Selenium Status is Associated with Colorectal Cancer risk in the European Prospective Investigation of Cancer and Nutrition Cohort

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Title Pages

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Abbreviations: Se, Selenium; CRC, colorectal cancer; SePP, Selenoprotein P; EPIC, European Prospective Investigation into Cancer and Nutrition; IRR, Incidence Rate Ratios; 95% CI, 95% confidence intervals; CRA, colorectal adenoma; NPC, Nutritional Prevention of Cancer; SELECT, Selenium and Vitamin E Cancer Prevention Trial; WHI, Women's Health Initiative; IARC, International Agency for Research on Cancer; HRT, hormonal replacement therapy; TXRF, total reflection X-ray fluorescence; BMI, body mass index; SD, standard deviation; NHANES, National Health and Nutrition Examination Survey.

Article category: Epidemiology

Brief description of the novelty and impact of the work: The association of Se status with CRC development is controversial. The present study shows that Se status is suboptimal for SePP saturation in many Western Europeans and that a higher Se status is inversely associated with CRC risk, which is more evident in women. The contrasting results of our study and those from the NPC and SELECT Se intervention trials may be due to differences in baseline Se levels of study participants. In populations where Se status is sub-optimal (e.g. Western Europe) increasing Se intake may reduce CRC risk.

ABSTRACT

Suboptimal intakes of the micronutrient selenium (Se) are found in many parts of Europe. Low Se status may contribute to colorectal cancer (CRC) development. We assessed Se status by measuring serum levels of Se and Selenoprotein P (SePP) and examined the association with CRC risk in a nested case-control design (966 CRC cases; 966 matched controls) within the European Prospective Investigation into Cancer and Nutrition. Se was measured by total reflection X-ray fluorescence and SePP by immunoluminometric sandwich assay. Multivariable incidence rate ratios (IRRs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression. Respective mean Se and SePP levels were 84.0 µg/L and 4.3 mg/L in cases and 85.6 µg/L and 4.4 mg/L in controls. Higher Se concentrations were associated with a non-significant lower CRC risk (IRR = 0.92, 95%CI: 0.82-1.03 per 25 µg/L increase). However, sub-group analyses by sex showed a statistically significant association for women (P_{trend} = 0.032; per 25 µg/L Se increase, IRR = 0.83, 95%CI: 0.70-0.97) but not for men. Higher SePP concentrations were inversely associated with CRC risk (P_{trend} = 0.009; per 0.806 mg/L increase, IRR = 0.89, 95%CI: 0.82-0.98) with the association more apparent in women (P_{trend} = 0.004; IRR = 0.82, 95%CI: 0.72-0.94 per 0.806 mg/L increase) than men (P_{trend} = 0.485; IRR = 0.98, 95%CI: 0.86-1.12 per 0.806 mg/L increase). The findings indicate that Se status is suboptimal in many Europeans and suggest an inverse association between CRC risk and higher serum Se status, which is more evident in women.

INTRODUCTION

In Europe, colorectal cancer (CRC) is the second leading cause of cancer related death[1]. Varying international CRC incidence rates and observations from migrant studies have long suggested that modifiable factors such as diet and lifestyle play an important role in CRC aetiology, however there is little knowledge on how dietary micronutrients affect disease risk[2].

Selenium (Se) is an essential micronutrient for human health whose biological activities and potential anti-carcinogenic properties likely result from its incorporation as the amino acid selenocysteine in selenoproteins encoded by 25 separate human genes with roles in cell protection from oxidative stress, redox control and the inflammatory response[3, 4]. Due to differing soil Se levels and resultant food content, there is great geographical variation in dietary Se intake worldwide[5]. As a result the Se status of many populations, including those across Europe is low compared with much of North America[5, 6]. Such relatively low intake has been associated with an increased risk of a number of major diseases[7, 8] .

There is much current debate as to whether Se influences development of CRC or its precursor colorectal adenoma (CRA) lesions. A recent randomized trial supplementing antioxidant nutrients including Se showed a significant protective effect on CRA recurrence[9]. Three other analyses based on subjects enrolled in randomized CRA prevention trials with Se alone have also considered the association of baseline Se levels and CRA recurrence. The first did not indicate any association[10], the second observed a significant inverse association[11], while the third observed a reduced risk in smokers only[12]. Data from the Nutritional Prevention of Cancer (NPC) intervention trial[13, 14] and case-control and cohort studies (see[15, 16] for reviews) suggest that a low Se intake is associated with a higher CRC risk. However, a subsequent intervention trial among men (Selenium and Vitamin E Cancer

Prevention Trial, SELECT)[17] and a prospective cohort among women (Women's Health Initiative, WHI)[18] have shown no associations. In the NPC study, Se supplementation had a significant effect on CRC risk in volunteers with a baseline plasma Se of <106 µg/L whereas the SELECT trial and the WHI prospective study included too few participants with levels within this range.

Differences in the range of Se or baseline Se status between these studies[19] and differences in risk of CRC by sex[12, 18, 20] are major possible explanations for these discrepant findings. Overall, these studies suggest that an association with cancer risk is more likely for individuals in populations with lower Se levels (possibly due to a lower Se availability[5, 6]). Effect modification by sex appears to be biologically plausible due to differences in metabolism, excretion, and interaction between Se and other factors (e.g., alcohol and smoking)[21-24].

However, there is no strong epidemiologic evidence available for the association of Se status with CRC risk in European populations. Existing data are compromised by the lack of robust markers of Se status and / or studies with small sample sizes[15, 19]. Selenoprotein P (SePP) is regarded as the best biomarker of functional Se as serum SePP protein is the major transporter of hepatic Se towards other tissues and reflects long-term intake that is less influenced by the chemical form of the ingested Se[25]. Nevertheless, the association of circulating SePP protein levels with CRC risk has not previously been studied in European populations.

