

Increased Oxidation of LDL in Patients With Coronary Artery Disease Is Independent From Dietary Vitamins E and C

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Abstract There is increasing experimental evidence that oxidation of LDL plays a major role in the pathogenesis of coronary artery disease (CAD). However, results from clinical studies on LDL oxidation and CAD are not consistent. In most studies only single plasma factors of LDL oxidation have been determined. We studied 207 patients who underwent coronary angiography. They were divided into subjects with CAD ($n=137$) and those without CAD ($n=70$). We determined the susceptibility of LDL to in vitro oxidation (lag phase), potential prooxidative and antioxidative plasma factors (plasma vitamin E, LDL vitamin E, ascorbate, iron, copper, ferritin, and ceruloplasmin), and markers of in vivo LDL oxidation (autoantibodies to malondialdehyde-modified LDL, oxidized LDL,

and thiobarbituric acid-reactive substances), plasma lipids and lipoproteins, smoking habits, and other coronary risk factors in both groups. The lag phase was significantly shorter in patients with CAD than in patients without CAD (101 ± 38.6 versus 119 ± 40.6 minutes, $P < .01$). There was no correlation between the lag phase and the other oxidation parameters or the coronary risk factors. In multivariate regression analyses the lag phase remained significant in all tested models. Our data suggest that a short lag phase of LDL oxidation might be an independent risk factor of CAD. (*Arterioscler Thromb Vasc Biol.* 1997;17:1432-1437.)

Key Words • coronary artery disease • oxidation • lipoproteins • lag phase • antioxidants

It is well established that hypercholesterolemia is the leading risk factor for CAD¹ and that LDL are the main atherogenic lipoproteins.^{2,3} However, at any given level of hypercholesterolemia there is a wide variation in the incidence and expression of CAD. Therefore, it has been proposed that LDL may undergo postsecretory modification, increasing their atherogenic potency.⁴ All major cell types of the artery wall can oxidize LDL,⁵⁻⁸ and oxLDL have been found in atherosclerotic plaques.^{9,10} OxLDL are rapidly taken up by macrophages via scavenger receptors, leading to foam cell formation.¹¹ They are cytotoxic¹² and can alter gene expression in cells of the vessel wall.¹³ Furthermore, oxidized LDL have immunogenic properties and thus induce the formation of autoantibodies, which have been demonstrated in plasma⁹ and atherosclerotic plaques¹⁴ of humans and rabbits.

Some clinical studies revealed a positive correlation between plasma factors of LDL oxidation and atherosclerotic diseases.¹⁵⁻¹⁷ However, others could not demonstrate this correlation.^{18,19} Information on LDL oxidation in patients with CAD is limited because only single plasma factors of LDL oxidation were determined or the study collectives were small.^{15,19,20}

The purpose of this study was to test whether the susceptibility of LDL to oxidation as well as prooxidative and antioxidative plasma factors and markers of in vivo LDL oxidation differ between patients with and those without CAD. We therefore measured the following variables in a collective of 207 patients who underwent coronary angiography: susceptibility of LDL to in vitro oxidation (lag phase), potential prooxidative and antioxidative plasma factors (plasma vitamin E, LDL vitamin E, ascorbate, iron, copper, ferritin, and ceruloplasmin), and markers of in vivo LDL oxidation (autoantibodies to MDA-LDL, oxLDL, and plasma TBARS). In addition, we determined plasma lipids and lipoproteins, smoking habits, and other coronary risk factors (diabetes and hypertension).

Methods

Patients and Samples

We studied patients who underwent diagnostic coronary angiography in the department of cardiology of our hospital between 1993 and 1994. Patients were asked about their smoking habits (never, ex-smoker, or current smoker), history of diabetes mellitus and hypertension, and their current medication. All patients had given informed consent to participate in the study. Fasting blood samples (serum and EDTA-plasma) were drawn immediately before angiography. The angiography was performed with the use of the Judkins technique through the right femoral artery. The angiograms were reviewed by experienced investigators who were independent from the study. Patients with one or more artery luminal stenoses of $>50\%$ were classified as cases; patients with no stenoses were classified as control subjects. Patients with stenoses $\leq 50\%$, heart transplant patients, and vitamin E users were excluded. Initially 239 patients were enrolled into the study (all were white); 32 patients were not eligible. The remaining 207 patients were 150 men and 57 women 18 to 80 years of age.

