Resistance of a very low density lipoprotein subpopulation from familial dysbetalipoproteinemia to in vitro lipolytic conversion to the low density lipoprotein density fraction

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Abstract In vitro lipolysis of very low density lipoprotein (VLDL) from normolipidemic and familial dysbetalipoproteinemic plasma by purified bovine milk lipoprotein lipase was studied using the combined single vertical spin and vertical autoprofile method of lipoprotein analysis. Lipolysis of normolipidemic plasma supplemented with autologous VLDL resulted in the progressive transformation of VLDL to low density lipoprotein (LDL) via intermediate density lipoprotein (IDL) with the transfer of the excess cholesterol to high density lipoprotein (HDL). At the end of 60 min lipolysis, 92–96% of VLDL triglyceride was hydrolyzed, and, with this process, >95% of the VLDL cholesterol and ^{125}I-labeled VLDL protein was transferred from the VLDL to the LDL and HDL density region. When VLDL from the plasma of an individual with familial dysbetalipoproteinemia was substituted for VLDL from normolipidemic plasma, less than 50% of the VLDL cholesterol and 65% of ^{125}I-labeled protein was removed from the VLDL density region, although 84–86% of VLDL triglyceride was lipolyzed. Analysis of familial dysbetalipoproteinemic VLDL fractions from pre- and post-lipolyzed plasma showed that the VLDL remaining in the postlipolyzed plasma (lipoprotein lipase-resistant VLDL) was richer in cholesteryl ester and tetramethylurea-insoluble proteins than that from prelipolysis plasma; the major apolipoproteins in the lipoprotein lipase-resistant VLDL were apoB and apoE. During lipolysis of normolipidemic VLDL containing trace amounts of ^{125}I-labeled familial dysbetalipoproteinemic VLDL, removal of VLDL cholesterol was nearly complete from the VLDL density region, while removal of ^{125}I-labeled protein was only partial. A competition study for lipoprotein lipase, comparing normolipidemic and familial dysbetalipoproteinemic VLDL to an artificial substrate ([^{3}H]triolein), revealed that normolipidemic VLDL is clearly better than familial dysbetalipoproteinemic VLDL in competing for the release of ^{3}H-labeled free fatty acids. The results of this study suggest that, in familial dysbetalipoproteinemic individuals, a subpopulation of VLDL rich in cholesteryl ester, apoB, and apoE is resistant to in vitro conversion by lipoprotein lipase to particles having LDL-like density. The presence of this lipoprotein lipase-resistant VLDL in familial dysbetalipoproteinemic subjects likely contributes to the increased level of cholesteryl ester-rich VLDL and IDL in the plasma of these subjects.—**Chung, B. H., and J. P. Segrest.** Resistance of a very low density lipoprotein subpopulation from familial dysbetalipoproteinemia to in vitro lipolytic conversion to the low density lipoprotein density fraction. *J. Lipid Res.* 1983. **24:** 1148–1159.

Supplementary key words very low density lipoprotein • intermediate density lipoprotein • low density lipoprotein • high density lipoprotein • lipoprotein lipase • lipolysis • familial dysbetalipoproteinemia

Familial dysbetalipoproteinemia (type III hyperlipoproteinemia or broad-β-disease) is a disorder of plasma lipoprotein catabolism associated with premature atherosclerotic vascular disease (1). The plasma lipoprotein profile of individuals with this disorder characteristically shows increased levels of very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) (2, 3) that are unusually rich in cholesteryl ester (4) and apolipoprotein E (5), and exhibit β-electrophoretic mobility (6). The β-migrating VLDL and IDL are considered to be lipolytic remnants of triglyceride-rich lipoproteins that accumulate in subjects with familial dysbetalipoproteinemia (F. Dys.) because of a defect in the catabolic removal of VLDL and/or IDL (7, 8).

It has been reported that, *a*) normal remnant lipoproteins are cleared initially by the liver (9, 10) and *b*) an important component of remnant lipoproteins responsible for hepatic uptake is apolipoprotein E (11). Utermann, Jaeschke, and Menzel (12) identified several apoE isoforms by one-dimensional isoelectric focusing,

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; Lp, lipoprotein; F. Dys., familial dysbetalipoproteinemia; TG, triglyceride; n-VLDL, Lp lipase-sensitive VLDL; r-VLDL, Lp lipase-resistant VLDL; TMU, tetramethylurea.

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and found that one of these isoforms, apoE-3, appeared to be missing in VLDL from a F. Dys. patient.

Zannis and Breslow (13) showed, using two-dimensional polyacrylamide gel electrophoresis, that plasma apoE isoforms were due to genetic variation in apoE with three alleles at a single genetic locus, as well as post-transitional modification of apoE carbohydrate chains containing sialic acid. They also showed strong association of F. Dys. with the apoE phenotype (E-2/E-2) which represents homozygosity for the apoE allele E-2. Weisgraber, Rall, and Mahley (14) have shown that heterogeneity of the three major isoforms of the human apoE is due to differences in primary structure and involves cysteine-arginine interchange in the apoE polypeptides. Havel et al. (15) demonstrated that the marker for the hepatic uptake of human apolipoprotein E is largely confined to apolipoproteins E-3 and E-4, and postulated that the accumulation of remnant lipoproteins in F. Dys. is caused by a defective hepatic clearance of remnant lipoprotein due to a lack of apolipoprotein E-3 and E-4 on the remnant lipoprotein surfaces. In earlier studies, Utermann et al. (16) proposed the concept that a necessary genotype for F. Dys. is E-2/E-2, but that some additional triggering factor, such as hypothyroidism or combined familial hypercholesterolemia, is required for expression of the F. Dys. phenotype. Recent studies by Weisgraber, Innerarity, and Mahley (17) indicate that the apoE-2 isoform, the most abundant specie of apoE in most subjects with F. Dys., has a defective binding to the cell surface receptor of LDL, and have suggested that this defective binding might result in a defective clearance of remnant lipoproteins. Earlier studies by Schneider et al. (18) showed a decreased binding of the E-2 isoform to the putative liver LDL receptor from most, but not all, F. Dys. subjects.

Studies of the in vivo metabolism of F. Dys. VLDL in normolipidemic and F. Dys. subjects have revealed that F. Dys. VLDL is cleared from the circulation, but at a much slower rate than the clearance of normal VLDL (19). It is not certain, however, whether delayed clearance of F. Dys. VLDL is due to a defective removal of lipolytic remnant particles by the liver, since the role of the human liver in metabolism of remnant lipoproteins is still unclear. A significant portion of the in vivo metabolism of VLDL occurs through multiple interactions with Lp lipase, producing a progressive change in the composition of remnants and in the affinity of remnants to Lp lipase (20). It may be that a progressive deficiency in the ability of remnant particles to serve as a substrate for Lp lipase may be a contributing factor in the accumulation of remnant particles in F. Dys. Ganesan et al. (21) have previously shown that apolipoprotein E is an irreversible inhibitor of apolipoprotein C-II-activated Lp lipase.

It therefore is reasonable to hypothesize that accumulation of apolipoprotein E-rich lypolytic remnant particles in F. Dys. might be associated with a defective lipolysis of these remnant particles. The present study was designed to test this hypothesis. We recently showed that VLDL from type IV hypertriglyceridemic plasma is defective in its ability to act as an in vitro Lp lipase substrate (22). However, the lipolytic kinetics of plasma from F. Dys. reported here are quite different from that of type IV plasma.

MATERIALS AND METHODS

Plasma

Fresh normolipidemic plasma (overnight fast) collected in sodium citrate was obtained from the Alabama Regional Red Cross Blood Center, Birmingham, AL. The lipoprotein cholesterol profiles of these samples were examined by the vertical autoprofile method (23) developed in this laboratory. Plasma from a 29-year-old fasted male subject with familial dysbetalipoproteinemia was collected by plasmaphoresis. F. Dys. in this subject was initially suspected during routine vertical autoprofile analysis of 160 sophomore medical students and was confirmed by strip electrophoresis (24). ApoE isoform phenotyping was further performed by two-dimensional gel electrophoresis by Drs. Zannis and Breslow, Childrens' Hospital Medical Center, Harvard Medical School, Boston, MA. The phenotype was identified as an E-2/E-2. Plasma from an untreated F. Dys. subject was obtained from Dr. Robert Lees, Massachusetts Institute of Technology, Cambridge, MA, and the vertical autoprofile analysis of this sample was found to be quite similar to that of the experimental subject.

