Lipoproteins and Their Genetic Variation in Subjects With and Without Angiographically Verified Coronary Artery Disease


To examine the concentration of serum lipoproteins and the association of their genetic variation with the occurrence of coronary artery disease (CAD), composite serum lipoprotein profiles including lipoprotein(a) (Lp[a]), apolipoprotein (apo) E phenotypes, and apo B Xba I genotypes were determined in patients with angiographically verified CAD (CAD+ group, n=111) and in subjects with no angiographic evidence of CAD (CAD- group, n=46). In addition, we determined the concentrations of serum lipids, lipoproteins, and apolipoproteins in 96 healthy controls. Both CAD- and CAD+ groups had lower concentrations of apolipoprotein A-I and A-II but higher concentrations of serum total and very low density lipoprotein triglyceride and very low density lipoprotein cholesterol than did healthy controls. The mean concentrations of serum total and low density lipoprotein cholesterol and the median values of Lp(a) were similar in the CAD+ and CAD- groups, both having higher concentrations of low density lipoprotein cholesterol and apo B than the healthy controls. Irrespective of gender, patients with CAD had significantly lower serum high density lipoprotein cholesterol than did those without CAD (1.48±0.40 versus 1.16±0.29 mmol/l, p<0.001). In women, the mean serum total and very low density lipoprotein triglyceride concentration was also higher in the CAD+ than in the CAD- group. The frequency of the apo E4 allele (e4) was significantly higher in the CAD+ group (0.193) than in the CAD- group (0.174; p<0.001). The frequencies of the two apo B alleles, X1 (Xba I restriction site absent) and X2 (Xba I restriction site present), were similar in the two groups. Stepwise discriminant analysis revealed that in men, serum high density lipoprotein cholesterol had the highest power to discriminate for CAD. In addition, the concentration of plasma apo B levels and the occurrence of apo E phenotypes were independently associated with CAD in men. In women, the only independent factor associated with CAD after adjustment for beta-blocker and diuretics usage was the concentration of serum triglycerides.

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Epidemiological and clinical studies have unequivocally demonstrated that elevated low density lipoprotein (LDL) cholesterol and apolipoprotein (apo) B concentrations are associated with an increased risk of coronary artery disease (CAD), whereas the relation of high density lipoprotein (HDL) cholesterol and plasma apo A-I concentrations to the risk of CAD is inverse. Whether elevated plasma triglyceride levels also act as an independent risk factor for CAD is controversial. This may be due to the fact that hypertriglyceridemia seldom occurs as an isolated entity but is usually associated with other risk factors such as low HDL or an increase of intermediate density lipoprotein (IDL). However, recent data reinforce the role of increased plasma triglyceride levels as an independent risk factor.

Molecular cloning of cDNAs encoding apolipoproteins, lipolytic enzymes, and other proteins involved in lipid metabolism has provided new tools to study the genetic background of CAD. Apo B, the main
protein of LDL, is required for synthesis, secretion, and metabolism of chylomicrons and very low density lipoproteins (VLDLs), and it also serves as a ligand for the interaction of LDL with its receptor.17 The apo B gene, which resides in the short arm of chromosome 2, is highly polymorphic at the DNA level.18-23 In some populations including the Finns, the Xba I restriction fragment length polymorphism (RFLP) of the apo B gene has been found to be associated with high serum cholesterol and triglyceride levels.20-22 This RFLP may influence susceptibility to myocardial infarction.24

Apo E is a constituent of VLDL and HDL and mediates the uptake of cholesterol ester–containing remnants of triglyceride-rich lipoproteins in the liver.25-26 Apo E displays genetic polymorphism that is determined by three common apo E alleles, e2, e3, and e4. The e4 allele is associated with hypercholesterolemia and increased levels of apo B, while the e2 allele has an opposite effect.25-29 In the Finnish population, the frequency of the e4 allele is higher than in other populations studied29 and may contribute to the increased prevalence of CAD in Finland,30 in accordance with previous observations.31

Elevated concentrations of plasma lipoprotein(a) (Lp[a]) are associated with CAD and acute myocardial infarction.16,32-36 Structural studies have demonstrated that Lp(a) is an LDL-like particle containing a glycoprotein, apo(a), which shares extensive homology with plasminogen.37-40 Because of its resemblance to plasminogen, Lp(a) has been suggested to interfere with the binding of plasminogen to endothelial cells and to impair fibrinolysis and thus promote atherosclerosis.41,42

The purpose of this study was to examine the interrelations between multiple lipoprotein phenotypes and genotypes as possible risk factors of CAD. We measured separately and in concert the associations of apo E phenotypes, the Xba I RFLP of the apo B gene, and the plasma concentration of Lp(a) with alterations of serum lipids, lipoproteins, and apolipoproteins and defined their discriminatory power for CAD as assessed by angiography. The control group comprised patients with no angiographically identified narrowing.

