

# Toenail selenium as an indicator of selenium intake among middle-aged men in an area with low soil selenium<sup>1-3</sup>

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**ABSTRACT** Toenail selenium concentration has been proposed as a long-term (6–12 mo) indicator of human selenium status. This study investigated the association between toenail selenium concentration and selenium intake and other dietary factors among 166 urban men aged 55–69 y. The dietary information was collected by food records covering a 6-mo period. Toenail clippings were collected by mail 9–10 mo after food recording. The mean selenium intake from food was 42.5 µg/d and the dietary intake was equal to that of users and nonusers of selenium supplements. The mean toenail selenium concentration was 0.47 mg/kg. The mean selenium intake from supplements was 29.7 µg/d among supplement users. In the analysis of covariance the best predictors of toenail selenium concentration were selenium intake from supplements and food, and among supplement users dietary β-carotene also. *Am J Clin Nutr* 1993;57:662–5.

**KEY WORDS** Selenium intake, diet, toenail selenium, middle-aged men, selenium supplements

## Introduction

Some epidemiological studies have indicated that a low selenium status may be involved in the development of cardiovascular disease and cancer (1–5). Assessment of selenium status is usually performed by measuring serum selenium (short-term status), erythrocyte glutathione peroxidase activity (intermediate-term status), or toenail selenium concentration (long-term status) (6).

Information about long-term selenium status is desirable in epidemiological studies on selenium and chronic disease. Measurement of toenail selenium seems to be useful for this purpose. Toenail growth takes ≈4–12 mo (2, 7, 8) or even longer (9), and the selenium content of the toenails has been suggested to reflect the average intake of selenium over this period (7). The comprehensive difficulties in assessing dietary intake, present or past, argue for the use of biochemical markers in addition to estimated nutrient intake in nutritional studies.

Toenail analyses have been considered a valuable tool in large epidemiological studies because of the ease of collection and storage of nail samples. Hadjimarkos and Shearer (8) were the first to publish results from studies that used toenail clippings to assess selenium intake and status. Relationships between toenail selenium concentration and selenium intake and other dietary factors have since been reported in several studies (7,

10–12), but little is known of the determinants of toenail selenium concentration.

Dietary and supplementary intakes of selenium have been proposed as the most important determinants of toenail selenium concentration (11, 12). Correlations between selenium supplementation and toenail selenium of 0.32–0.50 have been reported (10), whereas correlation between dietary intake and toenail selenium concentration has been as high as 0.69 (11). In some studies smoking has been associated with lowered toenail selenium concentrations, presumably because of homeostatic mechanisms and increased metabolic needs in other tissues (10, 12).

This study reports the association between selenium intake and toenail selenium concentration in a middle-aged male population in Finland. The effect of selenium supplementation and other dietary and nondietary predictors of toenail selenium were also examined.

## Subjects and methods

### Subjects

The study population consisted of 190 urban men aged 55–69 y. The men were primarily enrolled in a validity study of a new dietary assessment method (13). Approximately 420 men from four large work places and from a section of Helsinki's population register were invited. Of the 217 men who entered the study, 27 were excluded because of missing forms, unreliable record keeping, or major changes in diet.

Participants were asked to keep food-consumption records for 24 d over 6 mo from April to October 1984. The recorded days represented all days of the week equally (13). Daily food consumption was converted to nutrient intake by using a computer-program system developed at the National Public Health Institute, Helsinki. The database currently comprises ≈1400 foods (900 mixed dishes and 500 individual food items) and

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200 nutrients. The selenium composition of Finnish foods is based on recent analyses (14). The computed food consumption and nutrient intake of the men have been reported in detail earlier (13).

The use of selenium supplements (type, brand, and dosage) was inquired about at the beginning, middle, and end of the study. A total of 30 men, at one or more inquiry, reported taking selenium supplements; 17 of them reported using organic selenium preparations (selenium enriched yeast, consisting almost entirely of selenomethionine). Only seven men reported using selenium supplementation at all three inquiries. The selenium intake from supplements for each individual was calculated as the mean of all three measurement times.

The median age of the men was 59 y, and 22% of them had retired. The social class distribution was as follows: 20% executives and management employees, 36% clerical and service employees, 40% blue-collar workers, and 4% of unknown occupation. Thirty-two percent of the men were current smokers.

