Plasma oxidized LDL: a predictor for acute myocardial infarction?

Nordin Fredrikson, Gunilla; Hedblad, Bo; Berglund, Göran; Nilsson, J

Published in:
Journal of Internal Medicine

DOI:
10.1046/j.1365-2796.2003.01128.x

2003

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Plasma oxidized LDL: a predictor for acute myocardial infarction?

G. NORDIN FREDRIKSON1,3, B. HEDBLAD1,2, G. BERGLUND1 & J. NILSSON1
From the 1Departments of Medicine and 2Community Medicine, Malmö University Hospital, Lund University, and 3Department of Biomedical Laboratory Science, Malmö University, Sweden


Objectives. Oxidized LDL has been attributed a key role in the development of atherosclerosis. Previous studies have demonstrated increased plasma levels of oxidized LDL in patients with established coronary artery disease. The aim of the present study was to investigate if plasma oxidized LDL also predicts risk for development of coronary heart disease (CHD).

Design. We used a nested case–control design to study the association between plasma levels of oxidized LDL and risk for development of acute myocardial infarction (AMI) and/or death by CHD.

Results. Oxidized LDL correlated with total plasma and LDL cholesterol in both cases (r = 0.72, P < 0.01, r = 0.69, P < 0.01, respectively) and controls (r = 0.71, P < 0.01, r = 0.77, P < 0.01, respectively). The oxidized LDL/plasma cholesterol ratio was higher amongst cases (13.5, range 10.7–19.8) than in controls (12.6, range 9.5–15.8, P < 0.05) and hypercholesterolaemic controls (12.2, range 8.0–16.0, P < 0.01).

Conclusions. These findings identify high plasma oxidized LDL/total cholesterol ratio as a possible indicator of increased risk for AMI.

Keywords: atherosclerosis, hypercholesterolaemia, myocardial infarction, oxidized low density lipoproteins, plasma cholesterol.

Introduction

A growing body of evidence demonstrates that markers of inflammatory activity are increased in CHD patients [1]. High levels of inflammatory makers are also associated with an increased risk for development of CHD [2]. These associations are not surprising as inflammation of the arterial intima is one of the major characteristics of atherosclerosis. Accumulation, aggregation and oxidative modification of LDL are believed to play an important role in activation of this inflammation [3]. Oxidation of LDL is believed to occur mainly in the extracellular matrix of the arterial intima and to be cleared locally by macrophages through the scavenger receptor pathway. The recent development of high-sensitive ELISAs for oxidized LDL using monoclonal antibodies has made it possible to identify oxidized LDL also in the circulation [4]. Whether these particles become oxidatively modified in the circulation or whilst passing through vascular or other tissues remains to be fully clarified. Increased levels of oxidized LDL have been demonstrated in patients with coronary heart disease (CHD). Moreover, patients with acute coronary syndromes, such as acute myocardial infarction (AMI) and unstable angina, have been reported to have higher plasma levels of oxidized LDL than patients with stable angina [5]. In transplant-associated coronary artery disease plasma levels of oxidized LDL correlate with the severity of coronary stenoses [6]. Studies by Holvoet et al. [7] suggest that the plasma level of oxidized LDL is a more sensitive marker of the presence of coronary artery disease than the Global Risk Assessment Score (GRAS).
In the present study we recruited subjects from the population-based Malmö Diet Cancer Study cohort in a nested case–control design to analyse if increased plasma levels of oxidized LDL also predicts risk for development of CHD.

**Methods**

*Study population*

The study subjects, born between 1926 and 1945, belong to the ‘Malmö Diet and Cancer (MDC)’ study cohort. A random 50% of those who entered the MDC study between November 1991 and February 1994 were invited to take part in a study on the epidemiology of carotid artery disease [8]. Routines for ascertainment of information on morbidity and mortality following the health examination, and definition of traditional risk factors, have been reported [8]. The Ethics Committee at Lund University approved the study.

The first youngest 26 cases (below 60 years of age) with an acute coronary heart event, i.e. fatal or nonfatal myocardial infarction (MI) or deaths caused by CHD were identified. For each case two controls without a history of myocardial infarction or stroke was individually matched for age, sex, smoking habits and month of baseline examination and duration of follow-up. One of the two controls was selected on basis of having LDL cholesterol above 5.0 mmol L$^{-1}$.

*Laboratory analyses*

After overnight fasting blood samples were drawn for the determination of serum values of total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and whole blood glucose. C-reactive protein (CRP) was measured using a custom-made enzyme-linked immunosorbent assay (ELISA) using a rabbit anti-human CRP antibody (Dako, Glostrup, Denmark). LDL cholesterol in mmol L$^{-1}$ was calculated according to the Friedewald formula. Oxidized LDL was measured using ELISA (Mercordia, Uppsala, Sweden) in ethylene diaminetetraacetic acid (EDTA) plasma supplemented with the antioxidants butylhydroxytoluene and diethylenetriaminepenta-acetic acid. The plasma samples had been stored at $-80^\circ$C and not previously thawed. This oxidized LDL ELISA is a capture ELISA using the mAb4E6 antibody developed by Holvoet et al. [4]. The coefficient of variation for the assay is 8% and the recovery 95%. Two control plasmas with known oxidized LDL levels supplied by the manufacturer were used as internal control in the analysis.

