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# Circulating Lipid Hydroperoxides Predict Cardiovascular Events in Patients With Stable Coronary Artery Disease

## The PREVENT Study

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**Objectives** This study was designed to determine the predictive value of lipid hydroperoxide (LOOH) levels for adverse cardiovascular outcomes in patients with stable coronary artery disease (CAD).

**Background** Oxidative modification of circulating lipids contributes to inflammation and endothelial dysfunction, which are hallmark features of atherosclerosis. A serum biomarker of oxidation is LOOH, which is a primary product of fatty acid peroxidation.

**Methods** Serum LOOH levels were measured and correlated with clinical events over a 3-year period in 634 patients with angiographic evidence of CAD.

**Results** Baseline LOOH levels in the highest quartile were associated with hazard ratios of 3.24 (95% confidence interval [CI] 1.86 to 5.65; p = 0.0001) for nonfatal vascular events (n = 149), 1.80 (95% CI 1.13 to 2.88; p = 0.014) for major vascular procedures (n = 139), and 2.23 (95% CI 1.44 to 3.44; p = 0.0003) for all vascular events and procedures. Baseline LOOH levels correlated with serum levels of soluble intercellular adhesion molecule-1 (p = 0.001) and thiobarbituric acid reactive substances (p = 0.001) as well as the mean percent change in stenosis for large segments >50% stenosed (p = 0.048). A multivariate proportional hazards model, adjusted for traditional risk factors and inflammatory markers, showed an independent effect of LOOH on nonfatal vascular events, vascular procedures, and all events or procedures. Amlodipine treatment was associated with reduced cardiovascular events and changes in LOOH levels compared with placebo.

**Conclusions** Elevated LOOH levels were predictive of nonfatal vascular events and procedures in patients with stable CAD, independent of traditional risk factors and inflammatory markers. (J Am Coll Cardiol 2008;51:1196–202)


Oxidative modification of low-density lipoprotein (LDL) and cellular lipids contributes to foam cell formation, endothelial dysfunction, and destructive inflammatory processes associated with atherosclerosis (1–3). Lipid hydroperoxides (LOOH) are generated from polyunsaturated fatty acids and represent primary end products of the lipid peroxidation cascade. Circulating levels of LOOH have been shown to be significantly elevated in association with myocardial ischemia (4) and cardiovascular risk factors (5–9). With sustained exposure to reactive oxygen species, LOOH undergoes further decomposition to reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal. We recently reported that levels of MDA (measured as thiobarbituric acid reactive substances [TBARS]) were highly predictive of cardiovascular events, independent of traditional risk factors (10). In this earlier study, levels of TBARS did not predict changes in large stenotic lesions. This has led to our hypothesis that primary end products of lipid peroxidation may be useful, and perhaps more sensitive, diagnostic biomarkers of cardiovascular disease (CVD) progression.

This longitudinal study evaluated the predictive value of LOOH levels in 634 patients with stable CAD from PREVENT (Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial), a prospective, double-blind clinical trial. Patients in this study had angiographic evidence of coronary artery disease (CAD) and normalized blood pressure at baseline. The participants were random-

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ized to treatment with either amlodipine or placebo. The primary outcome was the average 36-month angiographic change in mean minimal diameters of segments with a baseline diameter stenosis of 30%. Amlodipine treatment (10 mg/day) did not slow angiographic progression of coronary atherosclerosis relative to placebo but significantly slowed carotid artery atherosclerosis, which was assessed ultrasonographically (11). Amlodipine was associated with fewer cases of unstable angina pectoris and coronary revascularization procedures, although treatment did not significantly decrease mortality or major vascular events (11). The benefits seen with amlodipine treatment may be attributed to various calcium-dependent and -independent mechanisms, including its reported antioxidant activity (12–15).