We hypothesized that a low Se status is associated with a higher CRC risk and that the influence of Se status on CRC risk is modulated by sex. In this study, we evaluated the association between pre-diagnostic Se and SePP concentrations in serum and CRC risk in

samples taken from 966 CRC cases and 966 matched controls nested within the large, European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

MATERIALS AND METHODS

Study population and data collection

EPIC is a multicentre prospective cohort study designed to investigate the associations between diet, lifestyle, genetic and environmental factors and various types of cancer. The rationale and methods of the EPIC design have been published previously[26, 27]. In summary, 521,448 participants (aged 25-70 years; approximately 70% women) were enrolled between 1992-2000 in 23 sub-cohorts in 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom). The present analysis is based on participant data from all centres except for Norway (blood samples only recently collected; few CRCs diagnosed after blood donation) and Sweden (no available serum samples). Detailed and validated dietary (country-specific questionnaires) and lifestyle data (standardized questionnaires), anthropometrics, and biological samples were collected at the time of enrolment. Serum samples are stored at the International Agency for Research on Cancer (IARC) at -196°C under liquid nitrogen for all countries except Denmark (-150°C, nitrogen vapour). Written informed consent was provided by all study participants. Ethical approval for the EPIC study was obtained from the review boards of the IARC and local participating centres.

Incident cancer cases were identified by follow-up based on population cancer registries (Denmark, Italy, Netherlands, Spain, United Kingdom) and other methods such as health insurance records, pathology registries and active contact of study subjects or next of kin (France, Germany, Greece). Complete follow-up censoring dates varied amongst centres, ranging between June 2002 and June 2003.

Selection of cases and control subjects

Case subjects were men and women who developed first incident, histologically-confirmed CRC after recruitment and latest follow-up date. Cancer incidence data were

coded using the 10th Revision of the International Classification of Diseases (ICD-10) and the second revision of the International Classification of Disease for Oncology (ICDO-2). Colon cancers were defined as tumours in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending and sigmoid colon (C18.0-C18.7), and overlapping or unspecified origin tumours (C18.8 and C18.9). Rectal cancers were defined as tumours occurring at the recto-sigmoid junction (C19) or rectum (C20). Anal canal cancers (C21) were excluded. Colorectal cancer is the combination of the colon and rectal cancer cases.

All subjects with prior cancer diagnoses at any site (except non-melanoma skin cancer) or with missing values on any Se status biomarkers were excluded. The total number of samples processed for Se and SePP measurements in the laboratory was 2192 (cases=1096, controls=1096), from which 130 case-control sets had missing biomarker measurements in either the case or matched control, due to insufficient availability of bio-sample for laboratory analysis, so that 1932 individuals (cases=966, controls=966) were included in the final dataset and utilized in the present analyses. Cases were matched 1:1 by study centre of enrolment, sex, age at blood collection time of blood collection and fasting status and among women, menopausal status. Premenopausal women were matched on phase of menstrual cycle and postmenopausal women were matched on current hormonal replacement therapy (HRT) use.

Serum selenium and SePP measurements

Total Se levels were measured from 20 µl of each blood serum sample by X-ray fluorescence, using a bench-top total reflection X-ray fluorescence (TXRF) spectrometer (PicofoxTM S2, Bruker Nano GmbH, Berlin, Germany) as described previously[28]. Concentrations of the SePP protein were measured from 20 µl of each blood serum sample

by an immunoluminometric sandwich assay[29] (Selenotest™, ICI GmbH, Berlin, Germany) essentially as described previously[30]. For quality-control, case-control status was blinded and two serum samples of known Se and SePP concentrations for intra-assay variability were used in each analysis plate. Se measurements were controlled with a commercial standard serum (Seronorm, Billingstad, Norway) and an atomic absorption standard (1000 mg/ml, Sigma, Taufkirchen, Germany). The samples were measured in duplicate and the mean concentration values, standard deviation and coefficient of variation were calculated. Duplicate samples with differences in concentration varying by more than 10% were measured again. The evaluation was performed using GraphPad Prism 6.01 (La Jolla, CA, USA) using a 4-parameter logistic function (4PL). The coefficient of variation was 7.3% and 7.2% for controls 1 (SePP: 1.5 mg/L) and 2 (SePP: 8.6 mg/L), respectively.

Statistical Analysis

Serum Se and SePP concentrations were compared by linear Pearson product-moment correlation. Analysis of covariance (values were natural logarithm transformed to approximate a normal distribution) was used to examine geometric mean differences in Se and SePP concentrations among the controls by baseline characteristics, with adjustment for study centre. *P*-values for tests of trend (for ordinal variables) or of heterogeneity were reported.

Two conditional logistic models, 1) with matching factors only, and 2) with adjustment for *a priori* selected confounders including smoking status/duration/intensity, physical activity (combined recreational and household activity; expressed as sex-specific categories of metabolic equivalents), education level, and continuous measures for body mass index (BMI; kg/m^2), total dietary energy consumption (kcal/d), and intakes of alcohol (g/d), calcium (g/d), fruits and vegetables (g/d), and red and processed meats (g/d) were

used to assess the strengths of association [incidence rate ratio (IRR) as estimated by odds ratio (OR)[31] with 95% confidence interval (CI) and tests for trend]. Adjustments for dietary fibre intake (instead of fruits and vegetables), and consumption of fish (in addition to and instead of red and processed meats) did not change the effect estimates. Se and SePP concentrations were included in models as continuous [per 25 μg/L and 0.806 mg/L, respectively; approximately 1 standard deviation (SD)] and as categorical variables, with quintile cut-points based on the distribution in the control subjects. All analyses were run separately for CRC and by anatomical sub-sites of colon and rectum, and for men and women separately and combined using the same categorical cut-points for all tests. To test dose-responses, trend variables were assigned the median values for Se or SePP quintiles and predefined categories. Heterogeneity of effects by sex and anatomical sub-site were assessed by χ^2 statistic.

Effect modification on the multiplicative scale for potential biologically plausible effect modifying variables (age at diagnosis, BMI, smoking, baseline alcohol consumption; and among women, menopausal status and HRT use) was tested by including interaction terms formed by the product of modifying variable categories and the value of categories of exposure of interest. The statistical significance of interactions was assessed using likelihood ratio tests based on the models with and without the interaction terms. In sensitivity analyses, analyses were limited to follow-up of >2 years after blood collection to assess possible reverse causation.

All statistical tests were two-sided, and P-values<0.05 were considered statistically significant. Analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC) statistical package.