Preparation of lipoprotein and plasma fractions

Normolipidemic plasma, containing <20 mg/dl VLDL cholesterol, and the F. Dys. plasma were dialyzed against 0.15 M NaCl–0.1% EDTA, pH 7.4, and VLDL was isolated at the plasma density by ultracentrifugation at 7°C in a Sorvall fixed-angle rotor, T-865, at 55,000 rpm for 18 hr using a Sorvall OTD-2 ultracentrifuge (25). A second portion of the F. Dys. plasma was adjusted to a density of 1.020 g/ml with solid KBr, and this density-adjusted sample was subjected to the same ultracentrifuge procedure as for the VLDL isolation.

The VLDL or VLDL plus IDL, d < 1.02 g/ml, fraction was collected by tube slicing and the infranate fractions were saved. Isolated VLDL was washed once by single vertical spin ultracentrifugation (26) and whole plasma, VLDL, VLDL infranate, and VLDL + IDL infranate fractions were dialyzed simultaneously against

four changes (4 liters each) of 0.01 M Tris–0.15 M NaCl, pH 7.4, for 16–18 hr.

Lipoprotein lipase

Lp lipase was purified from bovine raw milk, obtained from Bearden Dairy Farms, Helena, AL, by heparin-Sepharose affinity column chromatography (27). The Lp lipase activity was measured by the artificial substrate method (28). The specific activities of enzyme used in this study ranged from 10–20 mU lipolytic activity per μl of enzyme (1 mU = 1 nmol of free fatty acid released per min at 37°C).

Substrate

Reconstituted plasma or whole plasma was used as a substrate for Lp lipase; the reconstituted plasma was made by mixing preisolated normolipidemic VLDL or F. Dys. VLDL with either the normolipidemic VLDL infranate fraction (d > 1.006 g/ml) or the F. Dys. IDL infranate fraction (d > 1.02 g/ml) to yield a VLDL triglyceride level of 150 mg/100 ml. For the preparation of [^{3}H]triglyceride-labeled VLDL, isolated VLDL and free plasma proteins were added into a tube containing a thin dry film of [^{3}H]triolein ([9,10-^{3}H]triolein) (New England Nuclear, Boston, MA) and incubated at 7°C for 18 hr (29). The ^{3}H-labeled VLDL was then separated from free proteins by single vertical spin ultracentrifugation (26). Artificial substrate, an emulsion of triolein containing trace amounts of [^{3}H]triolein, was prepared according to the method of Nilsson-Ehle and Schotz (28).

In vitro lipolysis of plasma or reconstituted plasma

For studies involving kinetics of lipolysis, 6 ml of F. Dys. plasma or reconstituted plasma was mixed with 300 μl of enzyme solution, incubated at 37°C under nitrogen atmosphere, and 1.5-ml aliquots were withdrawn at 0, 10, 30, and 60 min of incubation. The reaction was stopped by adjusting the sample density to d 1.21 g/ml with desiccated solid KBr. Quantitation of plasma lipoprotein cholesterol fractions was achieved by the vertical autoprofiler associated with a computer deconvolution technique (23, 30).

In order to measure the rate and extent of hydrolysis of VLDL-triglyceride, 3 ml of reconstituted plasma containing trace amounts of ^{3}H-labeled VLDL (1–3 μCi/mg VLDL-triglyceride) was mixed with 150 μl of enzyme solution, incubated at 37°C, and 100-μl aliquots were withdrawn following 0, 5, 15, 30, and 60 min of incubation. The enzyme reaction was stopped by adding methanol–chloroform–heptane 1.45:1.25:1 (v/v/v) immediately after withdrawal of samples, and the levels of free fatty acids released were determined by the method of Nilsson-Ehle and Schotz (28). At the end of 60 min incubation, samples were extensively dialyzed against Tris buffer (0.01 M Tris–0.15 M NaCl, pH 7.4) and the net changes in triglyceride mass of the F. Dys. case and controls were determined.

Fate of VLDL components

A portion of isolated normolipidemic and F. Dys. VLDL was radiolabeled with ^{125}I by the iodine monochloride method of McFarlane (31). Trace amounts of ^{125}I-labeled VLDL were then added to plasma reconstituted with the homologous VLDL, and the mixtures were incubated with purified Lp lipase at 37°C for 60 min. The enzyme reaction was stopped by adjusting the sample density to d 1.30 g/ml with desiccated solid KBr. Plasma samples containing enzyme buffer only were used as controls. The lipolyzed and control samples were loaded into a vertical rotor (Sorvall TV 850, DuPont Co.) and ultracentrifuged at 50,000 rpm for 150 min at 7°C (26). At the end of a run the tubes were removed from the rotor, the bottoms were punctured, and 30 fractions were collected from each tube using a gradient fractionator (Hoefer Scientific Instruments, San Francisco, CA). The total ^{125}I activity in a 200-μl aliquot of each fraction was counted by a Packard Autogamma Counter 800 C (Packard Instruments, Pleasant Grove, IL); these aliquots were then treated with tetramethylurea (Sigma Co., St. Louis, MO), after adding an aliquot of 100 μl low density lipoprotein (LDL) (4 mg/ml) as a carrier, to determine the ^{125}I activity in the tetramethylurea-soluble (apolipoprotein C) and tetramethylurea-insoluble (apolipoprotein B) fractions (32). Levels of total cholesterol and free cholesterol were determined by an enzymatic method using Boehringer cholesterol test set No. 124079 (Bio-Dynamics/BMC, Indianapolis, IN).

Competition studies

In order to study the competition for Lp lipase lipolysis between normolipidemic and F. Dys. VLDL, normolipidemic VLDL or F. Dys. VLDL was combined with normolipidemic VLDL infranate to give 100 mg/100 ml VLDL-triglyceride. Trace amounts of ^{125}I-labeled normolipidemic or F. Dys. VLDL were added separately to each of the reconstituted plasmas and the mixtures were incubated with purified Lp lipase at 37°C for 30 min. Plasma samples with buffer only were used as controls. The densities of control and lipolyzed samples were brought to d 1.30 g/ml with solid KBr, and samples were divided into 26 fractions following the single vertical spin method using a Sorvall vertical rotor (TV 865) (26). Levels of ^{125}I activity and total cholesterol in each density gradient fraction were determined as described above.

In order to study the competition for Lp lipase lipolysis between normolipidemic or F. Dys. VLDL and artificial substrate, increasing amounts of F. Dys. or normolipidemic VLDL triglyceride (50–400 μg) were added to 1.2 μmol of triolein, and the mixtures were lipolyzed by addition of Lp lipase and incubation at 37°C for 30 min. The reaction was stopped by addition of methanol–chloroform–heptane 1.45:1.25:1 (v/v/v), and the amounts of free fatty acids (^{3}H-labeled) released were measured by the procedure of Nilsson-Ehle and Schotz (28).

Other methods

Levels of triglyceride were measured by the enzymatic assay method using Boehringer test set No. 126012 (Bio-Dynamics/BMD, Indianapolis, IN). The apolipoproteins of VLDL and/or IDL were examined by urea (32) and SDS gel electrophoresis (33). The protein content of lipoproteins was measured by the method of Lowry et al. (34), and phospholipid levels were determined from lipid-extracted samples (chloroform–methanol 2:1 (v/v)) by the method described by Stewart (35).

