Composition of Serum Very-Low-Density and High-Density Lipoproteins in Diabetes

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We examined the cholesterol/protein ratio and the apoprotein composition of serum lipoproteins in a randomly selected population of maturity-onset diabetics and in a group of nondiabetics of similar age. We found no differences in cholesterol distribution between the groups as a whole, but diabetics with above-normal low-density lipoproteins (LDL) had decreased concentrations of high-density lipoprotein (HDL) cholesterol. In the diabetics as a whole, there was an increase in the cholesterol/protein ratio in HDL, a negative correlation between the amounts of LDL cholesterol and HDL cholesterol, an increase in the proportion of apolipoprotein C in very-low-density lipoprotein (VLDL), and a decrease in the proportion of the apolipoprotein AI component of HDL. In diabetic subjects with increased VLDL, there was an increase in the relative amount of apolipoprotein CII, and a consequent decrease in the ratio of apolipoprotein CII/apolipoprotein CIII in the VLDL. In both diabetic and control subjects, apolipoprotein E and cholesterol content of VLDL were linearly related.

Additional Keyphrases: lipoprotein cholesterol • apolipoproteins • risk of atherosclerosis • serum glucose and lipid concentrations

Several serum lipid abnormalities are common in diabetes: hypercholesterolemia, hypertriglyceridemia, and hyperlipoproteinemia of the IIA, IIB, and IV phenotypes (1). There is also an increased incidence of atherosclerosis in diabetes (2, 3). This high incidence may be partly a consequence of the alterations in lipid concentrations, but changes in the apolipoprotein composition or concentration possibly also play a role. The amounts of apoVLDL and apoLDL, and of apoA, the major apoprotein of HDL, are normal in treated nonmalignant diabetes (4, 5), but no detailed study of the apoprotein compositions has been reported. To determine if there are changes characteristic of a diabetic population, we examined the apoprotein composition of VLDL and HDL by quantitative gel electrophoresis in a group of patients attending an outpatient diabetes clinic.

Materials and Methods

We studied 17 normal volunteers and 37 diabetics of comparable ages (55 ± 9 years for the controls, 64 ± 5 years for diabetics). The latter were randomly selected, without regard to severity of diabetes, from a group of non-obese, maturity-onset patients being treated by diet alone (six subjects) or by diet plus either insulin (nine subjects) or oral hypoglycemic agents (22 subjects). None was taking other medications known to affect serum lipids. No division was made into age groups; because all subjects were adult and all women were postmenopausal, age would be expected to have little effect on their lipoprotein concentrations (5, 6).

Blood, collected after a 12 to 24 h fast and mixed at once with a 15 g/L thimerosal solution, pH 8 (0.1 mL to 25 mL of blood), was allowed to clot at room temperature for 2 h. The serum was collected by centrifugation and EDTA solution was added to give a final concentration of 1 g/L. An aliquot was taken for measurement of serum cholesterol and triglycerides and for lipoprotein electrophoresis on thin agarose gel. The remainder was overlaid with NaCl with a relative density of 1.006 and centrifuged for 30 min at 15,000 rpm; any chylomicrons thus separated were discarded.

The other apolipoprotein species were separated by sequential ultracentrifugation in a Beckman 40.3 rotor, solid KBr being used for density adjustments; VLDL d <1.006; IDL, 1.006–1.019; LDL, 1.019–1.063; HDL, 1.063–1.210. Each fraction was washed once by recentrifugation at its own density, dialyzed for three days against EDTA (0.1 g/L, pH 8.6) to remove KBr, then diluted to a predetermined volume. An aliquot was then used for measurement of cholesterol and protein; the remainder was freeze-dried, then delipidated with ether/ethanol (7). The resulting protein residue was stored, desiccated, at −20 °C until gel electrophoresis was carried out.

Gel electrophoresis of VLDL and HDL was performed in 100 g/L acrylamide in the presence of 8 mol of urea per liter; samples were dissolved in tris(hydroxymethyl)methylamine buffer (0.2 mol/L, pH 8.2), containing 1 g of sodium dodecyl sulfate per liter. A current of 2.5 mA/gel was applied for 4 h. Gels were stained with Amido Black (2 g/L of dilute acetic acid, 70 mL/L) and destained in acetic acid/water (10/90 by vol). They were scanned in a Beckman spectrophotometer. Areas under the peaks were measured in arbitrary units by planimetry and expressed as a percentage of the total for the gel. Planimetry was reproducible to ±5% for areas down to 1 arbitrary unit. No correction was made for differences in chromogenicity among the apoproteins (8). Linearity of densitometric response for increasing amounts of VLDL peptides, as demonstrated by Kane et al. (8), was confirmed; in addition, we found linearity of response for apoAI and apoAII in HDL with a difference in chromogenicity between the two apoproteins (Figure 1). For VLDL, 100 μg of protein was applied. In the case of HDL, gels were run with 7 μg, to obtain relative amounts of apoAI and apoAII, and with 50 μg, to obtain the amounts of the minor components in relation to apoAI; from the combined information the proportions of all components were estimated. Duplicate gels were run in all cases: for each band, duplicates agreed within 5% of the mean; runs on two separate days agreed within 8%.

