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Abstract

Background—Oxidative stress (OS) may be a key mechanism underlying the development of atrial fibrillation (AF) in experimental studies, but data in humans remain limited.

Objective—Systemic OS can be estimated by measurements of circulating levels of the aminothiols including glutathione, cysteine, and their oxidized products. We tested the hypothesis that the redox potentials of glutathione (E_hGSH) and cysteine will be associated with prevalent and incident AF.

Methods—Plasma levels of aminothiols were measured in 1439 patients undergoing coronary angiography, of whom 148 (10.3%) had a diagnosis of AF. After a median follow-up of 6.3 years, 104 of 917 patients (11.5%) developed incident AF. Multivariate logistic regression and Cox regression models were used to determine whether OS markers were independent predictors of prevalent and incident AF after adjustment for traditional risk factors, heart failure, coronary artery disease, and high-sensitivity C-reactive protein level.

Results—For each 10% increase in E_hGSH, the odds of prevalent AF was 30% higher (odds ratio [OR] 1.3; 95% confidence interval [CI] 1.1–1.7; $P = .02$) and 90% higher (OR 1.9; 95% CI 1.3–2.7; $P = .004$) when the median was used as a cutoff. The E_hGSH level above the median was more predictive of chronic AF (OR 4.0; 95% CI 1.3– 12.9; $P = .01$) than of paroxysmal AF (OR 1.7; 95% CI 1.1–2.7; $P = .03$). Each 10% increase in E_hGSH level was associated with a 40% increase in the risk of incident AF (hazard ratio 1.4; 95% CI 1.1– 1.7; $P = .01$).

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Appendix Supplementary data: Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.hrthm.2017.07.028.

Conclusion—Increased OS measured by the redox potentials of glutathione is associated with prevalent and incident AF. Therapies that modulate OS need to be investigated to treat and prevent AF.

Keywords

Atrial fibrillation; Oxidative stress; Glutathione; Redox potentials; Inflammation

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in clinical practice. Although its pathophysiology is incompletely understood, oxidative stress (OS) and inflammation appear to be important triggers.[1–3] OS increases when the production of reactive oxygen species (ROS) overwhelms endogenous antioxidant defenses, causing tissue injury. ROS are derived from many sources including mitochondria, xanthine oxidase, uncoupled nitric oxide synthases, and nicotinamide adenine dinucleotide phosphate oxidases.[4] Increased oxidation results in cell dysfunction, necrosis, apoptosis, and alterations in cellular proteins and signaling pathways.[5] Because of the short half-life of ROS, the focus has been on measuring stable markers in the circulation that reflect cellular and systemic OS. These include markers of lipid peroxidation (isoprostanes), oxidized phospholipids, malondialdehyde, nitrotyrosine, myeloperoxidase, and aminothiol compounds. Aminothiols play a crucial role in redox signaling and can be quantified in plasma.[6] Of these, cysteine and glutathione and their oxidized disulfide compounds (cystine and glutathione disulfide) constitute the major extra- and intracellular reduced thiol pools, respectively. The redox potentials of both glutathione and cysteine pools can be calculated using the Nernst equation to estimate the OS burden in vivo[6] or simply calculating the ratio of cystine to oxidized glutathione, which has been shown to improve risk discrimination of adverse clinical outcomes.[7] Because the plasma glutathione disulfide concentration is low (<200 μM), we have developed a method using high performance liquid chromatography with fluorescence detection that directly measure the low nanomolar plasma concentration of glutathione.

We have shown that increased systemic OS, measured as higher levels of cystine, lower levels of reduced glutathione, or altered ratios of oxidized to reduced aminothiols, has been associated with aging, risk factors for cardiovascular disease including hypertension and smoking, endothelial dysfunction, arterial stiffness and thickness, prevalent and incident cardiovascular disease, and increased pulmonary arterial pressures.[7–9] Experimental studies have shown that increased systemic OS is associated with AF,[1,2] but the evidence in humans remains limited.[3] To investigate the role of OS in AF, we tested the hypothesis that increased OS, measured as changes in circulating aminothiol levels, will be associated with prevalent and incident AF.