DNA Analysis

DNA was isolated from 10 ml venous blood.45 DNA (5–10 μg) was digested with the restriction
enzyme Xba I (Promega, Madison, Wis.; 2–5 units/μg DNA), fractionated by gel electrophoresis on 0.6% agarose, and transferred to nitrocellulose filters that were hybridized with a phosphorus-32-labeled apo B cDNA probe as described.23 The probe, pB23,24 was washed and dried. Filters were carried out by exposing them to Kodak XAR film for 1–5 days at -70°C. Autoradiography of the DNA, fractionated by gel electrophoresis on 0.6% agarose, and transferred to nitrocellulose filters that were hybridized with a phosphorus-32-labeled apo B cDNA probe was described.23 The probe, pB23,24 was a generous gift of Jan L. Breslow (The Rockefeller University, New York, N.Y.). Autoradiography of the washed and dried filters was carried out by exposing them to Kodak XAR film for 1–5 days at -70°C. The apo B allele resulting in the formation of an 8.6-kb Xba I restriction fragment (polymorphic site present), X1, and that generating a 5-kb fragment (polymorphic site present), X2. The apo B allele frequencies in a random Finnish population sample (n=176) were as follows: X1 allele, 0.58 and X2 allele, 0.42.23

Lipid and Lipoprotein Analyses

Lipoprotein(a) measurement. The concentration of Lp(a) was measured by using the Pharmacia Apo-lipoprotein (a) RIA 100 assay system. This Lp(a) assay is a solid-phase, two-site immunoradiometric assay that employs two monoclonal antibodies directed toward different epitopes on apo(a).46 The standard curve range was 16.0–840 mg Lp(a)/l. The coefficients of variation (CV) were 4.5% (intra-assay) and 4.9% (interassay). All samples were stored at -20°C until assayed.

Apolipoprotein E phenotyping. The apo E phenotypes were determined from serum by isoelectric focusing.29,47 The apo E allele frequencies in a random Finnish population sample (n=615) were as follows: ε2, 0.041; ε3, 0.733; and ε4, 0.227.29

Lipid analysis. Serum lipid and lipoprotein analyses were carried out on blood samples collected after a 12-hour fast. Lipoprotein fractions were isolated by sequential ultracentrifugation in a Beckman L7-70 ultracentrifuge (Beckman Instruments, Palo Alto, Calif.) using a Kontron TZT 45.6 rotor (Kontron AG, Basel, Switzerland). VLDL and LDL were isolated at densities of 1.006 and 1.063 g/ml, respectively. The density fraction <1.063 g/ml was considered to represent HDL. Cholesterol and triglyceride concentrations were determined by enzymatic methods.49,50 The serum apo A-I and A-II determinations were performed by immunoturbidimetry using monospecific gamma globulins (Boehringer GmbH, Mannheim, FRG).51 The interassay CVs of the apo A-I and A-II assays were 4.3% and 5.3%, respectively. The density fraction <1.063 g/ml was considered to represent HDL. Cholesterol and triglyceride concentrations were determined by enzymatic methods.49,50 The serum apo A-I and A-II determinations were performed by immunoturbidimetry using monospecific gamma globulins (Boehringer GmbH, Mannheim, FRG).51 The interassay CVs of the apo A-I and A-II assays were 4.3% and 5.3%, respectively. The apo B concentration was determined by radial immunodiffusion (Behringwerke GmbH, Marburg, FRG). The interassay CV of this method was 4.7%.

Statistical Analysis

All statistical calculations were performed using Biomedical Data Processing Programs (BMDP).52 Quantitative variables were compared by using a t test (two groups). For multiple pairwise comparisons, analysis of variance was used, followed by pairwise comparisons with Scheffe's method. For variables with highly skewed distributions, logarithmic transformation or nonparametric tests (Kruskall-Wallis analyses of variance and Kruskall-Wallis test adjusted for multiple comparisons) were employed.

