

ORIGINAL ARTICLE

Glutathione Peroxidase 1 Activity and Cardiovascular Events in Patients with Coronary Artery Disease

Stefan Blankenberg, M.D., Hans J. Rupprecht, M.D., Christoph Bickel, M.D., Michael Torzewski, M.D., Gerd Hafner, M.D., Laurence Tiret, Ph.D., Marek Smieja, M.D., Ph.D., François Cambien, M.D., Jürgen Meyer, M.D., and Karl J. Lackner, M.D., for the AtheroGene Investigators*

ABSTRACT

BACKGROUND

Cellular antioxidant enzymes such as glutathione peroxidase 1 and superoxide dismutase have a central role in the control of reactive oxygen species. In vitro data and studies in animal models suggest that these enzymes may protect against atherosclerosis, but little is known about their relevance to human disease.

METHODS

We conducted a prospective study among 636 patients with suspected coronary artery disease, with a median follow-up period of 4.7 years (maximum, 5.4) to assess the risk of cardiovascular events associated with base-line erythrocyte glutathione peroxidase 1 and superoxide dismutase activity.

RESULTS

Glutathione peroxidase 1 activity was among the strongest univariate predictors of the risk of cardiovascular events, whereas superoxide dismutase activity had no association with risk. The risk of cardiovascular events was inversely associated with increasing quartiles of glutathione peroxidase 1 activity (P for trend <0.001); patients in the highest quartile of glutathione peroxidase 1 activity had a hazard ratio of 0.29 (95 percent confidence interval, 0.15 to 0.58; $P<0.001$), as compared with those in the lowest quartile. Glutathione peroxidase 1 activity was affected by sex and smoking status but retained its predictive power in these subgroups. After adjustment for these and other cardiovascular risk factors, the inverse association between glutathione peroxidase 1 activity and cardiovascular events remained nearly unchanged.

CONCLUSIONS

In patients with coronary artery disease, a low level of activity of red-cell glutathione peroxidase 1 is independently associated with an increased risk of cardiovascular events. Glutathione peroxidase 1 activity may have prognostic value in addition to that of traditional risk factors. Furthermore, increasing glutathione peroxidase 1 activity might lower the risk of cardiovascular events.

From the Departments of Medicine II (S.B., H.J.R., J.M.) and Clinical Chemistry and Laboratory Medicine (M.T., G.H., K.J.L.), Johannes Gutenberg University, Mainz, Germany; INSERM Unité 525, Faculté de Médecine Pitié-Salpêtrière, Paris (S.B., L.T., F.C.); Innere Abteilung, Bundeswehrzentral Krankenhaus, Koblenz, Germany (C.B.); and the Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ont., Canada (M.S.). Address reprint requests to Dr. Blankenberg at the Department of Medicine II or to Dr. Lackner at the Department of Clinical Chemistry and Laboratory Medicine, Johannes Gutenberg University Mainz, Langenbeckstr. 1, 55131 Mainz, Germany, or to stefan.blankenberg@uni-mainz.de or lackner@zentrallabor.klinik.uni-mainz.de.

*Members of the AtheroGene study group are listed in the Appendix.

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MANY ASPECTS OF THE PATHOGENESIS of atherosclerosis have been unraveled in recent years, and an important potential role of oxidative mechanisms has been elucidated.^{1,2} This research has led to the assumption that oxidation of low-density lipoprotein represents a key event in atherogenesis, even though the results of trials of antioxidants in the prevention of human atherosclerosis have been mostly negative.³

Oxidative stress may be defined as an imbalance between the production and degradation of reactive oxygen species such as superoxide anion, hydrogen peroxide, lipid peroxides, and peroxynitrite. Enzymatic inactivation of reactive oxygen species is achieved mainly by glutathione peroxidase, superoxide dismutase, and catalase.⁴ In mammalian cells, glutathione and the glutathione peroxidases constitute the principal antioxidant defense system.^{5,6} There are at least four different glutathione peroxidases, all of which contain selenocysteine at their active sites.⁷

Glutathione peroxidase 1, the ubiquitous intracellular form and key antioxidant enzyme within most cells, including the endothelium, uses glutathione to reduce hydrogen peroxide to water and lipid peroxides to their respective alcohols,⁸ and it also acts as a peroxynitrite reductase.⁹ In mice, glutathione peroxidase 1 deficiency results in abnormal vascular and cardiac function and structure.¹⁰ Similarly, superoxide dismutase is represented by three different ubiquitously expressed enzymes that convert superoxide anion to hydrogen peroxide: cytosolic copper- and zinc-containing superoxide dismutase, mitochondrial manganese-containing superoxide dismutase, and extracellular superoxide dismutase. Extracellular superoxide dismutase is most active in the vessel wall and has been shown to regulate the availability of nitric oxide by scavenging superoxide anion.¹¹

On the basis of the experimental evidence, we addressed the hypothesis that enhanced activity of cellular glutathione peroxidase 1 and superoxide dismutase would be protective against cardiovascular events in a large prospective cohort of patients with coronary artery disease.