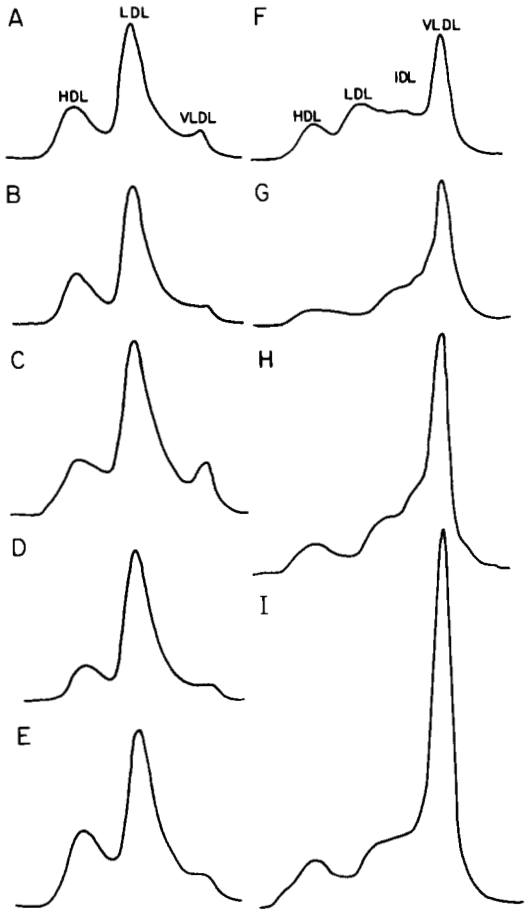

Fig. 1. Plasma lipoprotein cholesterol profile patterns determined by the vertical autoprofile method (23) for five normolipidemic (A–E) and four F. Dys (F–I) subjects.

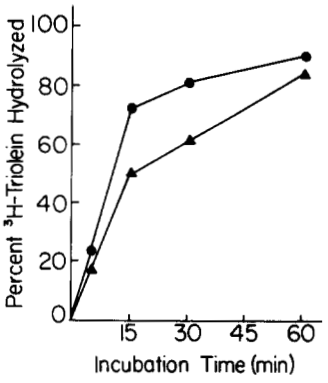

Fig. 2. Hydrolysis of [^{3}H]triolein-labeled VLDL by purified bovine milk lipoprotein lipase. Normolipidemic VLDL or F. Dys. VLDL containing trace amounts of [^{3}H]triolein-labeled VLDL was reconstituted with normolipidemic VLDL infranate (d > 1.006 g/ml) to yield a VLDL triglyceride level of 150 mg/100 ml. Three ml of reconstituted plasma was incubated with 0.15 ml of Lp lipase at 37°C and 0.1-ml aliquots (triplicate) were taken at 0, 5, 15, 30, and 60 min for determination of free fatty acid level. Normolipidemic VLDL (● —— ●), and F. Dys, VLDL (▲ —— ▲).

RESULTS

Lipoprotein cholesterol profiles of plasma from normolipidemic and F. Dys. subjects

Fig. 1 shows typical plasma lipoprotein cholesterol profiles obtained by the vertical autoprofile method for five normolipidemic (Fig. 1A–E) and four F. Dys. subjects (Fig. 1F–I). The patterns of the normolipidemic profiles are similar, although the levels of high density lipoprotein (HDL), LDL, and VLDL vary within limits. The characteristic feature of the F. Dys. profile is an increase in both VLDL and IDL, with a defined IDL peak, and a low level of LDL. The total plasma cholesterol and triglyceride levels for the F. Dys. subject studied (Fig. 1H) were 223 and 404 mg/100 ml, respectively, and the VLDL, IDL, LDL, and HDL cholesterol levels determined by vertical autoprofile analysis (26), were 88, 46, 49, and 40 mg/100 ml, respectively.

Kinetics of lipolysis of normolipidemic and F. Dys. VLDL

The kinetics of lipolysis of normolipidemic and F. Dys. VLDL in reconstituted plasma are shown in **Fig. 2** and **Fig. 3.** The time-dependent lipolysis of VLDL containing trace amount of [^{3}H]triolein-labeled VLDL indicates that the initial rate of release of ^{3}H-labeled free fatty acids from normolipidemic VLDL was considerably faster than that of F. Dys. VLDL (Fig. 2). However, the extent of hydrolysis of [^{3}H]triolein-la-

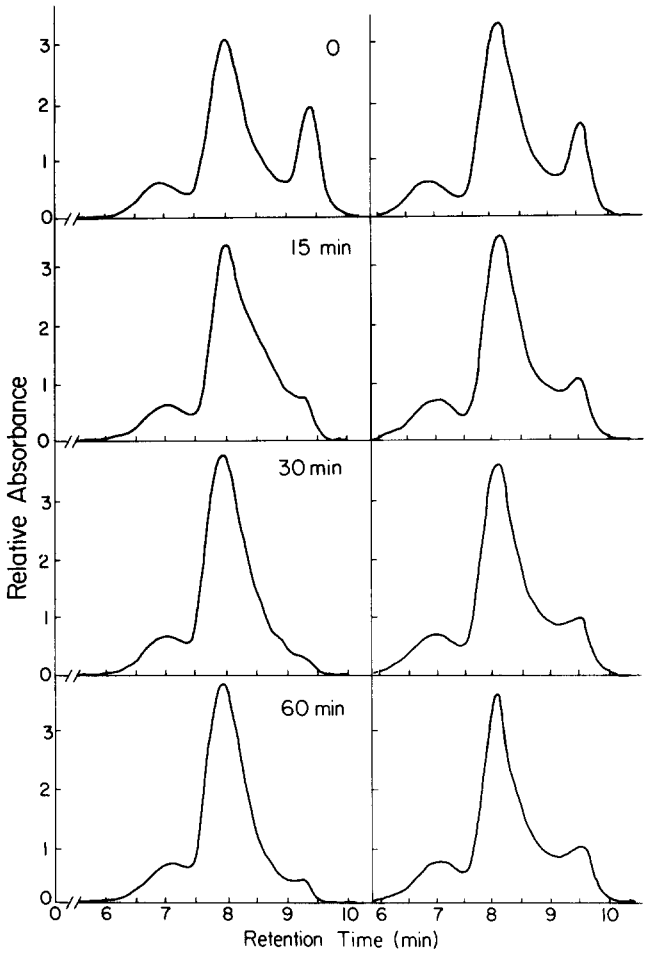

Fig. 3. Kinetics of in vitro lipolysis by purified bovine milk Lp lipase of normolipidemic VLDL (left) and F. Dys. VLDL (right). Normolipidemic VLDL or F. Dys. VLDL was reconstituted with normolipidemic VLDL infranate (d > 1.006 g/ml) to yield a VLDL triglyceride level of 150 mg/100 ml. Six ml of reconstituted plasma was incubated with 0.3 ml of Lp lipase at 37°C and 1.3-ml aliquots were taken at 0, 15, 30, and 60 min for vertical autoprofile analysis.

beled normolipidemic VLDL at the end of 60 min incubation was only slightly greater than that of F. Dys. VLDL (92% vs. 86%) (Fig. 2). When the total mass of triglyceride in the pre- and post-lipolyzed plasma was measured, the mass loss of triglyceride in reconstituted plasma containing normolipidemic and F. Dys. VLDL following lipolysis corresponded to 96.2% and 84.6% of added VLDL triglyceride, respectively. These small, but significant, differences are magnified by the fact, demonstrated in Fig. 3, that only a fraction of the F. Dys. VLDL is defective in its ability to be converted to LDL densities by incubation with Lp lipase.

The kinetics of the movement of VLDL cholesterol in normolipidemic and F. Dys. VLDL (152 and 148 mg/100 ml triglyceride, respectively) in reconstituted plasma are shown in Fig. 3. As we have reported elsewhere (22), normolipidemic VLDL during in vitro lipolysis in reconstituted plasma appears to be transformed initially to particles with a density corresponding to IDL and then ultimately to that of LDL (Fig. 3-left). In addition, the cholesterol level in the HDL density region progressively increased with time of incubation and the HDL peak shifted toward a light ("HDL_2") density region. By 15 min incubation, approximately 70% of the VLDL cholesterol was removed from the VLDL density region, and 53% and 35% of the displaced VLDL cholesterol were associated with the IDL and LDL density regions, respectively. Longer incubation resulted in further removal of VLDL cholesterol and transformation of accumulated IDL cholesterol into LDL, with some transfer to the HDL density fraction. Approximately 90% of VLDL cholesterol was removed from the VLDL density region by 60 min incubation, and the VLDL cholesterol transformed to LDL and transferred to HDL was 88% and 12% of the displaced VLDL cholesterol, respectively.

