Concentrations of Lipids and Apolipoproteins in Patients with Clinically Well-Controlled Insulin-Dependent and Non-Insulin-Dependent Diabetes

J. Joven, E. Vilella, B. Costa, P. R. Turner, C. Richart, and L. Masana

The triglyceride and cholesterol content of total, very-low-, intermediate-, low-, and high-density lipoproteins, and of apolipoproteins (apo) AI, AII, B, CII, CIII, and E were determined in plasma from 107 patients with clinically well-controlled diabetes and from 66 age- and weight-matched healthy normal subjects. The diabetic patients were separated into two groups: those with insulin-dependent diabetes mellitus (IDDM, type 1, n = 24) and those with non-insulin-dependent diabetes mellitus (NIDDM, type 2, n = 83). The latter group contained two subgroups: those treated by diet (type 2d, n = 42) or by insulin (type 2i, n = 41). High-density lipoprotein cholesterol was increased in IDDM patients, and decreased in NIDDM patients relative to control subjects. Mean apo AI values in IDDM patients were higher than in their respective controls and in NIDDM patients. Concentrations of apo B, CIII, and E were higher in all diabetic patients than in the healthy controls, but those of apo CII did not differ statistically between diabetics and nondiabetics. Although total plasma cholesterol and triglyceride concentrations were apparently near normal values in patients with good glycomic control, we found a persistent increase of intermediate-density lipoproteins (remnants) in all the diabetic groups studied. This factor may be related to the perceived increased cardiovascular risk in these individuals.

Lipoprotein abnormalities in diabetes mellitus are well established and might account for the increased frequency of atherosclerotic vascular disease observed in these patients despite good glycomic control (1, 2). Some workers (3, 4) have attempted to distinguish the lipoprotein and lipid changes observed in type 1 diabetes (insulin-dependent diabetes mellitus, IDDM) from those in type 2 diabetes (non-insulin-dependent diabetes mellitus, NIDDM). Although there is general agreement that the concentration of high-density lipoprotein (HDL) cholesterol is higher than normal in type 1, and normal or lower in type 2 diabetes, reported findings are inconsistent for low-density lipoprotein (LDL) cholesterol values (5-17) and little is known about changes in intermediate-density lipoproteins (IDL) in this disease (12). Reliable information about these particles may be especially important in view of their postulated atherogenicity (13).

Avogaro et al. (14) suggested that the apolipoproteins, mainly apo AI and apo B, or the ratio of the two, may be better than cholesterol and triglyceride concentrations alone as predictive factors for the development of coronary heart disease. This may well be true in conditions not exacerbated by confounding factors such as diabetes in general and diabetic sub-types in particular.

We present here an attempt to discriminate between clearly defined groups on the basis of their lipid profiles and of the apoproteins known to affect their concentrations in plasma.

Materials and Methods

Subjects. We recruited 107 well-controlled diabetic patients from a population of 450 diabetics regularly attending the Diabetes Clinic at the Hospital Joan XXIII (Tarragona). To carefully delineate the groups, we studied only male subjects. Other exclusion criteria were the use of thiazide diuretics, beta-blockers, or any other drug known to alter lipoprotein metabolism; clinical or laboratory evidence of renal or hepatic damage; and evidence of other metabolic disorders. Diabetic patients were assigned to two groups according to the criteria of the National Diabetes Data Group (15) and the World Health Organization (16): type 1 (n = 24) and type 2 (n = 83). From the latter, we formed two subgroups: type 2d, those treated with diet (n = 42) and type 2i, those treated with diet and insulin (n = 41); none of the patients had been treated with sulfonylureas in the previous six months. The type 2i group consisted of patients whose C-peptide concentration in plasma exceeded 0.60 nmol/L 6 min after intravenous administration of 1 mg of glucagon (17).

The patients were matched with male controls for age (±5 years), weight (±5 kg), smoking habits, and alcohol consumption. Because of the tendency of type 2 patients towards overweight and the difference in age of onset with respect to type 1 patients, two matching control groups were necessary: group A (n = 24) for type 1 patients, and group B (n = 42) for patients with type 2 diabetes.

