Toenail Selenium Levels and the Subsequent Risk of Prostate Cancer: A Prospective Cohort Study[1]

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Abstract

Results of a randomized controlled trial have suggested a protective effect of selenium against prostate cancer. Few other prospective studies have been conducted to confirm or refute this. The association between prostate cancer and baseline toenail selenium level was evaluated in the Netherlands Cohort Study, conducted among 58,279 men, aged 55–69 years at entry. In September 1986, the cohort members completed a questionnaire on risk factors for cancer and provided toenail clippings for determination of baseline selenium status. After 6.3 years of follow-up, 540 incident prostate carcinoma cases and 1,211 subcohort members with complete toenail selenium data were available for case-cohort analyses. In multivariate survival analysis, an inverse association between toenail selenium level and prostate cancer risk was observed. Incidence rate ratios in increasing selenium quintiles were 1.00 (ref), 1.05, 0.69, 0.75, and 0.69 (95% confidence interval, 0.48–0.99), respectively (P-trend = 0.008). This association persisted after exclusion of cases diagnosed during early follow-up. The inverse association was more pronounced in ex-smokers than current smokers, and unclear in never-smokers. Analysis of effect modification by intake of antioxidant vitamins C, E, and the carotenoids α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin showed a strong, significant interaction with β-cryptoxanthin, and to a lesser extent with vitamin C. These results confirm the hypothesis that higher selenium intake may reduce prostate cancer risk. Future research on optimum dose level is needed.

Introduction

It has been suggested that selenium may protect against cancer (1), particularly because of its role as component of glutathione peroxidase, an enzyme that is part of the cellular defense system against oxidative damage (2). Whereas animal studies have shown that selenium may have an inhibitory effect on carcinogenesis in various experimental models (3), ecological studies have also indicated an inverse association between dietary selenium levels and cancer mortality, among which was prostate cancer (4). After these early observations, prostate cancer was not as often investigated in additional analytical epidemiological studies as other cancer sites, but interest rose considerably when secondary results of a randomized controlled trial became available, showing a decreased risk of prostate cancer after supplementation with selenium-enriched yeast (5).

New controlled trials have been initiated to examine whether the result of the secondary analysis of Nutritional Prevention of Cancer Trial (5) can be confirmed (6, 7). Meanwhile, it is informative to find out whether the inverse association can also be found in case-control and cohort studies on selenium levels in serum or toenails and prostate cancer risk (8). Analytic epidemiological studies on selenium and cancer frequently use biological markers of selenium status, such as serum or toenail selenium levels, because estimation of dietary selenium is considered unreliable (9). Toenail selenium has gradually gained popularity as a biomarker of selenium status, after observations that this marker is an indicator of long-term selenium status (10, 11) and reflects differences in selenium intake (12, 13).

Several observational studies on selenium and prostate cancer have been reported since publication of the trial results by Clark *et al.* (5), including five cohort studies, all from the United States (14–18). Other recent studies were focused on dietary selenium and prostate cancer risk (19–21).

In the United States, generally high serum selenium levels are reported, whereas blood selenium levels in the Netherlands are intermediate between those reported from New Zealand and the United States, as is true for toenail selenium levels (22). We studied the relationship between toenail selenium levels and risk of prostate cancer in a cohort study in the Netherlands. The potential effect modification by smoking and intake of antioxidant vitamins C, E, and several carotenoids was also investigated.

Materials and Methods

The NLCS[3]. We will only briefly outline the study design because this has been reported in detail elsewhere (23). The NLCS was initiated in September 1986 and includes 58,279 men ages 55–69 years at the beginning of the study. The study population originated from 204 municipal registries throughout the country. At baseline, the cohort members completed a mailed questionnaire on usual diet and potential confounders, and provided toenail clippings. For reasons of efficiency in data

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[3] The abbreviations used are: NLCS, Netherlands Cohort Study; RR, rate ratio; CI, confidence interval.

processing and analysis, the case-cohort approach (24, 25) was used. In a case-cohort approach, cases are derived from the entire cohort (providing numerator information for calculation of cancer incidence rates), whereas accumulated person years at risk in the total cohort are estimated using a random subcohort sample (providing denominator information for the rates). In contrast to nested case-control sampling, this subcohort can be used for multiple disease end points. The subcohort ($n = 1{,}688$ men) was sampled directly after identification of all of the cohort members and has been followed up biennially for vital status information. Follow-up for incidence of prostate cancer was established by computerized record linkage with all nine cancer registries in the Netherlands and with the Dutch national database of pathology reports (PALGA; 26). No subcohort members were lost to follow-up, and completeness of follow-up of cancer has been estimated to be at least 96% (27). The present analysis is restricted to cancer incidence in 6.3 years of follow-up, from September 1986 to December 1992, because only information of toenail selenium status for cases diagnosed until 1992 were available at the time of the analysis. After excluding prevalent cases with cancer other than skin cancer, a total of 1,630 subcohort men and 704 incident, microscopically or histologically confirmed, primary prostate cancer cases were available after 6.3 years of follow-up.

Exposure Status. The self-administered questionnaire on diet has also been described in detail elsewhere (28). Mean daily nutrient intakes were calculated using the computerized Dutch food composition table (29). For carotenoids, a recently developed food composition database was used (30). About 7% of the subjects were excluded because of incomplete or inconsistent dietary questionnaire data (28). Toenail clippings had been provided by 565 patients with prostate cancer (80.3%) and by 1282 (78.7%) subcohort members.

