

Selenium supplementation does not improve vascular responsiveness in healthy North American men

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Hawkes WC, Laslett LJ. Selenium supplementation does not improve vascular responsiveness in healthy North American men. *Am J Physiol Heart Circ Physiol* 296: H256–H262, 2009. First published November 26, 2008; doi:10.1152/ajpheart.00935.2008.—Selenium is an essential trace nutrient required for the synthesis of selenoproteins such as glutathione peroxidase and thioredoxin reductase, the major forms of selenium in the endothelium that have important functions relevant to inflammation and cardiovascular disease. Selenium deficiency is associated with cardiomyopathy and sudden cardiac death in animals, and a low selenium status is associated with cardiovascular disease in humans. Endothelial dysfunction, measured as the impaired flow-mediated vasorelaxation of the brachial artery, is a reliable indicator of future cardiovascular disease risk in healthy individuals. To test whether selenium supplementation affects endothelial function, we conducted a randomized, placebo-controlled trial in healthy men who were administered 300 µg of selenium a day as high-selenium yeast for 48 wk. Brachial artery responsiveness to transient occlusion was assessed at baseline and after 24 and 48 wk of supplementation. The supplementation increased the selenium concentration by more than half in blood plasma and erythrocytes. However, there was no effect of selenium on arterial diameter or blood flow rate before or after transient occlusion or on the maximum dilated diameter after the administration of nitroglycerin. This study indicates that selenium supplementation is not likely to improve endothelial function or peripheral arterial responsiveness in healthy North American men receiving adequate selenium from their diets.

brachial artery; nutrition; endothelium

SELENIUM (Se) is an essential trace nutrient required in microgram amounts by humans and by all animals in which it has been tested (recommended dietary allowance = 55 µg/day) (42). Signs of Se deficiency include liver necrosis in rats, pancreatic atrophy in chickens, nutritional muscular dystrophy (“white muscle disease”) in sheep, and skeletal myopathy in patients on total parenteral nutrition without supplemental Se (15). Growing pigs fed rations deficient in Se and vitamin E develop a severe cardiomyopathy known as “mulberry heart disease” (60), and Se deficiency, along with infection by coxsackievirus B₃, is a causative factor in the fatal human cardiomyopathy known as Keshan disease (13, 48).

The essential functions of Se in humans are mediated by a group of 25 selenoproteins that contain Se in the form of selenocysteine (Sec), the Se-containing homolog of cysteine. The known human selenoenzymes include glutathione peroxidase (GPX), iodothyronine deiodinase, thioredoxin reductase,

and methionine sulfoxide reductase B. Sec is synthesized from serine-aminoacyl transfer RNA and incorporated at internal UGA codons. Recoding UGA from a stop codon to Sec requires several unique factors, including specific secondary structure in the mRNA, a unique tRNA, an RNA binding protein, and a specialized elongation factor (56). Se is the only element specified in the genetic code (“TGA”), and Sec has become recognized as the 21st protein amino acid (16).

A link between Se intake and cardiovascular disease was first suggested by the negative correlation between regional cardiovascular disease mortality and the Se content of drinking water and crops in a cross-sectional study (53). Subsequent prospective human studies have reported that serum Se is inversely related to risk of progression of carotid artery atherosclerosis (49), ischemic heart disease (59), coronary artery disease (65), cardiovascular disease mortality (22), and hypertension (43). Endothelial dysfunction is an early event in the development of atherosclerosis, hypertension, coronary artery disease, and chronic heart failure.

The available human data support a causative role for oxidative damage in the development of endothelial dysfunction. Hydrogen peroxide and other reactive oxygen species are significant in endothelial dysfunction because of their ability to react with and eliminate nitric oxide (NO), the main small molecule mediator of endothelium-dependent vasodilation. Endothelial dysfunction of the coronary arteries is an independent predictor for the progression of atherosclerosis and subsequent cardiovascular events (51). Patients with impaired endothelium-dependent flow-mediated dilation (FMD) of the brachial artery or with a greater endothelial response to infused vitamin C are at significantly greater risk of subsequent cardiovascular events (35). Plasma lipid peroxides were higher in patients with ischemic heart disease or peripheral arterial disease (58), and double-strand breaks in lymphocyte DNA were significantly negatively correlated with brachial artery FMD in a mixed population of patients with coronary artery disease and matched controls (68).

