Toenail selenium, plasma selenoprotein P and risk of advanced prostate cancer

A nested case-control study

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Title:

Toenail selenium, plasma selenoprotein P and risk of advanced prostate cancer: a nested case-control study

Short title:

Selenium and risk of advanced prostate cancer

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Key words:

Selenium, biomarkers, single nucleotide polymorphisms, prostate cancer, nested case-control
study,

Abbreviations:

BMI: body mass index

CI: confidence interval

FDR: false discovery rate

HWE: Hardy-Weinberg equilibrium

OR: odds ratio

PC: prostate cancer

SELENOP: selenoprotein P

SNP: single nucleotide polymorphism

TNM: tumor-node-metastasis

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Novelty and Impact:

The role of selenium in prostate cancer etiology remains unclear and the evidence is inadequate to formulate dietary intake recommendations for prostate cancer prevention, although studied for many years. Few previous studies, however, have investigated the selenium-prostate cancer risk association in low selenium status cohorts. In this case-control study nested within a Danish cohort, toenail selenium and plasma selenoprotein P levels were not associated with risk of advanced, high-grade or advanced-stage prostate cancer.

Abstract:

Low selenium status may be associated with increased risk of prostate cancer (PC), particularly aggressive PC, and variation in selenoprotein genes may constitute an important modifying factor. We aimed to investigate the association between two selenium status biomarkers [toenail selenium, plasma selenoprotein P (SELENOP)] and risk of advanced, high-grade, and advanced-stage PC. We further studied whether variations in selenoprotein genes were associated with PC risk and selenium biomarker concentrations. In the 'Diet, Cancer and Health' cohort, 27,178 men aged 50-65 years were enrolled during 1993-1997. Between baseline and 2012, 1160 cohort participants were diagnosed with advanced PC; among these 462 had high-grade and 281 had advanced-stage disease at diagnosis. Each case was risk set-matched to one control. Toenail selenium and plasma SELENOP concentrations were measured by neutron activation analysis and a SELENOP-ELISA, respectively, and genotyping was performed for 27 selected single nucleotide polymorphisms (SNPs) in 12 selenium pathway genes (including 7 selenoproteins) by allele-specific PCR. Toenail selenium and circulating SELENOP concentrations were not associated with advanced, high-grade or advanced-stage PC. After adjustment for multiple testing, none of the genes were associated with PC risk. Neither toenail selenium nor plasma SELENOP were associated with advanced, high-grade or advanced-stage PC.

Introduction

The role of selenium in prostate cancer (PC) prevention remains inconclusive.¹⁻³ Recently, data from existing studies associating blood or nail selenium concentrations to PC incidence were re-analyzed.² For plasma selenium (reflecting short-term selenium intake)⁴, a lower incidence of aggressive PC was indicated with higher selenium concentrations, whereas no associations were seen for any other PC classifications. For toenail selenium (reflecting previous 6-12 months selenium intake)^{5,6}, a considerably stronger picture was revealed, with higher selenium concentrations being associated with lower risk of all studied sub-classifications of PC.² This indicates that the exposure period, reflected by type of biomarker used for selenium status determination, is a critical aspect. Importantly, one Dutch case-cohort study by Geybels et al. contributed most cases for the pooled toenail selenium estimates and this finding therefore requires confirmation.⁷ Furthermore, variation in selenoprotein genes and genes related to selenoprotein expression levels, stability and activity,^{8,9} may constitute important factors to include when studying the potential role of selenium in PC prevention.

In the present study, we investigated the association between pre-diagnostic selenium status (measured as toenail selenium, plasma SELENOP concentrations and a combined measure of both) with risk of advanced, high-grade, and advanced-stage PC, while also considering relevant genetic variation and lifestyle factors. The case-control study was nested within a Danish cohort very similar to the one reported by Geybels et al.⁷ regarding selenium status. We expected to confirm that higher toenail selenium concentrations were associated with lower risk of advanced, high-grade, and advanced-stage PC. Additionally, our ability to study toenail selenium and plasma SELENOP concordantly may reveal new information about the usability of these two selenium biomarkers in isolation or as a combined parameter.

