Lipoprotein Composition as a Component in the Lipoprotein Clearance Defect in Experimental Diabetes*

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The hypertriglyceridemia associated with streptozotocin-induced diabetes in rats is largely reflected in the plasma lipoproteins of density < 1.006 g/ml. Analysis of the plasma apolipoproteins of these rats indicated marked alterations in both the total levels and in the lipoprotein distribution of the major apolipoproteins. In whole plasma, diabetes was associated with significant increases in apolipoprotein (apo)-AIV, apo-AI, and apo-B (mainly in the intestinally derived apo-B<sub>240</sub>) and a marked decrease in apo-E. In the d < 1.006 g/ml lipoprotein fraction (very-low-density lipoproteins (VLDL)), there were significant increases in apo-B<sub>240</sub>, apo-AI, and apo-AIV and decreased levels of apo-E and the C apolipoproteins. The decrease in apo-C was primarily due to lower levels of apo-CII, and the ratio of the lipoprotein lipase inhibitor, apo-CIII, to the lipoprotein lipase activator, apo-CII, was significantly increased over that in controls. The comparative clearance of triglycerides of VLDL particles from control and diabetic rat plasma was tested in recirculating heart perfusion in vitro. During 45-min perfusions of hearts from control donor rats, lipolysis of triglycerides of VLDL from diabetic rats was only 53% of that obtained when both the lipoprotein and the organ were from control rats. The data suggest that in addition to depressed lipoprotein lipase activity in the tissue from diabetic rats, there are also major compositional changes in circulating lipoproteins which may contribute to defective triglyceride clearance from the circulation.

In an earlier study from this laboratory (1), the intact rat heart in recirculating perfusion in vitro was employed to demonstrate the defective clearance of VLDL<sup>1</sup> triglycerides during streptozotocin-induced diabetes and to correlate this clearance defect with diminished levels of functional lipoprotein lipase in the heart. It was also reported that myocardial lipoprotein lipase activity was increased either by treatment of diabetic animals with insulin in vivo or by direct perfusion of intact heart tissue with insulin in vitro, and that this was associated with improved clearance of lipoprotein lipids (1). In these studies, VLDL was obtained from lymph or plasma of control donor rats, and contained the typical component of apolipoproteins, including apo-B, apo-AI, apo-E, and the C apolipoproteins (2–5).

The roles of apo-CII as an activator for lipoprotein lipase (6–8) and of apo-B and apo-E for hepatic recognition of peripherally metabolized lipoprotein remnant particles (9–12) have been extensively documented. Furthermore, major changes in the apolipoprotein pattern of circulating lipoproteins during experimental diabetes have been reported (13–17) and have been suggested as a possible factor in the lipoprotein clearance defect associated with experimental diabetes (16).

In the present study, the circulating lipoproteins from normal and streptozotocin-induced diabetic rats have been isolated and characterized for lipid and apolipoprotein composition. Cross-over perfusion studies using d < 1.006 g/ml VLDL and hearts from either control or diabetic rats suggest that, in addition to insulin-dependent changes in tissue lipoprotein lipase (1), a second lipoprotein clearance defect may be associated with the composition of the circulating lipoprotein particle per se. Preliminary reports of these findings have been presented earlier (18).

EXPERIMENTAL PROCEDURES<sup>2</sup>

RESULTS

Plasma Glucose and Lipids—By 48 h after streptozotocin administration, plasma glucose was elevated by 3.6-fold and plasma triglycerides by 3.0-fold over control levels, while plasma cholesterol was unchanged (Table I, Miniprint). The increase in plasma triglycerides in diabetic rats was distributed among the plasma lipoproteins in the same proportions as in control rats. However, a greater percentage of the unchanged plasma cholesterol levels in diabetes was associated with the d < 1.006 g/ml lipoproteins.

Apolipoprotein Levels and Distributions—Total plasma apo-

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<sup>1</sup>The abbreviations used are: VLDL, very-low-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; apo, apolipoproteins; SDS, sodium dodecyl sulfate; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid.

<sup>2</sup>Portions of this paper (including "Experimental Procedures," Figs. 1 and 3, and Tables 1, II, and IV) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-1656, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Lipoprotein Composition in Experimental Diabetes

Plasma VLDL protein levels in diabetics were twice those of controls, while LDL protein was unaltered and HDL protein was significantly lower than in controls.

