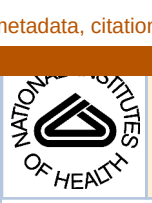

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Longitudinal association between toenail selenium levels and measures of subclinical atherosclerosis: the CARDIA trace element study

Pengcheng Xun[1,2], **Kiang Liu**[3], **J Steven Morris**[4], **Martha L Daviglus**[3], and **Ka He**[1,2]

[1] Department of Nutrition, Gillings School of Global Public Health and School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[2] Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[3] Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

[4] Research Reactor Center, University of Missouri-Columbia, Columbia, MO, USA

Abstract

Objectives—To examine the longitudinal association between toenail selenium levels and sub-clinical atherosclerosis over an 18-year period.

Methods—Toenail selenium concentrations were examined among 3112 Americans age 20–32 years in 1987 and measured by instrumental neutron-activation analysis. Subclinical atherosclerosis, including common, bulb and internal carotid intima-media thickness (CIMT), was measured in 2005 and coronary artery calcium (CAC) score in 2000 and 2005. General linear regression was developed examining the relation between toenail selenium levels and CIMTs, and logistic regression for repeated outcomes was employed estimating the risk of having CAC>0.

Results—After adjustment for potential confounders, no associations were observed between toenail selenium levels and CIMTs as well as CAC score. Comparing participants in the highest with the lowest quintile of selenium, the CIMT was 0.005mm (SE=0.008mm, P_{trend}=0.39), 0.018mm (SE=0.019mm, P_{trend}=0.49), and 0.017mm (SE=0.014mm, P_{trend}=0.21) thicker measured in common, bulb and internal carotid, respectively. The adjusted odds ratio of having CAC>0 was 0.95 (95%CI: 0.67–1.35; P_{trend}=0.999).

Conclusions—No associations were observed between toenail selenium and measures of subclinical atherosclerosis among American young adults. This study does not support an atherosclerotic mechanism of selenium for risk reduction of cardiovascular disease.

Keywords

toenail selenium; biomarker; sub-clinical atherosclerosis; carotid intima-media thickness; coronary artery calcium

Address for correspondence: Dr. Ka He, Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, 2221 McGavran-Greenberg #7461, Chapel Hill, NC 27599, USA, Tel: +1 919 843 2476, Fax: +1 919 966 7216, kahe@unc.edu.

Competing Interests Declaration: None declare.

1. Introduction

Because of its presence in selenocysteine and glutathione peroxidase, selenium, an essential trace element, has been recognized to play a role in protecting against oxidative damage.[1] Laboratory and observational studies indicate that antioxidants may protect cells from the adverse effects of free radicals and lipid peroxides. [2] Thus, it has been suggested that selenium may be beneficial in reducing the risk of cardiovascular diseases (CVD). [3]

Data from previous studies on the role of selenium in CVD risk were inconsistent. A systematic review published in 2006 summarizing 25 observational studies (11 case-control studies and 14 cohort studies) reported that selenium was inversely associated with coronary heart disease (CHD). However, the findings were not in agreement with those from 6 randomized clinical trials, in which no significant association was documented [4]. In addition, a recent secondary data analysis based on a large scale randomized trial also found no association between selenium supplementation (200 μg/d) and self-reported CVD endpoints [5]. Of note, data relating selenium to subclinical atherosclerosis are limited.

Literature suggests that selenium level in toenails is a reliable biomarker reflecting time-integrated body intake [6–11]. Also, computed tomography scanning of the coronary arteries for calcium (CAC) has been increasingly used as a noninvasive method of assessing early coronary artery diseases. The presence of CAC has been determined to be a predictor of future coronary events.[12] In addition, a number of studies have indicated that measures of carotid intima-media thickness (CIMT) including common CIMT (cCIMT), bulb CIMT (bCIMT) and internal CIMT (iCIMT) are powerful predicators of subclinical atherosclerosis.[13] However, few studies have used these measures simultaneously in a single cohort.

To determine the long-term association of selenium status with subclinical atherosclerosis, we prospectively examined toenail selenium levels in relation to measures of subclinical atherosclerosis in the Coronary Artery Risk Development in Young Adults (CARDIA) Trace Element Study.

