Increased Apoprotein B in Very Low Density Lipoproteins of Patients with Peripheral Vascular Disease

Guido Franceschini, Alighiero Bondioli, Manuela Mantero, Marina Sirtori, Guido Tattoni, Giorgio Biasi, and Cesare R. Sirtori

Lipoprotein compositional studies were carried out in 20 patients with atherosclerotic peripheral vascular disease. Twelve of these patients were normolipidemic, the other eight, hypertriglyceridemic. Ten normolipidemic and 10 hypertriglyceridemic age-matched subjects were used as controls. High density lipoprotein cholesterol levels were markedly reduced in the hypertriglyceridemic subjects, both with (35.1 ± 5.0 mg/dl) and without (36.2 ± 11.7 mg/dl) peripheral vascular disease, as compared to the normolipidemic patients (47.0 ± 6.3 mg/dl) and controls (48.1 ± 10.0 mg/dl). A decreased relative content of apo C-II in very low density lipoproteins in the hypertriglyceridemic subjects, as compared to the normolipidemics, was detected by isoelectric focusing. Hypertriglyceridemia in patients with peripheral vascular disease shows a typical Type IV lipoprotein and apoprotein profile.

Apoprotein B levels in very low and low density lipoproteins were determined by electroimmunodiffusion and selective precipitation with tetramethylurea (r = 0.981 between the two methods). All the patients with peripheral vascular disease showed an increased apo B content in very low density lipoproteins (VLDL) as compared to controls (apo B cholesterol in VLDL = 0.431 ± 0.124 for peripheral vascular disease patients and 0.236 ± 0.086 for controls, p < 0.001). A significant correlation between VLDL cholesterol and apo B levels was detected both in peripheral vascular disease patients and in controls; however, two distinct populations could be clearly separated (slopes of the regression lines: peripheral vascular disease patients = 0.350; controls = 0.215, p < 0.001). The data suggest a possible discriminatory power of VLDL-apo B levels in patients with peripheral vascular disease independent from other lipoprotein and lipid parameters. (Arteriosclerosis 2:74-80, January/February 1982)

Atherosclerotic peripheral vascular disease (PVD) is a frequent condition in Southern Europe; epidemiological data from Italy indicate that its incidence is more than twice as high as in the United States. Previous studies from our group indicated that approximately 50% of the patients show a Type IV hyperlipoproteinemia, apparently related to a high consumption of simple and complex carbohydrates and of ethanol.

Studies on the plasma lipoprotein and apolipoprotein composition of PVD patients have been relatively limited. The high incidence of Type IV hyperlipoproteinemia has been confirmed by some authors, whereas others using a smaller series found a relative prevalence of Type IIb hyperlipoproteinemia; more recently, reduced high density lipoprotein (HDL) cholesterol and protein levels have been described. The association between PVD and reduced HDL cholesterol has also been reported by other authors, either in aged subjects or in relatively younger patient groups.

The modes of plasma lipid, particularly cholesterol transport, have recently attracted consider-
able interest. The apoprotein B and apoprotein E contents, respectively, in low density lipoproteins (LDL) and very low density lipoproteins (VLDL) have been suggested as indicative of lipoprotein atherogenicity. A raised concentration of apoprotein B (apo B) in LDL was recently described in patients with coronary heart disease.11

We undertook a detailed study on the lipid and lipoprotein composition of PVD patients, in view of the considerable clinical significance of this disease in Italy. Particular attention was paid to the apo B content of different lipoprotein fractions because of a possible discriminative power of LDL- and VLDL-associated apo B in PVD patients, as compared to controls.

