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

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

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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

Subendothelial accumulation of foam cells plays a key role in the initiation of atherosclerosis.[1,2] These foam cells, which may be generated by the uptake of oxidized LDL and/or malondialdehyde (MDA)-modified LDL by macrophages via scavenger receptors,[3] accumulate in fatty streaks that evolve to more complex fibrofatty or atheromatous plaques.[4] Oxidized LDL may also be involved in atherogenesis by inducing smooth muscle cell proliferation[5] and smooth muscle foam cell generation.

An association between LDL oxidation and atherogenesis was first suggested by experiments showing that oxidized LDL caused injury to endothelial cells[6] and was further supported by studies showing a protective effect of antioxidants against progression of atherosclerosis.[7] With the use of a specific ELISA for oxidized LDL, an association between the extent of coronary artery disease (CAD) in heart transplant patients and plasma levels of oxidized LDL was recently established, suggesting that oxidized LDL may be a marker of CAD.[8] Previously, elevated levels of MDA-modified LDL were detected in the plasma of acute myocardial infarction (AMI) patients but not patients with stable angina.[9]

We wanted to compare plasma levels of oxidized and MDA-modified LDL in patients with acute coronary syndromes and patients with stable CAD and to study the association between oxidized LDL and MDA-modified LDL, respectively, and troponin I, a marker of ischemic syndromes,[10,11] and C-reactive protein, a marker of inflammation.[12]

Methods

Patients and Blood Sampling

A total of 270 individuals were studied: 63 consecutive patients with acute coronary syndromes, 35 patients with stable CAD, 107 posttransplant patients, and 65 control subjects. Patients with acute coronary syndromes had ischemic chest discomfort with ST-segment elevation or depression of >0.5 mm or T-wave inversion of >1 mm. In 45 patients, elevated creatine kinase (CK)-MB levels (and ≥3% of total CK) were present at entry or in the samples taken at 6 to 8 hours after enrollment, indicating AMI. In 18 patients, no CK-MB elevations were found, and these patients were classified as having

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

	Control Subjects (n=65)	Patients With Stable Angina (n=35)	Patients With Unstable Angina (n=18)	Patients With AMI (n=45)	Transplant Patients Without CAD (n=79)	Transplant Patients With CAD (n=28)
Age, y	52±1.30	64±1.98†	72±2.84*	63±1.58	56±1.23	58±1.41
Male/female ratio	31/34	27/8	10/8	28/17	73/6‡	22/6*
Total cholesterol, mg/dL	180±4.63	188±6.67	174±8.69	178±5.54	193±3.74	182±5.61
LDL cholesterol, mg/dL	105±5.40	125±5.73	109±7.86	112±4.83	117±3.40	103±4.79
HDL cholesterol, mg/dL	48±2.69	37±1.76	45±3.67	39±1.45	50±2.06	53±3.21
Triglycerides, mg/dL	130±7.50	128±7.43	103±13.05	128±8.45	136±7.06	124±8.02
Oxidized LDL, mg/dL	0.71±0.033	2.65±0.17‡	2.84±0.13‡	3.44±0.16‡	1.27±0.061†	2.49±0.18‡
MDA-modified LDL, mg/dL	0.37±0.017	0.43±0.023	0.98±0.054‡	1.14±0.053‡	0.38±0.016	0.39±0.038
Troponin I, ng/mL	0.025±0.0031	0.032±0.020	0.37±0.13‡	0.68±0.16‡	0.026±0.0029	0.041±0.0096
C-reactive protein, mg/dL	0.34±0.033	0.64±0.15	1.80±0.74†	2.20±0.44‡	0.40±0.056	0.41±0.033
D-Dimer, μg/dL	13±0.86	30±3.60	37±7.10*	57±9.40‡	21±2.30	21±2.80

Quantitative data represent mean±SEM. *P* values were determined by nonparametric multiple comparison test except for male/female ratios, which were compared by χ^2 analysis.

*$P<0.05$; †$P<0.01$; ‡$P<0.001$.

