Low Plasma Ascorbic Acid Independently Predicts the Presence of an Unstable Coronary Syndrome

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Objectives. This study sought to investigate the relations between plasma antioxidant status, extent of atherosclerosis and activity of coronary artery disease.

Background. Previous studies indicate that increased antioxidant intake is associated with decreased coronary disease risk, but the underlying mechanisms remain controversial.

Methods. Plasma samples were obtained from 149 patients undergoing cardiac catheterization (65 with stable angina, 84 with unstable angina or a myocardial infarction within 2 weeks). Twelve plasma antioxidant/oxidant markers were measured and correlated with the extent of atherosclerosis and the presence of an unstable coronary syndrome.

Results. By multiple linear regression analysis, age ($p < 0.001$), diabetes mellitus ($p < 0.001$), male gender ($p < 0.001$) and hypercholesterolemia ($p = 0.02$) were independent predictors of the extent of atherosclerosis. No antioxidant/oxidant marker correlated with the extent of atherosclerosis. However, lower plasma ascorbic acid concentration predicted the presence of an unstable coronary syndrome by multiple logistic regression (odds ratio [OR] 0.59, 95% confidence interval [CI] 0.40 to 0.89, $p = 0.01$). The severity of atherosclerosis also predicted the presence of an unstable coronary syndrome (OR 1.7, 95% CI 1.14 to 2.47, $p = 0.008$) when all patients were considered. When only patients with significant coronary disease were considered (at least one stenosis >50%), ascorbic acid concentration (OR 0.56, 95% CI 0.37 to 0.85, $p = 0.008$) and total plasma thiols (OR 0.52, 95% CI 0.34 to 0.80, $p = 0.004$) predicted the presence of an unstable coronary syndrome, whereas the extent of atherosclerosis did not.

Conclusions. These data are consistent with the hypothesis that the beneficial effects of antioxidants in coronary artery disease may result, in part, by an influence on lesion activity rather than a reduction in the overall extent of fixed disease.

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Prospective studies have demonstrated reduced risk of coronary artery disease in subjects with a greater intake of vitamin E (1,2) or ascorbic acid (3). Because these antioxidant vitamins inhibit oxidation of low density lipoprotein (LDL), a critical event in the pathogenesis of atherosclerosis, investigators have speculated that they reduce coronary artery disease by limiting the development or progression of atherosclerotic lesions. However, studies examining this issue have provided conflicting results (4–8). It has recently become apparent that antioxidants may favorably influence coronary artery disease

through alternative mechanisms, including improvement of endothelial function (9,10), inhibition of platelet aggregability (11) and a decrease in the risk of plaque rupture (12) (see Diaz et al. [8] for review). The clinical relevance of these alternative mechanisms is supported by the finding that vitamin E treatment reduces nonfatal myocardial infarction in patients with coronary disease after only 200 days of follow-up (13), a time span that is most likely too short for significant lesion regression.

It is difficult to assess the effect of antioxidants on lesion progression or regression and the relevance of such effects to primary or secondary prevention of coronary events in the available larger studies (1–3) because angiography was not performed. These studies are further limited because antioxidant levels were not directly measured. In smaller studies, increased LDL susceptibility to oxidation (14) and decreased LDL alpha-tocopherol content (15) have been shown to correlate with the angiographic extent of atherosclerosis. Leukocyte ascorbic acid concentration correlates inversely with the angiographically determined extent of coronary atherosclerosis (16). The presence of angina pectoris also correlates with lower alpha-tocopherol and ascorbic acid concentrations (17). In a recent prospective population study, a low plasma ascorbic acid level was associated with an increased risk of myocardial

Abbreviations and Acronyms	
CI	= confidence interval
CK	= creatine kinase
HDL	= high density lipoprotein
LDL	= low density lipoprotein
HPLC	= high pressure liquid chromatography
OR	= odds ratio

infarction (18). Thus, the available evidence suggests important links between coronary atherosclerosis, clinical symptoms of coronary artery disease and impaired antioxidant defenses. However, no previous study has related antioxidant status to both coronary artery disease extent and coronary disease activity. Therefore, this study sought to make such a comparison and determine whether antioxidant status relates independently to clinical symptoms of coronary artery disease.

Methods

Patients. Consecutive patients referred to the Boston Medical Center for cardiac catheterization were enrolled as required by the institutional review board. A 30-ml fasting blood sample was obtained at the time of catheterization.