Methods

Study design

Between 2004 and 2008, plasma levels of aminothiols were measured in 1439 subjects aged between 18 and 90 years who were enrolled in the Emory Cardiovascular Biobank, a

prospective registry of patients undergoing elective or emergent cardiac catheterization for suspected or known coronary artery disease (CAD) at 3 Emory Healthcare sites.[10] Subjects were excluded if they had a history of heart transplantation, immunosuppressant use, malignancy, or significant infections. Patients' demographic characteristics, medical history, medication use, and behavioral habits were documented with questionnaires and interview as previously described.[7] The study was approved by the Institutional Review Board of Emory University (Atlanta, GA). All subjects provided written informed consent.

Data collection

Diagnoses of prevalent and incident AF were obtained from patient questionnaire, phone follow-up, chart review, and *International Classification of Diseases codes* (*International Classification of Diseases* codes 427.3 and 427.31). Prevalent AF was classified into paroxysmal (spontaneously terminating AF sustained for <7 days) and chronic (includes persistent and permanent AF) AF. Etiology was documented as valvular or nonvalvular AF (in the absence of rheumatic mitral valve disease, a prosthetic heart valve, or mitral valve repair). Diagnosis of incident AF was defined as first known occurrence of AF during follow-up. Presence of CAD was defined as angiographic evidence of >50% luminal stenosis. A CHA_2DS_2-VASc score was calculated for each subject using medical history data acquired through both patient interview and medical record review.

Measures of OS

Plasma levels of aminothiols including cysteine and glutathione, and their oxidized forms cystine and glutathione disulfide, respectively, were measured using high performance liquid chromatography. The redox states (E_h) of the thiol/disulfide pools were calculated using the Nernst equation: $E_h = E_0 + (RT/nF)\ \ln([\text{disulfide}]/[\text{thiol}]^2)$, where E_0 is the standard potential for the redox couple, R is the gas constant, T is the absolute temperature, n is 2 for the number of electrons transferred, and F is the Faraday constant. E_hGSH and E_hCyS were expressed as redox potentials in millivolts, where a more positive numeric value implies a more oxidized state. Detailed methods for the measurements of aminothiols in plasma have been delineated previously and provided in the Supplemental Methods.[6,7] Inflammatory marker (high-sensitivity C-reactive protein [hsCRP]; Life Diagnostics) was measured using commercially available reagents.

Statistical analysis

All continuous variables are described as mean ± SD or median (interquartile range), while categorical variables are presented as proportions. Differences between groups were assessed using the *t* test for continuous variables and the χ^2 test for categorical variables. For nonnormally distributed variables, the Mann-Whitney *U* test was used to compare groups in unadjusted analyses. The Spearman correlation test was used to determine bivariate correlations between aminothiols and categorical variables. We used the Kolmogorov-Smirinov test to determine normality for continuous variables. Logarithmic transformation was performed when data were not normally distributed (Supplemental Figure 1). Multivariate logistic regression was used to determine independent predictors of prevalent AF after adjustment for age, sex, black race, body mass index, smoking, diabetes, hypertension, hyperlipidemia, renal function, heart failure history, CAD history, and hsCRP

level. Regression coefficients are presented as point estimates with 95% confidence intervals (CIs). The adjusted Cox regression model was used to investigate independent predictors of incident AF. Missing covariate data (range 0%–4%) were imputed, and sensitivity analysis with unimputed data found results to be similar. The proportional hazards assumption for Cox models was evaluated by plots of Schoenfeld residuals and formal testing (a χ^2 test calculated as the sum of Schoenfeld residuals), with no significant violations of the assumption found. A 2-tailed *P* value of .05 was considered statistically significant. Analyses were performed using SPSS version 24.0 (IBM Corp., Armonk, NY).

Results

The age of the cohort was 63.4 ± 11.3 years; 67% were men, 33% had diabetes, 69% had hypertension and/or hyperlipidemia, and 67.8% were smokers. Approximately three-quarters had significant CAD (≥50% luminal stenosis) on angiography and 10% presented with acute myocardial infarction (Table 1). Of 1439 patients enrolled in this study, 148 (10.3%) had a diagnosis of AF (86% nonvalvular and 77% paroxysmal). The relationship between markers of OS and clinical features are provided in the Supplemental Tables.