A meta-analysis of 16 case-control studies found a relative risk for coronary heart disease of 0.43 (95% confidence interval: 0.29, 0.66) in subjects with the highest Se concentrations compared with the lowest but found a nonsignificant risk reduction of 0.89 (95% confidence interval: 0.68, 1.17) from Se supplementation in six randomized trials (25). However, the lack of significance in the meta-analysis of Se supplementation trials should be viewed with caution. First, the pooled relative risk was consistent with a protective effect of Se supplement-

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tation. Second, the average dose of 112 $\mu\text{g}/\text{day}$ did not explore the full range of safe doses. Third, four of the six trials analyzed gave Se in combination with several other nutrients (e.g., coenzyme Q, vitamin C, vitamin E, β -carotene, and zinc), making it impossible to draw conclusions specific to Se. Fourth, of the two trials using only Se, one was only 6 mo in duration, too short to expect a benefit in a lifelong disease process, and the other was a reanalysis of the Nutritional Prevention of Cancer trial in which coronary heart disease was a secondary end point determined from death certificates and medical records instead of clinical examinations (57). Lastly, the reanalysis of the Nutritional Prevention of Cancer trial excluded all prevalent cases of coronary heart disease at baseline, and the subjects admitted to the study were age 62 yr on average, when cardiovascular disease risk is already largely determined, endothelial function is no longer an early indicator of risk, and nutritional prevention is unlikely to be effective.

FMD of the brachial artery measured with Doppler ultrasound is a noninvasive technique for assessing NO-mediated endothelial function (30). FMD of the brachial artery is the best independent predictor of long-term adverse cardiovascular events in healthy subjects with no apparent heart disease (54, 55). Dietary interventions can be important determinants of endothelial function, even in patients given intensive pharmacological therapy (12). FMD of the brachial artery is impaired in hypercholesterolemic, obese, and diabetic children and improves with therapy (1), indicating its potential usefulness for monitoring prediagnostic improvements in cardiovascular disease risk in young subjects. The present study was designed to test the hypothesis that long-term, high-dose Se supplementation (300 $\mu\text{g}/\text{day}$ for 48 wk) could improve endothelial function as reflected by brachial artery FMD in young disease-free subjects before clinically significant dysfunction is evident (average age, 31 yr). We did not observe any change in FMD or other hemodynamic parameters, suggesting that Se supplements up to the recommended maximum daily intake do not improve endothelial function or vascular responsiveness in healthy North American men.

MATERIALS AND METHODS

Subjects. The present study was part of a broader investigation into the health effects of long-term, high-level Se supplementation in healthy men aged 18–45 yr. Only the results pertaining to brachial artery responsiveness and cardiovascular markers in serum are presented here. Other aspects of the main study have been reported elsewhere (32, 34). Potential volunteers were examined by a nurse practitioner and determined to be in good health. Inclusion criteria were a self-reported absence of disease (hypertension, diabetes, sexually transmitted disease, cancer, etc.) and a clinically normal blood count, blood chemistries, and thyrotropin. Exclusion criteria were tobacco smoking; positive blood test for human immunodeficiency virus, hepatitis B, or syphilis; positive urine tests for drugs of abuse (barbiturates, benzodiazepines, cocaine metabolites, opiates, amphetamines, and cannabinoids); Se supplements providing more than 50 $\mu\text{g}/\text{day}$; and exercise or physical training in excess of three 1-h sessions per week. Subjects were paid for their participation. The study protocol was reviewed and approved by the Institutional Review Board of the University of California at Davis School of Medicine, and informed consent was obtained in writing from all subjects.

Experimental protocol. Potential subjects meeting the recruitment criteria were enrolled into a run-in period lasting 3–6 wk, during which baseline measurements were obtained and compliance was

assessed. Noncompliant subjects were dismissed before randomization and are not reported further. Two subjects at a time were randomized to treatment from July 2000 to November 2002, with one subject from each pair randomly assigned to each treatment group by coin flip. Fifty-four men satisfactorily completed the run-in period and were randomized to receive placebo yeast tablets or high-Se yeast tablets for 48 wk. Neither the subjects nor the study staff was aware of subjects' treatment assignments. Subjects took their first tablet the same day that all baseline measurements were completed. The subjects visited the Center every 6 wk during the 48-wk supplementation period. The visits were scheduled relative to each subject's first day of supplementation. When a visit was missed, the next visit was scheduled based on the first day of supplementation to restore the original schedule. Unused tablets were counted at each visit and were collected at the end of the treatment period to measure compliance. Subjects consumed $93 \pm 5.3\%$ of the pills assigned. Forty-two subjects completed the 48-wk supplementation period.

