Oxidative modification of LDL makes it more atherogenic than its native form in many ways; eg, it is more rapidly taken up by macrophages, and in addition, it is immunogenic.\(^1\) Oxidized LDL (oxLDL) is present in human atherosclerotic lesions,\(^2\) and autoantibodies against oxLDL have been detected in human and animal plasma and atherosclerotic lesions.\(^3,4\) It is not known whether these antibodies are just an indication of oxidative modification of LDL in the body or whether they contribute to the pathogenesis of atherogenesis.

Antiphospholipid antibodies, which contain antibodies against cardiolipin, phosphatidylserine, and phosphatidylethanolamine, have traditionally been linked with inflammatory and autoimmune diseases, such as systemic lupus erythematosus.\(^5\) The epitopic sites detected by the conventional anti-cardiolipin antibody assays include \(\beta_2\)-glycoprotein 1, cardiolipin, or a complex of \(\beta_2\)-glycoprotein 1 and cardiolipin.\(^5,8\) The major source of \(\beta_2\)-glycoprotein 1 in the assay is bovine \(\beta_2\)-glycoprotein 1 from the bovine serum or bovine serum albumin used in blocking and the sample diluent, and a minor source is human \(\beta_2\)-glycoprotein 1 from the test serum or plasma. It is also suggested that neoepitopes of oxidized phospholipids or adducts of oxidized phospholipids and associated proteins, including \(\beta_2\)-glycoprotein 1, could account for the antigenicity.\(^9\) Cross-reactivity between autoantibodies against cardiolipin and against oxLDL has been shown in patients with systemic lupus erythematosus.\(^10\)

Several studies have been conducted to investigate the role of autoantibodies against oxLDL (reviewed by Ylä-Herttuala\(^11\) in 1998) and cardiolipin in atherogenesis. However, the results are conflicting. In some prospective studies, antibodies against oxLDL have predicted myocardial infarction\(^12,13\) and progression of carotid atherosclerosis.\(^4\) There are also studies that have not found any association between anti-oxLDL antibodies and the extent of atherosclerosis\(^14–16\) or restenosis after balloon angiography.\(^17\) In addition to antibodies against oxLDL, those against cardiolipin have also predicted myocardial infarction in men\(^13,18\) and have been associated with ischemic heart disease,\(^19\) thrombosis,\(^20,21\) recurrent myocardial infarction,\(^22\) and restenosis after coronary bypass graft.\(^23\) On the contrary, some investigators\(^24–26\)
have not found an association between anti-cardiolipin antibodies and atherosclerosis or myocardial infarction. Because the presentation and severity of coronary heart disease (CHD) could affect the levels of antibodies against oxLDL and cardiolipin, we determined their levels at least 6 months after discharge from the hospital in 4 groups of patients with CHD: patients with (1) coronary artery bypass surgery, (2) balloon angioplasty, (3) myocardial infarction, and (4) myocardial ischemia. We also evaluated the effect of dietary variables on the levels of these antibodies.

### Methods

#### Subjects

European Action on Secondary Prevention through Intervention to Reduce Events (EUROASPIRE) was a survey involving the management of risk factors and the use of cardiovascular drugs in secondary prevention of CHD. According to the EUROASPIRE study protocol, consecutive male and female patients with CHD aged <71 years were identified from the hospital discharge lists and coronary angiography register of the Kuopio University Hospital from the following 4 diagnostic categories: (1) patients with their first elective or emergency coronary artery bypass surgery (CABG), (2) patients with their first elective or emergency percutaneous transluminal coronary angioplasty (PTCA) but with no previous CABG, (3) patients with their first or recurrent acute myocardial infarction (AMI) but with no previous CABG or PTCA, and (4) patients with acute myocardial ischemia (AMIS) but no previous CABG, PTCA, or AMI.

For each diagnostic category, 125 consecutive patients (with the exception of 156 patients for the AMI category) were identified. Patients hospitalized before November 1, 1994, were invited for an interview and examination at least 6 months after hospitalization. From the respective CABG, PTCA, AMI, and AMIS groups, 109, 106, 101, and 99 patients participated; 1, 4, 20, and 0 patients were dead; and 15, 15, 35, and 26 patients did not participate (there was no response to invitation, they refused, or travel was impractical). The median time interval between hospital admission and the interview and examination for the respective patient groups was 1.0 (range 0.8 to 1.3) years, 1.9 (range 0.9 to 4.0) years, 2.3 (range 0.9 to 3.7) years, and 2.2 (range 1.0 to 3.8) years. Extensive background information, including weight, height, smoking (breath carbon monoxide measurement), blood pressure, and diabetes, was collected as described. Fasting blood samples were collected for the analysis of serum lipids, fatty acid profile of serum lipids, and autoantibodies against oxLDL and cardiolipin. Standardized enzymatic methods were used for the analysis of serum total cholesterol, HDL cholesterol, and triglycerides (Boehringer GmbH kits 237574 and 701904). The study was approved by the Ethics Committee at the University of Kuopio. All patients gave their informed consent for the study.