Relationship between markers of OS and prevalent AF

In bivariate analysis, patients with prevalent AF were more likely to be men, older, have more cardiovascular risk factors including hypertension, worse renal function, heart failure with lower ejection fraction, and stroke, but less likely to be black. Among the OS markers, prevalent AF was associated with higher cystine level, cystine/glutathione ratio, and E_hGSH, all indicative of increased OS (Table 1). In unadjusted analysis, a 10% increase in E_hGSH (increased OS) was associated with a 40% increase in the odds of prevalent AF (odds ratio [OR] 1.4; 95% CI 1.1–1.6; *P* = .001) and 80% increase when the median was used as a cutoff (OR 1.8; 95% CI 1.3–2.6; *P* = .001). Moreover, E_hGSH was higher (increased OS) in those with chronic AF than in those with paroxysmal AF (median −135 mV vs −129 mV; *P* = .03). However, E_hGSH was not significantly different between patients with valvular AF and those with nonvalvular AF. The prevalence of AF did not correlate with either the hsCRP level or other markers of OS, including cysteine and glutathione and their oxidized disulfide compounds (cystine and glutathione disulfide) (Table 1).

In multivariate analysis including the aforementioned risk factors, hsCRP level, and markers of OS, predictors of prevalent AF included advanced age, male sex, history of heart failure, diabetes, and higher E_hGSH level (Table 2). The OR for prevalent AF was 1.9-fold (95% CI 1.3–2.7; *P* = .004) higher for those with E_hGSH level above the median than for those with a level below median; for each 10% increase in E_hGSH, the OR for prevalent AF was 1.3-fold higher (95% CI 1.1–1.7; *P* = .02) (Table 2). High E_hGSH level above the median was more predictive of chronic AF (OR 4.0; 95% CI 1.3–12.9; *P* = .01) than of paroxysmal AF (OR 1.7; 95% CI 1.1–2.7; *P* = .03) in the fully adjusted model.

Relationship between markers of OS and CHA_2DS_2-VASc score

According to CHA_2DS_2-VASc scores in those with prevalent or incident AF, 32 subjects (12.7%) were categorized as low risk (0 or 1), 175 (69.4%) as intermediate risk (2–4), and

45 (17.9%) as high risk (>4). Higher CHA_2DS_2-VASc scores significantly differed in oxidative markers including cystine, glutathione, glutathione disulfide, and E_hGSH (P <.01 for all) (Table 3). Correlations between CHA_2DS_2-VASc risk factors and OS markers are shown in Supplemental Table 2.

Relationship between markers of OS and incident AF

Follow-up for incident AF was available for 914 patients. During a median follow-up period of 6.2 years (interquartile range 3.3–7.7 years), 104 patients (11.5%) developed incident AF. Those with incident AF were more likely to be older and have a history of diabetes, myocardial infarction, revascularization, and heart failure with preserved ejection fraction (Table 1).

In unadjusted analyses, a 10% increase in E_hGSH was associated with a 40% increase in the risk of incident AF (95% CI 1.1–1.7; P = .002). There was also a trend for patients with higher E_hCyS (increased OS) to have more incident AF, but did not reach statistical significance (P = .08).

In a Cox regression model including the aforementioned covariates and markers of OS, the independent predictors of incident AF included age, diabetes, b-blocker use, and E_hGSH (Table 4). Thus, each 10% increase in E_hGSH level was associated with an adjusted 1.3-fold (95% CI 1.1–1.7; P = .03) increase in incident AF. Other markers of OS, including cysteine and glutathione and their oxidized disulfide compounds, or hsCRP levels were not independent predictors of incident AF.

Discussion

In this large prospective cohort study of patients undergoing evaluation for CAD, OS assessed as E_hGSH was associated with an increased risk of prevalent and incident AF. A 10% increase in E_hGSH level was associated with a 30% increase in prevalent and incident AF. This association was independent of hsCRP level and other clinical predictors of AF. In subjects with AF, OS markers correlated with CHA_2DS_2-VASc score, which is a power predictor of stroke risk. To our knowledge, this is the first study to show that OS, measured as E_hGSH, predicts the incidence of AF and lends support to the concept of a crucial pathophysiological role of OS in the development of AF.