The association between apo B Xba I polymorphism and the presence of CAD was assessed with the χ2 test. When the minimum estimated expected value was less...
than five, Fisher’s exact test was used. Bonferroni’s adjustment was used in multiple pairwise comparisons. Similar testing was applied to apo E. The frequency distributions of Lp(a) between subjects with and without CAD were compared by Kolmogorov-Smirnov’s knifed). The presence of the apo E phenotype and the median values of Lp(a) were compared by Kolmogorov-Smirnov’s test of the two-sample variety. The independent discriminatory power of each risk factor was assessed separately for both sexes by stepwise discriminant analysis. Variables significantly different in the univariate analyses (Table 2) were included in equations. The independent discriminatory power of each risk factor was assessed for both sexes, whereas the association of high serum triglyceride concentration with CAD was significant only for women. The mean concentrations of serum and LDL cholesterol and HDL cholesterol levels. Patients in the CAD+ group had significantly lower concentrations of serum HDL cholesterol, apo A-I, and apo A-II but higher concentrations of serum total cholesterol and VLDL triglyceride, VLDL cholesterol, and apo B than did the control group consisting of healthy volunteers. Similar trends were apparent for serum, LDL, and HDL cholesterol levels. Patients in the CAD+ group had significantly lower concentrations of serum HDL cholesterol, apo A-I, and apo A-II but higher concentrations of serum total and VLDL triglyceride than did either the CAD− subjects or the healthy controls. The difference in HDL cholesterol level between CAD− and CAD+ groups was significant for both sexes, whereas the association of high serum triglyceride concentration with CAD was significant only for women. The mean concentrations of serum and LDL cholesterol and the median values of Lp(a) were similar in the CAD− group had lower concentrations of apo A-I and A-II, whereas the patients in the CAD− group with a normal lipoprotein pattern (1.18±0.31 versus 1.56±0.41 mmol/l, p<0.001).

### Results

**Serum Lipids, Lipoproteins, Apolipoproteins, and Coronary Artery Disease**

The mean concentrations of serum lipids, lipoproteins, and apolipoproteins and the median concentration of Lp(a) in the CAD+ group, in subjects without angiographically detectable lesions (CAD− group), and in the population-based healthy controls are summarized in Table 2. Patients in the CAD− group had lower concentrations of apo A-I and A-II but higher concentrations of serum total and VLDL triglyceride, VLDL cholesterol, and apo B than did the control group consisting of healthy volunteers. Similar trends were apparent for serum, LDL, and HDL cholesterol levels. Patients in the CAD+ group had significantly lower concentrations of serum HDL cholesterol, apo A-I, and apo A-II but higher concentrations of serum total and VLDL triglyceride than did either the CAD− subjects or the healthy controls. The difference in HDL cholesterol level between CAD− and CAD+ groups was significant for both sexes, whereas the association of high serum triglyceride concentration with CAD was significant only for women. The mean concentrations of serum and LDL cholesterol and the median values of Lp(a) were similar in the CAD− group had lower concentrations of apo A-I and A-II, whereas the patients in the CAD− group with a normal lipoprotein pattern (1.18±0.31 versus 1.56±0.41 mmol/l, p<0.001).
TABLE 3. Apolipoprotein E Allele Frequencies in Patients With (CAD+) and Without (CAD-) Angiographically Verified Coronary Artery Disease

<table>
<thead>
<tr>
<th>Group/allele</th>
<th>CAD- patients</th>
<th>CAD+ patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>(n=46)</td>
<td>(n=111)</td>
</tr>
<tr>
<td>e4</td>
<td>0.174±0.041</td>
<td>0.293±0.032†</td>
</tr>
<tr>
<td>e3</td>
<td>0.751±0.064</td>
<td>0.676±0.040†</td>
</tr>
<tr>
<td>e2</td>
<td>0.076±0.058</td>
<td>0.031±0.035†</td>
</tr>
<tr>
<td>Men</td>
<td>(n=23)</td>
<td>(n=100)</td>
</tr>
<tr>
<td>e4</td>
<td>0.174±0.058</td>
<td>0.280±0.034†</td>
</tr>
<tr>
<td>e3</td>
<td>0.761±0.090</td>
<td>0.685±0.042†</td>
</tr>
<tr>
<td>e2</td>
<td>0.065±0.082</td>
<td>0.035±0.037</td>
</tr>
<tr>
<td>Women</td>
<td>(n=23)</td>
<td>(n=100)</td>
</tr>
<tr>
<td>e4</td>
<td>0.174±0.058</td>
<td>0.409±0.114†</td>
</tr>
<tr>
<td>e3</td>
<td>0.739±0.091</td>
<td>0.591±0.122*</td>
</tr>
<tr>
<td>e2</td>
<td>0.087±0.083</td>
<td>0.000±0.107†</td>
</tr>
</tbody>
</table>

