High oxidative stress in patients with stable coronary heart disease

Tanja Weinbrenner [a], Mercedes Cladellas [b], Maria Isabel Covas [a,c], Montserrat Fitó [a],
Marta Tomás [a], Mariano Sentí [a,d], Jordi Bruguera [b], Jaume Marrugat [a,e,*], The SOLOS
Study Investigators [1]

[a] Unitat de Lípids i Epidemiologia Cardiovascular, Institut d'Investigació Mèdica, Dr Aiguader 80, E-08003 Barcelona, Spain
[b] Servei de Cardiologia, Hospital del Mar, Barcelona, Spain
[c] Laboratori de Referència de Catalunya, Barcelona, Spain
[d] Universitat Pompeu Fabra, Barcelona, Spain
[e] Universitat Autònoma de Barcelona, Barcelona, Spain

Abstract

Oxidized low density lipoprotein (oxLDL) plays a pivotal role in the development of atherosclerosis. The aim of the study was to investigate the relationship between oxLDL and other oxidative stress biomarkers with stable coronary heart disease (CHD). We compared the degree of oxidative stress in patients with CHD and sex-matched healthy control subjects in a case-control study. The study included 64 male subjects: 32 patients with stable CHD and 32 normal control subjects. Levels of circulating oxLDL were measured by a monoclonal antibody 4E6-based competition ELISA. Comparison of oxidative stress marker levels between cases and controls, adjusted for age, revealed significantly higher plasma oxLDL levels (63.32 ± 25.49 vs. 37.73 ± 20.58 U/l, $P = 0.001$), lower serum levels of autoantibodies against oxLDL (341.53 ± 350.46 vs. 796.45 ± 1034.2 mU/ml, $P = 0.021$), higher activities of the antioxidant enzymes superoxide dismutase in erythrocytes (951 ± 70.2 vs. 771.6 ± 191.2 U/g, $P = 0.032$) and glutathione peroxidase in whole blood (GSH-Px: $10\ 714.4 \pm 3705.4$ vs. 5512.2 ± 1498.1 U/l, $P < 0.001$). The risk of having CHD was 20.6-fold greater (95% CI, 1.86–228.44, $P = 0.014$) in the highest tertile of the oxLDL distribution than in the lowest, determined by logistic regression analysis on the combined study population after adjustment for age and other potential confounding factors. When the risk associated with GSH-Px levels was calculated, the odds ratio was 305.3 (95% CI, 5.07–18369.95, $P = 0.006$) in the highest tertile compared with the lowest. Our results showed that an oxidative stress occurs in patients with CHD despite being clinically stable and under medical treatment. The combination of oxLDL levels and GSH-Px activity may be useful for the identification of patients with stable CHD.

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1. Introduction

Oxidative stress produced by free radicals has been linked to the development of several diseases such as atherosclerosis, cancer, and neurodegenerative diseases [1]. The biological oxidative effects of free radicals on lipids, DNA, and proteins are controlled by a spectrum of exogenous dietary antioxidants, including vitamin E, C, and phenolic compounds, and by endogenous antioxidants including the scavenger enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) [2]. Furthermore, serum paraoxonase1 (PON1), a high density lipoprotein (HDL) linked enzyme, appears to

have antioxidative properties [3]. The balance between free radical generation and antioxidant activity is critical to the pathogenesis of oxidative stress related disorders [1,2]. Elevated oxidative stress and superoxide anion formation in vascular cells could promote conversion of low density lipoprotein (LDL) to atherogenic oxidized LDL (oxLDL). One of the earliest steps in the generation of oxidatively modified LDL is the peroxidation of its polyunsaturated fatty acids. The oxidative breakdown of these fatty acids, such as malondialdehyde (MDA) and 4-hydroxynonenal, form covalent bands with lysine residues of apolipoprotein B (apoB). The modified apoB has immunogenic properties [4]. Several studies have been conducted to investigate the role of autoantibodies against oxLDL (OLAB), suggesting that OLAB reflect the degree of oxidative modification of LDL, but the results are controversial [5,6].

High levels of oxLDL are found in patients with different acute coronary syndromes, indicating that oxLDL might be a marker for atherosclerosis [7–9]. However, controversial data have been obtained in patients with stable coronary heart disease (CHD) [7,8]. The aim of this study was to compare the degree of oxidative stress in patients with stable CHD and healthy subjects, and to study the association between oxLDL and other oxidative stress biomarkers with stable CHD.

