Glutathione Peroxidase-1 Activity, Atherosclerotic Burden, and Cardiovascular Prognosis

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Recent findings suggest that erythrocyte intracellular glutathione peroxidase-1 (GPX-1) activity is related inversely to future cardiovascular events. The aim of this study is to evaluate the association of GPX-1 activity to extent of atherosclerosis, as well as its long-term prognosis in context with atherosclerotic burden. In a prospective study, we included 508 patients before coronary angiography. Atherosclerosis of carotid and leg arteries was documented using sonographic methods. Blood samples were drawn after an overnight fasting period, and GPX-1 activity was determined in washed erythrocytes. GPX-1 activity tended to decrease with increasing numbers of atherosclerotic vascular beds, so that patients without clinically relevant atherosclerosis had GPX-1 activity of 49.3 U/g hemoglobin compared with 46.0 U/g hemoglobin in patients with prevalent atherosclerosis in all 3 vascular beds (p = NS). Follow-up data (median 6.5 years) were available for 504 patients (99.2%), and 96 patients (19.0%) experienced cardiovascular events (cardiovascular death, infarction, and stroke). The event rate was inversely associated with level of GPX-1 activity divided into tertiles (hazard ratio 2.3, 95% confidence interval 1.4 to 4.0 for lowest vs highest tertile of GPX-1 activity, p = 0.002, adjusted). The highest event rate was found in persons with low GPX-1 activity and multivascular atherosclerosis (event rate 36.9%, p <0.0001). In conclusion, decreased red blood cell GPX-1 activity is associated with increased cardiovascular risk according to the extent of atherosclerosis. **(Am J Cardiol 2007;99:808–812)**

In recent years, there has been much interest in the role of free radicals and oxidative stress in the pathogenesis of atherosclerosis.[1–3] Central to the defense against oxidative stress are antioxidant enzymes, including superoxide dismutase, catalase, and gluthatione peroxidase (GPX).[4–6] To date, 4 different GPX forms are known, all of which contain selenocysteine at their active sites.[7] GPX-1 is the ubiquitous intracellular form that is a key antioxidant enzyme within most cells, including the endothelium.[8] Accumulating evidence suggests that alterations or disturbances in defensive enzyme systems may accelerate atherosclerosis.[9–12] Decreased GPX-1 activity was observed in patients with coronary artery disease (CAD) and those with acute myocardial infarction.[13,14] From a clinical perspective, we were able to show that a low level of red blood cell GPX-1 activity is associated independently with future cardiovascular events.[14] Atherosclerosis is a process that involves the entire arterial vessel tree, and there are known differences between the atherosclerotic process in various vascular beds.[15,16] Therefore, we aim to investigate whether GPX-1 activity is associated with atherosclerosis in different vascular regions and address the following questions. (1) Is there a correlation between decreased GPX-1 activity and extent of atherosclerosis, including coronary, carotid, and peripheral arteries? (2) Is enhanced activity of cellular GPX-1 protective against cardiovascular events in a cohort of patients with atherosclerosis, including multiple vascular territories?

Methods

Study population: Between November 1996 and December 1997, a total of 732 patients referred to the Medical Department II of the Johannes Gutenberg-University Clinic Mainz, Germany, with suspected CAD were enrolled in the Athero*Gene* registry. In a subgroup of 508 patients, additional examinations of the carotid and leg arteries were performed. The study design and patient selection are described in detail elsewhere.[15,17] Exclusion criteria were evidence of hemodynamically significant valvular heart disease, surgery or trauma within the previous month, known cardiomyopathy, known cancer, or febrile conditions. Patients presented with stable angina pectoris (57.1%) or acute coronary syndrome (30.1%). In 65 patients (12.8%), CAD was excluded. Cardiovascular risk factors, such as family history of cardiovascular disease, smoking, and hyperlipidemia, were defined according to previous publications.[15,17] Patients either presented in our clinic or were interviewed by telephone by trained medical staff. A total of 504 of 508 patients (99.2%) were followed up for a median of 6.5 years (minimum 2.8, maximum 7.4). Follow-up information was

