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Selenium supplementation improves antioxidant capacity in vitro and in vivo in patients with coronary artery disease: The SElenium Therapy in Coronary Artery disease Patients (SETCAP) Study

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Abstract

Background—Selenium is a central determinant of antioxidative glutathione peroxidase 1 (GPx-1) expression and activity. The relevance of selenium supplementation on GPx-1 in coronary artery disease (CAD) needs to be established. We assessed the effect of selenium supplementation on GPx-1 in cell culture and on endothelial function in a prospective clinical trial.

Methods—Human coronary artery endothelial cells were incubated with 5.78 to 578 nmol/L sodium selenite, Se-methyl-selenocysteine hydrochloride, or seleno-L-methionine. Glutathione peroxidase 1 mRNA and protein expression and activity were measured. Coronary artery disease patients (n = 465) with impaired endothelial function (flow-mediated dilation [FMD] <8%) were randomly assigned to receive 200 or 500 µg sodium selenite daily or matching placebo during a 12-week period. We tested the effect on red blood cell GPx-1 activity and brachial artery FMD. Furthermore, differences in biomarkers of oxidative stress and inflammation were measured.

Results—Sodium selenite and Se-methyl-selenocysteine hydrochloride increased GPx-1 protein and activity in a dose-dependent manner ($P < .0001$). The intention-to-treat groups comprised 433 CAD patients. Glutathione peroxidase 1 activity increased from 37.0 U/gHb (31.3–41.7) to 41.1 U/gHb (35.2–48.4) ($P < .0001$) in the 200 µg and from 38.1 U/gHb (33.2–43.8) to 42.6 U/gHb (35.0–49.1) ($P < .0001$) in the 500 µg sodium selenite group treated for 12-weeks. No relevant changes were observed for FMD or biomarkers of oxidative stress and inflammation.

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Conclusions—Sodium selenite supplementation increases GPx-1 activity in endothelial cells and in CAD patients. Future studies have to demonstrate whether long-term CAD outcome can be improved.

Oxidative stress is central to coronary artery disease (CAD). Glutathione peroxidases (GPx's) constitute the major antioxidant defense system^{1,2} and GPx-1 is one of the most abundant isoforms within eukaryotic cells.³ In vivo studies in knockout and transgenic mice have found that GPx-1 can modulate vascular function.^{3,4}

In this context, GPx-1-deficient mice are significantly more susceptible to oxidative challenge^{5,6} and have endothelial dysfunction and abnormal cardiac function after ischemia/reperfusion injury.⁷ Other studies suggest that increased expression of GPx-1 in transgenic mice decreases tissue damage after cerebral and myocardial ischemia/reperfusion.⁸

In clinical studies, high activity of GPx-1 protects from future cardiovascular events and modifies risk associated with conditions of heightened oxidative stress, such as smoking⁹ or elevated homocysteine levels.¹⁰ Consequently, increasing GPx-1 activity constitutes an attractive approach for cardiovascular risk modification.

Selenium is a key component of GPx-1 required for optimal enzyme activity. In Europe, the bioavailability of selenium is generally low.^{11,12} An inverse relationship between selenium concentration and incident cardiovascular disease and mortality was observed.^{13–16} In patients with acute coronary syndrome and ischemic cardiomyopathy, selenium concentrations are transiently decreased.^{17,18}

However, epidemiologic data supporting an association between circulating selenium levels and cardiovascular risk are inconsistent to date, and whether selenium supplementation promotes cardiovascular risk protection remains controversial.¹⁹

The pathways by which selenium may exert its potential protective effects in vivo are manifold. A direct link to GPx-1 suppression or activation, however, has not yet been established. Using a translational approach, we performed cell culture experiments to study GPx-1 expression and activity in human coronary artery endothelial cells (HCAEC) treated with different sources of selenium and also prospectively investigated the effect of sodium selenite supplementation on GPx-1 activity and endothelial function in patients with CAD.

Methods

Cell culture and cell treatment

Human coronary artery endothelial cells were maintained in endothelial basal medium supplemented with the endothelial basal medium–bullet kit (Lonza, Walkersville, MD) and used up to passage 10. Near-confluent HCAEC were incubated with 5.78 to 578 nmol/L sodium selenite, Se-methyl-selenocysteine hydrochloride, or seleno-L-methionine (Sigma-Aldrich, St. Louis, MO) for 4 days in complete medium. RNA was isolated (Qiagen Sciences, Germantown, MD), complementary DNA synthesized (Clontech, Mountain View, CA), and human GPx-1 transcript analyzed using quantitative reverse-transcription-polymerase chain reaction. Glutathione peroxidase 1 protein levels were determined by Western blot analysis and GPx-1 activity by an indirect assay as described elsewhere.²⁰ Laboratory details on the experiments are provided in Appendix A.