The kinetics of lipolysis of F. Dys. VLDL (Fig. 3-right) was demonstrably different from that of normolipidemic VLDL. Approximately 40% of VLDL cholesterol was rapidly removed from the VLDL density region within 15 min of incubation with Lp Lipase, while only an additional 7% of the VLDL cholesterol

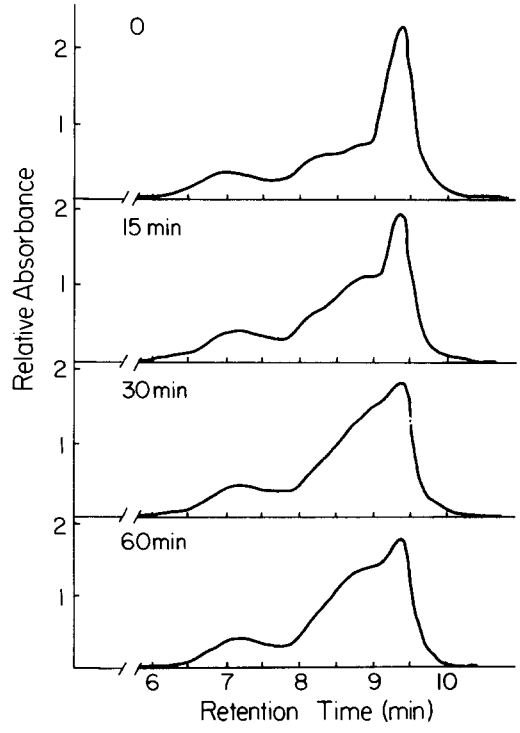

Fig. 4. Kinetics of in vitro lipolysis by purified bovine milk Lp lipase of F. Dys. plasma. Six ml of plasma was incubated with 0.3 ml of Lp lipase at 37°C, and 1.3-ml aliquots were taken at 0, 15, 30, and 60 min for vertical autoprofile analysis.

was removed with further incubation up to 60 min. Time-dependent lipolysis of whole plasma from the F. Dys. subject yielded similar kinetics to that of plasma reconstituted with F. Dys. VLDL. Less than half of the VLDL of the whole plasma was removed from the VLDL density region, and most of the displaced VLDL cholesterol was in the IDL density region (**Fig. 4**).

Fate of VLDL constituents during lipolysis

Further studies were performed to determine the movement of free and esterified cholesterol and the ^{125}I-labeled protein moieties of VLDL during in vitro lipolysis. In vitro lipolysis of reconstituted plasma containing unlabeled normolipidemic VLDL and trace amounts of ^{125}I-labeled normolipidemic VLDL resulted in the removal of >95% of the ^{125}I label and total cholesterol from the VLDL region after 60 min incubation, with a concomitant transformation of these components to LDL and transfer to HDL (**Fig. 5A**). Displaced VLDL free cholesterol was approximately equally associated with the LDL (54%) and the HDL (46%) density regions, while displaced VLDL cholesteryl ester was mostly associated with the LDL density region (86%). Following lipolysis, the tetramethylurea-soluble ^{125}I-radioactivity of VLDL (apolipoprotein C) was mostly (>80%) associated with the HDL density region, while the tetramethylurea-insoluble ^{125}I-radioactivity (apolipoprotein B) was predominantly (>90%) associated with the LDL density region.

Fig. 5B shows the results of a similar experiment performed on reconstituted plasma containing unlabeled F. Dys. VLDL and trace amounts of ^{125}I-labeled F. Dys. VLDL. In contrast to normolipidemic VLDL, only 70% of free cholesterol, 47% of cholesteryl ester, and 72% of ^{125}I-radioactivity were removed from the VLDL density region after 60 min incubation; however the distribution of these easily displaced VLDL components in the LDL and the HDL density regions was similar to those of normolipidemic VLDL (Fig. 5B). The Lp lipase-resistant VLDL remaining in the VLDL density

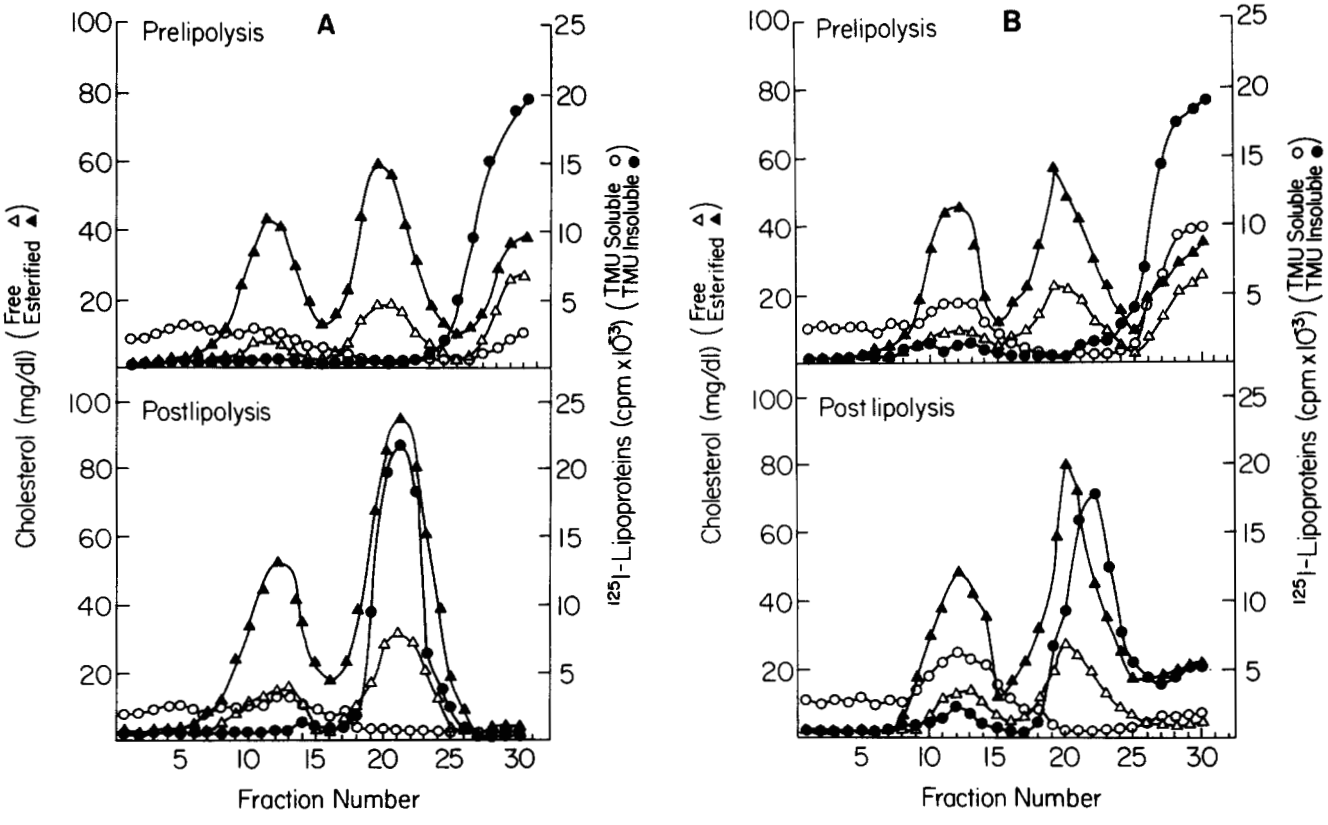

Fig. 5. Distribution of free cholesterol, cholesteryl ester, and radioactivity of ^{125}I-VLDL in the single vertical spin density gradient fractions of reconstituted normolipidemic and F. Dys. plasma. A, Ten ml of reconstituted plasma containing 150 mg/100 ml normolipidemic (^{125}I-labeled) VLDL triglyceride was incubated with 0.5 ml of Lp lipase at 37°C for 1 hr. Recovery of free cholesterol and cholesteryl ester following lipolysis was 101% and 104% of the prelipolysis (control) sample. B, Ten ml of reconstituted plasma containing 150 mg/100 ml F. Dys. (^{125}I-labeled) VLDL triglyceride was incubated with 0.5 ml of Lp lipase at 37°C for 1 hr. Recovery of free cholesterol and cholesteryl ester was 103% and 101% of the prelipolysis (control) sample. The prelipolysis (control) sample was incubated at 37°C for 1 hr without Lp lipase (buffer only).

region following lipolysis of the reconstituted F. Dys. plasma was richer in cholesteryl ester and tetramethylurea-insoluble apoproteins than prelipolyzed VLDL (Fig. 5B).

