Aging-Related Decline of Glutathione Peroxidase 3 and Risk of Cardiovascular Events in Patients With Atrial Fibrillation

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Background—Experimental studies demonstrated that glutathione peroxidase 3 (GPx3), an antioxidant enzyme that catabolizes hydrogen peroxide, protects against thrombosis. Little is known about its role in cardiovascular disease.

Methods and Results—A prospective cohort study was conducted in 909 atrial fibrillation patients. Serum activities of GPx3, superoxide dismutase (SOD), and catalase were measured at baseline to assess the risk of cardiovascular events during a mean follow-up of 43.4 months (3291 person-years). Serum Nox2 and urinary excretion of 11-deydro-thromboxane B_2 were also measured. During follow-up 160 cardiovascular events occurred (4.9%/year). Significantly lower values of GPx3 ($P<0.001$) and SOD ($P=0.037$) were detected in patients with, compared to those without, cardiovascular events. A lower survival rate was observed in patients with GPx3 ($P<0.001$) and SOD ($P=0.010$) activities below the median, as compared to those above. In a fully adjusted Cox regression model, GPx3 was the only antioxidant enzyme predictor of cardiovascular events (hazard ratio 0.647, 95% confidence interval 0.524-0.798, $P<0.001$). GPx3 was inversely associated with urinary 11-dehydro-thromboxane B_2 (B −0.337, $P<0.001$) and serum Nox2 (B: −0.423, $P<0.001$). GPx3 activity progressively decreased with decades of age ($P<0.001$), with a progressive reduction in people aged ≥70 years.

Conclusions—This study provides evidence that a low antioxidant status, as depicted by reduced levels of GPx3, increases the risk of cardiovascular events in patients with atrial fibrillation. The age-related decline of GPx3 may represent a mechanism for the enhanced cardiovascular risk in the elderly population. (*J Am Heart Assoc*. 2016;5:e003682 doi: 10.1161/JAHA.116.003682)

Key Words: atrial fibrillation • cardiovascular disease • cardiovascular events • catalase • glutathione • Nox • risk factor • superoxide dismutase • thromboxane

Cardiovascular disease is the main cause of morbidity and mortality in the Western countries. Aging largely contributes to this phenomenon, as it is independently associated with an increased risk of coronary and cerebrovascular disease.[1] As the size of the elderly population steadily increases in the next 2 to 3 decades, the number of subjects expected to experience cardiovascular disease, will substantially increase in the next future.

There is a growing body of experimental evidence in support of the free radical theory of aging, suggesting that oxidative stress is implicated in vascular damage, atherosclerosis, and its sequelae.[2] According to this theory, the formation of reactive oxygen species increases with age, ultimately resulting in damage to cells and their components such as DNA, lipids, and proteins. The balance between enzymes eliciting the formation of reactive species and antioxidants seems to have a negative impact on morbidity and life span. The relationship among aging, oxidative stress, and cardiovascular disease is essentially based on in vitro and experimental studies,[3] while data in humans are still undefined. A previous study[4] demonstrated that glutathione peroxidase 1 is predictive of cardiovascular events, suggesting that a low antioxidant status predisposes to poor vascular outcomes. However, the relationship between low glutathione peroxidase activity and oxidative stress, and the impact of aging on antioxidant status are still unclear. To address these issues, we investigated a population affected by atrial fibrillation (AF), which is closely related to oxidative stress[5] and aging. In particular, previous studies showed that oxidative stress related to the activity of NADPH oxidase (Nox) is increased in experimental atrial pacing-induced AF[6] and associated with an increased risk of postoperative AF

after cardiac surgery.[7] Moreover, Nox2 activity has been shown to be predictive of cardiovascular events in AF.[5] Despite the many efforts, the overall thrombotic risk is still high in AF, and, therefore, the clinical need remains to identify patients high-risk patients. Older age may contribute to lower antioxidant status, but the relationship between older age and antioxidant status in patients with AF is not well established. To this purpose, we measured the activity of three antioxidant enzymes, namely glutathione peroxidase 3 (GPx3) and catalase, which catabolize hydrogen peroxide,[8] and superoxide dismutase (SOD), which converts superoxide anion to hydrogen peroxide,[9] in an AF population prospectively followed up for about 3 years to assess the incidence of cardiovascular disease such as stroke, myocardial infarction, and cardiovascular death.