*B-mode ultrasound vasculography*

An Acuson 128 computed tomography system (Acuson, Mountain View, CA, USA) with a 7-MHz transducer was used for the assessment of carotid plaques in the right carotid artery as described previously [9].

*Statistics*

The SPSS was used for the statistical analyses. Distributions of risk factors are presented as median and range and as proportions when appropriate. Tolerance was computed in order to assess the collinearity between clinical and biochemical variables. One-way analysis of variance accompanied by a Tukey HSD posthoc test was used to assess plasma concentrations of oxidized LDL and the oxidized LDL to LDL cholesterol ratio in cases and controls. Potential confounders were identified by significance testing of other clinical and biochemical variables using a chi-square test and one-way analysis of variance accompanied by a Tukey HSD posthoc test. P-values <0.20 was used as criteria for potential confounding [Rothman KJ, Greenland S. Modern Epidemiology (2nd Edn) Lippincott-Raven, Philadelphia, PA, USA, pp. 255–259]. A general linear model was used to adjust the mean values of oxidized LDL cholesterol and oxidized LDL to LDL cholesterol ratio for potential confounders.

*Results*

The basic characteristics of the study groups are presented in the Table 1. The median time from the baseline investigation to development of AMI was 2.6 years (range 0.1–6.1 years) amongst the cases. Two groups of controls were included in the study. The first were matched against cases for age, gender, examination period and smoking habits. The subjects in the second control group were matched against cases for the same criteria but all were hypercholesterolaemic (LDL cholesterol > 5.0 mmol L$^{-1}$). Thus, the second control group

including high-risk subjects that did not develop clinical signs of CHD during the follow-up period. Diabetes was more common amongst cases and plasma oxidized LDL, oxidized LDL to LDL cholesterol ratio, body mass index (BMI), systolic blood pressure, LDL to HDL cholesterol ratio and triglycerides were higher in cases than in nonhypercholesterolaemic controls. Carotid plaques, as assessed by carotid ultrasonography, were more common amongst cases and hypercholesterolaemic controls.

Mean values of oxidized LDL cholesterol remained significantly higher in cases than in the nonhypercholesterolaemic control group after adjustment for potential confounders (BMI, systolic blood pressure, LDL to HDL cholesterol ratio, triglycerides, diabetes and carotid plaques) significantly higher in cases than in both control groups (Fig. 1b). A correlation between oxidized LDL and the inflammatory marker CRP was observed when all subjects were included in the analysis ($r = 0.33$, $P < 0.005$), but did not reach statistical significance in the individual groups.

**Discussion**

The present observations suggest that subjects with high plasma oxidized LDL/total cholesterol ratio is at increased risk for development of AMI. Plasma oxidized LDL was closely associated with total and LDL cholesterol levels and hypercholesterolaemic controls had higher oxidized LDL than cases. These findings indicate that the risk for AMI is associated with the relative degree of LDL oxidation rather than with the total level of oxidized LDL in plasma. In accordance, there is an association between the susceptibility of LDL to become oxidized by copper in vitro and severity of coronary artery disease (CAD) [10]. High level of small dense LDL, that is more susceptible to oxidation, is an established risk factor for CHD [11].

**Table 1** Baseline characteristics of subjects with myocardial infarction, and age, sex, smoking and examination period matched controls with and without LDL cholesterol $>5.0$ mmol L$^{-1}$. Values are expressed as median (range) or as proportions