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Selected Abbreviations and Acronyms

CAD	= coronary artery disease
MDA-LDL	= malondialdehyde-modified LDL
oxLDL	= oxidized LDL
TBARS	= thiobarbituric acid-reactive substances

The blood samples were immediately analyzed or stored at -80°C for further analysis. For the determination of the lag phase, EDTA-plasma was stored at 4°C , and isolation of LDL was performed within 3 days. For the analysis of ascorbate, plasma was immediately deproteinized with trichloroacetic acid (TCA) 10% (wt/vol) and stored at -80°C .²¹

Lipid and Lipoprotein Analyses

Total cholesterol and triglycerides were measured by enzymatic colorimetric methods with kits from Boehringer Mannheim (CHOD-PAP and GPO-PAP, respectively) on an automated analyzer Hitachi 705 (Boehringer Mannheim). LDL cholesterol and HDL cholesterol were determined by precipitation techniques (LDL, precipitation with dextran sulfate, Immuno GmbH; HDL, precipitation of the apolipoprotein B-containing lipoproteins with phosphotungstate/ MgCl_2). Apolipoproteins A1 and B and lipoprotein (a) were assayed by immunonephelometry with a nephelometer analyzer BNA, antisera, standards, and controls from Behring AG.

Susceptibility of LDL to Oxidation (Lag Phase)

LDL was isolated from fresh EDTA-plasma (1 mL) by sequential ultracentrifugation.²² Plasma with a density of 1.02 g/mL was centrifuged for 4 hours at 340 000g with the use of polycarbonate tubes, a TLA-100.1 rotor, and a TL-100 ultracentrifuge from Beckmann Instruments. The infranant was removed and adjusted to a density of 1.063 g/mL with NaCl and centrifuged for 4 hours at 340 000g. The LDL in the supernatant were used for the oxidation analysis. The susceptibility of LDL to in vitro oxidation was assessed according to Esterbauer et al.²³ EDTA was removed from LDL by desalting columns (Econo-Pac 10 DG, Bio-Rad Laboratories, Hercules). The LDL fraction was resuspended in oxygen-saturated phosphate-buffered saline (PBS) (10 mmol/L) at a final concentration of 0.26 mmol cholesterol/L (100 $\mu\text{g}/\text{mL}$). Oxidation was initiated by the addition of freshly prepared CuCl_2 at a final concentration of 1.68 nmol/mL. In a subgroup ($n=99$) the lag phase was determined in addition with 3.36 and 5.04 nmol/mL CuCl_2 . The absorbance at 234 nm was monitored at 30°C by a spectrophotometer (Shimadzu) in 5-minute intervals for 4 hours to follow the formation of conjugated dienes. The lag phase was defined as the intercept of the baseline and the slope of the absorbance curve in the propagation phase and was expressed in minutes.²³ The peak time was defined as the time until maximum absorbance was reached.

Determination of Autoantibodies to Modified LDL

Antibodies to MDA-LDL were determined by an enzyme-linked immunosorbent assay (ELISA). LDL was isolated from fresh plasma of healthy donors by sequential ultracentrifugation²² and incubated with malondialdehyde, generated from acid hydrolysis of 1,1,3,3-tetramethoxypropane (Sigma) to prepare MDA-LDL.¹¹ Microtiter plates (MaxiSorp F96, Nunc) were coated with 100 μL of MDA-LDL (5 μg protein per milliliter) in PBS 10 mmol/L containing 0.25 mmol/L EDTA and 20 $\mu\text{mol}/\text{L}$ butylated hydroxytoluene (BHT) for 16 hours at 4°C . The same LDL preparation was used in all assays. The wells were washed and remaining binding sites were blocked with 2% human albumin (Behring) in PBS for 2 hours at room temperature. To determine the unspecific binding, half of the wells were not coated with antigen but only blocked with albumin. The serum of a normal donor served as the standard

serum with the arbitrary concentration of 100 U/mL antibodies to MDA-LDL. One hundred microliters of standard (1:25, 1:50, 1:100, 1:200, 1:400 in 10 mmol/L PBS containing 0.25 mmol/L EDTA) and the samples (1:100) were added in duplicates to coated and uncoated wells. The antibodies to MDA-LDL were detected by the biotin-avidin system (biotinylated, polyvalent antibodies to human α -, γ - and μ -chains and avidin-peroxidase complex ExtrAvidin peroxidase; Sigma). Peroxidase activity was measured according to the supplier at 405 nm with the use of an MR 7000 spectrophotometer (Dynatech). Extinctions of the MDA-LDL-coated wells were corrected by subtraction of the corresponding value of the uncoated wells. Antibody concentrations were calculated by linear regression and expressed in arbitrary units per milliliter.