Methods

Patient Selection

The 20 males selected for the study represent a consecutive series of patients referred for metabolic evaluation to the E. Grossi Paoletti Center from the Department of Vascular Surgery of the Garbagnate Hospital, and from Division III of Surgical Pathology of the University of Milan, from November, 1979 until May, 1980. Patients ranged in age from 40 to 63 years and had histories of PVD documented by clinical symptoms and by angiography. Of the group, 17 were in Stage II according to Fontaine;12 the other three were in Stage III. A quantitative measurement of the reduction in blood flow to the lower extremities was obtained by strain gauge plethysmography (Periflow 4, Janssen, Beerse, Belgium). The "peak flow," determined after 3 minutes of ischemia (by compression of the thigh at 250 mm Hg), was used as an index of disease severity.13

None of the patients was following any specific dietary or drug therapy known to affect lipid and lipoprotein levels. Of the 20 patients, 12 were normolipidemic (PVD-N); the other eight were hypertriglyceridemic (PVD-HTG). Secondary causes of hyperlipidemia, such as liver, thyroid, and renal disease, were excluded; none of the patients had diabetes or impaired glucose tolerance. Table 1 reports age, body weight, plasma lipids, lipoprotein phenotype, disease location, and arterial flow data of the examined patients. For controls, 20 age-matched subjects were selected from persons referred to our Center for control of lipid levels, found to be abnormal in other laboratories. Ten controls were normolipidemic (C-N) and ten, hypertriglyceridemic (C-HTG). All had normal peripheral vascular function; the same exclusion criteria used for the patients were applied to the controls.

Table 1. Age, Plasma Lipids, and Clinical Characteristics of Patients with Peripheral Vascular Disease and of Controls

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Body weight (kg)</th>
<th>Plasma total</th>
<th>Hyperlipoproteinemia phenotype</th>
<th>Disease location*</th>
<th>Peak flow†</th>
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<tbody>
<tr>
<td>BE</td>
<td>57</td>
<td>73</td>
<td>260</td>
<td>369</td>
<td>IV</td>
<td>C</td>
</tr>
<tr>
<td>BG</td>
<td>56</td>
<td>68</td>
<td>224</td>
<td>382</td>
<td>IV</td>
<td>L</td>
</tr>
<tr>
<td>BM</td>
<td>51</td>
<td>63</td>
<td>233</td>
<td>133</td>
<td>N</td>
<td>C</td>
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<td>53</td>
<td>79</td>
<td>194</td>
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<td>N</td>
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</tr>
<tr>
<td>CN</td>
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<td>57</td>
<td>205</td>
<td>270</td>
<td>IV</td>
<td>L</td>
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<tr>
<td>DA</td>
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<td>65</td>
<td>224</td>
<td>103</td>
<td>N</td>
<td>C</td>
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<tr>
<td>FF</td>
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<td>N</td>
<td>C</td>
</tr>
<tr>
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<td>62</td>
<td>253</td>
<td>114</td>
<td>N</td>
<td>L</td>
</tr>
<tr>
<td>LG</td>
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<td>210</td>
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</tr>
<tr>
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<tr>
<td>ME</td>
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<td>78</td>
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<td>305</td>
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<td>C</td>
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<tr>
<td>NC</td>
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<td>76</td>
<td>190</td>
<td>130</td>
<td>N</td>
<td>L</td>
</tr>
<tr>
<td>OS</td>
<td>40</td>
<td>80</td>
<td>262</td>
<td>516</td>
<td>I</td>
<td>C</td>
</tr>
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<td>PF</td>
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<td>245</td>
<td>286</td>
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<tr>
<td>ZR</td>
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<td>81</td>
<td>195</td>
<td>164</td>
<td>N</td>
<td>L</td>
</tr>
<tr>
<td>ZV</td>
<td>52</td>
<td>77</td>
<td>224</td>
<td>130</td>
<td>N</td>
<td>L</td>
</tr>
<tr>
<td>Range</td>
<td>40-63</td>
<td>50-81</td>
<td>165-310</td>
<td>57-516</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (range)</td>
<td>38-61</td>
<td>56-79</td>
<td>164-335</td>
<td>50-475</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A = aortoiliac; C = combined; L = femoropopliteal.
† Maximal flow (ml/min per 100 ml of calf volume) occurring after 3 minutes of occlusion ischemia.
Biochemical and Statistical Analyses