2. Methods

2.1. Subjects

The present case-control study was nested within the Spanish Olive Oil Study (SOLOS) which is aimed at evaluating the antioxidant effect of olive oil in healthy volunteers and CHD patients. The study was conducted in 32 healthy non-smoking male volunteers aged from 23 to 92 years (mean, 57 years) and in 32 male patients aged from 42 to 82 years (mean, 68 years) with stable CHD and angiographically documented coronary stenosis. For the present study, serum samples drawn at entry (basal level) were available from the volunteers.

The inclusion criteria for stable CHD included (1) history of myocardial infarction (MI) or unstable angina pectoris without clinical signs of ischemia and any changes in treatments over 45 days prior to inclusion in the study and (2) significant coronary stenosis, defined as ⩾50% reduction of lumen area in any of the three coronary arteries. Patients with previous coronary revascularization were not excluded. Controls were healthy male non-smoking volunteers from a general population. Inclusion criteria for normal controls included: absence of any history of CHD, absence of hypertension, any condition limiting mobility, life-threatening diseases, or any other disease or condition
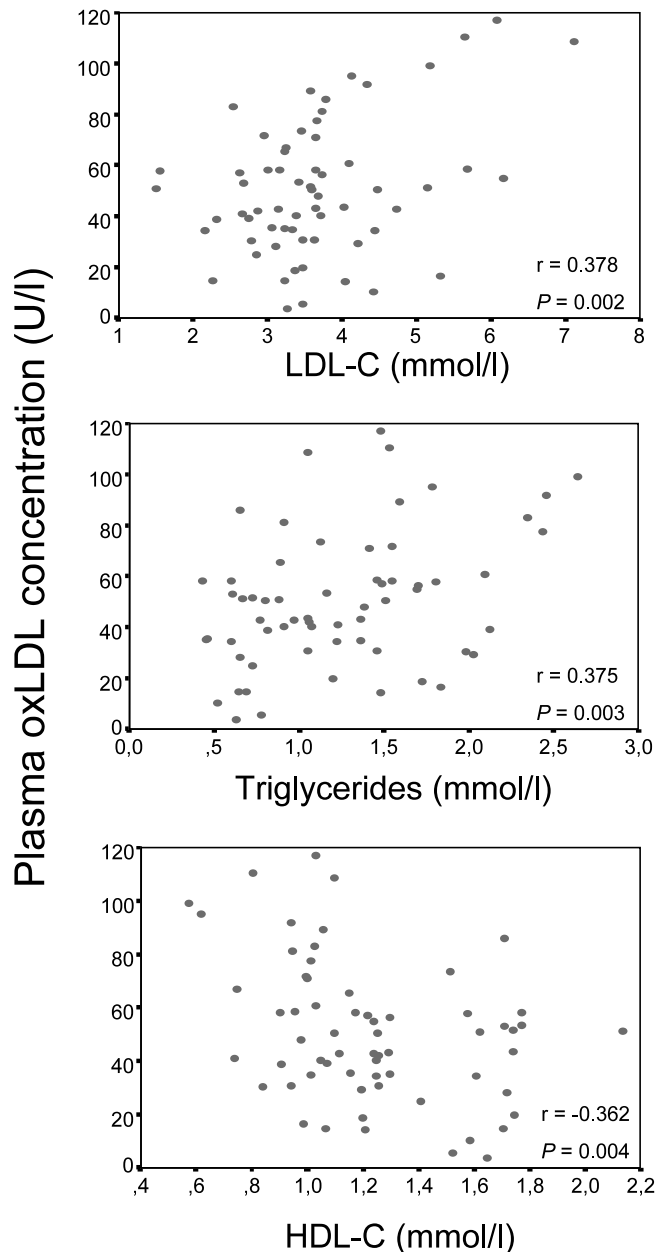
Fig. 1. Pearson's correlation coefficient between plasma lipids and plasma oxLDL levels for the combined study population. *Non-standardized Abbrevations:* oxLDL, oxidized LDL; *r*, Pearson's correlation coefficient; *P*, level of significance.

that would impair compliance. Exclusion criteria for both groups was the intake of antioxidant supplementation in the 2 months previous to their inclusion in the study.

Anthropometric variables, height and weight, of the volunteers were recorded by using standard methods, and body mass index (BMI) was calculated (kg/m^2).