Antibodies against oxidized LDL were determined by ELISA technique with the use of a commercial kit (oLAB-ELISA, Biomedica).²⁴

Determination of TBARS

TBARS were determined fluorometrically by high-performance liquid chromatography (HPLC) (Merck/Hitachi) according to Wong et al.²⁵ 1,1,3,3-tetramethoxypropane (Sigma) was used as standard.

Determination of α -Tocopherol

LDL for α -tocopherol determination was isolated from 100 μL plasma by precipitation with heparin-acetate buffer (0.3 mol/L sodium acetate, 100 IU/mL sodium heparinate, pH 4.85) and resuspension of the LDL pellet in 0.1 mol/L TRIS buffer (containing 10 g/L bovine serum albumin, 3 mmol/L NaN_3 , 1 mmol/L EDTA, and 154 mmol/L NaCl). α -Tocopherol in plasma and LDL was determined fluorometrically by HPLC (Merck/Hitachi) as described.²⁶

Determination of Ascorbate, Iron, Copper, Ferritin, Ceruloplasmin, and Cotinine

Ascorbate was determined by the method of Albanese et al²¹ with the use of microtiter plates (Sarstedt) and an MR 7000 spectrophotometer (Dynatech). Iron was measured by the FerroZine method on an automated analyzer Hitachi 747 (Boehringer Mannheim). Copper was determined by atom absorption spectrometry on a 3110-atom absorption spectrometer (Perkin-Elmer GmbH). Ferritin was determined by ELISA technique (Enzymun-Test Ferritin) on an ELISA analyzer ES 700 (Boehringer Mannheim). Ceruloplasmin was measured by nephelometry on a nephelometer analyzer BNA (Behring AG) with antisera, standards, and controls from Behring AG. Cotinine was determined with the use of a radioimmunoassay as described by Langone et al.²⁷

Statistical Methods

Data are summarized as mean \pm SD, and the 10th and 90th percentiles were added. Differences between cases and control subjects were tested for significance with the Wilcoxon two-sample test for continuous variables, with the χ^2 test for categorized variables, and additionally with the Mantel-Haenszel χ^2 test for categorized variables with adjustment for sex and age. Differences were considered significant when probability values were $<.05$. For correlation analyses, the Spearman correlation coefficient was used. Adjusted odds ratios were calculated by multivariate logistic regression analysis. All statistical analyses were performed with the computer program SAS 6.03.

Results

Plasma Factors of LDL Oxidation in Cases and Control Subjects

The susceptibility of LDL to in vitro oxidation was significantly increased in patients with CAD. The lag phase of copper-induced LDL oxidation was 18 min-

TABLE 1. Plasma Factors of LDL Oxidation in Patients With Angiographically Assessed CAD (Cases) and in Control Subjects

	Cases	Control Subjects	P
Lag phase, min	101±38.6 (50, 154)	119±40.6 (62, 182)	.007
Peak time, min	289±114 (165, 500)	299±97.4 (190, 500)	.160
Anti-MDA-LDL antibodies, U/mL	316±195 (150, 520)	329±174 (170, 570)	.378
Anti-oxLDL antibodies, mU/mL	503±372 (186, 1053)	543±471 (166, 966)	.857
TBARS, $\mu\text{mol/L}$	0.320±0.245 (0.01, 0.54)	0.327±0.168 (0.15, 0.51)	.355
Total α -tocopherol, $\mu\text{mol/L}$	32.4±10.1 (21.3, 48.7)	30.0±7.67 (22.0, 39.7)	.176
LDL α -tocopherol, $\mu\text{g/mg}$ LDL cholesterol	6.13±1.98 (4.2, 8.7)	5.56±1.50 (4.1, 7.5)	.045
Ascorbate, $\mu\text{mol/L}$	31.3±16.7 (14.7, 51.0)	35.9±15.4 (17.0, 56.7)	.029
Iron, $\mu\text{mol/L}$	15.5±6.30 (8.41, 24.7)	16.5±5.93 (8.95, 23.6)	.207
Copper, $\mu\text{mol/L}$	17.0±3.94 (12.9, 22.4)	17.6±5.01 (13.0, 25.3)	.868
Ferritin, $\mu\text{g/L}$	219±175 (47, 463)	226±228 (42, 442)	.466
Ceruloplasmin, mg/L	292±55 (228, 362)	293±82 (216, 418)	.651