A research nurse noted the presence of the following risk factors: 1) age; 2) male gender; 3) clinical history of diabetes (fasting blood glucose >140 mg/dl or treatment with insulin or an oral hypoglycemic agent); 4) clinical history of hypertension (blood pressure >90 mm Hg diastolic or treatment for hypertension); 5) clinical history of hypercholesterolemia (total cholesterol >200 mg/dl or previous drug treatment for hypercholesterolemia); 6) cigarette smoking (total pack-years and time since last cigarette); and 7) family history of coronary disease (first-degree relative with myocardial infarction or cardiac death before age 55). Patients were also questioned about medications, multivitamin use and minority status. Total cholesterol, high density lipoprotein (HDL) and triglycerides were measured in the hospital clinical laboratory. LDL cholesterol was calculated using the Friedewald formula (19).

Assessment of extent of coronary atherosclerosis. Coronary angiograms were analyzed off-line in a blinded manner. The extent of atherosclerosis was quantified using the "Hamsten extent score" (20), which reflects the extent of early coronary atherosclerosis and is expressed on a scale of 0 (no disease) to 9 (extensive disease in each of 15 coronary segments). The extent of advanced coronary disease was assessed by measuring stenosis severity using digital calipers. For each patient, the "global stenosis score" was calculated as described by Regnström et al. (15). In this score, the severity of stenoses is assessed in each of 15 coronary segments, and the severity of atherosclerosis is expressed on a scale of 0 (no stenoses >25%) to 16 (multiple advanced stenoses or occlusions in all 15 segments).

Assessment of clinical activity of coronary disease. We obtained a detailed angina history and reviewed the medical record for evidence of unstable angina as defined by Braunwald (21) or myocardial infarction as indicated by the presence of typical symptoms, ischemic electrocardiographic changes and a diagnostic elevation of creatine kinase, MB fraction (CK-MB). It is currently understood that unstable angina and acute myocardial infarction have a common pathophysiology (plaque rupture and consequent intracoronary thrombosis) (22). Therefore, we categorized each patient as having or not having an unstable coronary syndrome (unstable angina or myocardial infarction within 2 weeks of study).

Assessment of antioxidant/oxidant status. Blood samples were immediately transferred into Vacutainer tubes containing heparin (286 U/15 ml blood) (Becton Dickinson), placed on ice and protected from light. Within 2 h of collection, plasma was prepared at 4°C, and samples were processed or frozen at −70°C, as appropriate.

For assessment of LDL resistance to oxidation, LDL was isolated from plasma within 2 h of collection (23), and LDL resistance to oxidation was determined by incubating LDL (0.1 mg LDL protein/ml) with 3.3 μmol/liter $CuCl_2$ at 37°C and assaying lipid peroxidation as diene conjugation. LDL resistance to oxidation was quantified as the lag phase duration (in minutes) before the propagation phase of diene conjugation (23). Levels of LDL alpha-tocopherol and plasma alpha-tocopherol, beta-carotene, gamma-tocopherol and retinol were determined from frozen samples by reversed-phase high performance liquid chromatography (HPLC), as previously described (24). Ascorbic acid and uric acid concentrations in metaphosphoric acid-precipitated plasma were determined by HPLC with electrochemical detection (25). Total plasma thiols were determined by 5,5′-dithiobis(2-nitrobenzoic acid) (26). The plasma concentration of free F_2-isoprostanes, a stable marker of lipid peroxidation, was measured using a stable isotope dilution mass spectrometric assay (27). Plasma ceruloplasmin was determined by its oxidase activity using o-dianisidine dihydrochloride (Sigma), as previously described (28). Erythrocyte copper–zinc superoxide dismutase activity was determined as previously described (29).

Statistical analysis. First, univariate analysis was performed to examine the relations between the extent of atherosclerosis, antioxidant/oxidant markers and standard coronary risk factors. The Hamsten extent score (20) was correlated with each of 12 antioxidant/oxidant markers (Table 1) and seven coronary risk factors (age, male gender, history of diabetes mellitus, history of hypertension, history of hypercholesterolemia, cigarette smoking [pack-years] and family history of premature coronary disease). Normal distribution of the Hamsten extent score and each antioxidant/oxidant marker was confirmed using the Kolmogorov-Smirnov algorithm. We also examined the relation between selected antioxidant markers and coronary risk factors using two-sample t tests or the chi-square test for continuous and categoric variables, respectively. The univariate analysis was also repeated using the global stenosis score (15).