Blood was collected on EDTA (1 mg/ml) after an overnight fast. Plasma lipoproteins were isolated by ultracentrifugation, according to the procedure described by Havel et al. by the use of a Beckman L5-50 instrument (Beckman, Palo Alto, California) equipped with a 50 Ti rotor. Density adjustments were made with solid KBr. Following centrifugation at 40,000 rpm at 15° C, VLDL was recovered at 20 hours and LDL at 24 hours. For the isolation of HDL, the centrifugation was carried out for 40 hours at 15° C. All fractions except for VLDL were dialyzed exhaustively against 0.15 M NaCl, 1 mM EDTA, pH 7.4 at 4° C. Lipoproteins were delipidated by extraction with chloroform/methanol (2/1, vol/vol).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and polyacrylamide gel isoelectric focusing (PAGIF) of isolated apoproteins were carried out as previously described. Separated apoproteins were quantitated by densitometry of the stained gels using a computerized densitometer (Seroskop, Elvi, Milan, Italy). The density recorded for each apoprotein band increased in a linear fashion with increasing amounts of apoprotein loaded on the gel.

The apo B content of isolated lipoproteins was measured by two techniques: electroimmunodiffusion and tetramethylurea (TMU) precipitation. For the electroimmunodiffusion assay (EIA), we followed the procedure described by Laurell, using a commercial antiserum against β-lipoprotein (Behringwerke AG, Marburgh-Lahn, German Federal Republic) previously shown to specifically react only with apoprotein B. Precipitation of TMU was carried out according to Kane et al., using 4.2 M redistilled tetramethylurea. TMU precipitation gave slightly lower apo B levels than EIA; however, no immunoreactive apo B was found in the TMU supernatants, and a highly significant correlation was detected between the values recorded by the two methods (r = 0.981, p < 0.001) (figure 1). If not otherwise stated, values reported are from the TMU precipitation method.

Cholesterol and triglyceride concentrations in plasma and in separated lipoproteins were determined by standard automated procedures. Protein was measured by the Lowry method using bovine serum albumin standards.

Statistical analysis of the data was carried out by Student's t test and by calculating the r correlation coefficients.

Results

Plasma Lipids and Lipoproteins

The levels of plasma lipid and lipoprotein components are summarized in table 2; no major difference could be detected between patients with specific locations of the arterial lesions (i.e., aortoiliac, femoropopliteal, and combined). Both the HTG groups showed markedly raised VLDL lipids and significantly decreased HDL-cholesterol (HDL-C) levels as compared to the other two groups. No significant correlation, however, could be detected between HDL-C and plasma or VLDL-triglycerides (VLDL-TG) in the four groups. Furthermore, a decreased cholesterol/TG ratio was also noted in the PVD-N group as compared to normolipidemic controls, both in LDL (PVD-N = 3.442 ± 0.621 mg/dl; C-N = 4.343 ± 0.927 mg/dl, p < 0.01) and HDL (PVD-N = 4.125 ± 2.092 mg/dl; C-N = 6.266 ± 1.783 mg/dl, p < 0.001). All PVD, moreover, showed an increased protein (P) content in VLDL, as compared to controls. The VLDL-C/VLDL-P ratio was significantly reduced in the patients (PVD = 0.930 ± 0.230; controls = 1.447 ± 0.339, p < 0.001).

Apoprotein Composition

All the PVD patients had higher VLDL-apo B levels as compared to controls (table 3), and the apo B/cholesterol ratio in VLDL was significantly
Table 2. Plasma Lipid and Lipoprotein Composition in Patients with Peripheral Vascular Disease and in Controls