The individual apolipoprotein levels of plasma (Table III) indicated that in diabetic rats, there were significant increases in total apo-B (134% of control), apo-AI (165% of control), and apo-AIV (221% of control). Apo-E levels were less than one-half of the levels in controls while total apo-C (CII, CIIIa, and CIIIb) was not significantly altered.

The distributions of the apolipoproteins among the major plasma lipoproteins of control and diabetic rats are summarized in Fig. 1 (Miniprint) and Fig. 2. The increase in plasma apo-B in diabetic rats was almost entirely associated with the <d> <1.006 g/ml lipoproteins</d> (Fig. 2). Furthermore, whereas the apo-B of plasma VLDL consisted of both isoforms (apo-B240 and apo-B235) in approximately equal amounts in controls (Fig. 3, Miniprint), the increase in VLDL apo-B associated with diabetes was entirely due to the apo-B240 or the intestinally derived isoform (45).

Plasma apo-AI and apo-AIV in control and diabetic rats were largely associated with the HDL fraction (93 ± 6% and 80 ± 24%, respectively) (Fig. 1). In diabetic rats, a greater percentage of the increased levels of plasma apo-AIV was recovered with the VLDL fraction (33 ± 3% compared to 9 ± 1% in controls).

Over one-half of plasma apo-E was recovered in the HDL fraction in control rats, with the remainder being equally distributed between VLDL and LDL (Fig. 1). In diabetics, the marked reduction in plasma apo-E was due largely to decreased apo-E levels of HDL and LDL (Fig. 2). Thus, over 60% of the remaining plasma apo-E in diabetics was associated with the VLDL fraction compared to 23% in controls.

Although total plasma apo-C levels were not altered in diabetics, the percentage of total apo-C associated with VLDL fraction was significantly decreased (Fig. 2). Furthermore, as shown in Table IV (Miniprint), this decrease was also associated with an altered proportion of apo-CII relative to the apo-CIII isoforms. Thus, the apo-CIII/apo-CII ratio of VLDL was increased from 4.0 ± 0.1 in controls to 6.7 ± 0.3 in diabetics. In contrast, there was a relative increase in the proportion of apo-CII associated with plasma HDL (d = 1.080–1.21 g/ml) in diabetics (Table IV). Subfractionation of plasma HDL indicated that in the d = 1.080–1.125 g/ml fraction, the ratio of apo-CII/apo-CII was significantly decreased from 2.0 ± 0.2 in controls to 1.6 ± 0.01 in diabetics. In the d = 1.125–1.21 fraction, this ratio was also decreased from 1.8 ± 0.05 in controls to 1.0 ± 0.03 in diabetics.

**VLDL Triglyceride Clearance during Heart Perfusion—** Forty-five minute recirculating perfusion of plasma VLDL from control rats (6.4 μmol of triglyceride) through hearts from control rats resulted in the net disappearance of 4.7 ± 0.1 μmol or 71.9 ± 2.0% of the initial triglyceride level (Table V). In contrast, perfusion of the plasma VLDL fraction of diabetic rats (6.4 μmol of triglyceride) through hearts from control donors resulted in the removal of 3.0 ± 0.5 μmol or 46.8 ± 7.8% of the original triglyceride level. As reported earlier (1), perfusion of VLDL from control rats through hearts from diabetic donors resulted in clearance of only 2.5 ± 0.1 μmol of triglyceride (compared to 4.7 ± 0.1 μmol when hearts were from control donors). When studies were conducted with both plasma VLDL and hearts from diabetic rats, triglyceride clearance was 1.1 ± 0.2 μmol or only 17.2 ± 3.1% of the available VLDL triglyceride.

Since the major expression of a defective clearance of VLDL triglyceride particles from diabetic rats was observed during perfusion of hearts from control rats, a study was conducted to determine whether this effect was dependent on VLDL triglyceride concentration. Hearts from control donor rats were perfused with VLDL preparations from control and diabetic rats, and the levels of VLDL triglyceride were adjusted from 2.4 to 8.2 μmol in the 20-ml perfusate. At the lowest concentration of VLDL triglyceride, (2.4 μmol/20 ml) clearance of the two-donor particles was statistically similar (91 ± 6% of control). However, at triglyceride levels of 4.8 μmoles and above, clearance of triglyceride from plasma VLDL particles from diabetic rats was statistically less than that obtained with control VLDL (range 60 ± 24% of control to 74 ± 10% of control).