To identify independent predictors of the extent of coronary atherosclerosis, all variables with a univariate p value <

Table 1. Univariate Predictors of Extent of Atherosclerosis in 149 Patients

	Mean (±SD) or No. (%) of Pts	r*	p Value
Coronary risk factors			
Age (yr)	62 ± 12	0.33	0.001
Male gender	87 (58%)	0.24	0.005
Diabetes mellitus	36 (24%)	0.26	0.002
Hypertension	85 (57%)	0.21	0.01
Hypercholesterolemia	81 (54%)	0.20	0.02
Smoking (pack-years)	31 ± 33	0.05	0.57
Family history of premature CAD	61 (41%)	0.01	0.87
Lipid profile			
Total cholesterol (mg/dl)	202 ± 37	−0.04	0.66
HDL cholesterol (mg/dl)	39 ± 12	−0.001	0.99
Triglycerides (mg/dl)	153 ± 81	−0.06	0.52
Calculated LDL (mg/dl)	134 ± 35	−0.04	0.62
Lipid-soluble antioxidants			
Alpha-tocopherol (μmol/liter) (n = 146)	19.8 ± 14.3	−0.01	0.93
Beta-carotene (μmol/liter) (n = 41)	0.39 ± 0.30	0.02	0.92
Gamma-tocopherol (μmol/liter) (n = 60)	5.4 ± 3.3	0.03	0.83
Retinol (μmol/liter) (n = 41)	1.5 ± 0.4	−0.22	0.17
LDL alpha-tocopherol (nmol/mg protein) (n = 41)	4.1 ± 1.4	0.03	0.87
Water-soluble antioxidants			
Ascorbic acid (μmol/liter) (n = 143)	37.5 ± 19.5	0.02	0.85
Uric acid (μmol/liter) (n = 144)	312 ± 92	0.05	0.54
Total plasma thiols (μmol/liter) (n = 147)	241 ± 70	−0.06	0.48
Other markers			
Ceruloplasmin (U/liter) (n = 62)	112 ± 31	−0.09	0.48
Erythrocyte SOD (U/mg protein) (n = 41)	0.23 ± 0.14	−0.02	0.92
LDL oxidation lag phase (min) (n = 41)	68 ± 18	−0.15	0.35
F_2-isoprostanes (pg/ml) (n = 54)	43.4 ± 38.5	0.06	0.66

*Correlation coefficient for relation with Hamsten extent score (see text). CAD = coronary artery disease; HDL = high density lipoprotein; LDL = low density lipoprotein; Pts = patients; SOD = superoxide dismutase.

0.10 were then included in a multivariate analysis. Independent predictors of the Hamsten extent score were identified using stepwise linear regression analysis. The threshold $p < 0.10$ for inclusion in the analysis was selected because it has been recommended for detecting important predictive factors without including unimportant nonpredictors (30,31).

For clinical activity of coronary artery disease, patients were categorized as having or not having an unstable coronary syndrome on the basis of the presence or absence of unstable angina or a myocardial infarction within 2 weeks of the study. Antioxidant/oxidant markers, coronary risk factors and the extent of atherosclerosis were compared for the two groups using two-sample t tests or the chi-square test for continuous and categoric variables, respectively. The variables with a univariate p value < 0.10 were then included in a multivariate analysis using multiple logistic regression to identify independent predictors of the presence of an unstable coronary syndrome. The ability to predict an unstable coronary syndrome was expressed as the odds ratio with the 95% confidence interval for an increase of 1 SD in the variable.

All analyses were completed using SPSS for Windows, Release 6.0. Statistical significance was defined as $p < 0.05$.

Unless otherwise indicated, all results are expressed as mean value ± SD.