In this study, we evaluated LOOH levels in serum samples collected from patients at baseline and at the end of each 12-month period during the 3-year study. The levels of LOOH were measured spectrophotometrically (16,17). The association of LOOH with clinical events and procedures was evaluated in univariate and multivariate models adjusted for inflammatory markers (high sensitivity C-reactive protein [hs-CRP], soluble intercellular adhesion molecule [sCAM]-1, interleukin [IL]-6) and other risk factors (age, gender, lipids, body mass index [BMI], and blood pressure).

## Methods

**General design features.** The PREVENT was a multicenter, randomized, placebo-controlled, double-blind clinical trial of patients who had angiographic evidence of CAD (11). Men and women (30 to 80 years of age) were randomized if there was angiographic evidence of 1 focal coronary lesion of ≥30% diameter stenosis (nonintervened and noninfarcted) and the presence of ≥1 lesion with a 5% to 20% stenosis that was not in a vessel with a ≥60% lesion. Other eligibility criteria included diastolic blood pressure (DBP) of <95 mm Hg, total cholesterol of <325 mg/dl, and fasting blood glucose of <200 mg/dl. Randomization was stratified according to clinical center and history of percutaneous transluminal coronary angioplasty. Study medication was initiated at 5 mg daily and increased to 10 mg daily after 2 weeks if tolerated. The final study angiogram was scheduled 36 months after randomization, 7 to 10 days after the study medication was stopped.

**Monitoring for clinical events and adverse experiences.** The pre-specified clinical events were all-cause mortality and the occurrence of major fatal/nonfatal vascular events or procedures. An external events classification committee blinded to treatment assignment classified the events as death, myocardial infarction, stroke, hospitalized heart failure, or hospitalized episodes of unstable angina. Confirmation of unstable angina required hospitalization for typical chest pain and either evidence of myocardial ischemia (electrocardiogram or stress test evidence, or new angiographic findings of disease) or an indication that this pain was similar to that of previously documented ischemia.

**Angiographic methods and outcomes.** The progression of early atherosclerotic segments was determined on the basis of a change in mean minimal diameter with quantitative coronary angiography (18,19). Atherosclerotic segments were defined as coronary segments with a diameter stenosis of ≤30% at baseline. Up to 12 coronary segments were used in the analysis of disease progression (20). Vessels that underwent a procedure at or before baseline were excluded from the analyses. A certified reader who was blinded to treatment assignment and the temporal sequencing of films read pairwise the baseline and follow-up films.

**Measurement of serum LOOH levels.** Serum samples were available from 634 fasting participants at the beginning of the study (baseline) and at the end of each of the 3 years. Samples were stored at −70°C without the addition of exogenous antioxidants prior to LOOH analysis. After thawing the samples, measurements of LOOH were performed in triplicate for each of 2,975 samples using the ferrous oxidation of xylenol orange (FOX) 2 assay (16,17). Serum lipid hydroperoxides were measured by oxidation of ferrous iron in the presence of xylenol orange. In this method, ferrous iron is oxidized to ferric iron by hydroperoxides. This product then forms a complex with xylenol orange to yield a chromophore that can be detected spectrophotometrically at 560 nm. No lipid extraction step is necessary and the assay measures total plasma lipid hydroperoxides (i.e., the sum of cholesterol, triacylglycerol, and phospholipid hydroperoxides contained in all lipoprotein fractions).

**Measurement of TBARS.** Malondialdehyde levels were evaluated by measuring serum TBARS. These measurements were performed in duplicate for each of the 2,975 samples using the method of Carbonneau et al. (21), with slight modifications. Briefly, 50 μl of 10 mol/l sodium hydroxide (NaOH) was added to 0.5 ml of serum and incubated at 60°C for 30 min. The sample was then acidified to pH 1.0 with 500 μl of 586 g/l perchloric acid. After centrifugation, 300 μl of supernatant was added to 50 μl of thiobarbituric acid (TBA) (10 g/l in 50 mmol/l