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Table 1
Baseline characteristics of the study population

	No. of Vascular Beds With Documented Atherosclerosis				p Value for Trend
	0 (n = 65)	1 (n = 244)	2 (n = 145)	3 (n = 54)	
Age (yrs)	59.6 ± 8.9	61.1 ± 9.7	64.2 ± 8.9	66.2 ± 6.4	0.0001
Men (%)	36 (54.5%)	184 (75.7%)	110 (75.9%)	46 (85.2%)	0.0001
Body mass index (kg/m^2)	26.7 ± 4.3	26.6 ± 3.1	26.3 ± 3.3	26.3 ± 3.8	0.73
Diabetes mellitus (%)	6 (9.2%)	45 (18.4%)	37 (25.5%)	19 (35.2%)	0.002
Hypertension	35 (53.8%)	158 (64.8%)	109 (75.2%)	43 (79.6%)	0.002
Never smoked	38 (58.5%)	109 (44.7%)	50 (34.5%)	16 (29.6%)	0.005
Formerly smoked	8 (12.3%)	23 (9.4%)	20 (13.8%)	8 (14.8%)	
Currently smoked	19 (29.2%)	112 (45.9%)	75 (51.7%)	30 (55.6%)	
Low-density lipoprotein cholesterol (mg/dl)	139 ± 34	137 ± 36	137 ± 38	145 ± 39	0.54
HDL cholesterol (mg/dl)	55 ± 19	47 ± 16	45 ± 14	44 ± 12	0.0001
Triglycerides (mg/dl)	167 ± 146	173 ± 118	172 ± 110	195 ± 108	0.56
Platelet aggregation inhibitors	42 (64.6%)	213 (87.3%)	133 (91.7%)	45 (83.3%)	0.0001
β-blocker medication use	19 (29.2%)	140 (57.4%)	68 (46.9%)	28 (51.8%)	0.0001
Statin medication use	4 (6.2%)	58 (23.8%)	33 (22.8%)	10 (18.5%)	0.02
Angiotensin-converting enzyme inhibitor medication use	20 (30.8%)	101 (41.4%)	80 (55.2%)	33 (61.1%)	0.0001

Data presented as number (percent) of patients or mean ± SD.

obtained for death from cardiovascular causes, death from causes not related to heart disease, nonfatal myocardial infarction, and stroke. Information about causes of death and clinical events was obtained from hospital or general practitioner charts. The study was approved by the Ethics Committee of the University of Mainz. Participation was voluntary, and each study subject gave written informed consent.

Evaluation of vascular status: Coronary angiography was performed in all study patients. Patients were considered to have CAD after coronary artery bypass surgery or in there was ≥1 diameter stenosis >30% according to coronary angiography by visual assessment in a major coronary artery after sublingual administration of nitroglycerin 0.8 mg. Patients were defined as having carotid artery disease if they previously had undergone carotid surgery or significant stenosis could be detected using duplex sonography (7.5-MHz transducer, Ultramark 9, Advanced Technology Laboratories, Bothell, Washington). Percent diameter stenosis was estimated for each internal and external carotid artery using both color duplex imaging and Doppler peak systolic flow velocities. In accordance with previous reports, peak velocities >1.4 m/s were assumed to indicate a >50% diameter lumen stenosis.[18] Peripheral vascular status was investigated using Doppler flow velocity study with a 4-MHz transducer for the femoral and popliteal arteries and 8-MHz transducer for the foot arteries. Manifest peripheral artery disease was diagnosed after a previous peripheral revascularization or if an ankle–arm index (systolic blood pressure ankle/arm) <0.9 could be detected in ≥1 pedal artery with pathologic monophasic Doppler waves.[19] Patients were divided into 4 groups with regard to number of vascular beds with documented atherosclerosis: 0 = neither CAD nor carotid artery stenosis nor peripheral arterial disease; 1 = CAD without carotid artery or peripheral arterial disease; 2 = CAD and carotid artery stenosis or peripheral arterial disease; and 3 = CAD and carotid artery stenosis and peripheral arterial disease.