Toenail selenium analyses were carried out by the Interfaculty Reactor Institute at Delft University (Delft, the Netherlands) using Instrumental Neutron Activation Analysis. This method and the use in the NLCS have been described previously (31–33). Because of problems with the detection of selenium in samples weighing <10 mg, and sometimes in samples with very high calcium contents, 44 and 71 specimens were excluded from the prostate cancer and subcohort groups, respectively. Toenail selenium data were available for analysis from 522 prostate cancer cases and 1211 subcohort members.

Statistical Analyses. To evaluate the potential influence of prediagnostic cancer on toenail selenium levels we categorized the cases according to the year of follow-up in which the diagnosis was made. Mean toenail selenium levels of prostate cancer cases were compared according to year of follow-up; differences were tested using a *t* test. Toenail selenium levels were categorized into quintiles according to the distribution in the subcohort. Incidence RRs and corresponding 95% CIs for prostate cancer were estimated using exponentially distributed failure time regression models (34) with the Stata statistical software package (35). SEs were estimated using the robust Hubert-White sandwich estimator to account for additional variance introduced by sampling from the cohort. This method is equivalent to the variance-covariance estimator presented by Barlow (36), and Lin and Ying (37). Because of skewedness to the right, continuous data on toenail selenium concentrations were normalized with a $\log_e$ transformation. The RR was calculated per standard unit (z-score) of toenail selenium levels. This is comparable with an increment of 0.190 μg/g selenium.

Variables that were considered as potential confounders were age, a family history of prostate cancer, level of education, and alcohol intake after results from previous analyses on associations with prostate cancer risk in the NLCS (38, 39). Because smoking is an important predictor of decreased selenium levels (12, 31), analyses were additionally controlled for smoking. Total energy and total fat intake were not considered as potential confounding factors, because in this population no association with prostate cancer risk was observed (40). The same applies to vegetable and fruit consumption (41). Subgroup analyses conditional on cigarette smoking status (never/ex/current) were performed to evaluate interaction of selenium with smoking. Previous studies (32, 42–44) have suggested a potential interaction between vitamin levels and selenium regarding cancer risk. Therefore, we evaluated the interaction with intake of antioxidant vitamins C, E, and the carotenoids α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin, by stratifying the results on high *versus* low intake status of the subjects. Low and high were defined as the two lowest quintiles and the two highest quintiles of intake of the mentioned vitamins and carotenoids, respectively. Statistical tests for interaction were based on Wald statistics. Advanced prostate cancer cases (T_{3-4}, M_0; T_{0-4}, and M_1; Ref. 38) were evaluated separately to test the hypothesis that selenium is more strongly inversely related to advanced prostate tumors (14, 15).

Table 1 Toenail selenium levels (μg/g) in prostate cancer cases according to year of follow-up, NLCS 1986–1992

Mean (±SD) selenium levels in male subcohort members were 0.547 (±0.126) μg/g ($n = 1211$).

Year of follow-up	No. of subjects	Toenail selenium level (μg/g)		
		Mean	SD	*P*
All cases	540	0.530	0.090	
1	52	0.524	0.093	0.193
2	50	0.515	0.077	0.079
3	72	0.526	0.090	0.196
4	106	0.534	0.082	0.535
5	95	0.520	0.095	0.044
6	124	0.539	0.095	reference
7	41	0.551	0.087	

Results

The mean (± SD) toenail selenium level in prostate cancer cases was 0.530 (± 0.090) μg/g, whereas in the male subcohort members this value was 0.547 (± 0.126) μg/g (Table 1). When cases were categorized with respect to the year of follow-up in which they were diagnosed, mean toenail selenium levels did not show a trend across time. Early diagnosed cases did not have significantly lower selenium values than cases diagnosed in later years (Table 1).

Toenail selenium was inversely associated with prostate cancer risk in age-adjusted analyses and multivariate analyses (Table 2). The age-adjusted RR was 0.63 (95% CI, 0.45–0.90) comparing highest to lowest selenium quintiles. After adjustment for age, family history of prostate cancer, level of education, and smoking, this RR was 0.69 (CI, 0.48–0.99) comparing highest to lowest selenium quintile (*P*-trend <0.008). When toenail selenium was analyzed as continuous variable, the RR was decreased by 10% for each increment of one standard unit of toenail selenium (0.190 μg/g). The reduced prostate cancer risk appeared to be restricted to the three highest quintiles of toenail selenium. Additional correction for alcohol intake yielded similar results (data not shown); therefore, this variable was not included in additional regression models.

Table 2 RR of prostate cancer according to toenail selenium levels, NLCS 1986–1992

Quintile of toenail Se (boundaries in μg/g)	Quintile median (μg/g)	All years of follow-up				First year excluded
		Cases in cohort	Person years in subcohort	RR[a] (95% CI)	RR[b] (95% CI)	RR[b] (95% CI)
1 (≤0.467)	0.433	125	1444	1.00[c]	1.00[c]	1.00[c]
2 (0.467–≤0.514)	0.494	139	1457	1.03 (0.75–1.42)	1.05 (0.74–1.47)	1.05 (0.74–1.49)
3 (0.514–≤0.560)	0.537	104	1494	0.74 (0.53–1.03)	0.69 (0.48–1.00)	0.68 (0.47–1.00)
4 (0.560–≤0.616)	0.585	83	1437	0.71 (0.50–1.01)	0.75 (0.51–1.09)	0.76 (0.51–1.11)
5 (>0.616)	0.663	89	1462	0.63 (0.45–0.90)	0.69 (0.48–0.99)	0.70 (0.48–1.01)
P for linear trend increment in standard units[d]				0.001	0.008	0.012
				0.87 (0.79–0.95)	0.90 (0.81–0.99)	0.91 (0.82–1.00)

[a] Adjusted for age (years, continuous).
[b] Adjusted for age (years), family history of prostate cancer (no/yes), number of cigarettes/day, years of cigarette smoking, level of education (low, medium, high).
[c] Reference category.
[d] z-scores based on In-transformed toenail selenium levels (increment of 0.190 μg/g).