Standard deviations of apolipoprotein E allele frequencies were calculated as described. Significance of the difference between allele frequencies of CAD- and CAD+ patients was calculated by the χ² test.

CAD, coronary artery disease.
*Denotes χ²=1.28 (not significant).

Apolipoprotein E and Apolipoprotein B Xba I Polymorphisms, Serum Lipids, Lipoproteins, and Coronary Artery Disease

Only two subjects exhibited the apo E 4/2 phenotype. When the patients were divided according to angiographic lesions, the frequency of the e4 allele in the CAD+ group (0.293) was significantly increased compared with that observed in the CAD- group (0.174) (contingency table χ²=4.16, p<0.05; Table 3). In female CAD+ patients, this increase in the frequency of the e4 allele was even more pronounced (0.409) compared with the 0.174 value observed for CAD- women (p<0.001). The apo E 3/2 phenotype was not represented among CAD+ women. In patients who had undergone angiography, subjects with the apo E 4/4 or E 4/3 phenotype had a significantly higher frequency of CAD than did those with the apo E 3/2 phenotype (78.6% versus 41.7%, p<0.05). The group of subjects who had the apo E 4/4 or E 4/3 phenotype also had significantly higher concentrations of serum and LDL cholesterol (6.47±1.20 versus 5.33±0.71 mmol/l, p<0.05, and 4.46±1.06 versus 3.55±0.06 mmol/l, p<0.05, respectively) and apo B (1.24±0.36 versus 0.93±0.18 mg/dl, p<0.05) than did subjects with the apo E 3/2 phenotype.

There was no significant difference in the distribution of the different apo B Xba I genotypes (X1X1, X1X2, and X2X2) or in the frequencies of the two alleles (X1 and X2) among CAD- and CAD+ patients (Table 4). Subjects with at least one X2 allele had, on average, 9% higher LDL cholesterol (4.46±1.02 versus 4.05±0.88 mmol/l, p<0.05) than did those homozygotic for the X1 allele. In contrast, the concentration of serum apo B was not significantly associated to the apo B Xba I genotype (data not shown).

Lipoprotein(a), Serum Lipids, Lipoproteins, and Coronary Artery Disease

The concentration of Lp(a) was under the detection limit (<16 mg Lp[a]/l) in 14% of the samples. The concentration of serum Lp(a) displayed a skewed distribution in both the CAD+ and CAD− groups and in the healthy control group. The cumulative frequency distribution curve of Lp(a) for the CAD+ group was shifted to higher values compared with that for CAD− patients, but the distribution curves were not significantly different by Kolmogorov-Smirnov's test (Figure 1A); neither were the median values of Lp(a) in the CAD+ and CAD− groups different from those in the healthy controls (Table 2). There was no difference in the concentration of Lp(a) among patients with one-, two-, or three-vessel disease (data not shown). In CAD+ patients with previous myocardial infarction, the concentration of Lp(a) was similar to that observed in patients with previous myocardial infarction.

![Figure 1A](http://atvb.ahajournals.org/)  
Cumulative frequency distribution (%) of CAD− (−) and CAD+ (−−) subjects according plasma lipoprotein(a) (Lp[a]) concentrations (mg/dl) in the patient cohort (panel A) and in subjects with hypercholesterolemia (low density lipoprotein cholesterol ≥4.5 mmol/l) (panel B) (maximal difference, 11.8%; p=NS). CAD, coronary artery disease.