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,[8] were reincluded. 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[8] 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.[13] MDA-modified and copper-oxidized LDL was prepared as described elsewhere.[14,15]

Assays

An mAb-4E6–based ELISA was used for the quantification of oxidized LDL in plasma.[8,16–18] Plasma levels of MDA-modified LDL were measured in an mAb-1H11–based ELISA.[9] 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 D-dimer were measured in ELISA as described previously.[19]

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

Coronary arteries were collected from cardiac explants and treated as described elsewhere.[9] 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

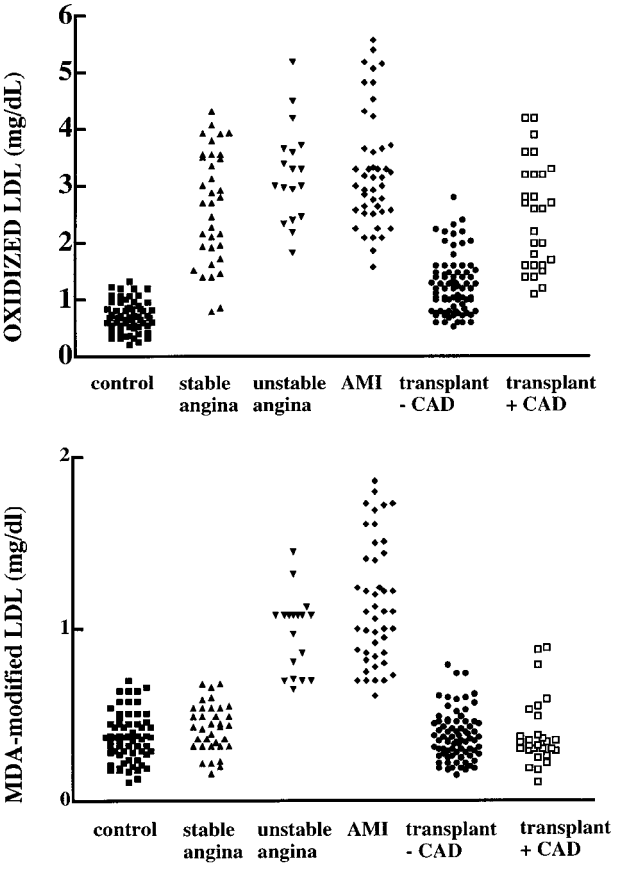

Figure 1. Individual values of oxidized LDL and MDA-modified LDL in control subjects; nontransplanted patients with stable angina and unstable angina and AMI; and heart transplant patients without and with posttransplant CAD.