2. Methods

2.1. Study participants

The CARDIA study is a multi-center, longitudinal study examining CVD risk factors in American young adults. Details of the study design have been published elsewhere [6]. Briefly, the cohort was established in 1985, comprised of 5115 Americans, age 18 to 30 years and roughly balanced by gender and ethnicity (African American and Caucasian) recruited from 4 metropolitan areas (Birmingham, AL; Chicago, IL; Minneapolis, MN and Oakland, CA). Follow-up examinations were conducted six times in 1987, 1990, 1992, 1995, 2000 and 2005. The average follow-up rate was 94.1%, and 69.4% participants in the original cohort returned in 2005. In the present study, 1987 was considered the study baseline since toenail specimens were collected then. A total of 4624 participants attended the examination in 1987. Of them, 4362 (94.3%) participants provided toenail clippings. In the analysis, we excluded 75 participants with missing data on selenium or toenail mass. We also excluded 28 participants with toenail selenium level ≥2 μg/g because this high concentration is very likely due to exogenous contamination based on our laboratory experience. We further excluded 282 participants who had any clinical CVD at baseline. In addition, we excluded 865 participants who had missing data on all 4 measures of subclinical atherosclerosis. After these baseline exclusions, a total of 3112 participants remained in the analysis.

All participants signed informed consent form. The study design, data collection and analyses were approved by the institutional review boards of the centers involved.

2.2. Exposure and outcome measures

2.2.1. Toenail selenium—Toenail clippings were collected with a stainless steel clipper from all 10 toes by the participants themselves during the clinical examination. All toenail clippings were processed with a washing procedure in a sonicator with deionized water. Selenium levels were analyzed by the instrumental neutron-activation analysis (NAA) at the University of Missouri Research Reactor. [14] Toenail specimens were treated in random order by the laboratory personnel who were blinded to other clinical measures. The average coefficient of variation in duplicated subsamples for the toenail selenium measure was 2.45% in the present study.

The toenail concentrations of selenium were suggested to be useful biomarkers of exposure in which a single sample was assumed to represent a long-term exposure since toenail clippings reflect a time period of 9 to 12 months of selenium exposure[10]. A study examined the reliability of toenail measure over six-year period. The Spearman correlation coefficient for the reproducibility of toenail selenium over 6 years was 0.48 [15]. In a pilot study conducted among 64 randomly selected CARDIA participants from Chicago Field Center, the Spearman correlation between toenail selenium concentrations in toenail clippings collected 20 years apart and measured by the same laboratory with same protocol was 0.56.

2.2.2. Subclinical atherosclerosis—High-resolution B-mode ultrasonography was used to capture images of the bilateral common, bulb and internal carotid arteries using a Logiq 700 ultrasound machine (General Electric Medical Systems) [16]. Five standardized B- mode images were acquired from each of the right and left carotid arteries in 2005. One measurement was made of the common carotid, two of the carotid bulb, and two of the internal carotid artery. Measurements were made at a central reading center by the readers blinded to all clinical information. The maximum of multiple measurements (4, 8 and 8, respectively) for cCIMT, bCIMT and iCIMT were defined as the maximum of the IMT of the near and far wall on both the left and right sides of common, bulb and internal carotid arteries. [17].

The CAC score was measured in 2000 and 2005 by computed tomography (CT) scan of the calcium in plaque on the walls of the arteries. At each examination, the means score of the 2 scans were obtained on the same occasion. The presence of CAC (Agatston score>0) was considered a marker of subclinical atherosclerosis [18].

2.3. Covariates

Demographic variables were collected via questionnaire. Smoking status was determined according to self report and serum cotinine [19]. Participants were classified into four groups: current smoker; former smoker; never smoker with passive exposure (self-report never smoker with serum cotinine concentrations 1 to 15 ng/ml); and never smoker without passive exposure (self-report never smoker with non-detectable serum cotinine). Serum cotinine concentration over 15ng/ml was used to determine current smokers who denied smoking. Alcohol intake was assessed through a self-administered questionnaire and interview-based dietary history. Fasting plasma high density lipoprotein (HDL) cholesterol was analyzed using enzymatic procedure after dextran sulfate-magnesium precipitation and low density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation [20]. Body weight and height were directly measured in light clothes without shoes, and body mass index (BMI; in kg/m^2) was classified into five groups: <21, 21–22.9, 23–24.9, 25–29.9 and ≥30 kg/m^2 [21]. Physical activity was assessed using the CARDIA Physical Activity History Questionnaire, an interviewer-based self-report of frequency of participation in each of 13 categories of sports and exercise over the previous 12 months [22,23].

2.4. Statistical analysis

Participants were divided into quintiles according to their toenail selenium levels. Group comparisons of characteristics at baseline were performed using analysis of variance, Kruskal-Wallis test or chi-square test as appropriate.

