subjected to equilibrium DGU, performed as described in Methods. Figure 4 illustrates the distribution...
METABOLISM OF IDL AND VLDL SUBSPECIES

Normal Subject

FRACTION

TIME

1a 3 5 7

1min

366 320 297 269

2hr

297 296 284 271

8hr

297 258 280 259

Figure 3. Densitometric scans of autoradiograms of 2% to 16% gradient gel-nitrocellulose blots showing the distribution of 125I-labelled lipoprotein products after infusion into rats of radioiodinated nonequilibrium DGU fractions 1a, 3, 5, and 7 from a normolipidemic subject. Particle diameters of the major peaks (in Å) were determined from calibration curves as described in Methods.

of 125I-apo B among the equilibrium DGU fractions at 8 hours for starting substrates 1a, 3, 5, and 7. The bulk of the LDL formed from fraction 1a was found in the equilibrium DGU fractions 1 and 2 (d = 1.025 to 1.033 g/ml). The LDL formed from nonequilibrium DGU fraction 3 was somewhat denser, peaking in equilibrium DGU fractions 2 and 3, corresponding to the density range of 1.030 to 1.040 g/ml. The LDL deriving from nonequilibrium DGU fraction 5 was again found predominantly in equilibrium DGU fractions 2 and 3. A larger portion of 125I-apo B, however, was now seen in equilibrium DGU fractions 0 and 1 (d < 1.030 g/ml). For the product formed from nonequilibrium DGU fraction 7, equilibrium DGU fraction 0 (d < 1.025 g/ml) contained the largest amount of 125I-apo B, with the remainder distributed among fractions 1 to 3.

Fraction 0 has been shown to include IDL. In fact, GGE autoradiograms (not shown) of fraction 0 derived from rats injected with nonequilibrium DGU fractions 5 and 7 contained a peak with particle diameter similar to the largest of the species present in whole plasma 8 hours after the injection of these two fractions (280 and 284 Å, respectively; Figure 4). On the other hand, the peak particle diameters of the lipoproteins in equilibrium fraction 1 from rats injected with the same two nonequilibrium fractions corresponded to the middle peaks seen on GGE autoradiograms of the 8-hour plasma (269 and 273 Å, respectively; Figure 3). Finally, lipoproteins in equilibrium fractions 2 and 3, deriving from all of the nonequilibrium DGU fractions, had peak particle diameters ≤ 263 Å, values similar to those for the smallest of the catabolic products observed on GGE autoradiograms of the uncentrifuged 8-hour plasma (Figure 3). Particles of similar diameter (260 Å) were also found in equilibrium fractions 0 and 1 from rats infused with nonequilibrium DGU fraction 1a, in contrast to the larger particles in these equilibrium DGU fractions deriving from the other nonequilibrium DGU fractions. Thus, a more buoyant, but not larger, LDL product was formed from large VLDL.

A separate equilibrium DGU run using fractions derived from a different normolipidemic subject gave essentially identical results.

Greater than 88% of the non-isopropanol-precipitable radioactivity in the 8-hour samples derived from fraction 1a was found in the d < 1.019 and d > 1.063 g/ml fractions. Estimation of the distribution of label among apolipoproteins by densitometric scanning of SDS-gel blot autoradiograms of delipidated d = 1.019 to 1.063 g/ml lipoproteins at 8 hours revealed detectable residual 125I-C apolipoproteins only in the products derived from fractions 1a and 2, where they comprised 13% and 3%, respectively, of the total radioactivity. SDS-gel blot analyses of the equilibrium DGU fractions, however, showed that most of this radioactivity was actually confined to fractions of d > 1.040 g/ml where increasing amounts of rat high density lipoprotein, (HDL,) may be found. Less than 2% of the radioactivity in the equilibrium DGU fractions containing the major labelled LDL products migrated with C-apolipoproteins, the remainder consisting of 125I-apo B.
Catabolism of VLDL and LDL Subfraction from a Patient with Dysbetalipoproteinemia

The d < 1.019 g/ml lipoproteins from a patient with dysbetalipoproteinemia were radioiodinated, fractionated by nonequilibrium DGU, and infused into rats, as described for the normal subjects above. Plasma decay of \(^{125}\)I-apo B for the different fractions averaged for three separate experiments is shown in Figure 5. The clearance of \(^{125}\)I-apo B from fractions 1a through 4 was considerably more rapid than observed with the corresponding fractions from normal subjects (Figure 2). Clearance of \(^{125}\)I-apo B following injection of dysbetalipoproteinemic fraction 3 averaged 50 minutes, compared with 6.5 hours for the corresponding fraction from normal subjects. Fraction 5 decayed at a slower rate, but still more rapidly than for this fraction from normal subjects. The more rapid clearance of these dysbetalipoproteinemic fractions was attributable to an early phase of rapid decay. After the first 2 hours, the rates of decay were comparable to those observed with normal fractions or normal human LDL (Figure 2B). Clearance of \(^{125}\)I-apo B after infusion of fraction 7 from the dysbetalipoproteinemic patient was monoexponential and similar to that seen with this fraction from normolipidemic subjects (compare Figures 2A and 5).

