＜循環器科＞

① Plasma levels of oxidized low density lipoprotein are associated with stable angina pectoris and modalities of acute coronary syndrome

② Imazu M*

③ Ono K*, Tadehara F*, Kajiwara K*, Hideya Y*, Sumii K, Tasaki N*, Oiwa J*, Shimohara Y*, Gomyo Y, Itabe H*

④ Int Heart J

⑤ 49(9):515-524, 2008

Plasma Levels of Oxidized Low Density Lipoprotein Are Associated With Stable Angina Pectoris and Modalities of Acute Coronary Syndrome

Michinori IMAZU, MD, Koichi ONO, MD, Futoshi TADEHARA, MD, Kenji KAJIWARA, MD, Hideya YAMAMOTO, MD, Kotaro SUMII, MD, Naohito TASAKI, MD, Jiro OIWA, MD, Yasuaki SHIMOHARA, MD, Yukihiko GOMYO, MD, *and* Hiroyuki ITABE, PhD

Reprinted from INTERNATIONAL HEART JOURNAL
Vol. 49, No. 5, Pages 515-524
September 2008

Plasma Levels of Oxidized Low Density Lipoprotein Are Associated With Stable Angina Pectoris and Modalities of Acute Coronary Syndrome

Michinori IMAZU,[1] MD, Koichi ONO,[1,2] MD, Futoshi TADEHARA,[1] MD, Kenji KAJIWARA,[3] MD, Hideya YAMAMOTO,[1,4] MD, Kotaro SUMII,[1,4] MD, Naohito TASAKI,[5] MD, Jiro OIWA,[6] MD, Yasuaki SHIMOHARA,[6] MD, Yukihiko GOMYO,[4] MD, *and* Hiroyuki ITABE,[7] PhD

SUMMARY

The role of plasma levels of oxidized low density lipoprotein (OxLDL) in the development of coronary heart disease (CHD) has not been fully elucidated. We examined the relationship among plasma levels of OxLDL, measured by an enzyme immunoassay using an antibody against OxLDL (FOH1a/DLH3) and apolipoprotein B, CHD, and modalities at the onset of acute coronary syndrome (ACS). A total of 115 individuals who underwent coronary angiography were studied. Of these, 21 patients complicated with extracoronary cardiovascular diseases were excluded. Consequently, 94 patients (63 men) (ACS: 23, stable angina pectoris (SAP): 46, and normal coronary artery (NCA):25) were eligible for inclusion in the study.

Elevated plasma levels of OxLDL were associated with CHD, especially with ACS. In patients with NCA, hypertension was associated with plasma OxLDL. Plasma levels of OxLDL were significantly higher in patients with new-onset type ACS than in those with worsening type ACS (2.98 versus 1.53 mg/dL, $P = 0.002$). In conclusion, plasma levels of OxLDL are associated with CHD and significantly higher in patients with new-onset ACS. The findings of the present study suggest that plasma OxLDL can be a marker of the development of CHD and modalities of ACS. (Int Heart J 2008; 49: 515-524)

Key words: Coronary heart disease, FOH1a/DLH3, New-onset type, Worsening type, Hypertension

ATHEROSCLEROSIS is characterized by accumulation of lipids within the artery wall, endothelial dysfunction, monocyte/macrophage infiltration, foam cell formation, and proliferation of smooth muscle cells.[1-3] Low density lipopro-

From the [1] Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, [2] Department of Internal Medicine, Innoshima Medical Associated Hospital, [3] Department of Internal Medicine, Masuda Medical Associated Hospital, [4] Department of Cardiology, Mazda Hospital, [5] Department of Internal Medicine, Higashi Hiroshima Medical Center, [6] Department of Internal Medicine, Kure Kyosai Hospital, Hiroshima, and [7] Department of Biological Chemistry, School of Pharmaceutical Sciences, Showa University, Tokyo, Japan.

Address for correspondence: Koichi Ono, MD, Department of Internal Medicine, Innoshima Medical Associated Hospital, 1962 Innoshima-Nakanosho-cho, Onomichi, Hiroshima 722-2211, Japan.

Received for publication March 13, 2008.