Methods

We performed a prospective single-center cohort study including 990 patients with nonvalvular AF who were referred to the Atherothrombosis Center of the Department of Internal Medicine and Medical Specialties of Sapienza University of Rome, from September 2007 to October 2015. All patients were treated with oral vitamin K antagonists (INR range 2.0-3.0) initially according to CHADS$_2$ score, and then to CHA$_2$DS$_2$ VASc score.

Exclusion criteria included the presence of prosthetic heart valves, cardiac stent placement or cardiac revascularization in the previous year, severe cognitive impairment, chronic infectious diseases, autoimmune systemic diseases, and active cancer.

Baseline medical history and anthropometric data were recorded, and blood and urine samples were collected. Electrocardiography and echocardiography were also performed. Cardiovascular risk factors were defined according to international criteria: arterial hypertension as repeatedly elevated blood pressure ($\geq140/\geq90$ mm Hg) or taking antihypertensive drugs[10]; diabetes as a casual plasma glucose ≥200 mg/dL (11.1 mmol/L), or fasting plasma glucose≥126 mg/dL (7.0 mmol/L), or 2-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test or taking antidiabetic drugs[11]; heart failure as the presence of signs and symptoms typical of congestive heart failure or reduced ejection fraction ($\leq40\%$).[12]

The primary endpoint of the study was a composite of cardiovascular events including fatal/nonfatal myocardial infarction or ischemic stroke, cardiac revascularization, cardiovascular death, and transient ischemic attack.

Diagnosis of myocardial infarction was made according to the definition proposed by the Joint ESC/ACCF/AHA/WHF Task Force.[13] The occurrence of ischemic stroke was determined on clinical manifestations and confirmed by radiological findings; transient ischemic attack was defined according to the Classification of Cerebrovascular Diseases III.[14] If a patient died within 4 weeks of myocardial infarction or ischemic stroke, these events were recorded as fatal myocardial infarction or fatal ischemic stroke, respectively. Death was classified as cardiovascular unless an unequivocal noncardiovascular cause of death was identified. Cardiovascular death included sudden death, progressive congestive heart failure, and procedure-related death.

Data on cardiovascular events were prospectively collected during follow-up, and only the first event was used for the analysis.

All patients provided a written informed consent before being included in the study. The study protocol was approved by the local ethical board of Sapienza-University of Rome (no. 1306/2007) and was conducted according to the principles of the Declaration of Helsinki.

Laboratory Analyses

A blood sample was collected for each AF patient in the morning after overnight fasting. Samples were centrifuged immediately after collection, aliquoted, and stored at $-80°C$ until use.

Glutathione peroxidase 3 activity was measured in serum by Colorimetric Assay Kit (Abcam, Cambridge, UK). In this assay, glutathione peroxidase reduces the probe cumene hydroperoxide while it converts reduced glutathione to its oxidized form, glutathione disulfide. The generated oxidized glutathione is converted into reduced glutathione by glutathione reductase, using nicotinamide adenine dinucleotide phosphate as reducing agent. GPx3 activity is proportional to the decrease in absorbance of nicotinamide adenine dinucleotide phosphate at 340 nm. One unit (U) of glutathione peroxidase is defined as the amount of enzyme that will cause the oxidation of 1 nmole of nicotinamide adenine dinucleotide phosphate (from NADPH to NADP$^+$) per minute at 25°C. GPx3 activity was expressed as units per milliliter. Intra- and interassay coefficients of variation (CVs) were 4.0% and 6.0%, respectively; sensitivity was 0.5 mU/mL. To assess temporal variability we measured the activity of GPx3 in 20 patients twice in 3 weeks and found no significant differences between the 2 determinations ($P=0.279$).