We and others have previously reported that aminothiol markers of OS are associated with cardiovascular risk factors including endothelial dysfunction, increased systemic arterial stiffness, increased carotid wall thickness, pulmonary systolic pressure, left atrial size, and CAD.[7–9] Importantly, we showed that high level of oxidized cystine and low level of reduced glutathione were predictors of mortality in a population with CAD.[7] This study extends our findings to AF. The link between AF and OS has been investigated previously.[1,2] One study demonstrated evidence of increased protein oxidation in the right atrial appendages from 7 patients with AF undergoing the Maze procedure.[1] Another study showed an increase in oxidized glutathione and lipid peroxidation after coronary bypass surgery, a procedure often complicated by AF.[11] Finally, a small study reported increased OS, measured as E_hGSH and E_hCyS, in 40 white subjects with permanent AF as compared

with a risk factor–matched control group.[3] Our study provides more robust evidence of an association of OS not only with prevalent AF but also with incident AF. Importantly, in our cohort, the hsCRP level was not correlated with incident or prevalent AF, a finding that is consistent with some[12,13] but not other previous[14,15] studies. There are few other studies that have examined other markers of OS in subjects with AF. Atrial nicotinamide adenine dinucleotide phosphate oxidase activity has been shown to be associated with an increased risk of postoperative AF, while plasma markers of lipid oxidation did not predict postoperative AF.[16] Another study did not find an association between AF and OS measured by F2-isoprostanes.[17] This could be explained by the fact that F2-isoprostanes and glutathione measurements may reflect different oxidative pathways. Abnormal levels of F2-isoprostanes indicate increases in oxidation of lipids, which occurs in lipid bilayers. In contrast, E_hGSH represents the oxidation of thiols that are most likely to occur in the cell cytoplasm. Therefore, it is important to recognize that different oxidative markers may reflect different oxidation events.

Plasma aminothiols are reliable measures of systemic oxidative burden, with glutathione representing intracellular and the cysteine/cystine pools reflecting extracellular oxidative burden. Glutathione is the primary intracellular redox buffer and is critical in maintaining reduced intracellular environment by scavenging ROS. In addition, glutathione has other biological functions including nutrient metabolism and regulation of cellular events including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation.[18] Moreover, glutathione is a substrate or a cofactor for a number of protective enzymes, such as glutathione peroxidase, glutathione S-transferases, and the glyoxalase.[19–21] Several conditions including diabetes, obesity, and heart failure are associated with glutathione depletion.[22–24] Under conditions of increased oxidant production, intra-and extracellular glutathione becomes oxidized, causing a state of glutathione depletion that can impact the regulation of ion channel activity. Atrial redox state is a critical determinant of atrial ion channel activity including the L-type calcium channel, the sodium channel, the transient outward potassium current, and the ryanodine receptor.[25] Altered atrial redox balance due to glutathione depletion may result in a significant reduction in contractile response to b-adrenergic stimulation.[26] Left atrial glutathione content also has been shown to be reduced in animal and human subjects with AF as compared with those without AF.[25] Accumulating experimental evidence from animal studies support the concept that risk factors for AF such as hypertension and heart failure increase the production of ROS that leads to pathologic atrial structural remodeling.[27,28] We have shown previously that increased OS, measured as plasma cystine level, is associated with increased left atrial size.[29]

The correlation between CHA_2DS_2-VASc scores and OS markers serves as an indirect evidence that OS might be predictive of stroke risk and explain the association of risk factors with risk of atheroembolism in AF. Further studies are needed to determine the added value of OS markers to stroke risk stratification models as well as the clinical utility of using OS markers as potential surrogates for complications or risk.

Currently, there are no overarching data to indicate the protective effect of supplemental antioxidants on the primary or secondary prevention of AF. This may have been partially secondary to the use of nontargeted antioxidant compounds. Antioxidant vitamins (A, C, B complex, and vitamin E) have been investigated in the prevention and treatment of AF. *N*-Acetylcysteine, a glutathione precursor, has been shown to increase L-type calcium channel current and reverse AF-induced remodeling of ion channels.[25] Multiple small clinical trials showed a protective effect of antioxidants and *N*-acetyl-cysteine in patients undergoing bypass surgery, although the effectiveness of these agents remains to be evaluated and confirmed in large nonoperative AF prevention and treatment.[30–34]

Our study has important strengths including its prospective design, large sample size, long-term follow-up with event confirmation by medical record review and exploration of the interaction with inflammation assessed by hsCRP level. Despite the novel finding of an association of OS markers with AF, our results should be interpreted in the context of certain limitations. It is also possible that paroxysmal cases of AF were missed during follow-up because of their time-dependent nature. Moreover, the effect of the duration of AF on the strength of association between OS and AF were not evaluated in this study. We did not have detailed information on diet, alcohol, and supplement use, as this may have an impact on glutathione and cysteine levels. Aminothiols represent mechanism-based biomarkers of oxidation rather than direct measurement of oxidation. Finally, although our study was observational that cannot infer causality, our longitudinal data lend credibility to the notion that OS may be associated with the development of AF. Future studies are needed to investigate whether targeted antioxidant compounds can prevent AF.