RESULTS

Baseline Characteristics of Participants

The baseline characteristics of participants are presented in **Table 1**. Colon and rectal cancer cases were diagnosed, on average, 3.7 and 3.9 years after blood collection, respectively. Cases were more likely to be current smokers, to have higher intakes of alcohol and red and processed meats per day, and were less likely to be physically active. Serum concentrations of SePP and Se correlate significantly among the participants (r = 0.60; P = <0.001). No statistically significant differences by case-control status in serum Se were detected (P_{CRC} =0.147; P_{colon} = 0.097; P_{rectum} = 0.816). Geometric mean serum SePP was lower in CRC cases *vs*. controls (4.2 vs. 4.3 mg/L; P = 0.027), and particularly in colon cancer cases *vs*. controls (4.1 vs. 4.3 mg/L; P = 0.008), but not in rectal cancer cases (4.2 vs. 4.3 mg/L; P = 0.922).

Selenium and SePP levels by Baseline Characteristics among Controls

Concentrations of Se and SePP did not differ statistically significantly by sex (see **Table 2**; P for Se = 0.079, P for SePP = 0.674). The mean serum Se and SePP concentrations varied significantly between countries and Western European geographic regions (grouped as Northern, Central or Southern; P < 0.001). Comparisons by country are compromised due to the small participant numbers in France and Greece. Considering the regional groupings the order of highest to lowest concentrations of both Se and SePP was Northern (represented by Denmark) > Southern (France, Italy, Spain, Greece) > Central (UK, the Netherlands, Germany) areas. Serum levels of Se differ by smoking status in men (P = 0.041), with the lowest values in current smokers. In men and in women, higher consumption of total fish and shellfish was statistically significantly associated with higher concentrations of SePP (P_{men} = 0.021; P_{women} = 0.051). Among women, higher total fruits and vegetables intake was positively associated with SePP (P = 0.017); whereas in men,

higher intake of red and processed meat was associated with higher concentrations of SePP (P = 0.011). No statistically significant differences were found between BMI, age, physical activity, alcohol intake and serum Se or SePP concentrations (**Table 2**).

Selenium Concentrations and CRC risk

The association between serum Se concentration and CRC risk is shown in **Table 3**. A higher Se concentration was not statistically significantly associated with an altered CRC risk in all participants (multivariable $IRR_{Q5\ vs.\ Q1}$ = 0.88, 95% CI: 0.64 – 1.21 for the fifth quintile versus the first quintile, P_{trend} =0.458; IRR = 0.92, 95% CI: 0.82-1.03 per 25 µg/L increase in Se concentration). Similarly, there were no notable effects of Se level when assessing the major anatomical sub-site classification of cancers located in the colon or rectum. Among men, serum Se concentration was not associated with CRC risk overall, or by anatomical sub-site. However, among women, participants in the highest quintile had a 36% lower risk of CRC that did those in the lowest quintile (P_{trend} = 0.032; per 25 µg/L, IRR = 0.83, 95% CI: 0.70-0.97; $P_{heterogeneity\ by\ sex}$ = 0.105), and a similar reduction in risk was found by anatomical sub-site (for colon P_{trend} = 0.045; per 25 ug/L, IRR = 0.84, 95% CI: 0.68-1.05; for rectum P_{trend} = 0.271; per 25 µg/L, IRR = 0.74, 95% CI: 0.57-0.98; $P_{heterogeneity\ by\ sub\text{-}site}$ = 0.474).

SePP Concentrations and CRC risk

The association of serum SePP concentration and CRC risk is shown in **Table 4**. Higher serum SePP level was associated with a statistically significant lower risk of CRC in all participants (multivariable $IRR_{Q5\ vs.\ Q1}$ = 0.60, 95% CI = 0.42-0.85; P_{trend}=0.009). There was a significant 11% reduction in CRC risk per 0.806 mg/L serum SePP increase (IRR = 0.89, 95% CI = 0.82-0.98). To further understand components involved in this association, we sub-divided our study according to anatomical sub-sites and sex. In the analysis by colon

sub-site, the association of SePP with disease risk was statistically significant in colon cancers (P_{trend} = 0.003; per 0.806 mg/L, IRR = 0.85, 95% CI = 0.75-0.95) but not in rectal cancers (P_{trend} = 0.806; per 0.806 mg/L, IRR = 0.96, 95% CI = 0.82-1.13; $P_{heterogeneity\ by\ sub\text{-}site}$ = 0.231). Among men, serum SePP concentration was not associated with CRC risk (P_{trend}=0.485; per 0.806 mg/L, IRR = 0.98, 95% CI: 0.86-1.12). Among women, higher SePP level was associated with a statistically significant lower risk of CRC (multivariable IRR $_{Q5\ vs.\ Q1}$ = 0.46, 95% CI: 0.28-0.78, P_{trend}=0.004; per 0.806 mg/L, IRR = 0.82, 95% CI: 0.72-0.94; $P_{heterogeneity\ by\ sex}$ = 0.230). The associations with SePP status for women were highly significant for cancers of the colon (P_{trend} = 0.008; per 0.806 mg/L, IRR = 0.82, 95% CI: 0.62-0.96) but not rectum (P_{trend} = 0.386; per 0.806 mg/L, IRR = 0.82, 95% CI: 0.63-1.08; $P_{heterogeneity\ by\ sub\text{-}site}$ = 0.710).

Interaction and Sensitivity Analyses

The results did not differ by sex and colon site (All *P*-values for heterogeneity >0.11). No multiplicative interactions were statistically significant (all *P*-values were ≥0.06). Selected results for age at blood collection, BMI and smoking status per 25 μg/L increase in Se and SePP are shown in **Supplemental Table 1**. The exclusion of cases with less than two years of follow-up did not change any of the results (data not shown).

DISCUSSION

The results of this study represent the largest prospective analysis of the association of serum Se status biomarkers (total serum Se levels and SePP protein concentrations) with risk of CRC in European populations. Our findings indicate that higher levels of serum Se are significantly associated with a lower CRC risk in women only and that higher concentrations of SePP, a functional biomarker of Se status, are significantly associated with a lower CRC risk. This suggests that Se intake/status is an important factor in affecting CRC risk in a population of marginally low Se status, such as in Europe[5, 6].