SOD activity was measured in serum samples by Activity Kit (Arbor Assay, Ann Arbor, MI). Samples were incubated with the substrate followed by xanthine oxidase reagent. The xanthine oxidase generates superoxide in the presence of oxygen, which converts a colorless substrate into a colored product. The colored product was read at 450 nm. SOD activity was expressed as units per milliliter. Intra- and interassay CVs were 4.6% and 6.1%, respectively.

Catalase activity was measured in serum samples with Catalase Activity Kit (Arbor Assay). Hydrogen peroxide was added to samples and standards. In the presence of hydrogen peroxide, horseradish peroxidase—conjugated antibody converts the colorless substrate into a colored product. The colored product was read at 560 nm. Increasing levels of catalase in the samples cause a decrease in H_2O_2. Catalase activity was expressed as units per milliliter. Intra- and interassay CVs were 3.5% and 9.8%, respectively.

The excretion of urinary 11-dehydro-thromboxane B_2 was measured by an ELISA commercial kit (Cayman, Ann Arbor, MI). Data were expressed as nanograms per milligram urinary creatinine. Intra- and interassay CVs were 4.0% and 3.6%, respectively.

Serum Nox2 activity was assessed by serum levels of Nox2 derived peptide, a marker of Nox2 activation, by ELISA method. The peptide was recognized by the specific monoclonal antibody against the amino acidic sequence (224—268) of the extramembrane portion of Nox2 that was released in the medium on cell activation. Values were expressed as picograms per milliliter. Intra- and interassay CVs were 5.2% and 6%, respectively.

Statistical Analysis

Categorical variables were reported as counts (percentage). The normal distribution of parameters was assessed by Kolmogorov-Smirnov test. Continuous variables were expressed as mean ± standard deviation or median and interquartile range. Independence of categorical variables was tested with the chi-squared test. Student unpaired t test and Pearson product-moment correlation analyses were used for normally distributed continuous variables. Appropriate nonparametric tests (Mann–Whitney U test and Spearman rank correlation test) were used for all the other variables. Kruskal-Wallis test was used to compare groups. The cumulative incidence of cardiovascular events was estimated using a Kaplan-Meier product-limit estimator for each antioxidant enzyme of interest. Survival curves were formally compared using the log-rank test. Cox proportional hazards analyses were used to calculate the adjusted relative hazards of cardiovascular events by each clinical variable. We built 4 different models, 1 for each of the above-mentioned antioxidant enzymes, and 1 fully adjusted model including all predictors. In addition to antioxidant enzymes, the

Table 1. Baseline Characteristics of the Study Cohort and Occurrence of Cardiovascular Events

Variables	Overall	Cardiovascular Events		P Value
	(n=909)	No (n=749)	Yes (n=160)	
Age, y	73.5±8.2	72.8±8.3	76.7±7.2	<0.001
Women, %	43.1	43.4	41.9	0.792
Smokers, %	9.5	9.5	9.4	0.967
CHA$_2$DS$_2$-VASc score*	4.0 [3.0-4.0]	3.0 [2.0-4.0]	4.0 [3.0-5.0]	<0.001
Arterial hypertension, %	94.6	94.4	95.6	0.700
Diabetes mellitus, %	19.9	17.9	29.4	0.001
Heart failure, %	16.1	13.6	27.5	<0.001
Ejection fraction, %	53.5±9.0	54.1±8.7	50.7±9.9	<0.001
History of stroke/transient ischemic attack, %	14.5	11.9	26.9	<0.001
History of myocardial infarction/cardiac revascularization, %	25.1	21.4	42.5	<0.001
Antiplatelet drugs, %	19.1	17.5	26.9	0.008
Statins, %	41.3	41.7	39.4	0.658
Glutathione peroxidase 3, U/mL*	20.0 [10.0–34.0]	21.0 [12.0–34.0]	12.0 [6.0–21.0]	<0.001
Superoxide dismutase, U/mL*	2.2 [1.4–3.2]	2.3 [1.4–3.2]	2.1 [1.5–3.2]	0.037
Catalase, U/mL*	25.0 [20.0–32.0]	25.0 [20.0–32.0]	26.0 [21.0–31.0]	0.954
Thromboxane B$_2$, ng/mg creatinine†	120.0 [70–196.5]	120.0 [68.5–186.0]	150.0 [90.0–283.0]	<0.001
Serum Nox2, pg/mL‡	10.0 [7.0–20.0]	10.0 [6.0–19.0]	13.0 [8.0–23.0]	0.002