Conclusion

Increased OS, measured as E_hGSH, was associated with a high risk of prevalent and incident AF. Whether reduction of OS will prevent AF needs to be investigated further.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline characteristics among patients with and without prevalent and incident AF

Characteristic	No AF (n = 1291)	AF (n = 148)	*p**	No incident AF (n = 813)	Incident AF (n = 104)	*p*†
Age (y)	62.7 ± 11.2	69.9 ± 10.1	**.0001**	62.5 ± 11.1	65.9 ± 10.9	**.001**
Male	848 (65.7)	111 (75)	**.02**	517 (63.6)	73 (70.2)	.19
Black	212 (16.4)	11 (7.4)	**.004**	125 (15.4)	13 (12.5)	.44
Body mass index (kg/m^2)	30.2 ± 6.3	29.6 ± 6.7	.11	29.9 ± 6.2	30.1 ± 6.3	.94
Estimated GFR (mL/(min,1.73 m^2))	74 ± 21.1	65.9 ± 21.4	**<.001**	74 ± 20.8	71.8 ± 19.4	.28
Smoking	879 (68.1)	95 (64.2)	.34	559 (68.8)	73 (70.2)	.77
Diabetes	434 (33.6)	38 (25.7)	.05	247 (30.4)	47 (45.2)	**.002**
Hypertension	881 (68.2)	114 (77)	**.03**	560 (68.9)	72 (69.2)	.94
Hypercholesterolemia	880 (68.3)	102 (68.9)	.87	555 (68.3)	73 (70.2)	.69
Acute myocardial infarction	131 (10.1)	15 (10.1)	1.00	15 (1.8)	0 (0)	.16
Obstructive CAD	879 (72.9)	109 (77.3)	.27	524 (69.1)	72 (74.2)	.30
History of stroke	98 (7.6)	23 (15.6)	**.001**	59 (7.3)	16 (15.4)	**.004**
History of heart failure	343 (26.6)	62 (41.9)	**<.001**	189 (23.2)	32 (30.8)	.09
Ejection fraction (%)	54 ± 11.5	49.2 ± 13.4	**<.001**	55.2 ± 10.5	53.4 ± 12.3	.24
ACE-I/ARB use	785 (60.8)	99 (66.9)	.15	474 (58.3)	69 (66.3)	.12
Aspirin use	1004 (77.8)	118 (79.7)	.59	613 (75.4)	90 (86.5)	**.01**
Plavix use	702 (54.4)	67 (45.3)	**.04**	410 (50.4)	62 (59.6)	.08
Statin use	950 (73.6)	109 (73.6)	.99	572 (70.4)	89 (85.6)	**.001**
β-Blocker use	848 (65.7)	103 (69.6)	.34	505 (62.1)	84 (80.8)	**.0001**
Cystine level (μmol/L)	97.1 (83.1 to 114.9)	103.3 (87 to 119.5)	**.01**	96 (82.9 to 112.9)	97.6 (85.6 to 113.4)	.54
Cysteine level (μmol/L)	12.2 (10.1 to 14.6)	12 (9.9 to 15)	.85	12.1 (10.1 to 14.5)	11.6 (9.9 to 14.4)	.52
Glutathione level (μmol/L)	1.2 (0.9 to 1.5)	1.1 (0.9 to 1.4)	.09	1.2 (0.9 to 1.5)	1.2 (0.9 to 1.4)	.35
Glutathione disulfide level (μmol/L)	0.02 (0.01 to 0.03)	0.03 (0.04 to 0.06)	.11	0.02 (0.02 to 0.03)	0.02 (0.02 to 0.04)	.06
Cystine/glutathione ratio	83.0 (60.9 to 112.8)	95.4 (70.3 to 125.6)	**.007**	1.5 (1.1 to 1.9)	1.7 (1.2 to 2.1)	.07
E_hGSH (mV)	**−138.2 (−144.4 to −131.1)**	**−134.6 (−142.9** to **−127)**	**.003**	**−138.5 (−144.9** to **−131.2)**	**−136.1 (−142.9** to **−128.8)**	**.01**
E_hCyS (mV)	−75.6 (−80.2 to −70.5)	−74.2 (−79.4 to −70.2)	.2	−75.8 (−79.9 to −70.6)	−74.1 (−80.6 to −70.8)	.31
High-sensitivity C-reactive protein level (mg/L)	1.4 (0.7 to 3.6)	1.3 (0.8 to 3.8)	.9	1.4 (0.7 to 3.9)	1.1 (0.6 to 1.6)	.26

Values are presented as mean ± SD, as n (%), or as median (interquartile range).