Each study participant signed an informed consent prior to enrollment, and the study was approved by the local ethics committee.

2.2. Blood sampling

Venous blood was obtained in the morning after an overnight fast for at least 12 h. Washed erythrocytes and whole blood were stored at $-80\ ^\circ$C for SOD and GSH-Px determinations, respectively. For lipids, PON1, and OLAB assays, serum was separated and kept frozen at $-40\ ^\circ$C until assayed. For the measurements of oxLDL concentrations, blood was drawn into tubes containing EDTA-2Na (1 mg/ml), chilled on ice, and plasma was separated by centrifugation at $4\ ^\circ$C.

2.3. Glucose and lipid assays

Serum glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels were determined using enzymatic kits (Roche Diagnostic, Basel, Switzerland) adapted to a Cobas Mira Plus autoanalyser (Hoffmann-La Roche, Basel, Switzerland). Low density lipoprotein cholesterol (LDL-C) was calculated by means of the Friedewald formula [10]. Lipoprotein(a) (Lp(a)) was determined by immunoturbidemetry (Roche Diagnostics, Basel, Switzerland).

2.4. Enzymatic assays

SOD (EC 1.15.1.1) activity in erythrocytes was measured by the rate of inhibition of I.N.T. [(2-(4iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride] reduction (Ransod SDE 125, Randox Laboratory, Crumlin, Northern Ireland) [11] and expressed in unit per gram of haemoglobin. Haemoglobin concentration was determined with a Sysmex K1000 haematological analyser (Toa Electronics, Kobe, Japan). GSH-Px in whole blood activity (EC 1.11.1.9) was measured by a modification of the method of Paglia and Valentine [12] using cumene hydroperoxide as an oxidant of glutathione (Ransel RS 505, Randox Laboratories, Crumlin, United Kingdom) and expressed in unit per liter. Intra-run imprecision was 4.74% and 3.6% for SOD and GSH-Px, respectively. Between-run imprecision was 5.64% and 5.43% for SOD and GSH-Px, respectively.

2.5. Lipid peroxides assay

The degree of oxidative stress was assessed by the generation of MDA equivalents, and measured by the thiobarbituric acid reactive substances method [13]. Lipid peroxides were adjusted for TG values. Values were expressed in micromole per liter. Intra-run and between-run imprecision were 4.24% and 6.87%, respectively.

2.6. Oxidized LDL

The concentration of oxLDL in plasma was measured by a sandwich ELISA procedure using the murine monoclonal antibody, mAb-4E6 as capture antibody bound to microtitration wells, and a peroxidase conjugated anti-apolipoprotein B antibody recognizing oxLDL bound to the solid phase (oxLDL, Mercodia AB, Uppsala, Sweden).

2.7. Antibodies against oxLDL

OLAB were measured in serum by ELISA using copper-oxLDL coated onto microtiter strips as antigen, and specific peroxidase conjugated anti human IgG antibodies to detect the presence of bound antibodies (OLAB, Biomedica, Vienna, Austria).

2.8. PON1 activity analysis

PON1 activity was measured by the hydrolysis of paraoxon as substrate, as previously described [14]. The intra-assay and interassay coefficients of variation were 0.78 and 1.69%, respectively.

2.9. Statistical analysis

Statistical analysis was done with the SPSS statistical package (SPSS Incorporated Co., USA). Normality of variable distribution was assessed by the Kolmogorov–Smirnov test and by analysis of skewness and kurtosis. For comparisons of continuous variables between cases and controls, univariate ANOVAs were performed adjusting for age. A P value of < 0.05 was regarded as significant. Comparisons of means of more than two groups were carried out with one-way-ANOVA and the post-hoc Tukey-test was used to identify significant differences between the groups. Relationships between parameters were determined by Pearson's correlation coefficient and Spearman rank correlation coefficient. Logistic regression analyses were performed to evaluate the association between angiographically detected CHD and the oxidative stress biomarkers, which showed significative differences between the two study groups. For the performance of the analyses, levels of the oxidative stress biomarkers of the complete study group were divided into tertiles, and the odds ratios were calculated using the lowest as the reference. The selection of potential confounders was done on the basis of a $P < 0.15$ between the 3rd tertile and the remaining tertiles of the oxidative stress biomarkers, determined by unpaired Student's t test. Each logistic regression analysis was also adjusted for age.