*Data expressed as median and interquartile range.
†Data available in 852 patients.
‡Data available in 742 patients.

multivariable analyses were performed with the following prespecified variables entered as covariates: age, female sex, smoking, arterial hypertension, diabetes, history of myocardial infarction, history of stroke/transient ischemic attack, heart failure, and treatment with antiplatelet drugs and statins. Multivariable linear regression analysis was used to determine factors associated with serum levels of GPx3.

Two distinct linear regression models were used to investigate the association of GPx3 with Nox2 and thromboxane B2, respectively.[5,15]

Statistical significance was set at a *P* value <0.05. All tests were 2-tailed, and analyses were performed using computer software packages (SPSS-18.0, SPSS Inc, Chicago, IL).

Results

According to the exclusion criteria, 59 patients were excluded, and 22 refused to be included in the study. Thus, 909 AF patients composed the final cohort.

Antioxidant Enzymes and Cardiovascular Events

Mean follow-up was 43.4 (±29.6) months, yielding 3291 person-years of observation; overall, 160 cardiovascular events were registered (4.9%/year): 19 nonfatal and 16 fatal myocardial infarctions, 19 cardiac revascularizations, 24 nonfatal and 12 fatal ischemic strokes, 5 transient ischemic attacks, and 65 cardiovascular deaths. Patients with cardiovascular events had lower GPx3 and SOD activities compared to patients free from events (see Table 1).

After the cohort had been divided according to the median value of GPx3, 113 cardiovascular events were in the group below and 47 in the group above the median (Log-rank test for GPx3 below the median, *P*<0.001; see Figure 1A). Similar results were obtained with SOD (*P*=0.010), with 94 cardiovascular events in the group below and 66 in the group above the median (see Figure 1B). Conversely, catalase levels were not associated with cardiovascular events (*P*=0.673).

Cox regression analysis showed that log GPx3 along with age, diabetes, history of stroke/transient ischemic attack, and myocardial infarction predicted cardiovascular events (see Table 2, Model 1). Similar results were found with log SOD (see Table 2 Model 2) but not with catalase (see Table 2, Model 3). In a fully adjusted model, only log GPx3 persisted as predictor of cardiovascular events (see Table 2, Model 4).

Determinants of GPx3 Activity

Because GPx3 was the strongest predictive antioxidant enzyme we investigated factors affecting its serum levels.