Analysis by GGE of the lipoprotein products after infusion of dysbetalipoproteinemic precursors is shown in Figure 6 for a representative experiment. Fraction 1a formed a relatively homogeneous LDL product measuring 259 Å in peak particle diameter, resembling in size the product formed from the corresponding starting fraction isolated from normal subjects. As expected from the rapid \(^{125}\)I-apo B decay curve for this fraction (Figure 5), only very small amounts of this material were present at 8 hours. Fractions 2 through 4 from dysbetalipoproteinemic plasma formed only minimal amounts of 260 Å LDL, with most of the lipoprotein product found in two overlapping bands in the 270 to 290 Å size range. This pattern resembled that seen with fraction 7 from normal subjects, as shown in the bottom panel of Figure 6. The major product formed from dysbetalipoproteinemic fraction 5 in this experiment was a somewhat broader intermediate-sized peak at 272 Å, although in another experiment a 280 to 288 Å band similar to that seen in the product of fraction 3 was the predominant product formed from this fraction as well. Dysbetalipoproteinemic fraction 7, however, gave rise to a more homogeneous peak with particle diameter 261 Å at 8 hours with little, if any, of the larger lipoproteins that were found after injection of fraction 7 from normal subjects (Figure 3).
The low levels of cholesteryl ester/triglyceride transfer activity in rat plasma may limit lipid "remodeling" of LDL, believed to occur in vivo in humans. On the other hand, low
lipid transfer protein activity makes it possible to investigate the direct conversion of subfractions of triglyceride-rich lipoproteins to LDL subspecies in an in vivo setting free from this complicating factor. The question of whether the precursor-product relationships defined in this study and in parallel in vitro studies correspond to physiologic human metabolic pathways, however, will require direct testing in humans.

We report here that a subpopulation of VLDL-containing particles of 300 to 330 Å mean peak diameter from normolipidemic human plasma were preferentially converted to LDL, while larger VLDL (>350 Å mean particle diameter) were cleared from the plasma much more rapidly and gave rise to only small amounts of an LDL-sized product. Since nonequilibrium DGU fraction 1a also contains variable amounts of 300 to 330 Å-sized VLDL in addition to the predominant larger VLDL, it is uncertain whether the small amounts of LDL that were formed derive from the large, the small, or possibly both VLDL subspecies. It is also uncertain to what extent large VLDL are degraded to smaller particles prior to rapid clearance.

Our findings are in agreement with those of Packard et al. who reported that small and large VLDL differ in the extent to which they give rise to LDL in normal and hypertriglyceridemic human subjects. This phenomenon may have a variety of metabolic consequences. It may well contribute to the reported differences in the extent to which VLDL is transformed to LDL in different animal species and in hypertriglyceridemic states in humans where large VLDL predominate.

IDL are generally thought of as transition products of VLDL metabolism. Our findings indicate that small VLDL do give rise to particles of intermediate size which, in turn, form products with size and density characteristic of the LDL-II subclass (Figure 3). Based on the observation that IDL-containing fractions also yield LDL-II products, it is likely that these IDL include metabolic intermediates of small VLDL catabolism. IDL-containing fractions, however, also yielded two additional subspecies not clearly identified in the product formed from catabolism of small VLDL. One of these corresponded in size and density to the LDL-I subclass previously reported in normal human plasma of diameter 260 to 275 Å and density 1.025 to 1.030 g/ml. The second of these products was still larger (275 to 285 Å) and overlapped the conventional LDL and IDL density intervals.

In a previous report we described two major forms of IDL in normal subjects: IDL-1, found predominantly in nonequilibrium DGU fraction 5 by the procedure used here; and IDL-2, isolated primarily in fractions 6 and 7, which include smaller, denser, and cholesterol-enriched particles. We have here shown that lipoproteins in fractions 6 and 7 yield higher proportions of the LDL-I and IDL-sized products than the larger, more triglyceride-enriched IDL in
fraction 5. It would therefore seem plausible that IDL-2 is metabolically related to at least one of these products. Since IDL-2 include particles that are smaller than the IDL-sized product,25 we surmise that IDL-2 form LDL-I, while the larger products may arise from a larger form of cholesterol-enriched IDL that is obscured within the smaller, denser end of the IDL-1 particle distribution. Firm conclusions as to these precursor-product relationships will require improved methodology for separating IDL-1 and IDL-2 subspecies.

