Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Sushma Umesh Date

Prediagnostic Selenium and Selenoprotein P and Survival in Colorectal Cancer Patients

By

Sushma Umesh
MPH

Epidemiology

_____

Dr. Veronika Fedirko
Committee Chair

Prediagnostic Selenium and Selenoprotein P and Survival in Colorectal Cancer Patients

By

Sushma Umesh

Bachelor of Arts
University of California, Berkeley
2012

Thesis Committee Chair: Dr. Veronika Fedirko, PhD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2016

Abstract

Prediagnostic Selenium and Selenoprotein P and Survival in Colorectal Cancer Patients
By Sushma Umesh

BACKGROUND: The dietary intake of selenium (Se) and blood concentrations of selenoprotein P (SePP), a biomarker of functional Se, varies significantly worldwide with lower levels observed in the European population. There is limited data on the association between Se and cancer survival and no studies on the association between Se, SePP and colorectal cancer (CRC) survival to our knowledge.

METHODS: To investigate whether pre-diagnostic circulating levels of Se and SePP are associated with overall and CRC-specific mortality after cancer diagnosis, we analyzed 1,021 patients (all deaths = 450, CRC deaths = 375) with CRC from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, which included Western European populations that are historically Se deficient. Cox proportional hazards models were used to calculate the hazard ratios and 95% confidence intervals, adjusting for dietary and lifestyle factors.

RESULTS: Multivariable analyses suggested a statistically non-significant inverse association between total Se and overall (HR for the fifth quintile versus the first quintile = 0.82, 95% CI: 0.56 – 1.16) and CRC-specific mortality (HR for the fifth quintile versus the first quintile = 0.76, 95% CI: 0.52 – 1.11). Higher SePP levels were associated with a statistically significantly lower overall (HR for the fifth quintile versus the first quintile = 0.70, 95% CI: 0.50 – 0.98), but not CRC-specific mortality. No statistically significant interactions by potential modifying factors related to CRC and overall survival were identified.

CONCLUSION: Our study suggests that higher pre-diagnostic total serum Se and SePP protein concentrations may be associated with lower mortality among patients with CRC in Western Europe. Se intake/status might be a potential factor affecting survival in CRC patients, particularly from a population with low Se status, such as in Europe.

IMPACT: This study is the first and largest prospective analysis of the association between pre-diagnostic levels of Se and SePP with mortality among CRC patients.

Prediagnostic Selenium and Selenoprotein P and Survival in Colorectal Cancer Patients

By

Sushma Umesh

Bachelor of Arts
University of California, Berkeley
2012

Thesis Committee Chair: Dr. Veronika Fedirko, PhD, MPH

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2016

Acknowledgements

I would like to acknowledge my advisor, Dr. Veronika Fedirko, for her patience, dedication and epidemiological expertise while completing this thesis. Her mentorship and guidance was invaluable throughout this process.

TABLE OF CONTENTS

CHAPTER I

BACKGROUND

Colorectal Cancer

Colorectal Cancer (CRC) remains as one of the leading causes of death both in the United States and worldwide. In the United States, CRC is the third leading cause of death in both men and woman while in Europe, CRC is the second leading cancer killer [1, 2]. The incidence rates for this cancer are similar in both sexes [3]. By 2030, the global cancer burden for CRC is expected to see a 60% increase in cases with over 2 million cases and over 1 million deaths [4]. However, the cancer distribution is not uniformly spread and differs geographically, with incidence rates varying up to 10-fold between countries with the highest and lowest rates [3]. Increases in CRC incidence and mortality are becoming more prominent in European, Asian and South American countries while these rates have started to stabilize in North American and Australian populations [4]. Reasons for this declining trend may include increased screening and early detection in addition to improved prevention measures and perioperative care [4].

CRC survival is dependent on several factors, including stage of diagnosis. According to Surveillance, Epidemiology, and End Results (SEER) data from 2005-2011, 39.5% of CRC cases are diagnosed at the localized stage. The 5-year survival rate for localized CRC is 90.1% and 13.1% for distant stage [5]. Survival for CRC continues to increase with improved diagnosis and effective treatments. These survival rates also vary geographically with the US reporting a 12-14% higher CRC survival rate than Europe in the late 1990s [6]. These differences may have been attributable to several factors

including improved screening programs, but more importantly, point out the differences in survival by geographical location. Positive family history of CRC, typically defined as the presence of CRC in one or more first and/or second-degree relatives, is also considered an indicator of cancer survival, although results are mixed [7]. In the Nurses' Health Study, women with colorectal cancer and a family history of CRC were at higher risk of dying from CRC or any cause compared to women with colorectal cancer and no family history of CRC (adjusted HR: 1.38, 95%CI: 1.02-1.86) [8]. Conversely, a study conducted using the survival arm of the Melbourne Colorectal Cancer Study suggested that 5-year survival of CRC was neither improved nor worsened by the presence of positive family history of CRC [9].

CRC has become a significant problem both in developed and in developing countries. Environmental factors are also major factors in disease etiology and survival. 5-10% of all CRCs are due to genetic defects and over 70% of cases have been associated with diet [10]. Modifiable risk factors including diet and lifestyle and their association with CRC development are also primary areas of interest for survival research [2].

Colorectal Carcinogenesis

CRC refers to a cancer that originates either in the colon or the rectum, which may transform the normal mucosa to adenomatous polyps, and can ultimately lead to invasive carcinoma. [10]. CRC carcinogenesis typically requires a normal cell to accumulate several mutations and establish clones that continue to proliferate. Genomic instability is a common characteristic of carcinogenesis and the two most common categories include chromosomal instability and microsatellite instability [11]. Advanced

metastatic cancer is generally considered incurable, emphasizing the need for early detection [12]. Historically, screening and detection of polyps have been used as the primary prevention strategy [2]. Screening is typically recommended for adults starting at 50 years of age [13]. Although primary prevention for CRC is increasing, many individuals still fail to meet the minimum recommendations [14].

Risk Factors in Colorectal Cancer

It has been well-characterized that high-fiber diets are associated with lower risk of CRC. A study conducted using EPIC data suggested a 40% reduced risk of CRC among those who had a high-fiber diet [15]. Similarly, several studies have indicated an inverse association between selenium (Se) levels and CRC. A study conducted by Hughes et al, showed a significant association between higher Se levels and lower CRC risk in women [1]. Furthermore, the researchers also showed that higher levels of Selenoprotein P (SePP) are also associated with lower CRC risk. Two North American Se intervention trials suggested opposing results however, it is speculated that differing baseline Se levels may be the logical explanation [1].

In addition to diet, several other lifestyle factors play an important role in the development of CRC. High alcohol intake and smoking have been associated with greater risk of CRC [16, 17]. Increased alcohol consumption and risk of CRC is especially prevalent in younger patients [3]. Varying rates of cancer incidence have suggested that diet and lifestyle are contributing factors to the onset of disease. High body mass index (BMI), obesity, lower levels of physical activity and presence of diabetes mellitus are also established factors that increase the risk of CRC [18]. Although there is limited

literature on the subject, dietary micronutrients have been implicated in the etiology of the disease [1]. However, the heterogeneous nature of the disease and difficulty in measuring dietary exposures makes definitive associations between cancer and dietary nutrients hard to characterize.

Prognostic Factors in Colorectal Cancer

Currently, stage information is most the most commonly used clinical prognostic factor and there is a need to continue identifying additional factors [19]. Early detection is especially important for CRC because prognosis and survival is highly dependent age of diagnosis, stage, and grade of tumor [3]. Poorly-differentiated tumors have been shown to be associated with bowel penetration [19]. A combination of chemotherapy and radiation is used to treat the disease, with surgical removal of the tumor and lymph vessels in advanced cases [4].

Selenium (Se)

The exposure of interest in this study, selenium (Se), an essential micronutrient, is a trace element that is involved in several major metabolic pathways. Biochemically, it forms a covalent bond within selenocysteine (Sec), the 21st amino acid, which is subsequently present in the mammalian protein, glutathione peroxidase (GPX) [20, 21]. The human genome contains twenty-five selenoproteins and five GPX selenoproteins have been identified in humans, spanning a variety of biological roles including their involvement with redox homeostasis [20, 22]. In addition to GPXs, other classes of selenoproteins including thioredoxin reductases (TRXRs) and iodothyronine deiodinases

(DIOs) have been widely researched [21]. Se is also contained in another amino acid, selenomethionine, which is biosynthesized in plants. Subsequent research studies have identified the important role of Se to human health and Se remains an important area of study today.

Dietary Selenium

Large amounts of Se is found in the soil that is used to grow plants for human consumption which greatly varies based on geography [22]. For example, in the European population and certain regions of China, it has been shown that there are significantly lower levels of dietary Se as compared to the North American and Australian population [21]. Soil management is also a key component of Se status as agricultural techniques such as irrigation and fertilization can greatly affect the bioavailability of the micronutrient [23]. Se is most commonly present in meats and fish in addition to plants and grain products. Animals that are raised for food consumption are typically fed a Se-supplemented diet to ensure greater Se concentrations in the meat. The recommended dietary allowance (RDA) for Se in the United States and Europe is 55µg/d for both men and women [22, 24]. Europe has an average dietary Se intake of 40µg/d compared with upwards of 90µg/d in the United States [25].

Early research on Se focused on understanding its toxic effects to humans. However, Se deficiency can also lead to several adverse outcomes both in humans and animals. The relationship between health outcomes and Se levels has typically been depicted by a U-shaped curve, suggesting that there are adverse health consequences for both Se deficiency and toxicity [24]. Historically, Se deficiency has been associated with

white muscle disease, common in livestock. In humans, endemic Se deficiency has been associated with many diseases including Chagas' disease, epilepsy, Keshan disease (cardiomyopathy), Kaschin-Beck disease (osteoarthropathy) and Myxedematous endemic cretinism [21-23]. The exact function of Se in these diseases have eluded researchers but Se deficiency is likely to be a pre-disposing factor. Se deficiency may also lead to muscle disorders in humans, particularly in areas with low levels of Se in the soil [21]. Conversely, Se toxicity, also referred to as selenosis, can also be a major problem. Selenosis is rather uncommon in humans but can lead to garlic breath, hair loss or liver cirrhosis in extreme cases [22]. Acute and chronic cases of Se deficiency and selenosis have varying consequences on human health and further research is needed to identify Se's role and mechanism of action in these cases.

Se is also critical for normal brain development and function as the brain strictly regulates Se homeostasis [22]. In addition, Se and selenoproteins are also thought to play a major role in several chronic diseases including cardiovascular disease, diabetes mellitus, neurodegeneration and cancer. Selenoproteins are important to the production of hormones and are involved with thyroid hormone activation [21]. Despite the fact that selenoproteins serve a variety of functions, it has been speculated that regulation of Se may serve as a therapeutic agent and prevent the onset of several life-threatening diseases.

Selenoprotein P (SePP)

As previously mentioned, humans contain twenty-five selenoproteins that perform a myriad of functions. Selenoprotein P (SePP) is a secreted glycoprotein that is

predominantly produced by the liver [26]. SePP contains over 50% of the total plasma Se, allowing for its unique property to serve as a biomarker of functional Se. SePP depletion leads to increased Se excretion in the urine and SePP and Se deficiency leads to decreased plasma concentrations of SePP [26]. The decreased Se expression when SePP is depleted suggests that SePP may also be responsible for Se transport and storage, although the exact role is still debated [27].