ACE-I = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin II receptor blocker; CAD = coronary artery disease; E_hCyS = redox potential of cysteine; E_hGSH = redox potential of glutathione; GFR = glomerular filtration rate.

*P <.05 for the comparison of AF vs no AF.

†P<.05 for the comparison of incident AF vs no incident AF.

Table 2
Multivariate logistic regression for predictors of prevalent AF

		95% CI		*P*
Variable	**OR**	**Lower**	**Upper**	
Age (10-y increase)	**2.2**	**1.7**	**2.9**	**<.001**
Male	**1.9**	**1.1**	**3.1**	**.02**
Black	0.5	0.2	1.0	.06
Body mass index (5-unit increase)	1.1	0.9	1.3	.53
Smoking	0.7	0.4	1.0	.07
Diabetes	**0.6**	**0.3**	**0.9**	**.02**
Hypertension	1.4	0.8	2.3	.22
Hyperlipidemia	1.0	0.6	1.6	1.00
Estimated GFR	1.0	0.9	1.2	.74
Coronary artery disease	0.8	0.4	1.3	.29
Heart failure history	**1.9**	**1.2**	**2.9**	**.01**
High-sensitivity C-reactive protein level (log)	1.1	1.0	1.3	.15
E_hGSH (log, 10% increase)	**1.3**	**1.1**	**1.7**	**.02**

CI = confidence interval; OR = odds ratio. Other abbreviations as in Table 1.

Table 3

Levels of oxidative stress markers (μmol/L) in patients with CHA_2DS_2-VASc scores

CHA_2DS_2-VASc score	**Cystine**	***P***	**Cysteine**	***P***
<2	87.57 (74.18 to 98.26)	<.001	11.54 (9.89 to 13.43)	.6
2–4	101.22 (86.96 to 116.07)		12.24 (9.92 to 14.89)	
>4	109.43 (92.19 to 136.95)		11.44 (9.89 to 13.96)	
CHA_2DS_2-VASc score	Glutathione	*P*	Glutathione disulfide	*P*
<2	1.3 (1.05 to 1.66)	.008	0.03 (0.02 to 0.04)	.01
2–4	1.11 (0.89 to 1.39)		0.02 (0.01 to 0.04)	
4	1.04 (0.83 to 1.38)		0.03 (0.02 to 0.05)	
CHA_2DS_2-VASc score	E_hGSH	*P*	E_hCyS	*P*
<2	−138.18 (−142.85 to −133.4)	.001	−74.73 (−80.3 to −71.5)	.11
2–4	−136 (−143.19 to −128.87)		−74.7 (−80.02 to −70.36)	
>4	−129.06 (−136.63 to −121.29)		−71.96 (−77.74 to −68.1)	

Values are presented as median (interquartile range). *P* values are for comparison.

Abbreviations as in Table 1.

Table 4
Cox regression model for predictors of incident AF

	HR	95% CI		*P*
Variable		**Lower**	**Upper**	
Age (10-y increase)	**1.3**	**1.03**	**1.6**	**.03**
Male	1.4	0.9	2.1	.19
Black	0.7	0.4	1.4	.36
Body mass index (5-unit increase)	0.9	0.8	1.1	.41
Smoking	1.2	0.8	1.9	.40
Diabetes	**1.7**	**1.1**	**2.5**	**.01**
Hypertension	0.9	0.6	1.4	.62
Hyperlipidemia	0.8	0.5	1.3	.41
Estimated GFR	1.0	1.0	1.0	.81
β-Blocker use	**1.8**	**1.1**	**2.9**	**.02**
Coronary artery disease	1.0	0.6	1.7	.91
Heart failure	**1.6**	**1.03**	**2.5**	**.04**
High-sensitivity C-reactive protein level (log)	1.0	0.8	1.1	.78
E_hGSH (log, 10% increase)	**1.3**	**1.1**	**1.7**	**.01**

CI = confidence interval; HR = hazard ratio. Other abbreviations as in Table 1.