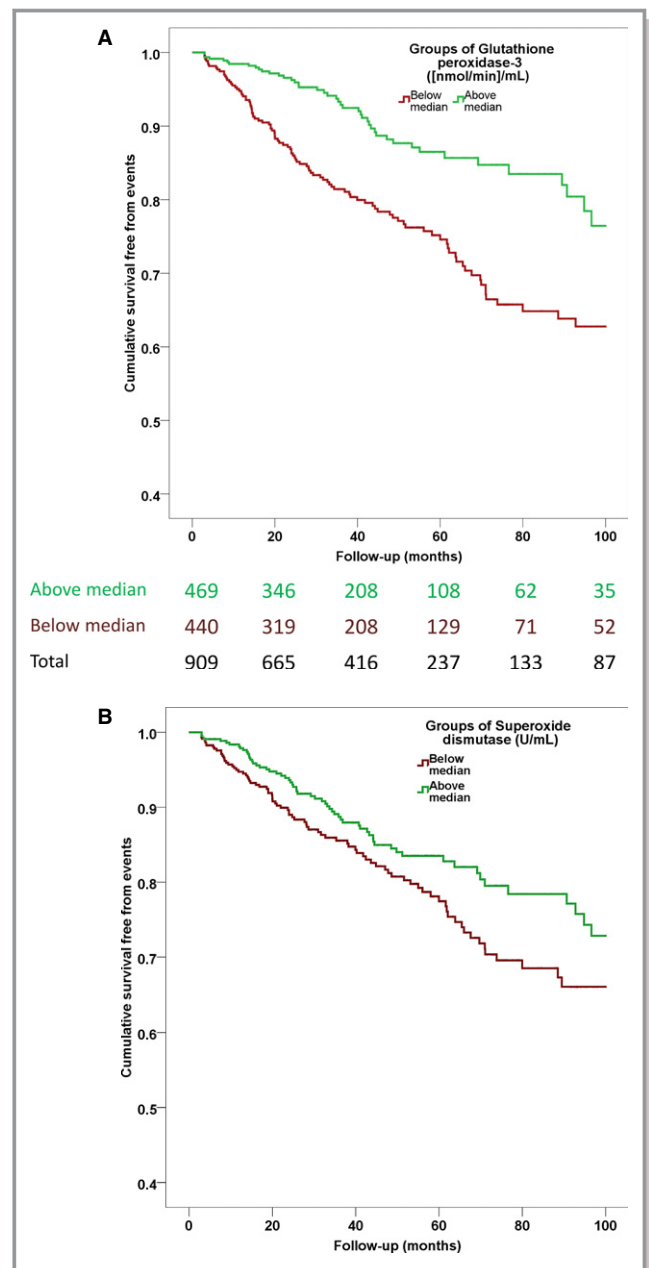|					
Above median	469	346	208	108	62	35
Below median	440	319	208	129	71	52
Total	909	665	416	237	133	87

Figure 1. Kaplan-Meier curves show estimates of cardiovascular events according to the median value of glutathione peroxidase 3 (A), and superoxide dismutase (B).

Gpx3 was weakly correlated with SOD (Rs 0.269, *P*<0.001) and not correlated with catalase (Rs −0.018, *P*=0.581).

A linear regression analysis showed an inverse association for age and heart failure, and smoking was positively associated (see Table 3). GPx3 activity was also inversely correlated with CHA_2DS_2 VASc score (Rs −0.187, *P*<0.001). Given that age was the most important determinant of GPx3, we further investigated the relationship between age and GPx3 by dividing our population into decades (ie, 50-60, 61-70, 71-80, >80 years). We found a progressive decrease of

Table 2. Adjusted Hazard Ratios, Based on Cox Proportional Hazards Models

	P Value	Hazard Ratio	95% CI	
Model 1 (glutathione peroxidase 3)				
Age	<0.001	1.072	1.048	1.096
Female sex	0.177	0.796	0.572	1.109
Arterial hypertension	0.860	1.073	0.491	2.344
Diabetes mellitus	0.009	1.595	1.126	2.258
Smoking	0.066	1.673	0.966	2.896
Heart failure	0.159	1.312	0.899	1.914
History of stroke/transient ischemic attack	0.002	1.773	1.244	2.526
History of myocardial infarction/cardiac revascularization	0.003	1.687	1.191	2.930
Antiplatelet drugs	0.496	1.132	0.792	1.617
Statins	0.549	1.108	0.792	1.551
Log-glutathione peroxidase 3	<0.001	0.625	0.515	0.759
Model 2 (superoxide dismutase)				
Age	<0.001	1.080	1.056	1.105
Female sex	0.242	0.822	0.591	1.142
Arterial hypertension	0.965	0.983	0.452	2.138
Diabetes mellitus	0.006	1.624	1.146	2.302
Smoking	0.176	1.455	0.845	2.507
Heart failure	0.054	1.448	0.993	2.110
History stroke/transient ischemic attack	0.001	1.775	1.247	2.527
History of myocardial infarction/cardiac revascularization	0.001	1.765	1.246	2.501
Antiplatelet drugs	0.504	1.130	0.790	1.614
Statins	0.741	1.058	0.757	1.480
Log-Superoxide dismutase	0.009	0.640	0.458	0.894
Model 3 (catalase)				
Age	<0.001	1.082	1.058	1.107
Female sex	0.197	0.805	0.579	1.119
Arterial hypertension	0.882	0.943	0.435	2.044
Diabetes mellitus	0.009	1.593	1.125	2.256
Smoking	0.180	1.451	0.842	2.502
Heart failure	0.038	1.487	1.023	2.161
History stroke/transient ischemic attack	0.001	1.799	1.264	2.562
History of myocardial infarction/cardiac Revascularization	0.002	1.759	1.240	2.495