Animal studies, utilizing Se deficient mice have been used to identify the different mechanisms of action and relationship between Se and SePP [28]. Dietary Se supplementation of SePP knockout mice (characterized by systemic Se deficiency), have restored the original phenotype [20]. It has been hypothesized that as SePP expression in the brain increases with age, it helps mediate oxidative stress [21].

Selenium, Selenoprotein P, and Colorectal Cancer

Although conflicting opinions in the literature exist, the association between Se and CRC has been well researched nonetheless. Findings from several cohorts and clinical trials suggest that the association between Se and CRC risk differs by sex. The Women's Health Initiative (WHI) study including women with sufficient Se levels, showed no association between Se and CRC risk: Adjusted OR comparing the fifth and first quintile was 1.26 (95% CI: 0.91 – 1.73) [29] . On the other hand, one of the largest prospective analyses conducted using the EPIC cohort data, a Se deficient population, suggested an increased CRC risk with lower Se levels for women: IRR per 25 µg/L Se increase was 0.83 (95% CI: 0.70 – 0.97) [1]. The varying body of literature around this subject show the importance of Se in the context of CRC risk.

Decreased levels of SePP mRNA have previously been reported in colon cancer tissues [30]. Four single nucleotide polymorphisms (SNPs) in the human SePP gene have been identified and linked to CRC risk or colorectal adenoma [31]. Understanding the association between SePP and diseases such as CRC continues to be a primary area of research today. More importantly, this association has not been well-characterized in European populations [32]. Measuring SePP levels in addition to Se levels provide a more accurate and better overall estimation of exposure to Se and nutritional status [33].

Selenium, Selenoprotein P, and Cancer Survival

There is less established evidence showing the effects of lifestyle factors and dietary factors including exposure to Se on CRC survival [34]. The literature on Se and survival for any cancer is generally limited and there are no studies on the association between Se and CRC survival. A small study of 62 renal cancer patients in Germany suggested that lower Se and SePP concentrations correlated with higher tumor grade and tumor stage at diagnosis [35]. A study conducted on 3,146 women diagnosed with invasive breast cancer in the Swedish Mammography Cohort, population with low exposure to Se, reported an inverse association between Se intake and breast cancer mortality with a HR of 0.64 (95% CI: 0.48 – 0.84) among women in the highest Se quartile as compared to women in the lowest Se quartile [36]. On the other hand, the Health Professionals Follow-Up study found that Se supplementation of 140 or more µg/day after diagnosis of non-metastatic prostate cancer, may increase the risk of prostate cancer mortality: HR of 140 or more µg/day of Se supplementation after diagnosis as compared to nonusers was 2.60 (95% CI: 1.44 – 4.70) [27]. Further research is needed to

examine the association between Se and CRC survivorship. Further research is needed in order to definitively make a conclusion about the association between SePP and CRC survival.

CHAPTER II

ABSTRACT

BACKGROUND: The dietary intake of selenium (Se) and blood concentrations of selenoprotein P (SePP), a biomarker of functional Se, varies significantly worldwide with lower levels observed in the European population. There is limited data on the association between Se and cancer survival and no studies on the association between Se, SePP and colorectal cancer (CRC) survival to our knowledge.

METHODS: To investigate whether pre-diagnostic circulating levels of Se and SePP are associated with overall and CRC-specific mortality after cancer diagnosis, we analyzed 1,021 patients (all deaths = 450, CRC deaths = 375) with CRC from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, which included Western European populations that are historically Se deficient. Cox proportional hazards models were used to calculate the hazard ratios and 95% confidence intervals, adjusting for dietary and lifestyle factors.

RESULTS: Multivariable analyses suggested a statistically non-significant inverse association between total Se and overall (HR for the fifth quintile versus the first quintile = 0.82, 95% CI: 0.56 – 1.16) and CRC-specific mortality (HR for the fifth quintile versus the first quintile = 0.76, 95% CI: 0.52 – 1.11). Higher SePP levels were associated with a statistically significantly lower overall (HR for the fifth quintile versus the first quintile = 0.70, 95% CI: 0.50 – 0.98), but not CRC-specific mortality. No statistically significant interactions by potential modifying factors related to CRC and overall survival were identified.

CONCLUSION: Our study suggests that higher pre-diagnostic total serum Se and SePP protein concentrations may be associated with lower mortality among patients with CRC in Western Europe. Se intake/status might be a potential factor affecting survival in CRC patients, particularly from a population with low Se status, such as in Europe.

IMPACT: This study is the first and largest prospective analysis of the association between pre-diagnostic levels of Se and SePP with mortality among CRC patients.

INTRODUCTION

Colorectal cancer (CRC) is the second most common cause of cancer death in Europe [37]. A number of prognostic factors contribute to disease progression including tumor stage and grade, and differences in lifestyle and diet vary across European countries [3, 38]. The epidemiologic literature is increasing on the association between dietary micronutrients and CRC risk, however, research is limited on how dietary micronutrients affect survival [1].

Selenium (Se), an essential micronutrient, is a trace element that is involved in several major metabolic pathways and it thought to have potential anti-carcinogenic properties [39]. The effects of Se are mediated by selenoproteins, which have a variety of biological roles including involvement with redox homeostasis [20, 22]. Selenoprotein P (SePP) is a secreted glycoprotein that is predominantly produced by the liver and is considered the best biomarker of functional Se [26]. SePP is a major transporter of hepatic Se in the serum and may have local Se storage functions [40]. It is an indicator of long-term Se intake and is less influenced by the chemical form of ingested Se [1].

The intake of Se and blood concentrations of selenoprotein P (SePP) varies significantly worldwide with lower levels observed in the European population [26]. European and Asian populations often experience Se deficiency as compared to North American populations where Se is abundant in the soil, and food system [23]. Se supplementation trials are frequently conducted in the US, where 50% of the population take dietary supplements and have sufficient Se levels [25]. Thus, it is important to understand the role of Se and its influence on survival after cancer diagnosis in a Se deficient population.

Many factors play a role in cancer progression including chronic inflammation, and oxidative stress, an imbalance between the production and safe elimination of free radicals and reactive oxygen species (ROS) [41]. During chronic inflammation, high levels of ROS can hinder cellular defense mechanisms and promote tumor initiation and progression [42]. It is biologically plausible that Se and SePP may influence prognosis due to their role in cellular growth and influence on cancer initiation [36, 43]. One possible explanation for its anticancer effects may be due to the micronutrient's ability to influence the transmission of "death signals" and trigger apoptosis [43]. Se and its metabolites have also been shown to inhibit progression of the cell cycle and inhibit blood vessel formation, a process necessary for tumor growth and metastasis [44]. Selenoproteins play a role in modifying inflammation and preventing further oxidative damage to other biomolecules with SePP specifically involved in antioxidant processes and transport functions [44].

Evidence from studies of Se and SePP levels and CRC risk suggest a possible association between the micronutrient and CRC survival. However, there is limited observational data on Se and SePP and cancer survival [34]. Tailored recommendations regarding lifestyle changes for patients post cancer diagnosis are scarce [45]. Cancer survivors are also at increased risk for comorbid conditions and often seek lifestyle changes to improve their long-term health [46, 47]. More specifically, there is little to no knowledge on the role diet and lifestyle factors during and after CRC treatment, and how they influence CRC survival [48]. To our knowledge, there is no data on the association between Se, SePP and CRC survival.

In consideration of the current gap in the literature, we investigated whether higher pre-diagnostic exposure to Se, as indicated by circulating levels of Se and SePP, is associated with lower overall and CRC-specific mortality in patients diagnosed with CRC within the context of a large Western European prospective cohort study.

METHODS

Study population and data collection

The European Prospective Investigation into Cancer and Nutrition (EPIC) is a multicenter 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 [15, 49]. Participating countries include France, Germany, Italy, Greece, the Netherlands, Spain, the United Kingdom, Sweden, Denmark and Norway. Between 1992 and 1998, standardized dietary and lifestyle/personal history questionnaires, anthropometric data, and blood samples were collected from most participants at recruitment, before disease onset or diagnosis. Diet over the previous 1 year was measured at baseline by validated country-specific dietary questionnaires developed to ensure high compliance and better measures of local dietary habits. Serum samples were stored at the International Agency for Research on Cancer (IARC) at -196°C under liquid nitrogen for all countries except Denmark (-150°C, nitrogen vapor). 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 centers.

Cancer incidence follow-up

Incident cancer cases were determined through record linkage with regional cancer registries (Denmark, Italy, the Netherlands, Spain, Sweden, and the United Kingdom) or through a combination of methods including the use of health insurance

records, contacts with cancer and pathology registries, and active follow-up through study subjects and their next-of-kin (France, Germany, Greece; complete up to June 2010).

Vital status follow-up

Vital status follow up was determined through record linkage with regional and/or national mortality registries (Italy, the Netherlands, Spain, the United Kingdom, Sweden, Denmark, and Norway) or active follow-up (France, Germany, and Greece). Censoring dates for complete follow-up varied amongst countries but were between December 2006 and June 2010 for Germany, Greece, and France and between December 2006 and December 2008 for the remaining countries. Mortality was coded using the 10th Revision of the International Classification of Diseases, Injuries, and Causes of Death (ICD-10) and the outcome was assigned based on underlying cause of death.

Case ascertainment and selection

Cancer data was coded using the tenth Revision of the International Classification of Diseases and the second revision of the International Classification of Disease for Oncology. CRC cases were selected from participants who developed colon (C18.0-C18.7), rectum (C19-C20), and overlapping or unspecified origin tumors (C18.8-C18.9). Anal cancers (C21) were excluded. CRC is defined as colon and rectal cancer cases. Case exclusions included 56 missing biomarker measurements due to insufficient availability of bio-sample, 4 missing date of death, and 11 diagnosed with CRC after date of censoring, resulting in 1,021 CRC cases. The present analysis is based on participant data

from all centers except for Norway (blood samples only recently collected; few CRCs diagnosed after blood donation) and Sweden (no available serum samples). Individuals who were eligible for the study were selected from the general population of a specific geographical area, town, or province. Exceptions included the French sub-cohort, which is based on members of the health insurance system or state-school employees, and the Utrecht (Netherlands) sub-cohort, which is based on women who underwent screening for breast cancer.

Selenium and Selenoprotein P measurements

Information regarding collection and measurement of Se and SePP has been previously published [34]. Briefly, Se concentrations were measured from 20µL sample of blood serum and total levels were quantified using X-ray fluorescence (TXRF spectrometer). SePP concentrations were measured from 20µL blood serum samples and quantified by conducting an immunoluminometric sandwich assay (Selenotest^{TM}). All sample measurements were conducted twice and mean concentration values were calculated and reported. Duplicate samples of known Se and SePP concentrations were used to account for intra-assay variability in each analysis. The coefficient of variation for control 1 was 7.3% (SePP: 1.5 mg/L) and 7.2% for control 2 (SePP: 8.6 mg/L).

Covariates

Several covariates were determined *a priori* and included in the statistical analyses. The following covariates were included in the analysis of Se, SePP and CRC survival: age at diagnosis, sex, stage, grade of tumor differentiation (well, moderately,

poorly differentiated, unknown), location of primary tumor (colon or rectum), smoking status (never smoker, former smoker, current smoker), BMI (kg/m^2), physical activity (combined recreational and household activity; expressed as sex-specific categories of metabolic equivalents), year of diagnosis and diabetes status. These variables were chosen based on evidence from the literature showing their association with CRC survival. Information regarding categorization and harmonization of tumor stage data has been previously published [34]. In short, a four-stage classification was used including localized, metastatic, metastatic regional and metastatic distant. Confounding assessment was conducted by evaluating if there was a sizeable change (> 10%) in hazard ratios (HR) after including the variable in the model.

