Oxidized LDL and Malondialdehyde-Modified LDL in Patients With Acute Coronary Syndromes and Stable Coronary Artery Disease

Paul Holvoet, PhD; Johan Vanhaecke, MD, PhD; Stefaan Janssens, MD, PhD; Frans Van de Werf, MD, PhD; Désiré Collen, MD, PhD

Background—The association between oxidative modifications of LDL and coronary artery disease (CAD) is suspected but not established. Therefore, the association between plasma levels of oxidized LDL and malondialdehyde (MDA)-modified LDL and acute coronary syndromes and stable CAD was investigated.

Methods and Results—The study population contained 63 patients with acute coronary syndromes (45 with acute myocardial infarction and 18 with unstable angina pectoris), 35 nontransplanted patients with angiographically confirmed stable angina, 28 heart transplant patients with posttransplant CAD, 79 heart transplant patients without CAD, and 65 control subjects. After correction for age, sex, and LDL and HDL cholesterol, plasma levels of oxidized LDL and MDA-modified LDL were significantly higher in patients with CAD than in individuals without CAD ($r^2=0.57$ and $r^2=0.26$, respectively; both $P<0.0001$). Plasma levels of MDA-modified LDL were significantly higher in patients with acute coronary syndromes than in individuals with stable CAD ($r^2=0.65; P=0.0001$) and were associated with increased levels of troponin I and C-reactive protein ($r^2=0.39$ and $r^2=0.34$, respectively; both $P=0.0001$). Plasma levels of oxidized LDL were not associated with increased levels of troponin I and C-reactive protein ($r^2=0.089$ and $r^2=0.063$, respectively).

Conclusions—Elevated plasma levels of oxidized LDL are associated with CAD. Elevated plasma levels of MDA-modified LDL suggest plaque instability and may be useful for the identification of patients with acute coronary syndromes. (Circulation. 1998;98:1487-1494.)

Key Words: lipoproteins • coronary disease • angina • diagnosis • plaque
TABLE 1. Characteristics of Control Subjects, CAD Patients, and Heart Transplant Patients

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=65)</th>
<th>Patients With Stable Angina (n=35)</th>
<th>Patients With Unstable Angina (n=18)</th>
<th>Patients With AMI (n=45)</th>
<th>Transplant Patients Without CAD (n=79)</th>
<th>Transplant Patients With CAD (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>52±1.30</td>
<td>64±1.98†</td>
<td>72±2.84*</td>
<td>63±1.58</td>
<td>56±1.23</td>
<td>58±1.41</td>
</tr>
<tr>
<td><strong>Male/female ratio</strong></td>
<td>31/34</td>
<td>27/8</td>
<td>10/8</td>
<td>28/17</td>
<td>73/6†</td>
<td>226*</td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dL</strong></td>
<td>180±4.63</td>
<td>188±6.67</td>
<td>174±8.69</td>
<td>178±5.54</td>
<td>193±3.74</td>
<td>182±5.61</td>
</tr>
<tr>
<td><strong>LDL cholesterol, mg/dL</strong></td>
<td>105±5.40</td>
<td>125±5.73</td>
<td>109±7.86</td>
<td>112±4.83</td>
<td>117±3.40</td>
<td>103±4.79</td>
</tr>
<tr>
<td><strong>HDL cholesterol, mg/dL</strong></td>
<td>48±2.69</td>
<td>37±1.76</td>
<td>45±3.67</td>
<td>39±1.45</td>
<td>50±2.06</td>
<td>53±3.21</td>
</tr>
<tr>
<td><strong>Triglycerides, mg/dL</strong></td>
<td>130±7.50</td>
<td>128±7.43</td>
<td>103±13.05</td>
<td>128±8.45</td>
<td>136±7.06</td>
<td>124±8.02</td>
</tr>
<tr>
<td><strong>Oxidized LDL, mg/dL</strong></td>
<td>0.71±0.033</td>
<td>2.65±0.17†</td>
<td>2.84±0.13†</td>
<td>3.44±0.16‡</td>
<td>1.27±0.061†</td>
<td>2.49±0.18‡</td>
</tr>
<tr>
<td><strong>MDA-modified LDL, mg/dL</strong></td>
<td>0.37±0.017</td>
<td>0.43±0.023</td>
<td>0.98±0.054‡</td>
<td>1.14±0.053‡</td>
<td>0.38±0.016</td>
<td>0.39±0.038</td>
</tr>
<tr>
<td><strong>Troponin I, ng/mL</strong></td>
<td>0.025±0.0031</td>
<td>0.032±0.020</td>
<td>0.37±0.13†</td>
<td>0.68±0.16‡</td>
<td>0.026±0.0029</td>
<td>0.041±0.0096</td>
</tr>
<tr>
<td><strong>C-reactive protein, mg/dL</strong></td>
<td>0.34±0.033</td>
<td>0.64±0.15</td>
<td>1.80±0.74†</td>
<td>2.20±0.44‡</td>
<td>0.40±0.056</td>
<td>0.41±0.033</td>
</tr>
<tr>
<td><strong>κ-Dimer, mg/dL</strong></td>
<td>13±0.86</td>
<td>30±3.60</td>
<td>37±7.10*</td>
<td>57±9.40†</td>
<td>21±2.30</td>
<td>21±2.80</td>
</tr>
</tbody>
</table>

