The β Very Low Density Lipoprotein Present in Hepatic Lipase Deficiency Competitively Inhibits Low Density Lipoprotein Binding to Fibroblasts and Stimulates Fibroblast Acyl-CoA:Cholesterol Acyltransferase*

Philip W. Connelly‡‡, Subramanian Ranganathan‡, Graham F. Maguire‡, Maureen Lee‡, John J. Myher§§, Bruce A. Kottke¶, Arnis Kuksis‡‡†, and J. Alick Little‡

From the ‡Department of Medicine, University of Toronto, Toronto, Canada and St. Michael's Hospital, 30 Bond Street, Toronto, Canada M5B 1W8, the §Department of Biochemistry, University of Toronto, Toronto, Canada, ¶Atherosclerosis Research Unit, Mayo Clinic and Foundation, Rochester, Minnesota 55905, and the ††Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L8

β very low density lipoprotein (VLDL) was isolated from a patient with hepatic lipase deficiency. The particles were found to contain apolipoprotein B-100 (apoB) and apolipoprotein E (apoE) and were rich in cholesterol and cholesteryl ester relative to VLDL with preβ electrophoretic mobility. These particles were active in displacing human low density lipoprotein (LDL) from the fibroblast apoB,E receptor and produced a marked stimulation of acyl-CoA:cholesterol acyltransferase. Treatment of intact β-VLDL with trypsin abolished its ability to displace LDL from fibroblasts. Incubation of trypsin treated β-VLDL with fibroblasts resulted in a significant stimulation of acyl-CoA:cholesterol acyltransferase activity.

β-VLDL isolated from a patient with Type III hyperlipoproteinemia and an apoE2/E2 phenotype had a higher cholesteryl ester/triglyceride ratio than the β-VLDL of hepatic lipase deficiency and contained apoB-48. It displaced LDL from fibroblasts to a small but significant extent. The Type III β-VLDL stimulated acyl-CoA:cholesterol acyltransferase to a level similar to that of trypsin-treated β-VLDL isolated from the hepatic lipase-deficient patient.

These results demonstrate that the cholesterol-rich β-VLDL particles present in patients with hepatic lipase deficiency are capable of interacting with fibroblasts via the apoB,E receptor and that this interaction is completely due to trypsin-sensitive components of the β-VLDL. These particles were very effective in stimulating fibroblast acyl-CoA:cholesterol acyltransferase. This stimulation was due to both trypsin-sensitive and trypsin-insensitive components.

Hepatic lipase is a phospholipase and triglyceride lipase located at the luminal surface of hepatic nonparenchymal cells that can be released into plasma by the intravenous administration of heparin (1). Familial hepatic lipase deficiency (2-4) is characterized by multiple lipoprotein abnormalities, including triglyceride-rich and phospholipid-rich low density lipoproteins (LDL)3 and high density lipoproteins. The very low density lipoproteins (VLDL) consist of a mixture of particles with preβ electrophoretic mobility and with β electrophoretic mobility (β-VLDL). The unusual lipid composition of the LDL and high density lipoproteins is consistent with the known lipid and lipoprotein substrate specificity of hepatic lipase (1, 5, 6).

The VLDL of hepatic lipase-deficient patients has a ratio of VLDL total cholesterol to VLDL triglyceride of 0.34, consistent with the presence of cholesterol-rich β-VLDL (1, 7). The lipolysis of VLDL triglyceride by lipoprotein lipase is known to produce a cholesterol-rich LDL remnant lipoprotein (8). It has been postulated that a physiological role of hepatic lipase is lipolysis of the triglyceride and phospholipid of the VLDL remnants, completing their conversion into LDL. It has been shown that lipoprotein lipase activity is significantly correlated with the rate of conversion of VLDL to remnant particles; however, hepatic lipase activity was not correlated with the rate of clearance of VLDL remnant particles (9). In a study including two of the hepatic lipase-deficient patients from the family described by this laboratory, it was concluded that the modification of VLDL remnant lipoproteins by hepatic lipase is important in the transfer of apolipoprotein E (apoE) from LDL to high density lipoproteins (10). ApoE occurs in the general population in three forms with different primary structure. ApoE4 and apoE3 function as ligands for the apoB,E receptor, while apoE2 binds to the apoB,E receptor with 1% of the affinity of apoE3 (11).

