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# Plasma oxidized LDL: a predictor for acute myocardial infarction?

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Abstract. Nordin Fredrikson G, Hedblad B, Berglund G, Nilsson J (Malmö University Hospital, Lund University; and Biomedical Laboratory Science, Malmö University, Sweden). Plasma oxidized LDL: a predictor for acute myocardial infarction? *J Intern Med* 2003; **253**: 425–429.

**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.

**Subjects.** Oxidized LDL was analysed by ELISA in cases (n = 26), controls (n = 26) and controls with LDL cholesterol >5.0 mmol L<sup>-1</sup> (n = 26).

**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 transplantassociated 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  $5.0 \text{ mmol L}^{-1}$ .

#### 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 enzymelinked immunosorbent assay (ELISA) using a rabbit anti-human CRP antibody (Dako, Glostrup, Denmark). LDL cholesterol in mmol L<sup>-1</sup> 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°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 cholersterol 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<sup>-1</sup>). Thus, the second control group

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<sup>-1</sup>. Values are expressed as median (range) or as proportions

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

BMI, body mass index.

included 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.

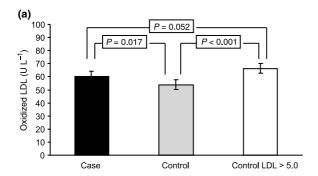
Mean values of oxidized LDL cholesterol remained significantly higher in cases than in the nonhyper-cholesterolaemic control group after adjustment for potential confounders (Fig. 1a). The hypercholester-olaemic controls had higher levels of oxidized LDL than cases (P=0.052) or nonhypercholesterolaemic controls (P<0.001).

In both cases and controls, oxidized LDL was significantly related to total plasma cholesterol (r=0.72 and r=0.71; P<0.01), LDL cholesterol (r=0.69 and r=0.77; P<0.01) and triglycerides (r=0.46; P<0.05 and r=0.48; P<0.01). In certain cases oxidized LDL showed an inverse relation to HDL cholesterol (r=-0.41; P<0.05). To compensate for the influence of LDL cholesterol levels, comparisons between the groups were also made using the oxidized LDL/plasma LDL cholesterol

ratio. This ratio was 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].



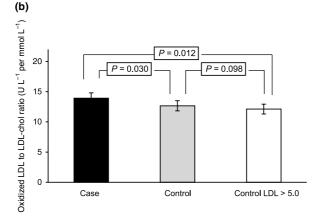


Fig. 1 Plasma oxidized LDL (a) and plasma oxidized LDL/total cholesterol ratio (b), in subjects with myocardial infarction and controls with or without LDL cholesterol >5.0 mmol L<sup>-1</sup> individually matched for age, sex, smoking habits and examination period. LDL-chol, LDL-cholesterol.

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 a more widespread, preexisting 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 plagues it is possible that in our study also plasma oxidized LDL reflects the severity of preexisting 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.

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#### References

- 1 Ridker P, Libby P. Nontraditional coronary risk factors and vascular biology: the frontiers of preventive cardiology. *J Invest Med* 1998; 46: 338–50.
- 2 Ridker P, Cushman M, Stampfer M, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *New Eng J Med* 1997; 336: 973–9.
- 3 Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell 2001; 104: 503–16.
- 4 Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 1998; 98: 1487–94.
- 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.
- 6 Holvoet P, Stassen JM, Van Cleemput J, Collen D, Vanhaecke J. Oxidized low density lipoproteins in patients with transplant-associated coronary artery disease. *Arterioscler Thromb Vasc Biol* 1998; 18: 100–7.
- 7 Holvoet P, Mertens A, Verhamne P et al. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2001; 21: 844–8.

- 8 Hedblad B, Nilsson P, Janzon L, Berglund G. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a crosssectional study in Malmo, Sweden. *Diabet Med* 2000; 17: 299–307.
- 9 Hedblad B, Wikstrand J, Janzon L, Wedel H, Berglund G. Low dose metropolol CR/XL and fluvastatin slow progression of carotid intima-media thickness (IMT). Main results from the beta-blocker cholesterol asymptomatic plaque study (BCAPS). Circulation 2001; 103: 1721–6.
- 10 Regnström J, Nilsson J, Tornvall P, Landou C, Hamsten A. Susceptibility to low density lipoprotein oxidation and coronary atherosclerosis in man. *Lancet* 1992; 339: 1183–6.
- 11 Krauss RM. Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. *Curr Opin Lipidol* 1994; 5: 339–49.
- 12 Vasankari T, Ahotupa M, Toikka J et al. Oxidized LDL and thickness of carotid intima-media are associated with coronary atherosclerosis in middle-aged men: lower levels of oxidized LDL with statin therapy. Atherosclerosis 2001; 155: 403-15.
- 13 Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. *Circulation* 2001; 103: 926–33.

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