Quantitative data represent mean±SEM. *P values were determined by nonparametric multiple comparison test except for male/female ratios, which were compared by χ² analysis.

Oxidatively Modified LDL and Coronary Disease

Table 1. Characteristics of Control Subjects, CAD Patients, and Heart Transplant Patients

Unstable angina. Thirty-five patients with angiographically documented CAD and no clinical signs of ischemia within the previous month were considered to have stable CAD.

One hundred seven posttransplant patients (47 patients got a heart transplant for dilated cardiomyopathy and 60 patients for end-stage CAD), who have been described in more detail elsewhere, were reinccluded. Twenty-eight of these patients had angiographically determined posttransplant CAD.

Sixty five control subjects (31 men, 34 women; age, 52±1.3 years) without a history of atherosclerotic cardiovascular disease were studied (Table 1).

Venous blood samples were taken in the fasting state in control subjects, patients with stable angina, and posttransplant patients. In patients with acute coronary syndromes, blood samples were taken on admission before the start of treatment.

Lipoproteins: Isolation and Modification

LDL was isolated from pooled plasma of fasting normolipidemic donors by density gradient ultracentrifugation. MDA-modified and copper-oxidized LDL was prepared as described elsewhere.

Assays

An mAb-4E6–based ELISA was used for the quantification of oxidized LDL in plasma. Plasma levels of MDA-modified LDL were measured in an mAb-1H11–based ELISA. Total cholesterol, HDL cholesterol, and triglycerides were measured by enzymatic methods (Boehringer Mannheim). LDL cholesterol values were calculated with the Friedewald formula. Troponin I levels were measured on a Beckman ACCESS immunoanalyzer by use of commercially available monoclonal antibodies (Sanofi). C-reactive protein levels were measured in a commercial immunoassay (Boehringer), and plasma levels of κ-dimer were measured in ELISA as described previously.

Immunodetection of Oxidized and MDA-Modified LDL in Coronary Atherosclerotic Lesions

Coronary arteries were collected from cardiac explants and treated as described elsewhere. Sections were developed with either mAb-4E6 or mAb-1H11 (final concentration, 1 μg/mL). Immunostaining of smooth muscle cells and monocytes or macrophages was performed with a murine monoclonal antibody against human α-actin (clone 1A4; Sigma Chemical Co) or a rat monoclonal antibody against the common leukocyte antigen/CD45 (clone 30F11.1; Pharmingen).