Clinical trial design

The double-blind, placebo-controlled, randomized, single center, 3-armed, clinical phase II trial Selenium Therapy in Coronary Artery Disease Patients (SETCAP) evaluated the effect

of a 12-week 200 or 500 µg sodium selenite supplementation against placebo on noninvasive measurement of endothelial function and GPx-1 activity in patients with stable CAD. The study protocol is outlined in Figure 1, and detailed inclusion/exclusion criteria are provided in Table I. In brief, the treatment was scheduled to last 12 ± 1 weeks. Patients were invited for a control visit after 6 ± 1 weeks. Data on potential side effects were documented and compliance was assessed. The study was approved by the local ethics committee of the University of Mainz (Mainz, Germany). Participation was voluntary; patients were enrolled after written informed consent was obtained.

Laboratory methods

Blood samples were drawn under standardized conditions after a minimum of 12 hours fast. Blood samples were obtained at the screening visit, at 6 ± 1 weeks (selenium [Biosyn Laboratories, Arzneimittel GmbH, Fellbach, Germany], GPx-1 [Randox Laboratories Ltd., Crumlin Co., Antrim, United Kingdom], and the safety parameters), and at 12 ± 1 weeks. Safety markers (creatinine, liver enzymes) and biomarkers for secondary analyses (blood lipids, C-reactive protein [CRP], fibrinogen) were measured by routine methods or by commercially available assays (Cu/Zn superoxide-dismutase, immunoluminometric assay, (BRAHMS [Hennigsdorf, Germany]), and 8-isoprostanes, competitive enzyme immunoassay; Cayman Chemical, Tallinn, Estonia).

Noninvasive endothelial function testing comprising flow-mediated dilation (FMD) and nitro-mediated dilation of the brachial artery as well as echocardiographic studies were performed at enrollment and after 12 ± 1 weeks as described previously.²¹ Additional information is provided in Appendix A.

Statistical considerations

Continuous variables were described using median and first and third quartiles, and categorical variables using absolute and relative frequencies. To investigate the change in continuous variables during the study, differences were calculated as the value at the last visit (12 weeks) minus the value at the baseline visit. Statistical details are provided in Appendix A.

There were 3 hierarchically ordered null hypotheses. Null hypothesis 1 was that there is no difference in the change of FMD between the group receiving 500 µg sodium selenite per day and the group receiving placebo. Null hypotheses 2 and 3 were formulated analogously for the comparison of the group receiving 200 µg sodium selenite with the placebo group and the comparison of both groups receiving supplemental selenium. The hypotheses were tested on a significance level of 5% (2-sided) with a Wilcoxon test in a confirmatory manner. The following null hypothesis was tested in a confirmatory manner only if a null hypothesis could be rejected; otherwise, it was tested in an exploratory manner. Other *P* values have not been corrected for multiple testing and must be considered descriptive. For experimental data, the effect of different selenium compounds on GPx-1 was tested by analysis of variance. Statistical analyses were performed with SAS 9.1 (SAS Institute, Cary, NC).

Results

Selenium treatment modifies expression and activity of GPx-1

Sodium selenite treatment increased GPx-1 protein expression dose-dependently, achieving a 2.4-fold maximal increase at 289 nmol/L in HCAEC (Figure 2, *A*); this increase was associated with a 1.9-fold increase in GPx-1 activity (203 ± 9.2 vs 109 ± 6.2 mmol min⁻¹ mg⁻¹, *P* < .0001) (Figure 2, *B*). The highest concentration of sodium selenite used, 578

nmol/L, was associated with a decrease in GPx-1 expression and accompanied by increased cell death. Selenium supplementation did not affect mRNA expression (data not shown). Selenomethyl-selenocysteine hydrochloride treatment significantly increased GPx-1 protein and activity compared to untreated HCAEC ($P < .0001$), achieving a maximal 1.6-fold increase at 57.8 nmol/L. Seleno-L-methionine treatment did not have a significant effect on expression or activity (Figure 2, C and D).

Clinical study—patient characteristics

We screened 669 patients with documented CAD, 465 of whom met the inclusion criteria (Figure 1). Baseline characteristics of the study population are given in Table II. The median age was 66 years, 18.5% were women, and the proportion of traditional risk factors was similar to that in a general CAD population.⁹ Randomization seemed to be intact.

Among the patients who were assigned to the treatment groups, 93.9% took sodium selenite supplementation as advised. No relevant differences were observed for side effects, dropout rates, or safety parameters in the sodium selenite intake treatment groups compared to the placebo group.

Sodium selenite and selenium concentration and GPx-1 activity

Circulating selenium concentrations were higher after the 12-week supplementation compared to placebo ($P < .0001$). Baseline selenium concentrations showed a median of 96.7 $\mu\text{g/L}$ (87.7–106.3). After 12 weeks, a dose-dependent increase in selenium levels was observed, with the highest selenium concentrations in the 500 μg group (141.0 $\mu\text{g/L}$ [127.5–152.0]) (Table III). The increase in selenium levels in the groups treated with sodium selenite was greater in the first half of the trial period than in the second half.

