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Serum selenium and prognosis in cardiovascular disease: results from the AtheroGene study

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Abstract

Objective—Experimental data suggest a protective role of the essential trace element selenium against cardiovascular disease (CVD), whereas epidemiological data remains controversial. We aimed to investigate the impact of serum selenium concentration in patients presenting with stable angina pectoris (SAP) or acute coronary syndrome (ACS) on long term prognosis.

Methods—Baseline selenium concentration was measured in 1731 individuals (852 with SAP, and 879 with ACS). During a median follow-up of 6.1 years, 190 individuals died from cardiovascular causes.

Results—In those ACS patients who subsequently died of cardiac causes, selenium levels were lower compared to survivors (61.0 µg/L versus 71.5 µg/L; $P < 0.0001$). In a fully adjusted model, patients in the highest tertile of selenium concentration had a hazard ratio of 0.38 (95% CI: 0.16–0.91; $P = 0.03$) as compared with those in the lowest. No association between selenium levels and cardiovascular outcome was observed in SAP.

Conclusions—Low selenium concentration was associated with future cardiovascular death in patients with ACS.

Keywords

Selenium; Stable angina pectoris; Acute coronary syndrome; Prognosis

1. Introduction

Ischemia and reperfusion (I/R) injury contribute to the size of myocardial necrosis, and reactive oxygen species (ROS), produced during ischemia, damage the stunned myocardium or the blood vessels themselves [1]. In animal experiments, selenium supplementation was

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used to reduce the production of ROS during I/R injury by increasing the activity of glutathione peroxidase (GPx)-1 [2]. Selenium is a trace element involved in protection against oxidative damage, and functions as a key determinant of selenoprotein synthesis and activity, such as the GPx [3]. The selenium bioavailability is a limiting factor in the synthesis of many of the antioxidant selenoproteins. A deficiency of GPx-1 activity has been related to the development of atherosclerosis in experimental studies and to adverse prognosis in patients with coronary artery disease (CAD) [4].

The association between blood selenium concentration and prevalence of CAD has been described controversially [5]. Despite initial prospective reports demonstrating an association between low selenium concentrations and incident cardiovascular events in a population-based setting [6] subsequent prospective studies evaluating the impact of blood or tissue selenium concentration on incident CAD, however, revealed conflicting data [7]. In addition, progression of atherosclerosis in general does not appear to be modified by selenium supplementation [8]. For these reasons, there is currently no general recommendation for selenium use for CAD prevention [7]. Especially the conflicting data concerning the narrow range of selenium between 70 and 90 ng/mL with beneficial use for prevention of CAD and the also reported adverse events [9,10] need further studies to investigate long term selenium supplementation. Further, the initial selenium supplementation has to be taken into account, as studies in selenium-replete populations report no beneficial effect of additional selenium supplementation in adults, when the 90 ng/mL are exceeded [11].

In acute coronary syndrome (ACS), oxidative stress constitutes an integral part of plaque rupture and platelet activation, as well as the extent and consequences of I/R injury. In animal models, pre-ischemic selenium status is a major determinant of outcome after myocardial ischemia *in vivo* [12]. In rats a decline in selenium concentrations correlated with a decrease in antioxidant capacity, aggravating the manifestations of experimental myocardial infarction [13]. Initial evaluations demonstrate that the prevalence of myocardial infarction is accompanied by depletion of total serum selenium [14]. Thus far, no prospective data are available to assess the impact of selenium concentration on cardiovascular outcome in ACS.

We evaluated the impact of serum selenium concentration in patients presenting with stable angina pectoris (SAP) or ACS on long term prognosis. In particular, we hypothesized that selenium deficiency in ACS patients leads to an adverse outcome; either based on increased risk of recurrent plaque rupture or enhanced I/R injury.

2. Methods

2.1. Study participants

Between June 1999 and March 2000, 1731 patients who presented with chest pain to the Department of Medicine II of the Johannes Gutenberg-University Mainz or the Federal Armed Forces Central Hospital, Koblenz, and who had at least one stenosis >30% in a major coronary artery were enrolled in the AtheroGene study registry. Exclusion criteria are described in the supplement and elsewhere [4]. Patients who had received antihypertensive treatment or who had a diagnosis of hypertension 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). Patients being on lipid lowering medication or with a previous diagnosis of hyperlipidemia or current total cholesterol above 240 mg/dL had been considered as hyperlipidemic.