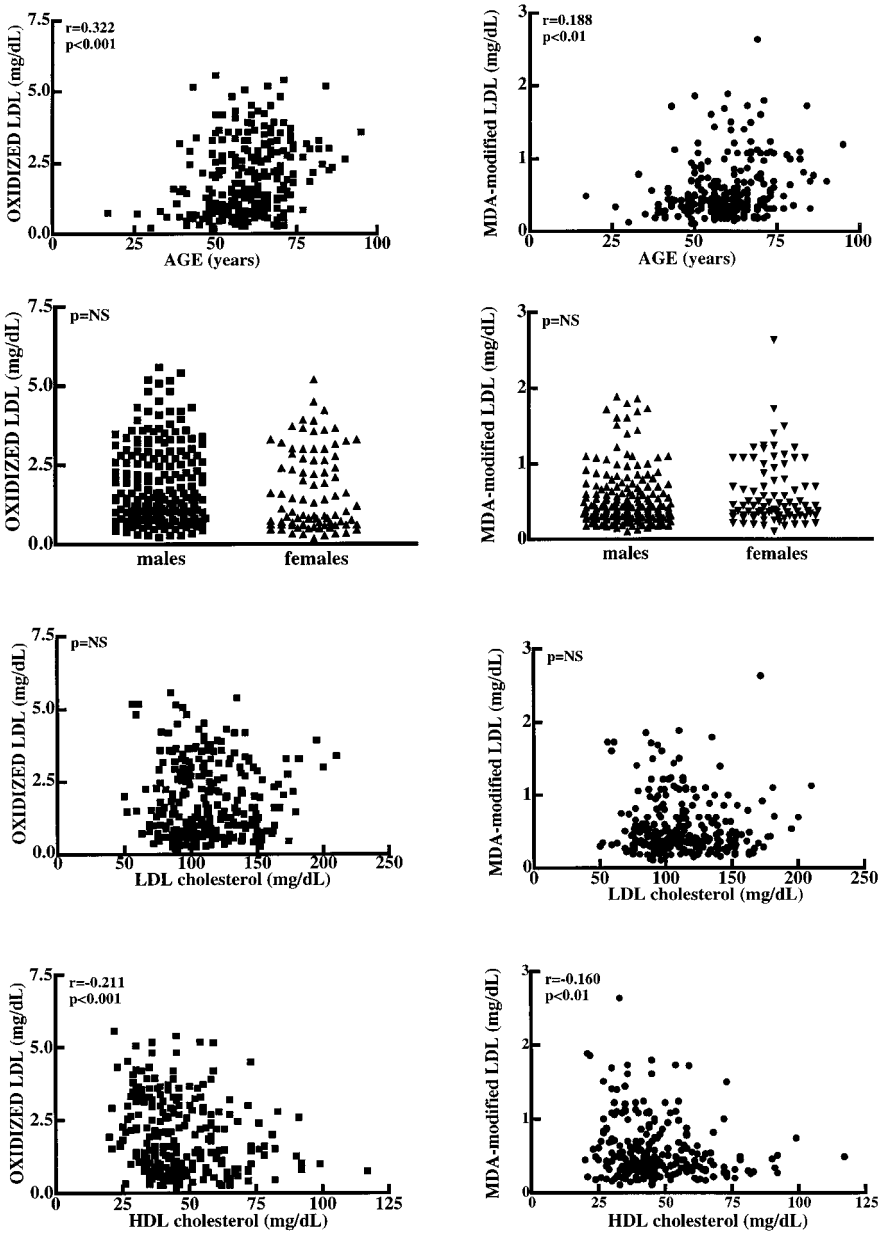

Figure 2. Plasma levels of oxidized LDL and MDA-modified LDL vs age, sex, LDL cholesterol, and HDL cholesterol, respectively.

D-dimer and in patients with and without peripheral vascular disease were compared by the Mann-Whitney test. Discontinuous parameters were compared by χ^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

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

	Patients With Acute Coronary Syndromes	Patients With Stable Angina	Patients With No CAD	χ^2	*P*
Troponin I	50/13	4/59	3/141	168	<0.0001
C-reactive protein	49/14	7/56	3/141	153	<0.0001
D-Dimer	27/36	13/50	5/144	52	<0.0001

Data represent ratios of number of individuals with elevated vs number of individuals with normal levels of troponin I, 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.

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 with 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, 40 of 45 AMI patients (89%) had increased troponin I levels compared with 10 of 18 patients with unstable angina (55%), 2 of 35 patients with stable CAD (5.7%), 2 of 28 patients with posttransplant CAD (7.1%), and 2 of 144 individuals without CAD (0.7%). In agreement with previously published data,[10,11] troponin I was found to be a marker of acute coronary syndromes (Table 2). Plasma levels of oxidized LDL were 3.26±0.14 mg/dL in CAD patients with increased troponin I levels compared with 2.66±0.11 mg/dL in CAD patients with normal troponin I levels ($P<0.01$) (Figure 3). Corresponding values of MDA-modified LDL were 1.10±0.061 and 0.54±0.039 mg/dL, respectively ($P<0.0001$) (Figure 3).

Plasma levels of C-reactive protein were 0.34±0.033 mg/dL in control subjects, similar in patients with stable CAD and heart transplant patients without and with posttransplant CAD, and 5.3- and 6.5-fold higher in patients with unstable angina and AMI, respectively (Table 1). At a cutoff value of ≥0.5 mg/dL, 39 AMI patients (97%), 10 patients with unstable angina (56%), 5 patients with stable angina (14%), 2 patients with posttransplant CAD (7.1%), and 2 individuals without CAD (1.4%) had increased levels of C-reactive protein. In agreement with previously published data,[12] 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 ($r^2=0.57$; $P=0.0001$) and, to a lesser extent, elevated plasma levels of MDA-modified LDL ($r^2=0.26$; $P=0.0001$). 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-