Revised and accepted June 30, 2008.

tein (LDL) was suggested to be the main source of the cholesterol that accumulates in developing foam cells.[3] Consequently, the concept that oxidative modification of LDL is a prerequisite for foam cells formation was reported. Oxidized LDL (OxLDL) is taken up by monocytes/macrophages specifically and can generate foam cells.[4,5] Moreover, several studies have found that oxidative modification of LDL can occur *in vivo* and is an important step of the atherosclerosis process.[6,7] An association between LDL oxidation and atherosclerosis was further supported by studies showing an autoantibody reacted with OxLDL in the serum[7-9] and antioxidant inhibition of atherogenesis in experimental models[3,10,11] and humans.[12,13]

The relationships between circulating OxLDL and coronary heart disease (CHD) have been demonstrated using a unique monoclonal antibody, FOH1a/DLH3, that reacts specifically against oxidized phosphatidylcholine (OxPC)[14] using a homogenate of atheromatous plaque of human aorta as an antigen in combination with an anti-apoprotein B antibody.[15] However, the role of plasma OxLDL in the development of CHD is not fully understood.

The purpose of this study was to clarify the relationship among plasma OxLDL, atherosclerotic cardiovascular disease, and the onset of acute coronary syndrome (ACS).

Methods

1. Study population: A total of 115 patients who underwent coronary angiography at Hiroshima University Hospital and affiliated hospitals were studied. Exclusion criteria were vasospastic angina and previous myocardial infarction. Twenty-one patients complicated with extracoronary cardiovascular diseases (ie, cerebral vascular disease, carotid artery stenosis, aortic stenosis, aortic aneurysms or arteriosclerosis obliterans) were excluded. Consequently, 94 patients (63 men and 31 women) (ACS: 23, stable angina pectoris (SAP): 46, and normal coronary artery (NCA):25) were eligible for enrollment.

2. Definitions: ACS was defined when patients had ischemic chest pain within 48 hours before admission with ST-segment elevation and/or elevated levels of creatine kinase MB isoenzymes. SAP was considered to be present when a patient had > 50% luminal stenosis in at least one vessel without ischemic chest pain recurring less than 48 hours before admission. NCA was when a patient showed no significant stenosis on angiography. The percent stenosis of each coronary lesion and Thrombolysis Infarction Myocardial Infarction (TIMI) flow grade[16] were determined by the physician performing the angiography. Angiographic characteristics of coronary plaques (including intracoronary thrombus and the presence of calcification) were recorded. Twenty-three patients with ACS under-

went urgent coronary angiography on admission. ACS patients were divided into two groups: 15 patients with new-onset type of ACS who had initial chest pain at rest within one week of admission, but did not experience a second chest pain event more than 48 hours after admission, and 8 patients with worsening type of ACS who had chest pain at rest more than one time within 48 hours of admission.

All patients gave written informed consent to participate in the study.

3. Blood Sampling: Venous blood samples were taken in the fasting state in patients with NCA and SAP. In patients with ACS, blood samples were taken on admission (day 1), one day after admission (day 2), and 2 days and 7 days after admission (day 3 and day 8). These samples collected in a tube containing EDTA, and plasma was stored in a refrigerator at 4°C.

4. Chemical Analysis: Plasma OxLDL was measured by a sandwich ELISA method as previously described[14] with minor modification. Briefly, plasma samples were diluted and added to the microtiter well precoated with 1.2 μg of an anti-OxLDL monoclonal antibody, FOH1a/DLH3, and incubated for 2 hours at 25°C. After extensive washing, the well was incubated for 60 min with a peroxidase-labeled goat anti-human apolipoprotein B at 25°C, followed by washing. After extensive washing, the remaining OxLDL was detected with a sheep anti-human apolipoprotein B antibody and an alkaline phosphatase-conjugated anti-sheep IgG antibody. In each ELISA plate, various concentrations of standard OxLDL, which was prepared by incubation of LDL with 5 μmol/L $CuSO_4$ at 37°C for 3 hours, were run simultaneously to determine a standard curve. Total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and LDL cholesterol were measured by enzymatic methods.