#### Autoantibodies Against oxLDL and Cardiolipin

LDL was isolated from pooled plasma of 2 healthy donors as described earlier, and oxLDL was prepared by oxidation of LDL with 20 μmol/L copper at 37°C for 48 hours. A modified ELISA was used to determine autoantibodies against oxLDL. One half of a flat-bottomed microtiter plate (Polyisorb, Nunc) was coated with

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>CABG (n=109)</th>
<th>PTCA (n=106)</th>
<th>AMI (n=101)</th>
<th>AMIS (n=99)</th>
<th>P, ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>87/22</td>
<td>66/40</td>
<td>75/26</td>
<td>57/42</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>61.5±7.3</td>
<td>57.2±7.5</td>
<td>61.9±8.2</td>
<td>63.5±8.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.2±3.6</td>
<td>28.0±4.0</td>
<td>28.2±4.3</td>
<td>28.2±4.3</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.62±0.91</td>
<td>6.03±1.16</td>
<td>6.39±1.18</td>
<td>6.50±1.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.15±0.25</td>
<td>1.25±0.27</td>
<td>1.25±0.33</td>
<td>1.27±0.32</td>
<td>0.015</td>
</tr>
<tr>
<td>Total triglycerides, mmol/L</td>
<td>1.86±0.87</td>
<td>1.89±1.15</td>
<td>2.09±2.31</td>
<td>1.96±1.38</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>140.0±21.1</td>
<td>139.6±19.6</td>
<td>142.6±24.8</td>
<td>141.5±24.0</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>81.6±11.4</td>
<td>81.4±11.0</td>
<td>83.1±11.8</td>
<td>82.2±13.9</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetics, n (%)</td>
<td>13 (11.9)</td>
<td>12 (11.3)</td>
<td>17 (16.8)</td>
<td>22 (22.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>10 (9.2)</td>
<td>17 (16.0)</td>
<td>14 (13.9)</td>
<td>12 (12.1)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD. E% indicates percentage of energy.

### Table 2. Nutrient Intake and Fatty Acid Profile of Serum CE

<table>
<thead>
<tr>
<th></th>
<th>CABG (n=106)*</th>
<th>PTCA (n=105)*</th>
<th>AMI (n=100)*</th>
<th>AMIS (n=91)*</th>
<th>P, ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, MJ/d</td>
<td>7.27±2.17</td>
<td>7.41±2.27</td>
<td>7.29±1.92</td>
<td>6.98±2.19</td>
<td>NS</td>
</tr>
<tr>
<td>Fat, E%</td>
<td>32.5±6.6</td>
<td>32.7±5.9</td>
<td>32.1±7.0</td>
<td>33.6±6.9</td>
<td>NS</td>
</tr>
<tr>
<td>Saturated fat, E%</td>
<td>12.7±4.1</td>
<td>12.8±3.6</td>
<td>12.6±3.8</td>
<td>13.5±3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Monounsaturated fat, E%</td>
<td>11.2±2.6</td>
<td>11.4±2.5</td>
<td>11.1±2.8</td>
<td>11.4±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Polyunsaturated fat, E%</td>
<td>5.9±1.9</td>
<td>5.7±1.5</td>
<td>5.7±1.6</td>
<td>5.9±2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin E, mg/d</td>
<td>9.5±4.2</td>
<td>9.2±3.9</td>
<td>9.2±3.5</td>
<td>8.9±4.0</td>
<td>NS</td>
</tr>
<tr>
<td>CE oleic acid, mol%</td>
<td>21.57±2.87</td>
<td>21.28±2.25</td>
<td>21.35±2.54</td>
<td>21.37±2.58</td>
<td>NS</td>
</tr>
<tr>
<td>CE linoleic acid, mol%</td>
<td>48.33±6.48</td>
<td>49.12±5.34</td>
<td>48.01±6.30</td>
<td>48.07±5.86</td>
<td>NS</td>
</tr>
<tr>
<td>CE γ-linolenic acid, mol%</td>
<td>0.67±0.25</td>
<td>0.68±0.31</td>
<td>0.70±0.32</td>
<td>0.65±0.26</td>
<td>NS</td>
</tr>
<tr>
<td>CE arachidonic acid, mol%</td>
<td>5.54±1.51</td>
<td>5.63±1.38</td>
<td>5.41±1.16</td>
<td>5.38±1.41</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD. E% indicates percentage of energy.