Table III shows the primary and secondary outcome variables. Median GPx-1 activity was 36.4 U/gHb (31.6–41.9) in the overall cohort at baseline. In both treatment groups, GPx-1 activity increased after 6 and 12 weeks ($P < .0001$). Although the 6-week value was higher in subjects receiving 500 μg selenium compared with those receiving 200 μg , this difference did not reach a P value of $< .05$, and after 12 weeks, GPx-1 activity did not differ relevantly among groups ($P = .624$). Median increases in GPx-1 activity from baseline values were 1.9 U/gHb (after 6 weeks, $P = .006$ vs placebo) and 3.6 U/gHb (after 12 weeks, $P < .0001$ vs placebo) in the 200 μg supplementation group, and 3.6 U/gHb (after 6 weeks, $P < .0001$ vs placebo, $P = .217$ vs 200 μg group) and 3.9 U/gHb (after 12 weeks, $P < .0001$ vs placebo, $P = .624$ vs 200 μg group) in the 500 μg intervention group.

Impact of sodium selenite supplementation on FMD

As demonstrated in Table III, sodium selenite supplementation did not have a significant effect on FMD compared to placebo ($P = .430$).

Secondary outcomes

None of the inflammatory (CRP and fibrinogen), oxidant stress markers (8-isoprostanes and Cu/Zn superoxide dismutase), or blood lipids were affected by sodium selenite (Table IV).

Linear regression analysis showed an association of sodium selenite with GPx-1 activity (Table V). The change in GPx-1 activity was further influenced by age (estimate 0.097) and body mass index (estimate 0.257). None of the other classic cardiovascular risk factors or left ventricular ejection fraction was relevantly related to changes in GPx-1 activity.

There was no relevant heterogeneity of GPx-1 increase among subgroups defined according to age, classic risk factors, or left ventricular ejection fraction. Specifically, there was no

relevant difference in the magnitude of the GPx-1 increase among patients with low and high selenium and low and high GPx-1 activities, respectively, although the increase in GPx-1 activity was greater in individuals with lower circulating selenium concentrations or GPx-1 activity below the median. There was no effect modification on the GPx-1 increase after 12 weeks with respect to treatment with statins, β -blockers, and angiotensin receptor antagonists (data not shown).

Discussion

Based on in vitro cell culture and in vivo clinical trial data, we have demonstrated that sodium selenite supplementation leads to an increase in GPx-1 activity. Although the administration of 500 μ g showed a steeper increase in GPx-1 activity during the first 6 weeks and higher blood selenium concentration than the 200 μ g dose, both doses led to a plateau of GPx-1 activity during long-term administration with no relevant difference in activity at the end of the study. Selenium supplementation did not influence endothelial function relevantly.

In HCAEC, we were able to induce GPx-1 protein expression and activity with sodium selenite and Se-methyl-selenocysteine hydrochloride. In contrast to these results, seleno-L-methionine treatment did not have any effect on GPx-1 expression or activity, likely owing to the nearby substitution of seleno-L-methionine for methionine in the translation of non-selenoproteins, thereby limiting availability of seleno-L-methionine for selenoprotein synthesis. Consistent with its role in translation, GPx-1 messenger RNA expression was not affected by sodium selenite treatment.

Selenium availability is the limiting factor in the biosynthesis of selenoproteins such as GPx-1. In states of selenium deficiency, the production of selenoproteins is reduced in a hierarchical order so that biologically more important proteins are expressed, whereas less frequently used enzymes are not expressed. By contrast, abundance in dietary selenium leads to increased induction of selenoprotein expression. The increase of GPx-1 by higher selenium availability was confirmed in the current clinical study.

Of importance, a plateau in GPx-1 activity was observed with both concentrations after the 12-week period. Although a dose-dependent increase in GPx-1 activity with lower concentrations of supplemental selenium has been described previously in individuals with selenium deficiency,²² we now demonstrate a ceiling effect with supplementation. This observation strengthens the postulate that GPx-1 levels could be used to adjust selenium supplementation in populations with relative selenium deficiency.^{12,23}

Despite considerable changes in selenium concentration and GPx-1 activity, no relevant improvement in noninvasively measured endothelial or vascular function was observed. Overall, a trend toward improved function was found even in the placebo group, which may be observed in patients taking part in a controlled trial with regular follow-up visits. With the present results, we cannot determine whether brachial artery function is adequate to capture the impact of selenium concentration and GPx-1 activity on vascular function, or whether the effect of higher GPx-1 on vascular performance is not sufficient to influence vascular function in the presence of a variety of risk factors that adversely affect vascular function itself.

Factors associated with the magnitude of change in GPx-1 activity were age and body mass index. An effect of age on body selenium status, although minor compared to nutritional factors, has previously been reported.^{24,25} An association of selenium concentration and body mass index has also been observed, most obviously in over-weight and obese subjects where a relative deficiency in micronutrients such as selenium has been observed. This

observation has been attributed to inadequate intake as well as alterations in selenium metabolism.^{23,26} Published data suggest that the impact of these factors on the relative increase of selenium concentration and GPx-1 activity may be due to a relatively lower baseline selenium concentration with older age and higher body mass index. A reduced effect of selenium supplementation on GPx-1 activity in participants treated with angiotensin-converting enzyme (ACE) inhibitors could be explained by findings that ACE inhibitors per se exhibit antioxidant characteristics and enhance glutathione-dependent antioxidant defense.²⁷ Thus, the additional effect achieved by selenium supplementation might be lower than in patients not taking ACE inhibitors.