Previous work from two major Se intervention trials in North America provides conflicting results with regards to Se intake and CRC risk. Differences in baseline Se levels of the participants may be the crucial factor in explaining this, while other important issues may include sex-specific CRC risks, the type of Se supplementation utilized and that CRC was only a secondary endpoint in both the NPC and SELECT studies (so that there was low power to see an effect of intervention for this cancer site)[19]. In the NPC trial[13], a decreased CRC incidence was only observed in participants with a baseline plasma Se level of <106 µg/L. In the SELECT trial[17], which did not show a significant cancer chemoprevention effect of Se supplementation, the baseline serum Se levels ranged from 122 to 152 µg/L (mean 136 µg/L) which has been shown to ensure optimal glutathione peroxidase 3 (GPx3) expression and SePP saturation[32-34]. A comparable range of ~~blood~~ serum Se concentration was reported in the WHI prospective study (111 - 162 µg/L; mean 134 µg/L), which also showed no association between Se concentration and CRC risk[18]. Recently, the large National Health and Nutrition Examination Survey (NHANES) III survey of over 16,000 adults in the United States reported a range of ~~blood~~ serum Se from 109 – 136 µg/L (mean 125 µg/L) along with an inverse association between all-cancer mortality and Se at levels above 126 µg/L[35]. Notably, all these studies had baseline Se levels comparable to the highest

quintile of our study (>101 µg/L) suggesting that the effects of Se on CRC risk may be negated if the baseline range is above this and at levels known to saturate selenoprotein biosynthesis, based on our current knowledge of those selenoproteins such as SePP and GPx3 that can be measured in humans (there is little known about saturation requirements of intracellular selenoproteins).

As serum SePP concentrations become maximally saturated when Se intake and Se status are replete, SePP is a particularly relevant biomarker for Se status assessment in subjects with Se deficient to borderline levels[36]. The estimated cut-off for Se sufficiency ensuring maximal expression of SePP is a ~~blood~~ Se concentration (ascertained in plasma) of 90–124 µg/L[33, 34]. Approximately 95% of the EPIC subjects had serum Se levels below <124 µg/L (and approximately 80% were below 100 µg/L). The correlation between Se and SePP levels was relatively high (r = 0.60; P = <0.001), indicating that most subjects were sub-optimal in Se when judged by previously published data on Se levels required for full expression of SePP and other selenoproteins as the criterion for Se sufficiency[22, 25, 33, 34]. This contrasts with data from a study of adequately Se supplied healthy individuals in the United States where the average plasma Se concentration was 142 µg/L and SePP and Se levels did not correlate[37], presumably because the surplus Se is present as selenomethionine in other serum proteins. Among lifestyle and dietary variables adjusted for in our analyses Se and SePP levels differed by smoking status, dietary intake of fish, meat, and fruit and vegetables (Table 2) as found for previous studies[21, 38, 39].

The association of lower CRC risk with increasing Se and SePP concentrations was more apparent for women than for men. The recommended dietary allowances for Se[8] are generally higher for men based primarily on data from animal studies showing Se requirements are higher in males; partly due to body mass and possibly also the requirement of Se for sperm production and sperm protection[40]. However, to date there has

been no study in humans showing an increased need for Se in men. Our findings are supported by previous studies showing a potential effect of estrogen on Se and selenoprotein levels, and a differential response to Se supplementation by sex[20, 41-43]. Additionally, there are clear differences between males and females in regard to Se biomarker outcomes[22, 24], e.g. urinary Se was higher in women after Se supplementation suggesting that men retain Se better than women[22], but the reasons for this are not well understood. Previously, a *GPX4* selenoprotein gene variant was reported to affect lymphocyte protein level in females only[42], and sex has been shown to influence the differential effects of polymorphic variants in the *SEPP1* gene (which codes for the SePP protein) on plasma SePP[44] and associations with particular selenoprotein genetic variants and CRC risk[45].

However, in contrast, several cohort studies and clinical trials suggest that Se status influences cancer risk in men more than women[14, 18, 20]. Possibly, women are more susceptible to the effects of lower Se intakes, which would not have been seen for most of these previous studies as their baseline Se levels were too high. Interestingly, significant interactions were reported between selenoprotein gene variants and oestrogen status with colon and rectal cancer risk[46]. The authors suggested that although this could possibly be due to the anti-inflammatory properties of oestrogen and the influence of oestrogen on the tissue distribution and metabolism of Se, as shown in animal models[47]. We do not see a statistically significant interaction of Se and CRC risk by HRT use and colon site among women, although we have limited power for this analysis. Differences in dietary factors may provide further insight into potential mechanisms of Se-associated colorectal carcinogenesis between the sexes. Although none of these factors were significant effect modifiers of the association of Se or SePP with CRC risk, it is interesting that SePP concentrations were associated with red and processed meat intake in males only and with fish intake in both

males and females. It can be speculated that as fish and meat represent a good source of Se supply, they could, in that respect, confer some protection against CRC, although for men this may be slightly masked by the adverse effects of a high consumption of red and processed meat[48].

The study strengths include an appreciable sample size within a large, prospective study with extensive data on lifestyle and other dietary factors, pre-diagnostic bloods. Use of blood samples taken at time points prior to CRC diagnoses and use of prospectively collected dietary and lifestyle data minimises recall and reverse causality biases. A second major strength lies in the determination of the two most meaningful biomarkers of Se status, i.e., total Se and SePP serum concentrations[30, 33]. The main limitations are the single time-point blood measure per subject, giving room for random error, and the relatively short follow-up time (~4 years). However, the presence of random error would rather bias the estimates towards null and exclusion of cases with less than two years of follow-up did not appreciably alter the findings. Despite the large sample size, some stratified analyses had limited power, particularly sub-group analyses by sex and anatomical sub-sites. Another potential limitation applicable to all observational studies is the possibility of residual confounding. However, in our models we adjusted for a large number of potentially relevant confounding variables. There was no information on CRC screening. However, in Western Europe there is no consistent CRC screening modality and only recently have several national screening programs been piloted or implemented, which mainly employ immunochemical faecal occult blood testing[49].

In conclusion, the present study provides significant prospective data indicating an association between high Se status and a lower risk of CRC and that in populations where Se status is sub-optimal (e.g. Western Europe) increasing Se intake may reduce risk of CRC,

especially for women. An optimum dietary Se level for CRC prevention may vary according to life-stage, sex, general state of health, colorectal sub-site, and genotype[15]. We are currently examining the potential modifying effect of common genetic variation in the selenoprotein gene pathway on the risk of CRC associated with Se status. An improved understanding of how individuals "respond" to Se and how this modifies CRC risk is crucial in designing targeted supplementation trials or a public health strategy, as Se supplementation is controversial although this requires further study[50]. Furthermore, as the next major step in resolving these issues, the applicability of a Se status biomarker oriented Se supplementation trial for CRC prevention needs to be examined in a population with sub-optimal Se availability.

