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Role of apolipoprotein E in the lipolytic conversion of β-very low density lipoproteins to low density lipoproteins in type III hyperlipoproteinemia

(lipoprotein metabolism/lipoprotein lipase)

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ABSTRACT **The β-very low density lipoproteins (β-VLDL) that accumulate in type III hyperlipoproteinemic subjects can be divided into two fractions (fraction I and fraction II), which differ in size, lipid composition, and the type of apolipoprotein B (apo-B) present in the particles. The apo-B48-containing particles (fraction I) are of intestinal origin, while apo-B100-containing particles (fraction II) are derived from the liver. Both fractions contain a defective form of apo-E referred to as apo-E2. Intravenous infusion of heparin into two subjects with type III hyperlipoproteinemia resulted in the complete removal of fraction II particles from density < 1.006 g/ml, while fraction I particles remained at this density. *In vitro* studies confirmed that fraction I particles did not change density when subjected to hydrolysis with lipoprotein lipase, while fraction II particles shifted to the intermediate density lipoprotein range (≈1.02 g/ml). When the β-VLDL were hydrolyzed by lipoprotein lipase in the presence of density > 1.21 g/ml lipoprotein-deficient plasma, the addition of normal apo-E (apo-E3), but not apo-E2, resulted in a shift of fraction II particles to the low density lipoprotein (LDL) range (≈1.05 g/ml). Fraction I particles did not undergo a shift to this higher density, supporting previous observations that apo-B48-containing particles are not converted to LDL. The demonstration that apo-B100-containing particles in type III hyperlipoproteinemic subjects could be converted to particles with the density of LDL suggests that apo-E plays a role in the normal conversion of VLDL to LDL. The mutant form of apo-E (apo-E2) found in the β-VLDL from type III hyperlipoproteinemic subjects appears to impede this conversion, whereas the addition of normal apo-E (apo-E3) allows the processing to occur.**

Type III hyperlipoproteinemia is an inherited disturbance of plasma lipoprotein metabolism, characterized by increases in plasma cholesterol and triglyceride levels and the presence of lipoproteins of abnormal composition. The principal abnormal lipoproteins present in dysbetalipoproteinemia are called β-very low density lipoproteins (β-VLDL), and they differ from normally occurring VLDL in their apolipoprotein content, β electrophoretic mobility, and higher content of cholesteryl ester relative to triglyceride (for review see refs. 1–3).

The β-VLDL include two distinct subclasses of lipoproteins (4, 5), which can be isolated by agarose chromatography; they are referred to as fraction I and fraction II. Fraction I consists of large particles (700–800 Å in diameter) containing apolipoprotein E (apo-E) and a low molecular weight form of apo-B referred to as B48. The fraction I β-VLDL appear to be of intestinal origin, and it is reasonable to assume that their accumulation reflects the impaired catabolism of chylomicron remnants (4). The second fraction (fraction II) consists of smaller, cholesterol-rich particles (≈400 Å in diameter), which contain apo-E and a high molecular weight form of apo-B referred to as B100. Since apo-B100 appears to be made exclusively by the liver (6), these particles probably represent remnants of cholesterol-rich VLDL of hepatic origin (4).

A primary defect at least partly responsible for the development of type III hyperlipoproteinemia is the presence of an abnormal form of apo-E (most commonly apo-E2) in the β-VLDL and other lipoproteins of affected subjects (for review see refs. 2 and 7). Apolipoprotein E occurs in the plasma in three major forms (2, 7–9); apo-E3 is the most prevalent form of the apolipoprotein, whereas the other two major forms, referred to as apo-E2 and apo-E4, represent genetic variants that differ from apo-E3 by single amino acid substitutions (10–12). An important role for apo-E in lipoprotein metabolism is to mediate the interaction of lipoproteins with specific cell surface receptors (13, 14), including both the low density lipoprotein (LDL) apo-B,E receptors of extrahepatic and hepatic tissues and the unique apo-E receptor of the liver (13, 15, 16). Apolipoproteins E3 and E4 possess normal receptor binding activity. However, the apo-E2 variants all display defective receptor binding (12, 17). Recent studies have localized the receptor binding domain of apo-E to the vicinity of amino acid residues 140–160 (18, 19).