5. Statistical Analysis: Values are expressed as the mean ± SEM. Differences between the means of the two groups were evaluated with Student's two-tailed *t* test. Logarithmic transformations of triglycerides, lipoprotein (a), and OxLDL were performed because these variables showed a skewed distribution in all statistical analyses including these variables as continuous variables. They were reconverted to their original form before presentation. Analysis of covariance was used to assess the relationship between CHD and plasma OxLDL levels adjusted for age, gender, and coronary risk factors, including body mass index (BMI), hypertension, diabetes, smoking, serum LDL cholesterol, triglycerides, HDL cholesterol, and lipoprotein (a) [Lp (a)]. Statistical analyses were carried out using the SAS program (JMP 3.0, SAS Institute Japan). A level of $P < 0.05$ was considered statistically significant.

RESULTS

1. Baseline characteristics: The baseline clinical characteristics are shown in Table I. There were no significant differences between the 3 groups with regard to gender, BMI, serum levels of total cholesterol, HDL cholesterol, LDL choles-

Table I. Baseline Characteristics

	NCA ($n = 25$)	SAP ($n = 46$)	ACS ($n = 23$)	P
Age (years)	60.7 ± 2.2	65.5 ± 1.5	66.8 ± 2.1	0.09
Men (%)	56.0	76.1	60.9	0.18
BMI (kg/m^2)	23.3 ± 6.1	24.3 ± 3.6	23.6 ± 2.7	0.62
TC (mg/dL)	202.2 ± 8.8	197.5 ± 5.1	208.2 ± 7.7	0.53
LDL-C (mg/dL)	128.3 ± 7.3	125.8 ± 4.4	143.0 ± 7.6	0.12
TG (mg/dL)*	122.8 (75.4-200.1)	140.5 (69.6-252.7)	81.7 (40.0-167.0)	0.003
HDL-C (mg/dL)	51.6 ± 3.3	44.6 ± 2.1	46.4 ± 2.0	0.13
Lp(a) (mg/dL)*	15.2 (9.1-25.3)	17.2 (7.6-39.0)	14.3 (6.5-31.5)	0.60
Ox-LDL (mg/dL)*	1.64 (1.16-2.30)	1.72 (1.18-2.50)	2.36 (1.39-4.02)	0.004
Hypertension (%)	48.0	56.5	34.8	0.23
Diabetes (%)	20.0	28.3	43.5	0.20
Smoking (%)	40.0	47.8	52.2	0.69

Values are given as the mean (± SEM) or (range of 1SD). *Values are back-transformed from log transformation. NCA indicates normal coronary artery; SAP, stable angina pectoris; ACS, acute coronary syndrome; BMI, body mass index; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; Lp (a), lipoprotein (a); and OxLDL, oxidized low density lipoprotein.

Table II. Correlations of Plasma Levels of Oxidized LDL With Baseline Characteristics

	Normal coronary artery ($n = 25$)		Stable angina pectoris (n=46)		Acute coronary syndrome (n=23)	
	r	P	r	P	r	P
Age	0.18	0.40	-0.33	0.03	0.08	0.73
Gender (men)	0.07	0.56	0.03	0.87	0.10	0.67
BMI	0.007	0.97	0.04	0.77	0.10	0.66
TC	0.37	0.08	0.33	0.03	-0.17	0.44
log TG	0.14	0.54	0.03	0.87	-0.24	0.28
HDL-C	0.04	0.85	0.12	0.42	-0.001	1.00
LDL-C	0.42	0.05	0.25	0.09	-0.24	0.27
log Lp(a)	0.44	0.03	0.57	0.001	0.54	0.01
FBS	-0.07	0.75	-0.06	0.73	-0.21	0.44
Leukocytes	0.44	0.03	0.13	0.44	-0.10	0.68
CK	0.03	0.91	0.30	0.04	0.005	0.98
Hypertension	0.43	0.03	0.20	0.14	-0.39	0.07
Diabetes	0.12	0.56	-0.12	0.41	-0.08	0.72
Smoking	0.03	0.86	0.14	0.26	0.05	0.81

BMI indicates body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Lp (a), lipoprotein (a); FBS, fasting blood sugar; and CK, creatine kinase.

terol, Lp (a), and glucose, and the prevalences of hypertension, diabetes, and smoking. Serum levels of triglycerides were lowest in patients with ACS. Plasma levels of OxLDL were the highest in patients with ACS and lowest in patients with NCA ($P = 0.004$). After adjustment for age, gender and coronary risk factors, plasma OxLDL levels were associated with CHD (ACS; 2.32, SAP; 1.83, NCA; 1.59 mg/dL, $P = 0.01$).