The precursor-product relationships among VLDL, IDL, and LDL subpopulations suggested by our data are represented schematically in Figure 8. The 1.019 g/ml density boundary conventionally separating IDL from LDL must be viewed as arbitrary. Thus, although the cholesterol-enriched IDL that give rise to LDL-I and the larger products are diagrammed as LDL “precursors” in Figure 8, they could also be viewed as particles contained within the more buoyant extremes of particle distributions overlapping the d = 1.019 g/ml boundary, which can be converted to particles in the conventional LDL density range (LDL-I or larger species) by hydrolysis of contained lipids. The origin of the cholesterol-enriched IDL precursors of LDL-I and the larger products remains uncertain. They could be secreted directly by the liver; alternatively, they could derive from subpopulations of VLDL representing a small part of the total VLDL. It is also not known to what extent the small VLDL precursor of LDL-II is directly secreted by the liver or formed in the circulation from larger VLDL precursors.

Recent studies of in vitro lipolysis of the VLDL and IDL subfractions studied here using bovine milk lipoprotein lipase have shown similar precursor-product relationships.
to those found in vivo in the rat.\textsuperscript{44} This suggests that the formation of LDL-I and LDL-II from VLDL and IDL precursors in vivo in the rat may occur by direct lipolysis.

While LDL-I has been shown to be polydisperse,\textsuperscript{24} a distinct LDL subspecies corresponding to the largest product observed here was not detected in previous gradient gel electrophoretic analyses.\textsuperscript{24} This may be a consequence of the fact that it overlaps the size and density range of IDL. It is also possible that large amounts of this product are not found in plasma because it is formed in small amounts or catabolized more rapidly in humans than other LDL species. In this regard, it may be relevant that human plasma contains substantial concentrations of LDL subclasses smaller and denser than the LDL species formed in these studies.\textsuperscript{21,24} and it may be that in normal humans, further catabolic processing of the larger primary products may give rise to one or more of the smaller, denser subclasses. Deckelbaum et al.\textsuperscript{46} have suggested that cycles of cholesterol-triglyceride exchange and subsequent lipolysis are responsible for formation of the small, dense LDL found in hypertriglyceridemic patients, and this process may be involved with formation of smaller LDL subspecies from LDL-I, LDL-II, or larger cholesterol-rich precursors in humans. As discussed above, the low levels of cholesterol ester/triglyceride exchange protein activity in rat plasma could limit "remodeling" of lipoproteins that normally result from lipid transfer during lipolysis. The role of this process in the formation of LDL subfractions in the setting of normotriglyceridemia, however, is speculative at the present time. Our findings that particles with properties of human LDL-I and LDL-II are readily produced from VLDL and IDL precursors in the rat as well as in vitro\textsuperscript{44} indicate that plasma lipid transfer activity is not required for the initial formation of these larger LDL subfractions from specific precursor lipoproteins.

The question arises whether the observed metabolic heterogeneity within the IDL might reflect an artifact introduced by methods of isolation. Ultracentrifugation may result in selective dissociation of apolipoproteins from lipid-protein particles.\textsuperscript{35,47} This might be particularly likely to occur with dysbetalipoproteinemic, compared with normal, d < 1.019 g/ml lipoproteins where initial apo E content is greater than normal. Could the loss of apo C-II, apo E, or other apolipoproteins from a portion of the IDL have given rise to a subpopulation of particles for which further hydrolysis by lipoprotein lipase or other possible metabolic transformations are impaired? Although difficult to exclude completely, it seems unlikely that this accounts for the metabolic heterogeneity observed because an ample pool of endogenous rat apo C-II, apo E, and other exchangeable apolipoproteins exists in rat plasma.