Data are shown as mean±SD, with the 10th and 90th percentiles in parentheses. Differences between cases and control subjects were tested with the Wilcoxon test.

utes shorter in patients with CAD than in patients without CAD (101 ± 38.6 versus 119 ± 40.6 minutes, $P < .01$). The enhanced oxidizability of LDL derived from CAD patients also has been confirmed with higher concentrations of copper in a subgroup of 99 patients (74 versus 90 minutes for $3.36 \text{ nmol/mL CuCl}_2$ and 64 versus 74 minutes for $5.04 \text{ nmol/mL CuCl}_2$). The difference of the lag phase between cases and control subjects was even stronger if the lag-phase was analyzed as a categorized variable with a threshold of 100 minutes: 53.7% of cases showed a lag phase <100 minutes, but only 21.7% of control subjects showed a lag phase of <100 minutes ($P < .001$; odds ratio, 4.2; 95% confidence interval, 1.9 to 9.1). This finding was independent from sex and age (data not shown). The peak time did not differ significantly between patients with and those without CAD.

The serum concentration of both autoantibodies, anti-MDA-LDL and anti-oxLDL, did not differ significantly between patients with and those without CAD. Plasma TBARS were not elevated in the case group. Both total α -tocopherol and LDL α -tocopherol were slightly higher in patients with CAD (total α -tocopherol, 32.4 versus $30.0 \mu\text{mol/L}$; LDL α -tocopherol, 6.13 versus $5.56 \mu\text{g/mg}$ LDL cholesterol). Ascorbate was significantly decreased in patients with CAD (31.3 versus $35.9 \mu\text{mol/L}$, $P < .05$). Metal-related variables (iron, copper, ferritin, and ceruloplasmin), which are supposed prooxidants, did not differ between cases and control subjects (see Table 1).

Correlations and Multivariate Analysis

Correlation analyses were performed with the oxidation parameters lag phase, TBARS, anti-MDA-LDL, and anti-oxLDL antibodies and with the variables that might influence oxidation. Cases and control subjects were included in the analyses. There was no correlation between the variables lag phase, TBARS, anti-MDA-LDL, and anti-oxLDL antibodies. Lag phase, TBARS, anti-MDA-LDL, and anti-oxLDL antibodies showed no correlation with the following variables: plasma vitamin E, LDL vitamin E, ascorbate, iron, copper, ferritin, and ceruloplasmin.

We also analyzed the correlation between plasma vitamin E and LDL vitamin E. There was a strong correlation between both parameters ($r = .730$, $P < .0001$).

To determine the association between parameters of LDL oxidation and risk factors of CAD, we formed groups of study participants who were positive for a certain risk factor. Each group was compared with the corresponding group, which consisted of the patients who were negative for the same risk factor. The following risk factors were used: male sex, age ≥ 60 years, current smoking, cotinine levels $\geq 10 \text{ ng/mL}$, diabetes, hypertension, and hypercholesterolemia (cholesterol $\geq 6.5 \text{ mmol/L}$). TBARS were significantly raised in the group of patients with hypercholesterolemia (0.376 versus $0.291 \mu\text{mol/L}$, $P < .05$). Susceptibility of LDL to oxidation and the autoantibodies to MDA-LDL and oxLDL were not increased in any of the risk factor groups.

Multivariate logistic regression analyses were performed with inclusion of the lag phase and the variables age, sex, diabetes, hypertension, current and former smoking, triglycerides, cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and lipoprotein (a). The lag phase remained significant and its odds ratio was ≈ 4 in all tested models. In one model that contained the lag phase, age, sex (male), diabetes, and hypertension, the odds ratios were 4.4, 4.8, 2.9, and 0.7, respectively. The probability value of the lag phase was $< .005$. The lag phase was used as a categorized variable in this model.