Statistical Analyses

Death from CRC was the primary endpoint and death from any cause was used as a secondary endpoint. Age of first tumor diagnosis and age at death or censor were used as the two-time interval points for patient follow-up time. Separate categories were created for categorical variables with missing values, and sex-specific median values were used for continuous variables with missing values. To evaluate the association between Se and SePP and CRC-death and all-cause mortality, a Cox proportional hazards model stratified by country of cancer diagnosis and adjusted for sex, stage of tumor, grade of differentiation, smoking status, location of tumor, year of diagnosis, age of diagnosis, physical activity, alcohol, and diabetes was used to calculate the hazard ratio (HR) and 95% confidence interval (CI). The proportional hazards assumption was graphically assessed by estimating “log-log” survival curves and checked for parallelism.

In addition, the proportional hazards assumption was verified using goodness of fit test methods. Correlations between Schoenfeld residuals and time dependent variables in the Cox model were evaluated to test for any violations of the proportional hazards assumptions. P_{trend} was calculated with the median value of each Se and SePP quintile as a continuous variable and adjusted for variables in the corresponding models. For the purposes of this analysis, the exposure was examined as follows: Se and SePP quintiles, per 22.38 µg/L (1 standard deviation) increase of Se and per 0.97 mg/L (1 standard deviation) increase of SePP, and predefined categories of Se (≤ 54, 54 - 140, >140 µg/L, and ≤ 106, 106 - 170, >170 µg/L; ref 25). In addition, analyses were also run separately for CRC specific and overall mortality by anatomical sub-sites of colon and rectum, and for men and women separately.

The effect of incomplete tumor stage information on effect estimates was assessed using several approaches. The first approach reclassified missing tumor stage values into a separate missing category and adjusted for the stage variable in the final model (included in the primary analysis). Second, a sensitivity analysis was conducted by excluding incomplete stage information and subsequently by assessing how the results were affected by the missing stage information. Finally, an imputation of missing stage values was conducted using the SAS PROC MI procedure [50]. The multiple imputation method was based on available data for the other covariates in the model and assumed that the stage data was missing at random.

We investigated the possibly non-linear relationship between Se, SePP and the HRs non-parametrically with restricted cubic splines [51, 52] fitted to a Cox proportional hazards model using the SAS macro “lgtphcurv9” [53]. Tests for non-linearity used the

likelihood ratio test, comparing the model with only the linear term to the model with the linear and cubic spline terms [53]. P-values of nonlinearity test from these models with 5 knots was 0.53 for Se and 0.10 for SePP, indicating a possible linear relationship, and thus linear spline analysis is presented (Supplementary Figure 1 and 2).

Subgroup analyses by categories of potentially biologically relevant effect modifiers (follow up, sex, age at diagnosis, site, grade, tumor stage, year of diagnosis, smoking status, BMI, physical activity, and alcohol) were conducted. Adjusted HRs and 95% CI were reported for a 22.4 µg/L and 0.97 mg/L increment in Se and SePP respectively. A cross product of Se and SePP as a continuous variable and the covariate of interest as a continuous or dichotomous variable was included in the model to test for statistical interaction. Likelihood ratios based on models with and without the interaction terms were used to test for statistical significance. All statistical tests were conducted using SAS version 9.2 (SAS Institute) and p-values of < 0.05 were considered statistically significant. A sensitivity analysis of Se and SePP quintiles and CRC specific and overall mortality was conducted by including complete CRC stage data or imputed stage data in the model (Supplementary table 7).

RESULTS

Characteristics of study participants

The distribution of selected baseline characteristics of CRC cases according to quintiles of serum Se and SePP are shown in Table 1 and Table 2, respectively. Among 1,021 eligible cases, there were 450 deaths (deaths from CRC = 375, other malignant neoplasms = 35, disease of the blood = 4, endocrine disorder = 1, mental disorders = 2, nervous system disorder = 1, cardiovascular disorders = 20, respiratory disorders = 3, gastrointestinal disorders = 3, kidney disease = 1, abnormal clinical findings = 2, injury = 1, and suicide = 1). Two observations were excluded from Se analysis (N = 1,019) and 5 observations were excluded from SePP analysis (N = 1,016) due to missing values. Mean follow up time was 65 months (SD = 44 months) and Se and SePP concentrations were measured on average of 44 months (SD = 25) before CRC diagnosis.

Selenium and mortality among colorectal cancer patients

The results of age-adjusted and multivariable adjusted Cox proportional hazard models for the association of Se and CRC cancer and all-cause mortality are shown in Table 3. Higher levels of Se were not associated with a statistically significant reduction in overall mortality or CRC mortality. For CRC mortality, the multivariable adjusted HR for the fifth quintile versus the first quintile was 0.76 (95% CI: 0.52 – 1.11, $P_{trend} = 0.10$). Similar results were obtained in analyses that were restricted to complete CRC stage data for CRC mortality: HR for the fifth quintile versus the first quintile was 0.83 (95% CI: 0.56 – 1.24, $P_{trend} = 0.21$). There also was a non-significant inverse association between Se and overall mortality: HR for the fifth quintile versus the first quintile was 0.82 (95%

CI: 0.56 – 1.16, P_{trend} = 0.14). A similar association was observed between Se and CRC mortality using pre-defined cutpoints where the HR for the fifth quintile versus the first quintile was 0.74 (95% CI: 0.28 – 1.98, P_{trend} = 0.71), and Se and overall mortality where the HR for the fifth quintile versus the first quintile was 0.57 (95% CI: 0.23 – 1.39, P_{trend} = 0.31). Similar results were obtained for pre-defined cutpoints (Table 6) and by tumor site (Supplementary table 10). A similar association was found by sex: HR for the fifth quintile versus the first quintile for men was 0.60 (95% CI: 0.35 – 1.01, P_{trend} = 0.07) and HR for the fifth quintile versus the first quintile for women was 0.84 (95% CI: 0.46 – 1.53, P_{trend} = 0.60) (Supplementary table 8).

Selenoprotein P and mortality among colorectal cancer patients

The results of age-adjusted and multivariable adjusted Cox proportional hazard models for the association of SePP and CRC cancer and all-cause mortality are shown in Table 3. Higher levels of SePP were not associated with a statistically significant reduction in CRC mortality: HR for the fifth quintile versus the first quintile was 0.83 (95% CI: 0.57 – 1.19, P_{trend} = 0.33). However, higher levels of SePP were associated with a statistically significant reduction in overall mortality: HR for the fifth quintile versus the first quintile was 0.70 (95% CI: 0.50 – 0.98, P_{trend} = 0.05). Similar results were obtained in imputed CRC stage data analyses for overall mortality: HR for the fifth quintile versus the first quintile was 0.69 (95% CI: 0.50 – 0.94, P_{trend} = 0.03). Similar results were also obtained for pre-defined cutpoints (Table 6), by tumor site (Supplementary table 11) and sex (Supplementary table 9).

Effect modifications and sensitivity analyses

Possible interactions across strata of potential effect modifiers related to survival showed that the inverse association between Se and SePP and CRC and overall mortality was unchanged. No statistically significant interactions were observed between Se, SePP and potential effect modifiers for CRC-specific and overall mortality, across subcategories (Table 4 and 5). Sensitivity analyses were conducted for missing stage data and complete stage data or imputed stage data was included. There was no considerable change in the estimates for the association between Se, SePP and CRC mortality. Complete CRC stage data sensitivity analyses suggested a stronger effect for the association between Se and overall mortality: HR was 0.89 (95% CI: 0.80 – 0.99, P_{trend} = 0.03).

DISCUSSION

This study is the first and largest prospective analysis of the association of pre-diagnostic serum Se status biomarkers (total serum Se levels and SePP protein concentrations) with mortality among CRC patients. The results of this study suggest that higher pre-diagnostic total serum Se levels and SePP protein concentrations may be associated with lower mortality among patients with CRC in Western Europe; however most effect estimates were statistically non-significant. This indicates that Se intake/status might be a potential factor affecting survival in CRC patients, particularly from a population with low Se status, such as in Europe [25].

The exact biological mechanism of Se and SePP function in relation to cancer survival has eluded researchers, however, the micronutrient has been shown to be involved in oxidative stress pathways and the defense system [54]. Selenoproteins have important enzymic functions and are commonly used as biomarkers of functional Se [26, 27]. These proteins have functions include modifying inflammation, protecting DNA from damage from oxygen radicals, and preventing the malignant transformation of normal cells into activating oncogenes [55]. Several mechanisms have been suggested for Se anticarcinogenesis including cell cycle regulation, apoptosis, immune surveillance and angiogenesis [56]. Anticarcinogenic properties of Se and its role in cellular defense makes this micronutrient a relevant candidate for cancer research, although further evidence is needed to establish biological plausibility [39].

To our knowledge, there have been no previous studies on Se, SePP and CRC survival, and evidence observing the association between Se and cancer survival is limited. At least two studies have been reported among breast cancer and renal cancer

patients. A study of 3,146 women diagnosed with invasive breast cancer in the Swedish Mammography Cohort, a population with low intakes of Se, reported an inverse association between dietary Se intake and breast cancer mortality with a HR of 0.64 (95% CI: 0.48 – 0.84) among women in the highest Se quartile as compared to women in the lowest Se quartile [36]. A small study of 62 renal cancer patients in Germany suggested that lower Se and SePP concentrations correlated with higher tumor grade and tumor stage at diagnosis [35]. Few studies have also reported the association between post-diagnosis Se supplementation and cancer survival. A study of the Health Professionals Follow-Up study found that Se supplementation of 140 or more µg/day after diagnosis of non-metastatic prostate cancer, may increase the risk of prostate cancer mortality: HR of 140 or more µg/day of Se supplementation after diagnosis as compared to nonusers was 2.60 (95% CI: 1.44 – 4.70) [27].

There are several potential explanations for the findings in this study including the possibility that higher Se levels do not meaningfully affect survival in CRC subjects. A U-shape between Se status and cancer has been proposed with those with low Se levels benefiting from supplementation [57]. Therefore, higher Se levels may not necessarily imply a greater protective effect. Nonetheless, the various metabolic pathways and role of Se in humans needs to be further explored.

Strengths of this study include the large prospective design and the measurement of both serum Se and SePP, a biomarker of functional Se. Samples were measured in duplicate for more accurate measurements. In addition, the European population reflected a group of people with known Se deficiencies, making this population a viable candidate for this type of analysis. Furthermore, we assessed for multiple potential confounders,

accounting for missing values for stage of cancer through various techniques including sensitivity analyses and imputation techniques.

Nonetheless, this study is not without limitations. An important limitation was the lack of CRC treatment data and to address this issue, we conducted our analyses by stratifying on country of CRC diagnosis, adjusting for year of diagnosis, and adjusting for tumor stage as a proxy for treatment. We further estimated the effect of missing CRC stage data using multiple validated approaches. As with other observational studies, there is the possibility for residual confounding despite controlling for relevant covariates. Finally, due to geographical differences in Se content, results from this type of study may be difficult to generalize to a population with sufficient Se levels.

In summary, the findings from this study suggest a statistically non-significant inverse association between total Se and overall and CRC specific mortality among CRC patients in a population that is historically Se deficient. Our results also suggest an inverse association between SePP levels and overall mortality, although further research is necessary to validate these findings in different populations and continue to understand the mechanism of action of Se and SePP in relation to survival among patients with CRC.