METHODS

STUDY POPULATION

Between November 1996 and December 1997, 732 patients referred to the Department of Medicine II

of the Johannes Gutenberg University in Mainz, Germany, with suspected coronary artery disease were enrolled in the AtheroGene registry. Fourteen patients with acute myocardial infarction and 75 patients in whom glutathione peroxidase 1 activity could not be determined immediately were excluded from the present analysis. Thus, the final study cohort consisted of 643 patients, 133 with symptoms of unstable angina and 510 with symptoms of stable angina. Coronary angiography was performed in all patients. Relevant coronary artery disease, defined by greater than 30 percent stenosis in at least one major coronary artery, was detected in 558 patients. The study design has been described in detail elsewhere.¹² The exclusion criteria were evidence of hemodynamically significant valvular heart disease, surgery or trauma within the previous month, known cardiomyopathy, known cancer, febrile conditions, or use of oral anticoagulant therapy within the previous four weeks.

We considered patients who were receiving dietary treatment or medication for diabetes or whose current fasting blood glucose level was above 125 mg per deciliter to have diabetes mellitus. Patients who had received antihypertensive treatment or who had received a diagnosis of hypertension (blood pressure above 160/90 mm Hg) were considered to have hypertension. Patients were classified as currently smoking, as having smoked in the past (if they had stopped more than 4 weeks and less than 40 years earlier), or as never having smoked (if they had never smoked or had stopped 40 or more years earlier).

Among the 643 patients, 636 (98.9 percent) were followed for a median of 4.7 years (maximum, 5.4). There were 64 deaths from cardiovascular causes, 21 deaths from other causes, and 19 nonfatal myocardial infarctions. Information about the causes of death and clinical events was obtained from hospital and general-practitioner charts.

The study was approved by the ethics committee of the University of Mainz. Participation was voluntary, and each patient gave written informed consent.

LABORATORY METHODS

Blood was drawn under standardized conditions before coronary angiography was performed. Glutathione peroxidase 1 activity and superoxide dismutase activity were determined in washed red cells obtained immediately after sampling from whole blood anticoagulated with EDTA. Hemolyzed cells

were stored frozen for up to one week; freezing does not lead to changes in enzyme activity. Glutathione peroxidase 1 was measured as previously described,¹³ with minor modifications (Ransel test kit, Randox). The intraassay and interassay coefficients of variation were 6.7 percent and 9.9 percent, respectively.

Superoxide dismutase activity was determined by the following method. Superoxide radicals generated by the xanthine oxidase reaction convert 1-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride quantitatively to a formazan dye (Ransod test kit, Randox). Conversion of superoxide radicals to hydrogen peroxide by superoxide dismutase inhibits dye formation and serves as a measure of superoxide dismutase activity. The intraassay and interassay coefficients of variation were 5.1 percent and 5.5 percent, respectively. Serum lipid levels (the levels of total cholesterol, triglycerides, and high-density lipoprotein cholesterol) were measured immediately by routine methods; low-density lipoprotein cholesterol was calculated by the Friedewald formula.

For all other biologic markers measured in the study, plasma and serum were stored at -80°C until analysis, which was performed after a mean of 1.5 years of storage time. C-reactive protein was measured with a highly sensitive, latex-particle-enhanced immunoassay (Roche Diagnostics; range of detection, 0.1 to 20 mg per liter; interassay coefficient of variation, 1.0 percent for values of 15 mg per liter and 6.5 percent for values below 4 mg per liter). Interleukin-6 and soluble intercellular adhesion molecule 1 were measured with commercially available immunoassays (EASIA, Biosource Europe). Homocysteine was measured by high-pressure liquid chromatography (interassay coefficient of variation, 7.1 percent) and selenium by carbon-furnace atomic-absorption spectrometry with Zeeman compensation, as previously described.¹⁴

STATISTICAL ANALYSIS

The mean levels and proportions of base-line cardiovascular risk factors were calculated for study participants in whom a cardiovascular event subsequently occurred and in those without such an event. The significance of differences between the means for the two groups was assessed with Student's *t*-test, and the significance of differences in proportions was tested with the chi-square statistic. Variables with a skewed distribution were presented as medians, and the Wilcoxon rank-sum test

was applied. The cumulative event plots according to quartile of glutathione peroxidase 1 activity were estimated by the Kaplan–Meier method and compared with use of the log-rank test. In all survival analyses, the end point was death from cardiovascular causes or nonfatal myocardial infarction. Data from patients who died from other causes were censored at the time of death. Hazard ratios for future coronary events according to quartile of glutathione peroxidase 1 activity were estimated by Cox regression models adjusted for potential confounders. Three adjusted models were constructed. We adjusted first for age and sex and second for other traditional risk factors. The final model included clinical and therapeutic variables as well as C-reactive protein, homocysteine, and creatinine.