General linear regression model was used to examine the relation between toenail selenium concentrations and three CIMT measures. We considered three sequential models in the analysis: model 1 adjusted for age, gender, ethnicity, and study center; model 2 additionally adjusted for education, smoking status, alcohol consumption and physical activity; and model 3 additionally adjusted for LDL/HDL, systolic BP, fasting glucose, BMI, family history of heart disease and toenail mass.

Continuous CAC scores were not used as an outcome in the analysis due to the relatively low prevalence of CAC in this young adult cohort and the highly skewed data. Since the time of the intermediate event (CAC>0) was left interval-censored, logistic regression model of correlated outcome for repeated measures was employed to estimate the perspective odds ratios (ORs) and 95% confidence intervals (CIs) of having CAC>0, with a sequential covariates-adjusted strategy as described in the CIMT analysis.

Because of the repeated measurements for some important covariates, we used quadratic random-effect growth curve model over unequally spaced repeated measurements to reduce the within-person random measure error and to better represent the long-term average level of the covariates in the sensitivity analysis. We computed the area under the curve (AUC) for each covariate, and replaced the baseline values in the primary analysis with the AUC values to test the robustness of our findings [24].

All analyses were performed by using SAS version 9.1.3 (SAS Institute Inc, Cary, NC). $P \leq 0.05$ was considered statistically significant.

3. Results

Baseline characteristics of the study population are shown in Table 1. The median values of toenail selenium across quintiles were 0.69, 0.78, 0.84, 0.92 and 1.04 μg/g. As compared with the participants in the lowest quintile of toenail selenium levels, those in the highest quintile were slightly older, and more likely to be females, Caucasians, and non-current smokers. They also had a higher education level, lower symbolic BP and lower alcohol consumption. Toenail selenium levels varied geographically by study center. Selenium levels were relatively lower in men, African Americans, current smokers, and participants with high alcohol consumption and non-supplement users (Table 2).

In the general linear regression analysis, toenail selenium levels were not associated with any of the three CIMT measures after adjustment for age, gender, ethnicity, study center and other potential confounding factors (Table 3). Gender did not appreciably modify the results. Although the value of bCIMT in the highest quintile of toenail selenium was higher among Caucasian, the interaction of selenium and ethnicity was not statistically significant (Figure 1).

Similarly, no association between toenail selenium and CAC score was found (Table 4). As compared with the participants in the lowest quintile of selenium, the odds ratios of having CAC score>0 for those in the higher four quintiles were 0.80 (95%CI: 0.58, 1.10), 0.86 (95% CI: 0.62, 1.20), 0.87 (95%CI: 0.62, 1.23) and 0.95 (95%CI: 0.67, 1.35; p for trend = 0.999), respectively. Neither gender nor ethnicity appreciably modified the findings (data not shown).

A few sensitivity analyses were conducted to test the robustness of our findings (data not shown). First, when used the AUC values computed by quadratic random-effect model for repeatedly measured or time-dependent covariates including systolic BP, BMI, LDL/HDL ratio, fasting glucose and smoking (cumulative dose, pack-years), the results were not considerably changed. Second, when further adjusted for other antioxidants such as total alpha-tocopherol and beta-carotene, the results were again not materially changed. Third, since data on selenium supplement use were available in 1992 and 2005, we stratified data according to selenium supplement use. The results were generally consistent between supplement users and non-users. Four, since one of major sources of selenium intake is marine food, we included long-chain omega-3 polyunsaturated fatty acids and/or non-fried (broiled steamed, baked or raw) fish in the analysis. Our findings remained. Also, no interactions between selenium and long-chain omega-3 fatty acids or fish were found in this cohort. Finally, we created two combined measures of CIMTs, i.e. the average of bCIMT and iCIMT, and the average of cCIMT, bCIMT and iCIMT. No statistically significant associations were found.

4. Discussion

In this 18-year prospective follow-up study among American young adults, no longitudinal associations between toenail selenium levels and multiple measures of subclinical atherosclerosis were observed.

Data from a systematic review published in 2006 summarizing 14 prospective cohort studies and 11 case-control studies suggested an inverse association between selenium concentrations and CHD risk [4]. The inconsistency with our findings may partially be explained by different biomarkers used for measuring selenium status. For example, the 25 observational studies included in the systematic review measured whole blood, serum, erythrocyte or toenail selenium, which reflect different time frame of selenium exposure. Only one cohort study and two case-control studies measured toenail selenium. Of note, these three studies also showed no association between toenail selenium levels and total risk of CHD [8,9,11], though a possible protective role for myocardial infarction was reported [11].