ACS patients presented with ST-elevation myocardial infarction (STEMI), non-STEMI (NSTEMI), and unstable angina. The latter was defined according to the Braunwald classification (classes I–IIIb). Ejection fraction was measured during cardiac angiography in 1344 patients. Since selenium is eliminated through renal excretion, renal function was assessed by calculation of the creatinine-clearance according to the Cockcroft–Gault formula ($[(140 - \text{age}) \times \text{weight}/72 \times \text{serum creatinine concentration}]$). The result is modified with the factor 0.85 in females.

1724 of 1731 patients (99.6%) were followed for a median of 6.1 years (maximum, 7.6 years). During follow-up, death from cardiovascular causes was confirmed in 190 patients and death from causes not related to heart disease in 74 patients. The survival data was censored at the end of the follow-up time. The endpoint of this study was cardiovascular death. Follow-up was conducted by questionnaires through scientific assistants. The study was approved by the local ethics committees. Participation was voluntary, and each subject gave written, informed consent.

2.2. Laboratory methods

Blood sampling was performed immediately after admission to hospital before coronary angiography was performed. The time between chest pain onset and clinical admission when blood sampling was performed is indicated as “chest pain onset time” (Table 1). Serum was immediately prepared from whole blood and stored at -80°C until analysis. Serum selenium concentrations were determined by a Spectra AA 220 Z (Varian) with carbon-furnace atomic-absorption spectrometry and Zeeman compensation [15]. Additional information is available in the supplement. Lipid serum concentrations were measured immediately by standardized methods. Troponin I concentration was determined using an immunofluorescence test on the ACCESS System (Beckman Coulter, Fullerton, California). C-reactive protein (CRP) was determined using a particle enhanced turbidimetric immunoassay (PETIA) format with the Dimension[®] Chemistry System (detection range of 0.05–25.0 mg/L, Dade Behring, Newark, USA).

2.3. Statistical analysis

Continuous variables are presented as median and 25th and 75th percentile if skewed distributed and analysed with Mann–Whitney *U*-test. If normally distributed, continuous variables are presented as mean and standard deviation and tested with Chi-Square test. Discrete variables are described through relative frequencies per category and tested with Student’s *t*-test. To test for bivariate correlation, Spearman’s correlation coefficient was calculated. Analyses were performed according to stratification by SAP and ACS patients. The survival curves were calculated according to the Kaplan–Meier method. The log-rank test was used to evaluate statistically significant differences.

To compare the prognostic value of baseline selenium concentrations on the primary endpoint of cardiovascular death and secondary endpoint of non-cardiovascular death or cardiovascular mortality and non-fatal myocardial infarction, respective concentrations were entered in a proportional hazard regression model as a continuous variable and as a categorical variable by increasing tertiles of selenium concentration. In the first model, adjustment was performed for age and sex; the second model was additionally adjusted for BMI, diabetes mellitus, smoking, hypertension, family history, β -blocker, ACE-inhibitor and statin use, number of diseased vessels, concentrations of triglycerides, LDL–HDL ratio, glucose, troponin I (ACS subgroup) concentrations and creatinine-clearance. The fully adjusted model included left ventricular ejection fraction.

To assess whether selenium concentration adds prognostic information beyond established risk factors, receiver operator characteristics (ROC) curves were calculated and area-under-the-curve (AUC) analyses were performed (supplement).

The hazard ratios (HR) and their 95% CI are reported. The *P*-values are two-sided; a *P*-value of less than 0.05 was considered statistically significant. All analyses were not adjusted for multiple testing; therefore results have to be regarded as descriptive. Analyses were performed using SPSS 12.0 (Chicago, IL, USA), and MedCalc for Windows, version 9.3.2.0 (Mariakerke, Belgium).

3. Results

Baseline characteristics of the study population are presented in Table 1. In the ACS group, patients with cardiovascular death had higher concentrations of troponin I and had lower selenium concentrations. No association was observed between selenium concentration and chest pain onset time (Table 1).