Sodium dodecyl sulfate gel electrophoresis of LDL was performed by the method of Laemmli (9); samples were dissolved in a mixture of 10 g/L sodium dodecyl sulfate, 2

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3 Nonstandard abbreviations used: VLDL, IDL, LDL, and HDL, very-low-, intermediate-, low-density-, and high-density lipoprotein; EDTA, ethylenediaminetetraacetate; LP, lipoprotein; and apo, apolipoprotein.

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mmol/L EDTA, and 5 mmol/L dithiothreitol by boiling for 3 min and then incubating at 37 °C for 18 h. Separation was effected with a current of 0.2 mA/gel for 18 h. Gels were stained with Coomassie Brilliant Blue G 250 (0.5 g/L of acetic acid/isopropanol/water, 2/5/2 by vol) and destained in a diffusion destainer with acetic acid/water (10/90).

Protein and cholesterol in the LP fractions were measured by conventional procedures (10, 11). Triglycerides and cholesterol in whole serum were measured by enzymic methods with a Technicon continuous-flow analyzer. Normal values in this laboratory are considered to be, per liter, <2.5 g of cholesterol and <1.5 g of triglycerides. apoB in VLDL was measured by difference between total protein and isopropanol-soluble protein (12).

Results

Cholesterol and Lipoproteins

Measurements were made on 17 control and 25 diabetics.

Table 1. Serum Glucose and Lipid Concentrations in Diabetic and Non-Diabetic Subjects

<table>
<thead>
<tr>
<th>Group (no. in group)</th>
<th>Glucose</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (17)</td>
<td>910 (20)</td>
<td>2120 (100)</td>
<td>800 (60)</td>
</tr>
<tr>
<td>Diabetic (25)</td>
<td>1520 (90)</td>
<td>2440 (100)</td>
<td>1690 (160)</td>
</tr>
<tr>
<td>Normolipidemic</td>
<td>1540 (140)</td>
<td>2080 (90)</td>
<td>1020 (70)</td>
</tr>
<tr>
<td>(15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ VLDL (6)</td>
<td>1410 (100)</td>
<td>2710 (170)</td>
<td>2930 (230)</td>
</tr>
<tr>
<td>↑ LDL (7)</td>
<td>1670 (250)</td>
<td>3909 (80)</td>
<td>2380 (390)</td>
</tr>
</tbody>
</table>

* p < 0.001 (vs control).
*b p < 0.025 (vs control).
* Three subjects had increased VLDL, four had increased LDL, and three had increases in both.

Of the latter, 15 were normolipoproteinemic (normal serum lipoprotein electrophoretic pattern, together with normal serum cholesterol and triglycerides) and 10 had increased values for VLDL (increased VLDL on serum electrophoresis together with increased serum triglycerides), LDL (increased LDL on serum electrophoresis together with increased serum cholesterol), or both VLDL and LDL (Table 1). None of the subjects' sera showed chylo micronemia on electrophoresis. The distribution of cholesterol among the lipoproteins in the normolipidemic subjects was normal in both absolute and relative terms (Table 2). As anticipated, in subjects with increased VLDL this fraction carried an increased cholesterol (absolute and relative), and in those with increased LDL the LDL cholesterol was increased. The HDL cholesterol was decreased in subjects with increased LDL, and in the group as a whole there was a significant negative correlation between HDL and LDL cholesterol levels (r = -0.45, p < 0.025). The cholesterol/protein ratios of the lipoprotein classes in the normal subjects were: VLDL, 1.00 (SD 0.02); IDL, 1.21 (SD 0.12); LDL, 0.84 (SD 0.06); HDL, 0.29 (SD 0.01). The values in the diabetic subjects did not differ significantly except for a small increase in the HDL to 0.34 (SD 0.09) (p < 0.05).