In the same systemic review, a meta-regression model pooling six clinical trials published between 1989 and 2004 including 8884 participants in active treatment group and 8904 controls, found that selenium supplementation was not related to CHD [4]. This finding was confirmed by a randomized placebo-controlled multi-center trial (Selenium and Vitamin E Cancer Prevention Trail [SELECT]) comprised of 35,533 men. In addition, low-dose supplementation of a combination of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg beta carotene, 100μg selenium, and 20 mg zinc) found no beneficial effects on atherosclerosis progression measured by cCIMT in a clinical trial [25]. Of note, almost all previous clinical trials on selenium and CVD risk were conducted in men and were secondary data analyses. For example, the largest clinical trial (SELECT) on selenium was primarily designed to study prostate cancer in men. Also, the previous studies were conducted in middle aged or older individuals who were likely to had already had disease onset. Our study adds additional data on women and young adults. Moreover, selenium was used in combination with other vitamins or minerals in most of the clinical trials, which makes it impossible to isolate the specific effects of selenium

In addition to the unique study population, a few strengths of our study should be highlighted. First, toenail selenium level was measured, which is recognized as the best indicator of long-term selenium status. Second, multiple clinical measures were employed to determine subclinical atherosclerosis. The consistent findings across these clinical measures strengthened our results. Our study provides a mechanistic explanation or morphological evidence for the non-beneficial effects of selenium on CVD events. Third, participants were followed for 18

years. It allows us to study the long-term association between selenium and subclinical arthrosclerosis.

Some limitations also need to be considered. In essence, this study may share some common weaknesses of any observational study, e.g. unknown confounders. It is possible that random measurement error could prevent the detection of any weak or moderate long-term association, though a good long-term reproducibility of toenail measurement was suggested by one published study and our pilot study. Nevertheless, our findings are generally consistent with some randomized clinical trials. Also, toenail selenium was only analyzed once at baseline. Thus, we were unable to study the effects of changes in selenium status on sub-clinical atherosclerosis over the 18 years of followed-up period. For example, if participants with low selenium levels at baseline increased intakes of selenium later on, any possible association would be attenuated. However, the results were not materially changed when we excluded the participants who were self-identified selenium supplement users. In addition, our findings were based on a young cohort from four urban areas, which may limit the generalizability to other populations.

Selenium can incorporate into selenoproteins through a complex genetic mechanism encoded by the UGA codon [26]. Through selenoproteins, selenium plays an in important role in protection against oxidative stress, which has been hypothesized to lower CVD risk. Our study does not support an atherosclerotic mechanism of selenium for risk reduction in CVD. However, the possibility of non-atherosclerotic mechanisms of selenium in relation to CVD risk can not be ruled out. In addition selenium has a narrow safety margin. It has been suggested that selenium may be a double-edged sword in that excess selenium could promote type 2 diabetes, which is an established risk factor of CVD, due to its over antioxidant activity [27]. In the present study, bCIMT values were significantly higher among Caucasian participants in the highest quintile of toenail selenium than those in the lowest quintile, though the interaction between selenium and ethnicity was not significant. However, this phenomenon was not seen in cCIMT and iCIMT. Although all of these measures are recognized as markers of subclinical atherosclerosis, they may be surrogates of different parts of the atherosclerotic process [28]. In addition, bCIMT is more difficult to accurately measure as compared to other CIMT measures (e.g. cCIMT) because it can only be measured in limited angles that may not reflect the thickest portions of vessel wall [29]. Further studies are warranted.

Since the null association between selenium and subclinical atherosclerosis was found in the present study, several possibilities should be taken into consideration: 1) Low statistical power may be a reason for null association. However, there were 3112 participants and continuous outcome variables were used in this study, which guaranteed about 94.5% power to detect a difference of 0.05 mm between the highest and the lowest selenium quintiles with a standard deviation of 0.2 mm (with a Bonferroni correction of alpha level to 0.005, two sided). 2) If the temporal relation between the exposure measure and outcome did not encompass the whole latent period, we may observe a null association. However, toenail selenium provides a time-ingredient measure, and the 18 years of follow-up should be long enough to capture the latent period of atherosclerosis. Particularly, atherosclerosis is usually not reversible. 3) A negative confounder, i.e. a factor with an opposite association with exposure and outcome, could bias any possible association towards the null. However, the likelihood should be small since we carefully considered a number of potential confounding factors. Also, we used biomarkers for exposure and outcome rather than dietary measure and self-report disease state. 4) Non-differential laboratory measure errors of toenail selenium may attenuate an existing association. Our results were unlikely to be substantially explained by the random measure error. In other studies, toenail selenium assessed by the same method as our coordinating laboratory successfully predicted chronic diseases [30]. 5) A null association can be explained by insufficient variation of exposure. However, the selenium levels in this cohort ranged from

0.48 to 1.98μg/g, which indeed provided a wide variation and enabled us to examine the potential association with high sensitivity. 6) If the exposure variation falls within a flat portion of the total dose-response relationship, we may not be able to detect any association. However, this is unlikely to explain our findings. We constructed a nonparametric spline analysis, and there was no nonparametric non-linear association such as a “threshold” phenomenon found.