Results

Patients. A total of 149 patients were enrolled in the study. The demographic profile, distribution of coronary risk factors and lipoprotein profile are presented in Table 1. The racial distribution of the patients was 137 white, 9 African American and 3 Hispanic. Regarding medications, 140 (94%) were taking some form of antianginal therapy (beta-adrenergic blocking agent, calcium channel blocking agent or nitrate); 128 (86%) were taking aspirin; 39 (26%) were receiving lipid-lowering therapy; 31 (21%) were taking an angiotensin-converting enzyme inhibitor; 4 (3%) were taking vitamin E supplements; 3 (3%) were taking ascorbic acid supplements; and 6 (4%) were taking multivitamins. The mean Hamsten extent score was 3.2 ± 1.9 (range 0.0 to 8.2), and the mean global stenosis score was 1.65 ± 1.45 (0 to 7.1). Twenty-one patients had normal coronary arteries or minimal coronary disease; 33 had one-vessel disease (>50% stenosis); 42 had two-vessel disease; and 53 had three-vessel disease. The Hamsten extent score corre-

Table 2. Multivariate Analysis for Predictors of Extent
of Atherosclerosis

	Adjusted r Value	Multivariate p Value
Included in model		
Age	0.44	0.0001
Hx of hypercholesterolemia	0.17	0.02
Hx of diabetes mellitus	0.27	0.0002
Male gender	0.36	0.0001
Excluded from model		
Hx of hypertension	0.06	0.47

*Multiple r^2 value for model = 0.58, p < 0.0001. Hx = history.

lated with the global stenosis score (r = 0.78, p < 0.0001). A total of 58 patients (39%) had unstable angina as defined by Braunwald (21), and 26 (17%) had a myocardial infarction within 2 weeks of the study; thus, 65 patients were classified as clinically stable, and 84 were classified as having an unstable coronary syndrome.

Antioxidant/oxidant status. The results of the assessment of plasma antioxidant status for the entire cohort are shown in Table 1. Patients with a history of cigarette smoking (n = 115 [77%]) had lower ascorbic acid concentrations (34.6 ± 18.5 μmol/liter) than those who never smoked (47.5 ± 20.1 μmol/liter, p = 0.001). Furthermore, patients with a history of smoking within 1 week of the study (n = 35 [24%]) had lower ascorbic acid concentrations (28.4 ± 18.7 μmol/liter) than smokers who had not smoked within 1 week of the study (37.3 ± 17.8 μmol/liter, p < 0.05). The lag phase of LDL oxidation was shorter in diabetic (60 ± 11 min) than in nondiabetic patients (74 ± 20 min, p = 0.02). Patients with a history of hypertension also had a shorter lag phase (61 ± 13 min) than nonhypertensive patients (75 ± 20 min, p = 0.008).

The ascorbic acid concentration in patients with a history of myocardial infarction within 2 weeks of the study (31.8 ± 14.8 μmol/liter) was not different from that in patients with unstable angina but no recent myocardial infarction (34.4 ± 17.5, p = 0.52). This finding argues against the possibility that myocardial infarction causes a fall in ascorbic acid concentration in this cohort, as has been previously reported (32–34). No significant differences were observed for any other plasma antioxidant/oxidant markers in patients with and without a history of tobacco use, diabetes mellitus or hypertension.

Predictors of extent of atherosclerosis. As presented in Table 1, age, hypercholesterolemia, diabetes mellitus, hypertension and male gender were significant univariate correlates of the Hamsten extent score. No other variables met the criteria for entry into the multivariate analysis (p < 0.10), and as presented in Table 2, the independent predictors of the extent of atherosclerosis were age, diabetes mellitus, hypercholesterolemia and male gender (multiple r^2 = 0.58, p < 0.0001). No antioxidant/oxidant marker correlated significantly with this measure of coronary atherosclerosis extent. As detailed in Table 1, not every antioxidant marker was examined in every

patient. For alpha-tocopherol, ascorbic acid and total thiols, the study provided 80% power to demonstrate statistical significance at the p < 0.05 level if the univariate correlation coefficient was >0.22.

Because a recent study (15) indicated a correlation between LDL alpha-tocopherol and global stenosis score, the univariate analysis was repeated using this measure of more advanced coronary atherosclerosis. A history of diabetes mellitus (r = 0.30, p = 0.0001), age (r = 0.28, p = 0.0006) and male gender (r = 0.21, p = 0.007) correlated with global stenosis score, but antioxidant/oxidant markers did not, including plasma alpha-tocopherol (r = −0.02, p = 0.83) and LDL alpha-tocopherol (r = −0.09, p = 0.56). Cholesterol-adjusted alpha-tocopherol, which was calculated as the ratio of alpha-tocopherol to total cholesterol concentration, also did not correlate with the Hamsten extent score (r = −0.03, p = 0.77) or global stenosis score (r = −0.01, p = 0.99).