Demographic Features in Cases and Control Subjects

Table 2 shows demographic data of the case group and the control group. The distribution of risk factors was different in both groups. There was a significant difference between both groups in sex, age, and in former or current smoking. There were also more study participants in the case group, who were positive for the risk factors diabetes, current smoking, raised plasma cotinine levels ($\geq 10 \text{ ng/mL}$, indicative of current smoking), and hypertension, but these differences were not significant. Drug intake of lipid-lowering drugs and anticoagulants or platelet aggregation inhibitors was higher in cases than in control subjects.

Lipid and Lipoprotein Values in Cases and Control Subjects

Table 3 provides plasma lipid and lipoprotein concentrations in the two groups. Triglycerides and VLDL

TABLE 2. Demographic Features of Patients With Angiographically Assessed CAD (Cases) and of Control Subjects

	Cases	Control Subjects	P
n	137	70	
Male sex, %*	78.1	61.4	.011
Age \pm SD, y†	60.7 \pm 10.0	57.4 \pm 12.1	.0468
Smoker, %*	21.2	15.4	.330
Former or current smoker, %*	79.6	56.9	.001
Cotinine, % \geq 10 ng/mL*	13.9	11.4	.622
Diabetes, %*	18.5	9.0	.076
Hypertension, %*	45.9	41.2	.520
Current medication, %*			
Lipid-lowering drugs	25.4	3.5	
Anticoagulants and platelet aggregation inhibitors	78.0	36.5	

*Differences between cases and control subjects were tested with the χ^2 test.

†Differences between cases and control subjects were tested with the Wilcoxon test.

cholesterol were normal in both groups but significantly higher in cases than in control subjects. Total cholesterol, LDL cholesterol, and apolipoprotein B were higher in patients with CAD, but only apolipoprotein B reached significance. It must be noted that more participants in the case group were treated with lipid-lowering drugs than in the control group (Table 2). HDL cholesterol and apolipoprotein A1 were both significantly lower in cases than in control subjects. Finally, lipoprotein (a) levels were significantly increased in cases.

Discussion

This study presents for the first time a wide range of prooxidative and antioxidative plasma factors and in vivo markers of LDL oxidation in patients with and without angiographically assessed coronary artery disease. The data obtained demonstrate that the susceptibility of LDL to oxidation is significantly higher in patients with CAD than in patients without CAD. Vitamin E was slightly higher in cases and vitamin C slightly lower. The in vivo markers of LDL oxidation and the prooxidative factors did not differ between cases and control subjects.

In our study autoantibodies against both oxLDL and MDA-LDL were not elevated in patients with CAD. This finding is consistent with recently published observations, which showed no significant correlation between anti-oxLDL and CAD,¹⁸ progression of carotid atherosclerosis,¹⁶ and restenosis after percutaneous translumi-

nal coronary angioplasty.¹⁹ However, Maggi et al²⁸ found increased anti-oxLDL and anti-MDA-LDL antibody ratios in patients with carotid atherosclerosis, and two prospective case-control studies identified anti-MDA-LDL as a predictor of the progression of carotid atherosclerosis¹⁶ and myocardial infarction,²⁹ respectively. The reason for these contradictory results might be the use of different methods for determination of the antibodies. Craig et al³⁰ reported that the choice of the blocking buffer and the method of data expression led to different results when they compared anti-MDA-LDL antibodies in women with and those without SLE, and Salonen et al¹⁶ reported in their study that there was a great variability in the antibody titers from the same serum sample when different preparations of oxLDL were used.

We observed slightly higher LDL vitamin E levels in patients with CAD. This is an unexpected finding because vitamin E is a major antioxidant of LDL.³¹ In addition, plasma vitamin E was reported to be inversely correlated with the regional CAD mortality in a large cross-sectional population study.³² However, in our study, there was no correlation between LDL vitamin E and the lag phase, a result that has also been described by others.³¹ Esterbauer and colleagues³¹ attributed this to a variation of the efficiency of vitamin E to increase the oxidation resistance and of a vitamin E-independent component of the oxidation resistance of LDL between individual subjects. Another explanation for this finding could be that in our study many case patients already had symptomatic CAD and—as a confounding factor—might have changed their diets. In other case-control studies there was also no correlation between vitamin E and cardiovascular mortality.^{33,34} However, other factors such as the density of LDL³⁵ or its fatty acid content³⁶ may play a role in the variability of LDL oxidation.