Table 3 RR of prostate cancer according to toenail selenium levels and smoking status, NLCS 1986–1992

Smoking status	No. of cases	Quintile of toenail selenium (boundaries in μg/g)					*P*
		1 (≤0.467)	2 (≤0.514)	3 (≤0.560)	4 (≤0.616)	5 (>0.616)	
Never[a]	72	1.00[b]	2.28 (0.80–6.47)	0.64 (0.23–1.76)	0.33 (0.09–1.18)	1.19 (0.48–2.92)	0.412
Ex[c]	300	1.00[b]	0.87 (0.51–1.49)	0.53 (0.31–0.92)	0.79 (0.45–1.37)	0.46 (0.27–0.79)	0.003
Current[c]	168	1.00[b]	0.88 (0.51–1.52)	0.95 (0.51–1.74)	0.53 (0.25–1.14)	0.97 (0.42–2.22)	0.383

[a] Adjusted for age (years), family history of prostate cancer (no/yes), level of education (low, medium, high).
[b] Reference category.
[c] Additionally adjusted for number of cigarettes/day, years of cigarette smoking.

Analysis excluding cases from the first year of follow-up resulted in essentially similar results (Table 2).

When analyses were conducted separately for subgroups according to smoking status, the inverse association between selenium and prostate cancer was significant among ex-smokers (*P*-trend = 0.003; Table 3). Whereas in current smokers the RRs for second to fifth selenium quintiles were all decreased, this inverse association was not as consistent as in ex-smokers (*P*-trend = 0.383). For never smokers, the RRs in quintiles 3 and 4 were appreciably lower than one, but this was not true for quintile 2 and 5. There was no clear direction of the association in this group. The interaction between selenium and smoking status was statistically significant ($P = 0.026$).

To evaluate effect modification of the association between toenail selenium and prostate cancer risk by intake level of antioxidant vitamins C and E, and several carotenoids, we also tested whether the association with prostate cancer depended on intake of these antioxidants (Table 4). The inverse selenium-prostate cancer association was significant (*P*-trend = 0.025) and stronger in those with a relatively low vitamin C intake than in those with a relatively high intake (*P*-trend = 0.249); the *P* for the test of interaction was 0.074. For vitamin E, there was no evidence for interaction with selenium (*P*-interaction = 0.555). For α-carotene and β-carotene, the association between selenium and prostate cancer was somewhat stronger in those men with a relatively low intake of these carotenoids, but *P*s for the interaction tests were 0.320 and 0.204, respectively. A strong interaction was found with intake of β-cryptoxanthin (*P*-interaction = 0.005). In those with a relatively low β-cryptoxanthin intake there was a strong inverse association, with the RR for highest *versus* lowest selenium quintile estimated at 0.25 (CI, 0.12–0.54; *P*-trend <0.001). On the other hand, in those with a relatively high β-cryptoxanthin intake, there was no significant association. For lutein/zeaxanthin, the association between selenium and prostate cancer was also stronger and more consistent in those with relatively low intakes of these carotenoids, but no evidence of interaction was found (*P*-interaction = 0.780). For lycopene, there was no clear difference between the two intake strata (*P*-interaction = 0.233). With the exception of vitamin E and lycopene, these analyses indicate that the selenium effect on prostate cancer was more apparent in those subjects with a relatively low intake of dietary antioxidants. Table 5 shows the RRs for toenail selenium in case subgroups of localized and advanced prostate tumors. The inverse association with selenium was seen in both groups (Table 5).

Discussion

In this prospective cohort study with many prostate cancer cases, a statistically significant inverse trend was found between the toenail selenium level and the risk of prostate cancer. Toenail clippings have been used as measures of long-term selenium intake. Toenail selenium level roughly reflected the selenium intake in the previous 3 months to 1 year in a 1-year feeding study (45), with a correlation coefficient of 0.67 between the two. In the NLCS, we could use prediagnostic toenail selenium levels as measure of exposure. We showed that selenium levels were not decreased in cases diagnosed during early follow-up. Therefore, information bias is not likely to have influenced our results. Furthermore, selection bias is also unlikely because of the high completeness of follow-up of cases and subcohort members (27, 46). The large number of prostate cancer cases in the NLCS resulted in a relatively high power in overall analyses, and we were also able to investigate different subgroups based on tumor characterization (advanced cases) or other characteristics of our study population (low and high intake of vitamins/carotenoids). There are also some limitations to our study. In the Nurses' Health Study, a correlation coefficient of 0.48 was found between toenail selenium levels for

Table 4 RR[a] of prostate cancer according to toenail selenium levels by category of vitamin or carotenoid intake, NLCS 1986–1992