Apoproteins

The electrophoretic pattern of the soluble apoVLDL (Figure 2) showed five major bands, as described by others (8,
Table 2. Distribution of Cholesterol in Lipoproteins

<table>
<thead>
<tr>
<th>Group</th>
<th>No. in group</th>
<th>% of total (and SEM)</th>
<th>Absolute distribution, mg/L serum (and SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VLDL</td>
<td>IDL</td>
</tr>
<tr>
<td>Control</td>
<td>17</td>
<td>12/5</td>
<td>6 (1)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>25</td>
<td>10/15</td>
<td>10 (3)</td>
</tr>
<tr>
<td>Normolipidemic</td>
<td>15</td>
<td>7/8</td>
<td>7 (1)</td>
</tr>
<tr>
<td>↑ VLDL</td>
<td>6c</td>
<td>2/4</td>
<td>16 (4)b</td>
</tr>
<tr>
<td>↑ LDL</td>
<td>7c</td>
<td>2/5</td>
<td>13 (3)</td>
</tr>
</tbody>
</table>

* Recovery of cholesterol in the ultracentrifugal fractions was 76–91% of the serum value; the absolute distribution is based on the relative distribution in the ultracentrifugal fractions, corrected to 100% recovery (disregarding chylomicrons).

b Differs from control value, p < 0.02.

c See Table 1.

d Differs from control value, p < 0.001.

Table 3. Apoprotein Composition of Soluble VLDL Protein, in Percent (±SEM)

<table>
<thead>
<tr>
<th>Group (no.)</th>
<th>(1) a</th>
<th>(2) a</th>
<th>(Cl)</th>
<th>E</th>
<th>Cll</th>
<th>CII</th>
<th>CIII</th>
<th>ClII</th>
<th>Minor components b</th>
<th>Cll/CIII1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (12)</td>
<td>5.2 (0.8)</td>
<td>10.6 (1.7)</td>
<td>32.4 (3.1)</td>
<td>11.4 (1.1)</td>
<td>18.2 (1.9)</td>
<td>15.8 (1.5)</td>
<td>6.1 (1.8)</td>
<td>0.65 (0.04)</td>
<td></td>
<td></td>
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<tr>
<td>Diabetic (27)</td>
<td>3.7 (0.7)</td>
<td>11.0 (1.4)</td>
<td>28.1 (2.1)</td>
<td>14.3 (0.8)c</td>
<td>24.4 (1.9)c</td>
<td>17.9 (0.9)</td>
<td>&lt;1c</td>
<td>0.66 (0.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normolipidemic</td>
<td>3.8 (0.9)</td>
<td>13.1 (1.8)</td>
<td>28.6 (2.9)</td>
<td>13.4 (1.1)</td>
<td>21.9 (5.8)</td>
<td>17.5 (1.5)</td>
<td>0.68 (0.06)</td>
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<tr>
<td>(14)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ VLDL (6)d</td>
<td>3.6 (1)</td>
<td>8.7 (2.2)</td>
<td>24.7 (3.8)</td>
<td>15.2 (1.9)</td>
<td>29.2 (4.5)*</td>
<td>18.6 (0.9)</td>
<td>0.56 (0.06)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ LDL (7)f</td>
<td>3.6 (0.7)</td>
<td>6.5 (1.2)</td>
<td>31.5 (3.8)</td>
<td>16.5 (1.2)e</td>
<td>23.4 (2.1)</td>
<td>18.4 (1.2)</td>
<td>0.71 (0.04)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Band 1 on the electrophoretic gel, near the origin; identity not determined.
b Includes Al, All, and one unidentified band moving between All and CII.
c Analysis of variance; difference between diabetics and controls significant at p < 0.025.
d Includes three subjects with increase in VLDL (phenotype IV) and three subjects with increase in both VLDL and LDL (phenotype IIB). There was no difference observed between these two subgroups.

* Within analysis of variance, differs from control with p < 0.05.

I3), and some minor components. However, as noted by Shore and Shore (I3), the relative amounts of the various apoproteins varied considerably from subject to subject. In general the apoprotein composition of the diabetic samples was similar to that for the normal, but it differed in several minor respects: the minor components (primarily Al and All) accounted for about 6% of the apoproteins in the normal but for less than 1% in the diabetic, and there were small but significant increases in the relative amounts of apoCII and the sum of ClI, CIII, and CIII2 (Table 3).

There were 12 sera from control individuals and 11 from diabetics for which we studied both cholesterol distribution and apoprotein electrophoresis, finding a positive correlation between the concentrations of cholesterol in serum and of apoE in VLDL (Figure 3); the correlation coefficients (r) were 0.593 for the control sera (p < 0.05), 0.711 for the diabetics (p < 0.05), and 0.697 in the combined data (p < 0.01). Such a relationship was not found for other components of apoVLDL. ApoB accounted for 62 (SD 8)% of the apoVLDL in normal subjects and for 65 (SD 8)% in diabetic subjects.

Gel electrophoresis of LDL was done in the case of those few normal subjects from whom large blood samples had been obtained; the pattern was very similar to that of VLDL (Figure 2), but there was insufficient material for accurate quantitation.