**Table 4. Apolipoprotein B Xba I Genotypes and Allele Frequencies in Patients With (CAD+) and Without (CAD−) Coronary Artery Disease**

<table>
<thead>
<tr>
<th>Patient group</th>
<th>X1X1</th>
<th>X1X2</th>
<th>X2X2</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD+ patients</td>
<td>36</td>
<td>64</td>
<td>11</td>
<td>0.613 0.387*</td>
</tr>
<tr>
<td>CAD− patients</td>
<td>13</td>
<td>24</td>
<td>9</td>
<td>0.543 0.457*</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease.
*Denotes χ²=1.28 (not significant).
Discriminatory Power of Serum Lipids, Lipoproteins, and Apolipoproteins in Coronary Artery Disease

To evaluate the independent association of genetic variation, serum lipids, and lipoproteins to angiographically assessed CAD, stepwise discriminant analyses were performed separately for both sexes in the CAD+ and CAD− groups (Tables 5 and 6).

Factors significantly different in univariate analyses (Table 2) were included in the model, together with apo E phenotype and apo B XbaI 1 genotype; the dependent variable was the presence of CAD. In men, HDL cholesterol, apo B, and apo E phenotype remained independently and significantly associated with CAD (Table 5). Adjustment for the effect of age, hypertension, and smoking particularly diluted the power of HDL and increased the power of apo B. The prevalence of hypertension was higher in the CAD+ group than in the CAD− group. The use of β-blockers was more common in the CAD+ group (Table 1). Adjusting for current use of β-blockers and diuretics had no significant effect on the statistical power of HDL cholesterol, apo B, and apo E phenotype as discriminants. In women logarithmically transformed triglyceride values were the only

### Table 5. Stepwise Discriminant Analysis Between Men With (CAD+) and Without (CAD−) Coronary Artery Disease

<table>
<thead>
<tr>
<th>Factor</th>
<th>Age F statistics</th>
<th>Hypertension F statistics</th>
<th>Smoking* F statistics</th>
<th>β-Blockers/ +diuretics† F statistics</th>
<th>All‡ F statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol</td>
<td>10.7</td>
<td>&lt;0.01</td>
<td>5.8</td>
<td>&lt;0.01</td>
<td>12.8</td>
</tr>
<tr>
<td>Apo B</td>
<td>9.8</td>
<td>&lt;0.001</td>
<td>6.7</td>
<td>&lt;0.001</td>
<td>15.6</td>
</tr>
<tr>
<td>Apo E phenotype§</td>
<td>8.1</td>
<td>&lt;0.001</td>
<td>6.2</td>
<td>&lt;0.001</td>
<td>11.2</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apo B X2 allele</td>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Percent correct</td>
<td>69</td>
<td>69</td>
<td>71</td>
<td>68</td>
<td>77</td>
</tr>
</tbody>
</table>

### Table 6. Stepwise Discriminant Analysis Between Women With (CAD+) and Without (CAD−) Coronary Artery Disease

<table>
<thead>
<tr>
<th>Factor</th>
<th>Age F statistics</th>
<th>Hypertension F statistics</th>
<th>Smoking* F statistics</th>
<th>β-Blockers/ +diuretics† F statistics</th>
<th>All‡ F statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>16.7</td>
<td>&lt;0.001</td>
<td>8.2</td>
<td>&lt;0.01</td>
<td>8.4</td>
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<tr>
<td>Apo E phenotype</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
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<tr>
<td>HDL cholesterol</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apo B X2 allele</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL triglycerides</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Percent correct</td>
<td>76</td>
<td>73</td>
<td>79</td>
<td>76</td>
<td>76</td>
</tr>
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</table>

Variables significantly different in the univariate analyses were included in equations (Table 2). The grouping variable was the presence of coronary artery disease (one to three vessels). F statistics and respective probability values are given for each variable in the equation. CAD, coronary artery disease; Apo, apolipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; NS, not significant.

**Smokers** include both current and former smokers.

†Includes users of β-blockers and/or diuretics.