Se supplements. Supplements were provided as high-Se Baker's yeast (*Saccharomyces cerevisiae*, SelenoPrecise, 300 μg Se per tablet, 3.81 μmol Se, Pharma Nord, Denmark). Placebo tablets were compounded identically, except using the same yeast grown without added Se (≤ 1.3 μg Se per tablet, ≤ 16.5 nmol Se). Tablets contained 0.5 g of spray-dried yeast in an inert binder and were coated with titanium dioxide for an identical appearance, smell, and taste. Tablets were provided in 28-tablet bubble packs.

Dietary intake assessment. The intake of nutrients from food was estimated from 3-day diet records. Twice during the run-in period and then at 24 and 48 wk, the subjects kept a written record of all foods eaten for a 3-day period, always including at least one weekday (Monday–Friday) and at least one weekend day (Saturday and Sunday). Records were analyzed for nutrient contents with the Minnesota Nutrition Data System 5.0 (Nutrition Coordinating Center, Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN), using food composition data derived primarily from the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference (63) to calculate dietary intakes of Se and other nutrients.

Physical activity measurements. During the same 3-day periods that food records were kept, subjects wore a heart-rate monitor (Polar Vantage NV, Polar Electro, Port Washington, NY), except when asleep, and carried a handheld computer that automatically prompted them every 60 min to record all physical activities in 10-min increments (37), based on a simplified version of the Bouchard 3-day physical activity record (9). At the end of each 3-day monitoring period, the digital activity logs were reviewed for completeness and accuracy by a physiologist. The estimated energy expended in each activity was summed to obtain a daily value (2), and the three daily values were averaged to estimate the energy expended per day in voluntary activities at each point in the study.

Laboratory measurements. Blood samples were collected in the mornings, after an overnight fast, and serum was separated by centrifugation and refrigerated until analyzed at a reference laboratory (University of California at Davis School of Medicine Pathology Laboratory, Sacramento, CA). Aliquots of serum, plasma, and washed erythrocytes were stored at -70°C until analyzed. Se concentrations were measured by HPLC of the fluorescent derivative formed from a reaction with diamionaphthalene after digestion in a nitric-perchloric acid mixture (33). GPX activity was measured using the glutathione reductase-coupled assay (46) with 1.36 mM glutathione and 0.5 mM cumene hydroperoxide as substrates, and one unit of activity was defined as oxidation of 1 μmol of NADPH/min. Hemoglobin was determined spectrophotometrically with Drabkin's reagent (21). Protein was measured by the Lowry method (40). Homocysteine was determined by competitive immunoassay on an Immulite analyzer ("LKH016", Diagnostics Products, Los Angeles, CA).

Flow-mediated arterial dilation. Brachial artery vasoreactivity was measured once during the run-in period and then again at 24 and 48

Table 1. Baseline characteristics of 42 subjects completing the study

Parameters	Placebo Group	High-Se Group
Age, yr	31±8.7	31±9.4
Height, cm	177±7.6	180±7.8
Weight, kg	77.5±12.4	76.0±9.5
Body mass index, kg/m ²	24.6±3.0	23.5±2.2
Pulse, beats/min	63±11	66±10
Systolic blood pressure, mmHg	120±11	121±11
Diastolic blood pressure, mmHg	70±6.1	70±9.2
Se intake, µg/day	138±39	137±42
Total energy intake, MJ/day	10.8±2.0	11.3±3.0
Polyunsaturated fat intake, g/day	15±5.3	19±9.6
Monounsaturated fat intake, g/day	29±10	34±14
Omega-3 fatty acid intake, g/day	1.56±0.57	1.85±0.96
Vitamin C intake, mg/day	245±403	169±121
Vitamin E intake (α-tocopherol equivalents), mg/day	43±90	16±6.7
Folic acid intake, µg/day	556±292	538±156
L-Arginine intake, g/day	5.2±1.6	4.7±1.4
Serum homocysteine, µmol/l	7.2±2.2	7.1±2.0
Serum glucose, mmol/l	4.83±0.59	4.97±0.54
Serum total cholesterol, mmol/l	4.60±0.98	4.53±0.91
Serum HDL cholesterol, mmol/l	1.24±0.44	1.14±0.23
Serum LDL cholesterol, mmol/l	2.82±0.78	2.87±0.75
Serum triacylglycerols, mmol/l	1.21±0.56	1.12±0.56
3,3',5-Triiodothyronine (T ₃), nmol/l	1.90±0.38	2.17±0.43
Thyroid-stimulating hormone, mU/l	2.30±1.31	2.10±0.85