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Cases $n = 26$</th>
<th>Controls $n = 26$</th>
<th>Controls with LDL $&gt; 5.0$ $n = 26$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55 (49–59)</td>
<td>55 (49–59)</td>
<td>55 (49–59)</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>69</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>Former smokers (%)</td>
<td>39</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>BMI (kg m$^{-2}$)</td>
<td>26.7 (18.6–36.0)</td>
<td>25.3 (19.9–33.0)</td>
<td>26.5 (20.3–39.4)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>27</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>148 (120–190)</td>
<td>143 (108–160)</td>
<td>144 (108–170)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>50</td>
<td>46</td>
<td>31</td>
</tr>
<tr>
<td>Blood pressure lowering medication (%)</td>
<td>27</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td>Total cholesterol (mmol L$^{-1}$)</td>
<td>6.25 (3.98–8.21)</td>
<td>5.97 (4.67–7.45)</td>
<td>7.70 (6.40–11.44)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol L$^{-1}$)</td>
<td>4.6 (2.4–6.0)</td>
<td>4.1 (2.5–5.1)</td>
<td>5.7 (5.1–9.1)</td>
</tr>
<tr>
<td>Oxidized LDL cholesterol (U L$^{-1}$)</td>
<td>59 (35–97)</td>
<td>48 (35–68)</td>
<td>68 (46–91)</td>
</tr>
<tr>
<td>Oxidized LDL to LDL cholesterol ratio (U L$^{-1}$/mmol L$^{-1}$)</td>
<td>13.5 (10.7–19.8)</td>
<td>12.6 (9.5–15.8)</td>
<td>12.2 (8.0–16.0)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol L$^{-1}$)</td>
<td>1.0 (0.7–2.5)</td>
<td>1.3 (0.8–2.4)</td>
<td>1.4 (0.7–2.3)</td>
</tr>
<tr>
<td>LDL cholesterol to HDL cholesterol ratio (mmol L$^{-1}$)</td>
<td>4.3 (1.3–7.2)</td>
<td>3.4 (1.5–6.2)</td>
<td>4.1 (2.4–7.6)</td>
</tr>
<tr>
<td>Triglycerides (mmol L$^{-1}$)</td>
<td>1.5 (0.5–10.0)</td>
<td>1.2 (0.5–2.6)</td>
<td>1.3 (0.5–3.0)</td>
</tr>
<tr>
<td>Lipid lowering medication (%)</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carotid plaques (%)</td>
<td>60</td>
<td>15</td>
<td>48</td>
</tr>
</tbody>
</table>

BMI, body mass index.
Based on the present observations it is not possible to determine if increased levels of oxidized LDL in itself contributes to the development of AMI or if it only serves as a marker of more widespread, pre-existing CAD. Holvoet et al. [6] showed that oxidized LDL correlates with the extent of CAD in heart transplanted patients suggesting that oxidized LDL may be a marker for atherosclerosis. Plasma oxidized LDL is also a more sensitive marker for the presence of CAD than scoring of traditional risk factors [7]. By studying the ratio of LDL diene conjugation to LDL cholesterol, Vasankari et al. [12] found that oxidized LDL correlates with both severity of CAD and common carotid intima-media thickness. As cases were characterized by an increased presence of carotid plaques it is possible that in our study also plasma oxidized LDL reflects the severity of pre-existing atherosclerosis. However, the observation that the two control groups had similar oxidized LDL/cholesterol ratios in spite of a higher incidence of carotid plaques in the hypercholesterolaemic control group suggest that other factors are also of importance.

Experimental studies have identified several mechanisms through which oxidized LDL may contribute to the development of atherosclerosis. Oxidized LDL may cause intimal inflammation by activating expression of adhesion molecules on endothelial cells, stimulate leucocyte chemotaxis and by inducing release of growth factors from macrophages [3]. Hence, it is possible that the increased levels of oxidized LDL in the cases may have contributed to a more aggressive development of CAD and AMI.

Several studies have reported increased levels of oxidized LDL in patients with acute coronary syndromes such as AMI and unstable angina suggesting that some of the oxidized LDL in plasma may originate from ruptured plaques [4, 5]. Oxidized LDL is abundant in advanced human plaques and may contribute to plaque rupture by inducing apoptosis or necrosis of plaque smooth muscle cells. In patients treated with pravastatin, a decrease in carotid plaque content of oxidized LDL is associated with increased smooth muscle cell viability, decreased matrix metalloproteinase activity and higher collagen content [13].

The difference in plasma oxidized LDL between cases and controls in the present study is much smaller than that previously reported between controls and patients with CAD/acute coronary syndromes [4, 5]. For example, Ehara et al. [5] reported that patients with stable angina pectoris had 50% higher oxidized LDL/LDL protein than controls versus a 10% higher level CHD cases than in controls in the present prospective case–control study. The smaller difference observed in the present study may be the result of the fact that all subjects were free of clinical signs of CHD at the time of oxidized LDL determination. However, it cannot be excluded that difference in the ELISA used could also be of importance.

In conclusion, this first prospective observation suggests plasma oxidized LDL/total cholesterol ratio as a possible indicator of increased risk for AMI. These observations should be confirmed in larger cohort studies.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council, the Swedish
Heart-Lung foundation, the King Gustaf V 80th Birthday foundation, the Bergqvist foundation, the Tore Nilsson foundation, the Crafoord foundation, the Swedish Society of Medicine, the Royal Physiographic Society, the Malmö University Hospital foundation and the Lundström foundation.

References

5 Ehara S, Ueda M, Naruko T et al. Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. *Circulation* 2001; 103: 1955–60.

Received 25 October 2002; revision received 18 December 2002; accepted 21 January 2003.

Correspondence: Gunilla Nordin Fredrikson, Wallenberg Laboratory, Ist floor, Malmö University Hospital, 205 02 Malmö, Sweden (fax: 46 40 332550; e-mail: gunilla.nordin_fredrikson@med.forsk.mas.lu.se).

© 2003 Blackwell Publishing Ltd *Journal of Internal Medicine* 253: 425–429