The defective binding of apo-E2-containing lipoproteins to hepatic and extrahepatic receptors represents at least one mechanism responsible for the accumulation of the β-VLDL in the plasma of affected subjects (for review see refs. 1 and 2). Population studies have indicated that about 1% of the population is homozygous for the E2 allele (16); however, many of these individuals do not manifest gross hyperlipoproteinemia—although they all display dysbetalipoproteinemia with the presence of β-VLDL in their plasma (20, 21). Therefore, it is necessary to postulate that other factors modulate the effects of the apo-E2 defect on lipoprotein metabolism. Chait *et al.* (22) showed that triglyceride removal during heparin infusion was delayed in type III hyperlipoproteinemic subjects, and they concluded that the β-VLDL in these subjects were not as susceptible to lipolysis as normal VLDL. In light of our current knowledge, this observation suggests that apo-E may play a role in modulating the lipolytic action of lipoprotein lipase and/or hepatic lipase. This role is investigated in the present study.

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Abbreviations: apo-, apolipoprotein; β-VLDL, β-very low density lipoproteins; HDL, high density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

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MATERIALS AND METHODS

Lipoprotein Preparation. Plasma containing Na_2EDTA (1 mg/ml) was prepared from the blood of fasting subjects. Normolipidemic VLDL were obtained by ultracentrifugation as described (4). The β-VLDL from type III hyperlipoproteinemic patients (D.R. and E.R.) were isolated by centrifugation, followed by Pevikon (Mercer Chemical, Amityville, NY) electrophoresis, and subfractionated into fraction I and fraction II by gel filtration (4). Both type III subjects were E2/2 homozygotes and possessed the (Arg → Cys at position 158) mutant form of apo-E2. Pertinent clinical data for subject D.R. has been published (16). Isolated human apo-E2 and apo-E3 were obtained as described (14). The apo-C-II was a gift from James Sparrow. The β-VLDL and VLDL were radiolabeled with ^{125}I by the iodine monochloride method (23). The specific activities of the iodinated lipoproteins ranged from 100 to 700 cpm/ng of protein (7–14% lipid labeling). Lipoprotein-deficient plasma of density $\rho > 1.21$ g/ml (all densities are in g/ml) was prepared by adjusting the density of whole plasma to 1.21 g/ml with solid KBr, followed by centrifugation at 59,000 rpm for 48 hr at 4°C in a 60 Ti Beckman rotor. The $\rho > 1.21$ fraction was exhaustively dialyzed against 0.15 M NaCl containing Na_2EDTA at 0.1 mg/ml, pH 7.4.

Intravenous Heparin Administration. Sterile heparin (100 international units/kg of body weight) (Elkins–Sinn, Cherry Hill, NJ) was injected intravenously (cephalic vein) into normolipidemic and type III hyperlipoproteinemic subjects who were fasted overnight. Type III subject E.M. had a plasma cholesterol of 226 mg/dl and was taking clofibrate, whereas B.M. was untreated and had a cholesterol of 302 mg/dl. Blood was obtained prior to the injection and 15 min after the injection and immediately put on ice.

Lipoprotein Characterization. Protein concentration was determined by the method of Lowry *et al.* (24). Paper electrophoretograms were prepared as previously described (4). Cholesterol and triglyceride concentrations were determined by enzymatic methods (Bio-Dynamics, Boehringer Mannheim), and phospholipid content was determined by the phosphorus content (25). Lipoproteins were delipidated by extraction with chloroform/methanol and electrophoresed under reducing conditions (2-mercaptoethanol) on 4–20% gradient polyacrylamide slab gels in the presence of sodium dodecyl sulfate (26). The gels were stained with Coomassie blue, and the proteins were identified according to known standards. Autoradiograms of gels were prepared with Kodak XAR-5 film. Gels were scanned with a Beckman DU-8 spectrophotometer. Negative staining electron microscopy was performed as described (4).