Analyses. The clinical status of the diabetic subjects was assessed, and body mass index (BMI) was calculated according to the formula BMI = weight (kg)/height2 (m). Blood was collected in EDTA (1 mg per milliliter of plasma) after an overnight fast, and the samples were centrifuged immediately at 4 °C to obtain the plasma.

One aliquot was stored at -30 °C for subsequent batched turbidimetric measurement of apo AI, AII, and B (Boehringer Mannheim, Marburg, F.R.G.). Apo CII, CIII, and E were measured by single radial immunodiffusion single (Daichi Pure Chemicals, Tokyo, Japan). Previous results obtained in our laboratory indicate no interferences in these analyses after storage of specimens at -30 °C for as long as three months.

Another aliquot was subjected to immediate analysis of glucose, cholesterol, and triglyceride in a Monarch 2000 centrifugal analyzer (IL Spa, Milan, Italy) by standard enzymatic procedures; glycated hemoglobin was assayed by microcolumn chromatography (Isolab Inc., Akron, OH). The same day, plasma was subjected to sequential preparative ultracentrifugation for lipoproteins (18) in a Kontron TFF

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45.6 angled rotor. Very-low-density lipoproteins (VLDL) were isolated at a density of less than 1.006 kg/L, IDL between 1.006 and 1.019 kg/L, and LDL between 1.019 and 1.063 kg/L. The lipids measured in the infranatant liquid of this last spin were considered to represent HDL.

Statistical analyses. We used the unpaired Student’s t-test to assess differences between the diabetic groups and their controls with respect to each of the variables measured; \( P < 0.05 \) was taken as significant. Pearson correlation coefficients were used to evaluate the degree of linear association between variables.

Results

The biometric and laboratory data of the groups are shown in Table 1.3 Diet- and insulin-treated type 2 patients were very similar in terms of age and weight, but tended to be older and with a higher BMI than type 1 patients. Glycated hemoglobin proportions were similar in all groups. The percentage of smokers (47% of subjects) and the alcohol intake (8.9 to 19.5 g/week) were comparable with that observed in the control group (48%, and 9.5 to 23 g/week, respectively).

Within-group comparison of cholesterol and triglyceride concentrations in plasma and in the isolated lipoprotein classes showed that in the type 1 group of patients, there was a significant increase of IDL cholesterol (\( P < 0.01 \)), IDL triglyceride (\( P < 0.01 \)), and HDL cholesterol (\( P < 0.05 \)) relative to the control group A. Similarly, in the type 2 groups of patients, increased concentrations of IDL cholesterol (\( P < 0.05 \), 2d vs control; and \( P < 0.01 \), 2i vs control), and IDL triglyceride (\( P < 0.05 \), 2d vs control; and \( P < 0.001 \), 2i vs control) were observed. The concentrations of IDL cholesterol and IDL triglyceride of the type 2i group of patients significantly exceeded those of the type 2d group (\( P < 0.05 \) and \( P < 0.001 \), respectively).

Cross-group comparisons of cholesterol and triglyceride values showed that in type 1 patients the mean plasma cholesterol concentration was lower than that in the type 2i group (\( P < 0.05 \)), whereas the mean VLDL cholesterol, VLDL triglyceride, and IDL triglyceride concentrations were lower than those observed in both of the type 2 groups of patients (\( P < 0.001 \), \( P < 0.01 \), and \( P < 0.001 \), respectively). Conversely, the mean HDL cholesterol concentration in type 1 patients was higher than that of type 2 patients, reaching statistical significance (\( P < 0.05 \)) relative to the type 2d group alone.