For end-point analysis, patients with CAD, peripheral artery disease, and/or carotid artery stenosis were defined as having multivascular atherosclerosis.

Laboratory methods: Blood samples were drawn from each subject after an overnight fast under standardized conditions before coronary angiography was performed. GPX-1 activity was determined in washed red blood cells obtained immediately after sampling from whole blood anticoagulated with ethylenediamintetraacetic acid. Hemolyzed cells were stored frozen for up to 1 week. GPX-1 was measured as previouly described with minor modifications (Ransel test kit, Randox, Belfast, Ireland).[14] Serum lipid levels (triglycerides and high-density lipoprotein [HDL] cholesterol) were measured immediately using routine methods; low-density lipoprotein cholesterol was calculated using the Friedwald formula.

Statistical analysis: Mean levels and proportions of baseline characteristics were calculated for study participants. Differences among the 4 patient groups were tested using the chi-square test for categorical variables and analysis of variance test for continuous variables. Survival was estimated using the Kaplan-Meier method and compared among different groups using the log-rank test. In all survival analyses, the end point was cardiovascular death, nonfatal myocardial infarction, or stroke. Data for patients who died from other causes were censored at the time of the event. Hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) for future cardiovascular death were estimated using Cox regression analysis adjusted for potential confounders. A p value ≤0.05 was considered to be locally significant. Computations were carried out using SPSS software, version 12.0 (SPSS Inc., Chicago, Illinois).

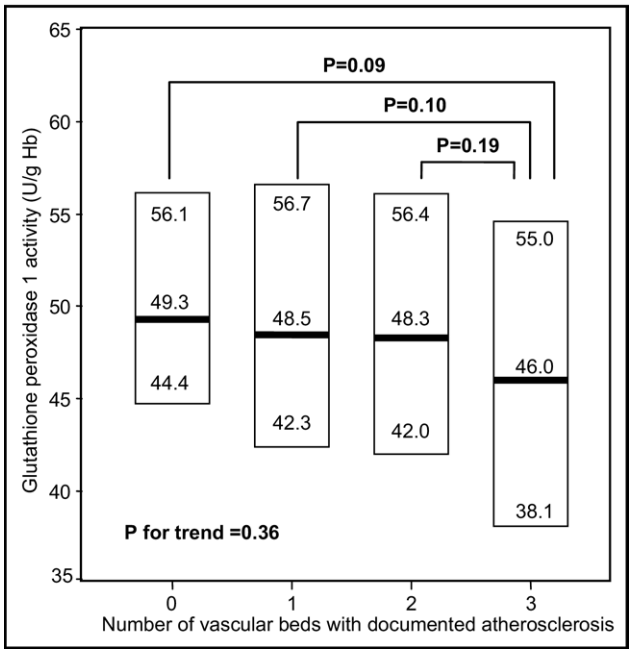

Figure 1. Box-plot analysis shows GPX-1 activity according to the number of vascular beds with atherosclerotic manifestations.

Results

Extent of atherosclerosis and glutathione peroxidase-1 activity: Relevant CAD in ≥1 major coronary artery was detected in 443 patients (87.2%). In 89 patients (17.5%), ≥1 carotid artery stenosis was detected, and in 169 patients (33.3%), peripheral artery disease was diagnosed. Patients were classified according to the extent of atherosclerosis. In 65 patients (12.8%), neither CAD nor carotid stenosis nor peripheral arterial disease was detected. In 244 patients, CAD was diagnosed; however, results of carotid duplex sonography and Doppler examination of the leg arteries were normal (CAD alone, 48.0%). One hundred forty-five patients (28.6%) had CAD and additional stenosis in 1 other vascular bed. In 54 patients (10.6%), ≥1 relevant stenosis was diagnosed in all 3 vascular beds. Table 1 lists baseline characteristics according to the number of vascular beds with atherosclerotic manifestations. GPX-1 activity was normally distributed among study participants. It ranged from 8.0 to 99.6 U/g hemoglobin (Hb) (mean ± SD 49.2 ± 11.4, median 48.4, interquartile range 42.0 to 56.3). Figure 1 shows the box plot analysis of GPX-1 activity according to number of vascular beds with documented atherosclerosis. We found a nonsignificant decrease in GPX-1 activity according to extent of atherosclerosis. In patients without clinically relevant atherosclerosis, median baseline GPX-1 activity was 49.3 U/g Hb (interquartile range 44.4 to 56.2) compared with patients with atherosclerosis in all 3 vascular beds with a median of 46.0 U/g Hb (interquartile range 38.1 to 55.0).