To evaluate the combined effect of glutathione peroxidase 1 activity and smoking status on cardiovascular risk, we divided the study participants into six groups according to whether the glutathione peroxidase 1 activity was above the median or at or below the median and according to their smoking-status category. In these analyses, Cox regression was used to assess simultaneously the risk of future cardiovascular events in each of the six groups, with the group of patients whose glutathione peroxidase 1 activity was above the median and who had never smoked as the reference group. The hypothesis that smoking and the level of glutathione peroxidase 1 activity had an interactive effect on the risk of future cardiovascular events was formally tested in a Cox regression model that included a term for the multiplicative interaction of smoking (across the three categories of smoking status) and glutathione peroxidase 1 activity. The hazard ratios and their 95 percent confidence intervals are reported. The *P* values are two-sided; a *P* value of less than 0.05 was considered to indicate statistical significance. All computations were carried out with SPSS software, version 10.07.

RESULTS

Table 1 gives the base-line characteristics of the 83 study participants who subsequently died from cardiovascular causes or had a nonfatal myocardial infarction and the 553 who did not have a cardiovascular event, including the results of base-line blood-chemistry analysis. Glutathione peroxidase 1 activity was normally distributed among the study participants. It ranged from 7.4 to 99.6 units per gram of hemoglobin, with a mean (\pm SD) of

Table 1. Base-Line Characteristics of the Study Patients.*

Characteristic	Patients without a Cardiovascular Event (N=553)	Patients with a Cardiovascular Event (N=83)	P Value†
Age (yr)	60.9±10.1	67.0±7.8	<0.001
Male sex (%)	72.0	73.5	0.77
Body-mass index‡	26.8±3.4	26.4±4.1	0.45
Diabetes (%)	23.5	42.2	<0.001
Hypertension (%)	68.0	68.7	0.90
Smoking status (%)			0.07
Never smoked	42.0	31.3	
Formerly smoked	45.2	51.8	
Currently smoke	12.8	16.9	
Disease in 2 or more vessels (%)	63.5	80.7	0.002
History of myocardial infarction (%)	46.1	45.8	0.95
Revascularization (%)§	53.3	59.0	0.33
Left ventricular ejection fraction (%)¶	63.4±14.0	53.0±20.2	<0.001
Beta-blocker medication (%)	51.9	43.4	0.15
Statin medication (%)	24.1	10.8	0.007
Antioxidant enzymes and cofactors			
Glutathione peroxidase 1 (U/g of hemoglobin)	49.8±11.3	45.3±12.9	<0.001
Superoxide dismutase (U/g of hemoglobin)			0.99
Median	9.9	9.7	
Interquartile range	8.7–11.4	8.5–11.6	
Selenium (ng/ml)	74.5±33.5	69.5±32.6	0.26
Lipid variables			
LDL cholesterol (mg/dl)	140.6±38.7	144.0±40.7	0.45
HDL cholesterol (mg/dl)	47.1±15.4	44.1±14.8	0.09
Triglycerides (mg/dl)			0.11
Median	147.0	159.0	
Interquartile range	107.0–205.5	122.0–223.0	
Inflammatory variables			
C-reactive protein (mg/liter)			0.03
Median	3.8	4.7	
Interquartile range	1.9–8.9	2.2–18.0	
Interleukin-6 (pg/ml)			0.005
Median	9.9	12.8	
Interquartile range	5.6–17.4	7.6–25.3	
sICAM-1 (ng/ml)			0.08
Median	258.6	308.4	
Interquartile range	194.3–364.3	216.9–420.6	
Metabolic variables			
Homocysteine (μmol/liter)			<0.001
Median	13.5	15.2	
Interquartile range	11.1–16.4	12.6–20.4	
Creatinine (mg/dl)**			<0.001
Median	1.03	1.13	
Interquartile range	0.93–1.16	0.99–1.30	

* Plus-minus values are means ±SD. LDL denotes low-density lipoprotein, HDL high-density lipoprotein, and sICAM-1 soluble intercellular adhesion molecule 1.

† For normally distributed variables, P values were computed with t-tests; for skewed variables, P values were computed with the Wilcoxon rank-sum test for the difference in medians.

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

§ Revascularization consisted of coronary-artery bypass surgery or percutaneous transluminal coronary angioplasty during follow-up.

¶ The left ventricular ejection fraction was available for 566 patients.

|| To convert values for cholesterol to millimoles per liter, multiply by 0.02586; to convert values for triglycerides to millimoles per liter, multiply by 0.01129.