Group	No. of cases	Quintile of toenail selenium (boundaries in μg/g)					*P*
		1 (≤0.467)	2 (≤0.514)	3 (≤0.560)	4 (≤0.616)	5 (>0.616)	
Vitamin C intake[b]							
Low	182	1.00[c]	0.71 (0.39–1.31)	0.36 (0.18–0.71)	0.66 (0.35–1.25)	0.46 (0.24–0.89)	0.025
High	220	1.00[c]	1.06 (0.62–1.82)	1.28 (0.71–2.29)	0.77 (0.43–1.38)	0.80 (0.45–1.40)	0.249
Vitamin E intake[b]							
Low	204	1.00[c]	1.23 (0.69–2.20)	0.55 (0.30–1.01)	0.80 (0.42–1.52)	0.70 (0.38–1.28)	0.083
High	180	1.00[c]	0.80 (0.46–1.41)	0.85 (0.46–1.57)	0.76 (0.40–1.44)	0.61 (0.33–1.13)	0.147
α-carotene intake[b]							
Low	213	1.00[c]	0.77 (0.43–1.37)	0.49 (0.27–0.89)	0.62 (0.35–1.13)	0.50 (0.27–0.92)	0.022
High	187	1.00[c]	1.33 (0.76–2.33)	1.19 (0.64–2.19)	0.93 (0.49–1.77)	0.62 (0.33–1.15)	0.058
β-carotene intake[b]							
Low	214	1.00[c]	0.69 (0.38–1.24)	0.38 (0.20–0.70)	0.51 (0.27–0.95)	0.50 (0.27–0.92)	0.019
High	185	1.00[c]	1.15 (0.67–1.96)	1.13 (0.62–2.08)	0.84 (0.45–1.58)	0.62 (0.34–1.13)	0.070
β-cryptoxanthin intake[b]							
Low	166	1.00[c]	0.89 (0.50–1.60)	0.44 (0.23–0.83)	0.53 (0.26–1.05)	0.25 (0.12–0.54)	<0.001
High	241	1.00[c]	1.25 (0.71–2.18)	1.42 (0.79–2.54)	1.12 (0.62–2.04)	1.21 (0.68–2.16)	0.696
Lycopene intake[b]							
Low	182	1.00[c]	0.75 (0.40–1.39)	0.39 (0.20–0.75)	0.53 (0.28–1.02)	0.41 (0.21–0.80)	0.005
High	196	1.00[c]	1.06 (0.62–1.80)	1.05 (0.57–1.92)	0.49 (0.25–0.94)	0.49 (0.27–0.92)	0.003
Lutein/zeaxanthin intake[b]							
Low	206	1.00[c]	0.76 (0.41–1.39)	0.57 (0.30–1.05)	0.64 (0.34–1.20)	0.64 (0.34–1.21)	0.146
High	202	1.00[c]	1.24 (0.72–2.13)	0.89 (0.48–1.65)	1.04 (0.57–1.87)	0.79 (0.43–1.44)	0.363

[a] Adjusted for age (years), family history of prostate cancer (no/yes), number of cigarettes/day, years of cigarette smoking, level of education (low, medium, high).
[b] Low and high are defined as the two lowest quintiles and the two highest quintiles of intake, respectively. For vitamin C, the cutoff values for low and high were: ≤82.20, >101.30 mg/day; for vitamin E the corresponding values were: ≤11.86, >15.50 mg/day, for α-carotene: ≤0.47, >0.67 mg/day, for β-carotene: ≤2.38, >2.98 mg/day, for β-cryptoxanthin: ≤0.07, >0.13 mg/day, for lycopene: ≤0.55, >0.89 mg/day and for lutein/zeaxanthin: ≤2.15, >2.60 mg/day.
[c] Reference category.

Table 5 RRs for prostate cancer according to toenail selenium levels in subgroups of localized (T_{0-2}, M_0) and advanced (T_{3-4}, M_0; T_{0-4}, M_1) prostate tumors, NLCS 1986–1992

Quintile of toenail selenium (boundaries in μg/g)	Localized tumors (*n* = 189)	Advanced tumors (*n* = 183)
	RR[a] (95% CI)	RR[a] (95% CI)
1 (≤0.467)	1.00[b]	1.00[b]
2 (0.467–≤0.514)	1.29 (0.79–2.10)	0.81 (0.50–1.33)
3 (0.514–≤0.560)	0.79 (0.46–1.35)	0.59 (0.35–1.00)
4 (0.560–≤0.616)	0.74 (0.42–1.33)	0.48 (0.27–0.86)
5 (>0.616)	0.72 (0.42–1.24)	0.62 (0.37–1.05)
P for linear trend	0.043	0.020

[a] Adjusted for age (years), family history of prostate cancer (no/yes), number of cigarettes/day, years of cigarette smoking, level of education (low, medium, high).
[b] Reference category.

specimens taken 6 years apart (47). This moderate reliability may have resulted in some misclassification of exposure. Another potential limitation is that we cannot exclude that residual confounding from unidentified confounders has influenced our results. By limiting our analysis to the follow-up period 1986–1992, when prostate-specific antigen testing was not a common screening routine in the Netherlands (48), we should not expect a confounding effect of this factor.