The toenail clippings were taken and delivered by mail 9–10 mo after the last food record. The men were asked to take one clipping from each toe. One hundred-forty men returned toenail clippings after the first inquiry. The others received a letter of reminder  $\approx$  1 mo later. Toenail specimens were obtained from a total of 169 men, all of whom had  $\geq$  20 d of acceptable food recording.

Two men were excluded because of extremely high toenail selenium concentrations ( $>$  1.0 mg/kg) unlikely to have been attained from the Finnish diet alone. Alfthan et al (15) have shown that toenail clippings can be irreversibly contaminated by the use of selenium-containing antidandruff shampoos. One man was excluded because of missing smoking information. Thus, 166 men were included in the analyses.

The study was approved by the Ethical Issues Committee of the National Public Health Institute.

#### Selenium analysis

Each individual's batch of toenail clippings was pretreated with a neutral detergent, 1% sodium dodecylsulphate for 1 h, followed by ultrasound for 1 min before rinsing with copious amounts of purified water. The samples were then dried at 105 °C to constant weight. Large samples were divided in two so that the original samples weighed between 30 and 110 mg.

The selenium concentration was determined by fluorimetry on nitric-perchloric-sulphuric acid digests with a single test tube method (16) by using 2, 3-diaminonaphthalene for piaseleole formation.

In 120 cases the original sample of toenails was sufficient to allow for duplicate analyses. The mean ( $\pm$  SD) selenium concentration of the first sample was  $0.46 \pm 0.09$  mg/kg and that of the second  $0.47 \pm 0.09$  mg/kg; the difference was statistically nonsignificant (paired *t* test). In each analysis series a certified reference material (Animal Muscle, H-4; IAEA, Vienna, Austria) was included. The mean ( $\pm$  SD) of 34 measurements on 10 different days was  $0.30 \pm 0.02$  mg/kg as compared with the certified value of  $0.28 \pm 0.08$  mg/kg of the reference.

#### Statistical analysis

Statistical analyses were performed with SAS software programs (17) by using means, SDs, *t* test, and Pearson and Spearman correlations.

The association between toenail selenium and dietary and supplementary selenium was studied by analysis of covariance.

Factors possibly influencing selenium balance and the growth of nails were included in the analysis as potential covariates. Because smokers have been shown to have lower concentrations of toenail selenium (10, 12), smoking status was added in all models as a potential confounder, as were energy intake and body mass index. In a forward stepwise procedure the *F* test ( $P < 0.05$ ) was used for inclusion of the following variables: 1) protein, magnesium, and calcium because they are needed for the growth of nails (6, 9); 2) certain heavy metals because they are known to be antagonistic to selenium (6); 3) alcohol because it may reduce the concentration of serum selenium (18); and 4) vitamins C and E,  $\beta$ -carotene, polyunsaturated fatty acids, and iron because of their involvement in oxidation processes (19).

## Results

The mean dietary intake of selenium was  $42.5 \mu\text{g/d}$  (Table 1). Supplements provided an additional  $29.7 \mu\text{g Se/d}$  with a median intake of  $20.6 \mu\text{g/d}$ . The toenail selenium concentration was significantly higher among men taking selenium supplements than in nonusers. Both forms of supplementation (organic or inorganic selenium) seemed to have a similar increasing effect on the toenail selenium concentration (data not shown).

There were no differences in selenium intake or toenail selenium concentration by age, smoking, body mass index, or alcohol consumption (Table 2).

The Pearson correlations between toenail selenium and dietary factors are shown in Table 3. Among men not taking selenium supplements the correlations were weak, the highest being for dietary mercury ( $r = 0.18$ ,  $P < 0.05$ ), whereas among selenium supplement users they were generally higher. The highest correlation was found between toenail selenium and selenium intake from supplements ( $r = 0.60$ ,  $P < 0.001$ ). Moderate correlations were also found between toenail selenium and dietary mercury,  $\beta$ -carotene, selenium, polyunsaturated fatty acids, and vitamin E ( $r = 0.50$ – $0.32$ ). The Spearman correlation coefficients (results not shown) were very similar to the Pearson correlation coefficients.