<table>
<thead>
<tr>
<th>Disease type</th>
<th>No. of patients</th>
<th>Plasma (mg/dl)</th>
<th>Very low density (mg/dl)</th>
<th>Low density (mg/dl)</th>
<th>High density (mg/dl)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Chol</td>
<td>TG</td>
<td>Chol</td>
<td>TG</td>
</tr>
<tr>
<td>PVD-N</td>
<td>12</td>
<td>212.9</td>
<td>114.6</td>
<td>15.0</td>
<td>56.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 24.1</td>
<td>29.1</td>
<td>± 6.6</td>
<td>28.9</td>
</tr>
<tr>
<td>PVD-HTG</td>
<td>8</td>
<td>252.3</td>
<td>323.9</td>
<td>54.0</td>
<td>221.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 32.3</td>
<td>96.9†</td>
<td>± 23.7†</td>
<td>± 90.0†</td>
</tr>
<tr>
<td>C-N</td>
<td>10</td>
<td>222.5</td>
<td>100.5</td>
<td>14.2</td>
<td>54.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 32.7</td>
<td>± 34.8</td>
<td>± 9.7</td>
<td>± 32.7</td>
</tr>
<tr>
<td>C-HTG</td>
<td>10</td>
<td>255.8</td>
<td>332.2</td>
<td>59.1</td>
<td>222.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 46.5</td>
<td>± 118.4†</td>
<td>± 17.0†</td>
<td>± 96.1†</td>
</tr>
</tbody>
</table>

*p < 0.02 HTG vs N.
†p < 0.001 HTG vs N.
‡p < 0.01 PVD-N vs C-N.
§p < 0.005 PVD-N vs C-N.
Results are expressed as means ± sd.

PVD-N = normolipidemic peripheral vascular disease; PVD-HTG = hypertriglyceridemic peripheral vascular disease; C-N = normolipidemic controls; C-HTG = hypertriglyceridemic controls; Chol = cholesterol; TG = triglycerides; Prot = protein.

Increased (PVD = 0.431 ± 0.124 mg/dl; controls = 0.236 ± 0.086 mg/dl, p < 0.001). A highly significant correlation was detected between VLDL-apo B levels and VLDL-C concentrations in all the studied subjects. The r correlation coefficients between VLDL-apo B and VLDL-C were 0.936 for controls and 0.928 for PVD patients (both p < 0.001).

However, two distinct populations (PVD patients and controls) could be well separated when VLDL-apo B was plotted against VLDL-C (figure 2). The slopes of these regression lines were, respectively, 0.215 for controls and 0.350 for PVD patients (p < 0.001), suggesting a more marked apo B enrichment in VLDL from patients, at corresponding lipoprotein cholesterol levels.

Table 3. Content of Apoprotein B in VLDL and LDL in Patients with Peripheral Vascular Disease and in Controls

<table>
<thead>
<tr>
<th>Disease type</th>
<th>No. of patients</th>
<th>VLDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mg/dl)</td>
<td>(mg/dl)</td>
</tr>
<tr>
<td>PVD-N</td>
<td>12</td>
<td>6.1 ± 2.0*</td>
<td>81.1 ± 18.0</td>
</tr>
<tr>
<td>PVD-HTG</td>
<td>8</td>
<td>21.3 ± 8.5†</td>
<td>94.9 ± 28.7</td>
</tr>
<tr>
<td>C-N</td>
<td>10</td>
<td>3.3 ± 1.6</td>
<td>88.7 ± 19.5</td>
</tr>
<tr>
<td>C-HTG</td>
<td>10</td>
<td>12.7 ± 4.3</td>
<td>88.3 ± 21.8</td>
</tr>
</tbody>
</table>

*p < 0.01 vs C-N.
†p < 0.02 vs C-HTG.

Apoprotein B levels were determined by TMU precipitation. Results are expressed as means ± sd. See table 2 for explanation of abbreviations.
The distribution of VLDL and HDL soluble apoproteins was determined by analytical isoelectric focusing. This procedure, yielding reproducible apoprotein patterns with excellent resolution, failed to show any significant difference between patients and controls (tables 4 and 5). A reduced relative content of apo C-II in VLDL could be detected in hypertriglyceridemic patients and controls, as compared to normolipidemics (table 4). These findings confirm similar results for hypertriglyceridemic subjects without PVD, reported by others.24