CHAPTER III

Public Health Significance

The literature suggests that the information about CRC incidence and risk has been well researched but gaps in the association between micronutrients and cancer survival still exist. This study is among the first studies to analyze the association between pre-diagnostic Se and SePP levels and CRC-specific and overall mortality. Previous studies have focused on the association between Se and other cancers, including breast, prostate, and renal cancers. This study aims to further understand the role of micronutrients and CRC survival and what implications that may have on diagnosis and treatment of this disease and improved lifestyle and dietary recommendations for cancer survivors.

Future Directions

As researchers continue to understand the relationship between Se and cancer survival, future studies should further explore the role of Se supplementation and cancer prognosis, specifically in Se deficient populations. Studies have published differences in CRC risk with selenium deficiencies and supplementation, and thus this would be an interesting area of research to further explore for CRC survivorship. Finally, expanding this research to analyze survival in populations with varying levels of Se may help understand the biological role of Se and selenoproteins in the progression of CRC.

REFERENCES

1. Hughes, D.J., et al., *Selenium status is associated with colorectal cancer risk in the European prospective investigation of cancer and nutrition cohort.* Int J Cancer, 2015. **136**(5): p. 1149-61.
2. Chan, A.T. and E.L. Giovannucci, *Primary Prevention of Colorectal Cancer.* Gastroenterology, 2010. **138**(6): p. 2029-2043.e10.
3. Haggar, F.A. and R.P. Boushey, *Colorectal Cancer Epidemiology: Incidence, Mortality, Survival, and Risk Factors.* Clinics in Colon and Rectal Surgery, 2009. **22**(4): p. 191-197.
4. Arnold, M., et al., *Global patterns and trends in colorectal cancer incidence and mortality.* Gut, 2016.
5. Surveillance, E., and End Results Program. *SEER Stat Fact Sheets: Colon and Rectum Cancer*. [cited 2016 March 30]; Available from: http://seer.cancer.gov/statfacts/html/colorect.html.
6. Allemani, C., et al., *Colorectal cancer survival in the USA and Europe: a CONCORD high-resolution study.* BMJ Open, 2013. **3**(9): p. e003055.
7. Zell, J.A., et al., *Survival after colorectal cancer diagnosis is associated with colorectal cancer family history.* Cancer Epidemiol Biomarkers Prev, 2008. **17**(11): p. 3134-40.
8. Bass, A.J., et al., *Family history and survival after colorectal cancer diagnosis.* Cancer, 2008. **112**(6): p. 1222-9.
9. Kune, G.A., S. Kune, and L.F. Watson, *The effect of family history of cancer, religion, parity and migrant status on survival in colorectal cancer.* European Journal of Cancer, 1992. **28**(8): p. 1484-1487.
10. Aggarwal, B., et al., *Prevention and Treatment of Colorectal Cancer by Natural Agents From Mother Nature.* Curr Colorectal Cancer Rep, 2013. **9**(1): p. 37-56.
11. Worthley, D.L., et al., *Colorectal carcinogenesis: road maps to cancer.* World J Gastroenterol, 2007. **13**(28): p. 3784-91.
12. Hammond, W.A., A. Swaika, and K. Mody, *Pharmacologic resistance in colorectal cancer: a review.* Therapeutic Advances in Medical Oncology, 2016. **8**(1): p. 57-84.
13. Smith, R.A., et al., *Cancer screening in the United States, 2016: A review of current American Cancer Society guidelines and current issues in cancer screening.* CA: A Cancer Journal for Clinicians, 2016: p. n/a-n/a.
14. Porter, N.R., et al., *Diabetes Status and Being Up-to-Date on Colorectal Cancer Screening, 2012 Behavioral Risk Factor Surveillance System.* Prev Chronic Dis, 2016. **13**: p. E19.
15. Riboli, E., et al., *European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection.* Public Health Nutr, 2002. **5**(6b): p. 1113-24.
16. Fedirko, V., et al., *Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies.* Ann Oncol, 2011. **22**(9): p. 1958-72.
17. Botteri, E., et al., *Smoking and colorectal cancer: A meta-analysis.* JAMA, 2008. **300**(23): p. 2765-2778.
18. *Colorectal Cancer 2011 Report: Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer*. World Cancer Research Fund.

19. Xu, B., et al., *Prognostic factors in the patients with T2N0M0 colorectal cancer.* World Journal of Surgical Oncology, 2016. **14**: p. 76.
20. Hatfield, D.L., et al., *Selenium and selenocysteine: roles in cancer, health, and development.* Trends Biochem Sci, 2014. **39**(3): p. 112-20.
21. Bellinger, F.P., et al., *Regulation and function of selenoproteins in human disease.* Biochem J, 2009. **422**(1): p. 11-22.
22. Kurokawa, S. and M.J. Berry, *Selenium. Role of the Essential Metalloid in Health.* Metal ions in life sciences, 2013. **13**: p. 499-534.
23. Combs, G.F., Jr., *Selenium in global food systems.* Br J Nutr, 2001. **85**(5): p. 517-47.
24. Reszka, E., et al., *Relevance of selenoprotein transcripts for selenium status in humans.* Genes & Nutrition, 2012. **7**(2): p. 127-137.
25. Rayman, M.P., *Selenium and human health.* Lancet, 2012. **379**(9822): p. 1256-68.
26. Burk, R.F. and K.E. Hill, *Selenoprotein P—Expression, functions, and roles in mammals.* Biochimica et Biophysica Acta (BBA) - General Subjects, 2009. **1790**(11): p. 1441-1447.
27. Davis, C.D., P.A. Tsuji, and J.A. Milner, *Selenoproteins and cancer prevention.* Annu Rev Nutr, 2012. **32**: p. 73-95.
28. Holben, D.H. and A.M. Smith, *The diverse role of selenium within selenoproteins: a review.* J Am Diet Assoc, 1999. **99**(7): p. 836-43.
29. Takata, Y., et al., *Serum selenium, genetic variation in selenoenzymes, and risk of colorectal cancer: primary analysis from the Women's Health Initiative Observational Study and meta-analysis.* Cancer Epidemiol Biomarkers Prev, 2011. **20**(9): p. 1822-30.
30. Murawaki, Y., et al., *Aberrant expression of selenoproteins in the progression of colorectal cancer.* Cancer Lett, 2008. **259**(2): p. 218-30.
31. Steinbrenner, H., B. Speckmann, and H. Sies, *Toward Understanding Success and Failures in the Use of Selenium for Cancer Prevention.* Antioxidants & Redox Signaling, 2013. **19**(2): p. 181-191.
32. Chen, Y.C., K.S. Prabhu, and A.M. Mastro, *Is selenium a potential treatment for cancer metastasis?* Nutrients, 2013. **5**(4): p. 1149-68.
33. Early, D.S., et al., *Selenoprotein levels in patients with colorectal adenomas and cancer.* Am J Gastroenterol, 2002. **97**(3): p. 745-8.
34. Fedirko, V., et al., *Prediagnostic 25-hydroxyvitamin D, VDR and CASR polymorphisms, and survival in patients with colorectal cancer in western European populations.* Cancer Epidemiol Biomarkers Prev, 2012. **21**(4): p. 582-93.
35. Meyer, H.A., et al., *Selenoprotein P Status Correlates to Cancer-Specific Mortality in Renal Cancer Patients.* PLoS ONE, 2012. **7**(10): p. e46644.
36. Harris, H.R., L. Bergkvist, and A. Wolk, *Selenium intake and breast cancer mortality in a cohort of Swedish women.* Breast Cancer Res Treat, 2012. **134**(3): p. 1269-77.
37. Ait Ouakrim, D., et al., *Trends in colorectal cancer mortality in Europe: retrospective analysis of the WHO mortality database.* BMJ, 2015. **351**.
38. Romaguera, D., et al., *Pre-diagnostic concordance with the WCRF/AICR guidelines and survival in European colorectal cancer patients: a cohort study.* BMC Medicine, 2015. **13**: p. 107.

39. Kornitzer, M., et al., *Serum selenium and cancer mortality: a nested case-control study within an age- and sex-stratified sample of the Belgian adult population.* Eur J Clin Nutr, 0000. **58**(1): p. 98-104.
40. RICHARDSON, Des R., *More roles for selenoprotein P: local selenium storage and recycling protein in the brain.* Biochemical Journal, 2005. **386**(2).
41. Reuter, S., et al., *Oxidative stress, inflammation, and cancer: How are they linked?* Free radical biology & medicine, 2010. **49**(11): p. 1603-1616.
42. Sosa, V., et al., *Oxidative stress and cancer: an overview.* Ageing Res Rev, 2013. **12**(1): p. 376-90.
43. McKenzie, R.C., J.R. Arthur, and G.J. Beckett, *Selenium and the regulation of cell signaling, growth, and survival: molecular and mechanistic aspects.* Antioxidants and Redox Signaling, 2002. **4**(2): p. 339-351.
44. Rayman, M.P., *Selenium in cancer prevention: a review of the evidence and mechanism of action.* Proc Nutr Soc, 2005. **64**(4): p. 527-42.
45. Patterson, R.E., et al., *Changes in diet, physical activity, and supplement use among adults diagnosed with cancer.* J Am Diet Assoc, 2003. **103**(3): p. 323-8.
46. Demark-Wahnefried, W., B.M. Pinto, and E.R. Gritz, *Promoting health and physical function among cancer survivors: potential for prevention and questions that remain.* J Clin Oncol, 2006. **24**(32): p. 5125-31.
47. Demark-Wahnefried, W., et al., *Riding the Crest of the Teachable Moment: Promoting Long-Term Health After the Diagnosis of Cancer.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2005. **23**(24): p. 5814-5830.
48. Winkels, R.M., et al., *The COLON study: Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life.* BMC Cancer, 2014. **14**(1): p. 1-8.
49. Bingham, S. and E. Riboli, *Diet and cancer--the European Prospective Investigation into Cancer and Nutrition.* Nat Rev Cancer, 2004. **4**(3): p. 206-15.
50. *SAS Institute Inc. 2015. SAS/STAT® 14.1 User's Guide. Cary, NC: SAS Institute Inc*
51. Durrleman, S. and R. Simon, *Flexible regression models with cubic splines.* Stat Med, 1989. **8**(5): p. 551-61.
52. Govindarajulu, U.S., et al., *Comparing smoothing techniques in Cox models for exposure-response relationships.* Stat Med, 2007. **26**(20): p. 3735-52.
53. [cited 2016 March 29]; Available from: http://www.hsph.harvard.edu/donna-spiegelman/software/lgtphcurv9/.
54. Laclaustra, M., et al., *Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004.* Atherosclerosis, 2010. **210**(2): p. 643-648.
55. Schrauzer, G., *Anticarcinogenic effects of selenium.* Cellular and Molecular Life Sciences CMLS, 2000. **57**(13-14): p. 1864-1873.
56. Jackson, M.I. and G.F. Combs, Jr., *Selenium and anticarcinogenesis: underlying mechanisms.* Curr Opin Clin Nutr Metab Care, 2008. **11**(6): p. 718-26.
57. Kenfield, S.A., et al., *Selenium Supplementation and Prostate Cancer Mortality.* JNCI Journal of the National Cancer Institute, 2015. **107**(1): p. dju360.