Compositional analyses of normolipidemic VLDL and F. Dys. VLDL from pre- and post-lipolyzed samples and IDL from F. Dys. plasma are given in **Table 1.** It is evident from these data that triglyceride is the major core lipid of both normolipidemic and F. Dys. VLDL. The F. Dys. VLDL, however, contains less triglyceride (45% vs. 55%) and more cholesteryl ester (18% vs. 10%) than normolipidemic VLDL. Following lipolysis of F. Dys. VLDL, the remnant lipoproteins remaining in the VLDL density region (Lp lipase-resistant VLDL) contain 13% residual triglyceride. Based on the mass of triglyceride present in each VLDL fraction per mg of cholesteryl ester, this corresponds to 87% hydrolysis of the triglyceride originally present in whole F. Dys. VLDL. The IDL isolated from F. Dys. plasma has a similar composition to that of Lp lipase-resistant VLDL, although it is somewhat richer in triglyceride and poorer in cholesteryl ester (Table 1).

Analysis of VLDL apolipoproteins of F. Dys. and normolipidemic VLDL by urea and/or SDS gel electrophoresis showed that F. Dys. VLDL is richer in apolipoprotein E than normolipidemic VLDL (**Fig. 6**-left A, B). The major apolipoproteins found in Lp lipase-resistant VLDL were apolipoproteins B and E (Fig. 6-left C and 6-right A, B). IDL isolated from the F. Dys. plasma contained apolipoproteins B and E as major apolipoproteins but also contained a minor amount of apolipoprotein C (Fig. 6-left D).

Lipolysis of ^{125}I-labeled normolipidemic VLDL or F. Dys. VLDL reconstituted with the IDL infranate of F. Dys. plasma ($d > 1.02$ g/ml) showed that the distribution of ^{125}I-VLDL label among the gradient fractions is similar to that for VLDL reconstituted with normolipidemic VLDL infranate (**Fig. 7**). Complete conversion of normolipidemic VLDL to LDL density in the presence of F. Dys. VLDL infranate (Fig. 7, upper) indicates that the F. Dys. VLDL infranate fraction has no apparent effect on the lipolysis of normolipidemic VLDL. This is in contrast to type IV hypertriglyceridemic VLDL infranate which has been shown to partially inhibit the lipolysis of normolipidemic VLDL (22). Lipolysis of ^{125}I-labeled VLDL from F. Dys. plasma again showed that a significant portion of ^{125}I-label was accumulated in the VLDL and IDL density region (Fig. 7, lower).

TABLE 1. Composition of normolipidemic VLDL, F. Dys. VLDL (pre- and postlipolyzed) and F. Dys. IDL[a]

	Normolipidemic VLDL[b] (n = 3)	F. Dys. VLDL[c]		F. Dys. IDL[c] $1.006 > d > 1.020$
		Pre-lipolysis	Post-lipolysis	
		% mass		
Triglyceride	55.0 ± 2.32	45.4	13.1	20.4
Cholesteryl ester	10.4 ± 2.60	17.6	40.0	28.9
Free cholesterol	6.8 ± 0.45	10.4	12.7	10.2
Phospholipid	15.8 ± 2.55	16.2	19.0	21.0
Proteins	11.6 ± 2.50	10.4	15.2	19.5

[a] Lipoproteins used for the compositional analysis were washed once by single vertical spin ultracentrifugation.
[b] Mean value (triplicates) of three normolipidemic VLDL.
[c] Mean value of triplicate.

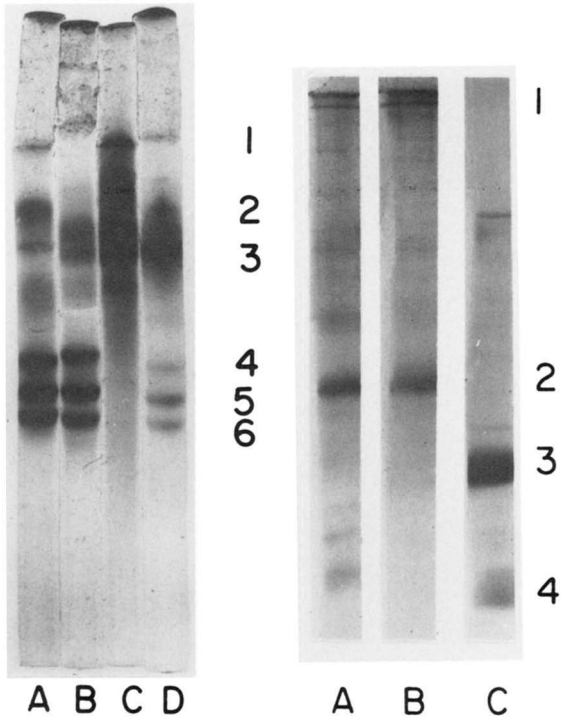

Fig. 6. Left, urea polyacrylamide gel electrophoretic patterns of (A) normolipidemic VLDL, (B) control F. Dys. VLDL (prelipolysis), (C) Lp lipase-resistant F. Dys. VLDL (postlipolysis), and (D) F. Dys. IDL (d 1.006–1.020 g/ml). Gel band: 1, apoB; 2, apoC-I; 3, apoE; 4, apoC-II; 5, apoC-III$_1$; 6, apoC-III$_2$. Right, SDS polyacrylamide gel electrophoretic patterns of (A) control F. Dys. VLDL (prelipolysis), (B) Lp lipase-resistant F. Dys. VLDL, and (C) HDL. Gel band: 1, apoB; 2, apoE; 3, apoA-I; 4, apoC and/or reduced apoA-II.

Competition studies

In order to evaluate further whether normolipidemic VLDL is a more effective substrate for LP lipase than F. Dys. VLDL, competition studies comparing ^{125}I-labeled normolipidemic VLDL with unlabeled F. Dys. VLDL and ^{125}I-labeled F. Dys. VLDL with unlabeled normolipidemic VLDL were performed. In order to maximize VLDL lipolysis, the level of triglyceride in the VLDL fractions was lowered to 100 mg/dl. Following lipolysis of F. Dys. VLDL containing trace amounts of

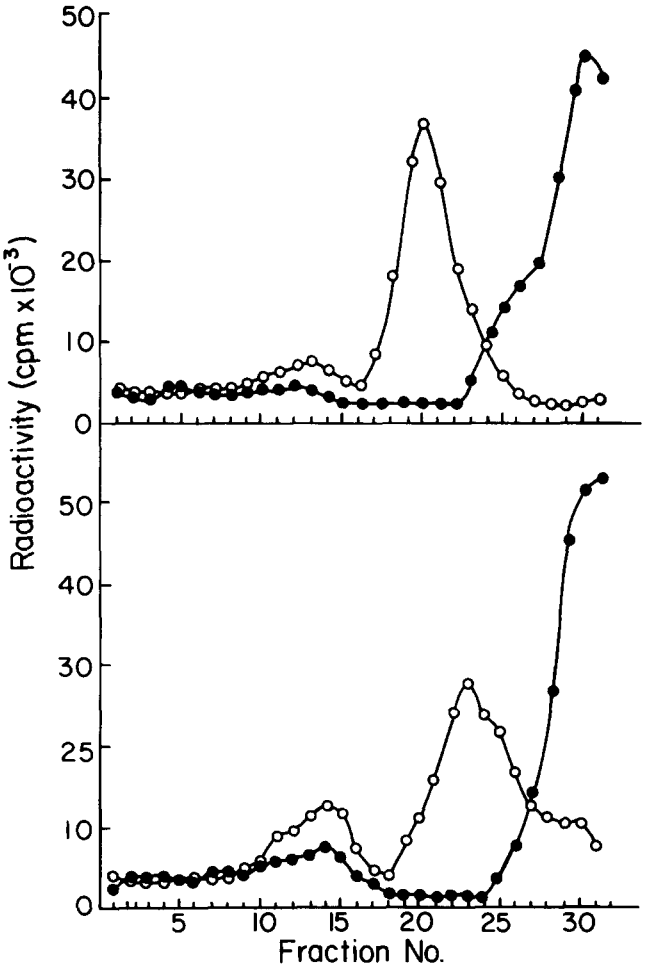

Fig. 7. Distribution of radioactivity of ^{125}I-labeled VLDL in the single vertical spin density gradient fractions of reconstituted plasma containing F. Dys. d > 1.02 g/ml plasma fractions (IDL infranate fraction) and normolipidemic VLDL (upper) or F. Dys. VLDL (lower). (● —— ●), Prelipolysis; (○ —— ○), postlipolysis. Reconstituted plasma (1.5 ml) (150 mg/100 ml VLDL triglyceride) containing trace amounts of autologous ^{125}I-labeled VLDL was incubated with 0.05 ml of Lp lipase at 37°C for 30 min.