Owing to its role in antioxidant defense, selenium has long been the focus of observational studies and interventional trials in cardiovascular disease.^{28,29} The overall effect of selenium concentration in long-term observational studies on cardiovascular outcome revealed a slight reduction in cardiovascular risk,³⁰ whereas clinical trials of selenium supplementation have yielded inconsistent results.^{19,31–35} Most trials used a combination of vitamins and trace elements and did not focus on GPx-1 as the major target variable. In patients with CAD, plasma selenium and GPx concentrations have been shown to be low after myocardial infarction, demonstrating that antioxidant defenses are challenged under such conditions.³⁶

In that atherosclerosis is an inflammatory disease, a decrease in plasma selenium in subjects with atherosclerosis has been explained by it being an acute-phase reactant.³⁷ If so, supplementation should overcome this deficiency. Our data suggest that the need for and the efficacy of selenium supplementation could be reflected in GPx-1 activity. Because it seems feasible to enhance GPx-1 activity with selenium supplementation, long-term intervention trials will be necessary to determine whether a benefit in cardiovascular outcome can be achieved.

Limitations

With the present data, we show an elevation of erythrocyte GPx-1 with sodium selenite supplementation in comparison to placebo, but we cannot prove that this higher activity contributes to a reduced rate of cardiovascular events and mortality. One limitation of the in vivo studies is that GPx-1 is measured in red blood cells rather than in vascular tissue. Our cell culture data indicate selenium supplementation can augment GPx-1 activity in endothelial cells; however, an in vivo marker for this effect is lacking. Whether treatment duration may have been inadequate to affect endothelial function remains to be determined.

One can argue that the supplementation with inorganic selenium is inferior to organified selenium (selenomethionine or methyl-selenocysteine) because inorganic selenium increases oxidant stress during its organification.³⁸ This effect may offset the benefits on endothelial cells. Blood selenium concentrations can be more effectively increased by the same molar concentration of selenomethionine, the major dietary form, than by sodium selenite.^{39,40} There is, however, evidence that selenomethionine does not provide the full amount of selenium for selenoprotein synthesis because much of it is shunted into normal protein synthesis routinely substituting for methionine. In support of this prior observation, we showed that, in vitro, seleno-L-methionine had no effect on GPx-1 activity in HCAEC; Se-methyl-selenocysteine hydrochloride, by contrast, was effective at increasing activity and expression of GPx-1.

In summary, our in vitro and in vivo findings consistently demonstrate that sodium selenite supplementation induces an increase in the activity of GPx-1, a selenoprotein prominent in antioxidant defense and cardiovascular protection. These data should spur further investigations to elucidate whether enhanced GPx-1 activity achieved by selenium supplementation has a protective role in cardiovascular disease during long-term follow-up.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A

RNA Isolation

RNA was extracted with the RNeasy kit (Qiagen Sciences), incorporating an optional DNase I step to remove residual DNA. Samples were quantitated and checked for purity and quality by A_{260}/A_{280} measurements and gel electrophoresis. Complementary DNA was synthesized from 0.4 μ g of each total RNA sample with oligo(dT) primers using the Advantage RT-for-PCR Kit (Clontech).

Quantitative real-time RT-PCR

All procedures, including data analysis, were performed on the Applied Biosystems PRISM 7900 HT Sequence Detector (Applied Biosystems 7900HT Fast Real-Time PCR Systems, Foster City, CA) using the TaqMan Gene Expression Assay (Applied Biosystems) for the human GPx-1 transcript (catalogue no. HS00829989_gH). Polymerase chain reaction products were analyzed by a method that compared the amount of glyceraldehyde 3-phosphate dehydrogenase, as an endogenous control. Cycle parameters were 95°C for 15 minutes to activate Taq, followed by 40 cycles of 95°C for 15 seconds, 58°C for 1 minute, and 72°C for 1 minute.

Western blot

For determination of GPx-1 protein levels, cells were washed in phosphate-buffered saline (1%), scraped from the plates, and pelleted at 300 × g for 5 minutes. Cell pellets were resuspended in ice-cold buffer (50 mmol/L Tris-HCl, pH 7.5, 5 mmol/L

ethylenediaminetetraacetic acid [EDTA], and 1 mmol/L dithiothreitol), lysed by the freeze-thaw method,⁴¹ and debris removed by centrifugation at $8000 \times g$ for 20 min. Samples were stored at -80°C . Protein samples (40 μg) were electrophoresed on 4% to 15% SDS-polyacrylamide gels (BioRad Laboratories, Hercules, CA), and transferred to nitrocellulose membranes (Hybond, Amersham Biosciences, Pittsburg, PA). To determine GPx-1 protein expression, the membranes were probed with monoclonal anti-GPx-1 antibody (Marine Biological Laboratory, Woburn, MA). The membranes were then stripped and reprobed with a polyclonal rabbit anti- β -actin antibody (Sigma). A Versa-doc system and the accompanying software (BioRad) were used to quantitate protein bands.