TABLES

Table 1. Characteristics of colorectal cancer cases in the EPIC cohort study categorized by dietary Selenium quintiles (N = 1,019)

	Selenium				
Characteristic	**Quintile 1 ≤ 67 µg/L (N= 205)**	**Quintile 2 67 - 77 µg/L (N= 204)**	**Quintile 3 77 - 87.7 µg/L (N= 203)**	**Quintile 4 87.7 - 99.8 µg/L (N= 204)**	**Quintile 5 > 99.8 µg/L (N= 203)**
Selenium (µg/L), mean (SD)	55.8 (9.2)	72.3 (2.9)	82.3 (2.9)	94.0 (3.5)	115.5 (19.0)
Age at diagnosis (years), mean (SD)	62.7 (7.9)	61.8 (7.1)	62.3 (7.0)	61.9 (7.5)	62.9 (7.0)
BMI (kg/m^2), mean (SD)	26.6 (4.4)	26.6 (4.1)	26.8 (4.5)	27.0 (4.5)	26.8 (4.1)
Women, N (%)	116 (56.6)	116 (56.9)	100 (49.3)	97 (47.6)	89 (43.8)
Location of primary tumor, N (%)					
Colon	142 (69.3)	135 (66.2)	122 (60.1)	105 (51.5)	127 (62.6)
Rectum	63 (30.7)	69 (33.8)	81 (39.9)	99 (48.5)	76 (37.4)
Stage of tumor, N(%)					
I	33 (16.0)	55 (27.0)	41 (20.2)	43 (21.1)	35 (17.2)
II	51 (24.9)	36 (17.7)	43 (21.2)	44 (21.6)	44 (21.7)
III	67 (32.7)	54 (26.5)	68 (33.5)	68 (33.3)	80 (39.4)
IV	25 (12.2)	32 (15.7)	25 (12.3)	25 (12.3)	20 (9.9)
Unknown	29 (14.2)	27 (13.2)	26 (12.8)	24 (11.8)	24 (11.8)
Tumor grade, N(%)					
Well-differentiated	15 (7.3)	7 (3.4)	17 (8.4)	12 (5.9)	7 (3.5)
Moderately differentiated	75 (36.6)	79 (38.7)	57 (28.1)	52 (25.5)	36 (17.7)
Poorly differentiated	20 (9.8)	11 (5.4)	14 (6.9)	17 (8.3)	10 (4.9)
Unknown	95 (46.3)	107 (52.5)	115 (56.7)	123 (60.3)	150 (73.9)
Smoking status, N(%)					
Never smoker	86 (42.0)	84 (41.2)	79 (38.9)	81 (39.7)	75 (37.0)
Former smoker	61 (29.5)	63 (30.9)	68 (33.5)	72 (35.3)	76 (37.4)
Current smoker	55 (26.6)	55 (27.0)	55 (27.1)	50 (24.5)	52 (25.6)
Physical activity, N(%)					
Inactive	26 (12.7)	34 (16.7)	37 (18.2)	27 (13.2)	35 (17.2)
Moderately inactive	64 (31.2)	54 (26.5)	59 (29.1)	62 (30.4)	64 (31.5)
Moderately active	97 (47.3)	88 (43.1)	82 (40.4)	96 (47.1)	85 (41.9)
Active	16 (7.8)	26 (12.8)	25 (12.3)	19 (9.3)	19 (9.4)
Diabetes, N(%)					
No	158 (77.1)	157 (77.0)	173 (85.2)	157 (77.0)	153 (75.4)
Yes	12 (5.9)	9 (4.4)	6 (3.0)	9 (4.4)	14 (6.9)
Alcohol (grams/day), mean (SD)	17.1 (24.7)	15.6 (19.6)	20.6 (24.2)	18.2 (21.0)	19.8 (21.8)
All-cause mortality, N(%)	84 (41.0)	93 (45.6)	88 (43.4)	89 (43.6)	83 (40.9)
Colorectal cancer mortality, N(%)	73 (35.6)	75 (36.8)	73 (36.0)	72 (35.3)	69 (34.0)

NOTE: Missing values of categorical variables were classified as a separate category and missing values for continuous variables were coded with sex-specific median values: Smoking status (N = 7), physical activity (N = 4), diabetes (N = 171), alcohol (N = 2). Percentages may not add up to 100% in each category due to the fact that missing values were not excluded from the frequency calculations.

Table 2. Characteristics of colorectal cancer cases in the EPIC cohort study categorized by dietary Selenoprotein P quintiles (N = 1,016)

	Selenoprotein P				
Characteristic	**Quintile 1 ≤ 3.51 mg/L (N= 204)**	**Quintile 2 3.51 - 4.03 mg/L (N= 203)**	**Quintile 3 4.03 - 4.48 mg/L (N= 204)**	**Quintile 4 4.48 - 5.04 mg/L (N= 202)**	**Quintile 5 > 5.04 mg/L (N= 203)**
Selenoprotein P (mg/L), mean (SD)	3.02 (0.4)	3.79 (0.2)	4.26 (0.1)	4.73 (0.2)	5.71 (0.6)
Age at diagnosis (years), mean (SD)	61.7 (7.8)	62.6 (7.3)	62.2 (8.1)	62.0 (6.9)	63.0 (6.2)
BMI (kg/m^2), mean (SD)	26.3 (4.5)	26.7 (4.3)	26.7 (4.2)	26.8 (4.2)	27.3 (4.4)
Women, N (%)	121 (59.3)	110 (54.2)	114 (55.9)	87 (43.1)	84 (41.4)
Location of primary tumor, N (%)					
Colon	137 (67.2)	126 (62.1)	118 (57.8)	129 (63.9)	121 (59.6)
Rectum	67 (32.8)	77 (37.9)	86 (42.2)	73 (36.1)	82 (40.4)
Stage of tumor, N(%)					
I	38 (18.6)	46 (22.7)	42 (20.6)	42 (20.8)	36 (17.7)
II	41 (20.1)	41 (20.2)	42 (20.6)	41 (20.3)	53 (26.1)
III	58 (28.4)	70 (34.5)	72 (35.3)	66 (32.7)	72 (35.5)
IV	33 (16.2)	17 (8.4)	24 (11.8)	30 (14.9)	24 (11.8)
Unknown	34 (16.7)	29 (14.3)	24 (11.8)	23 (11.4)	18 (8.9)
Tumor grade, N(%)					
Well-differentiated	11 (5.4)	13 (6.4)	12 (5.9)	14 (6.9)	9 (4.4)
Moderately differentiated	70 (34.3)	71 (35.0)	66 (32.4)	51 (25.3)	39 (19.2)
Poorly differentiated	16 (7.8)	17 (8.4)	14 (6.9)	17 (8.4)	8 (3.9)
Unknown	107 (52.5)	102 (50.3)	112 (54.9)	120 (59.4)	147 (72.4)
Smoking status, N(%)					
Never smoker	87 (42.7)	88 (43.4)	80 (39.2)	74 (36.6)	74 (36.5)
Former smoker	56 (27.5)	65 (32.0)	76 (37.3)	73 (36.1)	70 (34.5)
Current smoker	60 (29.4)	46 (22.7)	47 (23.0)	54 (26.7)	59 (29.1)
Physical activity, N(%)					
Inactive	24 (11.8)	38 (18.7)	31 (15.2)	35 (17.3)	32 (15.8)
Moderately inactive	70 (34.3)	69 (34.0)	47 (23.0)	56 (27.7)	61 (30.1)
Moderately active	92 (45.1)	76 (37.4)	101 (49.5)	91 (45.1)	85 (41.9)
Active	16 (7.8)	20 (9.9)	24 (11.8)	19 (9.4)	25 (12.3)
Diabetes, N(%)					
No	165 (80.9)	156 (77.0)	154 (75.5)	159 (78.7)	162 (79.8)
Yes	7 (3.4)	8 (4.0)	11 (5.4)	13 (6.4)	11 (5.4)
Alcohol (grams/day), mean (SD)	18.1 (24.8)	17.7 (22.0)	15.7 (20.1)	19.2 (23.0)	20.9 (21.5)
All-cause mortality, N(%)	99 (48.5)	79 (38.9)	84 (41.2)	85 (42.1)	89 (43.8)
Colorectal cancer mortality, N(%)	83 (40.7)	65 (32.0)	67 (32.8)	67 (33.2)	80 (39.4)

NOTE: Missing values of categorical variables were classified as a separate category and missing values for continuous variables were coded with sex-specific median values: Smoking status (N = 7), physical activity (N = 4), diabetes (N = 170), alcohol (N = 2). Percentages may not add up to 100% in each category due to the fact that missing values were not excluded from the frequency calculations.

Table 3. HRs and 95% CIs for overall mortality and CRC mortality based on quintiles of pre-diagnostic Selenium (N = 1,019) and Selenoprotein P (N = 1,016) levels in the EPIC cohort

	Selenium			Selenoprotein P		
	Event/Total	**µg/L**	**HR (95% CI)**	**Event/Total**	**mg/L**	**HR (95% CI)**
Overall mortality						
Age Adjusted[b]						
Quintile 1	84/205	≤ 67	1.00 (ref.)	99/204	≤ 3.51	1.00 (ref.)
Quintile 2	93/204	67 - 77	1.16 (0.85, 1.58)	79/203	3.51 - 4.03	0.75 (0.55, 1.03)
Quintile 3	88/203	77 - 87.7	0.96 (0.70, 1.31)	84/204	4.03 - 4.48	0.78 (0.57, 1.06)
Quintile 4	89/204	87.7 - 99.8	1.02 (0.74, 1.40)	85/202	4.48 - 5.04	0.80 (0.58, 1.08)
Quintile 5	83/203	> 99.8	0.83 (0.60, 1.15)	89/203	> 5.04	0.73 (0.53, 0.99)
P_{trend}[a]			*0.15*			*0.10*
Multivariable Adjusted[c]						
Quintile 1	84/205	≤ 67	1.00 (ref.)	99/204	≤ 3.51	1.00 (ref.)
Quintile 2	93/204	67 - 77	1.12 (0.87, 1.66)	79/203	3.51 - 4.03	0.81 (0.58, 1.13)
Quintile 3	88/203	77 - 87.7	1.11 (0.79, 1.52)	84/204	4.03 - 4.48	0.79 (0.57, 1.10)
Quintile 4	89/204	87.7 - 99.8	1.08 (0.77, 1.50)	85/202	4.48 - 5.04	0.76 (0.55, 1.06)
Quintile 5	83/203	> 99.8	0.82 (0.56, 1.16)	89/203	> 5.04	0.70 (0.50, 0.98)
P_{trend}[a]			*0.14*			*0.05*
CRC mortality						
Age Adjusted[b]						
Quintile 1	73/205	≤ 67	1.00 (ref.)	83/204	≤ 3.51	1.00 (ref.)
Quintile 2	75/204	67 - 77	1.04 (0.74, 1.46)	65/203	3.51 - 4.03	0.77 (0.54, 1.09)
Quintile 3	73/203	77 - 87.7	0.89 (0.63, 1.26)	67/204	4.03 - 4.48	0.76 (0.54, 1.07)
Quintile 4	72/204	87.7 - 99.8	0.92 (0.65, 1.31)	67/202	4.48 - 5.04	0.78 (0.55, 1.10)
Quintile 5	69/203	> 99.8	0.75 (0.52, 1.07)	80/203	> 5.04	0.81 (0.58, 1.13)
P_{trend}[a]			*0.08*			*0.31*
Multivariable Adjusted[c]						
Quintile 1	73/205	≤ 67	1.00 (ref.)	83/204	≤ 3.51	1.00 (ref.)
Quintile 2	75/204	67 - 77	1.07 (0.74, 1.54)	65/203	3.51 - 4.03	0.84 (0.58, 1.21)
Quintile 3	73/203	77 - 87.7	1.05 (0.73, 1.50)	67/204	4.03 - 4.48	0.79 (0.54, 1.14)
Quintile 4	72/204	87.7 - 99.8	0.98 (0.67, 1.42)	67/202	4.48 - 5.04	0.76 (0.52, 1.11)
Quintile 5	69/203	> 99.8	0.76 (0.52, 1.11)	80/203	> 5.04	0.83 (0.57, 1.19)
P_{trend}[a]			*0.10*			*0.33*