Materials and Methods

Study Population

Between December 1993 and May 1997, 80,996 men aged 50–64 years, born in Denmark, and without prior diagnoses registered in the Danish Cancer Registry¹⁰ were invited to participate in the Diet, Cancer and Health cohort¹¹ and 27,178 men were enrolled. At cohort entry, questionnaire data on social and lifestyle factors were collected. A non-fasting blood sample (collected in citrated and plain Venojects tubes) was taken from each participant and plasma, serum, lymphocytes and erythrocytes were isolated, and the participant brought toenail clippings in an envelope (collected for 99.7% of the cohort). Participants were advised to clip toenails from all toes if possible. Since data collection, blood samples have been stored in liquid nitrogen vapor at -150°C , and toenails have been stored in sealed envelopes at room temperature. Weight and height were measured by trained personnel.

Case Ascertainment and Classification

Information on vital status and emigration was obtained through linkage to the Central Population Registry using unique personal identification numbers. Information on cancer occurrence was obtained through record linkage to the Danish Cancer Registry.¹⁰ Each cohort member was followed for PC occurrence from the date of enrolment until the first date of diagnosis of any cancer (except for non-melanoma skin cancer), date of death or emigration, or 31 December 2012, whichever came first. 1909 cases of primary PC were identified, <1% were lost for endpoint assessment.

Information on clinical TNM (tumor/nodes/metastasis) stage and Gleason score at biopsy was obtained by medical record review for men diagnosed before 31/12 2008. From 1/1 2009 information on TNM was obtained from the Danish Cancer Registry, and Gleason score at diagnosis was extracted from The Danish Pathology Register.¹²

We defined 'advanced PC' as $\geq T3$ or Gleason score ≥ 7 or N1-3 or M1, or 'regional'/'distant' extent of disease. We defined 'high-grade PC' as Gleason score ≥ 8 , and 'advanced-stage PC' as T4 or M1, according to previous studies.^{2,7}

Each case was matched 1:1 with a control participant using risk set sampling from eligible cohort members who were alive and free of cancer at the age of diagnosis of the case. The matching criteria were age at blood collection (± 6 months), time of day of blood collection (± 1 h) and fasting status (time since last meal: <3 , $3-5$, >5 h).

Of the 1909 cases of primary PC, 1193 cases were defined as having advanced PC disease. We excluded 33 cases because of lack of toenail sample ($n=20$), very high toenail selenium concentration probably due to external contamination ($n=1$), very low toenail sample mass ($n=1$), and missing data on one or more potential confounders ($n=7$). Finally, incomplete case-control pairs were excluded ($n=4$), leaving 1160 complete case-control pairs.

Exposure Assessment

Case-control status was blinded in all exposure assessments. Toenail selenium concentration was measured by neutron activation analysis at the University of Missouri Research Reactor Center, Columbia, MO using methods previously described.^{5,6,13-17} Briefly, each analysis batch (n=100 subjects) consisted of toenail samples for matched case-control pairs. Where possible (52%) duplicates were prepared and analyzed along with selenium comparison standards (n=3), standard reference samples (n=3), batch factor samples (n=10), and cross-calibration check samples (n=5).

For 7 participants, the toenail selenium measurement was conducted on a low sample mass. We considered the measurements valid to retain them in the analyses based on a comparison of plasma SELENOP that did not indicate any signs of inconsistencies.

Plasma SELENOP concentrations were measured by a validated and CE-certified SELENOP-ELISA at Institute for Experimental Endocrinology, Charité–Universitätsmedizin Berlin, Germany.^{18,19} In every 96-well plate-based analysis, eight calibrators were used, and three controls with predefined low, middle or high SELENOP concentrations were included for quality assessment, yielding inter assay coefficients of variation of <20% as described.^{18,20}

Of the 1160 complete case-control pairs with toenail selenium measurements, 993 complete case-control pairs had plasma SELENOP measurements.