Table 2 summarizes the plasma apoprotein concentrations in all the study groups. The mean apo Al value in type

### Table 1. Biometric and Lipoprotein Data (Mean ± SD) for the Diabetic Patients and Their Respective Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic controls (A)</th>
<th>Type 1</th>
<th>Non-diabetic controls (B)</th>
<th>Type 2d</th>
<th>Type 2i</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (n = 24)</td>
<td>37.3 ± 9.6</td>
<td>36.1 ± 10.8</td>
<td>57.1 ± 8.9</td>
<td>55.9 ± 9.8</td>
<td>58.3 ± 8.7</td>
</tr>
<tr>
<td>Weight, kg (n = 24)</td>
<td>64 ± 6</td>
<td>65 ± 7</td>
<td>75 ± 10</td>
<td>77 ± 11</td>
<td>72 ± 11</td>
</tr>
<tr>
<td>Height, m (n = 24)</td>
<td>1.67 ± 0.05</td>
<td>1.69 ± 0.06</td>
<td>1.67 ± 0.06</td>
<td>1.66 ± 0.07</td>
<td>1.66 ± 0.06</td>
</tr>
<tr>
<td>BMI, kg/m² (n = 24)</td>
<td>23.1 ± 1.7</td>
<td>22.5 ± 1.9</td>
<td>26.9 ± 3.1</td>
<td>27.9 ± 3.2</td>
<td>26.0 ± 3.4</td>
</tr>
<tr>
<td>Hb A₁, % (n = 24)</td>
<td>6.9 ± 1.7</td>
<td>8.3 ± 2.6</td>
<td>7.2 ± 1.9</td>
<td>8.5 ± 2.3</td>
<td>8.7 ± 2.1</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>5.20 ± 0.70</td>
<td>5.27 ± 1.42</td>
<td>5.31 ± 1.42</td>
<td>5.54 ± 1.18</td>
<td>5.94 ± 1.56</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.12 ± 0.17</td>
<td>0.19 ± 0.11</td>
<td>0.39 ± 0.24</td>
<td>0.47 ± 0.81</td>
<td>0.40 ± 0.43</td>
</tr>
<tr>
<td>IDL</td>
<td>0.09 ± 0.09</td>
<td>0.20 ± 0.13</td>
<td>0.11 ± 0.09</td>
<td>0.21 ± 0.09</td>
<td>0.30 ± 0.27</td>
</tr>
<tr>
<td>LDL</td>
<td>3.60 ± 0.70</td>
<td>3.35 ± 1.22</td>
<td>3.55 ± 0.70</td>
<td>3.68 ± 1.28</td>
<td>3.98 ± 1.29</td>
</tr>
<tr>
<td>HDL</td>
<td>1.32 ± 0.24</td>
<td>1.52 ± 0.34</td>
<td>1.30 ± 0.25</td>
<td>1.18 ± 0.29</td>
<td>1.27 ± 0.38</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>0.89 ± 0.24</td>
<td>0.93 ± 0.25</td>
<td>1.25 ± 0.60</td>
<td>1.61 ± 2.01</td>
<td>1.43 ± 0.79</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.35 ± 0.16</td>
<td>0.43 ± 0.19</td>
<td>0.64 ± 0.44</td>
<td>1.14 ± 1.98</td>
<td>0.76 ± 0.56</td>
</tr>
<tr>
<td>IDL</td>
<td>0.09 ± 0.05</td>
<td>0.13 ± 0.04</td>
<td>0.12 ± 0.06</td>
<td>0.18 ± 0.11</td>
<td>0.46 ± 0.32</td>
</tr>
<tr>
<td>LDL</td>
<td>0.27 ± 0.09</td>
<td>0.23 ± 0.06</td>
<td>0.29 ± 0.10</td>
<td>0.25 ± 0.07</td>
<td>0.32 ± 0.16</td>
</tr>
<tr>
<td>HDL</td>
<td>0.17 ± 0.06</td>
<td>0.15 ± 0.03</td>
<td>0.19 ± 0.05</td>
<td>0.16 ± 0.04</td>
<td>0.18 ± 0.16</td>
</tr>
</tbody>
</table>

