Hypertriglyceridemic HyperapoB in Type 2 Diabetes

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OBJECTIVE — Much less attention has been paid to LDL in type 2 diabetes than to VLDL or HDL. In particular, there are few data on apoB levels in these patients. Moreover, most reports have focused on mean lipoprotein levels and consequently there is little information on the frequencies of the various dyslipidemic phenotypes.

RESEARCH DESIGN AND METHODS — Plasma and lipoprotein lipids, apoB and apoA1 were measured by standardized methods. LDL particle size was determined by PAGE. The total cohort was divided into phenotypes by two different methods. The first was based on triglycerides (≥ or <1.5 mmol/l) and LDL cholesterol (≥ or <4 mmol/l), whereas the second was based on triglycerides (≥ or <1.5 mmol/l) and apoB (≥ or <120 mg/dl).

RESULTS — For the overall cohort, plasma triglycerides were elevated (2.13 ± 1.6 mmol/l), total and LDL cholesterol were normal (5.34 ± 1.1 and 3.28 ± 0.88 mmol/l, respectively), and peak LDL size was reduced (252.9 ± 5.8 Å). HDL cholesterol was between the 25th and 50th percentiles of the general population (1.12 ± 0.36 mmol/l). The average level of apoB was 114 ± 29 mg/dl, a value that is between the 50th and 75th percentiles of the general population and is higher than that for LDL cholesterol, which was between the 25th and the 50th percentiles of the population.

The results of the phenotyping analysis were as follows. Using the conventional approach, only 23% has abnormal LDL, i.e., an elevated LDL cholesterol level. Using the new approach, almost 40% has an elevated apoB and therefore an elevated LDL particle number. Only 12.8% has combined hyperlipidemia based on the conventional approach, whereas almost one third had the equivalent, hypertriglyceridemic hyperapoB-based on the new algorithm. The severity of the dyslipoproteinemia in this group was noteworthy. Although the average LDL cholesterol was 3.91 mmol/l, a value just below the 75th percentile of the general population, the average apoB was 145 mg/dl, a value that approximates the 95th percentile of the population.

CONCLUSIONS — The dyslipidemic profile of patients with type 2 diabetes is not uniform. A substantial group have normal lipids and normal LDL particle number and size whereas others have markedly abnormal profiles. Diagnosis based on triglycerides and apoB rather than triglycerides and LDL cholesterol revealed that more than one in five had hypertriglyceridemic hyperapoB, which is characterized by hypertriglyceridemia, marked elevation of LDL particle number, small dense LDL, and low HDL, a constellation of abnormalities that is associated with markedly accelerated atherogenesis and therefore justifies intensive medical therapy.

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Abbreviations: apo, apolipoprotein; IDL, intermediate-density lipoprotein.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
studies suggest apoB levels are at least moderately increased on average (3–5). However, only one study has analyzed a cohort in terms of phenotypes, and the approach in that instance was based on lipoprotein lipids (3).

Accordingly, we measured plasma and lipoprotein lipids, plasma apoB and apoA1, and LDL size in a large cohort of patients with type 2 diabetes. We phenotyped our patients not only by the conventional approach but also based on triglycerides and apoB (2).

RESEARCH DESIGN AND METHODS — Lipid and apoprotein measurements were carried out at the Canadian External Quality Assessment Laboratory in Vancouver, British Columbia using standardized methods. HDL values were determined using a designated comparison method (6). Triglyceride levels were determined using an enzymatic free, glycerol-blanked assay, which is traceable to the Centers for Disease Control Chromatropic Acid Triglyceride Reference method. The between-day coefficients of variations (CVs) for all of these assays were <2.0%. LDL cholesterol was obtained by Friedewald calculation (7). ApoA1 and -B were immunochemically determined using a Dade Behring nephelometer. Calibration and IQC materials for this method were traceable to the World Health Organization/IFCC International Reference accuracy base for apoA1 (SP1-01) and apoB (SP3-07). The between-day CVs for the measurement of apoA1 were 3.34% at 129 mg/dl and 3.31% at 205 mg/dl. The between-day CVs for the measurement of apoB were 2.72 and 2.49% at 194 and 126 mg/dl, respectively. HbA1c was determined using a latex-enhanced turbidimetric immunoassay that was operated on a COBAS chemistry analytical system. LDL peak particle size was determined by non-denaturing 2–16% polyacrylamide gel electrophoresis using a modification of procedures previously described (8).

The patients were divided into four groups based on triglyceride and apoB levels. The cutoffs used to define the four phenotypes were 1.5 mmol/l for triglycerides and 120 mg/dl for apoB. A value of 1.5 mmol/l for triglycerides was chosen because it is at this level that small dense LDL particles become common (9). A value of 120 mg/dl for apoB was chosen because this approximately represents the 75th percentile value for individuals of this age (10,11), and it is the level at which subjects were separated and analyzed in regard to risk in the Framingham Study (11) and the Quebec Cardiovascular Study (12). For comparison, patients were also categorized by LDL cholesterol and triglycerides. For these analyses, levels of 1.5 mmol/l for triglycerides and 4.0 mmol/l for LDL cholesterol were chosen (the LDL cholesterol value approximately represents the 75th percentile of the study population) (13).