In the analysis of covariance, selenium from supplements explained most of the variation in toenail selenium (Table 4). The effects of dietary or supplementary selenium on toenail selenium seemed to be similar, as indicated by the equal regression coefficients. Beta-carotene was a significant predictor of toenail selenium concentration among supplement users. Energy intake

TABLE 1  
Selenium intake and toenail selenium in middle-aged men\*

	Selenium supplement		
	Users ( <i>n</i> = 30)	Nonusers ( <i>n</i> = 136)	All ( <i>n</i> = 166)
Selenium intake from food ( $\mu\text{g/d}$ )	$42.3 \pm 9.1$	$42.6 \pm 13.0$	$42.5 \pm 12.3$
Supplementary selenium ( $\mu\text{g/d}$ )	$29.7 \pm 33.5$	—	—
Total selenium intake ( $\mu\text{g/d}$ )	$72.0 \pm 35.9$	$42.6 \pm 13.0$	$47.9 \pm 22.2$
Toenail selenium (mg/kg)	$0.53 \pm 0.15$	$0.45 \pm 0.06^\dagger$	$0.47 \pm 0.09$

\*  $\bar{x} \pm \text{SD}$ .

† Significantly different from users,  $P < 0.01$ .

TABLE 2  
Toenail selenium, by age, smoking status, body mass index, and alcohol use\*

	Toenail selenium mg/kg
Age (y)	
55-59 (n = 87)	0.46 ± 0.08
60-69 (n = 79)	0.48 ± 0.10
Smoking (cigarettes/d)	
None (n = 113)	0.47 ± 0.08
< 15 (n = 26)	0.48 ± 0.11
≥ 15 (n = 27)	0.45 ± 0.12
Body mass index †	
< 24.4 (n = 55)	0.46 ± 0.09
24.4-26.4 (n = 55)	0.47 ± 0.07
> 26.4 (n = 55)	0.48 ± 0.11
Alcohol use (g ethanol/d)	
< 2.3 (n = 55)	0.46 ± 0.08
2.3-14.0 (n = 57)	0.45 ± 0.07
> 14.0 (n = 54)	0.49 ± 0.12

\*  $\bar{x} \pm SD$ . There were no significant differences from the reference category (data not shown).

† In kg/m<sup>2</sup>.

was a significant determinant of toenail selenium concentration among background variables; smoking and body mass index were not. The consumption of smoking relevant food groups (fish, meat products, and cereals) accounting for the majority of the dietary selenium were also included in the analysis of covariance. These variables did not give any further explanations on the variation of toenail selenium.

## Discussion

The toenail selenium concentration of our population was similar to that found in other low selenium areas (7). In seleniferous areas the toenail selenium is two to threefold higher (8-11). The dietary intake of selenium was also low in our population when compared globally (6), but very similar to that found in other Finnish studies (20, 21).

For the assessment of current nutrient intake, food diaries are recommended as long as the number of recorded days is sufficient (22). In Finland, Mutanen (20) has suggested that ≥ 20 recording days are needed to give an estimate within 20% of an individual's long-term selenium intake. In the present study the selenium intake from food was based on 20-24 food diaries.

We found a slight correlation between dietary selenium and toenail selenium ( $r = 0.18$ ), and in multivariate analysis dietary selenium explained 5.8% of the variation in toenail selenium. In a study of 677 nurses the dietary selenium score had no relation to toenail selenium either in bivariate- or multiple-regression analysis (10). The discrepancy in results compared with ours is probably because only a single selenium value was applied to foods in US states, although the nurses were living in 11 different US states with large variations in soil selenium concentrations. In our study the men were living in one rather limited area, and the dietary assessment took place over a fairly short time period. On the other hand, in seleniferous areas se-

TABLE 3  
Pearson correlation coefficients between toenail selenium and selected dietary variables

	Selenium supplement		
	Users (n = 30)	Nonusers (n = 136)	All* (n = 166)
Selenium intake from foods	0.35	0.17	0.18
Selenium intake from supplements	0.60	—	—
Energy	-0.03	0.04	0.07
Protein	0.15	0.08	0.09
Alcohol	-0.05	0.04	0.04
Polyunsaturated fatty acids	0.34	0.05	0.22
Vitamin E	0.32	0.07	0.21
Vitamin C	0.14	0.05	0.10
β-carotene	0.46	0.05	0.20
Cadmium	0.15	0.14	0.17
Mercury	0.50	0.18	0.21
Calcium	-0.04	-0.09	-0.05
Magnesium	0.11	0.05	0.07
Iron	0.05	0.11	0.11

\*  $P < 0.01$  for correlation coefficients ≥ 0.20.

lenium intake has explained ≤ 35% of the variation in toenail selenium (12).