Continued

Table 2. Continued

	P Value	Hazard Ratio	95% CI	
Antiplatelet drugs	0.534	1.120	0.784	1.601
Statins	0.805	1.043	0.744	1.463
Log-Catalase	0.292	0.834	0.595	1.169
Model 4 (fully adjusted)				
Age	<0.001	1.072	1.048	1.096
Female sex	0.177	0.796	0.571	1.109
Arterial hypertension	0.870	1.067	0.488	2.332
Diabetes mellitus	0.008	1.606	1.133	2.277
Smoking	0.058	1.701	0.982	2.947
Heart failure	0.183	1.294	0.885	1.892
History stroke/transient ischemic attack	0.001	1.789	1.255	2.550
History of myocardial infarction/cardiac revascularization	0.002	1.724	1.215	2.445
Antiplatelet drugs	0.452	1.147	0.803	1.639
Statins	0.637	1.085	0.773	1.522
Log-glutathione peroxidase 3	<0.001	0.647	0.524	0.798
Log-superoxide dismutase	0.289	0.824	0.577	1.178
Log-catalase	0.157	0.781	0.555	1.100

GPx3 across decades (Kruskal-Wallis test, $P<0.001$, see Figure 2).

GPx3 Activity and Oxidative Stress

To explore the relationship between GPx3 activity and oxidative stress, we measured the activity of serum Nox2. We found that GPx3 and Nox2 were inversely correlated (Rs −0.434, $P<0.001$). In a second model of linear regression analysis including Nox2 as covariate, we found that Nox2 and GPx3 were independently and inversely associated (B −0.423, $P<0.001$; Table 4).

Finally, when we investigated the relationship between GPx3 and urinary thromboxane B_2, we found an inverse association (Rs −0.365, $P<0.001$). Thromboxane B_2 and Nox2 were also mutually correlated (Rs 0.492, $P<0.001$). In a linear regression analysis, thromboxane B_2 (B −0.337, $P<0.001$), along with age and heart failure, was inversely associated with GPx3 (Table 4).

Discussion

The study provides evidence of an inverse relationship between GPx3 activity and cardiovascular events in a

Table 3. Linear Regression Analysis of Factors Associated With Log Glutathione Peroxidase 3 Levels

	B	SE	β	P Value	95% CI for B	
Female sex	−0.038	0.053	−0.024	0.464	−0.142	0.065
Age	−0.017	0.003	−0.179	<0.001	−0.023	−0.011
Diabetes	−0.010	0.065	−0.005	0.876	−0.137	0.117
Smoking	0.229	0.088	0.085	0.010	0.056	0.402
Heart failure	−0.194	0.072	−0.091	0.007	−0.335	−0.053
Previous stroke/transient ischemic attack	−0.123	0.072	−0.055	0.090	−0.265	0.019
Previous myocardial infarction/cardiac revascularization	−0.048	0.066	−0.026	0.473	−0.178	0.082
Arterial hypertension	0.051	0.113	0.015	0.652	−0.171	0.274
Antiplatelet drugs	0.011	0.068	0.006	0.871	−0.123	0.145
Statins	0.025	0.054	0.016	0.641	−0.080	0.131

population affected by AF. In addition, GPx3 progressively declines with aging, suggesting that the reduction of natural antioxidants may be a factor predisposing to cardiovascular complications in the elderly population.