‡Adjusted for age, hypertension, smoking, and β-blocker and diuretics usage.
TABLE 7. Stepwise Discriminant Analysis Between Male Random Controls and Men With Coronary Artery Disease

<table>
<thead>
<tr>
<th>Factor</th>
<th>F statistics</th>
<th>p</th>
<th>F statistics</th>
<th>p</th>
<th>F statistics</th>
<th>p</th>
<th>F statistics</th>
<th>p</th>
<th>F statistics</th>
<th>p</th>
<th>F statistics</th>
<th>p</th>
<th>All‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-I</td>
<td>104.4</td>
<td>&lt;0.001</td>
<td>53.3</td>
<td>&lt;0.001</td>
<td>59.8</td>
<td>&lt;0.001</td>
<td>74.0</td>
<td>&lt;0.001</td>
<td>224.0</td>
<td>&lt;0.001</td>
<td>97.9</td>
<td>&lt;0.001</td>
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<tr>
<td>VLDL triglycerides</td>
<td>92.5</td>
<td>&lt;0.001</td>
<td>61.4</td>
<td>&lt;0.001</td>
<td>66.0</td>
<td>&lt;0.001</td>
<td>71.4</td>
<td>&lt;0.001</td>
<td>161.1</td>
<td>&lt;0.001</td>
<td>85.3</td>
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<tr>
<td>LDL cholesterol</td>
<td>66.7</td>
<td>&lt;0.001</td>
<td>50.0</td>
<td>&lt;0.001</td>
<td>53.9</td>
<td>&lt;0.001</td>
<td>57.2</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td></td>
</tr>
<tr>
<td>Apo A-I</td>
<td>52.2</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>45.1</td>
<td>&lt;0.001</td>
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<tr>
<td>Triglycerides</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
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</tr>
<tr>
<td>HDL cholesterol</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>VLDL cholesterol</td>
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</tr>
<tr>
<td>Apo B</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tr>
</tbody>
</table>

Percent correct classifications 88 87 89 88 92 92

Variables significantly different in the univariate analyses were included in equations (Table 2). The grouping variable was the presence of coronary artery disease (one to three vessels). F statistics and respective probability values are given for each variable in the equation. Apo, apolipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; NS, not significant.

*Includes users of β-blockers and/or diuretics.
†Adjusted for age, hypertension, smoking, and β-blocker and diuretics usage.
‡Adjusted for the effect of hypertension, smoking, age, and β-blocker and diuretics usage.

independent discriminators for CAD in stepwise discriminant analyses (Table 6). The power of triglycerides remained significant after adjusting for age, hypertension, smoking, and current use of β-blockers and diuretics.

On stepwise discriminant analysis, apo A-I and A-II concentrations eliminated the power of HDL cholesterol to discriminate between healthy controls and CAD+ men (Table 7). In addition, VLDL triglyceride and LDL cholesterol also were significant independent discriminants for CAD. In women, only VLDL cholesterol and apo A-I, in this order, discriminated between patients and controls (Table 8). The concentrations of serum total and VLDL triglycerides were not significant discriminants for CAD in this grouping. It should be kept in mind that the genetic markers were not included in these analyses. Consequently, the discriminative power of different parameters may be variable when compared in CAD− versus CAD+ groups.

Discussion

This study is cross sectional in design and, thus, is unlike prospective studies that are vulnerable to bias selection due to the selection of an appropriate control group. In our study, the cases are representative of patients with symptomatic coronary disease who were selected for angiography entirely on a
clinical basis. We deliberately selected a control group comprising patients who had no significant lesions as detected by coronary angiography to discriminate unequivocally between patients with and without coronary disease. In addition, we also included another control group comprising healthy volunteers chosen to represent the Finnish population at large. Because these random controls were not assessed for coronary atherosclerosis by angiography, there is a possibility that a few of the subjects may have clinically undetected ischemia.

It is clear that environmental and behavioral risk factors modulate the concentration of serum lipids and lipoproteins.53-56 In this study, the prevalence of hypertension and smoking was higher in the CAD+ patients than in patients without CAD. The group of healthy controls was almost free of hypertension and the use of drugs and included fewer smokers. The impact of these factors on serum lipids and lipoproteins is emphasized by the differences observed between these two control groups (healthy controls versus CAD- patients). The association between lipid abnormalities and hypertension is well established, and elevation of serum triglyceride, serum total cholesterol, and LDL cholesterol concentrations and lowering of HDL cholesterol are observed in hypertensives.56-58 These changes are further aggravated by the use of thiazides and β-blockers,59,60 and even the selective β-blockers, which were the most commonly used among our patients, are not free of adverse effects on lipid metabolism.59-61 Consequently, comparison of groups of CAD+ patients with healthy volunteers from the general population overcomes the possible effect of these confounding factors commonly present in patients referred for angiography.