**Abbreviations and Acronyms**

BMI = body mass index
CAD = coronary artery disease
CV = coefficients of variation
CVD = cardiovascular disease
DBP = diastolic blood pressure
FOX = ferrous oxidation of xylenol orange
HDL = high-density lipoprotein
HPLC = high-performance liquid chromatography
hs-CRP = high sensitivity C-reactive protein
IL = interleukin
LDL = low-density lipoprotein
LOOH = lipid hydroperoxide
Lp-PLA$_2$ = lipoprotein-associated phospholipase A$_2$
MDA = malondialdehyde
MI = myocardial infarction
QC = quality control
SBP = systolic blood pressure
sICAM = soluble intercellular adhesion molecule
TBA = thiobarbituric acid
TBARS = thiobarbituric acid reactive substances

phosphate buffer, pH 7.0) and heated at 100°C for 30 min. The sample was cooled and 100 μl were removed for high-performance liquid chromatography (HPLC) analysis.

The MDA-TBA complex was separated from other possible TBA reactants using reverse-phase HPLC on a Varian ProStar system (Varian Inc., Palo Alto, California) coupled to a spectrophotometric detector (operating at 532 nm) and a fluorescence detector (excitation = 515 nm, emission = 553 nm) on a 150 mm × 4.6 mm Adsorbosphere (Alltech Associates Inc., Deerfield, Illinois) $C_{18}$ column with 5-μm particle size. The purpose for using reverse-phase HPLC followed by quantitation with both spectrophotometry and fluorescence was to eliminate other aldehydes that react with TBA and have absorbance characteristics at 532 nm (22–24). The flow rate was 1 ml/min and the mobile phase was 80% phosphate buffer (10 mmol/l, pH 5.8) with 20% methanol. A standard curve was run at the start, middle, and end of each sample set analysis using 1,1,3,3-tetraethoxypropane as a standard. Peak areas were determined using Varian Star Chromatography Workstation software.

**Measurement of serum hs-CRP, IL-6 and sICAM-1.** High sensitivity C-reactive protein levels were measured using the N Latex mono assay (Dade Behring Inc., Newark, Delaware) with a detection limit of 0.21 mg/l. Samples with values below the limit of detection were recorded as <0.21 mg/l; the value 0.20 mg/l was incorporated for statistical analyses. Intra- and interassay precision for the low quality control (QC) (0.46 mg/l) had coefficients of variation (CVs) of 9.9% and 14.8%, respectively. Serum soluble intercellular adhesion molecule-1 levels were measured with the Parameter Human sICAM-1 Immunoassay Kit (R&D Systems, Minneapolis, Minnesota), with a range of 0 to 588 ng/ml. Intra- and interassay precision for the middle QC (282.7 ng/ml) had CVs of 9.0% and 9.5%, respectively. Interleukin-6 levels were measured using the Quantikine HS IL-6 R&D Systems kit, which had an assay range of 0.156 to 10 pg/ml. Intra- and interassay precision for the low QC (0.338 pg/ml) had CVs of 9.9% and 14.4%, respectively. All measurements were made by Esoterix Coagulation (Aurora, Colorado).

**Statistical analysis.** All statistical analyses were performed using SAS Version 8.2 (SAS Institute Inc, Cary, North Carolina) with alpha set to 0.05. Simple descriptive statistics were used to describe the population. For clinical outcomes, proportional hazards regression models were used to obtain hazard ratios and associated 95% confidence intervals (CIs). The first proportional hazards model used baseline LOOH and treatment as covariates for each clinical outcome (major vascular events, hospitalizations for angina, coronary artery bypass grafting, PTCA, and major vascular procedures). To further investigate the impact of LOOH on clinical outcomes, a proportional hazards model was performed using quartiles of baseline LOOH with the reference group being those in the lowest quartile. The univariate effect of LOOH on major vascular events, nonfatal vascular events, vascular procedure, and all vascular events and procedures was also tested in a multivariate Cox proportional hazards regression model adjusted for inflammatory markers (sICAM-1, IL-6, hs-CRP) and known cardiovascular disease (CVD) risk factors, including age, gender, total cholesterol, high-density lipoprotein (HDL), LDL, triglycerides, systolic blood pressure (SBP), DBP, and BMI.