Predictors of an unstable coronary syndrome. As presented in Table 3, the significant univariate predictors of an unstable coronary syndrome were pack-years, lower ascorbic acid concentration and extent of atherosclerosis. The other variables that met criteria for entry into the multivariate

Table 3. Characteristics of Patients With and Without an Unstable Coronary Syndrome

	Stable (n = 65)	Unstable (n = 84)	p Value
Coronary risk factors			
Age (yr)	61 ± 12	63 ± 13	0.46
Family Hx of CAD	28 (43%)	33 (40%)	0.68
Hx of diabetes mellitus	15 (23%)	21 (25%)	0.79
Hx of hypertension	32 (49%)	53 (63%)	0.09
Hx of hypercholesterolemia	31 (48%)	37 (44%)	0.66
Smoking Hx (pack-years)	24 ± 27	35 ± 35	0.04
Smoking within 1 wk of study	12 (19%)	23 (27%)	0.20
Male gender	32 (49%)	55 (66%)	0.05
Lipid-soluble antioxidants			
Alpha-tocopherol (μmol/liter)	22.2 ± 16.1	18.0 ± 12.4	0.08
Beta-carotene (μmol/liter)	0.36 ± 0.28	0.43 ± 0.33	0.44
Gamma-tocopherol (μmol/liter)	5.6 ± 3.0	5.3 ± 3.6	0.72
Lycopene (μmol/liter)	0.93 ± 0.50	1.00 ± 0.56	0.70
Retinol (μmol/liter)	1.6 ± 0.3	1.5 ± 0.5	0.43
LDL alpha-tocopherol (nmol/mg protein)	4.2 ± 1.5	4.0 ± 1.4	0.61
Water-soluble antioxidants			
Ascorbic acid (μmol/liter)	42.5 ± 21.8	33.6 ± 16.7	0.007
Uric acid (μmol/liter)	297 ± 84	323 ± 96	0.10
Total plasma thiols (μmol/liter)	253 ± 74	231 ± 66	0.07
Other markers			
Ceruloplasmin (U/liter)	108 ± 34	115 ± 29	0.39
Erythrocyte SOD (U/mg protein)	0.25 ± 0.17	0.21 ± 0.09	0.31
LDL oxidation lag phase (min)	71 ± 18	66 ± 18	0.38
F_2-isoprostanes (pg/ml)	47.1 ± 34.6	40.4 ± 41.6	0.53
Atherosclerosis extent*			
Hamsten extent score	2.6 ± 1.9	3.6 ± 1.8	0.004
Global stenosis score	1.2 ± 1.3	2.0 ± 1.5	0.001

*See text for definition of Hamsten extent and Global stenosis scores. Abbreviations as in Tables 1 and 2.

analysis (p < 0.10) were male gender, history of hypertension, lower alpha-tocopherol concentration and lower plasma thiols. The independent predictors of an unstable coronary syndrome were a low ascorbic acid level (odds ratio [OR] 0.59, 95% confidence interval [CI] 0.40 to 0.89, p = 0.01) and the Hamsten extent score (OR 1.7, 95% CI 1.14 to 2.47, p = 0.008).

Because ascorbic acid levels have been reported to be low immediately after an acute myocardial infarction (32,34), the analysis was repeated excluding the 26 patients with a history of myocardial infarction within 2 weeks of study. In this analysis, a low ascorbic acid concentration (OR 0.63, 95% CI 0.40 to 0.98, p = 0.04) and the Hamsten extent score (OR 2.0, 95% CI 1.3 to 3.1, p = 0.003) remained independent predictors of an unstable coronary syndrome (unstable angina).

Because patients without significant coronary disease are unlikely to have an unstable coronary syndrome, and because antioxidant treatment is known to have beneficial effects on vascular function (9,10) and coronary events in patients with established coronary artery disease (13), we also examined the relation between antioxidant status and coronary disease activity in the subset of patients with at least one coronary artery stenosis >50% (n = 128). In this group of patients, a low plasma ascorbic acid concentration (OR 0.56, 95% CI 0.37 to 0.85, p = 0.008) and low plasma thiols (OR 0.52, 95% CI 0.34 to 0.80, p = 0.004) were independent predictors of an unstable coronary syndrome, whereas the Hamsten extent score and global stenosis score were not.