Data for Lp(a), glucose, lipid peroxides, and OLAB were transformed before analysis using a natural log transformation to achieve homogeneous variances.

Table 1
Baseline characteristics and oxidative stress biomarkers of patients with stable CHD (cases) and healthy control subjects

	Controls ($n = 32$)	Cases ($n = 32$)	P-value (ANOVA)[a]
Body weight (kg)	69.5 ± 13.1	78.8 ± 9.4	< 0.001
Height (m)	1.68 ± 0.007	1.68 ± 0.005	n.s.
BMI (kg/m^2)	24.5 ± 4.3	27.8 ± 3.3	0.01
TC (mmol/l)	5.71 ± 1.06	5.27 ± 1.01	< 0.1
LDL cholesterol (mmol/l)	3.76 ± 1.0	3.56 ± 1.1	n.s.
HDL cholesterol (mmol/l)	1.44 ± 0.3	1.05 ± 0.25	< 0.001
TG (mmol/l)	1.1 ± 0.048	1.4 ± 0.59	< 0.1
Lp(a) (g/l)	0.36 ± 0.36	0.47 ± 0.51	n.s.
Glucose (mmol/l)	4.71 ± 1.85	6.93 ± 2.45	< 0.001
oxLDL (U/l)	37.7 ± 20.6	63.3 ± 25.5	< 0.01
OLAB (mU/ml)	796.4 ± 1034.2	341.5 ± 350.5	< 0.05
Lipid Peroxides (μmol/l)	4.06 ± 2.7	5.03 ± 3.99	n.s.
SOD (U/g of Hemoglobin)	771.6 ± 191.2	950.96 ± 270.2	< 0.05
GSH-Px (U/l)	5512.2 ± 1498.1	$10\,714.4 \pm 3705.4$	< 0.001
PON1 (U/l)	235.7 ± 109.7	215.7 ± 88.9	n.s.

Data presented as mean $\pm$ S.D. *Non-standardized Abbrevations:* CHD, coronary heart disease; oxLDL, oxidized LDL; OLAB, antibodies against oxidized LDL; SOD, superoxide dismutase activity in erythrocytes; GSH-Px, glutathione peroxide in whole blood; PON1, paraoxonase1 activity; P, level of significance.

[a] Adjusted for effects of age.

However, only the original untransformed values are presented for ease of interpretation. All results are given as mean ($\pm$S.D.).

3. Results

3.1. Patient characteristics

CHD patients were older than the control group ($P < 0.01$). The clinical characteristics of the study group are presented in Table 1. CHD patients had a higher BMI than the control group. Plasma levels of TC, LDL-C, TG, and Lp(a) were similar, while the CHD patients had significantly lower HDL-C levels than the control group. Coronary angiograms of patients showed at least 50% stenosis of 1 ($n = 14$ patients), 2 ($n = 11$ patients), or 3 ($n = 7$ patients) vessels. Nine patients had CHD complicated by diabetes, and 13 had coronary artery bypass surgery (CABG). All patients received aspirin. Twenty seven of them received statins, 22 of them beta-blockers, 11 of them calcium antagonist, 18 of them angiotensin converting enzyme (ACE) inhibitors, and 10 of them nicotinic acid.

3.2. Oxidative stress biomarkers

Plasma levels of oxLDL were significantly higher and serum levels of OLAB were lower in CHD patients than in the control group (Table 1). Activities of the antioxidant enzymes, SOD in erythrocytes and GSH-Px in whole blood, were higher in CHD patients compared to healthy volunteers (Table 1). No significant differences were obtained between the two study groups in serum lipid peroxidation values and PON1 activity (Table 1).

oxLDL levels in patients with CHD did not differ, regardless of history of diabetes mellitus, CABG, intake of statins, beta-blockers, calcium antagonists, ACE inhibitors, and nitrates. Examination of the association between oxLDL and the extent of CHD showed that oxLDL levels did not differ significantly between 1-, 2- or 3-vessel disease.