Statistical Analysis

Control subjects and patients were compared by nonparametric Kruskal-Wallis ANOVA followed by Dunnet’s multiple comparison test in the Prism statistical program (Graph Pad Software). Plasma levels of oxidized LDL and MDA-modified LDL in patients with normal or elevated levels of troponin I, C-reactive protein, or κ-dimer were measured in ELISA as described previously.
D-dimer and in patients with and without peripheral vascular disease were compared by the Mann-Whitney test. Discontinuous parameters were compared by $\chi^2$ analysis. Multiple regression analysis, with SAS software, was performed to evaluate the association between angiographically detected CAD (independent variable) and oxidized LDL or MDA-modified LDL (response) after controlling for age, sex, LDL cholesterol, and HDL cholesterol; it was also used to study the interaction with heart transplantation. For patients with angiographically confirmed CAD, multiple regression analysis was performed to study the association between acute coronary syndromes, troponin I, C-reactive protein, or D-dimer (independent variables) and oxidized LDL or MDA-modified LDL (response) after controlling for age, sex, LDL cholesterol, and HDL cholesterol.

**Results**

Plasma levels of oxidized LDL were 0.71 ± 0.033 mg/dL (mean ± SEM) in 65 control subjects, 1.8-fold higher ($P < 0.01$) in heart transplant patients with angiographically normal coronary arteries, 3.7-fold higher ($P < 0.001$) in patients with stable angina pectoris, 4.0-fold higher ($P < 0.001$) in patients with unstable angina pectoris, 4.8-fold higher ($P < 0.001$) in patients with AMI, and 3.5-fold higher ($P < 0.001$) in patients with posttransplant CAD (Figure 1).

Plasma levels of oxidized LDL were independent of sex but correlated with age (Figure 2). In individuals with CAD, however, there was no correlation between plasma levels of oxidized LDL and age. Plasma levels of total cholesterol, LDL cholesterol, and triglycerides in control subjects and patients were very similar, whereas HDL cholesterol levels in nontransplanted CAD patients were significantly lower than in control subjects and the other patient groups (Table 1). Plasma levels of oxidized LDL were independent of LDL cholesterol levels but correlated inversely with HDL cholesterol levels (Figure 2). Plasma levels of oxidized LDL were

![Figure 2. Plasma levels of oxidized LDL and MDA-modified LDL vs age, sex, LDL cholesterol, and HDL cholesterol, respectively.](http://circ.ahajournals.org/)

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very similar in 21 CAD patients with clinical evidence of peripheral vascular disease and 105 patients without peripheral vascular disease (3.11± 0.27 and 2.89±0.095 mg/dL, respectively).

Plasma levels of MDA-modified LDL were 0.37±0.017 mg/dL in control subjects, similar in patients with stable angina pectoris and heart transplant patients without and with posttransplant CAD, 2.6-fold higher (P<0.001) in patients with unstable angina pectoris, and 3.1-fold higher (P<0.001) in AMI patients (Figure 1). Plasma levels of MDA-modified LDL were independent of sex and LDL cholesterol levels and correlated weakly with age (Figure 2). In individuals without CAD, however, there was no correlation between plasma levels of MDA-modified LDL and age. Plasma levels of MDA-modified LDL did not correlate with LDL cholesterol and correlated weakly with HDL cholesterol (Figure 2).

Plasma levels of MDA-modified LDL were very similar in 21 CAD patients with and 105 patients without clinical evidence of peripheral vascular disease (0.96± 0.14 versus 0.73±0.042 mg/dL).

Plasma levels of troponin I were 0.025±0.0031 ng/mL in control subjects, very similar in patients with stable angina and heart transplant patients without and with posttransplant CAD, and 14.8- and 27.2-fold higher in patients with unstable angina and AMI, respectively (Table 1). At a cutoff value of ≥0.1 ng/mL, exceeding the 99th percentile of distribution in individuals without CAD. Levels of troponin I increased 10-fold compared with 0.28 ng/mL in patients with clinical findings of acute coronary syndromes (P<0.001) (Figure 3). Corresponding values of MDA-modified LDL were 1.05±0.059 and 0.55±0.043 mg/dL, respectively (P<0.0001) (Figure 3).