Pearson's correlation coefficients were used to assess the correlation between LOOH and coronary angiography outcomes measurements (e.g., all segments, segments stenosed ≤30%, segments stenosed <30% and ≤50% and segments stenosed >50%) as well as LOOH and changes in patient characteristics (e.g., change in SBP, change in DBP, change in HDL, change in LDL and change in triglycerides). The p-values were calculated from the Spearman rank correlation coefficient.

## Results

**Baseline characteristics.** The baseline patient characteristics of the study cohort are shown in Table 1 and were well matched as a function of treatment with amlodipine or placebo, as previously described in detail (11). There were no clinically relevant differences in the baseline characteristics of the patients who did and did not participate in the biomarker study due to availability of adequate serum samples.

**Table 1** PREVENT: Baseline Description of Patients (n = 634)

| | Mean* |
|---|---|
| Variable | |
| Age (yrs) | 57.2 ± 9.6 |
| Male (%) | 80.9 |
| Female (%) | 19.1 |
| Total cholesterol (mg/dl) | 217.9 ± 39.1 |
| HDL cholesterol (mg/dl) | 45.7 ± 11.5 |
| LDL cholesterol (mg/dl) | 132.4 ± 36.1 |
| Triglycerides (mg/dl) | 203.7 ± 131.7 |
| Average SBP (mm Hg) | 129.0 ± 17.4 |
| Average DBP (mm Hg) | 78.6 ± 9.1 |
| BMI (kg/m × m) | 27.9 ± 4.6 |
| Current smoker (%) | 24.9 |
| Past smoker (%) | 54.9 |
| Prior MI (%) | 47.3 |
| Prior angina (%) | 51.6 |
| Medication use (%) | |
| Calcium channel blocker | 33.8 |
| ACE inhibitor | 9.1 |
| Diuretic | 11.8 |
| Beta-blocker | 62.8 |
| Nitrates | 63.8 |
| Lipid-lowering agent | 27.3 |

*Values are mean ± standard deviation unless indicated.

ACE = angiotensin-converting enzyme; BMI = body mass index; DBP = diastolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MI = myocardial infarction; PREVENT = Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial; SBP = systolic blood pressure.

**Clinical events.** During the study, there were 51 major vascular events, such as fatal/nonfatal myocardial infarction (MI) and stroke; 149 hospitalizations for nonfatal cardiovascular events (mainly unstable angina); and 139 patients also underwent a major vascular procedure, such as PTCA/CABG (11).

**Lipid hydroperoxide levels and clinical events.** Table 2 shows the relationship between measured baseline levels of LOOH and cardiovascular events in PREVENT. Baseline LOOH levels in the highest quartile were associated with hazard ratios of 3.24 (95% CI 1.86 to 5.65; p = 0.0001) for nonfatal vascular events (n = 149), 1.80 (95% CI 1.13 to 2.88; p = 0.014) for major vascular procedures (n = 139), and 2.23 (95% CI 1.44 to 3.44; p = 0.0003) for all vascular events and procedures. The absolute mean levels of LOOH in patients at baseline with and without specific vascular events and procedures events are shown in Table 3. At baseline, the overall mean absolute level of LOOH was 36.5 ± 28.1 μmol/l. The significant univariate effect of LOOH seen on major vascular events, nonfatal vascular events, vascular procedures, and all vascular events and procedures is also seen in a multivariate model, adjusting for inflammatory markers and known CVD risk factors. Specifically, a multivariate Cox proportional hazards regression analysis was performed in which the following variables were included: LOOH, inflammatory markers (sICAM-1, IL-6, hs-CRP), age, gender, total cholesterol, HDL, LDL, triglycerides, SBP, DBP, and BMI. After adjusting for all of these variables, LOOH levels showed an independent effect on nonfatal vascular events, vascular procedures, and all vascular events and procedures. The data presented in Figure 1 demonstrate that participants with the highest baseline LOOH levels were at increased risk for experiencing major events and/or procedures.