The range of selenium intake is rather wide in seleniferous areas and it is likely that our low association between dietary selenium and toenail selenium is at least partly due to a narrow range of selenium intake at a rather low intake.

The time lag between food recording and toenail clipping may have been too long to give the best estimate of association between dietary selenium and toenail selenium. Toenail samples were collected 9 mo after the last food records and 12 mo after the midpoint of the food-recording period. However, another study using the same data showed only slight seasonal variation in dietary selenium intake (CV 2%) and also that the ranking of individuals according to selenium intake was not considerably affected by season (23).

The effect of selenium supplementation on toenail selenium concentration was evident in this study. Measurement of duration and amount of supplementation is problematic, however. In our study only seven men reported supplement use on all

TABLE 4  
Determinants of toenail selenium concentration (mg/kg) in forward stepwise covariance analysis among 166 middle-aged men

Determinants	r	SE	P	Partial r <sup>2*</sup>
Intercept	0.413	0.060	< 0.001	—
Selenium from foods (μg/d)	0.002	0.001	0.002	0.058
Selenium from supplements among users (μg/d)	0.002	0.004	< 0.001	0.170
β-carotene intake (mg/d)				0.062
Selenium supplement nonusers	0.010	0.006	0.061	—
Selenium supplement users	0.026	0.008	0.002	—
Energy (MJ/d)†	-0.028	0.014	0.025	0.032
Smoking†	0.004	0.013	0.75	0.001
Body mass index†	0.001	0.002	0.72	0.001

\* Total  $r = 0.37$ .

† Included as potential confounders.

three occasions, but even irregular supplement use was associated with a considerable increase in the total intake. An effect of supplements has also been found at higher intakes of dietary selenium (10).

The correlation between dietary selenium and toenail selenium was slightly higher among selenium supplement users ( $r = 0.35$ ) than among nonusers ( $r = 0.17$ ), whereas among users the correlation between supplementary selenium and toenail selenium was moderate ( $r = 0.60$ ). These findings may indicate that at low intakes a significant amount of selenium is used for various metabolic demands, and thus the fluctuating surplus of selenium available for deposition in nails attenuates the correlation between dietary selenium and toenail selenium. At higher intakes the metabolic demands of selenium are likely to be saturated so that selenium accumulates in nails in a dose-response manner. This is partly supported by findings that plasma selenium and platelet glutathione peroxidase attain plateau concentrations in selenium supplementation studies (24, 25).

The bioavailability of selenium is influenced by the chemical form of selenium in the diet (19). Although we had no information on the chemical form in different foods, this factor presumably attenuated the association between dietary selenium and toenail selenium. In experiments on selenium-depleted rats the organic form increased toenail selenium concentrations twofold compared with inorganic selenium (26). In our study both organic and inorganic forms of supplementary selenium raised the toenail selenium concentration, but because of the small number of supplement users the effects of different selenium forms could not be reliably evaluated.

Dietary  $\beta$ -carotene intake was positively associated with toenail selenium concentration. Because this association was even stronger among selenium supplement users and it persisted after controlling for selenium intake from foods and supplements, it may be speculated that  $\beta$ -carotene competitively spares selenium in metabolic reactions that require antioxidants and thus leaves more selenium to be accumulated in the nails. The dietary sources of selenium and mercury were similar in Finland in 1984, and this probably explains the moderate univariate correlation between toenail selenium and dietary mercury intake. We could not confirm findings of others that smokers have lower toenail selenium concentrations than do nonsmokers (10, 12), possibly because of the low selenium intake of the men in our study.

The results of this study indicate that selenium intake contributed significantly to toenail selenium concentration. However, although toenail selenium concentrations do allow identification of individuals and groups with generally low dietary intake, they appear to be of limited value in distinguishing individuals in a population with low dietary intake who do not use supplements. Dietary  $\beta$ -carotene as a determinant of selenium status was an interesting finding because of their common antioxidant function, and should be further explored. Further studies should also examine if and when dietary selenium or toenail selenium concentrations should be preferred as the index of selenium status when studying the risk of disease. 

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