C-reactive protein was found to be a marker of acute coronary syndromes (Table 2). Plasma levels of oxidized LDL were 3.21±0.14 mg/dL in CAD patients with increased levels of C-reactive protein compared with 2.71±0.11 mg/dL in patients with normal levels (P<0.01) (Figure 3). Corresponding values of MDA-modified LDL were 1.05±0.059 and 0.55±0.043 mg/dL, respectively (P<0.0001) (Figure 3).

Plasma levels of d-dimer were 13±0.86 μg/dL in control subjects, similar in patients with stable angina and heart transplant patients without and with posttransplant CAD, 2.8-fold higher in patients with unstable angina, and 4.4-fold higher in AMI patients (Table 1). At a cutoff value of 40 μg/dL, 22 AMI patients (49%), 8 patients with unstable angina (44%), 11 patients with stable angina (31%), 1 patient with posttransplant CAD (3.6%), and 1 individual without CAD (0.7%) had increased plasma levels of d-dimer. In agreement with earlier published data,20 d-dimer was found to be a marker of acute coronary syndromes (Table 2). Plasma levels of oxidized LDL were 3.17±0.18 mg/dL in patients with increased d-dimer levels compared with 2.81±0.10 mg/dL in patients with normal d-dimer levels (P=NS). Corresponding values of MDA-modified LDL were 0.94±0.091 and 0.69±0.042 mg/dL, respectively (P<0.01) (Figure 3).

Multiple regression analysis was performed to evaluate the association between angiographically detected CAD and plasma levels of oxidized LDL and MDA-modified LDL. The analysis contained 144 patients without CAD (65 control subjects and 79 heart transplant patients with angiographically normal coronary arteries) and 126 individuals with CAD. After correction for age, sex, LDL cholesterol, and HDL cholesterol, CAD was associated with elevated plasma levels of oxidized LDL (r²=0.57; P=0.0001) and, to a lesser extent, elevated plasma levels of MDA-modified LDL (r²=0.26; P=0.0001). Multiple regression analysis was performed on the subgroups of CAD patients to study the association between acute coronary syndromes and plasma levels of oxidized LDL and MDA-modified LDL. The analysis contained 63 patients with stable CAD (35 nontrans-

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**Table 2.** Distribution of Troponin I, C-Reactive Protein, and d-Dimer in Patients With Acute Coronary Syndromes, Patients With Stable Angina, and Individuals Without CAD

<table>
<thead>
<tr>
<th></th>
<th>Patients With Acute Coronary Syndromes</th>
<th>Patients With Stable Angina</th>
<th>Patients With No CAD</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin I</td>
<td>50/13</td>
<td>4/59</td>
<td>3/141</td>
<td>168</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>49/14</td>
<td>7/56</td>
<td>3/141</td>
<td>153</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>d-Dimer</td>
<td>27/36</td>
<td>13/50</td>
<td>5/144</td>
<td>52</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data represent ratios of number of individuals with elevated troponin I levels, C-reactive protein, or d-dimer. Cutoff values were ≥0.1 ng/mL for troponin I, ≥0.5 mg/dL for C-reactive protein, and ≥40 μg/dL for d-dimer. These values exceeded the 99th percentile of distribution in individuals without CAD.
planted and 28 transplanted patients) and 63 patients with acute coronary syndromes. Elevated plasma levels of MDA-modified LDL were associated with acute coronary syndrome, increased troponin I, and increased C-reactive protein but not with increased D-dimer (Table 3). Differences between plasma levels of oxidized LDL in patients with stable CAD and patients with acute coronary syndromes were less pronounced (Table 3). Plasma levels of oxidized LDL and MDA-modified LDL were similar in nontransplanted and transplanted patients with stable CAD.

Figure 4 shows representative sections of coronary arteries obtained from the cardiac explants of CAD patients. mAb-4E6 immunostained oxidized LDL in nonthrombotic human coronary atherosclerotic plaques (Figure 4a, 4b, 4e, and 4f). Oxidized LDL was associated with smooth muscle foam cells in the fibrous cap (Figure 4b and 4f) and was present in the necrotic core (not shown). In contrast, mAb-1H11 detected only very small amounts of immunoreactive material associated with macrophages in the shoulder areas (Figure 4d), whereas no immunoreactive material was detected in the necrotic core of nonthrombotic plaques (Figure 4h).