Cardiovascular prognosis and glutathione peroxidase-1 activity. A total of 504 of 508 patients (99.0%) were followed up for a median of 6.5 years. Follow-up information was obtained about death from cardiovascular causes (n = 54, 10.7%), death from causes not related to heart disease (n = 27, 5.4%), nonfatal myocardial infarction (n = 21, 4.2%), and nonfatal stroke (n = 21, 4.2%). Ninety-six patients (19.0%) experienced ≥1 cardiovascular event. Patients were compared with regard to tertiles of GPX-1 activity. The unadjusted rate of cardiovascular events increased in a stepwise fashion across decreasing tertiles of baseline GPX-1 activity. The difference between the lowest and highest tertiles and the trend across all tertiles were significant (lowest vs highest tertile $p = 0.001$, trend across all tertiles $p < 0.001$). The event rate for patients in the lowest tertile of GPX-1 activity (27.4%) was approximately 2 times that for patients in the highest tertile (13.1%). This significant inverse relation between GPX-1 activity and relative risk remained nearly unchanged in a Cox regression analysis after adjustment for most potential confounders (adjusted for age, gender, body mass index, presence or absence of hypertension, presence or absence of diabetes, HDL cholesterol, family history of CAD, smoking, C-reactive protein, and statin, angiotensin-converting enzyme inhibitor, and β-blocker use). The HR was 2.3 (95% CI 1.4 to 4.0) for patients in the lowest tertile of GPX-1 activity compared with the highest tertile ($p = 0.002$, adjusted).

Cardiovascular prognosis, glutathione peroxidase-1 activity, and extent of atherosclerosis: In subjects without atherosclerotic manifestations, the cardiovascular event rate was 12.3% compared with 14.5% in subjects with CAD alone, 26.4% in subjects with CAD and documented atherosclerosis in 1 additional vascular bed, and 27.8% in subjects with documented atherosclerosis all 3 vascular beds ($p < 0.001$). To address the role of GPX-1 activity on cardiovascular risk depending on atherosclerotic burden, we performed a subgroup analysis comparing subjects with CAD alone and with CAD and peripheral manifestations of atherosclerosis. For this subgroup analysis, subjects with atherosclerotic manifestations in 2 or 3 vascular beds were considered to have multivascular atherosclerosis (n = 198, 39.3%). Figure 2 shows the Kaplan-Meier analysis for patients with CAD alone and those with multivascular atherosclerosis according to tertiles of GPX-1 activity. For subjects with CAD alone, there was a significant trend in comparing the 3 tertiles of GPX-1 activity (lowest vs highest tertile $p < 0.02$, trend across all tertiles $p < 0.03$). In patients with multivascular atherosclerosis, decreased GPX-1 activity significantly increased the cardiovascular event rate. The highest event rate was found in subjects with low serum GPX-1 activity and multivascular atherosclerosis (event rate 36.9%, lowest vs highest tertile $p < 0.04$, trend across all tertiles $p = 0.04$).