2. Correlations of OxLDL with clinical characteristics: Plasma levels of OxLDL were positively correlated with serum levels of LDL cholesterol, Lp (a), leukocyte counts, and the prevalence of hypertension in patients with NCA (Table II). In the SAP group, OxLDL was correlated with serum levels of total cholesterol, Lp (a), and CK. In the ACS group, the only correlation was between serum Lp (a) and OxLDL.

3. OxLDL, modality of onset, and angiographic data in patients with ACS: Plasma levels of OxLDL were significantly higher in patients with new-onset type ACS than in those with worsening type ACS ($P = 0.002$) and were also higher in patients with single vessel disease than in those with multivessel disease

Table III. Plasma Levels of Oxidized LDL, Modality of Onset, and Angiographic Data in Patients With Acute Coronary Syndrome

	n	Oxidized LDL (mg/dL)	*P*
Modality			
New-onset	15	2.98 (1.80-4.93)	
Worsening	8	1.53 (1.23-1.91)	0.002
Number of diseased vessels			
1	11	3.23 (2.01-5.29)	
2, 3	12	1.77 (1.18-2.68)	0.004
TIMI grade			
0	7	2.49 (1.66-3.73)	
1	5	1.51 (1.22-1.87)	
2	7	3.06 (1.53-6.09)	
3	4	2.42 (1.50-3.89)	0.15
Thrombus			
Present	16	2.22 (1.27-3.89)	
Absent	7	2.73 (1.72-4.32)	0.41
Calcification			
Present	8	1.99 (1.15-3.45)	
Absent	15	2.59 (1.55-4.34)	0.26
Lesion length			
> 20 mm	6	2.42 (1.13-5.18)	
≦ 20 mm	17	2.34 (1.49-3.69)	0.90

Values are given as the mean (range of 1SD). Values are back-transformed from log transformation. LDL indicates low density lipoprotein.

Table IV. Comparison of Clinical Characteristics in the New-Onset ACS Group With Those in the Worsening ACS Group

	New-onset ACS group (n = 15)	Worsening ACS group (n = 8)	P
Age (years)	66.5 ± 2.6	67.4 ± 3.6	0.84
Men (%)	66.7	50.0	0.44
Hypertension (%)	13.3	75.5	0.003
Hyperlipidemia (%)	53.3	50.0	0.88
Diabetes (%)	40.0	50.0	0.65
Smoking (%)	53.3	50.0	0.88
Lipoprotein (a) (mg/dL)*	17.6 (8.1-38.4)	9.9 (4.9-20.1)	0.10
Medication before onset (%)	73.3	100.0	0.11
Single vessel disease (%)	73.3	0.0	0.001
Lesion length > 20 mm	20.0	37.5	0.36
Calcification (%)	20.0	62.5	0.04

Values are given as the mean (± SEM), percentage, or (range of 1SD).
*Values are back-transformed from log transformation. ACS indicates acute coronary syndrome.