The distribution of risk factors between SAP and ACS patients and their association with selenium concentration are displayed in Table 2. None of the risk factors was associated with serum selenium concentration; however, lower selenium concentrations were apparent in nearly all subgroups of ACS patients (Table 2, Panel B). The highest correlation coefficient, indicating a very modest inverse correlation, was observed between age and selenium concentration ($r = -0.13$). Further, regarding markers of inflammation, selenium had a slight inverse correlation with CRP in both cohorts ($r = -0.12$). The time from chest pain onset to admission had neither an additional effect regarding mortality nor any influence on selenium concentration.

Fig. 1 displays the Kaplan–Meier survival curves according to tertiles of baseline selenium concentrations in patients with SAP (Fig. 1A) and ACS (Fig. 1B). The unadjusted survival decreased in a stepwise fashion across decreasing tertiles (P log-rank < 0.0001) in the ACS cohort, whereas only a trend could be observed among patients presenting with SAP.

To assess the independent strength of selenium for cardiovascular risk prediction in comparison with known cardiovascular risk factors, three Cox regression models were applied introducing selenium as categorical or continuous variable (Table 3). In ACS, increasing levels of selenium were associated with decreased risk for cardiovascular death; the age- and sex-adjusted hazard ratio revealed a 0.42-fold (95% CI: 0.24–0.72; $P = 0.002$) decrease in risk of cardiovascular mortality for the highest compared to the lowest tertile of selenium, respectively, and a 0.73-fold (95% CI: 0.60–0.90; $P = 0.002$) decrease of risk for an increase of one standard deviation. After adjustment for most potential confounders as well as left ventricular ejection fraction, this association remained independently significant (Table 3). No significant association with cardiovascular mortality was seen in SAP patients, irrespective of whether selenium concentration was included in the analysis as a categorical or continuous variable. In the conducted analyses, no significant association with non-cardiovascular mortality or cardiovascular mortality and non-fatal myocardial infarction was observed irrespective of whether selenium concentration was included as a continuous or categorical variable in three Cox regression models (data not shown).

All variables introduced into model 2 of the Cox regression analysis were included in the baseline model for the ROC analysis. In comparison to this baseline model, the second model additionally included serum selenium concentration as a continuous or categorical variable (Supplementary Table 1). In SAP patients, calculation of the *c*-statistic showed no statistically significant influence of serum selenium concentration, irrespective of whether selenium was included as a continuous or categorical variable in addition to the baseline

model. In ACS patients, inclusion of selenium in the model with the best predictive value increased the *c*-statistic from 0.85 to 0.86, again revealing no statistically significant difference.

4. Discussion

In this prospective cohort of CAD patients, baseline serum selenium concentration of individuals presenting with ACS was inversely associated with cardiovascular mortality, independent of classical risk factors, left ventricular ejection fraction, multivessel coronary disease, and biomarkers of necrosis. In contrast to ACS, serum selenium was not significantly associated with prognosis in chronic ischemic heart disease.

Several animal-based experimental studies investigating the effect of selenium administration in cardiac I/R injury prove benefits of selenium supplementation [12,16,17]. Selenium supplementation during I/R increased plasma activity of GPx-1, improved post-ischemic mean arterial pressure, and reduced infarct size in rats [12,17]. In humans admitted to cardiac surgery, supplementation of selenium during I/R time improved GPx-1 gene expression and activity and, thereby, protected against free radical-induced myocardial damage [18]. Administration of selenium induced a significant reduction in the severity of reperfusion arrhythmias and irreversible ventricular fibrillation [16,17]. The protective effect of selenium against arrhythmogenesis is seen in the reduction of oxidative stress, decreased connexin-43 dephosphorylation, and, as a result reduced electrical uncoupling at gap junctions [16]. This protective effect against I/R injury was also reported in the liver and the brain, showing reduced edema and microglial cell infiltration, and, possibly, salvaging the cells of the ischemic penumbra by reducing ROS flux [19,20].

In addition to these effects, selenium may affect inflammatory pathways crucial to atherosclerosis and ACS. Low selenium concentrations lead to an aggravation of the acute phase response in patients *in vivo*. This phenomenon has been attributed to activation of nuclear factor kappaB, which regulates inflammatory cytokine-encoding genes and is inhibited by physiological selenium concentrations [21]. In general, selenium is altered as part of the acute phase response [22]. In this study cohort, selenium had a slight inverse correlation with C-reactive protein, as sensitive inflammatory marker, in the patients with ACS and also the patients with SAP, suggesting an inhibitory effect of selenium on inflammatory pathways. In addition, elevation of selenium levels in ACS patients taking statin therapy has been reported, which might in part be explained by the antioxidant benefit of statins on selenium oxidation state and bioavailability in this condition. Another mechanism by which selenium affects atherosclerosis is its effect on arachidonic acid metabolism. In animal experiments, selenium deficiency increases the production of proaggregatory thromboxane B₂, while biosynthesis of antiaggregatory prostacyclin is impaired [23]; thereby promoting a prothrombotic microenvironment.