**Lipid hydroperoxide levels and inflammatory markers.** We measured serum levels of 3 different inflammatory markers at baseline from identical samples. The median (25th, 75th percentile) levels at baseline for IL-6, sICAM-1, and hs-CRP were 2.7 pg/ml (1.7, 4.3), 2.1 ng/ml (1.7, 2.5), and 2.5 mg/l (1.2, 5.5), respectively. A significant correlation of LOOH (p = 0.001) with levels of sICAM-1 was observed, with a correlation coefficient of 0.265; however, as reported in Table 4, baseline levels of LOOH did not correlate with either hs-CRP, an acute-phase reactant, or with levels of IL-6.

**Lipid hydroperoxide levels and advanced products of lipid peroxidation.** The decomposition of LOOH into more advanced products of lipid peroxidation, such as MDA, were measured and compared with levels of LOOH. As shown in Table 4, baseline levels of MDA (measured as TBARS) correlated with LOOH levels. The correlation coefficient was 0.158 and highly significant (p = 0.001).

**Lipid hydroperoxide levels and amlodipine treatment.** The median level at baseline for LOOH was 36.5 ± 28.1 μmol/l. Amlodipine treatment was associated with a smaller decrease in levels of LOOH (−16.4 μmol/l) versus placebo (−21.5 μmol/l) treated patients over the 3-year period (p = 0.032). Patients on amlodipine experienced a 31% reduction in the relative risk of a major documented vascular event or procedure (p = 0.01), as previously reported in detail (11).

**Lipid hydroperoxide levels and angiographic measurements.** In segments with >50% stenosis, baseline LOOH levels correlated significantly with the mean percent change in stenosis (n = 246; p = 0.048). By contrast, there was no association with the inflammatory markers (IL-6, sICAM-1, hs-CRP) or levels of TBARS. In segments with <30% or 30% to 50% stenosis (n = 540), there was no significant association with serum LOOH levels (p = 0.963).

**Lipid hydroperoxide levels and patient characteristics.** Levels of LOOH also did not correlate with baseline demographics including gender, age, history of smoking, previous angina, or MI. In addition, LOOH baseline levels did not associate significantly with the patient's family history of either MI or sudden death from CVD.

## Discussion

The key finding of this longitudinal study was that LOOH was a significant independent predictor of nonfatal cardiovascular events and procedures in patients with stable CAD. Levels of LOOH also correlated with a key marker of endothelial dysfunction (sICAM-1) and larger stenotic lesions (>50%) in these angiographically documented CAD patients. It was further observed that the association of LOOH levels and CVD risk was independent of traditional risk factors and inflammatory markers (hs-CRP and IL-6).

These data indicate prognostic utility with LOOH for cardiovascular events in patients with stable CAD, along with certain aspects of plaque progression and remodeling. As most unstable plaques are associated with smaller ste-

**Table 2** PREVENT: Predictive Value of LOOH Serum Levels (Baseline) in Highest Quartile

| Cardiovascular Event | Number With Event | HR | 95% CI for RR | p Value |
|---|---|---|---|---|
| Major vascular events (fatal/nonfatal MI, stroke) | 51 | 1.85 | 0.82–4.20 | 0.138 |
| Hospitalizations for angina | 149 | 3.24 | 1.86–5.65 | 0.0001 |
| Major vascular procedures (CABG/PTCA) | 139 | 1.80 | 1.13–2.88 | 0.014 |
| All major vascular events and procedures | 190 | 2.23 | 1.44–3.44 | 0.0003 |

CABG = coronary artery bypass graft; CI = confidence interval; HR = hazard ratio; PTCA = percutaneous transluminal coronary angioplasty; RR = relative risk; other abbreviations as in Table 1.