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Table Legends

Table 1. Selected baseline characteristics of incident colon and rectal cancer cases and their matched controls, the EPIC study.

Table 2. Geometric mean (95% CI) selenium and selenoprotein P concentrations in controls by age and other baseline characteristics.

Table 3. Incidence rate ratios (IRRs) and 95% confidence intervals (95%CI) for CRC and its sub-sites by quintiles of serum selenium concentration among all participants and by sex, EPIC cohort study, 1992-2003.

Table 4. Incidence rate ratios (IRRs) and 95% confidence intervals (95%CI) for CRC and its sub-sites by quintiles of serum SePP concentration, EPIC cohort study, 1992-2003.

Table 1. Selected baseline characteristics of incident colon and rectal cancer cases and their matched controls, the EPIC study

Characteristic*	Colon cancer			Rectal cancer		
	Cases	Controls	*P*-value**	Cases	Controls	*P*-value**
N	598	598		368	368	
Women, %	54.5	54.5	***	47.3	47.3	***
Age at blood collection, yrs	58.9 (7.2)	58.8 (7.3)	***	58.3 (6.9)	58.3 (6.9)	***
Years between blood collection and diagnosis	3.7 (2.1)	--		3.9 (2.1)	--	
Educational attainment, %			*0.396*			*0.672*
Primary	34.0	39.0		32.6	36.1	
Technical/professional school	23.9	24.9		27.5	27.7	
Secondary	16.2	13.2		13.0	11.7	
University degree	17.4	15.4		18.8	19.0	
Smoking status, %			*0.436*			*0.678*
Never smoker	39.6	43.5		38.0	40.8	
Former smoker	34.0	33.4		32.3	31.8	
Current smoker	25.6	22.1		29.1	26.4	
Physical activity, %			*0.102*			*0.677*
Inactive	16.2	11.5		15.5	14.7	
Moderately inactive	29.9	32.3		28.8	26.6	
Moderately active	43.5	43.7		44.3	42.4	
Active	9.7	11.5		11.4	14.1	
Among women						
Premenopausal, %	11.7	12.3	*0.824*	8.6	9.2	*0.887*
HRT use, %	25.5	23.9	*0.553*	19.0	26.4	*0.127*
Oral contraceptive use, %	40.5	43.6	*0.396*	44.3	51.2	*0.318*
BMI, kg/m^2	26.9 (4.6)	26.3 (3.8)	*0.017*	26.6 (4.0)	26.4 (3.8)	*0.607*
Baseline dietary intakes						
Total energy, kcal/d	2156.4 (753.1)	2134.5 (614.6)	*0.543*	2226.7 (698.1)	2183.3 (635.8)	*0.343*
Alcohol, g/d	16.0 (20.5)	15.0 (18.7)	*0.291*	20.5 (24)	17.6 (21.9)	*0.056*
Calcium, mg/d	1013.9 (425)	1042.9 (409.8)	*0.218*	1007.1 (413.4)	1057.2 (430.9)	*0.111*
Fiber, g/d	22.9 (8.2)	23.9 (8.2)	*0.047*	23.6 (7.8)	23.7 (7.8)	*0.790*
Folate, g/d	307.6 (113)	316.1 (112.9)	*0.139*	316 (110.9)	310.5 (98.5)	*0.459*

Fruit and vegetables, g/d	418.7 (262.9)	455.1 (261.4)	*0.007*	411.1 (264.2)	411.9 (226.3)	*0.964*
Fish and shellfish, g/d	34.9 (31.9)	37.9 (34.3)	*0.078*	36.8 (29.1)	37.7 (33.4)	*0.662*
Red and processed meat, g/d	91.4 (76.9)	85.6 (51.2)	*0.085*	100.3 (59)	96.5 (58.1)	*0.258*
Baseline serum biomarkers, geometric mean (5^{th}-95^{th} percentile)						
Selenium, µg/L	80.1 (49.5-118.3)	82.1 (52.1-125.1)	*0.097*	83.3 (52.9-121.3)	83.6 (53.4-126)	*0.816*
Selenoprotein P, mg/L	4.1 (2.7-6.0)	4.3 (2.9-6.1)	*0.008*	4.2 (3.0-5.9)	4.3 (2.9-6.0)	*0.922*

Abbreviations: HRT=hormone replacement therapy; BMI = body mass index.

*Data are given as means (SD) unless otherwise specified. Number of missing/unknown: smoking = 17, physical activity = 18, education=52, use of oral contraceptive = 7, HRT use=23. Missing values were not excluded from percentage calculations; therefore the sum of percent across subgroups may not add up to 100%.

** From conditional logistic regression (continuous variables) or chi-square test (categorical variables).

*** Matching factor.

Table 2. Geometric mean (95% CI) selenium and selenoprotein P concentrations in controls by age and other baseline characteristics.