We combined toenail selenium and plasma SELENOP (n=993 case-control pairs) into one exposure variable using Howe's score with ranks.²¹ The method has previously been shown to strengthen the investigation of diet-disease associations by increasing the statistical power when different exposure measures were combined.²¹ The principle is to rank each exposure

variable from 1 to n and then combine the two exposures by adding the two ranks given as points.

SNP selection and Genotyping

We selected 27 single nucleotide polymorphisms (SNPs) that had previously been reported as candidate selenoprotein SNPs, including functional SNPs not well represented in previous PC genome-wide association studies.²²⁻³¹

DNA for genotyping was extracted from buffy coat using Kleargene™ XL DNA extraction kit (LGC Genomics, Queens Road, Teddington, Middlesex, UK). Extracted DNA was stored at -20°C. Of the 1160 complete case-control pairs with a toenail selenium measurement, 1052 pairs had available DNA samples.

Genotyping for SNPs of the genes *SELENOP* (rs3877899, rs7579, rs11959466, rs13168440), *GPX1* (rs1050450, rs17650792, rs1800668), *SELENOK* (rs9880056), *SELENOF* (rs479341, rs561104), *GPX3* (rs4958872, rs8177426), *SOD1* (rs2070424), *SOD2* (rs7855, rs4880), *OGGI* (rs1052133, rs2304277), *TXNRD1* (rs7310505), *TXNRD2* (rs1005873, rs8141691, rs9605030, rs9605031), *SELENBP1* (rs10788804, rs2769264), and *CAT* (rs10836233, rs533425, rs7944397) were performed by LGC Genomics (Hoddesdon, UK) using a robust, competitive, allele-specific PCR system (KASP™). Genotyping was performed blinded to case-control status. Sample size varies by SNP as samples with unclear or failed genotype calls were excluded from the analysis.

The frequency distribution of genotypes was examined for cases and controls and deviation of genotype frequencies from the Hardy-Weinberg equilibrium (HWE) was

assessed in controls by Chi-square test (X^2 –test). Two SNPs (*SELENOP*: rs479341 and *CAT*: rs7944397) deviated from HWE (P -values < 0.001) and were excluded from the analyses.

Statistical Analysis

Associations between selenium status biomarkers (toenail selenium, plasma SELENOP, and combined biomarker measure) and risk of PC were estimated by conditional logistic regression stratified by case-control pair. Odds ratios (OR) and 95% confidence intervals (CI) were estimated from both crude (conditioned on the matching factors) and multivariable models. Analyses were conducted for both advanced, high-grade and advanced-stage PC.

The adjusted analyses included the following *a priori* selected potential confounders: Body Mass Index (BMI, continuous), education (low, medium, high), smoking status (never, former, current), and participation in sport (yes/no).

The associations were estimated both with selenium biomarkers as categorical (per quintile) and continuous (per increment of concentration) variables. Quintile cut-off points were based on the distribution among controls. Units used for linear associations corresponded approximately to the interquartile range of toenail selenium (per increment of 0.1 µg/g), plasma SELENOP (per increment of 0.8 mg/L) and combined selenium status biomarker (per increment of 500 points in score) levels among control participants. In sensitivity analyses, we excluded cases diagnosed within the first 5 years after blood collection to consider the effect of a potential undiagnosed PC at baseline.

For exposure variables (toenail selenium, SELENOP, and the combined biomarker score) and quantitative adjustment variables (BMI), the linearity of the associations was

evaluated graphically by linear splines with 3 knots placed at the quartile cut-off points for exposure distribution among cases.³² None of the associations showed signs of deflection or threshold values. Log-transformation of the selenium status biomarkers did not improve the model.

Correlation between toenail selenium and plasma SELENOP was evaluated using Pearson's correlations.