^{125}I-labeled normolipidemic VLDL, VLDL cholesterol was partially removed while the ^{125}I-radioactivity of VLDL was almost completely removed (**Fig. 8**-left). When ^{125}I-labeled F. Dys. VLDL was added in this mixture, the ^{125}I-label was only partially removed (Fig. 8-left). Following lipolysis of normolipidemic VLDL containing trace amounts of ^{125}I-labeled normolipidemic or F. Dys. VLDL, VLDL cholesterol was almost completely removed, as was the ^{125}I-label from labeled normolipidemic VLDL; however, there was only partial removal of ^{125}I-label from labeled F. Dys. VLDL (Fig. 8-right). These results indicate that within 30 min there is little, if any, exchange of the factors responsible for the Lp lipase resistance of F. Dys. VLDL.

Competition curves comparing normolipidemic and F. Dys. VLDL with an artificial substrate are shown in **Fig. 9.** When up to 200 μg of triglyceride of either normolipidemic or F. Dys. VLDL was added to 1.2 μmol of triolein, triolein hydrolysis was, if anything, slightly stimulated (Fig. 9). When the levels of normolipidemic VLDL-TG were increased to 400 μg, triolein hydrolysis was decreased, but F. Dys. VLDL at this triglyceride concentration had essentially no effect (Fig. 9), supporting the conclusions made above that lipolysis of triglyceride in the Lp lipase-resistant VLDL from F. Dys. is at least partially inhibited compared to normolipidemic VLDL.

DISCUSSION

Familial dysbetalipoproteinemia is a lipid transport disorder in which the metabolic conversion of VLDL to IDL and IDL to LDL is impaired (6). Because the metabolic conversion of VLDL to LDL involves an extrahepatic Lp lipase reaction (20), and because previous studies have shown that Lp lipase and hepatic lipase are normal in F. Dys. (36), the present study was initiated to find out whether the VLDL from F. Dys subjects shows any abnormalities in its interaction with Lp lipase. The results of the present study indicate that a subspecies, carrying approximately 50% of the VLDL cholesterol from the plasma of a well-documented subject with F. Dys., failed to be converted normally to LDL-like particles following in vitro lipolysis with Lp lipase. The resistance of this F. Dys. subspecies of VLDL to conversion to a higher density lipoprotein by Lp lipase action can be partially accounted for by inhibition of triglyceride lipolysis (80–85% lipolysis in the resistant VLDL subspecies vs. 92–96% lipolysis in normolipidemic VLDL). The increased levels of cholesteryl ester in the resistant VLDL subspecies also seem likely to play a role in this subspecies' failure to convert to an LDL density particle. Since only one F. Dys. subject was available for inclusion in this study and the precise amino acid sequence of the E-2/E-2 phenotype for this subject is not known (14), conclusions must remain somewhat tentative. Nevertheless, the defective lipolytic conversion of VLDL in this subject was consistently observed over a 4-month period, whereas VLDL from normolipidemic subjects (n = 10) was all converted to LDL like-particles with only narrow individual variations.

We recently reported lipolytic defects of VLDL from type IV patients with severe hypertriglyceridemia (22). The lipolytic kinetics of the VLDL from an individual with F. Dys. were different from type IV plasma in the following respects. *1*) The lipolytic defect of type IV VLDL, as yet not shown definitely to be due to triglyceride resistance, can be partially or completely over-

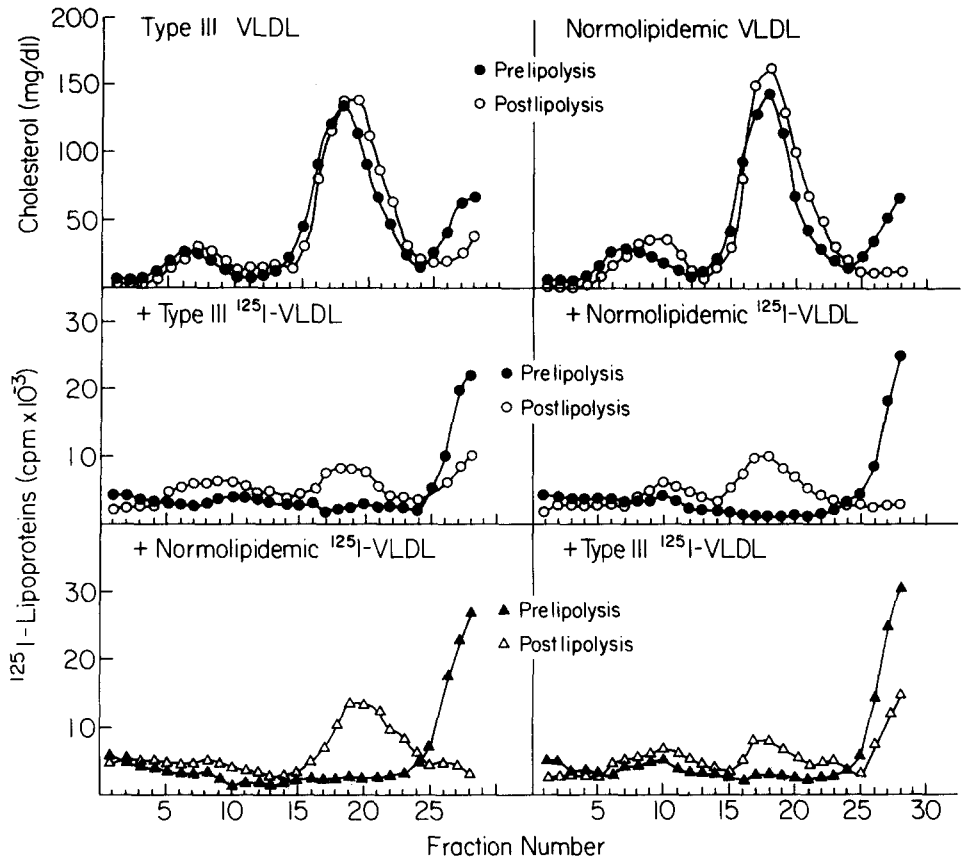

Fig. 8. Studies of the competition between normolipidemic VLDL and F. Dys. VLDL for Lp lipase-mediated lipolysis. Reconstituted plasma (1.5 ml) consisting of normolipidemic VLDL infranate (d > 1.006 g/ml) and F. Dys. VLDL (left) or normolipidemic VLDL (right), containing trace amounts of either ^{125}I-labeled F. Dys. or normolipidemic VLDL, was incubated with 0.05 ml of Lp lipase at 37° for 30 min. Reconstituted plasma gave 100 mg/100 ml VLDL triglyceride. Since the cholesterol changes were identical whether trace ^{125}I-labeled F. Dys. or normolipidemic VLDL was used, only one example is shown in the upper figures.

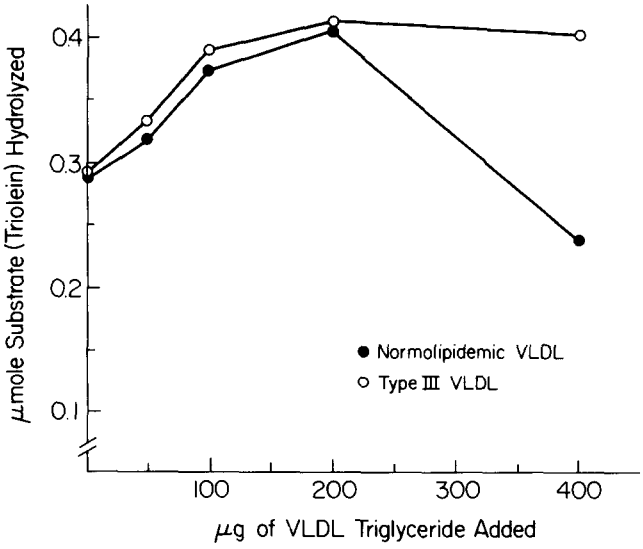

Fig. 9. Studies of the competition between normolipidemic and F. Dys. VLDL for Lp lipase-mediated lipolysis determined by ability to inhibit lipolysis of the artificial [^{3}H]triolein substrate.

come by some, as yet undetermined, exchangeable components present in normolipidemic VLDL,[2] while the substrate defect associated with VLDL in F. Dys. was not overcome by adding excess normolipidemic VLDL. *2)* The IDL infranate plasma fraction from the F. Dys. subject fully supported the lipolysis of normolipidemic VLDL, in contrast to the inhibitory effect of the VLDL infranate fraction from type IV VLDL.