Cox regression analysis was performed for both subgroups (Table 2). Patients in the highest tertile of GPX-1 activity were used as a control group. For subjects with CAD alone, the HRs were 2.5 (95% CI 0.9 to 7.2) for patients in the second and 2.9 (95% CI 1.0 to 8.0) for those in the lowest tertile of GPX-1 activity in a fully adjusted model. In the subgroup of subjects with multivascular atherosclerosis, HRs were 1.4 (95% CI 0.7 to 3.1) for patients in the second and 2.2 (95% CI 1.1 to 4.3) for patients in the lowest tertile of GPX-1 activity adjusted for possible confounders.

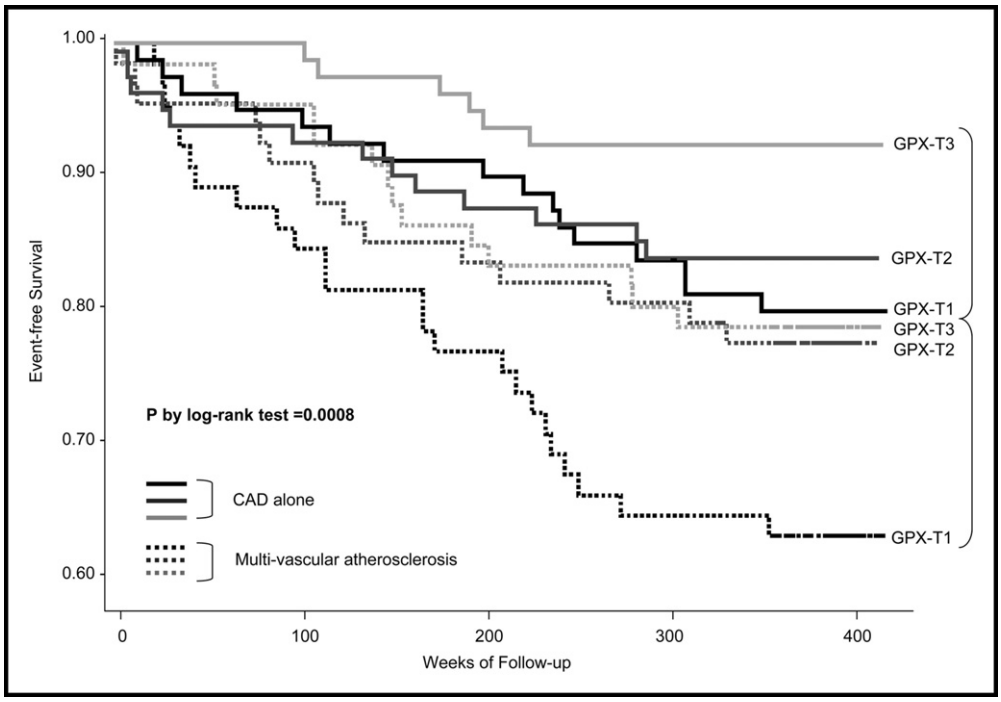

Figure 2. Kaplan-Meier curves for event-free survival of patients with CAD alone and multivascular atherosclerosis according to tertile (T) of GPX-1 activity.

Table 2
Hazard ratios for future cardiovascular events according to tertiles of glutathione peroxidase-1 activity and extent of atherosclerosis

	No. of Events/ No. of Patients	Age- and Gender-adjusted HR (95% CI)	p Value	Fully Adjusted Model* HR (95% CI)	p Value
Patients with CAD alone	n = 241				
Tertile 3 (≥54.0 U/g Hb)	6/80 (7.5%)	1.00		1.00	
Tertile 2 (>44.3–54.0 U/g Hb)	13/81 (16.3%)	2.2 (0.8–5.7)	0.12	2.5 (0.9–7.3)	0.09
Tertile 1 (≤44.3 U/g Hb)	16/80 (20.0%)	2.8 (1.1–7.2)	<0.03	2.9 (1.0–8.0)	0.04
p Value for trend	<0.03	<0.02		<0.02	
Patients with multivascular atherosclerosis	n = 198				
Tertile 3 (≥54.0 U/g Hb)	14/66 (21.2%)	1.00		1.00	
Tertile 2 (>44.3–54.0 U/g Hb)	15/67 (22.4%)	1.1 (0.5–2.3)	0.82	1.4 (0.7–3.1)	0.35
Tertile 1 (≤44.3 U/g Hb)	24/65 (36.9%)	2.5 (1.3–4.8)	<0.01	2.2 (1.1–4.3)	0.03
p Value for trend	<0.04	<0.0001		<0.0001	

* Adjusted for age, gender, body mass index, presence or absence of hypertension, presence or absence of diabetes, HDL cholesterol, family history of CAD, smoking, C-reactive protein, statin use, angiotensin-converting enzyme inhibitor use, and β blocker medication use.