Characteristic	Men (N=466)					Women (N=500)				
	N	Selenium, µg/L	*P**	Selenoprotein P,	*P**	N	Selenium, µg/L	*P**	Selenoprotein P,	*P**
	466	78.3 (75.1-81.7)		4.1 (4.0-4.3)		500	80.8 (77.8-83.9)		4.1 (4.0-4.3)	
Country										
Denmark	197	87.2 (84.2-90.3)	<0.001	4.6 (4.4-4.7)	<0.001	126	93.1 (89.1-97.3)	<0.001	4.7 (4.5-4.9)	<0.001
France	--	--		--		25	82.3 (74.6-90.8)		4.0 (3.7-4.4)	
Germany	62	74.3 (69.8-79.0)		4.1 (3.9-4.4)		35	73.2 (67.4-79.6)		4.1 (3.8-4.4)	
Greece	13	57.2 (50.0-65.4)		3.2 (2.9-3.6)		8	55.4 (46.5-65.9)		3.3 (2.8-3.8)	
Italy	48	77.9 (72.7-83.5)		4.2 (3.9-4.4)		67	84.1 (79.2-89.3)		4.3 (4.1-4.5)	
Spain	47	90.0 (83.8-96.5)		4.5 (4.3-4.8)		48	82.1 (76.5-88.2)		4.4 (4.1-4.6)	
The Netherlands	13	65.0 (56.9-74.4)		3.6 (3.2-4.1)		98	79.0 (75.2-83.0)		4.1 (3.9-4.3)	
United Kingdom	86	83.2 (79.0-87.7)		4.2 (4.0-4.4)		93	81.4 (77.4-85.7)		4.0 (3.8-4.1)	
Region**										
Southern	157	79.8 (76.6-83.1)	<0.001	4.3 (4.1-4.4)	<0.001	175	81.0 (78.0-84.2)	<0.001	4.2 (4.1-4.4)	<0.001
Central	112	77.4 (73.8-81.2)		4.0 (3.9-4.2)		199	79.0 (76.2-81.9)		4.0 (3.9-4.1)	
Northern	197	87.2 (84.1-90.4)		4.6 (4.4-4.7)		126	93.1 (89.1-97.4)		4.7 (4.5-4.9)	
Age at blood collection, years										
<55	125	78.2 (74.2-82.4)	0.566	4.2 (4.0-4.3)	0.290	159	80.9 (76.8-85.2)	0.466	4.1 (3.9-4.2)	0.078
55-59	135	78.8 (74.7-83.2)		4.1 (3.9-4.3)		124	79.5 (75.2-84.0)		4.1 (3.9-4.3)	
60-64	138	80.8 (76.6-85.2)		4.2 (4.0-4.4)		126	81.0 (76.3-85.8)		4.2 (4.0-4.4)	
≥65	68	75.5 (69.7-81.8)		3.9 (3.7-4.2)		91	82.8 (77.1-88.8)		4.3 (4.0-4.6)	
BMI, kg/m^2										
<25	141	74.8 (71.0-78.9)	0.525	4.0 (3.8-4.2)	0.692	231	79.7 (76.0-83.6)	0.604	4.1 (3.9-4.2)	0.138
25-30	259	80.6 (77.4-84.0)		4.2 (4.0-4.3)		200	81.8 (77.8-86.0)		4.2 (4.0-4.4)	
>30	66	76.6 (71.6-82.0)		4.0 (3.8-4.3)		69	81.3 (75.8-87.2)		4.3 (4.0-4.5)	
Smoking status										
Never	119	81.4 (77.2-85.8)	0.041	4.1 (3.9-4.3)	0.428	291	80.6 (77.1-84.2)	0.596	4.1 (4.0-4.3)	0.609
Former	201	79.0 (75.4-82.8)		4.2 (4.0-4.3)		116	81.7 (77.0-86.7)		4.1 (3.9-4.4)	
Current	139	75.3 (71.4-79.3)		4.0 (3.9-4.2)		90	78.8 (73.7-84.2)		4.0 (3.8-4.3)	
Physical activity										
Active	76	78.1 (73.1-83.5)	0.891	4.2 (4.0-4.4)	0.813	47	80.3 (74.0-87.1)	0.347	4.1 (3.8-4.4)	0.307
Moderately active	72	79.8 (75.8-84.0)		4.3 (4.1-4.4)		49	80.9 (77.1-84.9)		4.2 (4.0-4.4)	
Moderately inactive	10	77.7 (73.8-81.8)		4.1 (3.9-4.2)		4	82.0 (77.9-86.4)		4.1 (4.0-4.3)	

Inactive	136	77.2 (72.3-82.4)		4.0 (3.8-4.2)		155	76.2 (70.1-82.9)		3.9 (3.6-4.2)	
Alcohol at baseline, g/d										
None	10	74.3 (63.3-87.1)	0.398	3.8 (3.3-4.3)	0.101	25	79.7 (71.1-89.3)	0.301	4.1 (3.7-4.5)	0.353
0.1 - 6	109	75.5 (71.2-80.0)		4.0 (3.8-4.2)		236	80.8 (77.0-84.9)		4.1 (3.9-4.3)	
6.1-12	79	79.1 (74.3-84.3)		4.1 (3.9-4.3)		100	79.8 (74.9-84.9)		4.2 (4.0-4.4)	
12.1-24	95	82.4 (77.7-87.4)		4.3 (4.1-4.5)		89	84.2 (78.9-89.9)		4.3 (4.1-4.5)	
24.1-36	60	76.7 (71.4-82.3)		4.1 (3.9-4.4)		35	84.3 (76.7-92.7)		4.2 (3.9-4.6)	
>36	113	80.3 (75.8-85.0)		4.2 (4.0-4.4)		15	74.5 (65.0-85.3)		4.0 (3.6-4.5)	
Baseline dietary intakes:										
Total fish and shellfish, g/d										
None	13	80.4 (69.8-92.6)	0.405	3.7 (3.3-4.2)	0.021	14	80.6 (70.0-92.9)	0.488	3.8 (3.4-4.3)	0.051
0.1-15	74	75.0 (70.3-79.9)		4.0 (3.8-4.2)		140	76.3 (71.8-81.1)		4.0 (3.8-4.2)	
15.1-30	117	76.2 (72.0-80.6)		4.0 (3.8-4.2)		127	82.9 (78.3-87.8)		4.1 (3.9-4.3)	
30.1-50	125	80.4 (75.9-85.2)		4.1 (3.9-4.3)		106	83.1 (78.1-88.5)		4.3 (4.0-4.5)	
>50	137	82.9 (78.3-87.8)		4.3 (4.1-4.5)		113	81.7 (77.1-86.5)		4.2 (4.0-4.4)	
Fruits and vegetables, g/d										
≤260	150	75.6 (71.4-79.9)	0.121	4.0 (3.8-4.2)	0.137	91	80.3 (75.1-86.0)	0.586	4.0 (3.8-4.3)	0.017
260.1-400	116	78.0 (73.5-82.7)		4.1 (3.9-4.3)		129	78.3 (73.9-83.1)		4.0 (3.8-4.2)	
400.1-560	107	81.0 (76.7-85.6)		4.2 (4.0-4.4)		134	84.7 (80.0-89.7)		4.2 (4.0-4.4)	
>560	93	79.5 (75.0-84.2)		4.1 (3.9-4.3)		146	80.0 (76.0-84.1)		4.2 (4.0-4.4)	
Red and processed meats, g/d										
≤50	69	76.1 (71.1-81.4)	0.140	3.9 (3.6-4.1)	0.011	150	81.8 (77.6-86.2)	0.256	4.2 (4.1-4.4)	0.059
50-80	89	79.4 (74.8-84.2)		4.2 (3.9-4.4)		161	81.7 (77.4-86.2)		4.2 (4.0-4.4)	
80-120	135	77.0 (72.9-81.4)		4.2 (4.0-4.4)		139	79.2 (74.9-83.8)		4.0 (3.8-4.2)	
>120	173	82.3 (77.8-87.0)		4.3 (4.1-4.5)		50	78.6 (72.6-85.0)		4.0 (3.7-4.3)	