From a clinical perspective, initial data suggested an association of low selenium levels with cardiovascular outcome in a population-based setting [6]. Subsequent studies addressing the association of whole body selenium content with cardiovascular outcome, however, revealed conflicting results. Nevertheless, a recent meta-analysis showed a 24% reduction in CAD risk associated with a 50% increase of selenium concentration [7]. In this context, selenium supplementation did not prove beneficial in population studies, including apparently healthy individuals [24]. Other consistent studies demonstrate reduced total body selenium content during the acute phase of myocardial injury [14,25]. Current data confirm this observation and most importantly extend the analysis for the first time to a prospective setting showing that low selenium levels are associated with adverse outcome in ACS patients. Overall, blood selenium concentrations mirror trace element concentrations of a certain time span,

not only during the acute event; therefore, it is highly likely that low selenium status was present before the clinical event. This low selenium concentration, although amplified by the ACS itself, may thus be of etiologic relevance [25].

Supplementation with selenium in a population with low baseline concentrations of selenium increases plasma selenium concentration significantly, as well as GPx-1 activity in erythrocytes, platelets, and other blood cells [26]. In individuals with average selenium concentration of 80–140 μ the increase of GPx-1 activity and serum selenium may be modest with supplementation [27] as the effect of selenium on serum selenium concentration plateaus [4].

Thus, whether or not selenium supplementation is associated with cardiovascular outcome might be influenced by the baseline selenium status of the population tested. The important, as yet unresolved, question arising in this context is whether patients suffering from ACS can benefit from selenium supplementation. For further studies however, the narrow therapeutic range of selenium concentration has to be taken into account. In studies from the United States with a selenium-replete population, the number of patients with type 2 diabetes and increasing serum cholesterol was reported higher under supplementation with selenium [9,10]. However, this was not reported from the SETCAP trial [28]. In this study, we could not report an adverse effect regarding the supplementation with sodium selenite for 1 year, in addition, the number of patients with diabetes mellitus type 2 and the concentration of serum cholesterol was not statistically different. Thus, the data is still conflicting and further studies should be conducted in this field of research.

5. Limitations

First, we only performed baseline selenium measurement and, therefore, cannot clarify the variability of selenium during the course of the study nor the relation of this variability to myocardial injury. Second, measurement of all biomarkers was conducted on samples that were initially stored frozen; however, this should not affect the validity of our results because all samples were handled identically. Third, all patients included in the study suffered from manifest CAD, which limits the generalizability of the results. Fourth, it is known that selenium concentration shows a high variability in different persons, ethnicity, between certain compartments and different blood cells. In this study, the serum selenium concentration was determined as marker for whole body selenium concentration [27]. Finally, analyses using the *c*-statistic is based only on ranks and, therefore, less sensitive than likelihood or other measures of global risk assessment [29]. Inclusion of novel risk factors in risk prediction models could lead to better risk assessment despite little change in the *c*-statistic [29].

In conclusion, the present study data indicate that low serum selenium concentration is independently related to future cardiovascular mortality in patients with ACS. Information on selenium status may help to assess the risk of adverse outcome in ACS patients. Whether selenium supplementation becomes a target for prevention and therapy of ACS will be the aim of future investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.atherosclerosis.2009.09.008.

Fig. 1.

Kaplan–Meier curves for the proportional survival rate according to increasing tertiles of selenium concentrations for patients with stable angina pectoris (Panel A), acute coronary syndrome (Panel B) and cardiovascular mortality.

Baseline characteristics of the study population.