**Discussion**

Previous studies on human insulin-dependent diabetes and on streptozotocin-induced diabetes in experimental animals have suggested that the increase in circulating VLDL and...
TABLE V

<table>
<thead>
<tr>
<th>Lipoprotein Composition in Experimental Diabetes</th>
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<tbody>
<tr>
<td>Donor VLDL/</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>Control/control</td>
</tr>
<tr>
<td>Diabetic/control</td>
</tr>
<tr>
<td>Control/diabetic</td>
</tr>
<tr>
<td>Diabetic/diabetic</td>
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</table>

their associated triglycerides are largely due to defective clearance of these particles from the circulation (33–35). This defect appears to be related, at least in part, to a decreased activity of the enzyme, lipoprotein lipase (33, 36, 37), which is responsible for the peripheral degradation of triglycerides associated with VLDL (and chylomicrons) (38) prior to hepatic modification or clearance of the resulting remnant particles.

In the present studies, the hypertriglyceridemia observed in rats within 2 days after streptozocin administration was largely associated with the d < 1.006 g/ml lipoproteins. These particles are referred to as cholesterol and its esters by commercial manufacturers. There was no increase in plasma cholesterol in these animals although, as reported earlier (39), the cholesterol was redistributed toward the lower density lipoproteins.

It is also clear, however, that diabetes is also associated with marked alterations in the levels and distributions of the major apolipoproteins associated with circulating lipoproteins (14–17). The increases in plasma total apo-B, apo-AI, and apo-AIV and the decrease in plasma apo-E observed in the present study within 2 days after streptozocin administration are entirely consistent with earlier studies in rats 12 days after the drug (15), or those control or diabetic rats reported in male breeder rats (15). However, apo-C levels have been reported to be either unchanged or increased in diabetes (17, 40).

In addition to the overall changes in plasma apolipoproteins, there were major, consistent differences in the apolipoprotein compositions of the major plasma lipoproteins. The decreased level of HDL protein in diabetes was associated with a marked decrease in apo-E and a relative increase in apo-AI and apo-AIV. The unchanged LDL protein level in diabetes was a result of diminished apo-E and relative increases in apo-AI and apo-B<sub>240</sub>.

The major changes were, however, reflected in the increased protein levels of the d < 1.006 g/ml lipoproteins. Thus, the increased levels of plasma apo-B in diabetes were entirely associated with this lipoprotein fraction and this was largely due to apo-B<sub>240</sub> or the intestinally derived apo-B (41). There were also relative increases in apo-AI and apo-AIV, which are also, in part, of intestinal origin (42), and decreases in the levels of apo-E and apo-C (largely apo-CII).

REFERENCES

Lipoprotein Composition in Experimental Diabetes

Patricia D. O'Leary, David J. T. F. Irwin, Pamela A. O'tooney, David J. T. F. Irwin, and Bragdon (1983)

EXPERIMENTAL PROCEDURES

Materials - Bovine serum albumin (Fraction V; fasting albumin) and crystallized bovine serum albumin (Epitope, Leatherhead, England) were purchased from Biocytos Inc. and Horm bovine, respectively. Serum albumin was purchased from Amerham Biocytos Inc. All other chemicals and reagents were of highest purity.

Analysis - Adult male rats of the Wistar strain (Charles River, MA 250 g) were allowed standard laboratory chow and water prior to use. Diabetes was induced with a single intravenous dose of 0.5 mg/kg of streptozotocin (Sigma Chemical Co., St. Louis, MO) dissolved in 1.0% saline. Animals were maintained in a temperature-controlled environment (22°C) and provided fresh water and rat chow ad libitum to control the hypoglycemia occurring within 12 h after streptozotocin. Plasma glucose levels were determined with a Wako glucose analyzer at the time specified; levels in excess of 200 mg/dl were considered effective induction of diabetes. These animals were sacrificed at 68 h post-injection to harvest plasma lipoproteins.