Values are means ± SD. Se, selenium.

wk using Doppler ultrasonography. Fasting subjects were placed comfortably in a supine resting state. Blood pressure and heart rate were monitored every 3 min with an automated sphygmomanometer. The subject's left arm was immobilized in the extended position to allow consistent access to the brachial artery, and a sphygmomanometer cuff was positioned 2.5–5 cm below the antecubital fossa on the left lower arm. The cuff was inflated to 200 or 60 mmHg above systolic blood pressure around the lower left arm for 5 min. After a release of pressure, brachial artery flow velocity was immediately measured by range gating at an angle of incidence of 60° to allow the measurement of maximal laminar flow. The velocity was measured three times over the first minute of postocclusion. The maximum measured diameter was estimated by a continuous measurement of the brachial artery starting at 1 min postdeflation. Brachial artery diameter and flow velocity were imaged using a 7.5-MHz linear array transducer ultrasound system (Sonos 2500 or 5500; Hewlett-Packard) placed at a position 3–5 cm above the antecubital fossa and set at an image depth of 4 cm. Images were recorded on videotape for off-line calculations. After 5 min of recuperation, 0.4 mg of sublingual nitroglycerin were given and the measurements repeated. Percent FMD (%FMD) measured at 1 min after cuff deflation represented

endothelium-dependent dilation, and %FMD 3 min after nitroglycerin represented endothelium-independent dilation.

Statistical analysis. The effects of Se were tested with two-way repeated-measures analysis of variance or with a *t*-test of the within-subject changes. Statistical calculations were performed with Sigma Stat (version 2.03, SPSS, Chicago, IL). A probability of 0.05 or less was considered significant. When the Se main effect or the Se × time interaction was significant, the Student-Newman-Keuls multiple comparison test was used to identify significant differences between the groups at individual time points.

RESULTS

The baseline characteristics of the 42 subjects that completed the 48-wk intervention are shown in Table 1. Pulse, blood pressure, and serum metabolites were within normal ranges. None of the parameters in Table 1 was significantly different between groups, nor did any change during the study. Se status was increased significantly, as reflected in erythrocyte and plasma Se concentrations (Table 2). However, GPX enzyme activity was not changed in either blood compartment. Se concentration in plasma reached a plateau by 24 wk, when the first posttreatment FMD measurements were made. Because blood Se did not increase significantly after 24 wk, the post-treatment FMD measurements at 24 and 48 wk were averaged together into a single value for each subject for statistical testing.

FMD of the brachial artery in the study subjects averaged $5.7 \pm 4.6\%$ (range 0.7% to 15.7%) (Table 3), similar to values reported by others for healthy men (20, 45, 62). Nitroglycerin-induced vasodilation was also similar to previous reports (45, 62). FMD was not affected by Se supplementation, did not change in either group, and was not correlated with plasma Se. The only hemodynamic parameter that appeared to be affected by Se was the postocclusion peak velocity, which increased slightly in the high-Se group and tended toward statistical significance ($P = 0.066$, Table 3). Subjects with smaller-diameter brachial arteries tended to have larger %FMD changes (Fig. 1, $R = 0.334$, $P < 0.001$). Serum homocysteine was not changed by Se supplementation, and %FMD change was not significantly related to serum homocysteine or fasting serum triglycerides. %FMD was negatively correlated with alcohol intake (Fig. 2, $R = 0.354$, $P = 0.020$) but was not significantly associated with the intake of Se or total fat, omega-3 fatty acids, cholesterol, arginine, folic acid, vitamin B₁₂, vitamin A, vitamin C, vitamin D, vitamin E, iron, copper, zinc, or the soy isoflavones daidzein, glycitein, or genistein.