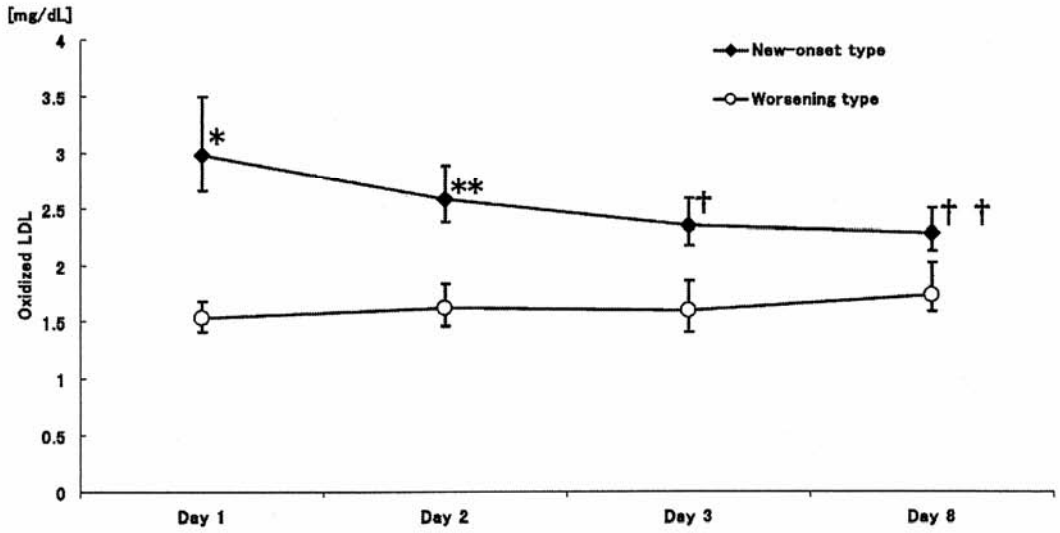

Figure. Time course of plasma levels of OxLDL in patients with new-onset and worsening type ACS. Values are given as the means ± SEM. Values are back-transformed from log transformation. *P = 0.002 versus worsening type; **P = 0.007 versus worsening type; †P = 0.02 versus worsening type; ††P = 0.09 versus worsening type.

(P = 0.004) (Table III). There were no significant differences with regard to TIMI grade, lesion length, the presence of thrombus, or calcification.

4. Clinical characteristics in patients with new-onset and worsening type ACS: The clinical characteristics of patients with new-onset ACS and those with worsening ACS are compared in Table IV. There were no significant differences with regard to age, gender, lesion length, medication before onset, serum levels of Lp (a), prevalence of hyperlipidemia, diabetes, and smoking. The prevalence of

hypertension was lower in patients with new-onset ACS than in those with worsening ACS. Patients with new-onset ACS had a higher prevalence of single vessel disease than those with worsening ACS (73.3% versus 0%, $P = 0.001$). However, patients with worsening ACS had more calcification than those with new-onset ACS (62.5% versus 20.0%, $P = 0.04$).

5. Time course of plasma levels of OxLDL in ACS patients: The distribution of plasma OxLDL on each day is shown in Figure. Plasma OxLDL levels did not change during the 7 day period after onset of ACS in the worsening ACS group, whereas in the new-onset ACS group the average plasma OxLDL levels decreased. In addition, average plasma OxLDL levels on day 1, day 2, and day 3 were significantly higher in the new-onset ACS group than in the worsening ACS group. Serum levels of lipids were not significantly different on each day between the two groups (data not shown).

DISCUSSION

Plasma levels of OxLDL increased more in patients with new-onset than in those with worsening ACS (Figure). These values in patients with worsening ACS were 1.53 mg/dL and similar in patients with NCA. Plasma levels of OxLDL were increased in patients with single vessel disease in the new-onset ACS group, but not in patients with multivessel disease in the worsening ACS group (Tables III and IV). It is not clear from these findings whether the increases in patients with ACS were due to coronary plaque instability. It is also suggested that previous angina pectoris may prevent the elevation of plasma levels of OxLDL in patients with ACS. Ischemic preconditioning triggers a late phase of protection 24 hours after the initial preconditioning, however, the mechanism of the late phase of preconditioning is unknown. Zhou, *et al*[17] reported that repetitive anoxia increased myocardial antioxidant activity 24 hours later and that it contributed to the late cardioprotective effect of preconditioning. The present findings may suggest that previous angina pectoris in patients with worsening ACS increases myocardial antioxidant activity and inhibits the elevation of plasma levels of OxLDL. No association between plasma levels of OxLDL and LDL cholesterol in patients with ACS suggested that leakage from arterial lesions might be a source of OxLDL in the plasma.

Using the same antibodies and a slightly different measuring technique, Ehara, *et al*[18] suggested that OxLDL levels increased gradually according to the severity of the coronary syndrome. In this study, we observed the same trend for OxLDL levels (Table I). Yamashita, *et al*[19] reported elevated OxLDL levels related to the presence of angiographically detected complex and thrombotic coronary artery lesion morphology, based on the Ambrose classification, in patients

with unstable angina. We also analyzed lesion morphology in this study, but there were no significant differences with regard to TIMI grade, lesion length, the presence of thrombus, or calcification. We cannot explain the discrepancy between the results of our study and those of Yamashita, *et al*. However, considering culprit lesion morphology based on the Ambrose classification, the small sample size or combination of risk factors in these two studies might be potentially misleading.