Associations between each of the 25 SNPs and risk of advanced, high-grade, and advanced-stage PC were estimated by conditional logistic regression. OR and 95% CI were estimated from crude models (conditioned on the matching factors). Risk estimates were presented with reference to the most frequent homozygous genotype. We merged rare homozygote with heterozygote when <5% of the study population was homozygous for the less common allele. To evaluate if selenium status was affected by genotype, a general linear model was applied in control subjects and a linear test was performed. Mean and 95% CI was estimated for each SNP, where genotype was described as 1: most frequent homozygote, 2: heterozygote, and 3: rare homozygote. *P*-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) approach.³³

Effect modification of the selenium status biomarkers and PC risk association was evaluated. Potential modifiers were factors related to oxidative stress, including BMI (≤ 25 kg/m², >25 – 30 kg/m², >30 kg/m²) and smoking status (current, former, never). Test for interaction was performed by comparing linear association estimates of selenium status biomarkers in groups by BMI and smoking, respectively, using a Wald test. Two-sided 95% CI for the OR was calculated and *P*-values < 0.05 were considered statistically significant. The procedure PHREG in the SAS software package (release 9.3; SAS Institute) was used to

perform the regression analyses. The TEST statement in PHREG was used to test the hypothesis of equal regression coefficients using Wald test. Other procedures used for statistical analyses were GLM, MEANS, RANK, CORR, and MULTTEST.

Results

Table 1 presents the baseline characteristics of the control group (n=1160), advanced (n=1160), high-grade (n=462), and advanced-stage (n=281) PC cases. No clear differences between controls and cases were seen for either the selenium status measures or the potential confounding factors. Toenail selenium and plasma SELENOP were only moderately correlated ($r=0.31$, $P<0.0001$) among control subjects (Figure 1).

Toenail selenium and plasma SELENOP concentrations were not associated with advanced, high-grade or advanced-stage PC (Table 2). Combining the two measures into a combined biomarker score did not reveal any associations.

Sensitivity analyses, excluding cases diagnosed within the first 5 years after baseline and mutually adjusting for the two selenium status biomarkers, revealed similar risk estimates (results not shown). No effect modification by BMI or smoking was found (results not shown).

Among selected selenoprotein or selenium-pathway gene variants, only rs533425 in the antioxidant gene *CAT* was associated with risk of advanced PC (CC: OR = 1.00 (referent), TC: OR = 1.20, 95% CI = 0.98 to 1.47 and TT: OR = 1.31, 95% CI = 1.02 to 1.70), high-grade PC (CC: OR = 1.00 (referent), TC: OR = 1.36, 95% CI = 0.98 to 1.89 and TT: OR = 1.52, 95% CI = 1.02 to 2.28), and advanced-stage PC (CC: OR = 1.00 (referent), TC: OR = 1.37, 95% CI = 0.91 to 2.07 and TT: OR = 1.73, 95% CI = 1.02 to 2.94) (Appendix Table 1). None of these associations remained statistically significant after adjustment for multiple testing. Appendix table 2 shows the baseline selenium biomarker levels in control subjects by genotype. Only rs9605031 in the *TXNRD2* selenoprotein gene was associated with toenail selenium concentrations ($P_{trend} = 0.008$) and rs2769264 in the selenium binding protein 1 gene

(*SELENBP1*) was associated with plasma SELENOP concentrations ($P_{trend} = 0.02$). For both, a higher biomarker concentration was seen with higher number of rare alleles. However, none of these findings remained statistically significant after adjustment for multiple testing.

Overall, the genotype frequencies in controls agreed with those reported for other

Caucasians.^{23-25,27,29,30}

Discussion

In this nested case-control study, we found no association between toenail selenium, SELENOP concentrations, or their combined score and risk of advanced, high-grade or advanced-stage PC. Selenoprotein or selenium-pathway related gene variants were not associated with PC risk after adjustment for multiple testing.

Major strengths of our study were the long and almost complete follow-up (loss to follow-up < 1%) and use of both TNM and Gleason score for disease classification. Another strength was our selenium status exposure variables including both toenail selenium and plasma SELENOP measurements. We were able to adjust for several potential confounding factors and to study the influence of relevant SNPs. The major limitation is that measurements of selenium status biomarkers were assessed from a single sample point, which may have caused exposure misclassification.