Population-based studies have defined HDL cholesterol as an inverse risk factor for coronary heart disease,5,6 and intervention trials have indicated that elevation of HDL can increase the reduction of CAD risk even beyond that caused by a lowering of LDL cholesterol alone.62-64 In the present study, CAD+ patients had lower concentrations of serum HDL cholesterol, apo A-I, and apo A-II, but the concentrations of serum total and VLDL triglycerides were higher than in CAD- subjects or in healthy volunteers. In this study, the serum concentrations of total and LDL cholesterol did not differ between patients with and without angiographically verified CAD.

In stepwise discriminant analyses including only CAD+ and CAD- groups, HDL cholesterol allowed the best separation between CAD- and CAD+ men, followed by apo B and apo E phenotype (Table 5). The discriminatory power of HDL cholesterol persisted even when apo B and genetic markers, which were predicted to be associated with the risk of CAD, were included in the model. However, the power of HDL cholesterol was particularly sensitive to the impact of confounding factors (Table 5). Interestingly, apo A-II and apo A-I were better than HDL cholesterol at discriminating between random controls and CAD+ men. The data allow no clear conclusion as to whether apo A-I and/or apo A-II are superior to HDL cholesterol for identifying subjects with CAD,65 although this does not alter the major conclusion regarding the role of HDL particles. The importance of low HDL as a risk factor was especially striking in those men who did not have elevations of serum triglyceride or cholesterol values. Recent data from the Framingham study also emphasize the increased risk for myocardial infarction and coronary death in subjects who are in the lowest quartile of HDL cholesterol,66,67 and in this cohort, HDL cholesterol emerges as the most important predictor of CAD risk. Data from the Prospective Cardiovascular Münster Study also identify HDL cholesterol as a strong predictor of CAD risk.68

In subjects who do not exhibit an elevation of serum total and LDL cholesterol, the role of HDL as a risk factor seems to be even more pronounced.66 In our study, one third of CAD+ patients had a normal lipoprotein phenotype. Even these patients, however, had one striking lipoprotein abnormality: their HDL cholesterol level was 25% lower than that in subjects without angiographically verified CAD. Although Holmes et al69 found no significant association between HDL cholesterol and CAD verified angiographically in men, the occurrence of hypoalphalipoproteinemia as the most common lipid abnormality in patients with CAD has been reported in several studies.5,70,71

The elevations of serum total and VLDL triglycerides in CAD patients suggest an association of elevated triglycerides with CAD. The difference between CAD+ patients and CAD- patients was clearer in women than in men. In stepwise discriminant analyses, the impact of serum triglycerides to distinguish between CAD- and CAD+ patients was confounded by HDL cholesterol in CAD- versus CAD+ men. Nevertheless, VLDL triglyceride emerged as a significant discriminant for CAD in analyses of CAD+ patients and random controls. In women, serum triglycerides emerged as the only significant discriminant for CAD. Similarly, Reardon et al72 demonstrated that in women with angiographically verified CAD, serum triglyceride was significantly related to the score for severity of CAD, but this association was not valid in men. In addition, the association in women persisted after controlling for HDL cholesterol. A positive univariate association between triglycerides and CAD existed for women but not for men in the study by Holmes et al.69 The observed difference in risk factors between the sexes is interesting, but the mechanism of the effect is unknown.

The role of serum triglycerides as a risk factor for CAD is stronger in people who have low or normal serum total cholesterol and low HDL cholesterol.8 Data from the Paris prospective study also emphasize the role of serum triglycerides as a predictor of CAD in subjects with low levels of serum cholesterol.14 In a recent study by Barbir et al,71 the serum concen-
istration of triglycerides was the best discriminator between male CAD patients and controls. In this study, abnormal values of both triglyceride and HDL cholesterol were six times more common in patients than in controls.71 Similarly, VLDL triglyceride was a significant discriminant between our CAD+ patients and random controls.