A number of studies have suggested that there are two VLDL populations in familial dysbetalipoproteinemia. Using starch block electrophoresis, Quarfordt, Levy, and Fredrickson (37) isolated two VLDL subspecies from patients with F. Dys. One subspecies was similar to VLDL from normal subjects in its physical and chemical characteristics. The other subspecies had a β-VLDL electrophoretic mobility, was not present in measurable quantities in normal subjects, and was

[2] Chung, B. H., and J. P. Segrest. Unpublished data.

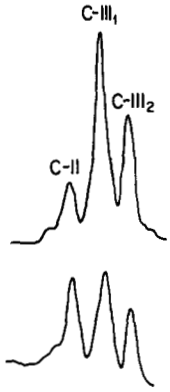

Fig. 10. Gel scans of urea gel of normolipidemic VLDL (upper) and F. Dys. VLDL (lower).

shown to be enriched in cholesteryl ester and apolipoprotein B. Pagman et al. (8) also separated VLDL from F. Dys. into subspecies with β and pre-β mobilities and suggested that β-VLDL, which contains increased amounts of cholesterol and apolipoproteins B and E, might represent remnant VLDL.

Our studies reported here are compatible with both the studies of Quarfordt et al. (37) and Pagman et al. (8), although we, as yet, do not have direct evidence that Lp lipase-resistant VLDL from the F. Dys. subject we studied is identical to β-VLDL, although this seems probable. Our data do not support the concept that β-VLDL is a simple remnant form of VLDL but rather may suggest that the VLDL lipolyzed normally (n-VLDL) and the VLDL (or VLDL-like particles) resistant to LpL (r-VLDL) represent entirely separate and essentially noninterchangeable populations. The inability of n-VLDL in large amounts to alter the Lp lipase resistance of r-VLDL raises the possibility that these two subspecies may arise by distinctly different biosynthetic pathways.

Berman et al. (38), on the basis of in vivo turnover studies, suggested that there are two metabolically distinct subspecies of VLDL in F. Dys. subjects. One of these, called α-VLDL, followed a precursor-product relationship from VLDL to IDL to LDL and the other, called β-VLDL, was present in measurable levels only in F. Dys. subjects and was cleared from circulation without being converted to either IDL or LDL. Our in vitro studies reported here are entirely compatible with this interpretation of the in vivo studies of Berman et al. (38).

It has been shown that *a*) removal of apolipoprotein C proteins from VLDL particles is proportional to the extent of triglyceride hydrolysis (39, 40) and, *b*) apolipoprotein E removal closely follows apolipoprotein C removal (41). In our studies, the selective retention of apolipoprotein E in the VLDL density region following lipolysis suggests that the r-VLDL is an apolipoprotein E-rich VLDL or VLDL-like subspecies.

The roles of apolipoproteins C-II and H as activators of Lp lipase (42, 43), and apolipoproteins C-I, C-III, and E as inhibitors of Lp lipase are well documented (21, 44). However, the possible role of variations in the ratio of activator to inhibitor apolipoproteins on the VLDL surface in the regulation of VLDL lipolysis of Lp lipase has not been determined. Based on gel scans, the F. Dys. VLDL used in our studies had a higher apolipoprotein C-II/C-III ratio than normolipidemic VLDL which exhibited full lipolytic conversion to LDL density (**Fig. 10**); while the r-VLDL remaining in the VLDL density region following lipolysis contained little or no apolipoprotein C-II, but was enriched in apolipoprotein E. The failure of r-VLDL to be lipolyzed completely could be the result of a deficiency of the Lp lipase activator apolipoprotein C-II and an abundance of an Lp lipase inhibitor, apolipoprotein E, on the VLDL surface.

A kinetic model to explain the F. Dys. genotype can be developed as follows. In genotypes homozygous or heterozygous for E-3 and for E-4, the rate of synthesis of r-VLDL (and/or chylomicrons) (K_s) is much less than its rate of clearance (K_c) in the liver. In the homozygous E-2/E-2 genotype, there is a decrease in K_c but K_s is still $\leq K_c$. However, if the E-2/E-2 genotype is also associated with some condition resulting in an increase in synthesis of r-VLDL, (and/or chylomicrons due, for example, to differences in fat absorption) then K_s becomes $>K_c$, resulting in an increase in r-VLDL to a new steady state that leads to the F. Dys. phenotype. This kinetic model can explain the well-known observation that F. Dys. patients respond particularly well to dietary intervention.

While the hypothesis of a receptor-mediated defect in removal of r-VLDL in F. Dys. (15, 17) is an attractive one, several findings seem to go against this being the sole basis for the etiology of F. Dys. *1*) The finding by Schneider et al. (18), that E-2/E-2 VLDL from several hypercholesterolemic F. Dys. patients is recognized normally by the putative liver apoE receptor, seems to require the contribution of additional defects in r-VLDL clearance. *2*) The fractional catabolic rates of VLDL from F. Dys. and type IV hyperlipoprotein subjects are reported to be the same (45). *3*) The role of apoE-mediated hepatic clearance of VLDL in humans is far from clear, since most, if not all, of normal human VLDL is converted to LDL and thus is not cleared by the putative apoE receptor. *4*) Infusion of normal plasma containing normal levels of various apoE isoforms into subjects with F. Dys. failed to correct the lipid abnormalities (46). Further, the resistance of the r-VLDL particle to Lp lipase must be one of these defects; otherwise the r-

VLDL in F. Dys. would be cleared by conversion to LDL via Lp lipase lipolysis. Whether this Lp lipase resistance is a primary or secondary defect is still to be established.