Hydrolysis of Lipoprotein Triglycerides. Hydrolysis of lipoproteins was conducted in a final volume of 1 ml. The reaction mixture was prepared by incubating iodinated lipoproteins with various combinations of apo-E2, apo-E3, apo-C-II, and/or $\rho > 1.21$ lipoprotein-deficient plasma at room temperature for 1 hr prior to the addition of lipoprotein and/or hepatic lipase. In some studies, the lipoproteins to be hydrolyzed were incubated with equal volumes of $\rho > 1.21$ lipoprotein-deficient plasma (protein, 70 mg/ml) for 12–16 hr at room temperature prior to the addition of apo-E2, apo-E3, or apo-C-II. Lipoprotein and/or hepatic lipase was added to this mixture along with 500 μl of 16% fatty-acid-free bovine serum albumin (Boehringer Mannheim) in 0.2 M Tris·HCl, pH 8.4. The volume was adjusted with 0.15 M NaCl to yield a final volume of 1 ml and a bovine albumin concentration of 8% in 0.1 M Tris·HCl. The final concentration of lipoproteins to be hydrolyzed was 40–226 μg/ml of protein. The apo-E2 and apo-E3 were added in weight ratios of 1:1 to 1:3 relative to the intact lipoproteins. The apo-C-II was added in a weight ratio of 1:4 relative to the intact lipoproteins (see figure legends for details). The hydrolysis was allowed to continue for 2–5 hr at room temperature in Beckman ultraclear tubes (14 × 89 mm).

The reaction was stopped by the addition of KBr to raise the density of the reaction mixture to 1.125 g/ml. A KBr density gradient from 1.006 to 1.125 g/ml was spun at 40,000 rpm for 16–24 hr at 4°C in a Beckman SW 41 rotor. The gradient was formed by overlayering the reaction mixture (adjusted to 3 ml, $\rho = 1.125$) with 3 ml of $\rho = 1.03$, 3 ml of $\rho = 1.02$, and 3 ml of $\rho = 1.006$. All solutions contained Na_2EDTA at 0.1 mg/ml. After centrifugation, the tubes were fractionated into 20 equal fractions, 590 μl each. The density of each fraction was determined by a measurement of the refractive index (Bausch and Lomb). The gradients were linear between 1.006 and 1.125 g/ml. There was >90% recovery of total radioactivity in these fractions. The distribution of the ^{125}I label was determined by using a Beckman γ counter. The chemical composition and electron microscopic appearance of lipoproteins were determined from pooled fractions taken from these density gradients. Fractions containing little radiolabel had no intact lipoprotein particles as determined by electron microscopy.

Bovine milk lipase (specific activity of ≈20,000 μmol of free fatty acid released/mg per hr) was obtained as described (27) or provided by Andre Bensadoun. The hepatic lipase used in the studies was purified from dog livers, essentially as previously described for rat hepatic lipase (28). The hepatic lipase preparation revealed one main protein band on sodium dodecyl sulfate/polyacrylamide gel electrophoretograms and had a specific activity of 800 μmol of free fatty acid released/mg per hr as measured by an assay system with gum arabic-emulsified triolein (29). The activity of the two enzymes added was ≈70 μmol of free fatty acid released per hr in each assay.

RESULTS

The intravenous injection of heparin (100 international units/kg of body weight) into two normolipidemic subjects resulted in the rapid clearance of almost all $\rho < 1.006$ lipoproteins within 15 min. Triglyceride and cholesterol levels in the $\rho < 1.006$ fraction decreased by >97% after the injection of heparin. Values for $\rho < 1.006$ triglyceride were 66 and 59 mg/dl, which fell to ≈1 mg/dl after heparin injection. The $\rho < 1.006$ cholesterol decreased from values of 14 and 18 mg/dl to less than 0.4 mg/dl after heparin. However, the intravenous injection of heparin into two type III hyperlipoproteinemic subjects resulted in an ≈85% decrease in triglyceride and only an ≈50–60% decrease in cholesterol in the $\rho < 1.006$ fraction. Values for $\rho < 1.006$ triglycerides for subjects E.M. and B.M. were 84 and 90 mg/dl, which fell to 15 and 9 mg/dl, respectively, after heparin. The $\rho < 1.006$ cholesterol decreased from values of 38 and 56 mg/dl for E.M. and B.M., respectively, to 21 and 20 mg/dl after the heparin injection. While no detectable lipoproteins were present in the $\rho < 1.006$ fraction of normolipidemic subjects after heparin injection, a specific subclass of $\rho < 1.006$ lipoproteins remained in the plasma of type III subjects after heparin injection.