** To convert values for creatinine to micromoles per liter, multiply by 88.4.

49.2±11.6, a median of 48.3, and an interquartile range of 42.0 to 56.3 units per gram of hemoglobin. The base-line level of glutathione peroxidase 1 activity was significantly lower among those who died from cardiac causes or had a nonfatal myocardial infarction than among those who did not. This result was stable when data from the subgroups with fatal events (64 patients) and nonfatal events (19 patients) were analyzed separately (45.7±13.5 and 44.1±10.4 units per gram of hemoglobin, respectively, vs. 49.8±11.3 units per gram of hemoglobin for those who did not have cardiovascular events or die from noncardiovascular causes; P=0.009 and P=0.03, respectively). The base-line level of glutathione peroxidase 1 activity in patients who died from noncardiovascular causes (21 patients) was the same as that in event-free patients (49.7±11.1 vs. 49.8±11.3 units per gram of hemoglobin).

Figure 1 shows the Kaplan–Meier curves for event-free survival according to quartile of glutathione peroxidase 1 activity. The unadjusted rate of cardiovascular events increased in a stepwise fashion across decreasing quartiles of base-line glutathione peroxidase 1 activity. The difference between the lowest and highest quartiles and the trend across all quartiles were significant (P<0.001 for both comparisons). The event rate for patients in the lowest quartile of glutathione peroxidase 1 activity (20.8 percent) was approximately three times that for patients in the highest quartile (7.0 percent). To place the effect in perspective, Table 2 presents the hazard ratios for cardiovascular events associated with an increase of 1 SD in various risk factors.

The strongest predictors of the level of glutathione peroxidase 1 activity were smoking status and sex. Significantly lower levels of glutathione peroxidase 1 activity were observed in current smokers than in those who had never smoked (45.7 vs. 51.6 units per gram of hemoglobin, P<0.001). Former smokers also had lower levels of enzyme activity than those who had never smoked (48.2 vs. 51.6 units per gram of hemoglobin); however, this difference was not statistically significant. Furthermore, the level of glutathione peroxidase 1 activity was lower in men than in women (48.5 vs. 51.1 units per gram of hemoglobin, P=0.009). Women below 55 years of age had higher levels of glutathione peroxidase 1 activity than older women (54.1 vs. 50.5 units per gram of hemoglobin, P=0.12). No difference in glutathione peroxidase 1 activity was detected between patients with stable angina and those with unstable angina.

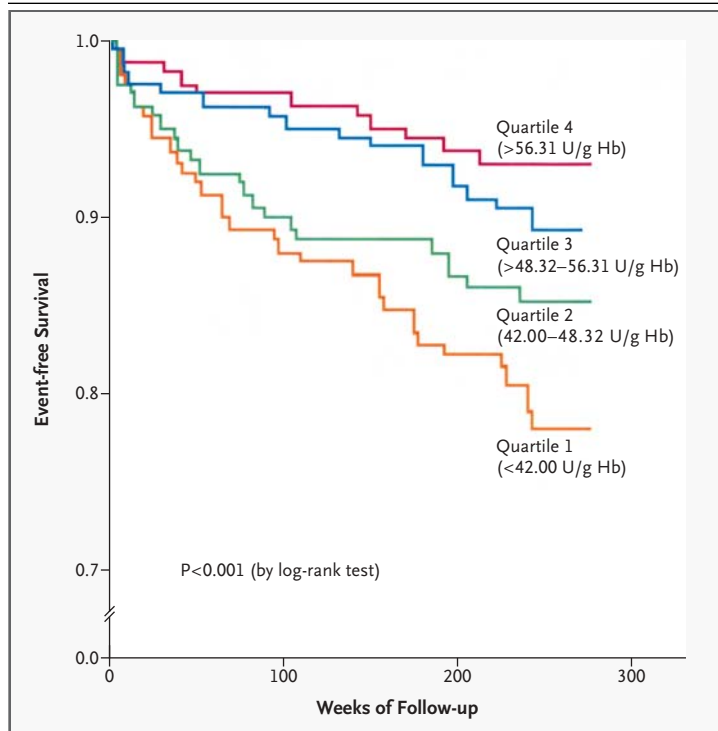


Figure 1. Kaplan–Meier Curves Showing Cardiovascular Events According to Quartile of Glutathione Peroxidase 1 Activity.

The numbers of cardiovascular events were 33, 23, 16, and 11 in quartiles 1, 2, 3, and 4, respectively. Glutathione peroxidase 1 activity is shown in units per gram of hemoglobin.