Glutathione peroxidase enzyme activity from cultured endothelial cells

Cellular GPx activity was determined by an indirect assay that links GPx-1-mediated oxidation of glutathione to the recycled reduction of oxidized glutathione to glutathione by glutathione reductase using nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH) as a reductant.⁴² The reaction was carried out in buffer containing 50 mmol/L TrisHCl, 5 mmol/L EDTA, 1 mmol/L glutathione, 0.4 U/mL glutathione reductase, and 0.2 mmol/L NADPH (pH 7.6), and initiated by the addition of *tert*-butyl-hydroperoxide (0.22 mmol/L final concentration). Enzyme activity was calculated from the change of absorbance at 340 nm over time, a measure of NADPH oxidation, using a molar extinction coefficient for NADPH of $6.220 \text{ mol L}^{-1} \text{ cm}^{-1}$. Enzyme activity was normalized to protein concentration (mU/mg).

Clinical trial design

Laboratory methods

For blinded determination of selenium at baseline, after 6 weeks, and after 12 weeks, measurements were made in whole blood anticoagulated with EDTA in a central laboratory (Biosyn). Additional selenium measurements were performed in cases of selenium levels higher than 170 $\mu\text{g/L}$. Treatment would have been stopped and patients would have been withdrawn from the study at selenium concentrations higher than 230 $\mu\text{g/L}$, but no patients achieved this predefined level. The reference values for whole blood selenium in the normal population are 100 to 140 $\mu\text{g/L}$. Glutathione peroxidase 1 activity was measured from washed red blood cells obtained immediately from whole blood anticoagulated with EDTA. Hemolyzed cells were stored frozen for up to 1 week, which did not alter enzyme activity. Glutathione peroxidase 1 was measured as described previously⁴³ with minor modifications (Randox). Intra- and interassay coefficients of variation were 6.7 and 9.9%, respectively. Serum creatinine, aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transferase, and CRP, as well as lipids, were measured immediately using routine methods. Low-density lipoprotein was calculated by the Friedewald formula. Fibrinogen was determined as derived fibrinogen.

Endothelial function

Noninvasive endothelial function testing was performed at enrollment and after 12 ± 1 weeks as described previously.⁴⁴ Briefly, endothelial function was measured as FMD and after administration of 8 mg sublingual nitroglycerin according to standardized procedures after a 12-hour fast and after a 20-minute rest period.⁴⁵ Two-dimensional high-resolution ultrasonic imaging of the right brachial artery was performed on a Philips HDI 5000 CV ultrasound machine (Philips, Best, Netherlands) with a linear array broadband probe, L12-5 (38 mm). All ultrasound scans were saved digitally and subsequently analyzed on an off-line reading station using the commercially available brachial tools software (Medical Imaging Applications LLC, Iowa City, IA). Nitroglycerin-mediated dilation was not determined in

patients with contraindications to nitroglycerin intake. Echocardiographic studies were performed on a Philips HDI 5000 (Philips) machine and comprised parasternal and apical routine M-mode and 2-dimensional image acquisition. Left ventricular ejection fraction was calculated by apical planimetry.

Image acquisition and analysis were performed blinded for selenium and GPx-1 concentrations. To eliminate interobserver variability, both enrolled and close out endothelial function tests of any given patient were performed by the same technician.

Statistical methods

Predefined analysis variables were change in GPx-1 activity (primary outcome) and absolute change of FMD (measured in percentage) from baseline to last visit (secondary outcome). Further analysis variables were the change in CRP, fibrinogen, and other biomarkers. In addition, GPx-1, selenium, and CRP were measured at the control visit (6 weeks), and for those variables, differences between value at control visit and the value at the baseline visit were calculated as the value at the last visit minus the value at the control visit. In addition, age, GPx-1, and selenium at baseline were dichotomized as below or above the median. The changes in FMD and GPx-1 were reported as median and quartiles in each category of dichotomized age, GPx-1, and selenium, as well as smoking habits.

There were 3 hierarchically ordered null hypotheses. Null hypothesis 1 was that there is no difference in the change of FMD between the group receiving 500 µg sodium selenite per day and the group receiving placebo. Null hypotheses 2 and 3 were formulated analogously for the comparison of the group receiving 200 µg sodium selenite with the placebo group and the comparison of both groups receiving supplemental selenium. The hypotheses were tested on a significance level of 5% (2-sided) with a Wilcoxon test in a confirmatory manner. The following null hypothesis was tested in a confirmatory manner only if a null hypothesis could be rejected; otherwise, it was tested in an exploratory manner. For analysis of cell culture on GPx-1 activity and mRNA levels, the effect of different selenium compounds on GPx-1 was tested by analysis of variance with pairwise post hoc analysis by Fisher's protected least-square difference test. Statistical analyses were performed with SAS 9.1.

Figure 1.
Study design and patient sample of the SETCAP trial. ITT indicates intention to treat.

Figure 2.

A, Glutathione peroxidase 1 protein in HCAEC after 4 days of sodium selenite treatment. **B**, Glutathione peroxidase 1 activity levels in HCAEC after 4 days of sodium selenite treatment. * $P < .0001$, # $P < .05$ vs no treatment ($n = 8$). **C**, Glutathione peroxidase 1 protein levels in HCAEC after 4 days of Se-methyl-selenocysteine hydrochloride or seleno-L-methionine. **D**, Glutathione peroxidase 1 activity levels in HCAEC after 4 days of Se-methyl-selenocysteine hydrochloride or seleno-L-methionine. * $P < .0001$ vs no treatment ($n = 9$). Data are expressed as mean $\pm$ standard error of the mean.