Discussion

Patients with documented multivascular atherosclerosis usually experience rapid progression of atherosclerotic disease and a high rate of cardiovascular events. In this prospective cohort of patients with suspected atherosclerotic disease, patients with multivascular atherosclerosis had the lowest GPX-1 activity. This result supports the hypothesis that oxidative mechanisms are involved in atherosclerotic processes in the entire arterial vessel wall, and GPX-1 activity seems to protect against oxidative stress in different vascular beds. Low GPX-1 activity increased the cardiovascular event rate independently of the severity of atherosclerotic disease. Prognosis was worst in patients with the lowest serum GPX-1 activity and multivascular atherosclerosis with a twofold increase in HR compared with patients with multivascular disease and high GPX-1 activity and a more than threefold increase in HR compared with patients without clinical manifest atherosclerosis. Therefore, patients with multivascular atherosclerosis and decreased GPX-1 activity were identified as a high-risk population. This significant finding was not changed after adjustment for most potential confounders, such as age, gender, body mass index, cardiovascular risk factors, C-reactive protein, or different medication with known protective influence on cardiovascular prognosis (statins, angiotensin-converting enzyme inhibitors, and β blockers).

Because GPX-1 is a ubiquitous intracellular form of GPX and a key antioxidant enzyme in the prevention of oxidative stress, it also may play an important role as an antiatherosclerotic enzyme.[8] In mice, GPX-1 deficiency resulted in such abnormal vascular and cardiac function as increased inflammation, neointimal formation, and collagen

deposition.[12] Moreover, GPX-1 deficiency apparently decreased bioavailable nitric oxide in mice.[20] Furthermore, GPX-1 inhibited 5-lipoxygenase in monocytic cells, which might constitute an antiatherosclerotic effect of the enzyme in addition to its antioxidant functions.[21] Several investigations also described a possible protective effect of GPX-1 in men. Lapenna et al[22] described decreased GPX-1 activity in carotid atherosclerotic plaques and postulated an association between the lack of GPX-1 and development of severe atherosclerotic lesions. In addition, an association between decreased GPX-1 activity in patients with coronary heart disease and individuals with acute myocardial infarction has been previously described.[13] These results are in line with our findings that GPX-1 activity tends to decrease with increasing numbers of atherosclerotic vascular beds. Patients with atherosclerosis in multiple vascular regions experience a higher percent of cardiovascular events than patients with limited disease. In a previous report, we described a correlation between low GPX-1 activity and cardiovascular events in patients with atherosclerosis.[14] In this evaluation, we extend our results, such that GPX-1 activity modulates cardiovascular risk according to the number of vascular beds involved in the atherosclerotic process.

Several limitations of this study should be considered. Erythrocyte GPX-1 activity probably is a suitable surrogate marker for cellular GPX-1 activity in general, but this has not been proved in systematic analysis. We evaluated a patient cohort with a high prevalence of coronary artery disease and a mean age of 63 years because we included only patients scheduled for coronary angiography, which is the gold standard for evaluating CAD. Therefore, the study describes changes in a highly selected patient group, and this population may not necessarily represent other populations.

1. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med* 1999;340:115–126.
2. Glass CK, Witz JL. Atherosclerosis: the road ahead. *Cell* 2001;104: 503–516.
3. Steinberg D, Witztum JS. Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to data refute the hypothesis? *Circulation* 2002;105:2107–2011.
4. Forsberg L, de Faire U, Morgenstern R. Oxidative stress, human genetic variation and disease. *Arch Biochem Biophys* 2001;389:84–93.
5. Ursini F, Maiorino M, Brigelius-Flohe R, Aumann KD, Schomburg D, Flohe L. Diversity of glutathione peroxidases. *Methods Enzymol* 1995; 252:38–53.
6. Raes M, Michiels C, Remacle J. Comparative study of the enzymatic defense systems against oxygen-derived free radicals: the key role glutathione peroxidase. *Free Radic Biol Med* 1987;3:3–7.
7. Arthur JR. The glutathione peroxidase. *Cell Mol Life Sci* 2000;57: 1825–1835.
8. Frank L, Massaro D. Oxygen toxicity. *Am J Med* 1980;201:875–880.
9. Porter M, Pearson DJ, Suarezmendez VJ, Blann AD. Plasma, platelet and erythrocyte glutathione peroxidases as risk factors in ischemic heart disease in man. *Clin Sci* 1992;83:343–352.
10. Candra M, Chandra N, Agrawal R, Kumar A, Ghatak A, Pandly VC. The free radical system in ischemic heart disease. *Int J Cardiol* 1994;43:121–125.
11. Hill MF, Singal PK. Antioxidant and oxidative stress changes during failure subsequent to myocardial infarction in rats. *Am J Pathol* 1996; 148:291–300.
12. Forgione MA, Cap A, Liao R, Moldovan NI, Eberhardt RT, Lim CC, Jones J, Goldschmidt-Clermont PJ, Loscalzo J. Heterozygous cellular glutathione peroxidase deficiency in the mouse: abnormalities in vascular and cardiac function and structure. *Circulation* 2002;106:1154–1158.
13. Loeper J, Goy J, Rozensztazn L, Bedu O, Moisson P. Lipid peroxidation and protective enzymes during myocardial infarction. *Clin Chim Acta* 1991;196:119–125.
14. Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, Smielja M, Cambien F, Meyer J, Lackner KJ. Gluathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. *N Engl J Med* 2003;349:1603–1613.
15. Espinola-Klein C, Rupprecht HJ, Blankenberg S, Bickel C, Kopp H, Rippin G, Victor A, Hafner G, Schlumberger W, Meyer J. Impact of infectious burden on extent and long-term prognosis of atherosclerosis. *Circulation* 2002;105:15–21.
16. Grobbe DE, Bots ML. Carotid artery intima-media thickness as an indicator of generalized atherosclerosis. *J Intern Med* 1994;236: 567–573.
17. Rupprecht HJ, Blankerberg S, Bickel C, Rippin G, Hafner G, Prellwitz W, Schlumberger W, Meyer J. Impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease. *Circulation* 2001;104:25–31.
18. Arbeille P, Desombre C, Aesh B, Philippot M, Lapierre F. Quantification and assessment of carotid artery lesions: degree of stenosis and plaque volume. *J Clin Ultrasound* 1995;23:113–124.
19. Newman AB, Shemanski L, Manolio TA, Cushman M, Mittelmark M, Polak JF, Powe NR, Siscovick D. Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol* 1999;19:538–545.
20. Forgione MA, Weiss N, Heydrick CJ, Cap A, Klings ES, Bierl C, Eberhardt RT, Farber HW, Loscalzo J. Cellular glutathione peroxidase deficiency and endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 2002;282:H1255–H1261.
21. Mehrabian M, Allayee H, Wong J, Shi W, Wang XP, Shaposhnik Z, Funk CD, Lusis AJ. Identification of 5-lipoxigenase pathway as a major gene contributing to atherosclerosis susceptibility in mice. *Circ Res* 2002;91:120–126.
22. Lapenna D, de Gioia S, Ciofani G, Mezzetti A, Ucchino S, Calafiore AM, Napolitano AM, Di Ilio C, Cussurullo F. Glutathione-related antioxidant defenses in human atherosclerotic plaques. *Circulation* 1998;97:1930–1934.