Few studies investigated the predictive role of glutathione peroxidases in patients at risk of cardiovascular events. Among the 5 glutathione peroxidase isoforms, glutathione peroxidase 1, contained in red cells, has been prospectively analyzed in 636 patients with suspected coronary heart disease. The study showed an inverse relationship between the activity of the enzyme and the risk of cardiovascular events in a follow-up period of 4.7 years.[4] Among the glutathione peroxidase isoforms, GPx3 is the only one detected in the extracellular space and represents a major antioxidant in

Figure 2. Decrease of glutathione peroxidase 3 across decades of age.

plasma that serves to scavenge oxidant species. Human and experimental studies demonstrated an important role of GPx3 in the thrombotic process.[16,17] Thus, reduced activity of this enzyme has been detected in patients at high risk of thrombotic events. Furthermore, animals deficient in GPx3 showed enhanced platelet activation, platelet-rich thrombi, and occluded vessels to a greater extent than wild-type ones.[17] The relationship between GPx3 and thrombosis is likely to be attributable to hydrogen peroxide, which is a stimulus for production of thromboxane A_2,[18] a potent aggregating molecule derived from COX1 activation. To best of our knowledge, there is only 1 study that analyzed the relationship between GPx3 and cardiovascular events in a small cohort including 130 participants who died of cardiovascular events after 5 to 12 years of follow-up.[19] This retrospective study showed an inverse relationship between glutathione peroxidase 3 activity and cardiovascular mortality in subjects with low high-density lipoprotein values.[19]

In the context of AF, a low antioxidant status, as demonstrated by an impaired GPx activity, has been described to promote AF onset in an experimental model of atrial pacing and in patients undergoing cardiac surgery.[6,7] However, no study investigated the relationship between GPx activity and the incidence of cardiovascular disease in AF.

Indeed, this is the first prospective study investigating the relationship between GPx3 and cardiovascular events in a large cohort of patients with AF; this clinical setting is characterized by a high risk of ischemic complications including not only thromboembolic stroke but, as evidenced by recent studies, myocardial infarction[20,21] and peripheral artery disease.[22]

Our analysis demonstrated an independent association between GPx3 and the risk of cardiovascular events, after
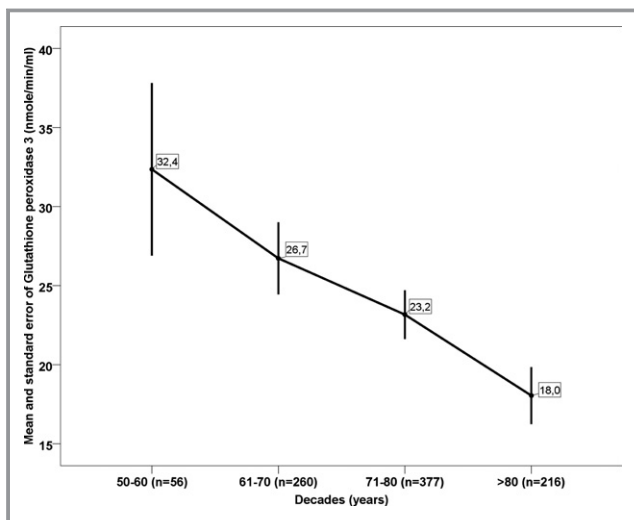
Table 4. Linear Regression Analysis of Factors Associated With Log Glutathione Peroxidase 3 Levels