Table I**Inclusion and exclusion criteria of the SETCAP trial***Inclusion criteria*

- 1 Documented CAD treated by a standard therapy in accordance with current guidelines
- 2 FMD<8%
- 3 Male and female patients 18 y old
- 4 Written informed consent

Exclusion criteria

- 1 Left ventricular ejection fraction <30%
- 2 Uncontrolled hypertension (blood pressure >180/100 mm Hg) or systolic blood pressure <100 mm Hg
- 3 Renal impairment (serum creatinine ≥2.5mg/dL)
- 4 Known hepatic disease or γ -glutamyl transferase >3x upper limit of normal range
- 5 Manifest inflammation or infectious disease with clinical symptoms
- 6 Malignancy with treatment indication
- 7 HIV infection
- 8 Initiation of therapy with any of the following medications within the last 12 weeks:
 - Aspirin
 - Lipid-lowering therapy
 - Calcium-channel blockers
 - ACE inhibitors
 - AT1 receptor antagonists
 - Hormone replacement therapy
 - Clopidogrel
 - Vitamins or other antioxidant substances, the daily dose of which exceeds the recommendations of the German Society of Nutrition
 - High-dose corticosteroid therapy or therapy with cytostatic substances within the last year
 - Long-term medication with NSAIDs except for aspirin
 - Concomitant therapy with selenium-containing substances exceeding 50 μ g/d
- 9 Current pregnancy or breastfeeding
- 10 Participation in another clinical trial within the 4 wk before study entry
- 11 Former participation in this clinical trial
- 12 Concomitant participation in another clinical trial
- 13 Planned participation in another clinical trial within 4 wk after termination of the current trial
- 14 Alcohol, medication, or drug dependency
- 15 Unreliability as a participant in a clinical trial as assessed by the clinician
Outcomes assessed during 12 wk of sodium selenite supplementation

Primary outcome

- 1 Change in whole blood selenium concentration
- 2 Change in GPx-1 activity

Secondary outcome

- 1 Change in FMD (measured in percentage)

- 2 Change in nitroglycerin-mediated dilation
 - 3 Change in inflammatory biomarkers
 - 4 Change in serum lipid parameters
-

NSAID, Nonsteroidal anti-inflammatory drug.

Table II

Baseline characteristics of the study population according to treatment arm

	Placebo (n = 144)	Sodium selenite 200 µg (n = 148)	Sodium selenite 500 µg (n = 141)	P
Age (y)	66 (61–70)	66 (61–70)	66 (58–72)	.99
Male sex (%)	115 (79.9)	118 (79.7)	120 (85.1)	.41
Body mass index (kg/m ²)	28.1 (26.1–30.8)	28.4 (25.7–30.7)	27.8 (25.4–30.7)	.78
Hypertension (%)	124(86.1)	124 (83.8)	116 (82.3)	.67
Systolic blood pressure (mm Hg)	140 (120–150)	130 (120–150)	140 (120–150)	.83
Heart rate (beat/min)	61 (56–67)	61 (55–67)	63 (58–70)	.12
Smoking				.55
Former (%)	60 (42)	56 (38)	64 (45)	
Current (%)	17 (12)	21 (14)	12 (9)	
Diabetes (%)	30 (21)	34 (23)	29 (21)	.86
Cardiac medication				
ACE inhibitors (%)	97 (67)	98 (66)	74 (53)	.02
β-Blockers (%)	116 (81)	124 (83)	111 (79)	.54
Angiotensin receptor antagonists (%)	23 (16)	25 (17)	36 (26)	.08
Metabolic parameters*				
Total cholesterol (mg/dL)	182 (163–204)	179 (158–205)	184 (162–210)	.33
HDL cholesterol (mg/dL)	53 (46–64)	53 (45–62)	58 (49–64)	.06
LDL cholesterol (mg/dL)	99 (78–118)	92 (77–115)	100 (77–117)	.71
Triglycerides (mg/dL)	136 (91–200)	134 (99–188)	142 (99–200)	.78
Serum creatinine (mg/dL)	1.0 (0.9–1.1)	1.0 (0.9–1.2)	1.0 (0.9–1.1)	.59
Glucose (mg%)	104 (97–117)	104 (96–119)	103 (96–119)	.97
Inflammatory parameters				
Fibrinogen (mg/dL)*	288 (259–330)	285 (249–338)	287 (255–328)	.75
CRP (mg/L)	2.2 (0.5–4.5)	2.0 (1.1–3.5)	1.5 (0.5–3.0)	.1
Selenium and GPx's				
Selenium (µg/L)*	93.4 (87.0–105.9)	97.4 (87.7–104.9)	99.0 (89.3–109.1)	.11
GPx-1 (U/gHb)	34.9 (31.0–40.3)	37.0 (31.3–41.7)	38.1 (33.2–43.8)	.02
Echocardiographic parameters				
EDV (mL)	93 (70–109)	96 (79–123)	98 (81–120)	.02
ESV (mL)	39 (30–53)	42 (32–60)	44 (34–57)	.06
LV ejection fraction (%)	54 (48–61)	54 (49–60)	55 (49–60)	.95
Measures of vascular function				
FMD (%)	4.9 (3.2–6.7)	5.5 (3.3–7.2)	4.8 (2.6–6.3)	.1
Nitroglycerin-mediated dilation (%) (n=354) [†]	10.8 (6.8–14.2)	11.2 (7.5–15.1)	10.9 (6.4–14.3)	.46
Baseline brachial artery diameter (FMD; mm)	5.0 (4.5–5.4)	4.8 (4.3–5.5)	5.0 (4.7–5.4)	.27
Hyperemic brachial artery diameter (FMD; mm)	5.2 (4.7–5.6)	5.0 (4.5–5.8)	5.3 (4.9–5.6)	.45
Baseline brachial artery diameter (NMD; mm)	5.0 (4.5–5.4)	4.8 (4.3–5.4)	5.1 (4.7–5.4)	.05