Characteristics		Stable angina pectoris		Acute coronary syndrome		
	Patients without CV mortality	Patients with CV mortality	P-Value	Patients without CV mortality	Patients with CV mortality	P-Value
No. of patients, <i>n</i> (%)	752 (88.6)	97 (11.4)	–	782 (89.4)	93 (10.6)	–
Age, years	61.3 ± 9.4	65.8 ± 9.2	<0.001	60.8 ± 10.7	67.6 ± 8.0	<0.001
Sex, male (%)	76.9	71.1	0.21	72.5	72.0	0.93
Classical risk factors						
BMI(kg·m ²)	27.3 ± 3.7	27 ± 4.4	0.46	27.4 ± 3.7	26.9 ± 3.9	0.26
Hypertension (%)	75.0	82.5	0.11	66.5	77.4	0.03
Family history (%)	38.4	33	0.30	38.1	32.3	0.27
Diabetes (%)	22.2	36.1	0.003	19.3	34.4	0.001
Dietary measures	5.6	5.2	0.86	5.0	2.2	0.22
Oral medication	8.5	11.3	0.36	7.5	14.0	0.03
Insulin dependent	8.1	19.6	<0.001	6.8	18.3	<0.001
Currently smoking (%)	35.6	37.1	0.78	29.9	37.6	0.13
Hyperlipidemia (%)	70.5	53.6	0.001	61.1	58.1	0.57
Lipid variables						
Cholesterol (mg/dL)	216.6 ± 44.1	218.2 ± 47.8	0.75	211.8 ± 46.3	208.9 ± 48.5	0.59
LDL-cholesterol (mg/dL)	136.8 ± 39.4	140.4 ± 40	0.40	134.7 ± 40.9	130.5 ± 38.5	0.33
HDL-cholesterol (mg/dL)	46 (38/57)	43 (36/56)	0.06	45 (39/55)	45 (37/54.5)	0.27
Triglycerides (mg/dL)	140 (103/194.8)	152 (106.5/196)	0.32	135 (98.8/187)	153 (104/211)	0.08
Clinical variables						
L V-ejection fraction (%)	65.6 ± 14	53 ± 19	<0.001	60.7 ± 13.8	49 ± 17.3	<0.001
Multivessel disease (>1 vessel) (%)	71.9	74.2	0.64	74.2	92.5	<0.001
Creatinine-clearance (Cockcroft–Gault formula) (ml/min)	92.06 ± 30.04	94.52 ± 35.36	0.67	85.92 ± 28.67	90.94 ± 36.27	0.49
Medication						
Statin (%)	45.1	35.1	0.06	34.3	32.3	0.70
Beta-blocker (%)	56.9	38.1	<0.001	68.5	57	0.03
ACE-inhibitor (%)	49.5	68.0	0.001	49.1	65.6	0.003

Characteristics	Stable angina pectoris		Acute coronary syndrome	
	Patients without CV mortality	Patients with CV mortality	Patients without CV mortality	Patients with CV mortality
<i>Laboratory markers</i>				
Selenium (µg/L)	74.8 ± 28.1	73 ± 28.1	71.5 ± 22.3	61.0 ± 22.5
Troponin I (µg/L)	–	–	0.3 (0.01/2.7)	0.6 (0.1/6.8)
C-reactive protein (mg/dL)	1.83 (0.93/4.02)	3.39 (1.47/6.95)	4.47 (1.65/11.88)	13.6 (5.62/29.75)
<i>Relation of chest pain onset time to selenium concentration (only ACS cohort)</i>				
Time (h)	0–3	3–6	12–24	>24
Selenium (µg/L)	77.9	68.5	72.5	74.2
				0.98 ^a

See section statistical analysis.

^a *P*-Value was calculated with the Kruskal–Wallis test.

Table 2

Mean concentration of selenium according to categorical risk factor distribution and clinical presentation.