HDL showed the expected pattern: two major bands (Al and AllI) and some minor bands, including the apoC peptides. For the sera from the diabetics, we saw small (5%) but statistically significant decrease in apoAl, and more of the minor components (apoAIII and apoC) than normal (Table 4). There were no differences between normolipoproteinemic diabetics and those with increased LDL or VLDL.

When apoLDL was reduced with dithiothreitol it was completely soluble in sodium dodecyl sulfate and gave rise to many bands on electrophoresis. There was no apparent difference between the normal and diabetic subjects in the patterns obtained (not shown).

Discussion

There have been numerous studies of lipoprotein composition and concentration, but the modes of measurement and of expression are so varied that comparisons among them frequently cannot be made. Nevertheless, changes have been...
reported in the cholesterol content and the composition of apoproteins in persons on a high cholesterol diet (14-17), or with atherosclerosis (18, 19), the nephrotic syndrome (20), or diabetes (4, 5), or who are being treated with sex steroids (21).

It was not our purpose to examine the distribution of cholesterol among the lipoprotein classes, but the data are of interest in this regard. In several previous studies (4, 5, 22) a decreased HDL cholesterol was found in normolipidemic diabetes; in another study, the HDL cholesterol was mildly decreased in women but not in men (23), while in three additional reports it was normal in both men and women (24-26). Our results agree with these last observations. The explanation for these discrepancies in results in not evident, but it seems clear that the HDL cholesterol concentration is not related to the degree of diabetic control (22, 25) or to obesity (25). HDL was decreased, however, in patients with increased LDL and hypercholesterolemia, a combination of changes found by Howard et al. (27) in Pima Indian diabetics. In our diabetics as a whole, the apoHDL was reduced from 1.96 (SE 0.16) to 1.51 (SE 0.16) g/L but the difference was not quite significant at \( p = 0.05 \).

The apoprotein pattern of VLDL in normal subjects was generally similar to that described by others, but there were distinct quantitative differences in composition from that reported by Kane et al. (8); we found a considerably higher proportion of apoE and less apoCIII. These differences persisted, even after our data were corrected for differences in apoprotein chromatography (Table 5). The similarity of the amounts of apoCII, apoCIII1, and apoCIII2 found here agrees with the results of Carlson and Ballantyne (28). We also found much more apoB than did Kane et al. (8). Our values are similar to those obtained by Kostner et al. (29).

The VLDL of diabetics showed an increased proportion of apoC, particularly marked in those with increased VLDL because of the increase in apoCIII1. Reportedly, increased VLDL triglyceride is related to a decrease in the ratio of CII/CIII1 in VLDL (28). In support of this, our results show that diabetics with increased VLDL and serum triglyceride concentrations had a decreased ratio of CII/CIII1 as compared to all other groups. Although a strict comparison cannot be made with the observations of Bar-On et al. (30), these authors reported an increase in CIII as well as an increase in total apoC in the serum of rats with streptozotocin-induced diabetes.

The apoprotein composition of HDL agrees closely with that observed by Kostner et al. (29) for normal subjects; the apoAI/apoAII ratio in the total HDL fraction, estimated from their data, is 4.9/1, while our results, corrected for differences in chromogenicity, give a ratio of 5.5/1 for normal subjects and 4.9/1 for the diabetics. There were small but statistically significant (\( p < 0.05 \)) changes in the relative amounts of the apoprotein classes in diabetes; these changes may reflect alterations in the relative amounts of HDL subfractions because these differ in apoprotein composition (31).

An incidental observation of interest is the relationship between the concentrations of apoE and VLDL cholesterol. Diet-induced hypercholesterolemia in man and animals is associated with the appearance and progressive increase of B-VLDL and HDLc (32). Both these lipoproteins have a high cholesterol content and contain apoE as a major apoprotein. Our data show a continuous relationship between the amounts of cholesterol and apoE in VLDL; thus B-VLDL may be an extreme form of this continuum and not a qualitatively distinct lipoprotein. Because VLDL is taken up by vascular smooth-muscle cells (33) and causes cell proliferation (34), and because apoE binds to the same cell-surface receptor as apoB (35), and with even greater affinity (36), it is possible that the atherogenic effect of dietary hypercholesterolemia is mediated by an increased apoE content of VLDL as well as by increased LDL and HDLc.

The diabetic subjects studied here constitute a heterogeneous population, broadly comparable to the large groups found to be at higher risk for atherosclerosis (2, 3). The results demonstrate several abnormalities in apoprotein compositions of VLDL and HDL, all quantitatively small. It remains to be determined whether these abnormalities are related to the increased risk of atherosclerosis in this disease.

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References