Our observations that persons with an elevated toenail selenium level have significantly lower prostate cancer risk are in agreement with results of several other recent cohort studies, analyzed in a nested case-control manner (14–16, 18), but not all (17). Whereas these publications from United States studies specifically dealt with prostate cancer, in earlier cohort studies prostate cancer was studied with much smaller case numbers as part of an initial overall cancer risk assessment. In these studies, nonsignificant inverse associations were reported with prostate cancer in United States studies (44, 49, 50) but not in a Finnish cohort (1). Whereas most of the aforementioned studies were based on serum selenium, the study of Yoshizawa *et al.* (14), Helzlsouer *et al.* (16), and the NLCS were based on toenail selenium. One case-control study using toenail selenium found no association (51), whereas another case-control study on plasma selenium observed an inverse association (52).

Our cohort study, which entails the largest number of prostate cancer cases studied until now, did not find such a strong inverse association as the other large cohort studies; the relative risk in our upper selenium quintile was 0.69, whereas relative risks of 0.4–0.5 have been reported in other studies for the highest quintile (14–16). This may be the result of a smaller exposure range and of the moderate selenium exposure in the Netherlands compared with the United States (22, 31, 53). It might be that stronger inverse associations can be found with higher selenium exposure, although not all of the United States studies support this (17). One cohort study (19) and most case-control studies (20, 21, 54, 55) have investigated selenium intake with respect to prostate cancer. Overall, no association was found, but estimation of dietary selenium intake is considered unreliable (9, 31).

Several potential mechanisms have been put forward to explain the anticarcinogenic effects of selenium. These include antioxidant effects, inhibition of normal and malignant cell proliferation and tumor growth, increased apoptosis, and stimulation of the immune system and of DNA repair (56–61). The antioxidant role of selenium has received most attention. Therefore, we evaluated the effect of modification of smoking, because smoking induces oxidative stress (62, 63). Our finding that the inverse association between selenium and prostate cancer was present in smokers (particularly ex-smokers) was partially concordant with results of Nomura *et al.* (15), although they found a stronger association among current smokers. However, no association between serum selenium and prostate can-

cer was observed in the beta-Carotene and Retinol Efficacy Trial, conducted among past and current smokers (17). Yoshizawa *et al.* (14) reported that the inverse association with selenium persisted among never and ex-smokers. Recently, results from the Nutritional Prevention of Cancer Trial (53) showed that the protective effect of selenium on total cancer risk was more pronounced in ex-smokers. Unfortunately, they did not specifically analyze prostate cancer in this respect.

Also from the antioxidant viewpoint, we evaluated possible effect modification by other dietary antioxidants, given previous results on these interactions with respect to lung and bladder cancer (32, 42). Overall, with the exception of vitamin E, these analyses indicated that the decreased prostate cancer risk associated with high toenail selenium was more apparent in subjects with a relatively low intake of dietary antioxidant vitamins and carotenoids than in those with higher intakes of these nutrients. The interaction was most prominent with vitamin C and β-cryptoxanthin. The lack of interaction with vitamin E is of interest because a new large prostate cancer prevention trial (Selenium and Vitamin E Cancer Prevention Trial, SELECT) has been started in which efficacy of selenium, α-tocopherol, and their combination in preventing prostate cancer will be studied, following promising results from separate trials (5, 64). Our data indicate that the association of selenium with prostate cancer does not depend on vitamin E intake level, as was found by others (14, 19). Helzlsouer *et al.* (16) reported dependency of the selenium effect on γ-tocopherol instead of α-tocopherol.

Whereas the observed interactions did not reach statistical significance for most of the considered vitamins/carotenoids (which can also be attributable to still low numbers in subgroups), the interaction with β-cryptoxanthin intake was significant. We reported earlier a positive association between β-cryptoxanthin and prostate cancer (65), as was found in other studies (55, 66, 67). The currently observed interaction between selenium and β-cryptoxanthin in the NLCS suggests that selenium only shows a protective effect regarding prostate cancer when β-cryptoxanthin intake is low. The data suggest also that when β-cryptoxanthin intake is high, selenium levels are not influencing prostate cancer risk. Because only few studies have evaluated specific carotenoids besides β-carotene in relation to prostate cancer (*e.g.* Ref. 68), and none have looked at interactions between selenium and other carotenoids, it is difficult to draw firm conclusions at this point. Additional epidemiological studies are needed to study this observed interaction and, if confirmed, laboratory studies are warranted to investigate the possible mechanisms behind this interaction. Altogether, our analyses of interaction with dietary antioxidants support the role of selenium as antioxidant in anticarcinogenesis.

The selenium-prostate cancer association was observed with both localized and advanced tumors. Nomura *et al.* (15) found a stronger inverse association with advanced prostate tumors than with localized tumors. In another cohort study, only advanced prostate tumors were considered (14).

In conclusion, this study from the Netherlands confirms earlier reports that subjects with elevated toenail selenium levels have a reduced risk of prostate cancer. More randomized controlled trials are needed to determine optimal dose levels and to evaluate whether selenium may only be protective among ex-smokers. Additional studies are also needed on interactions with antioxidant vitamins and carotenoids, particularly β-cryptoxanthin.

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References

1. Knekt, P., Aromaa, A., Maatela, J., Alfthan, G., Aaran, R. K., Hakama, M., Hakulinen, T., Peto, R., and Teppo, L. Serum selenium and subsequent risk of cancer among Finnish men and women. J. Natl. Cancer Inst., *82:* 864–868, 1990.

2. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., and Hoekstra, W. G. Selenium: biochemical role as a component of glutathione peroxidase. Science (Wash. DC), *179:* 588–590, 1973.

3. Combs, G. F. Selenium. *In:* T. E. Moon and M. S. Micozzi (eds.), Nutrition and Cancer Prevention, Investigating the Role of Micronutrients, pp. 389–420. New York, NY: Marcel Dekker, 1989.