The paper electrophoretogram of the $\rho < 1.006$ fraction of type III subjects contains pre-β- and β-migrating lipoproteins, as well as lipoproteins that remain at the origin (Fig. 1). As shown previously (4), fraction I β-VLDL remain at the origin on paper electrophoretograms; they contain primarily apo-B48 and apo-E and appear to represent cholesteryl ester-rich chylomicron remnants. Fraction II β-VLDL migrate to the β position on paper electrophoretograms; they primarily contain apo-B100 and apo-E and probably represent cholesteryl ester-rich VLDL or VLDL remnants of hepatic origin. After the intravenous injection of heparin, only lipoproteins remaining at the origin on the electrophoreto-

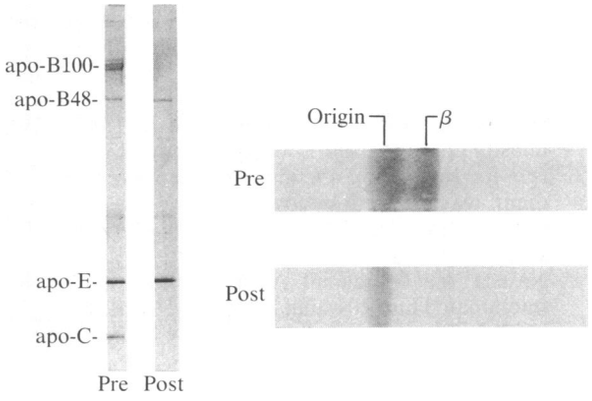

FIG. 1. Effects of intravenous heparin administration on the $\rho <$ 1.006 lipoprotein fraction in type III hyperlipoproteinemia. (*Left*) Sodium dodecyl sulfate/polyacrylamide gradient gel (4–20%) electrophoresis of the $\rho < 1.006$ fraction from a type III patient before (Pre) and 15 min after (Post) the intravenous injection of heparin (100 international units/kg of body weight). (*Right*) Paper electrophoresis of the $\rho < 1.006$ fraction from a type III subject before and after heparin injection.

gram were present in the $\rho < 1.006$ fraction (Fig. 1). Gradient gel electrophoresis (sodium dodecyl sulfate) also revealed that the apo-B48-containing particles were selectively retained in the $\rho < 1.006$ fraction (Fig. 1). These results suggested that the fraction I subclass of β-VLDL was resistant to post-heparin lipolytic activity, whereas the fraction II subclass of β-VLDL could be converted to $\rho > 1.006$ lipoproteins or cleared from the plasma.

To investigate further the resistance of type III β-VLDL to lipolytic hydrolysis, the subclasses of type III β-VLDL (fractions I and II) were isolated and incubated *in vitro* with bovine milk lipoprotein lipase and/or canine hepatic lipase. When fraction I β-VLDL were incubated with milk lipoprotein lipase in the presence of apo-C-II, with or without the addition of apo-E3 and hepatic lipase, there was no conversion to particles with a greater density—i.e., they remained at $\rho < 1.006$ (Fig. 2). Although significant quantities of triglyceride remained within these particles, there was an increase in the cholesterol-to-triglyceride (wt/wt) ratio from 0.42 prior to hydrolysis to 2.9 after hydrolysis. Despite the variety of conditions tested (see Fig. 2), it was not possible to convert the fraction I lipoproteins to particles of $\rho > 1.006$ ("IDL" or "LDL"). ["IDL" and "LDL" are shorthand notations for particles of these densities and are not meant to imply metabolic identity with *in vivo* intermediate density lipoproteins (IDL) and low density lipoproteins (LDL).] The conversion of normal VLDL to lipoproteins with the density of LDL is shown in Fig. 2*A* (●) for comparison. Likewise, it was possible to convert type III pre-β-migrating VLDL (which were isolated from the $\rho < 1.006$ fraction by Pevikon electrophoresis) to "LDL" by the addition of lipase and apo-C-II (Fig. 2*B*, ○). These VLDL have a lipid composition very similar to VLDL from normolipidemic subjects (triglyceride ≈ 60%; data not shown).