**Significantly different from control group A: aP < 0.01, bP < 0.05.**

### Table 2. Concentrations of Apolipoproteins in Plasma of Diabetic Patients and Control Subjects

<table>
<thead>
<tr>
<th>Apolipoproteins</th>
<th>Non-diabetic controls (A)</th>
<th>Type 1 patients</th>
<th>Non-diabetic controls (B)</th>
<th>Type 2d</th>
<th>Type 2i</th>
</tr>
</thead>
<tbody>
<tr>
<td>A I</td>
<td>1.11 ± 0.20</td>
<td>1.23 ± 0.18</td>
<td>1.10 ± 0.25</td>
<td>1.05 ± 0.19</td>
<td>1.12 ± 0.21</td>
</tr>
<tr>
<td>A II</td>
<td>0.37 ± 0.09</td>
<td>0.36 ± 0.08</td>
<td>0.35 ± 0.09</td>
<td>0.33 ± 0.08</td>
<td>0.36 ± 0.09</td>
</tr>
<tr>
<td>B</td>
<td>0.84 ± 0.21</td>
<td>0.94 ± 0.20</td>
<td>0.87 ± 0.22</td>
<td>1.14 ± 0.47</td>
<td>1.19 ± 0.31</td>
</tr>
<tr>
<td>C II</td>
<td>0.040 ± 0.015</td>
<td>0.044 ± 0.012</td>
<td>0.042 ± 0.014</td>
<td>0.046 ± 0.020</td>
<td>0.043 ± 0.016</td>
</tr>
<tr>
<td>C III</td>
<td>0.090 ± 0.030</td>
<td>0.100 ± 0.020</td>
<td>0.099 ± 0.030</td>
<td>0.113 ± 0.039</td>
<td>0.108 ± 0.034</td>
</tr>
<tr>
<td>E</td>
<td>0.037 ± 0.016</td>
<td>0.043 ± 0.008</td>
<td>0.038 ± 0.015</td>
<td>0.047 ± 0.013</td>
<td>0.049 ± 0.012</td>
</tr>
</tbody>
</table>

All values are expressed in g/L, mean ± SD.

**Significantly different from control group A: aP < 0.05, bP < 0.001.**

**Significantly different from control group B: cP < 0.01, dP < 0.05.**

**Significantly different from type 2d: eP < 0.001, fP < 0.05.**

**Significantly different from type 2i: gP < 0.001, hP < 0.05.**

Hb A₁, glycated hemoglobin.
1 patients significantly exceeded that of the control subjects ($P < 0.05$) and the two type 2 groups (2d, $P < 0.001$; and 2i, $P < 0.05$). Significantly increased values for apoproteins B ($P < 0.05$), CII ($P < 0.001$), and E ($P < 0.005$) were observed in all diabetic patients relative to their respective controls. No statistical significance was observed, for apo CII concentrations, between diabetic patients and their non-diabetic controls, nor between diabetic groups. However, the type 2 patient group had a significantly lower apo CII to triglyceride ratio than that of the type 1 patients.

Plasma triglycerides were strongly correlated with apo CII and apo CIII concentrations in all patient groups (type 1 patients: $r = 0.61$, and 0.62, $P < 0.01$; type 2d patients: $r = 0.76$, and 0.71, $P < 0.001$; type 2i patients: $r = 0.35$, $P < 0.05$, and $r = 0.78$, $P < 0.001$, respectively). Apo CII correlated positively with apo CIII in the type 2d patients ($r = 0.77$, $P < 0.001$) but not in the groups treated with insulin (type 1: $r = 0.34$, $P$ not significant; type 2i: $r = 0.28$, $P$ not significant). The same trend was observed for the correlation between total plasma cholesterol and apoprotein E (type 1: $r = 0.23$; type 2i: $r = 0.28$, $P$ not significant; and type 2d: $r = 0.43$, $P < 0.01$). Apoprotein AI values were strongly correlated with HDL cholesterol in the type 1 ($r = 0.86$, $P < 0.001$), type 2d ($r = 0.67$, $P < 0.001$), and type 2i patients ($r = 0.69$, $P < 0.001$). In the type 2d and type 2i groups HDL cholesterol was negatively correlated with plasma triglycerides ($r = -0.40$, $P < 0.01$; and $r = -0.42$, $P < 0.01$, respectively).