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REFERENCES

1. Morganroth, J., R. I. Levy, and D. S. Fredrickson. 1975. The biochemical, clinical and genetic features of type III hyperlipoproteinemia. *Ann. Intern. Med.* **82:** 158–174.
2. Fredrickson, D. S., J. Morganroth, and R. I. Levy. 1975. Type III hyperlipoproteinemia: an analysis of two contemporary definitions. *Ann. Intern. Med.* **81:** 150–157.
3. Patsch, J. R., S. Sailer, and H. Braunsteiner. 1975. Lipoprotein of the density 1.006–1.020 in the plasma of patients with type III hyperlipoproteinemia in the postabsorptive state. *Eur. J. Clin. Invest.* **5:** 45–55.
4. Hazzard, W. R., D. Porte, Jr., and E. L. Bierman. 1972. Abnormal lipid composition of very low density lipoproteins in diagnosis of broad-β disease (type III hyperlipoproteinemia). *Metabolism.* **21:** 1009–1019.
5. Kushwaha, R. S., W. R. Hazzard, P. W. Wahl, and J. J. Hoover. 1977. Type III hyperlipoproteinemia: diagnosis in whole plasma by immunoassay of apolipoprotein E. *Ann. Intern. Med.* **87:** 509–516.
6. Fredrickson, D. S., R. I. Levy, and R. I. Lees. 1967. Fat transport in lipoproteins: an integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276:** 32–44.
7. Havel, R. J., and J. P. Kane. 1973. Primary dysbetalipoproteinemia: predominance of a specific apoprotein species in triglyceride-rich lipoproteins. *Proc. Natl. Acad. Sci. USA.* **70:** 2015–2019.
8. Pagnan, A., R. J. Havel, J. P. Kane, and L. Kotite. 1977. Characterization of human very low density lipoproteins containing two electrophoretic populations: double prebeta lipoproteinemia and primary dysbetalipoproteinemia. *J. Lipid Res.* **18:** 613–622.
9. Faergeman, O., T. Sata, J. P. Kane, and R. J. Havel. 1975. Metabolism of apoprotein B of plasma very low density lipoproteins in the rat. *J. Clin. Invest.* **56:** 1396–1403.
10. Bergman, E. N., R. J. Havel, B. M. Wolfe, and T. Bohmer. 1971. Quantitative studies of the metabolism of chylomicron triglycerides and cholesterol by liver and extrahepatic tissues of sheep and dogs. *J. Clin. Invest.* **50:** 1831–1839.
11. Windler, E., Y. S. Chao, and R. J. Havel. 1980. Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. *J. Biol. Chem.* **255:** 5475–5480.
12. Utermann, G., M. Jaeschke, and J. Menzel. 1975. Familial hyperlipoproteinemia type III: deficiency of a specific apolipoprotein (apoE-III) in the very low density lipoproteins. *FEBS Lett.* **56:** 352–355.
13. Zannis, V., and J. L. Breslow. 1981. Human very low density lipoprotein apolipoprotein E isoprotein polymorphism is explained by genetic variation and post-translation modification. *Biochemistry.* **20:** 1033–1041.
14. Weisgraber, K. H., S. C. Rall, and R. W. Mahley. 1981. Human E apoprotein heterogeneity: cysteine-arginine interchanges in the amino acid sequence of the apoE isoforms. *J. Biol. Chem.* **256:** 9077–9083.
15. Havel, R. J., Y. W. Chao, E. E. Windler, L. Kotite, and L. S. S. Guo. 1980. Isoprotein specificity in the hepatic uptake of apolipoprotein E and the pathogenesis of familial dysbetalipoproteinemia. *Proc. Natl. Acad. Sci. USA.* **77:** 4349–4353.
16. Utermann, G., K. H. Vogelberg, M. Hees, and H. Canzler. 1979. Polymorphism of apolipoprotein E-II. Genetics of hyperlipoproteinemia type III. *Clin. Genet.* **15:** 37–62.
17. Weisgraber, K. H., T. L. Innerarity, and R. W. Mahley. 1982. Abnormal lipoprotein receptor binding activity of the human E apoprotein due to cysteine-arginine interchange at a single site. *J. Biol. Chem.* **257:** 2518–2521.
18. Schneider, W. J., P. T. Kovanen, M. S. Brown, J. L. Goldstein, G. Utermann, W. Weber, R. J. Havel, L. Kotite, J. P. Kane, T. L. Innerarity, and R. W. Mahley. 1981. Familial dysbetalipoproteinemia: abnormal binding of mutant apoprotein E to low density lipoprotein receptors of human fibroblasts and membranes from liver and adrenals of rats, rabbits and cows. *J. Clin. Invest.* **68:** 1075–1085.
19. Gregg, R. E., L. A. Zech, E. J. Schaefer, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia: defective metabolism of an abnormal apolipoprotein E. *Science.* **211:** 584–586.
20. Eisenberg, S. 1979. Very low density lipoprotein metabolism. *Prog. Biochem. Pharmacol.* **15:** 139–165.
21. Ganesan, D., H. B. Bass, W. J. McConathy, and P. Alaupovic. 1976. Is decreased activity of C-II activated lipoprotein lipase in type III hyperlipoproteinemia (broad-β-disease) a cause or an effect of increased apolipoprotein E levels? *Metabolism.* **25:** 1189–1195.
22. Chung, B. H., J. T. Cone, and J. P. Segrest. 1982. Defective in vitro lipolysis of type IV hyperlipidemic human plasma by purified milk lipoprotein lipase. *J. Biol. Chem.* **257:** 7472–7480.
23. Chung, B. H., J. P. Segrest, J. T. Cone, J. Pfau, J. C. Geer, and L. A. Duncan. 1981. High resolution plasma lipoprotein cholesterol profiles by a rapid, high volume semiautomated method. *J. Lipid Res.* **22:** 1003–1014.
24. Papadopoulos, N. M. 1978. Hyperlipoproteinemia phenotype determination by agarose gel electrophoresis updated. *Clin. Chem.* **24:** 227–229.
25. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoprotein in human serum. *J. Clin. Invest.* **34:** 1345–1353.
26. Chung, B. H., T. Wilkinson, J. C. Geer, and J. P. Segrest. 1980. Preparative and quantitative isolation of plasma lipoproteins: rapid, single discontinuous density gradient ultracentrifugation in a vertical rotor. *J. Lipid Res.* **21:** 284–291.
27. Iverius, P. H., and A. M. Ostlund-Lindqvist. 1976. Lipoprotein lipase from bovine milk: isolation procedure,

chemical characterization, and molecular weight analysis. *J. Biol. Chem.* **251:** 7791–7795.
28. Nilsson-Ehle, P., and M. C. Schotz. 1976. A stable, radioactive substrate emulsion for assay of lipoprotein lipase. *J. Lipid Res.* **17:** 536–541.
29. Thomas, M. S., and L. L. Rudel. 1982. Rapid incorporation of radiolabeled cholesterol esters into whole plasma lipoproteins. *Federation Proc.* **41:** 1216 (Abstract).
30. Cone, J. T., J. P. Segrest, B. H. Chung, J. B. Ragland, S. M. Sabesin, and A. Glasscock. 1982. Computerized rapid high resolution quantitative analysis of plasma lipoproteins based upon single vertical spin centrifugation. *J. Lipid Res.* **23:** 923–935.
31. McFarlane, A. S. 1975. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53.
32. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
33. Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli. 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. Biol. Chem.* **252:** 1102–1106.
34. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
35. Stewart, J. C. M. 1980. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Anal. Biochem.* **104:** 10–14.
36. Krauss, R. D., R. I. Levy, and D. S. Fredrickson. 1974. Selective measurement of two lipase activities in postheparin plasma from normal subjects and patients with hyperlipoproteinemia. *J. Clin. Invest.* **54:** 1107–1124.
37. Quarfordt, S., R. I. Levy, and D. S. Fredrickson. 1971. On the lipoprotein abnormality in type III hyperlipoproteinemia. *J. Clin. Invest.* **50:** 754–761.
38. Berman, M., M. Hall III, R. I. Levy, S. Eisenberg, D. W. Bilheimer, R. D. Phair, and R. H. Goebel. 1978. Metabolism of apoB and apoC lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **19:** 38–56.
39. Eisenberg, S., J. R. Patsch, J. T. Sparrow, A. M. Gotto, and T. Olivecrona. 1979. Very low density lipoprotein: removal of apolipoproteins C-II and C-III during lipolysis in vitro. *J. Biol. Chem.* **254:** 12603–12608.
40. Catapano, A. L., A. Capurso, and P. K. J. Kinnunen. 1981. In vitro catabolism of human very low density lipoproteins: fate of apolipoproteins B and C. *Artery.* **9:** 87–95.
41. Tam, S. P., L. Dory, and D. Rubinstein. 1981. Fate of apolipoproteins C-II, C-III, and E during lipolysis of human very low density lipoproteins in vitro. *J. Lipid Res.* **22:** 641–651.
42. LaRosa, J. C., R. I. Levy, P. Herbert, S. E. Lux, and D. S. Fredrickson. 1970. A specific apoprotein activator for lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **41:** 57–62.
43. Nakaya, Y., E. J. Schaefer, and H. B. Brewer, Jr. 1980. Activation of human post heparin lipoprotein lipase by apolipoprotein H (β_2-glycoprotein I). *Biochem. Biophys. Res. Commun.* **95:** 1168–1172.
44. Schrecker, O., and H. Greten. 1979. Activation and inhibition of lipoprotein lipase. Studies with artificial lipoproteins. *Biochim. Biophys. Acta.* **572:** 244–256.
45. Kushwaha, R. S., A. Chait, and W. R. Hazzard. 1982. Catabolism of very low density lipoproteins in subjects with broad β disease: comparison with endogenous hypertriglyceridemia. *J. Lab. Clin. Med.* **99:** 15–24.
46. Stigt, P. M. J., P. N. M. Demacker, and A. J. Laar. 1981. Effect of infusion of normal plasma on lipoproteins in type III hyperlipoproteinemia. *Horm. Metab. Res.* **13:** 715–716.