In conclusion, no longitudinal association between toenail selenium levels and subclinical atherosclerosis was observed among American young adults. Our findings do not support an atherosclerotic mechanism of selenium for risk reduction in CVD. We should be cautious to recommend selenium supplementation for CVD prevention.

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References

1. Rayman MP. The importance of selenium to human health. Lancet 2000;356:233–41. [PubMed: 10963212]
2. Lapenna D, de Gioia S, Ciofani G, et al. Glutathione-related antioxidant defenses in human atherosclerotic plaques. Circulation 1998;97:1930–4. [PubMed: 9609086]
3. Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair lo ngitudinal study. Lancet 1982;2:175–9. [PubMed: 6123886]
4. Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, Guallar E. Selenium and coronary heart disease: a meta-analysis. Am J Clin Nutr 2006;84:762–73. [PubMed: 17023702]
5. Lippman SM, Klein EA, Goodman PJ, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2009;301:39–51. [PubMed: 19066370]
6. Friedman GD, Cutter GR, Donahue RP, et al. CARDIA: study design, recruitment, and some characteristics of the examined subjects. J Clin Epidemiol 1988;41:1105–16. [PubMed: 3204420]
7. Hunter DJ, Morris JS, Chute CG, et al. Predictors of selenium concentration in human toenails. Am J Epidemiol 1990;132:114–22. [PubMed: 2356804]
8. Kardinaal AF, Kok FJ, Kohlmeier L, et al. Association between toenail selenium and risk of acute myocardial infarction in European men. The EURAMIC Study. European Antioxidant Myocardial Infarction and Breast Cancer. Am J Epidemiol 1997;145:373–9. [PubMed: 9054242]
9. Kok FJ, Hofman A, Witteman JC, et al. Decreased selenium levels in acute myocardial infarction. JAMA 1989;261:1161–4. [PubMed: 2915438]
10. Longnecker MP, Stampfer MJ, Morris JS, et al. A 1-y trial of the effect of high-selenium bread on selenium concentrations in blood and toenails. Am J Clin Nutr 1993;57:408–13. [PubMed: 8438776]
11. Yoshizawa K, Ascherio A, Morris JS, et al. Prospective study of selenium levels in toenails and risk of coronary heart disease in men. Am J Epidemiol 2003;158:852–60. [PubMed: 14585763]
12. Greenland P, LaBree L, Azen SP, Doherty TM, Detrano RC. Coronary artery calcium score combined with Framingham score for risk prediction in asymptomatic individuals. JAMA 2004;291:210–5. [PubMed: 14722147]
13. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation 2004;109:1089–94. [PubMed: 14993130]
14. Cheng TP, Morris JS, Koirtyohann SR, Spate VL, Baskett CK. The analysis of human nails for 24 elements via k0 and cyclic neutron activation analysis. Nuclear Instruments and Methods in Physics

Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment 1994;353:457–460.