Panel A			
Parameters	Stable angina pectoris Selenium concentration (µg/L)	Acute coronary syndrome Selenium concentration (µg/L)	
<i>Arterial hypertension</i>			
Yes	75.3 ± 27.6	70.3 ± 22.2	
No	72.5 ± 29.8	70.7 ± 23.3	
<i>P</i> -Value	0.24	0.81	
<i>Diabetes mellitus</i>			
Yes	76.0 ± 28.4	72.2 ± 22.6	
No	74.2 ± 28.1	70.0 ± 22.5	
<i>P</i> -Value	0.46	0.23	
<i>Currently smoking</i>			
Yes	72.9 ± 28.5	69.9 ± 22.4	
No	75.8 ± 27.8	70.7 ± 22.2	
<i>P</i> -Value	0.14	0.60	
<i>Family history</i>			
Yes	75.8 ± 29.1	70.6 ± 23.3	
No	73.9 ± 27.5	70.4 ± 21.1	
<i>P</i> -Value	0.35	0.89	
<i>BMI</i>			
<30	74.3 ± 28.9	70.5 ± 22.8	
>30	75.7 ± 25.3	70.2 ± 21.7	
<i>P</i> -Value	0.52	0.86	
<i>Statin therapy</i>			
Yes	73.8 ± 26.5	72.7 ± 21.3	
No	75.3 ± 29.4	69.3 ± 23.1	
<i>P</i> -Value	0.46	0.03	
<i>β-Blocker therapy</i>			
Yes	73.1 ± 26.9	70.9 ± 22.4	
No	76.5 ± 29.5	69.5 ± 22.8	
<i>P</i> -Value	0.08	0.43	

Panel B Spearman correlation coefficients: selenium concentration and continuous risk factors

	Selenium			
	ACS	<i>P</i> -Value	SAP	<i>P</i> -Value
Age	−0.13	<0.0005	−0.001	0.98
Body mass index	0.02	0.67	0.07	0.05
Sex	0.05	0.05	0.003	0.93
LDL–HDL ratio	0.01	0.89	0.005	0.89
Glucose	0.07	0.04	0.06	0.10
Triglycerides	0.04	0.26	0.02	0.53
Troponin I	−0.04	0.33	–	–

Panel B Spearman correlation coefficients: selenium concentration and continuous risk factors

	Selenium			
	ACS	<i>P</i> -Value	SAP	<i>P</i> -Value
Creatinine-clearance (Cockcroft–Gault)	0.07	0.03	0.03	0.32
Chest pain onset time	−0.04	0.33	–	–
C-reactive protein	−0.12	0.01	−0.12	0.05

Table 3

Hazard ratios for primary outcome to tertiles and increment of one standard deviation of selenium concentration in the AtheroGene study participants.

Pattern A		Stable angina pectoris		
Variable		Primary outcome (cardiovascular death)		
	Cardiovascular events	HR (95% CI) Age and sex adjusted	HR (95% CI) Model 2	HR (95% CI) Fully adjusted
Tertiles of selenium concentration (µg/L)				
<64.0 (<i>n</i> = 282)	33 (11.7%)	1.00	1.00	1.00
64.0–84.4 (<i>n</i> = 286)	35 (12.2%)	0.94 (0.58–1.52)	0.89 (0.54–1.45)	0.67 (0.38–1.17)
>84.4 (<i>n</i> = 284)	29 (10.7%)	0.82 (0.50–1.32)	0.81 (0.49–1.34)	0.63 (0.37–1.10)
<i>P</i> -Value ^a	0.66 ^b	0.41	0.42	0.10
Continuous variable (increment SD)		0.93 (0.77–1.13)	0.90 (0.74–1.11)	0.83 (0.66–1.06)
<i>P</i> -Value ^c		0.49	0.33	0.13
Pattern B		Acute coronary syndrome		
Variable		Primary outcome (cardiovascular death)		
	Cardiovascular events	HR (95% CI) Age and sex adjusted	HR (95% CI) Model 2	HR (95% CI) Fully adjusted
Tertiles of selenium concentration (µg/L)				
<61.6 (<i>n</i> = 294)	49 (16.8%)	1.00	1.00	1.00
61.6–80.0 (<i>n</i> = 294)	27 (9.2%)	0.69 (0.43–1.11)	0.68 (0.38–1.19)	1.07 (0.50–2.30)
>80.0 (<i>n</i> = 291)	17 (5.8%)	0.42 (0.24–0.72)	0.47 (0.25–0.89)	0.38 (0.16–0.91)
<i>P</i> -Value ^a	<0.001 ^b	0.002	0.02	0.03
Continuous variable (increment SD)		0.73 (0.60–0.90)	0.76 (0.61–0.96)	0.72 (0.54–0.96)
<i>P</i> -Value ^c		0.002	0.02	0.03

See section statistical analysis for candidate covariates for stepwise selection.

^a *P*-Value was calculated introducing selenium as categorical variable (increasing tertiles).

^b *P*-Value was calculated with the Chi-Square test.

^c *P*-Value was calculated introducing selenium as continuous variable (corrected for 1 SD).