In contrast to fraction I β-VLDL, the fraction II β-VLDL could be converted to particles of $\rho > 1.006$ by incubation with lipoprotein lipase. The addition of apo-C-II to the incubation mixture resulted in further hydrolysis of the fraction II lipoproteins to $\rho = 1.02$ (Fig. 3*A*, ●). These results indicate that fraction II β-VLDL may be relatively deficient in apo-C-II, as suggested by inspection of the apoproteins of unfractionated β-VLDL (30). The "IDL" (ρ = 1.015–1.03) were enriched in cholesterol (cholesterol-to-triglyceride ratio: 1.0 for fraction II prior to hydrolysis vs. 3.1 after hydrolysis; in contrast, the cholesterol-to-phospholipid ratio was 1.1 vs. 1.4) and had a composition similar to "IDL-LDL" produced

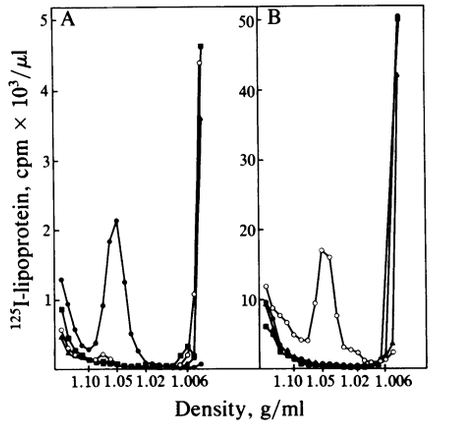

FIG. 2. Distribution of ^{125}I-labeled lipoproteins by density gradient ultracentrifugation after the *in vitro* incubation of ^{125}I-labeled fraction I β-VLDL or ^{125}I-labeled pre-β-VLDL. The apoproteins and lipoproteins were incubated with or without hepatic and/or lipoprotein lipase for 2 hr with a final concentration as follows: fraction I β-VLDL, 40 μg/ml; pre-β-VLDL from a normolipidemic individual, 180 μg/ml; pre-β-VLDL from a type III subject, 75 μg/ml; apo-E3, 54 μg/ml; and apo-C-II, 9 μg/ml. (*A*) Fraction I β-VLDL (no lipase) (○); fraction I β-VLDL after incubation with lipoprotein lipase, hepatic lipase, apo-C-II, and apo-E3 (■); pre-β-VLDL from a normolipidemic individual (no lipase) (▲); pre-β-VLDL from a normolipidemic individual after incubation with lipoprotein lipase and apo-C-II (●). (*B*) Fraction I β-VLDL incubated with $\rho > 1.21$ lipoprotein-deficient plasma for 1 hr and then subjected to lipolysis for 4 hr under the following conditions: fraction I β-VLDL after incubation with hepatic lipase, apo-C-II, and apo-E3 (▲); fraction I β-VLDL after incubation with lipoprotein lipase, hepatic lipase, and apo-C-II (●); fraction I β-VLDL after incubation with lipoprotein lipase, hepatic lipase, apo-C-II, and apo-E3 (■); pre-β-VLDL from a type III subject after incubation with lipoprotein lipase, hepatic lipase, and apo-C-II (○).