* All P-values are based on a test of linear trend, except P-values for heterogeneity by country, geographical region, sex, smoking status and educational level.

**Geographical regions: South = France, Italy, Spain, Greece; Central = UK, the Netherlands, Germany; Northern = Denmark.

All analyses were adjusted for study center, except analysis for country/region.

Number of missing/unknown among controls: smoking = 10, physical activity = 14.

Table 3. Incidence rate ratios (IRRs) and 95% confidence intervals (95% CI) for CRC and its sub-sites by quintiles of serum selenium concentration among all participants and by sex, EPIC cohort study, 1992-2003.

Cancer site Se, μg/L	All participants			Men			Women			*P* heterogeneity by sex†
	No. of ca/co	Matching factors* IRR (95% CI)	Multivariable adjusted† IRR (95% CI)	No. of ca/co	Matching factors* IRR (95% CI)	Multivariable adjusted† IRR (95% CI)	No. of ca/co	Matching factors* IRR (95% CI)	Multivariable adjusted† IRR (95% CI)	
Colorectal cancer										
<67.7	203/193	ref.	ref.	86/96	ref.	ref.	117/97	ref.	ref.	*0.105*[&]
67.7 – 78.3	201/193	0.99(0.75-1.30)	0.98(0.74-1.30)	86/91	1.06(0.70-1.59)	0.94(0.60-1.45)	115/102	0.91(0.63-1.33)	0.95(0.64-1.41)	
78.31-88.2	185/193	0.90(0.67-1.20)	0.93(0.69-1.26)	89/89	1.14(0.75-1.73)	1.14(0.73-1.78)	96/104	0.73(0.48-1.09)	0.84(0.54-1.30)	
88.3-100.6	195/193	0.95(0.71-1.27)	0.96(0.71-1.31)	111/102	1.25(0.83-1.89)	1.25(0.80-1.97)	84/91	0.73(0.48-1.11)	0.75(0.48-1.17)	
>100.6	182/194	0.87(0.64-1.18)	0.88(0.64-1.21)	94/88	1.23(0.79-1.91)	1.18(0.73-1.90)	88/106	0.63(0.41-0.97)	0.64(0.40-1.01)	
P_{trend}		*0.381*	*0.458*		*0.246*	*0.262*		*0.020*	*0.032*	
Per 25 μg/L		0.91(0.82-1.02)	0.92(0.82-1.03)		1.03(0.88-1.2)	1.02(0.86-1.22)		0.81(0.70-0.95)	0.83(0.70-0.97)	
Colon cancer										
<67.7	142/123	ref.	ref.	58/58	ref.	ref.	84/65	ref.	ref.	*0.613*[&]
67.7 – 78.3	130/116	0.96(0.68-1.35)	0.94(0.66-1.35)	55/49	1.12(0.66-1.89)	1.01(0.56-1.81)	75/67	0.85(0.54-1.34)	0.86(0.52-1.41)	
78.31-88.2	114/128	0.75(0.52-1.07)	0.75(0.51-1.10)	53/54	0.97(0.57-1.65)	0.96(0.53-1.72)	61/74	0.60(0.37-0.99)	0.65(0.38-1.12)	
88.3-100.6	99/116	0.71(0.48-1.04)	0.73(0.49-1.10)	54/62	0.85(0.49-1.48)	0.86(0.46-1.59)	45/54	0.62(0.37-1.05)	0.62(0.35-1.12)	
>100.6	113/115	0.82(0.55-1.20)	0.81(0.54-1.23)	52/49	1.05(0.59-1.87)	1.11(0.58-2.12)	61/66	0.66(0.39-1.13)	0.61(0.34-1.09)	
P_{trend}		*0.103*	*0.154*		*0.789*	*0.963*		*0.052*	*0.045*	
Per 25 μg/L		0.88(0.76-1.02)	0.90(0.77-1.05)		0.94(0.76-1.16)	0.97(0.77-1.23)		0.84 (0.69-1.02)	0.84 (0.68-1.05)	
Rectal cancer										
<67.7	61/70	ref.	ref.	28/38	ref.	ref.	33/32	ref.	ref.	
67.7 – 78.3	71/77	1.04(0.65-1.66)	1.24(0.74-2.08)	31/42	0.94(0.48-1.84)	1.03(0.44-2.39)	40/35	1.10(0.56-2.15)	1.52(0.70-3.29)	*0.273*[&]
78.31-88.2	71/65	1.28(0.78-2.10)	1.49(0.86-2.60)	36/35	1.50(0.75-2.96)	1.41(0.59-3.37)	35/30	1.09(0.52-2.28)	1.68(0.67-4.25)	
88.3-100.6	96/77	1.46(0.91-2.33)	1.61(0.95-2.72)	57/40	2.06(1.08-3.94)	2.39(1.01-5.67)	39/37	0.99(0.49-1.99)	1.26(0.55-2.87)	
>100.6	69/79	1.01(0.61-1.67)	1.09(0.63-1.89)	42/39	1.53(0.76-3.06)	1.32(0.55-3.19)	27/40	0.62(0.29-1.31)	0.76(0.32-1.80)	
P_{trend}		*0.516*	*0.568*		*0.039*	*0.170*		*0.199*	*0.271*	
Per 25 μg/L		0.95(0.80-1.12)	0.93(0.78-1.11)		1.16(0.91-1.48)	1.14(0.84-1.55)		0.78(0.61-1.0)	0.74(0.57-0.98)	
$P_{heterogeneity\ by\ sub\text{-}site}$†			*0.097*[&]			*0.219*[&]			*0.474*[&]	

Abbreviations: Se = selenium; No = Number; Ca = Cases; Co = controls; IRR = incidence rate ratio; CI = confidence interval; ref = reference.