[a]P_{trend} was calculated using the median value of each Se or SePP quintile as a continuous variable, adjusted for variables in the corresponding models.
[b]adjusted for age and stratified on country
[c]adjusted for age, sex, stage, grade, smoking status, BMI, site of primary tumor, year of diagnosis, sex-specific physical activity, diabetes and alcohol, and stratified on country

Table 4. Adjusted HRs and 95% CIs for an increment of 22.38 µg/L of Selenium for CRC and overall mortality across strata of effect modifiers

Risk Factor	CRC mortality			Overall mortality		
	Event/ Total	Multivariable[a] HR (95% CI)	P_{trend}[b] or interaction	Event/ Total	Multivariable[a] HR (95% CI)	P_{trend}[b] or interaction
All participants	362/1019	0.89 (0.80, 1.00)	0.06[b]	437/1019	0.90 (0.81, 1.00)	0.04[b]
Sensitivity analyses						
Complete CRC stage data	328/889	0.90 (0.80, 1.02)	0.10[b]	390/889	0.89 (0.80, 0.99)	0.03[b]
Imputed CRC stage data	362/1019	0.90 (0.81, 1.01)	0.07[b]	437/1019	0.90 (0.82, 1.00)	0.04[b]
Complete diabetes data	296/848	0.91 (0.79, 1.03)	0.14[b]	350/848	0.90 (0.80, 1.02)	0.09[b]
Follow-up (years)						
≥ 2	274/784	0.91 (0.79, 1.04)	0.88[b]	332/784	0.91 (0.80, 1.02)	0.70[b]
≥ 3	213/628	0.93 (0.79, 1.09)	0.74[b]	257/628	0.93 (0.80, 1.08)	0.79[b]
≥ 4	150/473	0.97 (0.79, 1.20)	0.69[b]	187/473	0.92 (0.76, 1.11)	1.00[b]
Sex						
Women	180/518	0.91 (0.76, 1.08)	0.45	226/518	0.93 (0.79, 1.09)	0.31
Men	182/501	0.85 (0.71, 1.01)		211/501	0.85 (0.73, 0.99)	
Age at diagnosis (years)						
< 62.5	165/509	0.90 (0.74, 1.09)	0.20	190/509	0. 92 (0.77, 1.10)	0.09
≥ 62.5	197/510	0.81 (0.69, 0.95)		247/510	0. 81 (0.70, 0.93)	
Site						
Colon	223/631	0.84 (0.72, 0.99)	0.91	270/631	0.88 (0.76, 1.00)	1.00
Rectum	139/388	0.95 (0.77, 1.16)		167/388	0.95 (0.79, 1.14)	
Grade[c]						
Well or moderately differentiated	92/357	1.06 (0.79, 1.44)	0.24	108/357	1.12 (0.86, 1.46)	0.26
Poorly differentiated	34/72	1.16 (0.47, 2.85)		41/72	1.04 (0.47, 2.27)	
Stage[c]						
I-III	225/762	0.97 (0.84, 1.11)	0.62[b]	279/762	0.96 (0.85, 1.09)	0.52[b]
Stage[c]						
I-II	62/425	1.16 (0.88, 1.53)	0.10	88/425	1.13 (0.90, 1.42)	0.07
III-IV	266/464	0.82 (0.71, 0.94)		302/464	0.82 (0.72, 0.93)	
Year of diagnosis						
1993-1999	157/428	0.87 (0.72, 1.05)	0.66	189/428	0.91 (0.78, 1.05)	0.26
1999-2003	205/591	0.89 (0.74, 1.06)		248/591	0.86 (0.73, 1.01)	
Smoking Status[c]						
Never smoker	135/405	0.84 (0.65, 1.09)	0.97	161/405	0.90 (0.72, 1.13)	0.27
Former smoker	122/340	0.95 (0.74, 1.21)		144/340	0.98 (0.79, 1.22)	
Current smoker	102/267	0.82 (0.66, 1.01)		128/267	0.81 (0.67, 0.98)	
BMI (kg/m^2)						
< 25	129/372	0.90 (0.72, 1.13)	1.00	153/372	0.90 (0.74, 1.10)	1.00
25-30	159/462	0.85 (0.70, 1.03)		189/462	0.83 (0.70, 0.98)	
≥ 30	74/185	0.88 (0.63, 1.22)		95/185	0.92 (0.68, 1.23)	
Physical Activity[c]						
Inactive	57/159	0.77 (0.52, 1.15)	0.10	66/159	0.80 (0.56, 1.16)	0.29
Moderately inactive	107/303	1.01 (0.76, 1.35)		130/303	0.97 (0.77, 1.22)	
Moderately active or active	196/553	0.84 (0.72, 0.99)		239/553	0.89 (0.77, 1.02)	
Alcohol (grams/day)						
< 10.3	174/508	0.88 (0.72, 1.09)	0.94	217/508	0.89 (0.74, 1.07)	0.68
≥ 10.3	188/511	0.87 (0.73, 1.02)		220/511	0.89 (0.77, 1.03)	

[a]adjusted for age of diagnosis, sex, stage, grade, smoking status, BMI, site of primary tumor, year of diagnosis, sex-specific physical activity, diabetes and alcohol, and stratified on country where appropriate.
[b]Ptrend
[c]Missing data was not included in the analysis.

Table 5. Adjusted HRs and 95% CIs for an increment of 0.97 mg/L of Selenoprotein P for CRC and overall mortality across strata of effect modifiers

Risk Factor	CRC mortality			Overall mortality		
	Event/ Total	Multivariable[a] HR (95% CI)	P_{trend}[b] or interaction	Event/ Total	Multivariable[a] HR (95% CI)	P_{trend}[b] or interaction
All participants	362/1016	0.98 (0.87, 1.11)	0.79[b]	436/1016	0.94 (0.85, 1.05)	0.27[b]
Sensitivity analyses						
Complete CRC stage data	328/888	1.02 (0.90, 1.15)	0.81[b]	390/888	0.93 (0.83, 1.05)	0.25[b]
Imputed CRC stage data	296/846	0.95 (0.85, 1.07)	0.39[b]	349/846	0.92 (0.83, 1.02)	0.10[b]
Complete diabetes data	296/846	0.98 (0.86, 1.12)	0.80[b]	349/846	0.93 (0.82, 1.05)	0.24[b]
Follow-up (years)						
≥ 2	274/782	1.01 (0.88, 1.16)	0.51[b]	331/782	0.96 (0.85, 1.09)	0.52[b]
≥ 3	213/627	0.99 (0.84, 1.16)	0.56[b]	256/627	0.97 (0.84, 1.12)	0.88[b]
≥ 4	150/473	1.14 (0.94, 1.38)	0.48[b]	186/473	1.03 (0.86, 1.23)	0.76[b]
Sex						
Women	180/516	0.96 (0.80, 1.16)	0.97	211/516	0.95 (0.80, 1.13)	0.52
Men	182/500	0.98 (0.82, 1.16)		225/500	0.91 (0.78, 1.06)	
Age at diagnosis (years)						
< 62.5	166/509	0.86 (0.71, 1.04)	0.89	191/509	0. 88 (0.74 , 1.05)	0.23
≥ 62.5	196/507	1.01 (0.86, 1.19)		245/507	0. 90 (0.78 , 1.04)	
Site						
Colon	223/631	0.99 (0.85, 1.16)	0.42	269/631	0.98 (0.85, 1.12)	0.25
Rectum	139/385	1.00 (0.79, 1.23)		167/385	0.86 (0.72, 1.09)	
Grade[c]						
Well or moderately differentiated	91/356	1.10 (0.82, 1.48)	0.69	107/356	1.08 (0.83, 1.40)	0.74
Poorly differentiated	34/72	0.84 (0.28, 2.55)		41/72	0.90 (0.31, 2.63)	
Stage[c]						
I-III	224/760	1.11 (0.95, 1.30)	0.22[b]	278/760	1.01 (0.88, 1.15)	0.91[b]
Stage[c]						
I-II	61/422	1.20 (0.90, 1.61)	0.41	87/422	1.02 (0.80, 1.30)	0.71
III-IV	267/466	0.95 (0.82, 1.09)		303/466	0.91 (0.79, 1.04)	
Year of diagnosis						
1993-1999	157/425	0.88 (0.72, 1.07)	0.30	189/425	0.85 (0.72, 1.01)	0.46
1999-2003	205/591	1.08 (0.91, 1.28)		247/591	1.01 (0.86, 1.18)	
Smoking Status[c]						
Never smoker	135/403	0.97 (0.75, 1.25)	0.58	160/403	0.99 (0.79, 1.23)	0.13
Former smoker	122/340	1.15 (0.92, 1.45)		144/340	1.06 (0.87, 1.31)	
Current smoker	102/266	0.86 (0.68, 1.09)		128/266	0.80 (0.65, 0.99)	
BMI (kg/m^2)						
< 25	129/370	1.01 (0.79, 1.31)	1.00	152/370	0.98 (0.78, 1.24)	1.00
25-30	159/461	0.97 (0.80, 1.17)		189/461	0.88 (0.74, 1.06)	
≥ 30	74/185	1.01 (0.69, 1.46)		95/185	0.98 (0.72, 1.34)	
Physical Activity[c]						
Inactive	57/160	0.87 (0.58, 1.30)	0.38	66/160	0.94 (0.66, 1.33)	0.34
Moderately inactive	107/303	1.07 (0.81, 1.40)		130/303	0.94 (0.74, 1.19)	
Moderately active or active	196/549	0.97 (0.82, 1.16)		238/549	0.95 (0.81, 1.11)	
Alcohol (grams/day)						
< 10.3	174/506	0.98 (0.81, 1.19)	0.81	217/506	0.96 (0.81, 1.13)	0.62
≥ 10.3	188/510	0.96 (0.81, 1.14)		219/510	0.92 (0.79, 1.08)	

[a]adjusted for age of diagnosis, sex, stage, grade, smoking status, BMI, site of primary tumor, year of diagnosis, sex-specific physical activity, diabetes and alcohol, and stratified on country where appropriate.
[b]Ptrend
[c]Missing data was not included in the analysis.

Table 6. HRs and 95% CIs for overall mortality and CRC mortality based on pre-specified Selenium cutpoints (N = 1,019) in the EPIC cohort.