In the present study, plasma levels of OxLDL were slightly but not significantly higher in patients with SAP than in patients with NCA. These values tended to be higher in patients with angina pectoris than in normotensive patients with NCA (data not shown). Moreover, in patients with a stable status (NCA or SAP), these values were higher in patients with than in patients without extracoronary cardiovascular disease (data not shown). Thus, these findings confirmed previous observations[15,18-20] that plasma levels of OxLDL were associated with atherosclerosis. Minor differences between the present and previous findings from Holvoet, *et al*[21] were dependent on the different antibodies against OxLDL. We measured OxLDL using monoclonal antibody, FOH1a/DLH3, not reacted with native, acetylated, or malonaldehyde-treated LDL. This antibody cross-reacted with oxidized high density lipoprotein. The epitope of this antibody resides in oxidized products of phosphatidylcholine that can form complexes with polypeptides, including apolipoprotein B that was detected in foam cells in human atherosclerotic lesions,[14] and atherectomized lesions from patients with coronary stenosis and restenosis.[22] Holvoet, *et al*[21] measured OxLDL using a murine monoclonal antibody, mAb4E4, that is specific for acetylated LDL and malondialdehyde-treated LDL, and binds specifically to modified LDL present in human atherosclerotic lesions.

Plasma levels of OxLDL were correlated with serum levels of LDL cholesterol, the prevalence of hypertension, and leukocyte counts in patients with NCA and serum levels of total cholesterol, and CK in patients with SAP. However, these values were not correlated with leukocyte counts (a marker of ACS), CK values (a marker of necrosis), LDL cholesterol levels, or the prevalence of hypertension in the ACS group, suggesting that the mechanisms of elevation of plasma OxLDL levels in patients with ACS appear to be different from the stable status (SAP or NCA). The increase in the plasma level of OxLDL was significantly related to the prevalence of hypertension. The present findings confirmed those of previous studies that reported LDL from hypertensive patients was more susceptible to oxidation than control LDL.[23,24] Scheidegger, *et al*[25] demonstrated that angiotensin II increases macrophage-mediated modification of LDL via a lipoxygenase dependent pathway. Moreover, Keidar[26] reported that angiotension II administration to mice enhanced their macrophage OxLDL uptake via its stim-

ulating effect on cellular proteoglycan content, leading to foam cell formation and atherosclerosis. Therefore, it is suggested that angiotensin II may play an important role in the development of atherogenesis via LDL oxidation in hypertensive patients. Essential hypertension is associated with enhanced LDL oxidation and impaired endothelium-dependent vasodilation. The antioxidant status is linked to the nitric oxide (NO) pathway. Angiotensin-converting enzyme (ACE) inhibitors with a sulfhydryl (SH) group have been shown to inhibit oxidative stress and atherogenesis in experimental models.[27] Napoli, *et al*[28] reported the sulfhydryl ACE inhibitor zofenopril reduces oxidative stress and improves the NO pathway in patients with essential hypertension.

Our analyses have several limitations. Normal coronary artery is defined based on angiography but an exact observation (e.g. IVUS or MDCT) of atheroma burden is lacking in order to clarify the normality of coronary arteries. The ACS group was small and was divided into smaller groups. The conclusion of the study is based on a subgroup analysis of a small number of patients. This result should be proved in a larger patient population.

In conclusion, plasma levels of OxLDL are associated with atherosclerotic cardiovascular disease, and those in patients with new-onset type of ACS are significantly elevated and then decrease during the first week.

ACKNOWLEDGMENTS

We gratefully thank Mr. Junji Kimura and Ms. Kyoko Shimamura of Vessel Research Laboratory Co., Ltd for their technical assistance.