Table 2. Blood parameters of healthy men consuming Se supplements or placebo

	Placebo Yeast			High-Se Yeast			Se Effect (P)*
	Baseline	24 wk	48 wk	Baseline	24 wk	48 wk	
<i>n</i>	20						
Plasma Se, µg/l (1 µg Se = 12.7 nmol)	146±19	141±15	141±18	142±19	223±47	228±63	<0.001
Erythrocyte Se, µg/l	264±49	258±32	262±36	261±35	502±105	524±141	<0.001
Plasma GPX, U/ml	2.91±0.43	3.00±0.57	2.99±0.50	3.16±0.37	3.08±0.42	3.22±0.69	NS
Erythrocyte GPX, U/g hemoglobin	431±93	426±102	424±96	427±132	423±128	421±119	NS
Serum homocysteine, µmol/l	7.2±2.2	ND	6.4±1.9	7.1±2.0	ND	7.2±1.3	NS
Serum triacylglycerols, mmol/l	1.19±0.58	1.05±0.45	1.13±0.52	1.08±0.55	1.16±0.55	1.06±0.50	NS

Values are means ± SD; *n*, number of men. Baseline, values at the end of the baseline period before starting supplementation; GPX, glutathione peroxidase; NS, not significant ($P > 0.05$); ND, not determined. **t*-test of within-subject changes (average of 24- and 48-wk values minus baseline).

Table 3. Brachial artery ultrasound measurements before and after occlusion and nitroglycerin treatment in healthy men consuming Se supplements or placebo

	Placebo Yeast			High-Se Yeast			Se Effect (P)*	
	Baseline ²	24 wk	48 wk	Baseline	24 wk	48 wk		
<i>n</i>		20			22			
Preocclusion diameter, mm	4.14±0.47	4.18±0.49	4.21±0.42	4.19±0.48	4.22±0.50	4.20±0.51	0.385	
Postocclusion diameter, mm	4.34±0.44	4.41±0.47	4.45±0.41	4.44±0.49	4.41±0.49	4.47±0.52	0.454	
Occlusion diameter change, mm	0.21±0.24	0.24±0.22	0.24±0.17	0.25±0.14	0.19±0.19	0.27±0.15	0.782	
Occlusion diameter change, %	5.2±5.8	5.8±5.4	5.8±4.3	6.1±3.3	4.8±4.8	6.6±4.0	0.818	
Nitroglycerin diameter change, mm	0.63±0.20	0.60±0.28	0.60±0.24	0.65±0.18	0.58±0.17	0.64±0.23	0.512	
Nitroglycerin diameter change, %	15.5±5.5	14.9±7.8	14.5±6.0	15.6±4.3	14.2±5.2	15.4±6.0	0.462	
Preocclusion velocity, cm/s	108±30	104±27	104±21	105±24	107±26	110±26	0.116	
Postocclusion velocity, cm/s	174±38	165±43	174±30	165±38	181±41	181±40	0.066	
Occlusion velocity change, cm/s	66±22	61±24	71±23	60±28	74±24	71±28	0.122	
Nitroglycerin velocity change, cm/s	-11±11	-7.0±14	-9.4±11	-9.6±13	-10±8.8	-11±9.3	0.276	

Values are means ± SD; *n*, number of men. Baseline, values at the end of the baseline period before starting supplementation. **t*-test of within-subject changes (average of 24- and 48-wk values minus baseline).

There were no changes in the 24-h heart rate or voluntary physical activity within or between groups (32).

DISCUSSION

Human vascular function is regulated by a plethora of pathophysiological mechanisms, some of which are influenced by diet. Although reactive oxygen species and antioxidants clearly have roles in endothelial NO metabolism, the potential health benefits of antioxidant supplementation are far from clear. The significance of dietary Se in the etiology of cardiovascular disease is still uncertain after more than 20 years of research. Recent meta-analyses of randomized supplementation trials failed to find a significant protective effect of antioxidants against the progression of atherosclerosis (8) or of low-dose Se against coronary heart disease (25). On the other hand, observational studies have consistently found an elevated risk of cardiovascular disease associated with low Se status (22, 43, 49, 59, 65), and a meta-analysis found a significantly higher risk of coronary heart disease associated with low Se status (25). These findings suggest that a suboptimal intake of Se and antioxidants can impair endothelial function and increase the risk of cardiovascular disease but that a supranutri-

tional intake confers no additional benefit. This is consistent with the essential function of Se in oxidation-reduction enzymes, the requirements of which for Se appear to be fully satisfied at low nutritional doses. Additional Se intake beyond the nutritional requirement may not increase the activities of these selenoenzymes and thus may confer no additional benefit. The epidemiological evidence is consistent with a saturation effect of Se on cardiovascular health (19), in which Se status predicts cardiovascular disease risk in populations with lower Se status but is not associated with risk in populations with higher Se status (3).