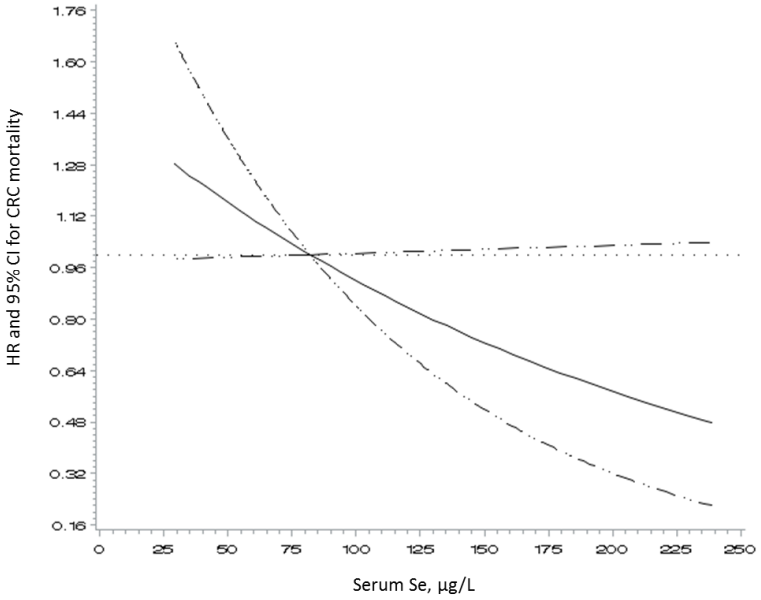
	Selenium			Selenium		
	Event/Total	**µg/L**	**HR (95% CI)**	**Event/Total**	**µg/L**	**HR (95% CI)**
Overall mortality						
Age Adjusted[b]						
Tertile 1	32/67	≤ 54	1.00 (ref.)	383/883	≤ 106	1.00 (ref.)
Tertile 2	398/939	54 – 140	0.72 (0.48, 1.07)	52/132	106 – 170	0.72 (0.54, 0.97)
Tertile 3	7/13	> 140	0.68 (0.29, 1.62)	2/4	> 170	1.08 (0.26, 4.48)
P_{trend}[a]			*0.13*			*0.09*
Multivariable Adjusted[c]						
Tertile 1	32/67	≤ 54	1.00 (ref.)	383/883	≤ 106	1.00 (ref.)
Tertile 2	398/939	54 – 140	0.88 (0.58, 1.33)	52/132	106 – 170	0.72 (0.53, 0.97)
Tertile 3	7/13	> 140	0.57 (0.23, 1.39)	2/4	> 170	0.88 (0.21, 3.73)
P_{trend}[a]			*0.31*			*0.07*
CRC mortality						
Age Adjusted[b]						
Tertile 1	26/67	≤ 54	1.00 (ref.)	317/883	≤ 106	1.00 (ref.)
Tertile 2	330/939	54 – 140	0.79 (0.50, 1.24)	44/132	106 – 170	0.85 (0.56, 1.29)
Tertile 3	6/13	> 140	0.90 (0.35, 2.31)	1/4	> 170	1.48 (1.39, 1.58)
P_{trend}[a]			*0.40*			*0.09*
Multivariable Adjusted[c]						
Tertile 1	26/67	≤ 54	1.00 (ref.)	317/883	≤ 106	1.00 (ref.)
Tertile 2	330/939	54 – 140	0.97 (0.61, 1.55)	44/132	106 – 170	0.74 (0.53, 1.04)
Tertile 3	6/13	> 140	0.74 (0.28, 1.98)	1/4	> 170	0.64 (0.09, 4.81)
P_{trend}[a]			*0.71*			*0.09*

[a]P_{trend} was calculated using the median value of each Se tertile as a continuous variable, adjusted for variables in the corresponding models.

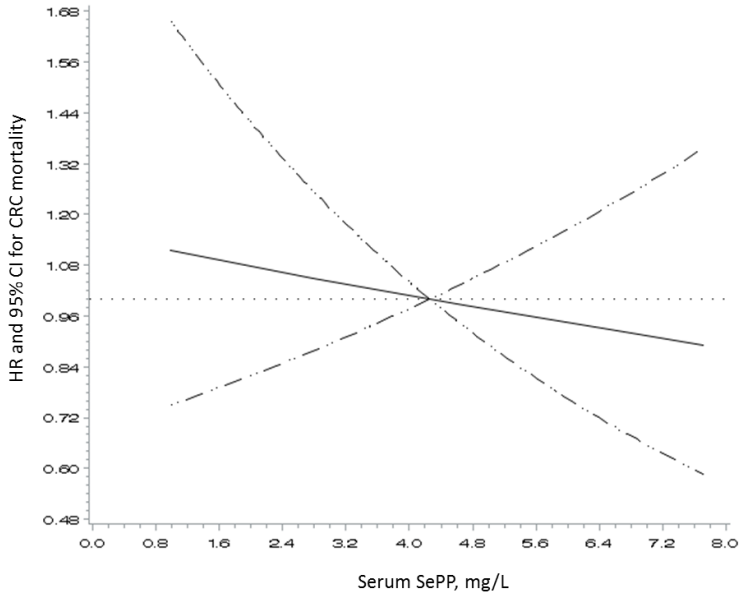
[b]adjusted for age and stratified on country

[c]adjusted for age, sex, stage, grade, smoking status, BMI, site of primary tumor, year of diagnosis, sex-specific physical activity, diabetes and alcohol, and stratified on country

SUPPLEMENTARY FIGURES AND TABLES



Supplemental Figure 1. Linear spline analysis showing the relationship between serum selenium levels and HR for CRC specific mortality P-value for linearity = 0.06.



Supplemental Figure 2. Linear spline analysis showing the relationship between serum selenoprotein P levels and HR for CRC specific mortality P-value for linearity = 0.59.

Supplementary Table 7. HRs and 95% CIs for overall mortality and CRC mortality based on quintiles of Selenium and Selenoprotein P for complete CRC stage data and imputed CRC stage data in the EPIC cohort

	Selenium				Selenoprotein P			
	No.	**Event**	**µg/L**	**HR (95% CI)**	**No.**	**Event**	**mg/L**	**HR (95% CI)**
Overall mortality								
Complete CRC stage data[b]								
Quintile 1	176	74	≤ 67	1.00 (ref.)	170	84	≤ 3.51	1.00 (ref.)
Quintile 2	177	82	67 - 77	1.20 (0.85, 1.71)	174	72	3.51 - 4.03	0.84 (0.59, 1.20)
Quintile 3	177	80	77 - 87.7	1.14 (0.81, 1.62)	180	77	4.03 - 4.48	0.82 (0.58, 1.16)
Quintile 4	180	80	87.7 - 99.8	1.08 (0.76, 1.54)	179	75	4.48 - 5.04	0.73 (0.51, 1.04)
Quintile 5	179	74	> 99.8	0.83 (0.58, 1.19)	185	82	> 5.04	0.73 (0.51, 1.03)
P_{trend}[a]				*0.16*				*0.07*
Imputed CRC stage data[b]								
Quintile 1	205	85	≤ 67	1.00 (ref.)	204	99	≤ 3.51	1.00 (ref.)
Quintile 2	204	93	67 - 77	1.17 (0.85, 1.61)	203	79	3.51 - 4.03	0.78 (0.57, 1.08)
Quintile 3	203	88	77 - 87.7	1.13 (0.82, 1.56)	204	84	4.03 - 4.48	0.82 (0.60, 1.12)
Quintile 4	204	89	87.7 - 99.8	1.05 (0.76, 1.44)	202	85	4.48 - 5.04	0.74 (0.54, 1.01)
Quintile 5	203	83	> 99.8	0.80 (0.58, 1.12)	203	89	> 5.04	0.69 (0.50, 0.94)
P_{trend}[a]				*0.08*				*0.03*
CRC mortality								
Complete CRC stage data[b]								
Quintile 1	176	84	≤ 67	1.00 (ref.)	170	68	≤ 3.51	1.00 (ref.)
Quintile 2	177	93	67 - 77	1.13 (0.77, 1.67)	174	60	3.51 - 4.03	0.92 (0.62, 1.36)
Quintile 3	177	88	77 - 87.7	1.08 (0.74, 1.59)	180	62	4.03 - 4.48	0.86 (0.58, 1.28)
Quintile 4	180	89	87.7 - 99.8	0.98 (0.66, 1.46)	179	61	4.48 - 5.04	0.77 (0.51, 1.14)
Quintile 5	179	83	> 99.8	0.83 (0.56, 1.24)	185	77	> 5.04	0.92 (0.63, 1.36)
P_{trend}[a]				*0.21*				*0.62*
Imputed CRC stage data[b]								
Quintile 1	205	73	≤ 67	1.00 (ref.)	204	83	≤ 3.51	1.00 (ref.)
Quintile 2	204	75	67 - 77	1.05 (0.74, 1.49)	203	65	3.51 - 4.03	0.80 (0.56, 1.14)
Quintile 3	203	73	77 - 87.7	1.07 (0.76, 1.53)	204	67	4.03 - 4.48	0.78 (0.55, 1.11)
Quintile 4	204	72	87.7 - 99.8	0.95 (0.67, 1.36)	202	67	4.48 - 5.04	0.72 (0.51, 1.02)
Quintile 5	203	69	> 99.8	0.76 (0.53, 1.88)	203	80	> 5.04	0.80 (0.56, 1.13)
P_{trend}[a]				*0.08*				*0.17*

[a]P_{trend} was calculated using the median value of each Se or SePP quintile as a continuous variable, adjusted for variables in the corresponding models.
[b]adjusted for age of diagnosis, sex, stage, grade, smoking status, BMI, site of primary tumor, year of diagnosis, sex-specific physical activity, diabetes and alcohol, and stratified on country

Table 8. HRs and 95% CIs for overall mortality and CRC mortality based on quintiles of Selenium (N = 1,019) by sex in the EPIC cohort

	Men (N = 501)			Women (N = 518)		
	Event/Total	**µg/L**	**HR (95% CI)**	**Event/Total**	**µg/L**	**HR (95% CI)**
Overall mortality						
Age Adjusted[b]						
Quintile 1	43/89	≤ 67	1.00 (ref.)	41/116	≤ 67	1.00 (ref.)
Quintile 2	39/88	67 - 77	0.85 (0.54, 1.33)	54/116	67 - 77	1.37 (0.87, 2.13)
Quintile 3	42/103	77 - 87.7	0.69 (0.43, 1.09)	46/100	77 - 87.7	1.25 (0.79, 1.96)
Quintile 4	55/107	87.7 - 99.8	0.96 (0.62, 1.47)	34/97	87.7 - 99.8	0.99 (0.61, 1.62)
Quintile 5	47/114	> 99.8	0.70 (0.45, 1.09)	36/89	> 99.8	0.91 (0.55, 1.50)
P_{trend}[a]			*0.25*			*0.38*
Multivariable Adjusted[c]						
Quintile 1	43/89	≤ 67	1.00 (ref.)	41/116	≤ 67	1.00 (ref.)
Quintile 2	39/88	67 - 77	0.94 (0.58, 1.53)	54/116	67 - 77	1.36 (0.83, 2.22)
Quintile 3	42/103	77 - 87.7	0.77 (0.47, 1.25)	46/100	77 - 87.7	1.32 (0.81, 2.15)
Quintile 4	55/107	87.7 - 99.8	0.93 (0.58, 1.48)	34/97	87.7 - 99.8	1.30 (0.75, 2.24)
Quintile 5	47/114	> 99.8	0.64 (0.40, 1.04)	36/89	> 99.8	0.98 (0.57, 1.67)
P_{trend}[a]			*0.09*			*0.75*
CRC mortality						
Age Adjusted[b]						
Quintile 1	38/89	≤ 67	1.00 (ref.)	35/116	≤ 67	1.00 (ref.)
Quintile 2	28/88	67 - 77	0.69 (0.41, 1.15)	47/116	67 - 77	1.31 (0.80, 2.12)
Quintile 3	34/103	77 - 87.7	0.62 (0.38, 1.02)	39/100	77 - 87.7	1.21 (0.74, 2.00)
Quintile 4	41/107	87.7 - 99.8	0.77 (0.47, 1.24)	31/97	87.7 - 99.8	1.08 (0.64, 1.84)
Quintile 5	41/114	> 99.8	0.62 (0.38, 1.01)	28/89	> 99.8	0.83 (0.47, 1.44)
P_{trend}[a]			*0.15*			*0.36*
Multivariable Adjusted[c]						
Quintile 1	38/89	≤ 67	1.00 (ref.)	35/116	≤ 67	1.00 (ref.)
Quintile 2	28/88	67 - 77	0.76 (0.43, 1.34)	47/116	67 - 77	1.24 (0.73, 2.12)
Quintile 3	34/103	77 - 87.7	0.74 (0.43, 1.27)	39/100	77 - 87.7	1.20 (0.70, 2.04)
Quintile 4	41/107	87.7 - 99.8	0.72 (0.42, 1.23)	31/97	87.7 - 99.8	1.42 (0.78, 2.59)
Quintile 5	41/114	> 99.8	0.60 (0.35, 1.01)	28/89	> 99.8	0.84 (0.46, 1.53)
P_{trend}[a]			*0.07*			*0.60*