	Placebo (n = 144)	Sodium selenite 200 µg (n = 148)	Sodium selenite 500 µg (n = 141)	P
Post nitro brachial artery diameter (NMD; mm)	5.5 (5.1–5.9)	5.4 (4.9–6.0)	5.6 (5.3–6.0)	.07

Data are number and percentage of patients or median and 25th/75th percentiles. *P* values were computed with the Kruskal-Wallis test. For comparison of categorical variables, a χ^2 test was used. *LDL*, Low-density lipoprotein; *HDL*, high-density lipoprotein; *ESV*, end-systolic volume, *EDV*, end-diastolic volume; *LV*, left ventricular.

* Lipid variables and fibrinogen were not obtained in one patient in the placebo group. Baseline selenium concentrations were not available in 6 patients in the placebo group, 7 patients in the 200 µg group, and for 6 patients in the 500 µg group. Glutathione peroxidase 1 baseline activity was not valid in 2 patients in the 200 µg group and 1 patient in the 500 µg group.

[†] Nitro-mediated dilation was only available in 354 patients.

Table III

Absolute values and difference of parameters according to visit for selenium, GPx-1, FMD, and NMD

	Placebo (n = 144)	Sodium selenite 200 µg (n = 148)	Sodium selenite 500 µg (n= 141)	P
Selenium concentration				
Selenium _{V0}	93.4 (87.0–105.0)	97.4 (87.7–104.9)	99.0 (89.3–109.1)	.11
Selenium _{V1}	95.2 (87.8–103.9)	118.6 (111.5–126.2)	137.5 (128.1–149.4)	<.0001
Selenium _{V2}	95.9 (88.8–104.2)	122.9 (113.5–131.1)	141.0 (127.5–152.0)	<.0001
Change in selenium concentration				
Selenium _{V1-V0}	0.2 (–5.8 to 5.1)	22.8 (13.3–30.5)	37.6 (28.8–48.1)	<.0001
Selenium _{V2-V1}	0.4 (–4.9 to 7.2)	3.3 (–4.3 to 9.5)	4.1 (–4.6 to 11.9)	.13
Selenium _{V2-V0}	0.2 (–6.6 to 9.5)	24.2 (15.3–35.6)	41.2 (29.9–51.3)	<.0001
GPx-1 activity				
GPx-1 _{V0}	35.0 (31–40.3)	37 (31.3–41.7)	38.1 (33.2–43.8)	.03
GPx-1 _{V1}	35.7 (30.9–42.1)	39.1 (33.2–46.0)	40.9 (35.8–46.9)	<.0001
GPx-1 _{V2}	35.5 (30.8–41.2)	41.1 (35.2–48.4)	42.6 (35.0–49.1)	<.0001
Change in GPx-1 activity				
GPx-1 _{V1-V0}	0 (–3.1 to 3.5)	1.9 (–1.3 to 6.2)	3.6 (–1.1 to 6.8)	.0004
GPx-1 _{V2-V1}	0.2 (–3.4 to 4.2)	1.9 (–2.2 to 7.1)	1.0 (–3.0 to 6.0)	.02
GPx-1 _{V2-V0}	–0.1 (–2.7 to 3.9)	3.6 (0.0–8.9)	3.9 (–0.2 to 10.2)	<.0001
FMD				
FMD _{V0}	4.6 (3.2–6.7)	5.5 (3.3–7.2)	4.8 (2.6–6.3)	.1
FMD _{V2}	5.9 (4.2–7.9)	6.1 (4.2–8.3)	5.5 (3.6–7.8)	.23
Change in FMD				
FMD _{V2-V0}	1.1 (–0.6 to 3.4)	0.9 (–1.0 to 3.6)	0.9 (–1.4 to 3.3)	.71
Nitroglycerin-mediated dilation				
NMD _{V0}	10.8 (6.8–14.2)	11.2 (7.5–15.1)	10.9 (6.4–14.3)	.47
NMD _{V2}	10.8 (8.1–13.5)	11.0 (7.5–14.9)	11.4 (8.1–13.8)	.77
Change in nitroglycerin-mediated dilation				
NMD _{V2-V0}	0.1 (–2.5 to 3.1)	–0.4 (–2.6 to 2.9)	0.6 (–2.5 to 4.0)	.79

Data are expressed as median and 25th/75th percentile range.