Previous studies have observed significant associations between blood Se and blood lipids in free-living adults (17, 62). A study of hyperlipidemic Portuguese women reported that plasma Se was positively correlated with the HDL cholesterol-to-total cholesterol ratio (64). Serum Se was positively correlated with serum total cholesterol in a study of healthy men and women in the United Kingdom (29) and in Korean women under the age of 40 yr (38). Two studies in Europe reported significant positive associations of serum Se with serum polyunsaturated fat (10, 44). However, we observed no effect of Se

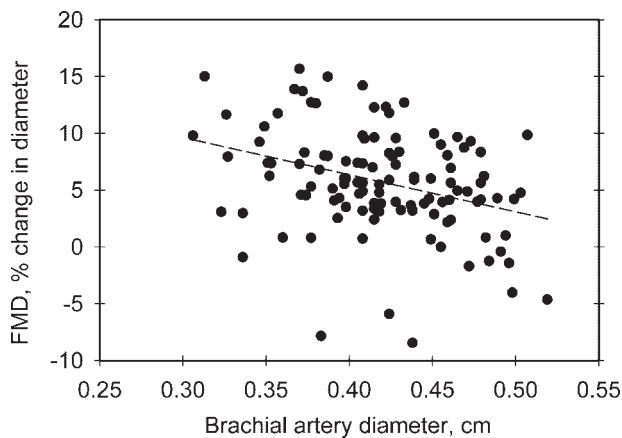


Fig. 1. Relationship between flow-mediated dilation (FMD) and resting diameter of the brachial artery. Percent change in diameter is plotted against pretreatment diameter. All measurements are shown. Dashed line shows result of linear regression: *n* = 117 measurements, *R* = 0.334, *P* = 0.0002.

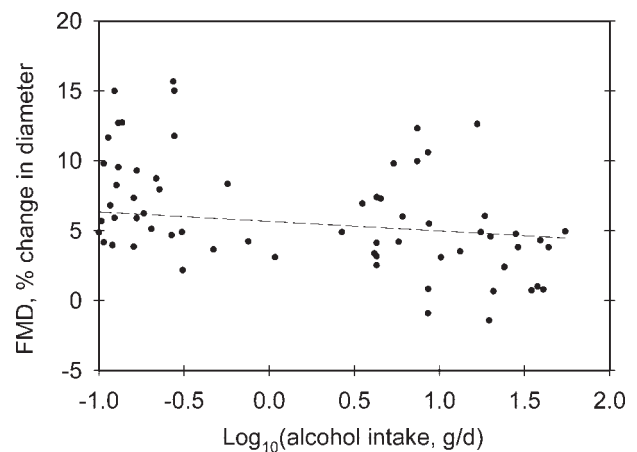


Fig. 2. Relationship between FMD of the brachial artery and alcohol intake. Percent change in diameter is plotted against the logarithm of alcohol intake [in g/day (g/d)] estimated from self-reported food records. Estimates of "zero" alcohol intake are not shown. Dashed line shows result of linear regression: *n* = 85 measurements, *R* = 0.201, *P* = 0.022.

supplementation on serum lipids, nor any significant correlation of plasma Se with serum lipids in the present study.

In vitro studies have shown that Se supplementation protects human endothelial cells from oxidized LDL, due to an increased expression of GPX1 and thioredoxin reductase (TR) (39). TR, which regenerates active thioredoxin at the expense of NADPH and is the most abundant selenoprotein in human endothelial cells (5), may be even more important than GPX1 for protecting human endothelial cells from oxidative damage (11). NO exposure decreases thioredoxin and TR expression, and an overexpression of thioredoxin prevents NO-induced decreases in NO synthase in cultured porcine pulmonary endothelial cells, providing a mechanism for the Se regulation of endothelial NO metabolism (70). TR is upregulated in human atherosclerotic plaques (28), and an endothelial cell-specific overexpression of thioredoxin improves vasodilation and decreases atherosclerotic lesions in transgenic mice (69). The enhanced relaxation of aortic rings in Se-supplemented rabbits (41) might therefore be due to an increased expression of TR, which responds to supranutritional Se (6).