4. Schrauzer, G. N., White, D. A., and Schneider, C. J. Cancer mortality correlation studies–III: statistical associations with dietary selenium intakes. Bioinorg. Chem., *7:* 23–31, 1977.

5. Clark, L. C., Combs, G. F., Jr., Turnbull, B. W., Slate, E. H., Chalker, D. K., Chow, J., Davis, L. S., Glover, R. A., Graham, G. F., Gross, E. G., Krongrad, A., Lesher, J. L., Jr., Park, H. K., Sanders, B. B., Jr., Smith, C. L., and Taylor, J. R. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. JAMA, *276:* 1957–1963, 1996.

6. Clark, L. C., and Marshall, J. R. Randomized, controlled chemoprevention trials in populations at very high risk for prostate cancer: Elevated prostate-specific antigen and high-grade prostatic intraepithelial neoplasia. Urology, *57:* 185–187, 2001.

7. Klein, E. A., Thompson, I. M., Lippman, S. M., Goodman, P. J., Albanes, D., Taylor, P. R., and Coltman, C. SELECT: the next prostate cancer prevention trial. Selenum and Vitamin E Cancer Prevention Trial. J. Urol., *166:* 1311–1315, 2001.

8. Platz, E. A., and Helzlsouer, K. J. Selenium, zinc, and prostate cancer. Epidemiol. Rev., *23:* 93–101, 2001.

9. Levander, O. A. Considerations on the assessment of selenium status. Fed. Proc., *44:* 2579–2583, 1985.

10. Willett, W. Nutritional Epidemiology. New York: Oxford University Press, 1990.

11. Morris, J. S., Stampfer, M. J., and Willett, W. Dietary selenium in humans; toenails as an indicator. Biol. Trace. Elem. Res., *5:* 529–537, 1983.

12. Hunter, D. J., Morris, J. S., Chute, C. G., Kushner, E., Colditz, G. A., Stampfer, M. J., Speizer, F. E., and Willett, W. C. Predictors of selenium concentration in human toenails. Am. J. Epidemiol., *132:* 114–122, 1990.

13. Swanson, C. A., Longnecker, M. P., Veillon, C., Howe, M., Levander, O. A., Taylor, P. R., McAdam, P. A., Brown, C. C., Stampfer, M. J., and Willett, W. C. Selenium intake, age, gender, and smoking in relation to indices of selenium status of adults residing in a seleniferous area. Am. J. Clin. Nutr., *52:* 858–862, 1990.

14. Yoshizawa, K., Willett, W. C., Morris, S. J., Stampfer, M. J., Spiegelman, D., Rimm, E. B., and Giovannucci, E. Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. J. Natl. Cancer Inst., *90:* 1219–1224, 1998.

15. Nomura, A. M., Lee, J., Stemmermann, G. N., and Combs, G. F., Jr. Serum selenium and subsequent risk of prostate cancer. Cancer Epidemiol. Biomark. Prev., *9:* 883–887, 2000.

16. Helzlsouer, K. J., Huang, H. Y., Alberg, A. J., Hoffman, S., Burke, A., Norkus, E. P., Morris, J. S., and Comstock, G. W. Association between α-tocopherol, γ-tocopherol, selenium, and subsequent prostate cancer. J. Natl. Cancer Inst., *92:* 2018–2023, 2000.

17. Goodman, G. E., Schaffer, S., Bankson, D. D., Hughes, M. P., and Omenn, G. S. Predictors of serum selenium in cigarette smokers and the lack of association with lung and prostate cancer risk. Cancer Epidemiol. Biomark. Prev., *10:* 1069–1076, 2001.

18. Brooks, J. D., Metter, E. J., Chan, D. W., Sokoll, L. J., Landis, P., Nelson, W. G., Muller, D., Andres, R., and Carter, H. B. Plasma selenium level before diagnosis and the risk of prostate cancer development. J. Urol., *166:* 2034–2038, 2001.

19. Hartman, T. J., Albanes, D., Pietinen, P., Hartman, A. M., Rautalahti, M., Tangrea, J. A., and Taylor, P. R. The association between baseline vitamin E, selenium, and prostate cancer in the α-tocopherol, β-carotene cancer prevention study. Cancer Epidemiol. Biomark. Prev., *7:* 335–340, 1998.

20. Key, T. J., Silcocks, P. B., Davey, G. K., Appleby, P. N., and Bishop, D. T. A case-control study of diet and prostate cancer. Br. J. Cancer, *76:* 678–687, 1997.

21. Lee, M. M., Wang, R. T., Hsing, A. W., Gu, F. L., Wang, T., and Spitz, M. Case-control study of diet and prostate cancer in China. Cancer Causes Control, *9:* 545–552, 1998.

22. van 't Veer, P., van der Wielen, R. P., Kok, F. J., Hermus, R. J., and Sturmans, F. Selenium in diet, blood, and toenails in relation to breast cancer: a case-control study. Am. J. Epidemiol., *131:* 987–994, 1990.

23. van den Brandt, P. A., Goldbohm, R. A., van 't Veer, P., Volovics, A., Hermus, R. J., and Sturmans, F. A large-scale prospective cohort study on diet and cancer in The Netherlands. J. Clin. Epidemiol., *43:* 285–295, 1990.

24. Prentice, R. L. A case-cohort design for epidemiologic cohort studies and disease prevention trials. Biometrika, *73:* 1–11, 1988.