Means and SDs are reported for continuous variables, whereas frequencies and percentages are reported for categorical variables. Student’s t tests were used to test for differences in continuous variables between two groups, such as men and women. F tests were used to test for differences in continuous variables among more than two groups, such as the four phenotypes. \( \chi^2 \) tests were used to test for differences in categorical variables. P values associated with the tests are reported. Tukey’s test was conducted to test for differences in the variables between pairs of phenotypic groups. Statistical analyses were conducted in SAS and S-plus.

RESULTS — Of the 249 subjects, 132 (54%) were men and 113 (46%) were women. None were taking any medication that was known to affect lipoprotein levels. The characteristics of the cohort are given in Table 1. All were being treated with insulin, though not optimally, as indicated by HbA1c levels. Obesity was common, even more so in women than in men. Otherwise, there were no significant differences between the sexes.

The mean values for lipids, lipoprotein lipids, apoproteins, and LDL peak size are also shown in Table 1. Total and LDL cholesterol were normal, triglycerides were elevated, and HDL cholesterol was within normal limits. The average LDL cholesterol and apoB levels are discordant with respect to those found in the general population. Thus, the average LDL cholesterol level is between the 25th and 50th percentile of the general population (10). This discordance suggests that small, dense LDL particles are present, and this hypothesis was confirmed by the estimates of LDL peak size. Of interest, dyslipidemia is more pronounced in women than in men, as evidenced by significantly higher total cholesterol, total triglycerides, and apoB. On the other hand, there was no significant difference in LDL cholesterol and HDL cholesterol was significantly lower in men than in women. On the other hand, HDL cholesterol was below the 25th percentile in women but not in men (13).

Based on plasma triglycerides and LDL cholesterol, the cohort was divided into four phenotypes: normal, normotriglyceride-increased LDL cholesterol, hypertriglyceride-normal LDL cholesterol, and hypertriglyceride-increased LDL cholesterol. Using the conventional classification, these correspond to normal, type IIA hypercholesterolemia, type IV hyperlipoproteinemia, and type IIB or combined hyperlipidemia. These findings were compared with those obtained with phenotypes based on plasma triglycerides and apoB. Again, there were four phenotypes: normal, normotriglycerideremic hyperapoB, hypertriglycerideremic normoapoB, and hypertriglycerideremic hyperapoB (Fig. 1B).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Total</th>
<th>Women</th>
<th>Men</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>249</td>
<td>114</td>
<td>135</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.0 (10.0)</td>
<td>58.5 (10.5)</td>
<td>59.5 (9.6)</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.087 (0.013)</td>
<td>0.088 (0.014)</td>
<td>0.86 (0.013)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.34 (1.1)</td>
<td>5.61 (1.2)</td>
<td>5.11 (0.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.13 (1.6)</td>
<td>2.33 (1.6)</td>
<td>1.87 (1.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.28 (0.88)</td>
<td>3.38 (0.99)</td>
<td>3.21 (0.77)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.12 (0.36)</td>
<td>1.19 (0.42)</td>
<td>1.07 (0.29)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>114 (29)</td>
<td>119 (32)</td>
<td>110 (25)</td>
<td>0.02</td>
</tr>
<tr>
<td>ApoA1 (mg/dl)</td>
<td>142 (27)</td>
<td>149 (29)</td>
<td>137 (24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL (peak Å)</td>
<td>252.9 (5.8)</td>
<td>253.2 (6.1)</td>
<td>252.9 (5.2)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.9 (4.7)</td>
<td>31.8 (4.8)</td>
<td>30.3 (4.4)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data are means (SD).
Fig. 1A shows the phenotype frequencies, which are based on triglycerides and apoB, whereas Fig. 1B shows the phenotype frequencies, which are based on triglycerides and LDL cholesterol. Using the conventional approach, 35.7% were normal, 10.2% had type IIA, 41.3% had type IV, and 12.8% had type IIB. In total, 23% had abnormal LDL, as evidenced by increased LDL cholesterol. The corresponding results using triglycerides and apoB to identify phenotypes are shown in Fig. 1A: 34.1% of the total cohort were normal and 9.2% were normotriglyceridemic hyperapoB. Just over one-quarter had hypertriglyceridemic normoapoB, whereas just under one-third had hypertriglyceridemic hyperapoB. Thus, 40% of the cohort had an elevated LDL particle number and therefore abnormal LDL. Thus, whereas the conventional approach suggested that only 23% of the cohort as having abnormal LDL particles, a major difference in diagnostic outcome. In this regard, the present study supports the recent report of Wagner et al. (3), who were the first to examine dyslipidemic phenotypes in type 2 diabetes while incorporating apoB.