The presence of β-VLDL and homozygosity for apoE2 are characteristic features of Type III hyperlipoproteinemia (7, 11). This results in the accumulation of intestinal remnant lipoproteins containing apoB-48 and hepatic remnant lipoproteins containing apoB-100. ApoB-100 contains an apoB,E receptor-binding domain (12-14) which is apparently not expressed by the apoB-100 of β-VLDL. ApoB-48 does not contain the apoB,E receptor-binding domain (12-14).

One laboratory has estimated that 91% of the probands that they have studied with hyperlipidemia and β-VLDL are homozygous for apoE2 (11). Unusual cases of hyperlipidemia with β-VLDL have been reported in which the patients were either apparently homozygous for apoE3 (15) or heterozygous

* The abbreviations used are LDL, low density lipoprotein; apo, apolipoprotein; VLDL, very low density lipoprotein; I-VLDL, intermediately dense lipoprotein; SLDL, sodium deoxycholate HDL; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; apo, apolipoprotein.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom reprint request should be addressed: Core Laboratory, Lipid Research Clinic, Room 230, 1 Spadina Crescent, Toronto, Canada M5S 2J5.
β Very Low Density Lipoprotein of Hepatic Lipase Deficiency

for apoE3 and a nonfunctional mutant of apoE3 (16). Hyperlipidemia in combination with β-VLDL is of significance because β-VLDL has been shown to promote accumulation of cholesterol by macrophages in culture, and it has been postulated that this process is important in the development of foam cells in the atherosclerotic lesions (17). Studies of this process in culture have included β-VLDL induced by cholesterol feeding in the dog (18) and rat (19). The accumulation of cholesterol by macrophages has been thought to be due to the existence of a β-VLDL receptor genetically distinct from the apoB,E receptor (17). Recent investigations using murine cholesterol feeding in the dog have suggested that β-VLDL internalization is due to the apoB,E receptor expressed by these cells in culture (20, 21).

VLDL subfractions from hypertriglyceridemic patients have been reported to bind to fibroblast apoB,E receptors due to the expression of both apoE- and apoB-binding determinants (22). The presence of β-VLDL and the apoE/E3 phenotype in our patients with hepatic lipase deficiency suggested that either the apoE- and apoB-binding determinants were not expressed or that the receptor-dependent clearance of the β-VLDL was saturated. The former possibility can be readily tested by isolation of the β-VLDL and the study of its interaction with fibroblast apoB,E receptors. We decided to determine whether this lipoprotein would compete with normal LDL for binding to the fibroblast apoB,E receptors and whether any binding was sensitive to trypsin, an attribute of apoE (22). To monitor the metabolic relevance of any binding of this β-VLDL to fibroblasts, the activity of acyl-CoA:Cholesterol acyltransferase was assayed.

EXPERIMENTAL PROCEDURES

Materials—Sodium [125I]iodide (carrier free) was obtained from Amersham Corp. [3H]Oleic acid and [3H]cholesterol oleate were obtained from Du Pont-New England Nuclear. Sodium oleate and cholesteryl oleate were purchased from Sigma. All tissue culture supplies were purchased from Grand Island Biological. Pevikon C-870 was obtained from Mercer Chemical Corp. (Amityville, NY).

Patients—The proband (B1) and his brother (B2) with hepatic lipase deficiency have been previously described (2). At the time of this study, B2 was a 54-yr-old male, 175 cm tall, and 103 kg in weight. He was taking L-thyroxine, 0.25 mg/day, diltiazem HCl, 240 mg daily in four divided doses and hydrochlorothiazide, 25 mg/day. The concentrations of plasma cholesterol and triglyceride were 6.20 mg/dl and 4.87 mg/dl, respectively. For comparison in these studies, a patient with Type III hyperlipoproteinemia was identified following the Lipid Research Clinic's protocol, standardized with the Lipid Standardization Laboratory, Centers for Disease Control, Atlanta, GA (23).

Agarose and SDS-Glycerol Electrophoresis—Agarose electrophoresis was performed as described by Maguire et al. (28). The apolipoproteins of the total VLDL and the isolated Pevikon fractions were separated using 3.5% SDS-polyacrylamide gels and a modification of the published procedure (29).