Discussion

In the present study, we evaluated the extent of coronary atherosclerosis, the clinical activity of coronary artery disease, coronary risk factors and 12 markers of plasma antioxidant/ oxidant status in patients referred for cardiac catheterization. The extent of coronary atherosclerosis correlated independently with several established risk factors for coronary disease (older age, diabetes mellitus, hypercholesterolemia and male gender) but not with any marker of antioxidant/oxidant status. Although antioxidant/oxidant status failed to predict atherosclerosis extent, a lower plasma ascorbic acid concentration did predict coronary disease activity (the presence of unstable angina or acute myocardial infarction). When the entire group of patients with and without coronary atherosclerosis was considered, we observed that the extent of atherosclerosis also predicted the presence of an acute coronary syndrome. However, when only patients with at least one coronary stenosis >50% were considered, two markers of antioxidant status (low plasma ascorbic acid and thiols) predicted the presence of an unstable coronary syndrome, whereas the extent of atherosclerosis did not. These associations could not be explained by the presence of recent myocardial infarction and were independent of the assessed risk factors and antioxidant markers, including previous or recent cigarette smoking, which may have a direct effect on ascorbic acid level. These findings support the hypothesis that antioxidant status may influence coronary artery disease through an effect on lesion activity rather than by a reduction of the overall extent of fixed disease.

Antioxidants and atherogenesis. The idea that antioxidants might prevent coronary events by limiting lesion formation is based on emerging evidence linking oxidation of LDL to human atherogenesis (14,35) and atherosclerosis progression (36). Because vitamins E and C can limit LDL oxidation (37), one would expect that increased availability of these antioxidant vitamins would slow the atherosclerotic process. In support of this hypothesis, previous studies have shown that 1) the severity of carotid atherosclerosis correlates inversely with dietary ascorbic acid and vitamin E intake (7); 2) the extent of coronary atherosclerosis correlates inversely with LDL alpha-tocopherol content (15); and 3) increased vitamin E intake in combination with lipid-lowering therapy is associated with less progression of coronary atherosclerosis (5). However, in other studies the relation between antioxidant status and lesion severity is less clear. For example, Hodis et al. (5) reported less progression of coronary atherosclerosis in patients with higher vitamin E intake, but the effect of vitamin E was modest (regression in mean stenosis severity of 0.8% vs. progression of 2.0% over 2 years) and was limited to the subgroup of patients receiving lipid-lowering therapy. In the only available prospective, placebo-controlled trial examining this issue (6), probucol (a potent synthetic antioxidant) had no effect on femoral artery lesion severity, although interpretation of this finding is complicated by a reduction in HDL cholesterol with probucol treatment.

In the current study, we observed no significant correlation between any of the measured markers of plasma antioxidant/ oxidant status and extent of coronary atherosclerosis. This finding contrasts with the work of Regnström et al. (14), who reported a significant correlation between LDL oxidation lag phase and the extent of early atherosclerosis (Hamsten extent score). In a more recent study by the same group (15), LDL alpha-tocopherol and lipid-adjusted alpha-tocopherol correlated with the extent of atherosclerosis (global stenosis score), whereas LDL oxidation lag phase did not. The reason for these apparent discrepancies is unclear but may relate to differences in study patients. Those two previous studies examined young men who were clinically stable several months after their first myocardial infarction and a larger group of normal patients, whereas our study examined older patients with more advanced disease and both stable and unstable symptoms.

Antioxidants and coronary disease activity. In contrast to the lack of significant correlation between disease extent and antioxidant/oxidant status, our study demonstrated a strong independent association between low plasma ascorbic acid concentration and a recent unstable coronary syndrome. In the patients with significant coronary artery disease, low plasma thiols were also predictive of an unstable coronary syndrome. Several previous studies also support the importance of antioxidant status in the clinical activity of coronary disease. A prospective population study (18) demonstrated that Finnish men with ascorbic acid deficiency (<11.4 μmol/liter) have an increased risk of myocardial infarction. In another study (17),

lower plasma levels of ascorbic acid and cholesterol-adjusted alpha-tocopherol correlated with angina, although the correlation between ascorbic acid and angina was lost after adjustment for the effect of smoking on ascorbic acid concentration. We also observed lower ascorbic acid concentrations in smokers, but in our study, the correlation of ascorbic acid with angina was independent of smoking as well as other risk factors. One previous study (38) also demonstrated an association of lower plasma levels of reduced thiols with unstable angina.