	B	Standard Error	Beta	*P* Value	95% CI for B	
Model A* including log-soluble Nox2-derived peptide						
Female sex	−0.033	0.054	−0.020	0.547	−0.139	0.074
Age	−0.012	0.003	−0.119	<0.001	−0.018	−0.005
Diabetes	0.038	0.067	0.019	0.570	−0.094	0.170
Smoking	0.196	0.089	0.073	0.028	0.021	0.372
Heart failure	−0.170	0.072	−0.079	0.019	−0.311	−0.028
Previous stroke/transient ischemic attack	−0.130	0.073	−0.058	0.074	−0.273	0.013
Previous myocardial infarction/cardiac revascularization	−0.056	0.066	−0.031	0.395	−0.185	0.073
Arterial hypertension	−0.077	0.115	−0.022	0.505	−0.302	0.149
Antiplatelet drugs	0.007	0.067	0.004	0.912	−0.124	0.139
Statins	0.064	0.056	0.039	0.251	−0.045	0.173
Log-serum Nox2	−0.481	0.037	−0.423	<0.001	−0.555	−0.408
Model B† including thromboxane B_2						
Female sex	−0.017	0.051	−0.011	0.739	−0.118	0.084
Age	−0.015	0.003	−0.153	<0.001	−0.021	−0.008
Diabetes	0.017	0.063	0.009	0.790	−0.107	0.141
Smoking	0.236	0.085	0.088	0.006	0.069	0.404
Heart failure	−0.155	0.068	−0.074	0.024	−0.289	−0.021
Previous stroke/transient ischemic attack	−0.105	0.070	−0.048	0.132	−0.243	0.032
Previous myocardial infarction/cardiac revascularization	−0.015	0.064	−0.008	0.815	−0.140	0.110
Arterial hypertension	0.046	0.112	0.013	0.682	−0.174	0.265
Antiplatelet drugs	−0.034	0.066	−0.017	0.601	−0.164	0.095
Statins	0.057	0.052	0.035	0.281	−0.046	0.159
Log-thromboxane B_2	−0.349	0.033	−0.337	<0.001	−0.414	−0.285

*Data available in 742 patients.
†Data available in 852 patients.

adjusting for potential confounding variables. Further confirmation of the role of an impaired antioxidant status in facilitating cardiovascular disease was provided by the analysis of SOD activity, although catalase showed no predictive value on cardiovascular events. Among the 3 antioxidants, GPx3 showed the best predictive value, as it was the only enzyme significantly associated with cardiovascular events in the fully adjusted model.

In accordance with previous experimental study,[11] we found that GPx3 activity was inversely associated with urinary excretion of thromboxane B_2, a marker of in vivo platelet activation,[23] which suggests that the relationship between GPx3 and cardiovascular events may be mediated by enhanced platelet activation. In addition to platelet activation, age was another strong predictor of GPx3 activity. This issue has already been investigated by previous studies, with equivocal results; of note, those studies measured the total activity of the glutathione peroxidase family but not that of specific isoforms. A novel finding of our study is that GPx3 progressively declines with advancing age with a significant reduction in people aged ≥70 years, but the reason for such an association cannot be deduced from the present study. Endogenous mechanisms related to a progressive imbalance between production of oxygen free radicals and antioxidant defenses cannot be excluded, as suggested by the inverse relationship between GPx3 and Nox2 activity. In addition,

exogenous factors, such as nutrition, may be implicated in this progressive decay of GPx3.

We also found a direct association between smoking and GPx3 activity. This finding is apparently in contrast with 2 previous works that found no association between GPx activity and smoking.[6,7] The positive association between smoking and GPx3 activity in our study may reflect a reactive enhanced GPx3 activity to the increased production of hydrogen peroxide, which is induced by smoking.[24] However, this association deserves further investigation.

The study has implications and limitations. The progressive decay of GPx3 by aging and the interplay between GPx3 and cardiovascular events may help understanding previous experimental data indicating that aging-related thrombosis is associated with enhanced platelet production of hydrogen peroxide.[25] However, a cause-effect relationship cannot be established from this observational study. These findings may help to identify a novel category of patients at high risk of cardiovascular events who could benefit from specific antioxidant treatment. The present data can apply only to a white population with AF, and results must be confirmed by a multicenter study; also, another limitation is represented by the lack of a control group of patients not affected by AF. The role of heart failure in cardiovascular events may be underestimated given its relatively low prevalence in our cohort. Finally, even if GPx3 is the only described circulating GPx isoform,[26,27] we cannot exclude that other isoforms may contribute to the circulating GPx activity.

In conclusion, the study provides evidence that GPx3 declines with aging, and its decline is associated with an increased risk of cardiovascular events in patients with AF. This finding might give novel insight into the mechanism linking aging with cardiovascular risk.

Disclosures

None.

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