*P<0.001.

### References

27. EUROASPIRE II: A survey involving the management of risk factors and the use of cardiovascular drugs in secondary prevention of CHD.循证医学欧洲计划 II：关于使用心血管药物管理风险因素的调查研究。
Autoantibodies against cardiolipin were also determined by ELISA. One half of a flat-bottomed microtiter plate (Maxisorp, Nunc) was coated with absolute ethanol, and the other half was coated with cardiolipin (Sigma Chemical Co, 50 μg/mL in absolute ethanol) at 25 μL per well. Ethanol was evaporated to dryness under a stream of nitrogen, and plates were incubated at room temperature for 24 hours to oxidize the cardiolipin. The plates were blocked with PBS containing 2% BSA, 0.27 mmol/L EDTA, and 20 μmol/L BHT at 150 μL per well for 2 hours. Plates were washed 3 times with PBS containing 0.05% Tween 20 (Wellwash 4 MK II, Labsystems Oy). Serum samples were diluted (1:50) in PBS containing 1% BSA, 0.05% Tween 20, 0.27 mmol/L EDTA, and 20 μmol/L BHT and pipetted on plates at 50 μL per well. Plates were incubated for 2 hours and washed as described above. Horseradish peroxidase–conjugated anti-human IgG (Cappel) diluted 1:4000 in the sample buffer was placed on the plates at 50 μL per well and incubated for 2 hours. After the plates were washed, adding the substrate (3,3',5,5'-Tetramethylbenzidine [Riedel de Haën] as chromogen), stopping the color reaction, and measuring the absorbances were performed as described for the oxLDL ELISA, but the reaction volume was 50 μL per well. All the incubations were carried out at room temperature. The results were calculated by subtracting the binding to ethanol-coated wells from the binding to cardiolipin-coated wells after subtracting the mean background binding to the wells. On each plate, 2 standard serum samples were analyzed, and their absorbances should be at a limit of mean ±10% to accept the results of unknown samples on the plate. A narrower acceptance limit was used for the anti-cardiolipin than for the anti-oxLDL antibody assay because of the more homogeneous antigen.

Dietary Assessment

Dietary intake was measured by a 4-day food record. The nutrient intake was calculated by using the Micro-Nutra dietary analysis program (version 2.0, Finnish Social Insurance Institute, Turku, Finland), which is based on the national database of the Finnish Social Insurance Institute.

The fatty acid profile of serum cholesteryl esters (CEs) was used as a biomarker of dietary fat quality. Lipids were extracted from the serum sample with chloroform-methanol (2:1, vol/vol). Lipid fractions were separated with an aminopropyl column. Fatty acids were analyzed with a gas chromatograph (Hewlett-Packard 5890 series II, Hewlett-Packard Co) equipped with an FFAP column (Hewlett-Packard), with helium as a carrier gas. Fatty acids are presented as molar percentages of total fatty acids.

Statistical Analyses

Statistical analyses were performed by use of the SPSS for Windows program (version 6.0.1, SPSS Inc). Normal distribution of variables was checked with the Kolmogorov-Smirnov (Lilliefors) test, and logarithmic transformation was used for those not normally distributed. The basic characteristics, nutrient intakes, and fatty acids among the groups were tested by ANOVA. Differences in the autoantibody levels among the groups and among the quartiles of dietary intake were analyzed by ANOVA. When differences in autoantibodies between 2 groups (eg, men versus women) were analyzed, a t test for independent samples was used. Pearson correlation coefficients were calculated between autoantibodies and dietary and clinical variables. The χ² test was used when categorical variables were compared. The results for continuous variables are expressed as mean ±SD, except for Figure 2, for which mean ±SEM is used. A value of P<0.05 (2-tailed) was considered statistically significant.