**Discussion**

The present study demonstrates that plasma levels of oxidized LDL are significantly elevated in CAD patients and that these levels are very similar in patients with stable CAD and in patients with acute coronary syndromes, suggesting that their increases are independent of plaque instability. The presence of oxidized LDL in nonthrombotic plaques and the lack of correlation between plasma levels of oxidized LDL and LDL cholesterol suggest that increased plasma levels of oxidized LDL in association with CAD may be due to their back-

<table>
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<tr>
<th>TABLE 3. Multiple Regression Analysis of Association Between Acute Coronary Syndromes and Plasma Levels of Oxidized LDL or MDA-modified LDL</th>
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<tbody>
<tr>
<td><strong>Oxidized LDL</strong></td>
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<tr>
<td><strong>F</strong></td>
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<tr>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>Troponin I</td>
</tr>
<tr>
<td>C-reactive protein</td>
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<tr>
<td>D-dimer</td>
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</tbody>
</table>

Analysis included 45 patients with AMI, 18 patients with unstable angina, and 35 nontransplanted and 28 transplanted patients with angiographically detected stable CAD. Partial r^2 values were obtained after correction for age, sex, LDL cholesterol, and HDL cholesterol.
Figure 4. Representative sections of coronary arteries isolated from cardiac explants of patients with end-stage ischemic heart disease. mAb-4E6 immunostained oxidized LDL in nonthrombotic human coronary atherosclerotic plaques (a, b, e, f). Oxidized LDL was associated with smooth muscle foam cells (immunostained with cell-specific mAb-1A4) in fibrous cap (b, f) or was present in necrotic core (not shown). In contrast, mAb-1H11 detected only very small amounts of immunoreactive material in nonthrombotic atherosclerotic lesions (c, d, g, h). Immunostaining was associated with macrophage foam cells (immunostained with cell-specific mAb-30F11) in shoulder areas (d) or was absent (h).
diffusion from the vessel wall. In contrast, plasma levels of MDA-modified LDL were significantly higher in patients with acute coronary syndromes than in patients with stable CAD, suggesting that increases in plasma levels of MDA-modified LDL are dependent on the ischemic syndromes in patients with unstable angina pectoris or AMI. The association between MDA-modified LDL and troponin I, a marker of ischemic syndromes, further supports this hypothesis. Furthermore, the increase in MDA-modified LDL was associated more with inflammation (with C-reactive protein as marker) than with thrombotic syndromes (with n-dimer as marker). These data thus suggest that elevated levels of MDA-modified LDL may be markers of plaque instability.

Different mechanisms for the oxidation of LDL have been proposed. Copper ion–induced oxidation of LDL results in the release of hydroperoxides that are converted to reactive aldehydes (eg, MDA and 4-hydroxynonenal). Interaction of these aldehydes with lysine residues in the apolipoprotein B-100 moiety renders the LDL more negatively charged, which results in a decreased affinity for the LDL receptor and an increased affinity for scavenger receptors. Endothelial cells, monocytes, macrophages, lymphocytes, and smooth muscle cells are all capable of enhancing the rate of metal ion–induced oxidation of LDL, and different enzymes may be involved. Myeloperoxidase, secreted by activated phagocytes, may be a catalyst for the initiation of lipid peroxidation in LDL independent of free metal ions. Previous studies have shown that isolated LDL is not released continuously from atherosclerotic plaques. Hammer et al recently characterized a monoclonal antibody, mAb-OB/O9, that is specific for LDL modified with aldehydes that can be released by activated platelets. It may be used to further investigate the role of activated platelets in the oxidative modification of LDL.

In conclusion, the present study demonstrates the association between elevated plasma levels of oxidized LDL and CAD clinically expressed in stable CAD and acute coronary syndromes. Elevated levels of MDA-modified LDL, however, are associated with acute coronary syndrome. A prospective investigation of the role of MDA-modified LDL and/or oxidized LDL in the progression of coronary atherosclerosis and/or the development of atherothrombosis appears to be warranted.

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