REFERENCES

1. Ylä-Herttuala S. Is oxidized low-density lipoprotein present in vivo? Curr Opin Lipidol 1998; 9: 337-44.
2. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993; 362: 801-9.
3. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997; 272: 20963-6.
4. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320: 915-24.
5. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 1991; 88: 1785-92.
6. Ylä-Herttuala S, Palinski W, Rosenfeld ME, *et al*. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest 1989; 84: 1086-95.
7. Palinski W, Rosenfeld ME, Ylä-Herttuala S, *et al*. Low density lipoprotein undergoes oxidative modification in vivo. Proc Natl Acad Sci USA 1989; 86: 1372-6.
8. Salonen JT, Ylä-Herttuala S, Yamamoto R, *et al*. Autoantibody against oxidized LDL and progression of carotid atherosclerosis. Lancet 1992; 339: 883-7.
9. Palinski W, Hörkkö S, Miller E, *et al*. Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from apolipoprotein E-deficient mice. Demonstration of epitopes of oxidized low density lipoprotein in human plasma. J Clin Invest 1996; 98: 800-14.

10. Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Proc Natl Acad Sci USA 1987; 84: 7725-9.
11. Kita T, Nagano Y, Yokode M, *et al.* Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. Proc Natl Acad Sci USA 1987; 84: 5928-31.
12. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993; 328: 1450-6.
13. Stampher MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 1993; 328: 1444-9.
14. Itabe H, Takeshima E, Iwasaki H, *et al.* A monoclonal antibody against oxidized lipoprotein recognizes foam cells in atherosclerotic lesions. Complex formation of oxidized phosphatidylcholines and polypeptides. J Biol Chem 1994; 269: 15274-9.
15. Toshima S, Hasegawa A, Kurabayashi M, *et al.* Circulating oxidized low density lipoprotein levels: A biochemical risk marker for coronary heart disease. Arterioscler Thromb Vasc Biol 2000; 20: 2243-7.
16. TIMI Study Group. The Thrombolysis in Myocardial Infarction (TIMI) trial. Phase I findings. N Engl J Med 1985; 312: 932-6.
17. Zhou X, Zhai X, Ashraf M. Direct evidence that initial oxidative stress triggered by preconditioning contributes to second window of protection by endogenous antioxidant enzyme in myocytes. Circulation 1996; 93: 1177-84.
18. 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.
19. Yamashita H, Ehara S, Yoshiyama M, *et al.* Elevated plasma levels of oxidized low-density lipoprotein relate to the presence of angiographically detected complex and thrombotic coronary artery lesion morphology in patients with unstable angina. Circ J. 2007; 71: 681-7.
20. 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.
21. Holvoet P, Collen D. Oxidized lipoproteins in atherosclerosis and thrombosis. FASEB J 1994; 8: 1279-84. (Review)
22. Jimi S, Saku K, Kusaba H, Itabe H, Koga N, Takebayashi S. Deposition of oxidized low-density lipoprotein and collagenosis occur coincidentally in human coronary stenosis: an immunohistochemical study of atherectomy. Coron Artery Dis 1998; 9: 551-7.
23. Maggi E, Finardi G, Poli M, *et al.* Specificity of autoantibodies against oxidized LDL as an additional marker for atherosclerotic risk. Coron Artery Dis 1993; 4: 1119-22.
24. Keidar S, Kaplan M, Shapira C, Brook JG, Aviram M. Low density lipoprotein isolated from patients with essential hypertension exhibits increased propensity for oxidation and enhanced uptake by macrophages: a possible role for angiotensin II. Atherosclerosis 1994; 107: 71-84.
25. Scheidegger KJ, Butler S, Witztum JL. Angiotensin II increases macrophage-mediated modification of low density lipoprotein via a lipoxygenase-dependent pathway. J Biol Chem 1997; 272: 21609-15.
26. Keidar S. Angiotensin, LDL peroxidation and atherosclerosis. Life Sci 1998; 63: 1-11.
27. de Nigris F, D'Armiento FP, Somma P, *et al.* Chronic treatment with sulfhydryl angiotensin-converting enzyme inhibitors reduce susceptibility of plasma LDL to in vitro oxidation, formation of oxidation-specific epitopes in the arterial wall, and atherogenesis in apolipoprotein E knockout mice. Int J Cardiol 2001; 81: 107-15.
28. Napoli C, Sica V, de Nigris F, *et al.* Sulfhydryl angiotensin-converting enzyme inhibition induces sustained reduction of systemic oxidative stress and improves the nitric oxide pathway in patients with essential hypertension. Am Heart J 2004; 148: e5.