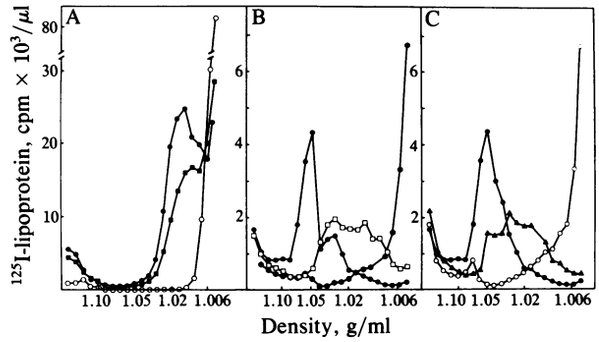

FIG. 3. Distribution of ^{125}I-labeled lipoproteins by density gradient ultracentrifugation after incubation of ^{125}I-labeled fraction II β-VLDL or ^{125}I-pre-β-VLDL *in vitro*. The apoproteins and lipoproteins were incubated with or without hepatic and/or lipoprotein lipase for 3 hr with a final concentration as follows: fraction II β-VLDL, 226 μg/ml; apo-E3, 84 μg/ml; apo-E2, 84 μg/ml; apo-C-II, 50 μg/ml. (*A*) Fraction II β-VLDL (no lipase) (○); fraction II β-VLDL after incubation with lipoprotein lipase (■); fraction II β-VLDL after incubation with lipoprotein lipase and apo-C-II (●). (*B* and *C*) Fraction II β-VLDL preincubated with $\rho > 1.21$ lipoprotein-deficient plasma for 16 hr and then subjected to lipolysis for 5 hr under the following conditions: (*B*) fraction II β-VLDL after incubation with apo-C-II and apo-E3 (no lipase) (●); fraction II β-VLDL after incubation with lipoprotein lipase, hepatic lipase, and apo-C-II (□); fraction II β-VLDL after incubation with lipoprotein lipase, hepatic lipase, apo-C-II, and apo-E3 (●); (*C*) fraction II β-VLDL after incubation with hepatic lipase, apo-C-II, and apo-E3 (○); fraction II β-VLDL after incubation with lipoprotein lipase, hepatic lipase, apo-C-II, and apo-E2 (▲); fraction II β-VLDL after incubation with lipoprotein lipase, apo-C-II, and apo-E3 (●).

in vitro, as reported by others (31).

In an additional study, purified canine hepatic lipase (specific activity, 800 μmol/mg per hr) was added to the incubation mixture to determine if fraction II β-VLDL could be converted to higher density "LDL" particles. Hepatic lipase, without the addition of lipoprotein lipase, caused no shift in the density of the fraction II β-VLDL (Fig. 3*C*, ○). Furthermore, the combination of lipoprotein lipase and hepatic lipase did not cause any greater shift in the density of the lipase-generated particles than did lipoprotein lipase alone (data not shown). Consequently, we have concluded that hepatic lipase, at least in this *in vitro* experimental system, does not convert these lipoprotein particles to the heavier "LDL"-like particles that float in the 1.05 g/ml density region.

Since previous work has suggested that the transformation of "IDL" particles into "LDL"-like particles (32) is dependent upon factors present in the lipoprotein-deficient plasma fraction, we included the $\rho > 1.21$ fraction in our incubations. All experiments illustrated in Fig. 3 *B* and *C* were conducted with fraction II β-VLDL that were preincubated overnight with the $\rho > 1.21$ lipoprotein-deficient plasma. When fraction II β-VLDL were incubated with $\rho > 1.21$ lipoprotein-deficient plasma (without other additions), no changes in the ultracentrifugal pattern were observed. Furthermore, the addition of apo-C-II or apo-E3 to the preincubated fraction II β-VLDL did not alter the density of the particles (Fig. 3*B*, ●). The addition of lipoprotein lipase plus hepatic lipase and apo-C-II resulted in the production of "IDL" and the limited production of "LDL" (Fig. 3*B*, □). However, when apo-E3 was added to this same incubation mixture, there was a marked conversion of the fraction II β-VLDL to "LDL" (Fig. 3*B*, ●). Similar results were obtained in three independent studies. The chemical composition of the "LDL" (ρ = 1.045–1.055) revealed a further decrease in triglyceride content as compared with the "IDL" (cholesterol-to-triglyceride ratio: 6.0 for "LDL" vs. 3.1 for "IDL"; cholesterol-to-phospholipid ratio: 1.2 vs. 1.4). In an additional study not shown, similar results were obtained by preincubating the lipoproteins with $\rho > 1.21$ plasma for 1 hr only (in the presence of apo-E3). This suggests that the overnight preincubation (12–16 hr) with $\rho > 1.21$ was not essential.