### Discussion

In this study, the patients who had proven peripheral vascular disease, showed a highly significant enrichment of apo B in the VLDL fraction. Our previous studies, as well as those by other investigators, had shown that high VLDL, and particularly TG levels, are a frequent finding in patients with PVD.3,4 however, further studies on the characterization of the TG-rich lipoprotein fractions were not carried out. The hypertriglyceridemia of PVD patients, about 50% in both this and our previous study,5 is characterized by lipoprotein and apoprotein modifications (i.e., reduced HDL-C and VLDL- apo C-II) similar to those reported in Type IV patients with and without clinical atherosclerosis.24,25 Raised HDL levels are an established protective factor against atherosclerosis26 and, from a statistical point of view, raised TG are negatively correlated with reduced HDL-C.27 The HDL-C levels were in the normal range in the PVD-N patients in the present series; in contrast, Bradby et al.6 showed a reduction of HDL-C levels and particularly of apoproteins A-I and A-II in their PVD patients. The similarity of the apoprotein pattern in PVD-HTG patients with that previously described in Type IV hyperlipoproteinemia suggests that other lipoprotein changes (i.e., the increased VLDL-apo B) may be specific and independent.

Changes of lipoprotein composition have been recently indicated in clinical and experimental atherosclerosis. Modified lipoproteins may be more easily taken up by the scavenger pathway of tissue receptors.28 In animal models, apo E-rich VLDL may be the lipoprotein class with the highest affinity for tissue macrophages,29 whereas LDL may more specifically interact with human monocytes.30 An increased apo B content of chylomicron remnants has been recently related to an enhanced cholesterol esterification and stor-
age in fibroblasts. Experience derived from the in vitro studies may, of course, have a limited applicability to clinical findings. Increased plasma apo B levels, together with reduced apo A-I, are considered a better index than total and LDL-cholesterol for the prediction of severity of coronary artery disease. The validity of plasma apo B levels as a risk factor for coronary artery disease, was recently confirmed in patients with relatively low cholesterolemia. Studies on the apoprotein composition of selected lipoprotein fractions in clinical atherosclerosis have been more limited: the enrichment of LDL with apo B seemed, however, to provide a helpful discriminant in the analysis of metabolic risk factors for coronary atherosclerosis. The mechanism underlying variations in the apo B content of different lipoproteins is difficult to establish. An independent regulation of the TG and apo B secretion in VLDL has been recently suggested.

The role of lipoprotein changes in the induction of peripheral atherosclerotic lesions requires, of course, further evaluation. Other authors previously failed to demonstrate any direct correlation between apoprotein changes (reduced plasma total A-I and A-II) and the severity of arterial disease. In our series, plasma lipoproteins and hemodynamic parameters were not significantly correlated. Postischemic peak flow, although a quantitatively reliable index of the severity of disease, may be influenced by a variety of environmental and anatomic factors.

Data recently reported on increased apo B in LDL of coronary patients suggested that these have a large load of apparently "atherogenic" apoproteins in a slowly turning over lipoprotein fraction, whereas the apo B enriched VLDL of PVD patients are likely to have a faster turnover and are quantitatively less represented in plasma. An early conclusion from studies on the lipoprotein composition of PVD patients had suggested that arterial changes were clinically less severe than those occurring in coronary patients and less likely to induce a more serious, possibly lethal form of the disease.

It may be, therefore, concluded that the hypertriglyceridermia frequently observed in PVD patients has a lipoprotein and apoprotein pattern not different from that previously reported by others in Type IV patients. On the other hand, the enrichment of apo B in VLDL seems to be a typical change in these patients, possibly leading to an atherogenic condition of less ominous clinical prognosis than the LDL-apo B enrichment detected in coronary disease.

References


Index Terms: atherosclerotic peripheral vascular disease • type IV hyperlipoproteinemia • apoprotein B • very low density lipoproteins • plethysmography • HDL cholesterol • apoprotein C-II
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G Franceschini, A Bondioli, M Mantero, M Sirtori, G Tattoni, G Biasi and C R Sirtori

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