**TABLE 3. Autoantibodies Against Cardiolipin and oxLDL**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CABG Men</th>
<th>CABG Women</th>
<th>PTCA Men</th>
<th>PTCA Women</th>
<th>AMI Men</th>
<th>AMI Women</th>
<th>AMIS Men</th>
<th>AMIS Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-cardiolipin</td>
<td>0.49±0.25</td>
<td>0.38±0.26</td>
<td>0.44±0.23</td>
<td>0.44±0.23</td>
<td>0.52±0.25</td>
<td>0.53±0.25</td>
<td>0.53±0.31</td>
<td>0.43±0.21</td>
</tr>
<tr>
<td>Anti-oxLDL*</td>
<td>1.91±1.41</td>
<td>1.86±0.91</td>
<td>2.11±2.19</td>
<td>1.59±0.89</td>
<td>2.52±2.05</td>
<td>1.66±0.67</td>
<td>1.96±1.78</td>
<td>1.85±1.39</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.022 among the groups for men (ANOVA).
Results

Table 1 shows the basic characteristics of the patients. The mean age of all patients was 61 (range 33 to 74) years. Serum total cholesterol was lower in patients with CABG than in patients with AMI or AMIS, and it was also lower in patients with PTCA than in patients with AMI because of the more frequent use of lipid-lowering drugs in patients in the operated groups (56%) than in patients with AMI or AMIS (21%). Serum HDL cholesterol was lower in patients with CABG than in patients with AMIS. Serum triglycerides, body mass index, blood pressure, and prevalence of diabetes and smoking did not differ among the groups. There were no differences in dietary intake of energy, different fatty acids, or vitamin E among the groups. The distributions of autoantibodies against cardiolipin and oxLDL in the study population were skewed to the right (Figure 1). Antibodies against oxLDL were higher in men (Table 2). The proportions of oleic, linoleic, \( \gamma \)-linolenic, and arachidonic acids in CEs were similar in all groups. Of the whole study group, 18% used vitamin supplements, and the use did not differ among the groups.

The distributions of autoantibodies against cardiolipin and oxLDL in the study population were skewed to the right (Figure 1). Antibodies against oxLDL were higher in men with AMI than in other groups \((P=0.022, \text{Table 3})\). Controlling for age, serum cholesterol, diastolic blood pressure, smoking, and diabetes in the ANOVA did not change the statistical significance of the difference in anti-oxLDL antibodies among the groups in men \((P=0.023)\). In women, there was no difference in antibodies against oxLDL among the groups. The titers of anti-cardiolipin antibodies did not differ among the groups in men or in women. The relation of recurrent coronary events after the index hospitalization to autoantibody titers was also analyzed. Of the patients with CABG, 5 had experienced CABG, PTCA, AMI, or AMIS after the hospitalization; the corresponding figures were 35 in patients with PTCA, 31 in patients with AMI, and 43 in patients with AMIS. Antibody titers against oxLDL and cardiolipin did not differ between those patients who had experienced recurrent events and those patients who had not (data not shown). The autoantibodies against cardiolipin and oxLDL did not differ between diabetic and nondiabetic patients or between patients with and without elevated blood pressure (systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg; data not shown).

Correlation coefficients were calculated between antibody titers, serum lipids, and fatty acids in CEs for men and women separately (Table 4). Autoantibodies against cardiolipin and oxLDL were not correlated with each other \((r=0.082, P=0.097)\). There were no significant correlations between autoantibodies and serum lipids, except that triglyceride concentration was positively correlated with autoantibodies against oxLDL in women \((P=0.057, \text{Table 4})\). Dietary intake of polyunsaturated fat (PUFA) was inversely correlated with autoantibodies against oxLDL and cardiolipin in men but not in women. Also, dietary intake of vitamin E was inversely correlated with anti-cardiolipin autoantibodies in men. The intakes of vitamin E and PUFA were highly correlated \((r=0.588, P<0.001)\). The proportion of linoleic acid in CE was inversely related and that of \( \gamma \)-linolenic acid was positively related to autoantibodies against cardiolipin.

The relations of PUFA and vitamin E with autoantibody levels were further studied by analyzing the antibody titers against oxLDL and cardiolipin in sex-specific quartiles of intakes of vitamin E and PUFA (Figure 2). The autoantibody levels against cardiolipin were higher in men in the lowest quartiles of vitamin E or PUFA intake than in men in the highest quartiles of intakes.