To elucidate further the role of apo-E in the conversion of "IDL" to "LDL," fraction II β-VLDL (preincubated with $\rho > 1.21$ lipoprotein-deficient plasma) were incubated with lipoprotein lipase and/or hepatic lipase either in the presence of normal apo-E3 or apo-E2 from a patient with type III hyperlipoproteinemia (Fig. 3C) or in the absence of added apo-E (Fig. 3*B*, □). It was evident that in the presence of lipoprotein lipase nearly complete conversion could be brought about with the addition of normal apo-E3 (Fig. 3*C*, ●) and that the mutant form of apo-E, apo-E2, did not mediate this conversion (Fig. 3*C*, ▲) as effectively as apo-E3. Furthermore, it could be seen that hepatic lipase, either in the presence of (Fig. 3*C*, ○) or in the absence of (data not shown) apo-E3, did not convert fraction II β-VLDL to "LDL"-like particles. These results show that lipoprotein lipase is involved in the processing of fraction II β-VLDL to "LDL"-like particles in the presence of normal apo-E3 and $\rho > 1.21$ lipoprotein-deficient plasma.

The lipoproteins formed by *in vitro* lipolysis were characterized by electron microscopy. The fraction I β-VLDL had a mean diameter of 860 Å, identical to that previously described (4). After hydrolysis with lipoprotein lipase and apo-C-II, the particles floating at $\rho < 1.006$ were reduced in size to ≈700 Å. Occasional particles were irregular in shape and displayed redundant surface material not dissimilar to that seen in the morphology of chylomicrons after hydrolysis. The fraction II β-VLDL were similar to those previously described and had a mean diameter of ≈400 Å (4). After hydrolysis with lipoprotein lipase in the absence of apo-E3 or $\rho > 1.21$ lipoprotein-deficient plasma, the "IDL" particles had a mean diameter of 380 Å, only slightly smaller than the original fraction II β-VLDL. However, after the addition of apo-E3 to the reaction mixture, the "LDL" particles found in the 1.05 g/ml density range were significantly smaller, with a diameter ranging from 260 to 360 Å (mean ≈300 Å). In this fraction, there was also a small number of flat, discoidal particles that may have represented excess surface material.

To assess the changes in apoprotein content, autoradiograms of polyacrylamide gels were made of fractions from a study similar to that shown in Fig. 3*B*. The material remaining at the top of the density gradient ($\rho < 1.006$), which was not treated with lipase, served as a control. Densitometric scanning of the autoradiograms of this fraction showed that the distribution of radiolabeled apoproteins was approximately 50%, 4.5%, and 23% for the apo-B100, apo-E, and apo-C bands, respectively. The LDL-like particles generated from fraction II β-VLDL by lipoprotein lipase hydrolysis in the presence of apo-E3 and the $\rho > 1.21$ fraction possessed approximately 60%, 3.5%, and 13% of the radiolabel in the apo-B100, apo-E, and apo-C bands, respectively. Likewise, the "IDL," generated in the presence of lipoprotein lipase, apo-E2, and $\rho > 1.21$ fraction, possessed the B, E, and C apoproteins with a distribution similar to that observed in the "LDL." Furthermore, Coomassie blue-stained gels revealed that both the "IDL" and "LDL" particles contained similar amounts of the various apoproteins, including apo-E.

DISCUSSION

Several lipoprotein abnormalities characteristic of subjects with type III hyperlipoproteinemia need to be explained. It is clear that a common feature of β-VLDL hyperlipoproteinemia is the occurrence of receptor-defective apo-E, usually apo-E2 (1, 2). However, not all individuals with defective apo-E who display abnormal receptor binding activity develop gross hyperlipoproteinemia; yet they all have variable amounts of β-VLDL in their plasma regardless of whether they display hyperlipidemia or not (20, 21).

One characteristic of type III hyperlipoproteinemia is the presence of two distinct subclasses of β-VLDL in the plasma (4, 5). Other lipoprotein changes seen in this disease include an increase in cholesterol-rich IDL in the plasma and a low level of plasma LDL (1, 2). This spectrum of abnormalities has led to speculation that there are several defects involved (for review see ref. 2). These include (*i*) the impaired catabolism of cholesterol-rich lipoproteins, particularly the chylomicron remnants (fraction I β-VLDL); (*ii*) the overproduction of hepatic lipoproteins, possibly representing the fraction II β-VLDL; and (*iii*) the impaired lipase conversion of β-VLDL and IDL to LDL. This delay in normal conversion could account, at least in part, for the low level of LDL in these patients. The present studies were undertaken to explore the defect in the conversion of β-VLDL to LDL and to determine if the presence of apo-E2 is involved directly or indirectly in preventing normal processing.