Our observation that neither serum total nor LDL cholesterol had any discriminatory power between CAD+ and CAD− patients was somewhat unexpected. LDL cholesterol discriminated between random controls and CAD+ men as shown in the majority of studies,65 but this was absent after adjustment for use of β-blockers and diuretics. Our observation that apo B was better than LDL cholesterol as a risk marker agrees with those from another Finnish study, in which survivors of myocardial infarction had similar total and LDL cholesterol levels as the controls but differences in LDL proteins.72 In contrast, Aro et al.,74 who studied a population from eastern Finland, observed that both LDL cholesterol and apo B were predictors of the severity of CAD. The lack of power for serum total and LDL cholesterol to discriminate between CAD− and CAD+ groups may have several explanations. First, the mean level of serum cholesterol in Finland, particularly in the Helsinki area, has declined significantly during 1980s. Second, changes in lifestyle and dietary habits may have preferentially taken place in patients with symptoms or signs of CAD. Third, the responses of serum and LDL cholesterol levels to changes in cholesterol and fat content in the diet seem to be more striking in people who have either the apo E 4/4 or the apo E 4/3 phenotype,75,76 that is, in those who were over-represented among our CAD patients.

The genetic variety of apo E has effects on serum lipid and lipoprotein levels that may either directly or indirectly influence susceptibility to atherosclerosis.25,26,29 The apo E polymorphism exerted similar effects on serum total cholesterol in men with and without CAD, with the average difference in total cholesterol being about 1.1 mmol/l between apo E 4/4 and E 4/3 phenotypes combined and the apo E 3/2 phenotype. These data agree well with the results of Lenzen et al.77 Stepwise discriminant regression analyses including CAD+ and CAD− groups demonstrated that in men, apo E phenotype distribution was an independent predictor of CAD irrespective of plasma apo B level. Thus, the genetic variation at the apo E gene locus contributes to CAD risk independently of its effect on apo B and LDL cholesterol level. This observation is in agreement with some previous studies.26,30,31

An association between elevated serum Lp(a) concentration and risk of atherosclerosis has been demonstrated in several studies.16,32–36 The relative risk associated with elevated Lp(a) levels has varied from 1.6 to 3.6.35 In the present study, there was no significant difference between the medians of Lp(a) in patients with and without CAD in comparison with the control group. It is possible that other genetic factors, such as the strong impact of the apo E phenotypes, mask the association of Lp(a) with CAD in the Finnish population. Furthermore, the lack of difference in Lp(a) concentration between CAD cases and controls is not a unique finding.35,78,79 Evidence has been presented that the predictive value of Lp(a) is related to the concentration of LDL cholesterol. According to the study of Armstrong et al,80 the association of Lp(a) with CAD is not apparent at concentrations of serum total and LDL cholesterol that are below the median values for CAD patients. The fact that neither serum total nor LDL cholesterol levels were an independent risk factor for CAD in the present study may mask the impact of Lp(a).

We have previously demonstrated that in the Finnish population, the serum cholesterol level is 11% higher in subjects who are either heterozygous or homozygous for the X2 allele compared with those who are homozygous for the X1 allele.23 The association between the apo B Xba I polymorphism and serum LDL cholesterol level may be population dependent24,81,82 and possibly explained by changes in LDL metabolism. Individuals with the X2X2 genotype have a significantly lower fractional removal rate of LDL than do those with the genotype X1X1.83,84 The change in LDL catabolism is particularly reflected in its plasma levels in those individuals who exhibit no overproduction of LDL.84 In one study, the frequency of the X1 allele was increased in patients with previous myocardial infarction.24 In the present study, there was a trend for a higher frequency of the X1 allele in patients with CAD, although the difference was not significant. Likewise, the X1 allele was slightly but not significantly more frequent in CAD patients than in controls in a recent study by Myant et al.85 Furthermore, Monsalve et al86 reported that the X1 allele occurred significantly more often in a London population of patients with carotid and/or coronary artery disease.

The present data emphasize the multifaceted nature of coronary disease with the clustering of several independent risk factors. The practical conclusion from this observation is that measurements of both HDL cholesterol and triglycerides, in addition to that of total cholesterol, are necessary in the evaluation of individuals at risk for CAD. This is particularly important in men with normal serum cholesterol levels.

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References


58. Roberts WC: Recent studies on the effects of beta blockers on blood lipid levels. *Am Heart J* 1989;117:709-714


76. Ololason BO, Dahlen G, Nilsson TK: Evidence for increased levels of plasminogen activator inhibitor and tissue plasminogen activator in plasma of patients with angiographically verified coronary artery disease. *Eur Heart J* 1988;10:77-82


between the apolipoprotein B XbaI polymorphism and blood lipid levels in a Swedish population. *Atherosclerosis* 1989;75:183–188


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