Our findings question whether selenium status alone is related to PC risk. Allen and colleagues pooled and re-analyzed almost all available data from 15 prospective studies on selenium in blood and nails in relation to PC incidence,² including data from the Dutch study by Geybels et al.⁷ They concluded that *‘investigations in large, representative populations that have selenium measurements from both blood and nail samples and that include information on screening history, as well as stage and grade of tumors, is needed to examine these possible associations in more detail’*.² In the present study, we have a population where selenium status is very similar to the Dutch study; the toenail selenium quintile medians in our study ranged from 0.42 to 0.67 µg/g compared with 0.43 to 0.67 µg/g in the Dutch study.⁷ Unexpectedly, we did not find associations between selenium status and PC, despite our ability to 1) investigate advanced-stage and high-grade of the disease, 2) include both toenail

selenium and plasma SELENOP measurements, and 3) take both genetic variability and a range of lifestyle factors into account. In the Dutch study, the risk estimate for the highest vs lowest quintile was: HR=0.37, (95% CI=0.27 to 0.51), and like our study, confounder adjustment only altered the association to a minor extent. In general, the Dutch cohort population and ours are expected to be similar according to potential risk factors for PC,³⁴ where for example BMI and height have been shown comparable in Dutch and Danish cohort participants,³⁵ and the two populations have similar age-standardized rates incidence and mortality rates from PC.³⁶ The two studies differed with respect to advanced disease which accounted for 35% and 62% of total PC cases in the Dutch and our cohort, respectively.⁷ A possible explanation for the large difference in advanced disease rates between the cohorts is that PC screening is not recommended in Denmark. It cannot be excluded that men with higher selenium levels are more often opportunistically screened and thus diagnosed at an earlier stage of disease, which can explain the highly different risk estimates. However, a recent Mendelian randomization study also indicated no association between selenium levels and PC incidence.³⁷ Altogether, the null findings in the present study imply that a higher selenium status may not prevent advanced prostate cancer. The relevance of future research on the hypothesized chemopreventive role of selenium in prostate cancer are to be considered in the light of this.

Within the 'Diet, Cancer and Health' cohort we have previously studied the association between plasma selenium and SELENOP and advanced and high-grade PC risk in a nested case-control study with follow-up until 2008.³⁸ For high-grade disease, we observed a lower risk with higher concentrations of plasma selenium (OR=0.77, 95% CI=0.64 to 0.94, $P=0.009$, $n=170$ case-control pairs) and SELENOP (OR=0.85, 95% CI=0.74 to 0.97, $P=0.01$,

n= 170 case-control pairs).³⁸ These cases were included in the present study and on the same case-control pairs with new exposure assessments, we found very similar associations for toenail selenium (OR=0.74, 95% CI=0.55 to 0.99, $P=0.05$, n= 127 case-control pairs) and SELENOP (OR=0.78, 95% CI=0.64 to 0.93, $P=0.007$, n= 127 case-control pairs). We were however unable to confirm the associations on the much larger complete data set with follow-up to 2012 evaluated in the present study (Table 2).

The moderate correlation between the two measures of selenium status in control subjects (Figure 1) indicates that they represent different exposure measures, and thus contribute with different exposure information. Combining the two biomarkers did not provide additional information.

In conclusion, our study did not confirm an association between selenium status and advanced PC and argues against a population relevant preventive effect of selenium on PC incidence. Including information on genetic variation in specific selenoprotein genes did not appear to appreciably alter this conclusion.

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Conflict of interest: LS holds shares in selenOmed GmbH, a company involved in selenium status assessment and supplementation. Authors not named here have disclosed no conflicts of interest.

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Ethics Statement

The study protocol was approved by the regional ethics committees on human studies in Copenhagen and Aarhus and by the Danish Data Protection Agency. All participants provided written informed consent.

Data Availability Statement

The data that support the findings of our study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1. Correlation between toenail selenium ($\mu\text{g/g}$) and plasma SELENOP (mg/L) in controls, $n=993$ (Pearson's correlation, $r=0.31$, $P<0.0001$).

Table 1. Baseline characteristics of advanced, high-grade, and advanced-stage prostate cancer cases and their matched controls.