25. Self, S. G., and Prentice, R. L. Asymptotic distribution theory and efficieny results for case-cohort studies. Ann. Stat., *16:* 64–81, 1988.

26. van den Brandt, P. A., Schouten, L. J., Goldbohm, R. A., Dorant, E., and Hunen, P. M. Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. Int. J. Epidemiol., *19:* 553–558, 1990.

27. Goldbohm, R. A., van den Brandt, P. A., and Dorant, E. Estimation of the coverage of Dutch municipalities by cancer registries and PALGA based on hospital discharge data. Tijdschr. Soc. Gezondheidsz., *72:* 80–84, 1994.

28. Goldbohm, R. A., van den Brandt, P. A., Brants, H. A., van 't Veer, P., Al, M., Sturmans, F., and Hermus, R. J. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. Eur. J. Clin. Nutr., *48:* 253–265, 1994.

29. Nevo-Table. Dutch Food Composition table 1986–1987; Nederlands voedingsstoffenbestand 1986–1987. The Hague: Voorlichtingsbureau voor de Voeding, 1986.

30. Goldbohm, R. A., Brants, H. A. M., Hulshof, K. F. A. M., and van den Brandt, P. A. The contribution of various foods to intake of vitamin A and carotenoids in the Netherlands. Int. J. Vit. Nutr. Res., *68:* 378–383, 1998.

31. van den Brandt, P. A., Goldbohm, R. A., van 't Veer, P., Bode, P., Hermus, R. J., and Sturmans, F. Predictors of toenail selenium levels in men and women. Cancer Epidemiol. Biomark. Prev., *2:* 107–112, 1993.

32. Zeegers, M. P., Goldbohm, R. A., Bode, P., and Van Den Brandt, P. A. Prediagnostic toenail selenium and risk of bladder cancer. Cancer Epidemiol. Biomark. Prev., *11:* 1292–1297, 2002.

33. Bode, P. Automation and quality assurance in the Neutron Activation Facilities in Delft. J. Radioanal. Nucl. Chem., *245:* 127–132, 2000.

34. Volovics, A., and van den Brandt, P. A. Methods for the analyses of case-cohort studies. Biom. J., *2:* 195–214, 1997.

35. StataCorp. Stata Statistical Software: Release 7.0: College Station, TX: Stata Corporation, 1999.

36. Barlow, W. E. Robust variance estimation for the case-cohort design. Biometrics, *50:* 1064–1072, 1994.

37. Lin, D. Y., and Ying, Z. Cox regression with incomplete covariate measurements. JASA, *88:* 1341–1349, 1993.

38. Schuurman, A. G., Zeegers, M. P., Goldbohm, R. A., and van den Brandt, P. A. A case-cohort study on prostate cancer risk in relation to family history of prostate cancer. Epidemiology, *10:* 192–195, 1999.

39. Schuurman, A. G., Goldbohm, R. A., and van den Brandt, P. A. A prospective cohort study on consumption of alcoholic beverages in relation to prostate cancer incidence (The Netherlands). Cancer Causes Control, *10:* 597–605, 1999.

40. Schuurman, A. G., van den Brandt, P. A., Dorant, E., Brants, H. A., and Goldbohm, R. A. Association of energy and fat intake with prostate carcinoma risk: results from The Netherlands Cohort Study. Cancer (Phila.), *86:* 1019–1027, 1999.

41. Schuurman, A. G., Goldbohm, R. A., Dorant, E., and van den Brandt, P. A. Vegetable and fruit consumption and prostate cancer risk: a cohort study in The Netherlands. Cancer Epidemiol. Biomark. Prev., *7:* 673–680, 1998.

42. van den Brandt, P. A., Goldbohm, R. A., van 't Veer, P., Bode, P., Dorant, E., Hermus, R. J., and Sturmans, F. A prospective cohort study on selenium status and the risk of lung cancer. Cancer Res., *53:* 4860–4865, 1993.

43. Nomura, A., Heilbrun, L. K., Morris, J. S., and Stemmermann, G. N. Serum selenium and the risk of cancer, by specific sites: case-control analysis of prospective data. J. Natl. Cancer Inst., *79:* 103–108, 1987.

44. Willett, W. C., Polk, B. F., Morris, J. S., Stampfer, M. J., Pressel, S., Rosner, B., Taylor, J. O., Schneider, K., and Hames, C. G. Prediagnostic serum selenium and risk of cancer. Lancet, *2:* 130–134, 1983.

45. Longnecker, M. P., Stram, D. O., Taylor, P. R., Levander, O. A., Howe, M., Veillon, C., McAdam, P. A., Patterson, K. Y., Holden, J. M., Morris, J. S., Swanson, C. A., and Willett, W. C. Use of selenium concentration in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake. Epidemiology, *7:* 384–390, 1996.

46. van den Brandt, P. A., van't Veer, P., Goldbohm, R. A., Dorant, E., Volovics, A., Hermus, R. J., and Sturmans, F. A prospective cohort study on dietary fat and the risk of postmenopausal breast cancer. Cancer Res., *53:* 75–82, 1993.

47. Garland, M., Morris, J. S., Rosner, B. A., Stampfer, M. J., Spate, V. L., Baskett, C. J., Willett, W. C., and Hunter, D. J. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer Epidemiol. Biomark. Prev., *2:* 493–497, 1993.