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**Table 3** PREVENT: Comparative Absolute LOOH Serum Levels With Specific Vascular Events and Procedures at Baseline

| Cardiovascular Event | Patients With Events/Procedures | | Patients Without Events/Procedures | | p Value* |
|---|---|---|---|---|---|
| | Mean, μmol/l | SD | Mean, μmol/l | SD | |
| Major vascular events (MI, stroke) | 41.2 | 28.6 | 35.8 | 27.8 | 0.251 |
| Nonfatal vascular events (angina/CHF) | 45.1 | 26.1 | 34.1 | 27.9 | 0.001 |
| Major vascular procedures (CABG/PTCA) | 43.0 | 26.8 | 34.7 | 28.0 | 0.004 |
| All vascular events and procedures | 42.0 | 26.5 | 34.2 | 28.1 | 0.002 |

*Lipid hydroperoxide (LOOH) levels for patients with versus without events/procedures (2-sample *t* test).
CHF = congestive heart failure; other abbreviations as in Tables 1 and 2.

notic lesions, we speculate that LOOH levels reflect the chronic phase of CAD and may not become elevated in patients with acute coronary syndrome in which inflammatory biomarkers (e.g., hs-CRP) are commonly high. The ability to determine CVD risk with LOOH in these patients was similar to that observed for MDA (measured by TBARS), as previously reported (10). However, levels of MDA did not correlate with the percent change in large stenotic lesions and were unaffected by amlodipine treatment (10). Thus, there may be differences in the predictive value of primary versus secondary end products of oxidation with respect to plaque progression and stability. This is further supported by the low degree of correlation between these markers of oxidation (Table 4).

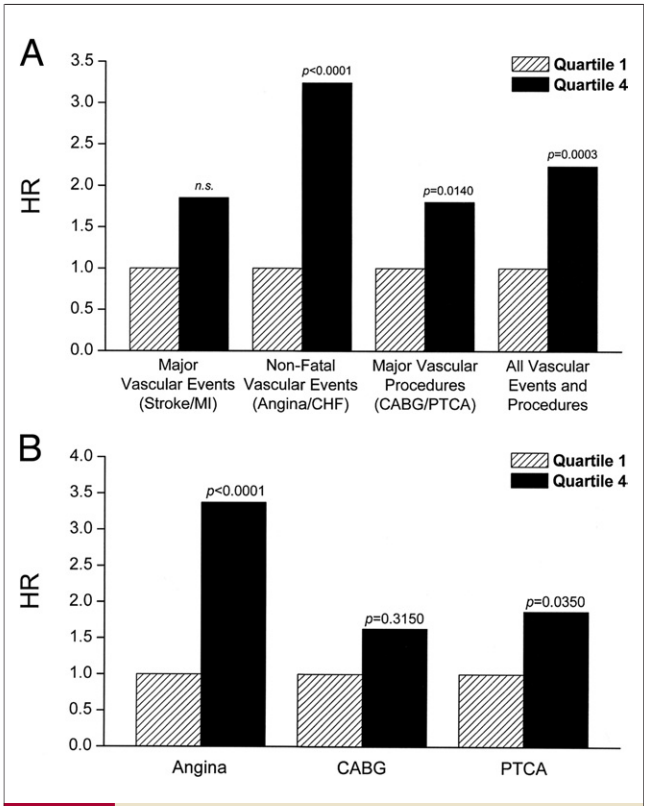


**Figure 1** Quartile Analysis of Serum LOOH Levels and Risk for Cardiovascular Events

Analysis of baseline lipid hydroperoxide (LOOH) levels in patients with documented coronary artery disease for combined **(A)** and individual **(B)** events and procedures. CABG = coronary artery bypass graft; CHF = congestive heart failure; HR = hazard ratio; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.

Oxidative modification of lipids associated with LDL and cellular constituents contribute to endothelial dysfunction and inflammatory pathways associated with atherosclerosis and plaque development (1–3,25). A correlation between oxidative stress markers, including LOOH, and angiographic measurements had been observed in an earlier study of 1,200 patients (26). The mechanism of in vivo LDL and protein oxidation in human atherosclerotic lesions has been attributed to myeloperoxidase activity (27). Other enzymatic sources of reactive oxygen species include reduced NAD(P)H, lipoxygenases, xanthine oxidases, uncoupled endothelial nitric oxide synthases, cyclooxygenases, and mitochondria. Approaches that have been used to assess oxidative stress levels include monoclonal antibodies against oxidized LDL, quantitation of protein oxidation markers, and measurement of isoprostanes (28–30).