Digestion of β-VLDL with Trypsin—β-VLDL was incubated with trypsin at a protein ratio of 200:1 for 18 h at 37 °C. Control β-VLDL was incubated under identical conditions without trypsin (30, 31).

Fibroblasts—Human skin fibroblasts (GM 49) derived from normal subjects were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ. The cells were grown in monolayers and used for experiments between the 8th and 20th passages. The cultures were maintained in Dulbecco's modified Eagle's medium supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), HEPES (25 mM, pH 7.4), and fetal calf serum (10%) in 75-cm2 tissue culture flasks. Cells were incubated in a humidified incubator with 5% CO2 at 37 °C. Subcultures for the experiments were obtained by trypsin treatment (37 °C, 3–4 min) of the confluent stock monolayers using a solution of 0.05% trypsin in Dulbecco's phosphate-buffered saline containing 0.5 mM EDTA. The cells were suspended in trypsin medium, and then dispersed in plastic dishes (60 × 15 mm) at a concentration of 1.0 × 105 cells/dish in 3 ml of medium. On alternate days the medium was removed and fresh medium added.

LDL Binding to Fibroblasts and Competition with β-VLDL—The binding of LDL to fibroblasts was determined using the method described by Brown and Goldstein (32). Briefly, the fibroblasts were incubated in a medium containing lipoprotein-deficient serum for 48 h. The cells were cooled to 5 °C and incubated with 10 μg of 125I-LDL and the indicated amounts of unlabeled lipoproteins in 2 ml of medium for 3 h. The cell-associated 125I-LDL was determined after washing the cells extensively.

Acyl-CoA:Cholesterol Acyltransferase—After 48 h of incubation of the monolayers in Dulbecco's Modified Eagle's medium containing lipoprotein-deficient serum, they were incubated with 0.1 mM [14C]oleate bound to albumin for 20 h at 37 °C, and radioactivity incorporated in cholesteryl ester was determined as described by Goldstein et al. (33).

RESULTS

Characterization of VLDL and Its Subfractions—The VLDL from hepatic lipase-deficient patient B2 was fractionated by Pevikon electrophoresis into particles with β mobility, particles with preβ mobility, and LDL, particles with a mobility intermediate between these two fractions (Fig. 1). The chemical composition of the VLDL, the β-VLDL, I-VLDL, and preβ-VLDL subfractions is shown in Table I. The cholestery ester/triglyceride mole ratio was higher in the β-VLDL than in the preβ-VLDL. The phospholipid/cholesterol mole ratio

---

**Fig. 1.** Agarose electrophoresis of VLDL and Pevikon fractions of VLDL. VLDL was isolated from plasma by ultracentrifugation and separated into fractions with β mobility or preβ mobility by Pevikon electrophoresis. A, VLDL, from the hepatic lipase-deficient patient B2. B, VLDL from a patient with Type III hyperlipoproteinemia. V, total VLDL; β, β-VLDL; I, intermediate VLDL; preβ, preβ-VLDL.
was higher in the preβ-VLDL than in the β-VLDL. Thus, the β-VLDL were rich in cholesterol and cholesteryl ester relative to the preβ-VLDL.

The apolipoprotein composition was examined by SDS-glycerol gel electrophoresis (Fig. 2). Preβ-VLDL contained apoB-100, apoE, and the apoCs. The relative amount of the apoCs was decreased in the I-VLDL. The β-VLDL contained apoB-100 and apoE, while the apoCs were below the limit of detection. The apoCs were detected in the β-VLDL when 150 µg of β-VLDL protein was examined by isoelectric focusing (data not shown). There were small amounts of apoB-48 in the preβ-VLDL and I-VLDL fractions from the hepatic lipase-deficient patient. ApoB-48 was not detected in the β-VLDL of this patient.