48. Post, P. N., Kil, P. J., Crommelin, M. A., Schapers, R. F., and Coebergh, J. W. Trends in incidence and mortality rates for prostate cancer before and after prostate-specific antigen introduction. A registry-based study in southeastern Netherlands, 1971–1995. Eur. J. Cancer, *34:* 705–709, 1998.

49. Criqui, M. H., Bangdiwala, S., Goodman, D. S., Blaner, W. S., Morris, J. S., Kritchevsky, S., Lippel, K., Mebane, I., and Tyroler, H. A. Selenium, retinol, retinol-binding protein, and uric acid. Associations with cancer mortality in a population-based prospective case-control study. Ann. Epidemiol., *1:* 385–393, 1991.

50. Coates, R. J., Weiss, N. S., Daling, J. R., Morris, J. S., and Labbe, R. F. Serum levels of selenium and retinol and the subsequent risk of cancer. Am. J. Epidemiol., *128:* 515–523, 1988.

51. Ghadirian, P., Maisonneuve, P., Perret, C., Kennedy, G., Boyle, P., Krewski, D., Lacroix, A., Hardell, L., Degerman, A., Tomic, R., Marklund, S. L., and Bergfors, M. A case-control study of toenail selenium and cancer of the breast, colon, and prostate. Cancer Detect. Prev., *24:* 305–313, 2000.

52. Hardell, L., Degerman, A., Tomic, R., Marklund, S. L., and Bergfors, M. Levels of selenium in plasma and glutathione peroxidase in erythrocytes in patients with prostate cancer or benign hyperplasia. Eur. J. Cancer Prev, *4:* 91–95, 1995.

53. Duffield-Lillico, A. J., Reid, M. E., Turnbull, B. W., Combs, G. F. J., Slate, E. H., Fischbach, L. A., Marshall, J. R., and Clark, L. C. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. Cancer Epidemiol. Biomark. Prev., *11:* 630–639, 2002.

54. West, D. W., Slattery, M. L., Robison, L. M., French, T. K., and Mahoney, A. W. Adult dietary intake and prostate cancer risk in Utah: a case-control study with special emphasis on aggressive tumors. Cancer Causes Control, *2:* 85–94, 1991.

55. Jain, M. G., Hislop, G. T., Howe, G. R., and Ghadirian, P. Plant foods, antioxidants, and prostate cancer risk: findings from case-control studies in Canada. Nutr. Cancer, *34:* 173–184, 1999.

56. Seo, Y. R., Sweeney, C., and Smith, M. L. Selenomethionine induction of DNA repair response in human fibroblasts. Oncogene, *21:* 3663–3669, 2002.

57. Thompson, H. J., Wilson, A., Lu, J., Singh, M., Jiang, C., Upadhyaya, P., el Bayoumy, K., and Ip, C. Comparison of the effects of an organic and an inorganic form of selenium on a mammary carcinoma cell line. Carcinogenesis (Lond.), *15:* 183–186, 1994.

58. el Bayoumy, K., Chae, Y. H., Upadhyaya, P., Meschter, C., Cohen, L. A., and Reddy, B. S. Inhibition of 7, 12-dimethylbenz(a)anthracene-induced tumors and DNA adduct formation in the mammary glands of female Sprague-Dawley rats by the synthetic organoselenium compound, 1, 4-phenylenebis(methylene)selenocyanate. Cancer Res., *52:* 2402–2407, 1992.

59. Medina, D. Mechanisms of selenium inhibition of tumorigenesis. Adv. Exp. Med. Biol., *206:* 465–472, 1986.

60. Griffin, A. C. Role of selenium in the chemoprevention of cancer. Adv. Cancer Res., *29:* 419–442, 1979.

61. Redman, C., Scott, J. A., Baines, A. T., Basye, J. L., Clark, L. C., Calley, C., Roe, D., Payne, C. M., and Nelson, M. A. Inhibitory effect of selenomethionine on the growth of three selected human tumor cell lines. Cancer Lett., *125:* 103–110, 1998.

62. Nakayama, T., Kaneko, M., Kodama, M., and Nagata, C. Cigarette smoke induces DNA single-strand breaks in human cells. Nature (Lond.), *314:* 462–464, 1985.

63. Church, D. F., and Pryor, W. A. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ. Health Perspect., *64:* 111–126, 1985.

64. Anonymous. The effect of vitamin E and β carotene on the incidence of lung cancer and other cancers in male smokers. The α-Tocopherol, β Carotene Cancer Prevention Study Group. N. Engl. J. Med., *330:* 1029–1035, 1994.

65. Schuurman, A. G., Goldbohm, R. A., Brants, H. A. M., and van den Brandt, P. A. A prospective cohort study on intake of retinol, vitamins C and E, and carotenoids and prostate cancer risk (Netherlands). Cancer Causes Control, *13:* 573–582, 2002.

66. Kolonel, L. N., Hankin, J. H., and Yoshizawa, C. N. Vitamin A and prostate cancer in elderly men: enhancement of risk. Cancer Res., *47:* 2982–2985, 1987.

67. Nomura, A. M., Stemmermann, G. N., Lee, J., and Craft, N. E. Serum micronutrients and prostate cancer in Japanese Americans in Hawaii. Cancer Epidemiol. Biomark. Prev., *6:* 487–491, 1997.

68. Giovannucci, E., Rimm, E. B., Liu, Y., Stampfer, M. J., and Willett, W. C. A prospective study of tomato products, lycopene, and prostate cancer risk. J. Natl. Cancer Inst., *94:* 391–398, 2002.

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