Table 2. Age- and Sex-Adjusted Analysis of the Association between Novel and Traditional Risk Factors and the Risk of a Cardiovascular Event.*

Variable	Hazard Ratio (95% CI)†	P Value
Antioxidant enzymes and cofactors		
Glutathione peroxidase 1	0.69 (0.57–0.85)	<0.001
Superoxide dismutase	0.92 (0.72–1.19)	0.54
Selenium	0.87 (0.68–1.11)	0.26
Lipid variables		
LDL cholesterol	1.06 (0.85–1.33)	0.60
HDL cholesterol	0.76 (0.59–0.98)	0.03
Triglycerides	1.17 (0.98–1.40)	0.09
Inflammatory variables		
C-reactive protein	1.18 (1.02–1.36)	0.03
Interleukin-6	1.15 (0.96–1.37)	0.13
sICAM-1	1.25 (0.99–1.58)	0.06
Metabolic variables		
Homocysteine	1.27 (1.11–1.44)	0.001
Creatinine	1.23 (1.08–1.40)	0.002

* CI denotes confidence interval, LDL low-density lipoprotein, HDL high-density lipoprotein, and sICAM-1 soluble intercellular adhesion molecule 1.

† The hazard ratio is the age- and sex-adjusted risk associated with an increase of 1 SD in the variable.

Although statin medication had no significant association with glutathione peroxidase 1 activity in the whole study population, the percentage of patients receiving statins was significantly higher among those in the highest quartile of glutathione peroxidase 1 activity than among those in the other three quartiles (30.6 percent vs. 19.5 percent, $P=0.003$). With this exception, no association was observed between the use of any cardiovascular medication and glutathione peroxidase 1 or superoxide dismutase activity. There was a weak but significant correlation of glutathione peroxidase 1 activity with homocysteine ($r=-0.09$, $P=0.03$) and selenium ($r=0.09$, $P=0.04$). Of all the inflammatory markers measured in this study, only soluble intercellular adhesion molecule 1 showed a moderate inverse correlation with glutathione peroxidase 1 activity ($r=-0.11$, $P=0.02$).

To assess the independent predictive value of glutathione peroxidase 1 activity, we used a series of Cox predictive models (Table 3). The inverse relation between glutathione peroxidase 1 activity and

relative risk remained nearly unchanged after adjustment for cardiovascular risk factors and clinical features (model 2). Further adjustment for therapeutic variables as well as C-reactive protein (as a cluster representative of the inflammatory markers), homocysteine, and creatinine (model 3) also did not attenuate the relative risk associated with glutathione peroxidase 1 activity; patients in the highest quartile of glutathione peroxidase 1 activity had a hazard ratio of 0.29 (95 percent confidence interval, 0.14 to 0.60; $P=0.001$) as compared with those in the lowest quartile. Inclusion of interleukin-6 instead of C-reactive protein in the Cox predictive model had no effect on the hazard ratios. Similarly, in a subgroup of 566 patients in whom the ejection fraction had been measured, adjustment for this variable did not alter the hazard ratio associated with increasing quartiles of glutathione peroxidase 1 activity (data not shown).

In previous reports from the entire AtheroGene cohort, the levels of several inflammatory markers, such as interleukin-18 and soluble vascular adhe-

Table 3. Hazard Ratios for Future Cardiovascular Events According to Quartile of Base-Line Glutathione Peroxidase 1 Activity.*

Variable	Quartile				P Value for Trend
	1 (<42.00 U/g)	2 ($42.00-48.32$ U/g)	3 ($>48.32-56.31$ U/g)	4 (>56.31 U/g)	
Total no. of patients	159	159	161	157	
Cardiovascular events — no. (%)	33 (20.8)	23 (14.5)	16 (9.9)	11 (7.0)	<0.001
Adjusted for age and sex (model 1)					
Hazard ratio	1.0	0.65	0.42	0.29	<0.001
95% CI	—	0.38–1.11	0.23–0.77	0.15–0.58	
P value	—	0.13	0.005	<0.001	
Adjusted for risk factors (model 2)†					
Hazard ratio	1.0	0.71	0.49	0.32	<0.001
95% CI	—	0.41–1.21	0.26–0.90	0.16–0.65	
P value	—	0.20	0.02	0.001	
Fully adjusted (model 3)‡					
Hazard ratio	1.0	0.70	0.38	0.29	<0.001
95% CI	—	0.40–1.21	0.20–0.74	0.14–0.60	
P value	—	0.20	0.004	0.001	

* Values for glutathione peroxidase 1 are given in units per gram of hemoglobin. CI denotes confidence interval.

† Further adjustment was made for body-mass index, presence or absence of hypertension, presence or absence of diabetes, smoking status, extent of vessel disease, presence or absence of acute coronary syndrome, and high-density lipoprotein (as a continuous variable).