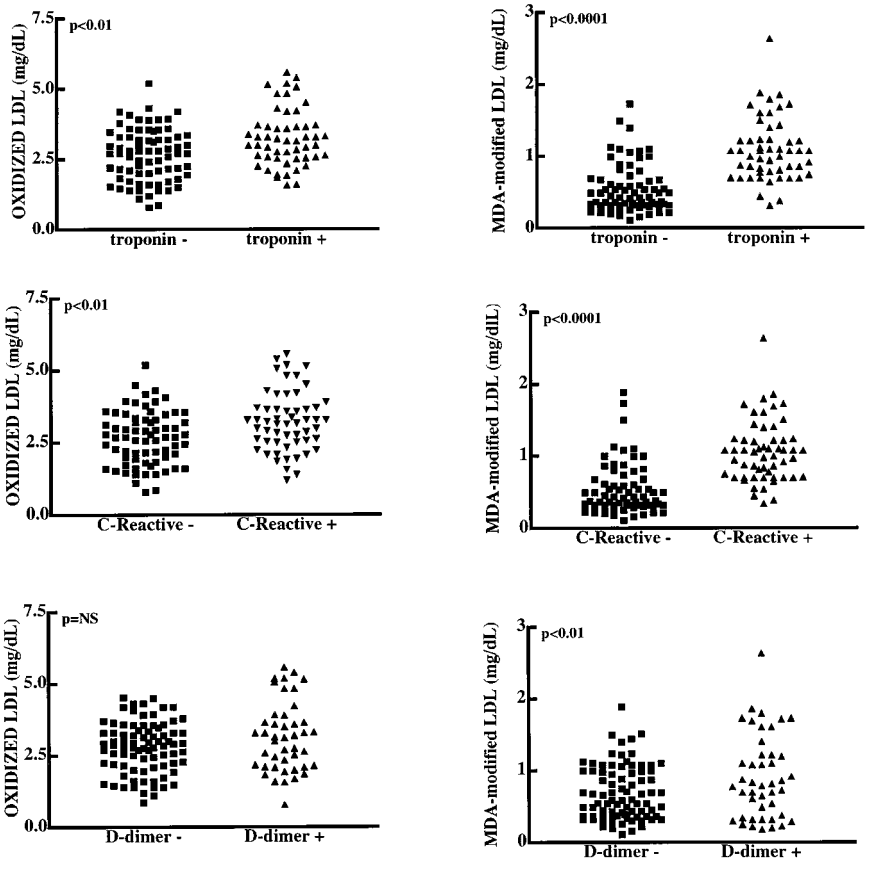

Figure 3. Plasma levels of oxidized LDL and MDA-modified LDL in CAD patients with normal and elevated levels of troponin I, C-reactive protein, and D-dimer, respectively.

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 3. Multiple Regression Analysis of Association Between Acute Coronary Syndromes and Plasma Levels of Oxidized LDL or MDA-modified LDL

	Oxidized LDL			MDA-Modified LDL		
	F	r^2	*P*	*F*	r^2	*P*
Acute coronary syndrome	18	0.14	0.0001	136	0.65	0.0001
Troponin I	11	0.086	0.0014	69	0.39	0.0001
C-reactive protein	7.7	0.063	0.0068	56	0.34	0.0001
D-Dimer	2.3	0.023	0.13	4.0	0.056	0.110

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.