Table IV

Absolute values and difference of parameters by visit

	Placebo (n = 144)	Sodium selenite 200 µg (n = 148)	Sodium selenite 500 µg (n = 141)	P
<i>Inflammatory markers</i>				
CRP (mg/L) V ₀	2.2 (0.5–4.5)	2.0 (1.1–3.5)	1.5 (0.5–3.0)	.1
CRP V ₁	1.7 (0.5–3.4)	1.7 (0.5–4.1)	1.5 (0.5–3.1)	.76
CRP V ₂	2.0 (1.0–3.6)	2.0 (0.5–4.2)	1.5 (0.5–3.3)	.14
<i>Change in CRP concentration</i>				
CRP V ₁ -V ₀	-0.1 (-1.1 to 0.2)	0.0 (-0.8 to 0.8)	0.0 (-0.6 to 0.7)	.04
CRP V ₂ -V ₁	0.0 (-0.6 to 0.7)	0.0 (-0.5 to 1.2)	0.0 (-0.6 to 0.6)	.45
CRP V ₂ -V ₀	0.0 (-1.3 to 0.7)	0.0 (-0.65 to 0.8)	0.0 (-0.6 to 0.7)	.34
Fibrinogen (mg/dL) V ₀	288 (259–330)	285 (249–338)	287 (255–328)	.75
Fibrinogen V ₂	310 (272–372)	317 (263–375)	310 (263–361)	.77
<i>Change in fibrinogen concentration</i>				
Fibrinogen V ₂ -V ₀	17 (-20 to 74)	28 (-16 to 74)	19 (-22 to 70)	.73
<i>Serum lipids</i>				
HDL cholesterol (mg/dL) V ₀	53 (46–64)	53 (45–62)	58 (49–64)	.06
HDL cholesterol V ₂	53 (46–65)	54 (46–63)	55 (49–64)	.33
<i>Change in HDL cholesterol</i>				
HDL cholesterol (mg/dL) V ₂ -V ₀	0.0 (-3.0 to 4.0)	1.0 (-3.0 to 4.0)	-2.0 (-4.5 to 3.0)	
LDL cholesterol V ₀	99 (78–118)	92 (77–115)	100 (77–117)	
LDL cholesterol V ₂	94 (74–118)	92 (77–115)	95 (72–123)	
<i>Change in LDL cholesterol</i>				
LDL cholesterol V ₂ -V ₀	-1 (-10 to 12)	1 (-11 to 10)	0 (-14 to 13)	.03
Triglycerides (mg/dL) V ₀	136 (91–200)	134 (99–188)	142 (99–200)	.71
Triglycerides V ₂	140 (98–197)	135 (102–187)	148 (111–221)	.92
<i>Change in triglycerides</i>				
Triglycerides V ₂ -V ₀	9 (-36 to 33)	0.5 (-30 to 32)	8.5 (-19 to 43)	
8-Isoprostanes (pg/mL) V ₀	379 (211–596)	436 (265–673)	427 (255–749)	.12
8-Isoprostanes V ₂	534 (333–950)	613 (339–1091)	672 (378–1097)	.43
<i>Change in isoprostanes</i>				
8-Isoprostanes V ₂ -V ₀	0185 (-49 to 508)	217 (-128 to 483)	193 (-34 to 577)	.85
Cu/Zn SOD nmol/L V ₀	4.70 (3.91–5.70)	4.87 (4.15–5.62)	4.77 (3.96–6.61)	.50
Cu/Zn SOD V ₂	4.53 (3.84–5.11)	4.66 (3.96–5.29)	4.415 (3.560–5.350)	.27
<i>Change in Cu/Zn SOD</i>				
Cu/Zn SOD V ₂ -V ₀	-0.26 (-0.94 to 0.52)	-0.11 (-1.00 to 0.59)	-0.245 (-1.020 to 0.490)	.89

Data are expressed as median and 25th/75th percentile. *SOD*, Superoxide dismutase.

Table VLinear regression for change in GPx-1 activity, visit₂-visit₀ (n = 410)^{*}

Variable	Coefficient	95% CI		P
		Lower	Upper	
Change in selenium V ₂ -V ₀	0.098	0.067	0.129	<.0005
Age	0.097	0.007	0.187	.035
Gender (female)	0.358	-1.488	2.204	.704
Smoking				.785
Formerly	0.298	-1.307	1.193	.716
Currently	0.879	-1.626	3.384	.492
Diabetes	-1.292	-3.057	0.473	.151
Hypertension	0.231	-1.740	2.202	.818
Body mass index	0.257	0.067	0.446	.008
LV ejection fraction	-0.048	-0.129	0.034	.251

Data are expressed as linear regression coefficients and 95% CIs for change. Sex, diabetes, and hypertension were entered into the model as dichotomized variables. The variable smoking consisted of 3 categories.

^{*} Complete parameters were available in 410 individuals.