Transfer of rat apolipoproteins must be considered in interpreting the fate of the injected human subfractions. Although it is not possible to directly quantitate the apo E associated with the labelled subfractions after injection into rats, apo E is known to exchange rapidly between lipoproteins. Reassociation of apo E with the larger VLDL subfractions is suggested by the rapid clearance of these particles despite prior large preparative losses of apo E. The still more rapid clearance of both large and small VLDL from the dysbetalipoproteinemic subject presumably also reflects exchange or transfer of rat apo E to these lipoproteins, permitting uptake mediated by rat liver receptors that do not interact with the E2 isoform.\textsuperscript{48} It would appear that the observed differences in plasma decay of different subfractions injected into rats probably reflect intrinsic properties of these particles determining their capacity to associate with apo E and that losses of this apolipoprotein during ultracentrifugal isolation are rapidly replenished in vivo by transfer of rat apo E. Our data show that LDL subfractions by all measured criteria identical to the major LDL-I and LDL-II subfractions found in normal human plasma are formed from specific human precursors in vivo in the rat despite depletion in apo E prior to the injection. Nevertheless, both loss of apo E during preparation of lipoproteins, as well as acquisition of rat apolipoproteins following injection, could have influenced these results.

Both normal and dysbetalipoproteinemic plasma contain lipoprotein precursors of particles with characteristics of LDL-I and LDL-II, as well as larger IDL-sized products. In normals, the precursors of the latter are found predominantly in the IDL size and density range (nonequilibrium DGU fraction 7), while in dysbetalipoproteinemia these precursors are in fractions which contain β-VLDL. Despite some resemblance to β-VLDL in size, composition, and density,\textsuperscript{25} the small VLDL species in normals, as noted above, are preferentially converted to an LDL-II-like product after injection into rats. It seems likely that the β-VLDL in dysbetalipoproteinemic plasma that form IDL-sized products correspond metabolically to the smaller and denser LDL subpopulation in normals which behaves similarly. The fact that, in contrast to normals, only small proportions of the largest product are formed from dysbetalipoproteinemic nonequilibrium DGU fractions 6 and 7 is consistent with the idea that the precursors for this product have shifted to larger size and lower buoyant density in dysbetalipoproteinemia (Figure 8). This shift may be a consequence of lipid enrichment of lipoproteins whose residence time in plasma is abnormally prolonged as a result of the impaired apo E-receptor interaction characteristic of this disorder.

The present findings are consistent with the hypothesis that the cholesterol content of a given precursor (relative to protein or apo B) is the major determinant of the size of the final LDL product formed in the rat. It is noteworthy in this regard that the cholesterol/protein ratio of dysbetalipoproteinemic β-VLDL is considerably higher than that of small VLDL from normals, but only slightly higher than the cholesterol-enriched IDL found in equilibrium DGU fraction 7 from normals.\textsuperscript{25} The resemblance of the product formed from type 3 β-VLDL to that formed from a component of normal IDL, therefore, might result from similar degrees of cholesterol enrichment of a homologous lipoprotein particle.

Other investigators have observed that a subpopulation of the d < 1.006 g/ml lipoproteins from subjects with dysbetalipoproteinemia is resistant to conversion to an LDL product both in vivo\textsuperscript{45} and in vitro upon incubation with lipoprotein lipase.\textsuperscript{49} Ehnholm et al.\textsuperscript{30} recently confirmed the latter result specifically for an apo B-100-containing B-VLDL subfraction isolated from the plasma of the same subject (D.R.) studied in this report. They also found that
when this fraction of β-VLDL was hydrolyzed by milk lipo-protein lipase in the presence of d > 1.21 g/ml lipoprotein-deficient plasma and apolipoproteins C-II and E3 (but not E2), a product in the LDL density range (d = 1.045 to 1.055 g/ml) was formed. Our data show that in vivo in the rat, a subpopulation of dysbetalipoproteinemic small VLDL is converted to a product outside the size and density range of most normal LDL, regardless of the presence of normal rat apo E, while other dysbetalipoproteinemic triglyceride-rich subpopulations (e.g., large VLDL or subpopulations of IDL) which also contain apo E2, form products of LDL-II size and density.

A variety of clinical and experimental data suggest that lipoproteins in the IDL size and density range are associated with atherosclerosis. This is most evident in patients with dysbetalipoproteinemia. Increased levels of IDL and cholesterol enrichment of VLDL have also been reported in populations of myocardial infarction survivors in Italy and Japan. Evidence for a strong association between Sj 10 to 20, particularly Sj 10 to 14, is most strongly associated with coronary artery disease progression, compared with other lipoprotein subfractions. The IDL species shown here to have metabolic characteristics analogous to β-VLDL are found in an ultracentrifugal fraction (nonequilibrium DGU fraction 7) that contains lipoproteins of Sj 10 to 14. This, it seems likely that these cholesterol-enriched particles isolated as IDL subpopulations in normal and hypercholesterolemic subjects and as β-VLDL in dysbetalipoproteinemia have properties that render them relatively atherogenic.

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Metabolism of human intermediate and very low density lipoprotein subfractions from normal and dysbetalipoproteinemic plasma. In vivo studies in rat.

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