*Model based on matching factors only.

†Model based on matching factors plus further adjustments for smoking status/duration/intensity, body mass index, total physical activity, education level, total dietary energy consumption, and intake of total calcium, fruits and vegetables, red and processed meats, and alcohol.

[&] *P*-value for heterogeneity for serum selenium concentration categorized into quintiles.

Table 4. Incidence rate ratios (IRRs) and 95% confidence intervals (95% CI) for CRC and its sub-sites by quintiles of serum SePP concentration, EPIC cohort study, 1992-2003.

Cancer site SePP, mg/L	All participants			Men			Women			*P* heterogeneity by sex†
	No. of ca/co	Matching factors* IRR (95% CI)	Multivariable adjusted† IRR (95% CI)	No. of ca/co	Matching factors* IRR (95% CI)	Multivariable adjusted† IRR (95% CI)	No. of ca/co	Matching factors* IRR (95% CI)	Multivariable adjusted† IRR (95% CI)	
Colorectal cancer										
< 3.617	236/193	ref.	ref.	96/84	ref.	ref.	140/109	ref.	ref.	*0.230*&
3.618 – 4.113	175/193	0.71(0.53-0.95)	0.72(0.53-0.97)	79/91	0.74(0.48-1.14)	0.67(0.42-1.07)	96/102	0.68(0.47-1.00)	0.71(0.47-1.07)	
4.114 – 4.558	219/193	0.91(0.69-1.20)	0.89(0.67-1.20)	100/92	0.95(0.63-1.43)	0.81(0.51-1.29)	119/101	0.88(0.61-1.28)	0.94(0.62-1.41)	
4.589 – 5.150	168/193	0.67(0.50-0.91)	0.69(0.51-0.94)	97/99	0.84(0.55-1.28)	0.82(0.52-1.29)	71/94	0.53(0.35-0.81)	0.56(0.35-0.89)	
> 5.151	168/194	0.62(0.44-0.86)	0.60(0.42-0.85)	94/100	0.78(0.49-1.24)	0.73(0.43-1.22)	74/94	0.48(0.30-0.78)	0.46(0.28-0.78)	
P_{trend}		*0.008*	*0.009*		*0.501*	*0.485*		*0.002*	*0.004*	
Per 0.806 mg/L		0.90(0.83-0.98)	0.89(0.82-0.98)		0.99(0.88-1.12)	0.98(0.86-1.12)		0.82(0.73-0.93)	0.82(0.72-0.94)	
Colon cancer										
< 3.617	154/116	ref.	ref.	57/47	ref.	ref.	97/69	ref.	ref.	*0.421*&
3.618 – 4.113	114/117	0.69(0.48-0.99)	0.71(0.48-1.04)	46/49	0.75(0.43-1.32)	0.70(0.38-1.31)	68/68	0.63(0.39-1.02)	0.73(0.43-1.24)	
4.114 – 4.558	123/117	0.77(0.54-1.10)	0.76(0.52-1.12)	54/53	0.82(0.47-1.43)	0.82(0.44-1.56)	69/64	0.74(0.46-1.18)	0.85(0.50-1.44)	
4.589 – 5.150	105/124	0.58(0.40-0.85)	0.63(0.42-0.94)	61/59	0.82(0.47-1.42)	0.89(0.48-1.65)	44/65	0.42(0.25-0.71)	0.48(0.27-0.87)	
> 5.151	102/124	0.52(0.34-0.79)	0.49(0.31-0.76)	54/64	0.62(0.33-1.14)	0.53(0.26-1.06)	48/60	0.45(0.25-0.80)	0.44(0.23-0.84)	
P_{trend}		*0.002*	*0.003*		*0.232*	*0.232*		*0.002*	*0.008*	
Per 0.806 mg/L		0.86(0.77-0.96)	0.85(0.75-0.95)		0.92(0.79-1.08)	0.89(0.74-1.07)		0.81(0.70-0.94)	0.82(0.69-0.96)	
Rectal cancer										
< 3.617	82/77	ref.	ref.	39/37	ref.	ref.	43/40	ref.	ref.	*0.657*&
3.618 – 4.113	61/76	0.72(0.45-1.15)	0.71(0.43-1.18)	33/42	0.70(0.35-1.37)	0.61(0.26-1.44)	28/34	0.75(0.39-1.43)	0.66(0.31-1.41)	
4.114 – 4.558	96/76	1.21(0.78-1.88)	1.27(0.78-2.06)	46/39	1.16(0.62-2.18)	1.10(0.49-2.47)	50/37	1.26(0.68-2.33)	1.28(0.60-2.74)	
4.589 – 5.150	63/69	0.86(0.53-1.38)	0.93(0.55-1.57)	36/40	0.83(0.43-1.59)	0.89(0.40-1.96)	27/29	0.88(0.42-1.84)	0.82(0.34-1.98)	
> 5.151	66/70	0.85(0.49-1.47)	0.80(0.43-1.48)	40/36	1.10(0.53-2.27)	0.95(0.37-2.43)	26/34	0.55(0.23-1.33)	0.53(0.19-1.48)	
P_{trend}		*0.784*	*0.806*		*0.721*	*0.805*		*0.394*	*0.386*	
Per 0.806 mg/L		0.98(0.85-1.13)	0.96(0.82-1.13)		1.09(0.90-1.31)	1.08(0.85-1.37)		0.84(0.67-1.06)	0.82(0.63-1.08)	
$P_{heterogeneity\ by\ sub\text{-}site}$†			*0.231*&			*0.632*&			*0.710*&	

Abbreviations: SePP = selenoprotein P; No = Number; Ca = Cases; Co = controls; IRR = incidence rate ratio; CI = confidence interval; ref = reference.

*Model based on matching factors only.

†Model based on matching factors plus further adjustments for smoking status/duration/intensity, body mass index, total physical activity, education level, total dietary energy consumption, and intake of total calcium, fruits and vegetables, red and processed meats, and alcohol.

&*P*-value for heterogeneity for serum SePP concentration categorized into quintiles.