**Discussion**

Several studies describing lipoprotein abnormalities in patients with diabetes mellitus have been reported (3–12). The findings have not been consistent. Classification of diabetics, considerations of treatment regimens, and the degrees of metabolic control—though with appropriate control groups for comparison—are all important in assessing qualitative and quantitative differences in lipoprotein patterns.

In none of the previous studies were detailed lipid profiles, including IDL (or VLDL remnants), combined with plasma apoproteins A1, AII, B, CII, CIII, and E. Although sex-related differences, in conjunction with diabetes mellitus, in lipoprotein distributions have been described (19), in the present study we selected only diabetic males because, at least within our diabetic population, they constituted a more stable group with regard to treatment adherence and the degree of glycemic control. All diabetics selected had comparable values for glycated hemoglobin, which allowed comparisons between type 1 and type 2 patients. The BMI of our type 2 patients was greater than that of type 1 patients; reflecting the known close association between type 2 diabetics and obesity (20).

Type 1 patients had higher apo A1 and HDL cholesterol concentrations than their controls, whereas type 2 patients showed normal apo A1 values with normal, or decreased in the case of type 2d, HDL cholesterol values. The decreased HDL cholesterol concentration in type 2d diabetic patients confirmed earlier reports (3, 4, 7); near normal values in our type 2i patients together with its elevation in type 1 patients would suggest that the original peripheral insulin resistance in diabetic subjects is a causative factor in determining low HDL cholesterol concentrations in untreated diabetics.

In all our diabetic groups, plasma apo B concentrations were higher than in the controls. This factor may be important, because the risk of coronary heart disease with increased apo B, independent of its associated cholesterol, has been documented (21). Of further note was that the apoprotein A1 to B ratio in the type 1 group was no different from its control group ($1.30 \pm 0.25$ vs $1.32 \pm 0.20$), whereas in the type 2 groups the A1:B ratios were significantly lower than in controls (type 2d, $0.92 \pm 0.30$, and type 2i, $0.94 \pm 0.25$, vs $1.26 \pm 0.23$ in the control group; $P < 0.001$). Such ratios for assessing coronary heart disease risk under other circumstances must, in diabetics, be used cautiously.

In all diabetic groups, concentrations of apolipoproteins CIII and E in plasma exceeded control values. These apoproteins, together with apo B, are known to constitute the greater apoprotein proportion of the VLDL and IDL fractions (22), so these findings would suggest an accumulation of these particles in plasma. This is further evidenced by the increased IDL cholesterol concentrations we observed. Such apo E-enriched particles (synonymously termed "remnant") were shown by Fielding et al. (23) to be associated with a marked decrease in cholesterol efflux from fibroblasts that had been cultured in plasma from patients with type 2 diabetes. Such a finding is consistent with cholesteryl ester accumulation in cells and possible atherosclerotic sequelae. An explanation for the phenomenon of remnant accumulation in diabetes is not immediately apparent. A possible explanation could be the inhibitory effect of apo CIII on the lipase-mediated catabolism of lipoproteins (24). However, in all our diabetic patients, plasma triglyceride, VLDL cholesterol, and VLDL triglyceride concentrations were unremarkable and, moreover, because the concentrations of the lipase activator, apo CII (25), were similar to those of the control subjects, this would suggest no striking abnormalities in the enzyme's function of catabolism of the triglyceride-rich VLDL. Indeed, the finding that the insulin-treated type 1 patients have a significantly higher CII to triglyceride ratio (data not shown) suggests an enhancement of extrahepatic lipoprotein lipase activity (26) hepatic lipase activity, remaining unaffected or decreased (27), may result in the accumulation, in plasma, of the triglyceride-depleted "remnant" particles that we have observed.

In conclusion, our results indicate that in the overall clinical and laboratory management of diabetes, it may not suffice, in relation to premature cardiovascular risk, to aim for a "normalization" of total plasma cholesterol and (or) triglyceride concentrations. One should also address the question of the distribution of lipoprotein subfractions and apoproteins in plasma.

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References