It is interesting that coronary disease activity relates to the plasma concentrations of these two important water-soluble antioxidant species. Ascorbic acid is known to be the most effective water-soluble antioxidant in plasma (25). As was recently suggested by Levine et al. (39), some previous studies (1) may have failed to detect an association between coronary disease and ascorbic acid intake because they failed to examine a sufficiently low intake level, given that tissue stores are saturated with a daily intake of only 100 mg/day (39). Thiol species also act as antioxidants in plasma (25), and intracellularly, the thiol compound glutathione is the predominant low molecular weight antioxidant species that acts in concert with ascorbic acid to regulate cellular redox status (40). Thus, a deficiency of thiols or ascorbic acid is likely to have important implications for arterial homeostasis.

The precise mechanisms of how decreased plasma antioxidant levels could be associated with more severe symptoms or unstable coronary syndromes independent of an effect on atherosclerosis extent remain controversial. It is currently recognized that acute coronary syndromes occur after activation of atherosclerotic lesions, a process that leads to plaque rupture, thrombosis and vasospasm (22). Other interventions that have been clearly shown to reduce coronary artery disease risk, such as lipid-lowering therapy, most likely act in large part by inhibiting these processes (41). There is growing evidence that increased oxidative stress and impaired antioxidant protection are relevant to plaque activation and the other events associated with acute coronary syndromes (8). For example, oxidized LDL stimulates expression of adhesion molecules and impairs effective release of nitric oxide, leading to accumulation of leukocytes in the vessel wall and thus promotes plaque rupture (12,42,43). Furthermore, oxidized LDL induces expression of prothrombotic factors, such as plasminogen activator inhibitor-1 and tissue factor, and thus increases the risk of coronary thrombosis (8,12). By inhibiting nitric oxide action, oxidized LDL also promotes platelet adhesion and vasospasm. By opposing formation of oxidized LDL, antioxidants may limit all these processes (8,12,43).

In addition to limiting LDL oxidation, antioxidants may further oppose acute coronary events by direct effects on vascular cell function. Such effects include increased resistance to the cytotoxic effects of oxidized LDL, decreased production of reactive oxygen species and increased effective production of nitric oxide (8,43). In human studies, lipid-soluble antioxidants have been shown to inhibit platelet aggregation (11) and improve endothelium-dependent vasomotor function (10).

Furthermore, ascorbic acid treatment acutely improves endothelial nitric oxide action in patients with coronary artery disease (9) and decreases monocyte adhesiveness to endothelial cells in cigarette smokers (44). Ascorbic acid may also increase prostacyclin production (45) and lower blood pressure (46). There also is growing evidence that thiol status importantly regulates nitric oxide bioactivity (47). The findings of the present study demonstrating an inverse association between plasma thiol and ascorbic acid concentrations and the presence of an unstable coronary syndrome fit well with these previous observations.

Study limitations. This study has a number of limitations. The analysis involved multiple comparisons and a relatively small number of patients; thus, the possibility remains that the observed association between low ascorbic acid concentration and an unstable coronary syndrome is a chance finding. Furthermore, the relatively high prevalence of unstable coronary syndromes in the study patients could have led to an overestimation of relative risk. It is important to point out that the associations observed in this study do not necessarily imply causality. It is possible that myocardial infarction is associated with an inflammatory state or some other process that consumes antioxidants and reduces their concentration in plasma. However, such an effect is unlikely to explain our findings because they were unchanged after patients with a recent myocardial infarction were excluded. In addition, patients with a recent myocardial infarction did not have lower ascorbic acid levels than patients with unstable symptoms without a recent infarction. It is also possible that higher antioxidant levels might reflect a behavioral or dietary pattern that reduces risk by other unmeasured factors such as level of physical activity. Furthermore, it is possible that additional correlations between antioxidant status and coronary artery disease might have been demonstrated in a larger study or a different population. Despite these limitations, our results support the hypothesis that antioxidant status may be relevant to plaque activation and the clinical expression of coronary artery disease rather than affecting the overall extent of fixed disease. The results provide a further rationale for ongoing trials of antioxidant therapy in patients with coronary artery disease.

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