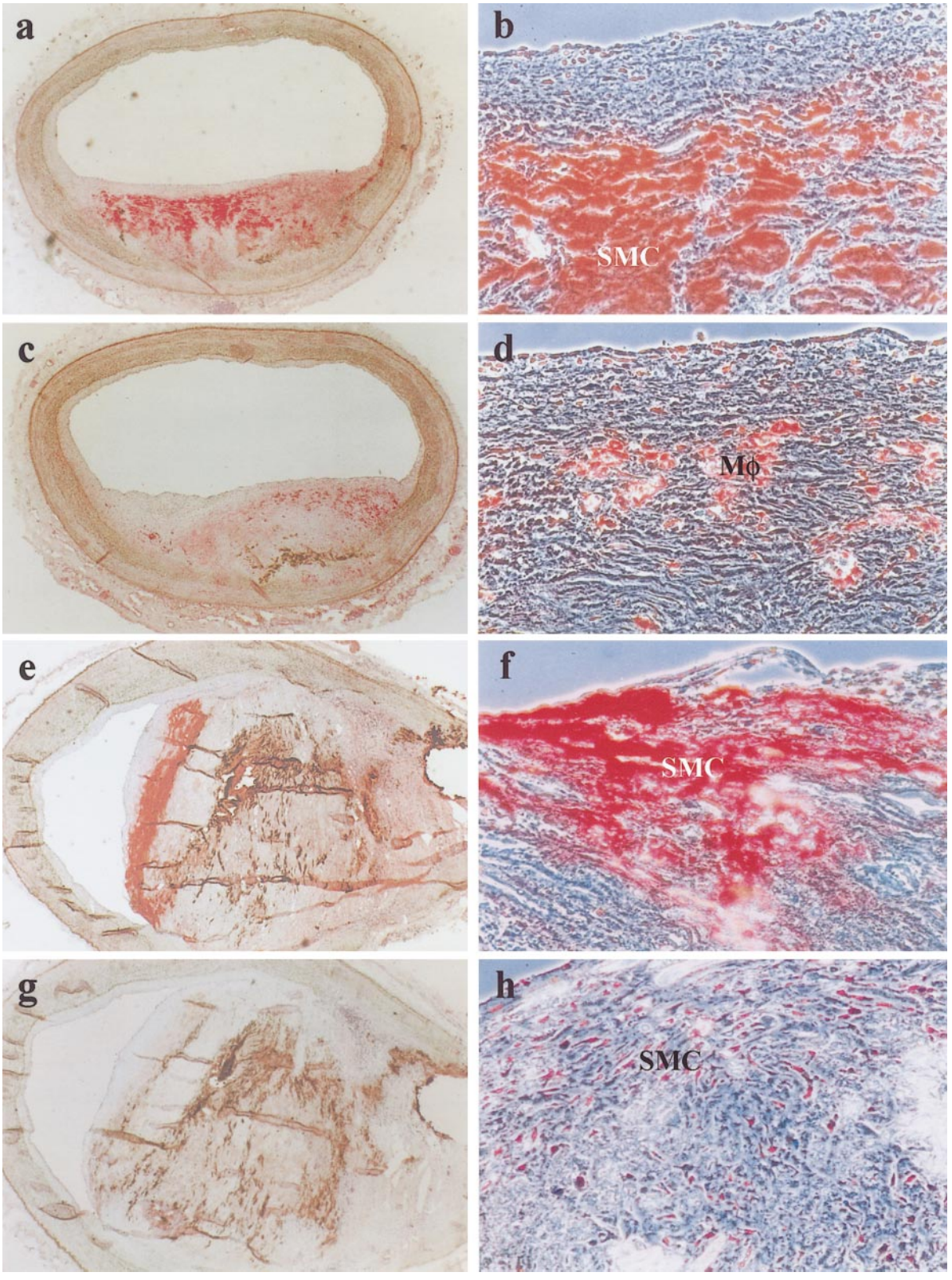

Figure 4. Representative sections of coronary arteries isolated from cardiac explants of patients with end-stage ischemic heart disease. mAb-4E6 immunostained oxidized LDL in nonthrombotic human coronary atherosclerotic plaques (a, b, e, f). Oxidized LDL was associated with smooth muscle foam cells (immunostained 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 D-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 in vitro oxidation of LDL results in the release of hydroperoxides that are converted to reactive aldehydes (eg, MDA and 4-hydroxynonenal).[21,22] 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.[3] Endothelial cells, monocytes, macrophages, lymphocytes, and smooth muscle cells are all capable of enhancing the rate of metal ion–induced in vitro oxidation of LDL,[23] and different enzymes may be involved.[24–28] Myeloperoxidase, secreted by activated phagocytes, may be a catalyst for the initiation of lipid peroxidation in LDL independent of free metal ions.[29] Previously, we isolated oxidized LDL from the plasma of patients with posttransplant CAD.[8] The characteristics suggested that it did not originate from extensive metal ion–induced oxidation of LDL but that it might be generated by cell-associated oxidative enzymatic activity in the arterial wall. Previously, it was demonstrated in animal models that the oxidation of LDL indeed occurs in the arterial wall and not in the blood.[17,18]

The causal role of oxidized LDL is suspected but not established.[30–33] The observed association between CAD and plasma levels of oxidized LDL, measured in a specific ELISA, suggests that the assay may be a useful tool to investigate the causal role of oxidized LDL in atherosclerotic cardiovascular disease in a prospective study.

Previously, we have also isolated MDA-modified LDL from the plasma of AMI patients.[9] It was concluded that it did not originate from extensive metal ion–induced oxidation of LDL but that it may be generated by MDA released by oxidation of arachidonic acid present in LDL.[34–36] Ischemic injury may result not only in the activation of the cyclooxygenase-dependent pathway of prostaglandin synthesis in endothelial cells[37] but also in increased production of F_2-isoprostanes, non–cyclooxygenase-derived prostaglandin F_2–like compounds,[38,39] that are strong inducers of platelet activation. Activated platelets may then produce large amounts of aldehydes, further enhancing the modification of LDL. The present very significant association between plasma levels of MDA-modified LDL and markers of necrosis (troponin I) or inflammation (C-reactive protein) further supports the hypothesis that the generation of MDA-modified LDL is associated with ischemic injury rather than with the extent of coronary atherosclerosis. The very low reactivity of the monoclonal antibody mAb-1H11 with nonthrombotic atherosclerotic plaques indeed suggests that MDA-modified LDL, in contrast with oxidized LDL, is not released continuously from atherosclerotic plaques. Hammer et al[40] 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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