‡ Additional adjustment was made for vessel revascularization, statin and beta-blocker therapy, plasma homocysteine level, serum creatinine level, and C-reactive protein level (all treated as log-transformed continuous variables). Because of missing data on homocysteine and C-reactive protein, this model considered only 80 cardiovascular events.

sion molecule 1, were found to be elevated among those at risk for subsequent coronary events. No statistically significant correlations were observed between glutathione peroxidase 1 activity and these two proteins. Inclusion of these variables in multivariate analyses did not attenuate the risk of future coronary events associated with decreased levels of glutathione peroxidase 1 activity.

Because smoking status was associated with base-line glutathione peroxidase 1 activity as well as with coronary risk, the interaction between smoking status and glutathione peroxidase 1 activity was analyzed in more detail. The association between smoking and cardiovascular events was observed predominantly in patients with levels of glutathione peroxidase 1 activity below the median. As illustrated in Figure 2, among those with low levels of glutathione peroxidase 1 activity (at or below the median value of 48.32 units per gram of hemoglobin), former smokers were at significant risk for cardiovascular events (hazard ratio, 3.0, as compared with patients who had never smoked and had high glutathione peroxidase 1 activity; 95 percent confidence interval, 1.5 to 5.9; $P=0.001$), as were current smokers (hazard ratio, 5.6; 95 percent confidence interval, 2.4 to 13.5; $P<0.001$). To a lesser extent, a significant increase in cardiovascular risk was also observed in former or current smokers in the subgroup of those with levels of glutathione peroxidase 1 activity above the median.

DISCUSSION

In this prospective cohort of patients with angiographically documented coronary artery disease, we demonstrated that erythrocyte intracellular glutathione peroxidase 1 activity is inversely associated with future fatal and nonfatal cardiovascular events. Erythrocyte glutathione peroxidase 1 is probably a suitable surrogate marker for cellular glutathione peroxidase 1 activity in general, but this has not been proved by a systematic analysis. The risk of a cardiovascular event among those in the highest quartile of glutathione peroxidase 1 activity was approximately 30 percent of that among those in the lowest quartile. This difference did not change appreciably after adjustment for most potential confounders, indicating that the relation between glutathione peroxidase 1 and future cardiovascular events is independent of other risk factors and clinical features.

Because glutathione peroxidase 1 appears to have a major role in the prevention of oxidative stress,

it may also be an important antiatherogenic enzyme.² In fact, reduced expression of glutathione peroxidase 1 has been shown to increase cell-mediated oxidation of low-density lipoprotein in mice.¹⁵ Furthermore, mice that are heterozygous for glutathione peroxidase 1 deficiency have endothelial dysfunction combined with structural vascular abnormalities, such as increased periadventitial inflammation, neointimal formation, and collagen deposition surrounding the coronary arteries.¹⁰ Glutathione peroxidase 1 deficiency apparently decreases bioavailable nitric oxide in mice,¹⁶ an effect that can be aggravated by hyperhomocysteinemia.¹⁷ In addition, glutathione peroxidase 1 activity is decreased or absent in carotid atherosclerotic plaques, and the lack of glutathione peroxidase 1 activity in atherosclerotic lesions appears to be associated with the development of more severe lesions in humans.¹⁸ Since catalase activity is absent from human vascular cells¹⁹ and superoxide dismutase is poorly effective against cellular oxidant damage,⁶ the most important antioxidative shield is reduced in atherosclerotic plaque.

The variability in glutathione peroxidase 1 activity within an individual patient has been reported to be about half the variability between patients,²⁰ which is in accordance with our own observations (unpublished data). The causes of variability be-

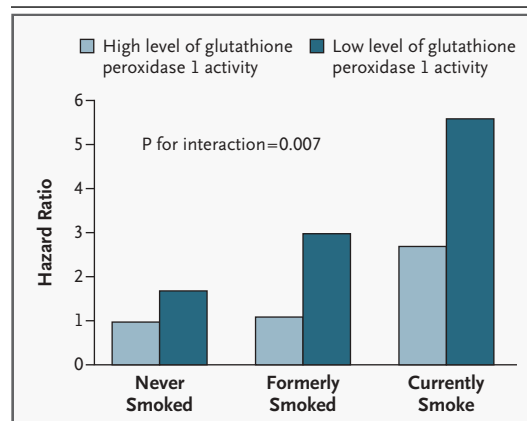


Figure 2. Age- and Sex-Adjusted Hazard Ratios for Cardiovascular Events According to the Level of Glutathione Peroxidase 1 Activity and Smoking Status.