The VLDL from a patient with Type III hyperlipoproteinemia and the apoE2/E2 phenotype was fractionated by Pevikon electrophoresis into β-VLDL, 1-VLDL, and preβ-VLDL, (Fig. 1). The chemical composition of the VLDL and the β-VLDL, 1-VLDL, and preβ-VLDL subfractions is shown in Table I. The cholesteryl ester/triglyceride mole ratio was higher in the β-VLDL than in the preβ-VLDL, while the phospholipid/cholesterol mole ratio was higher in the preβ-VLDL than in the β-VLDL, as was observed for the VLDL subfractions isolated from the hepatic lipase-deficient patient. The highest cholesterol ester/triglyceride mole ratio was observed for the β-VLDL from the Type III patient. This fraction also had the lowest phospholipid/cholesterol mole ratio.

SDS-glycerol gel electrophoresis of the apolipoproteins of the VLDL subfractions from the Type III patient is shown in Fig. 2. The preβ-VLDL consisted of apoB-100, the apoCs and apoE and small amounts of apoB-48. The amount of the apoCs was decreased in I-VLDL relative to the other apolipoproteins. The apolipoproteins of the β-VLDL consisted of apoB-100, apoB-48, and apoE. The apoCs were detectable when 150 µg of β-VLDL protein was examined by isoelectric focusing (data not shown). The differences in the lipid and apolipoprotein composition between the preβ-VLDL and β-VLDL in this patient are consistent with previous reports of the lipid and apolipoprotein composition of human β-VLDL (5, 8).

The weight percent of protein in β-VLDL from either patient was similar. However, since the β-VLDL from the Type III patient had a higher content of cholesterol compared to the β-VLDL from the hepatic lipase-deficient patient, the total cholesterol/protein ratios were different.

**Competition of β-VLDL with LDL for Binding to Fibroblasts**—The results of the competition between β-VLDL from hepatic lipase-deficient patient B2 and normal LDL for binding to the fibroblast receptor are shown in Fig. 3. The β-VLDL displaced 50% of the LDL at -1.2 µg of protein/ml. Unlabeled LDL displaced 50% of the labeled LDL at ~8 µg of protein/ml. The results of the competition of trypsin treated β-VLDL with LDL for the binding sites is also shown in Fig. 3. With up to 50 µg of protein/ml of trypsinized β-VLDL, there was no displacement of normal LDL from fibroblasts. Thus, the β-VLDL from the hepatic lipase-deficient patient was more effective than normal LDL as a competitive inhibitor of binding, and this competition was entirely due to trypsin-sensitive sites on the β-VLDL.

The results of binding studies using β-VLDL isolated from a patient with E2/E2 dysbetalipoproteinemia are also shown in Fig. 3. There was a very low but detectable level of competition between this β-VLDL and LDL within the range of 1–12 µg of β-VLDL protein/ml. Due to the low levels of competition, further studies with trypsin-treated E2/E2 β-VLDL were not carried out.

**The Effect of β-VLDL on Fibroblast Acyl-CoA:Cholesterol Acyltransferase Activity**—The activity of acyl-CoA:cholesterol acyltransferase was determined to test whether the results of the competition studies corresponded to changes in cellular cholesterol metabolism (Table II). The β-VLDL from the

---

**TABLE I**

**Chemical composition of VLDL, β-VLDL, and preβ-VLDL**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fraction</th>
<th>PROT</th>
<th>CHOL</th>
<th>PL</th>
<th>CE</th>
<th>TG</th>
<th>PL/CHOL</th>
<th>CE/TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL DEF</td>
<td>VLDL</td>
<td>11.3</td>
<td>6.0</td>
<td>16.3</td>
<td>18.3</td>
<td>48.1</td>
<td>1.33</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>12.3</td>
<td>7.7</td>
<td>23.0</td>
<td>17.7</td>
<td>39.3</td>
<td>1.47</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>9.7</td>
<td>6.3</td>
<td>20.8</td>
<td>15.9</td>
<td>47.3</td>
<td>1.67</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>preβ</td>
<td>7.7</td>
<td>5.1</td>
<td>17.9</td>
<td>9.2</td>
<td>60.0</td>
<td>1.78</td>
<td>0.21</td>
</tr>
<tr>
<td>TYPE III</td>
<td>VLDL</td>
<td>14.1</td>
<td>8.1</td>
<td>19.1</td>
<td>24.3</td>
<td>34.3</td>
<td>1.15</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>12.5</td>
<td>9.2</td>
<td>19.8</td>
<td>32.1</td>
<td>26.4</td>
<td>1.05</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>9.6</td>
<td>7.5</td>
<td>20.8</td>
<td>19.2</td>
<td>42.9</td>
<td>1.17</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>preβ</td>
<td>10.0</td>
<td>5.8</td>
<td>19.3</td>
<td>14.3</td>
<td>50.5</td>
<td>1.63</td>
<td>0.38</td>
</tr>
</tbody>
</table>

* Patient: HL DEF, hepatic lipase-deficient patient B2; TYPE III, patient with Type III hyperlipoproteinemia.