Discussion

The aim of the present study was to evaluate the ability of the autoantibodies against oxLDL and cardiolipin to distinguish patients with different manifestations of CHD. The main finding for a large number of CHD patients was that men with AMI had higher titers of antibodies against oxLDL than did men with CABG, PTCA, or AMIS. The antibodies against cardiolipin did not differ among the patient groups nor in men or in women. The dietary intakes of vitamin E and PUFA were inversely associated with antibodies against cardiolipin in men.

Men with AMI had higher antibody titers against oxLDL than did men in the other groups, even though the analysis was controlled for known cardiovascular risk factors. The time interval between the hospitalization and autoantibody...
measurement was 0.8 to 4.0 years, and it is possible that the severity and presentation of the disease could have changed before the measurement of antibodies. However, recurrent events after the index hospitalization were not associated with autoantibodies against cardiolipin or cardiolipin.

To date, normal values for anti-oxLDL antibody measurements have not been determined in any studies, but based on an unrelated control population (n=341) analyzed independently in our laboratory, the mean value for anti-oxLDL antibody titer was 1.83 (SD 1.17), and titers >4.17 (mean±2 SD) could be regarded as high (O.N. et al, unpublished data, 1999). Similarly, the mean value for anti-cardiolipin antibodies was 1.83 (SD 1.17), and titers >1.47 (mean±2 SD) could be regarded as high. The distribution of the antibody titers in the control population did not differ from that observed in the CHD patients.

As analyzed together, all men had higher anti-oxLDL antibody titers and a tendency to higher anti-cardiolipin antibodies than did all women (oxLDL 2.13±1.87 [men] versus 1.73±1.05 [women], P=0.026; cardiolipin 0.49±0.26 [men] versus 0.44±0.23 [women], P=0.073). However, this finding needs to be confirmed in other studies, because in most previous studies, there is no mention of sex differences in antibody levels, and either sex may be included in the results.12–20,29–31

There were no significant correlations between serum lipids and autoantibodies, except for a positive one between serum triglycerides and antibodies against oxLDL in women. Results of earlier studies have been contradictory but suggest an inverse relation of HDL cholesterol12,14 and a positive relation of total or LDL cholesterol12 and antibodies against oxLDL, but several studies have not found any association between lipid and lipoprotein concentrations and antibodies against oxLDL.4,13,33 Based on the present study and earlier studies, serum lipid concentrations do not seem to be a major determinant of autoantibodies against oxLDL and cardiolipin.

The dietary intakes of PUFA and vitamin E were inversely associated with antibodies against cardiolipin in men. Fats and oils are the major source of vitamin E in the average diet of the Finnish population42; thus, the intakes of vitamin E and PUFA were highly correlated in the present study. The intake of vitamin E in the present study corresponded well to the average intake in the Finnish population (men, 10 mg/d; women, 8 mg/d).35 Autoantibodies against malondialdehyde-derivatized LDL or copper oxLDL have been inversely associated with α-tocopherol concentrations in serum or in LDL,15,29,36 although 1 study found no correlation between antibodies against oxLDL and plasma LDL vitamin E concentration.37 In a supplementation trial, autoantibodies against oxLDL were decreased after 4 months of vitamin E treatment in hypercholesterolemic smokers.38 To our knowledge, there are no studies reporting an association between antibodies against cardiolipin and dietary vitamin E.

Dietary fatty acids are reflected in the fatty acid profile of serum lipids.39 In the present study, the inverse correlation between the proportion of linoleic acid in CEs and anti-cardiolipin antibodies is consistent with the inverse association between dietary intake of PUFA and anti-cardiolipin antibodies. In previous studies, it has been shown that the dietary fatty acids affect the susceptibility of LDL to oxidation, with the linoleic acid–enriched diet enhancing the susceptibility of LDL to oxidation compared with the oleic acid–enriched diet.40 However, high intake of vitamin E was connected to high intake of PUFA in the present study and may affect the associations of PUFA and antibodies.

Autoantibodies against cardiolipin and oxLDL were not correlated with each other, which is corroborated by an earlier follow-up study in 50-year-old men.13 On the other hand, significant correlations between antibodies against oxLDL and cardiolipin18,33 and cross-reactivity between the 2 antibodies10 have also been reported. It may imply that in addition to possible differences in the ELISA assays used in the present study, other patient groups. The antibodies also showed no association with recurrent coronary events.
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Erkkilä et al Autoantibodies Against oxLDL and Cardiolipin in CHD 209
Autoantibodies Against Oxidized Low-Density Lipoprotein and Cardiolipin in Patients With Coronary Heart Disease
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