The interaction tested is between smoking category and glutathione peroxidase 1 activity (as a continuous variable). A high level of glutathione peroxidase 1 activity was defined as more than the median value of 48.32 units per gram of hemoglobin, and a low level as 48.32 or fewer units per gram of hemoglobin.

tween patients are not well established. The few genetic polymorphisms identified so far do not show an association with glutathione peroxidase 1 activity.²¹ Smoking consistently reduces glutathione peroxidase 1 activity, whereas the effect of commonly used drugs appears to be negligible.²⁰ The level of glutathione peroxidase 1 activity is higher in women than in men. In our study this difference was most pronounced in premenopausal women, as has been described previously.²²

Cigarette smoking is strongly associated with dysfunctional vasomotor responses, diminished nitric oxide levels, and time-dependent decreases in the content of endothelial nitric oxide synthase messenger RNA.²³ In accordance with previous studies,²⁴ glutathione peroxidase 1 activity was decreased in smokers and former smokers. However, the association between low levels of glutathione peroxidase 1 activity and high cardiovascular risk was also observed in smokers. Therefore, measurement of glutathione peroxidase 1 should identify smokers who are at highest risk for cardiovascular events.

Glutathione peroxidase 1 was recently shown to inhibit 5-lipoxygenase in monocytic cells.²⁵ Since 5-lipoxygenase is induced in monocytes and macrophages within progressing atherosclerotic lesions²⁶ and strongly contributes to atherosclerotic susceptibility in mice,²⁷ the interference of glutathione peroxidase 1 with 5-lipoxygenase might constitute a protective function of the enzyme, in addition to its antioxidant activity.

The role of selenium, which is known to increase glutathione peroxidase 1 gene expression and activity,^{28,29} in cardiovascular disease is controversial. Epidemiologic studies have been inconclusive,³⁰⁻³² and prospective, controlled trials of selenium supplementation are lacking.³³ In the present study, a weak but statistically significant association was observed between selenium levels and glutathione peroxidase 1 activity. The weakness of this association might be due to the fact that the average selenium level was above 70 ng per milliliter, which is probably within the range where the effect of selenium on glutathione peroxidase 1 activity plateaus.^{34,35}

From a clinical perspective, our data show that low erythrocyte glutathione peroxidase 1 activity identifies patients with coronary artery disease who are at the highest risk for cardiovascular events. Adjustment for inflammatory markers such as soluble adhesion molecules or interleukin-18, which have been shown to be predictive of cardiovascular events in this study population,^{36,37} does not affect the association between glutathione peroxidase 1 and prognosis. This observation suggests that measurement of glutathione peroxidase 1 activity provides additional information on risk and might be useful in identifying patients who would benefit from preventive antioxidative treatment.

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APPENDIX

The other members of the AtheroGene study group are as follows: Department of Medicine II, Johannes Gutenberg University, Mainz, Germany—C. Espinola-Klein; INSERM Unité 525, Paris—O. Poirier, V. Nicaud, D. Tregouet, and J.-L. Georges. AtheroGene recruitment centers were the Department of Medicine II, Johannes Gutenberg University, Mainz, Germany, and Innere Abteilung, Bundeswehrzentralrankenhaus, Koblenz, Germany.

REFERENCES

- Ross R. Atherosclerosis — an inflammatory disease. *N Engl J Med* 1999;340:115-26.
- Glass CK, Witztum JL. Atherosclerosis: the road ahead. *Cell* 2001;104:503-16.
- Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? *Circulation* 2002;105:2107-11.
- Forsberg L, de Faire U, Morgenstern R. Oxidative stress, human genetic variation, and disease. *Arch Biochem Biophys* 2001;389:84-93.
- Ursini F, Maiorino M, Brigelius-Flohe R, et al. Diversity of glutathione peroxidases. *Methods Enzymol* 1995;252:38-53.
- Raes M, Michiels C, Remacle J. Comparative study of the enzymatic defense systems against oxygen-derived free radicals: the key role of glutathione peroxidase. *Free Radic Biol Med* 1987;3:3-7.
- Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci* 2000;57:1825-35.
- Flohe L. Glutathione peroxidase. *Basic Life Sci* 1988;49:663-8.
- Sies H. Glutathione and its role in cellular functions. *Free Radic Biol Med* 1999;27:916-21.
- Forgione MA, Cap A, Liao R, et al. Heterozygous cellular glutathione peroxidase deficiency in the mouse: abnormalities in vascular and cardiac function and structure. *Circulation* 2002;106:1154-8.
- Fukai T, Folz RJ, Landmesser U, Harrison DG. Extracellular superoxide dismutase and cardiovascular disease. *Cardiovasc Res* 2002;55:239-49.
- Rupprecht HJ, Blankenberg S, Bickel C, et al. Impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease. *Circulation* 2001;104:25-31.
- Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70:158-69.
- Oster O, Prellwitz W. A methodological comparison of hydride and carbon furnace atomic absorption spectroscopy for the determination of selenium in serum. *Clin Chim Acta* 1982;124:277-91.