Lipoprotein isolation and characterization. - Blood, obtained by cardiac puncture, was centrifuged (3000 x g for 20 min) and the supernatant was harvested at 0°C g/75°. Plasma lipoprotnes were isolated by preparative ultracentrifugation (15) as with the following modified modifications. Triglycerides were isolated by ultracentrifugation for 20 h at 30,000 rpm (Schwartzman) in a Ti 70 rotor at 1°C (16). Lipoproteins were isolated by ultracentrifugation for 20 h at 30,000 rpm (Schwartzman) in a Ti 70 rotor at 1°C (16).

For lipid analysis, all of the lipoprotein fractions were extracted in 15 volumes of 2:1 dichloromethane/methanol, and lipids were extracted from individual lipoprotein fractions by the method of Bligh and Dyer (27). Lipids were then saponified and neutralized by the Bligh and Dyer (27) method. Triacylglyceride and phospholipid were individually isolated and eluted from silica gel with methanol/acetone (80:20, v/v) as described earlier (28). The origin of the lipoprotein fractions was confirmed by ultracentrifugation and isolated lipids were quantitated by gas-liquid chromatography. The conditions for extraction, neutralization, and chromatography were the same as used for plasma lipoproteins.

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Protein was determined by the method of Lowry (29). Data are expressed as means ± SEM. Differences between means were analyzed by Student's t-test.

Table I: Lipoprotein Composition and Lipid Content

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Plasma Levels (mg/dl)</th>
<th>Lipid Content (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td>LDL</td>
<td>80 ± 4</td>
<td>37 ± 5</td>
</tr>
<tr>
<td>HDL</td>
<td>14 ± 1</td>
<td>460 ± 44</td>
</tr>
</tbody>
</table>

Distribution of lipoproteins. -

- **LDL:**
  - d < 1.006 g/ml: 85
  - 1.006 - 1.080 g/ml: 8
  - 1.080 - 1.21 g/ml: 5

- **HDL:**
  - d < 1.006 g/ml: 27
  - 1.006 - 1.080 g/ml: 25
  - 1.080 - 1.21 g/ml: 24
TABLE I
Lipoprotein Composition in Experimental Diabetes

Molten plasma samples (4 pools of 7 animals) from control and diabetic rats were subjected to ultracentrifugal separation of major plasma lipoproteins as described under "Experimental Procedures". Each fraction was desalted and total protein was determined.

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Total Lipoprotein Protein (mg/100 ml)</th>
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<tbody>
<tr>
<td>LDL</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>HDL</td>
<td>83 ± 9</td>
</tr>
</tbody>
</table>

TABLE II
Distribution of Apo-apolipoproteins and Ratio of apo AIV/Apo E in Plasma Lipoproteins from Control and Diabetic Rats

Plasma samples from control and diabetic rats were subjected to ultracentrifugal separation of major plasma lipoproteins. These were desalted and subjected to isoelectric focusing electrophoresis at pH 4.6 and the C and apo AIV lipoproteins were estimated by densitometry. Figures represent means ± SEM for 6 analyses in each group.

<table>
<thead>
<tr>
<th>Lipoprotein Type</th>
<th>Percentage Distribution</th>
<th>apo AIV/ apo E</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>C11</td>
</tr>
<tr>
<td>Control</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>Diabetic</td>
<td>13</td>
<td>44</td>
</tr>
<tr>
<td>LDL</td>
<td>100</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>163</td>
<td>42</td>
</tr>
<tr>
<td>Diabetic</td>
<td>44</td>
<td>53</td>
</tr>
</tbody>
</table>

Figure 1A: Distribution of apo-apolipoproteins among plasma lipoproteins from control rats. Data represent means ± SEM for 6 pools of 7 animals.

Figure 1B: Distribution of apo-apolipoproteins among plasma lipoproteins from diabetic rats. Data represent means ± SEM for 6 pools of 7 animals.

Figure 1C: Levels of total apolipoprotein B and AIV in the VLDL and LDL of control and diabetic rats. Lipoprotein fractionation and apolipoprotein electrophoresis techniques are described under "Experimental Procedures". Data represent means ± SEM for 6 pools of 7 rats in each group. Total apo B [ ], apo AIV [ ].