The finding in this study that LOOH, but not hs-CRP, predicted revascularization procedures in patients with stable CAD is similar to that observed with an enzymatic mediator of oxidative stress known as lipoprotein-associated phospholipase $A_2$ (Lp-$PLA_2$). The Lp-$PLA_2$ enzyme is associated with circulating LDL that hydrolyzes oxidized phospholipids. The formation of downstream inflammatory mediators derived from these oxidized phospholipids accounts for the pro-atherogenic effects of Lp-$PLA_2$. Recently, Lp-$PLA_2$ was measured in 3,766 patients with stable CAD from the PEACE (Prevention of Events with Angiotensin-Converting Enzyme Inhibition) clinical trial. Although both hs-CRP and Lp-$PLA_2$ predicted acute coronary syndromes, only Lp-$PLA_2$ was a significant predictor of coronary revascularization after adjustment for baseline characteristics (31). This is consistent with other large studies that indicate a prognostic role for oxidized lipids and Lp-$PLA_2$, independent of inflammatory markers and traditional risk factors (32).

Although amlodipine treatment produced a 31% reduction in the risk of major vascular events in these patients, a larger decrease in serum LOOH levels was observed in placebo-treated subjects. To explain this apparent paradox, we hypothesize that amlodipine may interfere with LOOH

**Table 4** PREVENT: Correlation Between LOOH and Various Biomarkers at Baseline

| Biomarker | R Value | p Value |
|---|---|---|
| hs-CRP | 0.000 | 0.722 |
| sICAM-1 | 0.265 | 0.001 |
| IL-6 | 0.055 | 0.168 |
| TBARS | 0.158 | 0.001 |

hs-CRP = high sensitivity C-reactive protein; IL = interleukin; sICAM = soluble intercellular adhesion molecule; TBARS = thiobarbituric acid reactive substances; other abbreviations as in Tables 1 and 3.

decomposition to more atherogenic products such as dicarboxylic acids. The benefits of such an antioxidant mechanism was described by Sakuma et al. (33) who suggested that an effective treatment for CAD would be one that stabilized lipid peroxidation primary end products, thereby interfering with formation of secondary products such as reactive aldehydes. These highly reactive aldehydes modify lysine residues associated with apolipoprotein B, resulting in oxidized LDL particles that are more readily recognized by scavenger receptors. Franzoni et al. (15) have shown that hydroxyl radicals generated by the Fenton reaction were rapidly and efficiently scavenged by amlodipine in a manner that was far superior to Trolox or glutathione acid.

Serum levels of LOOH correlated directly with levels of sICAM-1, but not IL-6 or hs-CRP. Expression of sICAM-1 promotes the adherence and migration of new monocytes through the endothelial barrier and is associated with increased CVD risk (34,35). Additionally, sICAM-1 is effectively stimulated by oxidized LDL (36,37). Given the interrelationships between cytokine expression, angiotensin II, and reactive oxygen species, the correlation between levels of sICAM-1 and LOOH was not unexpected.

The implication of these data for antioxidant therapy remains an open question because the balance of studies in this area suggest that oxidized lipids serve as biomarkers of CAD with no clear causative role in atherogenesis. Indeed, epidemiological studies indicate that low levels of antioxidants are associated with increased risk for cardiovascular events; but, as recently reviewed (38,39), several large prospective clinical trials have failed to show any benefits of antioxidant treatment. Possible explanations for this paradox may be provided by variations in trial design, baseline antioxidant status of participants, dosage and source of the antioxidants, and time of intervention relative to disease progression. It is also possible that vitamin E does not neutralize relevant oxidants, such as those produced by myeloperoxidase (2-electron oxidants) (40), and/or is less efficient at penetrating into the atherosclerotic plaque (41,42). Alternatively, antioxidants that act at different steps in the lipid peroxidation cascade mechanism (i.e., primary versus secondary end products) may have distinct effects on the course of the disease.

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