15. Garland M, Morris JS, Rosner BA, et al. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer Epidemiol Biomarkers Prev 1993;2:493–7. [PubMed: 8220096]
16. Green D, Foiles N, Chan C, Schreiner PJ, Liu K. Elevated fibrinogen levels and subsequent subclinical atherosclerosis: the CARDIA Study. Atherosclerosis 2009;202:623–31. [PubMed: 18602107]
17. O'Leary DH, Polak JF, Kronmal RA, et al. Thickening of the carotid wall. A marker for atherosclerosis in the elderly? Cardiovascular Health Study Collaborative Research Group. Stroke 1996;27:224–31. [PubMed: 8571414]
18. Yan LL, Liu K, Daviglus ML, et al. Education, 15-year risk factor progression, and coronary artery calcium in young adulthood and early middle age: the Coronary Artery Risk Development in Young Adults study. JAMA 2006;295:1793–800. [PubMed: 16622141]
19. Houston TK, Person SD, Pletcher MJ, Liu K, Iribarren C, Kiefe CI. Active and passive smoking and development of glucose intolerance among young adults in a prospective cohort: CARDIA study. BMJ 2006;332:1064–9. [PubMed: 16603565]
20. Bachorik PS, Albers JJ. Precipitation methods for quantification of lipoproteins. Methods Enzymol 1986;129:78–100. [PubMed: 3724552]
21. He K, Liu K, Daviglus ML, et al. Magnesium intake and incidence of metabolic syndrome among young adults. Circulation 2006;113:1675–82. [PubMed: 16567569]
22. Pereira MA, FitzerGerald SJ, Gregg EW, et al. A collection of Physical Activity Questionnaires for health-related research. Med Sci Sports Exerc 1997;29:S1–205. [PubMed: 9243481]
23. Schmitz KH, Jacobs DR Jr, Leon AS, Schreiner PJ, Sternfeld B. Physical activity and body weight: associations over ten years in the CARDIA study. Coronary Artery Risk Development in Young Adults. Int J Obes Relat Metab Disord 2000;24:1475–87. [PubMed: 11126345]
24. Cook NR, Rosner BA, Chen W, Srinivasan SR, Berenson GS. Using the area under the curve to reduce measurement error in predicting young adult blood pressure from childhood measures. Stat Med 2004;23:3421–35. [PubMed: 15505884]
25. Zureik M, Galan P, Bertrais S, et al. Effects of long-term daily low-dose supplementation with antioxidant vitamins and minerals on structure and function of large arteries. Arterioscler Thromb Vasc Biol 2004;24:1485–91. [PubMed: 15217803]
26. Papp LV, Lu J, Holmgren A, Khanna KK. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal 2007;9:775–806. [PubMed: 17508906]
27. Stranges S, Marshall JR, Natarajan R, et al. Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. Ann Intern Med 2007;147:217–23. [PubMed: 17620655]
28. Mackinnon AD, Jerrard-Dunne P, Sitzer M, Buehler A, von Kegler S, Markus HS. Rates and determinants of site-specific progression of carotid artery intima-media thickness: the carotid atherosclerosis progression study. Stroke 2004;35:2150–4. [PubMed: 15243147]
29. Casella IB, Presti C, Porta RM, Sabbag CR, Bosch MA, Yamazaki Y. A practical protocol to measure common carotid artery intima-media thickness. Clinics 2008;63:515–20. [PubMed: 18719764]
30. Michaud DS, De Vivo I, Morris JS, Giovannucci E. Toenail selenium concentrations and bladder cancer risk in women and men. Br J Cancer 2005;93:804–6. [PubMed: 16175184]