Ferritin, a marker of stored iron, has been reported to be associated with the risk of myocardial infarction³⁷ in eastern Finnish men. Copper was associated with the classic risk factors of CAD in the population of Northern Ireland³⁸ and with the progression of carotid atherosclerosis.³⁹ In a recent study, ceruloplasmin levels were increased in male patients with CAD compared with control subjects.⁴⁰ These findings support a role of iron ions and copper ions in the oxidation of LDL. However, we could not find elevated levels of the metal-related variables iron, copper, ferritin, and ceruloplasmin in the group of patients with CAD, and there was also no correlation between these variables and the lag phase in

TABLE 3. Lipids and Lipoproteins in Patients With Angiographically Assessed CAD (Cases) and of Control Subjects

	Cases	Control Subjects	P
Cholesterol, mmol/L	5.59 \pm 1.11 (4.09, 6.49)	5.40 \pm 1.05 (4.04, 6.79)	.226
Triglycerides, mmol/L	1.86 \pm 0.968 (0.866, 3.41)	1.49 \pm 1.04 (0.638, 2.41)	.001
HDL cholesterol, mmol/L	1.09 \pm 0.307 (0.777, 1.50)	1.31 \pm 0.434 (0.803, 1.89)	.0002
LDL cholesterol, mmol/L	3.76 \pm 0.921 (2.62, 5.02)	3.50 \pm 1.00 (2.02, 4.69)	.101
VLDL cholesterol, mmol/L	0.724 \pm 0.391 (0.362, 1.42)	0.606 \pm 0.370 (0.337, 0.952)	.012
Apolipoprotein A1, mg/100 mL	131 \pm 24.4 (104, 160)	145 \pm 27.6 (104, 182)	.0002
Apolipoprotein B, mg/100 mL	134 \pm 28.6 (95, 170)	121 \pm 27.3 (85, 154)	.0073
Lipoprotein (a), mg/100 mL	26.9 \pm 27.7 16.0* (5.0, 63)	17 \pm 18.7 8.0* (5.0, 38)	.006

Data are shown as mean \pm SD, with the 10th and 90th percentiles in parentheses. Differences between cases and control subjects were tested with the Wilcoxon test.

*Median (for lipoprotein [a] shown in addition).

our study. Therefore our data cannot confirm the assumption that the concentration of metal ions in plasma is associated with CAD. This is consistent with the findings of two recent studies, which showed no correlation between serum-ferritin levels and myocardial infarction⁴¹ and between iron intake and CAD,⁴² respectively.

Among all tested plasma factors of LDL oxidation, only the in vitro susceptibility of LDL to oxidation was increased in patients with CAD. The lag phase was 18 minutes shorter in patients with coronary artery disease than it was in control subjects. This finding is consistent with the results of two studies: Regnström et al¹⁵ observed a correlation between the lag phase and the severity of CAD in a small group of patients, and Cominacini et al²⁰ demonstrated recently a shorter lag phase in patients with CAD than in control subjects. In addition, we found that the difference between patients with and those without CAD was stronger if the lag phase was analyzed as a categorized variable. This might lead to the suggestion that the lag phase is a variable with a "threshold" and that subjects with a lag phase below a certain value have a particularly high risk of developing atherosclerosis of the coronary arteries. With a threshold of 100 minutes, for example, the odds ratio was 4.2. The lag phase was independent from the risk factors sex, age, smoking, diabetes, hypertension, and hypercholesterolemia because the lag phase remained significant in the multivariate regression analysis and it showed no correlation with these risk factors. In conclusion, our data support the suggestion that the susceptibility of LDL to oxidation is an independent risk factor of CAD, which varies between individuals and could therefore partly explain the different incidence and manifestation of CAD at similar cholesterol levels. However, the role of the lag phase as a risk factor cannot be proven by a case-control study. Prospective studies in CAD patients are necessary to confirm these findings. Recently, the Cambridge Heart Antioxidant Study (CHAOS)⁴³ reported a significant reduction of nonfatal myocardial infarctions in CAD patients who were treated with a pharmacological dose of vitamin E. However, oxidation parameters of LDL were not determined. The lag phase should be measured in further intervention studies with antioxidants to determine their individual effect on the susceptibility of LDL to oxidation.

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