GPX1 is ubiquitously expressed in the cytosol of all human tissues and is the main catalyst for the removal of physiological levels of hydrogen peroxide. GPX1 has consistently been associated with a lower risk of cardiovascular disease. The relative risk of cardiac complications was 0.29 in patients with the highest quintile of blood GPX1 activity compared with the lowest quintile (7), and blood GPX activity was the strongest univariate predictor of subsequent cardiovascular events during 7.1 years of follow-up (52). The importance of GPX1 in maintaining endothelial function has been clarified in studies with transgenic mice deficient in or overexpressing GPX1. Mesenteric arterioles from mice heterozygous (26) or homozygous (27) for deletion of the GPX1 gene exhibited paradoxical vasoconstriction in response to acetylcholine or β -methacholine, respectively, instead of the normal vasodilation observed in wild-type mice. Relaxation to acetylcholine was markedly impaired in angiotensin II-treated carotid arteries from GPX^{+/-} transgenic mice (14) and in untreated carotid arteries from homocysteine-treated GPX^{-/-} transgenic mice (18). Moreover, the overexpression of GPX1 restored the normal endothelium-dependent vasodilator response in hyperhomocysteinemic mice (66). Significantly, the beneficial effect of physical training on vascular endothelial function in patients with chronic heart failure is associated with an increased expression of GPX1 (23). Endothelium-dependent vasorelaxation in high fat-fed rats is protected by a mixture of dietary antioxidants (50). GPX1 activity in aortic arterial walls was increased by a Se supplementation of rats (67), suggesting that supplemental Se above the dietary requirement may be able to improve endothelial function.

To our knowledge, the present study is the first test of Se supplementation on vasoreactivity in humans. There was no evidence of altered endothelial function in the Se-supplemented subjects. Our failure to detect an effect of Se in the present study is unlikely to be due to our methodology because we were able to detect previously reported associations between FMD and arterial diameter and alcohol intake. It is generally considered that FMD of the brachial artery after 5 min of distal occlusion reactive hyperemia without ischemic handgrip exercise in healthy subjects, as used in the present study, is a valid index of NO-mediated vasodilation (61). Se

supplementation also had no effect on vasodilation of the brachial artery induced by nitroglycerin, an index of endothelium-independent vascular responsiveness (4). Thus we conclude that Se supplementation did not improve endothelium-dependent or -independent vasoreactivity. The prestudy Se intakes of our subjects was well above the recommended daily allowance, and their average plasma Se was greater than the mean value for men in the Third National Health and Nutrition Examination Survey (24, 36), indicating that our subjects were already receiving adequate amounts of Se from their diets. Our results indicate that Se supplementation does not affect endothelium-dependent vasoreactivity in healthy, well-nourished men. The 60% increase we observed in plasma Se corresponds to an approximate doubling of whole body Se stores (31). The combined Se intake of our Se-supplemented subjects (ca. 437 μ g/day) exceeded the "tolerable upper intake level" of 400 μ g/day defined by the National Academy of Sciences (47), suggesting that Se intakes within the current recommended range do not impair vasoreactivity.

Overall, the experimental manipulation of Se and antioxidant status in animals have shown significant effects on endothelial function and/or cardiovascular health, whereas the experimental variation of Se and antioxidant intake in humans have not. These disparate trends may be reconciled by the following considerations. First, animal experiments typically compare low or deficient intakes of Se and antioxidant nutrients to superadequate intakes, whereas human experiments are constrained logistically and ethically to compare existing (usually adequate) intakes to those same intakes plus supplements. Thus animal experiments frequently examine the effects of nutrient deficiency and human studies (at least those in developed countries where cardiovascular diseases are of greatest concern) usually examine effects of additional nutrient intake in excess of dietary requirements. Second, experiments in mice, rats, and rabbits typically use young animals whose nutritional status is varied over a considerable portion of their life span, thus including the potential effects of nutrition at every stage of the disease process, whereas human experiments usually involve relatively short-term studies of high-risk individuals with late-stage or occult disease. Since much of an individual's lifetime risk for cardiovascular disease may be accrued decades before biomarkers or clinical signs of disease are detectable, the opportunity for dietary intervention in most human studies may be long since past. The present study was designed to address some of these issues by studying young men free of any signs of disease and increasing Se intake as much as allowable and long enough to ensure significantly increased tissue Se levels. Our failure to observe any effects on endothelial function in the present study suggests that the role of Se in human endothelial function is either critical only in younger subjects, is expressed only when intake is deficient, or is significant only in individuals with preexisting disease.

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