	Matched controls (n=1160)	Advanced prostate cancer cases ($\geq$ T3 or Gleason score $\geq$ 7 or N1-3 or M1) (n=1160)	High-grade prostate cancer cases (Gleason score $\geq$ 8) (n=462)	Advanced-stage prostate cancer cases (T4 or M1) (n=281)
Age at baseline ¹ , y	57.0 (50.5-64.0)	57.0 (50.5-64.0)	58.0 (51.0-64.0)	59.0 (51.0-64.0)
Years between baseline and diagnosis ¹ , y	-	11.9 (3.6-16.4)	12.2 (4.9-16.5)	10.1 (1.6-15.7)
Body mass index ¹ , kg/m ²	25.9 (21.6-32.7)	26.1 (21.6-32.0)	26.2 (21.8-32.4)	26.4 (21.8-33.0)
Smoking status, % (n)				
Never	28.4 (330)	27.9 (324)	27.9 (129)	23.8 (67)
Former	36.0 (418)	35.2 (408)	36.4 (168)	34.9 (98)
Current	35.5 (412)	36.9 (428)	35.7 (165)	41.3 (116)
Education, % (n)				
Low school	35.3 (409)	34.2 (397)	35.1 (162)	40.6 (114)
Medium school	41.5 (481)	40.4 (469)	40.9 (189)	37.0 (104)
High school	23.3 (270)	25.3 (294)	24.0 (111)	22.4 (63)
Participation in sport, % (n)				
Yes	49.1 (569)	47.2 (547)	50.0 (231)	38.8 (109)
No	50.9 (591)	52.8 (613)	50.0 (231)	61.2 (172)
Baseline selenium biomarkers ¹				
Toenail selenium, μ g/g	0.510 (0.394-0.717)	0.509 (0.391-0.706)	0.511 (0.390-0.706)	0.503 (0.390-0.710)
Plasma SELENOP, mg/L	5.5 (3.5-8.0) ²	5.4 (3.4-8.0) ²	5.5 (3.6-8.0) ³	5.4 (3.2-8.1) ⁵
Toenail selenium + plasma SELENOP, points ⁴	2049.5 (397.0-3502.5) ²	2033.0 (363.0-3467.5) ²	2084.0 (380.5-3316.5) ³	2264.5 (380.5-3767.5) ^{5,6}

* SELENOP, selenoprotein P

¹ Median (5th and 95th percentiles).² n = 993.³ n= 405.⁴ The combined selenium biomarker (toenail selenium + plasma SELENOP) conducted using the method *Howe's score with ranks*.⁵ n=219.⁶ Based on the combined selenium biomarker ranking conducted for the advanced prostate cancer cases (n=1160).

Table 2. Selenium biomarkers associated with risk of advanced, high-grade, and advanced-stage prostate cancer (Odds ratios (OR) and 95% confidence intervals).