† Abbreviations: PROT, protein; CHOL, free cholesterol; PL, phospholipid; CE, cholesteryl esters; TG, triglyceride; β, β-VLDL; I, VLDL of intermediate mobility; preβ, preβ-VLDL.
The present studies compared the lipid and apolipoprotein composition of β-VLDL from an hepatic lipase-deficient patient with an apoE3/E3 phenotype with that of β-VLDL from a Type III hyperlipoproteinemic patient with an apoE2/E2 phenotype. The β-VLDL from both types of patients was relatively enriched in cholesteryl ester, cholesterol, and apoE. The distribution of apoB-48 in the VLDL fractions of the hepatic lipase-deficient patient and the Type III patient was different. The β-VLDL of the Type III patient clearly contained apoB-48. In contrast, apoB-48 was not detected in the β-VLDL of the hepatic lipase-deficient patient. This may have been due to the different level of lipemia in the patients at the time of this study or to a difference in the metabolism of intestinal remnant lipoproteins in hepatic lipase deficiency compared to Type III hyperlipoproteinemia.

The apoE-rich β-VLDL of the hepatic lipase-deficient patient was an effective competitive inhibitor of binding of normal LDL to the fibroblast apoB,E receptors. All of this activity was abolished by trypsin. Thus, the apoproteins of the β-VLDL of the hepatic lipase-deficient patient are functional as ligands for the apoB,E receptor. This implies that the presence of these β-VLDL particles is not due to an inability to bind to the apoB,E receptor. A mutation in apoE that reduces its in vitro binding to 40% of normal has been described in association with β-VLDL (16). These investigators noted that this extent of reduction in binding was not sufficient to account for the presence of β-VLDL. The hepatic lipase-deficient patients have apoE3 with a single cysteine residue as determined by chemical derivatization and isoelectric focusing (data not shown). It is possible that these patients may have a mutant apoE that was not detected by these methods; however, the competitive binding data are not consistent with the presence of a mutant nonfunctional apoE. We speculate that the presence of β-VLDL in hepatic lipase deficiency is due to abnormal function of the apoB,E receptor. The combination of hepatic lipase deficiency and β-VLDL suggests that, either hepatic lipase has a role in the receptor-dependent uptake of VLDL remnant lipoproteins by the liver, or that the primary defect that has resulted in a deficiency of hepatic lipase has also affected a mechanism for the clearance of β-VLDL particles.

It has been shown with VLDL from hypertriglyceridemic patients that trypsin inactivates apoE but not apoB-100 (22). The results of this study suggest that the major lipoprotein-binding determinant of the β-VLDL in hepatic lipase deficiency is apoE. Definitive distinction between apoE- and apoB-100-binding determinants will require the use of specific reagents, such as monoclonal antibodies (34).

The metabolic significance of the competition studies was established by the observation that β-VLDL was effective in stimulating fibroblast acyl-CoA:cholesterol acyltransferase. It was of interest that trypsin-treated β-VLDL was also effective in stimulating fibroblast acyl-CoA:cholesterol acyltransferase in spite of the absence of detectable competitive inhibition with the binding of normal LDL. This suggests that in the absence of specific binding to the apoB,E receptors these particles deliver cholesterol to fibroblasts. The cellular uptake of cholesterol, independent of the apoB,E receptor, is known to be significant in the atherogenic process of cellular accu-
mulation of cholesterol (35, 36). It is conceivable that β-VLDL could also participate in an apoB,E receptor-independent delivery of cholesterol to cells in vivo.

The metabolic properties of the β-VLDL suggest that this lipoprotein promotes the formation of cholesterol-rich plaques and the premature ischemic vascular disease that has been documented in these patients with hepatic lipase deficiency.

REFERENCES