3.3. Correlation between oxLDL and other variables

Significant positive correlations between oxLDL and age ($r = 0.319$, $P = 0.011$), and TC ($r = 0.349$, $P = 0.005$) were observed when the 64 study participants were analyzed together. Plasma oxLDL levels also correlated positively with LDL-C and TG, and negatively with HDL-C (Fig. 1). When these relationships were examined separately in each group, plasma levels of oxLDL of individuals with CHD were independent of age, correlations of oxLDL with TC and LDL-C were stronger and remained significant with TG (oxLDL with TC: $r = 0.747$, $P < 0.001$; oxLDL with LDL-C: $r = 0.709$, $P < 0.001$; oxLDL with TG: $r = 0.371$, $P = 0.039$). In the control group, these relationships lost their significance with exception of the positive correlation of oxLDL with age ($r = 0.402$, $P = 0.002$). A positive relationship was observed between oxLDL levels and SOD ($r = 0.343$, $P = 0.009$) in the total study group, and the correlations between oxLDL and other oxidative stress biomarkers reached borderline positive significance in the case of GSH-Px ($r = 0.245$, $P = 0.060$) and negative with OLAB ($r = -0.211$, $P = 0.100$).

Table 2
Differences in age, BMI, glucose, and lipid profile between the 1st and 2nd tertiles and the 3rd tertile of the oxidative stress biomarkers

	oxLDL tertiles			OLAB tertiles			SOD tertiles			GSH-Px tertiles		
	3rd	1st+2nd	P	3rd	1st+2nd	P	3rd	1st+2nd	P	3rd	1st+2nd	P
Age (years)	68.6 (11.4)	59.2 (16.9)	<0.05	65.2 (16.6)	61.3 (15.4)	n.s.	67.4 (14.0)	58.9 (16.6)	<0.1	66.8 (8.6)	59.6 (18.1)	<0.05
BMI (kg/m^2)	27.1 (3.9)	25.6 (4.3)	n.s.	25.2 (3.6)	26.6 (4.4)	n.s.	27.4 (3.1)	25.1 (4.4)	<0.05	28.1 (3.2)	25.1 (4.4)	<0.05
TC (mmol/l)	5.85 (1.15)	5.28 (1.02)	<0.05	5.67 (0.84)	5.38 (1.20)	n.s.	5.99 (1.24)	5.29 (0.95)	<0.05	5.05 (0.74)	5.59 (1.05)	<0.05
LDL cholesterol (mmol/l)	4.09 (1.18)	3.44 (0.93)	<0.05	3.66 (0.88)	3.65 (1.15)	n.s.	4.22 (1.24)	3.43 (0.81)	<0.05	3.39 (0.72)	3.66 (1.0)	n.s.
HDL cholesterol (mmol/l)	1.05 (0.30)	1.33 (0.31)	<0.001	1.37 (0.33)	1.18 (0.34)	<0.05	1.11 (0.31)	1.31 (0.33)	<0.05	1.04 (0.24)	1.35 (0.35)	<0.001
TG (mmol/l)	1.50 (0.65)	1.13 (0.47)	<0.05	1.40 (0.56)	1.15 (0.53)	<0.1	1.43 (0.56)	1.19 (0.54)	n.s.	1.34 (0.48)	1.22 (0.59)	n.s.
Lp(a) (g/l)	0.40 (0.40)	0.43 (0.46)	n.s.	0.41 (0.45)	0.43 (0.44)	n.s.	0.45 (0.48)	0.38 (0.38)	n.s.	0.38 (0.45)	0.42 (0.48)	n.s.
Glucose (mmol/l)	5.82 (1.78)	5.84 (2.72)	n.s.	5.60 (2.29)	5.90 (2.54)	n.s.	6.85 (2.88)	5.34 (2.19)	<0.05	6.83 (2.38)	5.35 (2.42)	<0.01

Data for the combined study population are presented as mean ±S.D. *Non-standardized Abbrevations*: CHD, coronary heart disease; oxLDL, oxidized LDL; OLAB, antibodies against oxidized LDL; SOD, superoxide dismutase activity in erythrocytes; GSH-Px, glutathione peroxidase in whole blood; OR, odds ratio; CI, confidence interval; *P*, level of significance.