Our data extend their results in two regards. First, in our study, LDL particle size was directly measured, and the data indicate that small dense LDL particles were present in both hypertriglyceridemic groups. Second, we analyzed phenotypes based on triglycerides and apoB as well as on the conventional approach to further characterize the overall cohort. Using the method based on triglycerides and apoB, we observed that 30% of our cohort had hypertriglyceridemic hyperapoB and that the average apoB in this group was at the 95th percentile of the general population.

The extent of the abnormalities in type 2 diabetic subjects with hypertriglyceridemic hyperapoB is noteworthy. Not only is LDL particle number markedly increased, but LDL composition is altered in a proatherogenic direction, in that small dense LDL particles are present in this syndrome (2). In addition, in this group, HDL cholesterol was low and plasma tri-

Table 2—Variables by triglycerides/ApoB phenotype

<table>
<thead>
<tr>
<th></th>
<th>Hypertriglyceridemic hyperApoB</th>
<th>Hypertriglyceridemic normoApoB</th>
<th>Normotriglyceridemic hyperApoB</th>
<th>Normotriglyceridemic normoApoB</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.24 (0.94)</td>
<td>4.81 (0.72)</td>
<td>6.33 (0.59)</td>
<td>4.69 (0.67)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>3.13 (0.75)</td>
<td>2.77 (1.9)</td>
<td>1.16 (0.23)</td>
<td>1.02 (0.30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.91 (0.75)</td>
<td>2.70 (0.64)</td>
<td>1.45 (0.49)</td>
<td>2.91 (0.57)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.99 (0.23)</td>
<td>0.95 (0.23)</td>
<td>1.36 (0.30)</td>
<td>1.31 (0.42)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoA (mg/dl)</td>
<td>145 (21)</td>
<td>101 (14)</td>
<td>136 (12)</td>
<td>91 (15)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoA1 (mg/dl)</td>
<td>138 (25)</td>
<td>133 (22)</td>
<td>161 (26)</td>
<td>147 (30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL (peak Å)</td>
<td>250.7 (5.1)</td>
<td>249.9 (5.8)</td>
<td>256.1 (3.7)</td>
<td>256.4 (4.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.3 (4.5)</td>
<td>31.9 (4.4)</td>
<td>30.0 (3.9)</td>
<td>29.3 (4.6)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means (SD). *A vs. B, †A vs. D, ‡B vs. C, §C vs. D, ||B vs. D, ¶A vs. C.
glycerides were high. On their own, such abnormalities would be expected to increase the risk of vascular disease. It is of interest that significantly more men than women were normal, a difference that might explain, at least in part, the higher coronary event rates in women with type 2 diabetes than in men (14).

The extent to which these results can be extended to other patients depends on whether this cohort is characteristic of type 2 diabetes in general. In the sense that all the subjects were treated with insulin, it is not. However, given that insulin, at least acutely, reduces VLDL secretion (2), there is no a priori reason to believe that dyslipidemia should be more extreme than usual. More importantly, a number of studies have shown that small dense LDL particles are common in type 2 diabetes (2) and that apoB levels, on average, are elevated in type 2 diabetes (3–5). The overall results of our study are in accord with these reports, and the subgroup analysis, we believe, extends them.

Because the amount of cholesterol per LDL particle can vary substantially, LDL cholesterol is not a reliable index of LDL particle number (2). By contrast, each VLDL particle contains one molecule of apoB, which remains with that particle for its biologic lifetime. Therefore, plasma apoB is an exact measure of the total number of VLDL and LDL particles (2). Of the total apoB, >90% are LDL particles or, more precisely, intermediate-density lipoprotein (IDL) and LDL particles, just as LDL cholesterol is the sum of the cholesterol in the IDL and LDL fractions. This remains true even in hypertriglyceridemia; therefore, total plasma apoB is a reliable surrogate for LDL particle number (2).

Statins are becoming an increasingly popular therapy for patients with type 2 diabetes because their LDL cholesterol levels, although normal, are above target levels. It is important to note, therefore, that on-treatment LDL cholesterol was predictive of outcome in only one of the clinical trials or observational studies of statin therapy. That study was the Scandinavian Simvastatin Survival Study (15), in which the patients had the highest levels of LDL cholesterol in any of the clinical trials. By contrast, on-treatment apoB was predictive in the four clinical trials and observational studies from which data were available (15–18).

In summary, the present results strongly indicate that major abnormalities in LDL particle number and composition are frequently present in patients with type 2 diabetes, and the extent of the abnormality is only evident if apoB is measured in addition to the standard lipid profile. These findings may explain, at least in part, the markedly accelerated atherogenesis that occurs in some patients with type 2 diabetes.

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References