15. Guo Z, Van Remmen H, Yang H, et al. Changes in expression of antioxidant enzymes affect cell-mediated LDL oxidation and oxidized LDL-induced apoptosis in mouse aortic cells. *Arterioscler Thromb Vasc Biol* 2001;21:1131-8.
16. Forgione MA, Weiss N, Heydrick S, et al. Cellular glutathione peroxidase deficiency and endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 2002;282:H1255-H1261.
17. Dayal S, Brown KL, Weydert CJ, et al. Deficiency of glutathione peroxidase-1 sensitizes hyperhomocysteinemic mice to endothelial dysfunction. *Arterioscler Thromb Vasc Biol* 2002;22:1996-2002.
18. Lapenna D, de Gioia S, Ciofani G, et al. Glutathione-related antioxidant defenses in human atherosclerotic plaques. *Circulation* 1998;97:1930-4.
19. Shingu M, Yoshioka K, Nobunaga M, Yoshida K. Human vascular smooth muscle cells and endothelial cells lack catalase activity and are susceptible to hydrogen peroxide. *Inflammation* 1985;9:309-20.
20. Andersen HR, Nielsen JB, Nielsen F, Grandjean P. Antioxidative enzyme activities in human erythrocytes. *Clin Chem* 1997;43:562-8.
21. Forsberg L, de Faire U, Marklund SL, Andersson PM, Stegmayr B, Morgenstern R. Phenotype determination of a common ProLeu polymorphism in human glutathione peroxidase 1. *Blood Cells Mol Dis* 2000;26:423-6.
22. Massafra C, Gioia D, De Felice C, Muscettola M, Longini M, Buonocore G. Gender-related differences in erythrocyte glutathione peroxidase activity in healthy subjects. *Clin Endocrinol (Oxf)* 2002;57:663-7.
23. Su Y, Han W, Giraldo C, De Li Y, Block ER. Effect of cigarette smoke extract on nitric oxide synthase in pulmonary artery endothelial cells. *Am J Respir Cell Mol Biol* 1998;19:819-25.
24. Mulholland CW, Elwood PC, Davis A, et al. Antioxidant enzymes, inflammatory indices and lifestyle factors in older men: a cohort analysis. *QJM* 1999;92:579-85.
25. Straif D, Werz O, Kellner R, Bahr U, Steinhilber D. Glutathione peroxidase-1 but not -4 is involved in the regulation of cellular 5-lipoxygenase activity in monocytic cells. *Biochem J* 2000;349:455-61.
26. Spanbroek R, Grabner R, Lotzer K, et al. Expanding expression of the 5-lipoxygenase pathway within the arterial wall during human atherogenesis. *Proc Natl Acad Sci U S A* 2003;100:1238-43.
27. Mehrabian M, Allayee H, Wong J, et al. Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. *Circ Res* 2002;91:120-6. [Erratum, *Circ Res* 2002;91:e27.]
28. Hussein O, Rosenblat M, Refael G, Aviram M. Dietary selenium increases cellular glutathione peroxidase activity and reduces the enhanced susceptibility to lipid peroxidation of plasma and low-density lipoprotein in kidney transplant recipients. *Transplantation* 1997;63:679-85.
29. Liu D, Liu S, Huang Y, Liu Y, Zhang Z, Han L. Effect of selenium on human myocardial glutathione peroxidase gene expression. *Chin Med J (Engl)* 2000;113:771-5.
30. Salonen JT, Salonen R, Seppanen K, et al. Relationship of serum selenium and antioxidants to plasma lipoproteins, platelet aggregability and prevalent ischaemic heart disease in Eastern Finnish men. *Atherosclerosis* 1988;70:155-60.
31. Suadicani P, Hein HO, Gyntelberg F. Serum selenium concentration and risk of ischaemic heart disease in a prospective cohort study of 3000 males. *Atherosclerosis* 1992;96:33-42.
32. Salvini S, Hennekens CH, Morris JS, Willett WC, Stampfer MJ. Plasma levels of the antioxidant selenium and risk of myocardial infarction among U.S. physicians. *Am J Cardiol* 1995;76:1218-21.
33. Rayman MP. The importance of selenium to human health. *Lancet* 2000;356:233-41.
34. Alissa EM, Bahijri SM, Ferns GA. The controversy surrounding selenium and cardiovascular disease: a review of the evidence. *Med Sci Monit* 2003;9:RA9-RA18.
35. Kardinaal AF, Kok FJ, Kohlmeier L, et al. Association between toenail selenium and risk of acute myocardial infarction in European men: the EURAMIC Study: European Antioxidant Myocardial Infarction and Breast Cancer. *Am J Epidemiol* 1997;145:373-9.
36. Blankenberg S, Tiret L, Bickel C, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. *Circulation* 2002;106:24-30.
37. Blankenberg S, Rupprecht HJ, Bickel C, et al. Circulating cell adhesion molecules and death in patients with coronary artery disease. *Circulation* 2001;104:1336-42.

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