Table 3
Adjusted odds ratios and 95% confidence intervals of CHD for tertiles of oxidized low density lipoprotein, oxidized low density lipoproteins antibodies, SOD, and GSH-Px models

	OR	95% CI	P
OxLDL (U/l)			
< 38.8	1.00		
38.9–57.9	5.75	0.73–45.12	0.096
> 57.9	20.6	1.86–228.44	0.014
OLAB (mU/ml)			
< 171	1.00		
172–415	0.46	0.076–2.74	n.s.
> 415	0.57	0.10–3.33	n.s.
SOD (U/g of Hb)			
< 714	1.00		
715–953	4.59	0.27–77.42	n.s.
> 953	23.05	0.72–734.68	0.076
GSH-Px (U/l)			
< 5942	1.00		
5943–9407	13.55	0.58–314.0	n.s.
> 9407	305.32	5.07–18 369.95	0.006

Models for the combined population study were adjusted for possible confounding variables. The selection of potential confounders for each oxidative stress biomarker was done on the basis of differences ($P < 0.15$) in baseline characteristics by tertiles (Table 2). Age was included in each model. *Non-standardized Abbreviations:* oxLDL, oxidized LDL; OLAB, antibodies against oxidized LDL; SOD, superoxide dismutase activity in erythrocytes; GSH-Px, glutathione peroxidase in whole blood; OR, odds ratio; CI, confidence interval; *P*, level of significance.

3.4. Differences in baseline characteristics by tertiles of oxidative stress biomarkers

In oxidative stress biomarkers which showed significant differences between CHD patients and controls, differences in baseline characteristics by tertiles of oxidative stress biomarkers were assessed in the whole group (Table 2). Age, TC, LDL-C, and TG were higher in the 3rd tertile of oxLDL compared with the remaining tertiles, while HDL-C was lower. HDL-C and TG levels significantly differed between the two groups of OLAB tertiles. Age, glucose, BMI, TC, LDL-C, and HDL-C significantly differed between the two tertile groups of the SOD distribution. Age, BMI, and HDL-C were higher in the 3rd tertile of the distribution of GSH-Px, and TC was lower, compared with the remaining tertiles.

3.5. Logistic regression analysis

Table 3 shows the results of the logistic regression analyses, performed to evaluate the adjusted odd ratios of CHD and plasma levels for oxLDL, OLAB, SOD, and GSH-Px, respectively. Levels of each oxidative stress biomarker were divided into tertiles, using the lowest as reference to calculate the odd ratios of the

other two. To adjust the logistic regression model of each biomarker, its confounding variables were selected on the basis of the differences in baseline characteristics by tertiles shown in Table 2. Every model was adjusted for age. Significant odds ratios of CHD for the highest tertiles of oxLDL and GSH-Px distribution were observed. A marginally significant odds ratio was observed for the highest tertile of SOD (Odds ratio, 23.1; 95% CI, 0.7–734.7). The risk of CHD was 20.6-fold greater (95% CI, 1.9–228.4) in the highest tertile of the oxLDL distribution compared with the lowest. Concerning GSH-Px, the odds ratio of CHD in the highest tertile was 305.3 (95% CI, 5.1–18 369.9).

4. Discussion

In the present study, we have determined several oxidative stress biomarkers in stable CHD patients and healthy volunteers. Our results showed oxidative stress to be high in CHD patients despite being clinically stable and under medical treatment. This oxidative stress was reflected by the increase of oxLDL levels together with high levels of the antioxidant enzymes SOD and GSH-Px.

Free radical production induces antioxidant enzyme activity as a protective mechanism against free radical injury. A decrease in antioxidant enzymes followed by an increase in their activity levels after free-radical generation conditions, such as myocardial injury followed by reperfusion [15], has been reported. Induction of SOD and GSH-Px activity has been proposed as the mechanism by which preconditioning exerts protection in MI [15]. The protective effect of GSH-Px activity on lipid peroxidation is reinforced by the fact that this enzyme not only detoxifies the H_2O_2 produced by SOD action, but also converts lipid hydroperoxide to non-toxic alcohols, acting as a chain-breaking antioxidant [2]. Therefore, SOD and GSH-Px activity levels may serve as indicators of the balance between free radical production and the bioscavenging capability of superoxide.

Several previous studies showed that oxLDL levels were significantly higher in patients with acute coronary syndromes such as acute myocardial infraction (AMI), unstable angina, and also in heart transplant patients with mild or severe coronary artery stenosis, and patients with post-transplant coronary artery disease (CAD) [7,16]. The few human studies comparing stable CHD with healthy subjects yielded opposite results [7–9]. We observed increased plasma levels of oxLDL in patients with stable CHD. Our results agree with those obtained by Holvoet et al. [17] using the same monoclonal antibody (4E6) to determine oxLDL [7,9].