	Advanced prostate cancer ($\geq$ T3 or Gleason score $\geq$ 7 or N1-3 or M1)			High-grade prostate cancer (Gleason score $\geq$ 8)		Advanced-stage prostate cancer (T4 or M1)	
	Cases/controls (n)	Unadjusted ²	Adjusted ³	Cases/controls (n)	Adjusted ³	Cases/controls (n)	Adjusted ³
		OR (95% CI)	OR (95% CI)		OR (95% CI)		OR (95% CI)
Quintiles of toenail selenium ¹ in µg/g							
Q1 ($\leq$ 0.447)	247/234	1.0 (ref.)	1.0 (ref.)	101/95	1.0 (ref.)	65/53	1.0 (ref.)
Q2 ($>$ 0.447 $\leq$ 0.488)	211/230	0.86 (0.67, 1.12)	0.87 (0.67, 1.12)	79/90	0.84 (0.56, 1.26)	52/56	0.78 (0.44, 1.39)
Q3 ($>$ 0.488 $\leq$ 0.533)	239/235	0.96 (0.74, 1.24)	0.97 (0.75, 1.27)	92/77	1.13 (0.73, 1.75)	51/54	0.86 (0.49, 1.52)
Q4 ($>$ 0.533 $\leq$ 0.599)	232/229	0.95 (0.73, 1.24)	0.98 (0.74, 1.30)	106/96	1.05 (0.68, 1.62)	53/57	0.91 (0.49, 1.70)
Q5 ($>$ 0.599)	231/232	0.94 (0.72, 1.21)	0.95 (0.72, 1.26)	84/104	0.75 (0.48, 1.18)	60/61	1.01 (0.56, 1.82)
Per 0.1 µg/g	1160/1160	1.00 (0.93, 1.08)	1.01 (0.93, 1.09)	462/462	0.97 (0.85, 1.10)	281/281	1.01 (0.85, 1.21)
P-value		0.99	0.88		0.62		0.89
Quintiles of plasma SELENOP ¹ in mg/L							
Q1 ($\leq$ 4.4)	200/199	1.0 (ref.)	1.0 (ref.)	74/78	1.0 (ref.)	45/45	1.0 (ref.)
Q2 ($>$ 4.4 $\leq$ 5.2)	226/199	1.13 (0.86, 1.49)	1.14 (0.86, 1.50)	88/83	1.09 (0.71, 1.66)	49/45	1.27 (0.67, 2.41)
Q3 ($>$ 5.2 $\leq$ 5.8)	209/199	1.03 (0.78, 1.37)	1.04 (0.78, 1.37)	95/75	1.27 (0.82, 1.96)	45/33	1.72 (0.85, 3.50)
Q4 ($>$ 5.8 $\leq$ 6.7)	184/198	0.90 (0.68, 1.20)	0.91 (0.68, 1.21)	76/80	0.95 (0.60, 1.51)	38/50	0.83 (0.42, 1.61)
Q5 ($>$ 6.7)	174/198	0.83 (0.61, 1.13)	0.83 (0.61, 1.14)	72/89	0.79 (0.48, 1.30)	42/46	1.02 (0.51, 2.04)
Per 0.8 mg/L	993/993	0.96 (0.91, 1.01)	0.96 (0.91, 1.01)	405/405	0.99 (0.91, 1.07)	219/219	0.97 (0.85, 1.10)
P-value		0.11	0.13		0.74		0.58
Quintiles of combined biomarker score ¹ in points							
Q1 ($\leq$ 1095.0)	208/199	1.0 (ref.)	1.0 (ref.)	79/85	1.0 (ref.)	46/36	1.0 (ref.)
Q2 ($>$ 1095.0 $\leq$ 1747.5)	191/199	0.90 (0.69, 1.19)	0.91 (0.69, 1.20)	73/67	1.15 (0.73, 1.81)	34/38	0.79 (0.41, 1.53)
Q3 ($>$ 1747.5 $\leq$ 2288.0)	207/198	1.01 (0.76, 1.33)	1.01 (0.76, 1.35)	90/78	1.20 (0.77, 1.87)	39/38	0.96 (0.50, 1.83)
Q4 ($>$ 2288.0 $\leq$ 2893.0)	204/199	0.98 (0.74, 1.30)	0.99 (0.74, 1.32)	84/82	1.06 (0.66, 1.70)	45/42	0.93 (0.47, 1.81)
Q5 ($>$ 2893.0)	183/198	0.86 (0.64, 1.16)	0.87 (0.64, 1.19)	79/93	0.87 (0.53, 1.42)	55/65	0.94 (0.49, 1.80)
Per 500 points in score	993/993	0.98 (0.93, 1.03)	0.98 (0.93, 1.03)	405/405	0.97 (0.89, 1.06)	219/219	1.00 (0.90, 1.12)
P-value		0.40	0.46		0.54		1.00

* CI, confidence interval; OR, odds ratio; Q, quintile; ref., reference; SELENOP, selenoprotein P

¹ Quintiles based on range among control subjects.² Conditioned on matching factors: age at baseline, time of day of blood collection, fasting status (time since last meal).³ Further adjusted for BMI (continuous), smoking status (never, former, current), education (low, medium, high), participation in sport (yes/no).

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