The intravenous injection of heparin into type III subjects demonstrated that the fraction I β-VLDL did not shift density to >1.006 g/ml. (Presumably, these remnant lipoproteins accumulate in the plasma of type III subjects as a result of impaired clearance due to the presence of receptor-defective apo-E.) By contrast, the fraction II β-VLDL were converted into lipoproteins of $\rho > 1.006$.

The *in vitro* studies confirmed and extended the studies obtained with the intravenous injection of heparin into type III subjects. The fraction I β-VLDL could not be converted into lower density lipoproteins. It is of interest that even under *in vitro* conditions with the addition of both lipoprotein

and hepatic lipases, apo-E3, apo-C-II, and $\rho > 1.21$ lipoprotein-deficient serum it was not possible to produce an "IDL-LDL"-like particle from fraction I β-VLDL. These observations are consistent with those of other researchers, who showed that apo-B48-containing chylomicrons and chylomicron remnants do not get converted into lipoproteins in the LDL density range (33).

Recently, Chung and Segrest (34) demonstrated that a subpopulation of the $\rho < 1.006$ lipoproteins from type III subjects are resistant to lipolytic hydrolysis. Because the $\rho < 1.006$ fraction from type III patients contains at least three different types of lipoproteins, pre-β-VLDL, fraction I β-VLDL, and fraction II β-VLDL, it is somewhat difficult to compare their data with those reported here. However, it is reasonable to conclude that the lipolysis-resistant subfraction probably represents fraction I.

In contrast to the results obtained with fraction I β-VLDL, the fraction II β-VLDL could be converted into particles of "IDL" density *in vitro* with the addition of lipoprotein lipase and apo-C-II. However, to be converted into particles with the density of LDL required the addition of both $\rho > 1.21$ lipoprotein-deficient plasma and apo-E3. The presence of either the $\rho > 1.21$ fraction or apo-E2 or the combination of both did not result in the dramatic conversion of fraction II particles into "LDL." In these *in vitro* studies, hepatic lipase did not appear to affect the conversion of the β-VLDL into higher density subclasses.

The particles found in the LDL density range after hydrolysis of fraction II β-VLDL are clearly not identical to native LDL. They are similar in density and chemical composition. However, they still contain significant amounts of apo-E and apo-C. In addition, electron microscopy reveals that they are larger and more heterogeneous than native LDL and contain some discoidal particles. They are similar to the *in vitro* "LDL" described by Deckelbaum *et al.* (31). These authors showed that VLDL from normal subjects could readily be converted to LDL-like particles by hydrolysis with bovine milk lipoprotein lipase. Their *in vitro* "LDL" were also larger than native LDL (270 vs. 215 Å) and contained apo-E and apo-C. The chemical composition and appearance in electron micrographs were also similar to those described in the present study. It is possible that both the morphology and chemical composition of the "LDL" would be altered by the presence of other plasma lipoproteins capable of accepting surface constituents.

It is difficult to ascertain the precise mechanism responsible for the impaired conversion of the hepatic β-VLDL into "LDL" or how apo-E3 appears to correct the defect. It is not likely that the presence of apo-E2 directly influences the hydrolytic activity of lipoprotein lipase, since type III pre-β-VLDL (containing apo-B100 and apo-E2) can be converted to "LDL" by the addition of lipoprotein lipase and apo-C-II alone. Furthermore, *in vitro* incubations of triglyceride emulsions with lipoprotein lipase in the presence of the different isoforms of apo-E either have no effect on lipolysis or inhibit hydrolysis to the same extent in each case (unpublished observation). The necessity for the addition of $\rho > 1.21$ lipoprotein-deficient plasma and apo-E3 suggests that the conversion of fraction II β-VLDL to LDL may require a remodeling of the particle, possibly through a reduction of the cholesteryl ester or phospholipid content. The possible relationship between transfer proteins (phospholipid and cholesteryl ester) and apo-E in this system needs to be explored. A relationship between apo-E and cholesteryl ester transfer proteins has been suggested previously (35).

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