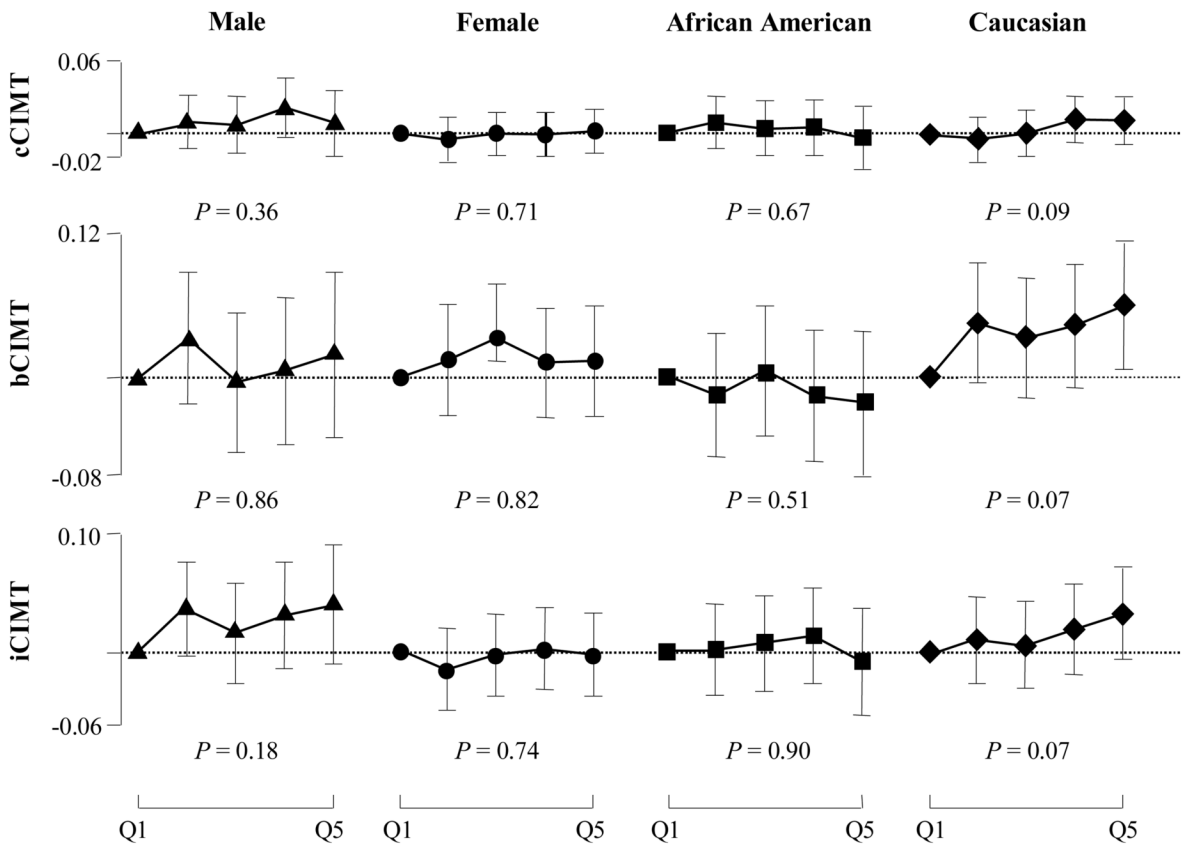

Figure 1.

Regression coefficients and 95% CIs according to toenail selenium levels stratified by gender and ethnicity.

cCIMT, bCIMT and iCIMT are common, bulb and internal carotid intima-media thickness, respectively. *P* value is test for trend across quintiles of toenail selenium levels.

Table 1

Baseline characteristics of study population according to quintiles of toenail selenium levels.[a]

	Quintiles of toenail selenium levels					
Characteristics	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	***P* –value[b]**
No. of participants	626	619	627	621	619	
Selenium levels, μg/g						
Median	0.69	0.78	0.84	0.92	1.04	
Range	0.48–0.74	0.74–0.81	0.81–0.88	0.88–0.96	0.96–1.98	
Age (year)	26.8±3.6	27.0±3.5	26.8±3.5	27.3±3.6	27.3±3.5	0.04
Female (%)	43.1	49.0	53.6	61.2	70.0	<0.01
African American (%)	60.7	45.6	43.7	41.9	34.7	<0.01
Education (year)	13.8±2.2	14.3±2.4	14.5±2.3	14.4±2.3	14.7±2.3	<0.01
Smoke status (%)						
Current	43.7	29.1	25.6	21.3	17.9	<0.01
Former	10.9	13.5	12.9	15.4	18.3	
Never, passive exposure	12.3	13.2	15.3	17.1	15.3	
Never, no passive exposure	33.1	44.3	46.2	46.2	48.5	
Alcohol consumption (ml/d)						
Median	11.0	6.8	6.1	6.1	5.1	<0.01
Inter-quartile range	2.0–25.5	0–17.6	0–15.2	0–16.2	0–13.2	
Physical activity (score)						
Median	328.0	312.0	316.0	312.0	313.0	0.93
Inter-quartile range	162.0–576.0	180.0–518.0	167.0–528.0	171.5–540.0	165.5–512.0	
LDL-C, mg/dL[c]	112.5±35.7	113.7±34.2	113.1±30.0	113.5±31.9	113.0±32.4	0.97
HDL-C, mg/dL[c]	54.5±15.1	54.0±13.8	54.7±13.9	56.0±14.4	55.3±13.5	0.12
LDL-C/HDL-C[c]	2.3±1.1	2.3±1.0	2.2±0.9	2.2±0.9	2.2±0.9	0.28
SBP (mmHg)	109.8±11.1	107.6±10.5	107.4±10.1	106.9±10.2	106.1±10.3	<0.01
Glucose, mg/dL	83.3±23.6	82.5±11.8	81.9±12.3	81.9±10.1	81.6±7.7	0.29
Body mass index	25.3±5.0	25.2±5.0	25.1±4.9	25.2±5.7	24.6±5.0	0.16
Toenail mass (g)	0.024±0.007	0.023±0.007	0.024±0.007	0.024±0.006	0.024±0.006	0.67

[a] Mean ± SD (all such values).

[b] P values were for any difference across all quintiles, obtained by using Analysis of Variance, Kruskal-Wallis test or Chi-square test as appropriate.

[c] LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.

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Table 2

Toenail selenium levels (μg/g) in subgroups.