The question arises as which mechanisms lead to the higher oxLDL levels in plasma of patients with stable CHD. We found a significant correlation between LDL-C and oxLDL in plasma in the total study group which was even stronger in CHD patients. This might indicate that LDL-C concentration itself influences the oxidation of the lipoproteins. However, this hypothesis is not probable because LDL-C levels were lower in patients in this study. Furthermore, other studies could not show a direct relationship between oxLDL levels and hypercholesterolemia [8,17]. The possibility exists that LDL particles of CHD patients are more susceptible to oxidation than LDL from healthy volunteers [18], suggesting that the quality of LDL (i.e. LDL composition) influences the oxidation process. This hypothesis is supported by studies of the research group of Hamsten et al. [19,20] reporting a tendency of enrichment in TG of LDL particles in subjects with more severe CAD, whereby LDL-TG levels correlated with overall severity and rate of progression of coronary atherosclerosis [20], as well as with their susceptibility to oxidation [19]. A recent report has revealed a significant association between the degree of in vivo oxLDL and the TG content of LDL in CHD patients [21] which may indicate that TG-rich particles are prone to oxidative modification. In our study, LDL composition and size could not be determined, but plasma TG levels correlated significantly with oxLDL both in the total study group and in the stable CHD patients when examined alone.

Serum paraoxonase1 (PON1), an HDL-linked enzyme, appears to have a role in the protection of LDL from oxidative stress [14]. PON1 activity was shown to be lower in MI patients compared to control subjects [14]. In this study, and despite a positive correlation between PON1 activity and HDL, we did not observe differences between CHD patients and controls. The high individual variability of PON1 levels, together with the limited sample size, could account for this fact.

OLAB exist in the atherosclerotic lesions of humans and rabbits, this fact reflects oxLDL immunogenicity [22,23]. Several case-control studies showed elevated OLAB titers in patients with early-onset peripheral vascular disease [24] and patients with angiographically verified CAD [25]. OLAB was reported to be predictive for the progression of carotid atherosclerosis [5] and AMI occurrence [26]. In the present study, significantly lower OLAB levels were observed in stable CHD patients. Other studies have reported low levels of OLAB in patients with hypercholesterolemia and signs of early atherosclerosis [6], borderline hypertension [27], ischemic stroke [28], AMI [29], and in diabetic patients who developed CAD [30]. A third group of studies, however, did not show differences in the levels of OLAB in patients with CHD and stroke [31,32]. The discrepancies among these studies are difficult to interpret due to differences in the techniques and antigens used, the populations studied, the stage of the atherosclerotic

disease of CHD patients investigated, and the time elapsed since the last CHD acute event.

Several hypotheses have been raised to explain the inverse relationship between antibody against oxLDL titers and atherosclerosis. It has been hypothesized that the physiological role of OLAB is to remove oxLDL from the circulation by mean of soluble antigen-antibody complexes, which may interfere with OLAB determination [30]. In accordance, an inverse correlation has been observed between OLAB and oxLDL in healthy individuals [33] using the same antibody and method for OLAB analysis as in the present study. The role of the complexes, however, remains to be elucidated, since on one hand, immunization of laboratory animals with oxLDL leads to dramatically enhanced IgG levels that inhibit the progression of atherosclerosis [23], but on the other, these immune complexes activate macrophages in the arterial wall and promote atherosclerosis [22].

Low levels of antibodies against oxLDL IgG titers in hypercholesterolemic patients compared with those in healthy controls have been observed, IgG titers being directly correlated with cell adhesion molecule levels and phospholipase A2 in the patient group [6]. The same authors [6] reported earlier that patients with a history of MI had significantly lower IgM titers of antibodies against oxLDL compared with patients without a history of MI, and with controls [34]. Thus, the humoral immune response could play a different role in different stages of the development of atherosclerosis, as well as in the presence of various other risk factors.

OxLDL and GSH-Px activity were independently and intensely associated with stable CHD in our study group. Thus, the combination of both oxidative stress biomarkers might be useful for the identification of preclinical CHD. Further population cohort studies are needed to confirm our findings.

In summary, the lower oxLDL, SOD, and GSH-Px, and the higher OLAB levels in healthy controls suggest an enhanced oxidative stress situation in CHD patients. The independent association of oxLDL and GSH-Px levels with stable CHD indicate a possible role of these parameters as biomarkers of CHD.

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