Subgroup	No. of participants	Mean ± SD	*P*-value[a]
Center			
Birmingham	752	0.78±0.13	<0.01
Chicago	647	0.85±0.13	
Minneapolis	848	0.91±0.15	
Oakland	865	0.88±0.15	
Gender			
Male	1390	0.83±0.15	<0.01
Female	1722	0.88±0.15	
Ethnicity			
African American	1411	0.83±0.15	<0.01
Caucasian	1701	0.88±0.15	
Smoking Status			
Current	854	0.81±0.14	<0.01
Former	439	0.88±0.15	
Never, passive exposure	454	0.87±0.16	
Never, no passive exposure	1352	0.87±0.15	
Alcohol consumption (ml/d)			
0	1230	0.87±0.15	<0.01
0.1–11.9	910	0.87±0.16	
12.0–23.9	512	0.85±0.15	
≥24	460	0.82±0.15	
Selenium Supplementation			
No	2066	0.85±0.15	<0.01
Yes	1046	0.87±0.15	

[a]P values were for any difference across all subgroups obtained by using Analysis of Variance.

Table 3

Multivariable-adjusted relations of toenail selenium levels and measures of carotid intima-media thickness.[a]

	Quintiles of toenail selenium levels					
	1	**2**	**3**	**4**	**5**	***P* for trend**
cCIMT (*n*=2654)						
Median (mm)	0.801	0.787	0.774	0.773	0.771	
Model 1[b]	0	0.001±0.007	0.001±0.007	0.003±0.007	−0.002±0.008	0.90
Model 2[c]	0	0.004±0.007	0.005±0.007	0.009±0.008	0.005±0.008	0.49
Model 3[d]	0	0.002±0.007	0.003±0.007	0.008±0.007	0.005±0.008	0.39
bCIMT (*n*=2593)						
Median (mm)	0.980	0.982	0.981	0.963	0.962	
Model 1[b]	0	0.007±0.017	0.008±0.018	−0.004±0.018	0.005±0.019	0.99
Model 2[c]	0	0.015±0.018	0.019±0.018	0.009±0.018	0.021±0.019	0.43
Model 3[d]	0	0.013±0.017	0.016±0.018	0.007±0.018	0.018±0.019	0.49
iCIMT (*n*=2498)						
Median (mm)	0.760	0.758	0.774	0.784	0.781	
Model 1[b]	0	0.008±0.013	0.006±0.013	0.011±0.013	0.013±0.014	0.37
Model 2[c]	0	0.011±0.013	0.009±0.013	0.015±0.013	0.019±0.014	0.19
Model 3[d]	0	0.008±0.013	0.005±0.013	0.013±0.013	0.017±0.014	0.21

[a] Data are β coefficients ± standard errors, the p values range from 0.182 to 0.915. All models were constructed using general linear regression model. cCIMT: common carotid intima-media thickness; bCIMT: bulb carotid intima-media thickness; iCIMT: internal carotid intima-media thickness.

[b] Model 1: adjusted for age, gender, ethnicity (Caucasian, African American) and study center.

[c] Model 2: additionally adjusted for education (<12, 12, 13–15, 16, >16 year), smoking status (current smokers, previous smokers, never smokers with passive smoke exposure, and never smokers without passive smoke exposure), alcohol consumption (0, 0.1–11.9, 12.0–23.9, ≥24 ml/d) and physical activity (quintile).

[d] Model 3: additionally adjusted for LDL-C/HDL-C ratio (quintile), systolic blood pressure (quintile), glucose (quintile), body mass index (<21, 21–22.9,23–24.9,25–29.9, ≥30; kg/m^2), family history of heart disease (yes or no) and toenail mass (quintile).

Table 4

Multivariable-adjusted odds ratios and 95% CIs of having coronary artery calcium (CAC>0) by quintiles of toenail selenium levels[a]

	Quintiles of toenail selenium levels					
	1	**2**	**3**	**4**	**5**	***P* for trend**
No. of participants	600	596	602	597	596	
No. of events (CAC>0)	131	104	103	92	95	
Model 1[b]	1.00	0.75(0.55,1.01)	0.75(0.55,1.01)	0.72(0.52,1.00)	0.76(0.55,1.06)	0.15
Model 2[c]	1.00	0.84(0.61,1.14)	0.91(0.66,1.25)	0.90(0.64,1.26)	0.99(0.70,1.41)	0.86
Model 3[d]	1.00	0.80(0.58,1.10)	0.86(0.62,1.20)	0.87(0.62,1.23)	0.95(0.67,1.35)	0.999

[a] All models were constructed using logistic regression model of correlated outcome for repeated measures.

[b, c, d] Adjusted for covariates cited in table 2.