[a]P_{trend} was calculated using the median value of each Se or SePP quintile as a continuous variable, adjusted for variables in the corresponding models.
[b]adjusted for age and stratified on country
[c]adjusted for age, sex, stage, grade, smoking status, BMI, site of primary tumor, year of diagnosis, sex-specific physical activity, diabetes and alcohol, and stratified on country

Table 9. HRs and 95% CIs for overall mortality and CRC mortality based on quintiles of Selenoprotein P (N = 1,016) by sex in the EPIC cohort

	Men (N = 500)			Women (N = 516)		
	Event/Total	**mg/L**	**HR (95% CI)**	**Event/Total**	**mg/L**	**HR (95% CI)**
Overall mortality						
Age Adjusted[b]						
Quintile 1	41/83	≤ 3.51	1.00 (ref.)	58/121	≤ 3.51	1.00 (ref.)
Quintile 2	36/93	3.51 - 4.03	0.73 (0.45, 1.18)	43/110	3.51 - 4.03	0.78 (0.51, 1.21)
Quintile 3	42/90	4.03 - 4.48	1.00 (0.63, 1.59)	42/114	4.03 - 4.48	0.60 (0.39, 0.95)
Quintile 4	53/115	4.48 - 5.04	0.94 (0.60, 1.47)	32/87	4.48 - 5.04	0.69 (0.43, 1.10)
Quintile 5	53/119	> 5.04	0.82 (0.53, 1.28)	36/84	> 5.04	0.58 (0.36, 0.94)
P_{trend}[a]			*0.67*			*0.02*
Multivariable Adjusted[c]						
Quintile 1	41/83	≤ 3.51	1.00 (ref.)	58/121	≤ 3.51	1.00 (ref.)
Quintile 2	36/93	3.51 - 4.03	0.73 (0.43, 1.23)	43/110	3.51 - 4.03	0.88 (0.54, 1.44)
Quintile 3	42/90	4.03 - 4.48	0.93 (0.56, 1.54)	42/114	4.03 - 4.48	0.68 (0.42, 1.12)
Quintile 4	53/115	4.48 - 5.04	0.75 (0.46, 1.24)	32/87	4.48 - 5.04	0.76 (0.45, 1.29)
Quintile 5	53/119	> 5.04	0.64 (0.40, 1.04)	36/84	> 5.04	0.75 (0.44, 1.29)
P_{trend}[a]			*0.10*			*0.25*
CRC mortality						
Age Adjusted[b]						
Quintile 1	34/89	≤ 3.51	1.00 (ref.)	49/121	≤ 3.51	1.00 (ref.)
Quintile 2	27/88	3.51 - 4.03	0.72 (0.41, 1.25)	38/110	3.51 - 4.03	0.83 (0.52, 1.34)
Quintile 3	32/103	4.03 - 4.48	0.91 (0.53, 1.55)	35/114	4.03 - 4.48	0.66 (0.40, 1.08)
Quintile 4	41/107	4.48 - 5.04	0.92 (0.55, 1.52)	26/87	4.48 - 5.04	0.70 (0.41, 1.81)
Quintile 5	48/114	> 5.04	0.93 (0.58, 1.52)	32/84	> 5.04	0.64 (0.38, 1.07)
P_{trend}[a]			*0.04*			*0.07*
Multivariable Adjusted[c]						
Quintile 1	34/89	≤ 3.51	1.00 (ref.)	49/121	≤ 3.51	1.00 (ref.)
Quintile 2	27/88	3.51 - 4.03	0.70 (0.38, 1.28)	38/110	3.51 - 4.03	0.96 (0.56, 1.63)
Quintile 3	32/103	4.03 - 4.48	0.76 (0.42, 1.37)	35/114	4.03 - 4.48	0.77 (0.45, 1.33)
Quintile 4	41/107	4.48 - 5.04	0.69 (0.38, 1.23)	26/87	4.48 - 5.04	0.73 (0.41, 1.33)
Quintile 5	48/114	> 5.04	0.74 (0.43, 1.27)	32/84	> 5.04	0.92 (0.51, 1.66)
P_{trend}[a]			*0.45*			*0.57*

[a]P_{trend} was calculated using the median value of each Se or SePP quintile as a continuous variable, adjusted for variables in the corresponding models.
[b]adjusted for age and stratified on country
[c]adjusted for age, sex, stage, grade, smoking status, BMI, site of primary tumor, year of diagnosis, sex-specific physical activity, diabetes and alcohol, and stratified on country

Table 10. HRs and 95% CIs for overall mortality and CRC mortality based on quintiles of Selenium (N = 1,019) by tumor site in the EPIC cohort

	Colon (N = 631)			Rectum (N = 388)		
	Event/Total	**µg/L**	**HR (95% CI)**	**Event/Total**	**µg/L**	**HR (95% CI)**
Overall mortality						
Age Adjusted[b]						
Quintile 1	59/142	≤ 67	1.00 (ref.)	25/63	≤ 67	1.00 (ref.)
Quintile 2	66/135	67 - 77	1.22 (0.84, 1.78)	27/69	67 - 77	1.03 (0.55, 1.94)
Quintile 3	58/122	77 - 87.7	1.08 (0.73, 1.60)	30/81	77 - 87.7	0.91 (0.50, 1.67)
Quintile 4	39/105	87.7 - 99.8	0.88 (0.57, 1.35)	50/99	87.7 - 99.8	1.28 (0.73, 2.24)
Quintile 5	48/127	> 99.8	0.82 (0.55, 1.23)	35/76	> 99.8	0.97 (0.53, 1.80)
P_{trend}[a]			*0.25*			*0.79*
Multivariable Adjusted[c]						
Quintile 1	50/142	≤ 67	1.00 (ref.)	25/63	≤ 67	1.00 (ref.)
Quintile 2	55/135	67 - 77	1.15 (0.77, 1.72)	27/69	67 - 77	1.32 (0.66, 2.65)
Quintile 3	47/122	77 - 87.7	1.09 (0.72, 1.64)	30/81	77 - 87.7	1.29 (0.66, 2.53)
Quintile 4	31/105	87.7 - 99.8	0.92 (0.58, 1.46)	50/99	87.7 - 99.8	1.39 (0.76, 2.56)
Quintile 5	40/127	> 99.8	0.86 (0.55, 1.32)	35/76	> 99.8	0.89 (0.46, 1.74)
P_{trend}[a]			*0.28*			*0.58*
CRC mortality						
Age Adjusted[b]						
Quintile 1	50/142	≤ 67	1.00 (ref.)	23/63	≤ 67	1.00 (ref.)
Quintile 2	55/135	67 - 77	1.16 (0.77, 1.76)	20/69	67 - 77	0.75 (0.37, 1.50)
Quintile 3	47/122	77 - 87.7	1.00 (0.65, 1.54)	26/81	77 - 87.7	0.84 (0.45, 1.59)
Quintile 4	31/105	87.7 - 99.8	0.79 (0.49, 1.28)	41/99	87.7 - 99.8	1.11 (0.61, 2.03)
Quintile 5	40/127	> 99.8	0.72 (0.46, 1.31)	29/76	> 99.8	0.84 (0.44, 1.62)
P_{trend}[a]			*0.06*			*0.91*
Multivariable Adjusted[c]						
Quintile 1	50/142	≤ 67	1.00 (ref.)	23/63	≤ 67	1.00 (ref.)
Quintile 2	55/135	67 - 77	1.01 (0.64, 1.59)	20/69	67 - 77	0.92 (0.42, 1.98)
Quintile 3	47/122	77 - 87.7	0.99 (0.62, 1.58)	26/81	77 - 87.7	1.09 (0.53, 2.25)
Quintile 4	31/105	87.7 - 99.8	0.82 (0.48, 1.40)	41/99	87.7 - 99.8	1.13 (0.58, 2.20)
Quintile 5	40/127	> 99.8	0.72 (0.44, 1.18)	29/76	> 99.8	0.74 (0.36, 1.53)
P_{trend}[a]			*0.12*			*0.49*

[a]P_{trend} was calculated using the median value of each Se or SePP quintile as a continuous variable, adjusted for variables in the corresponding models.
[b]adjusted for age and stratified on country
[c]adjusted for age, sex, stage, grade, smoking status, BMI, site of primary tumor, year of diagnosis, sex-specific physical activity, diabetes and alcohol, and stratified on country

Table 11. HRs and 95% CIs for overall mortality and CRC mortality based on quintiles of Selenoprotein P (N = 1,016) by tumor site in the EPIC cohort

	Colon (N = 631)			Rectum (N = 385)		
	Event/Total	**mg/L**	**HR (95% CI)**	**Event/Total**	**mg/L**	**HR (95% CI)**
Overall mortality						
Age Adjusted[b]						
Quintile 1	63/137	≤ 3.51	1.00 (ref.)	36/67	≤ 3.51	1.00 (ref.)
Quintile 2	50/126	3.51 - 4.03	0.80 (0.54, 1.21)	29/77	3.51 - 4.03	0.83 (0.48, 1.43)
Quintile 3	52/118	4.03 - 4.48	0.95 (0.64, 1.41)	32/86	4.03 - 4.48	0.68 (0.39, 1.17)
Quintile 4	53/129	4.48 - 5.04	0.86 (0.58, 1.28)	32/73	4.48 - 5.04	0.86 (0.50, 1.46)
Quintile 5	51/121	> 5.04	0.80 (0.54, 1.19)	38/82	> 5.04	0.69 (0.41, 1.17)
P_{trend}[a]			*0.37*			*0.24*
Multivariable Adjusted[c]						
Quintile 1	63/137	≤ 3.51	1.00 (ref.)	36/67	≤ 3.51	1.00 (ref.)
Quintile 2	50/126	3.51 - 4.03	0.92 (0.59, 1.43)	29/77	3.51 - 4.03	0.95 (0.52, 1.73)
Quintile 3	52/118	4.03 - 4.48	0.86 (0.55, 1.33)	32/86	4.03 - 4.48	0.81 (0.45, 1.46)
Quintile 4	53/129	4.48 - 5.04	0.88 (0.57, 1.35)	32/73	4.48 - 5.04	0.79 (0.43, 1.45)
Quintile 5	51/121	> 5.04	0.79 (0.51, 1.24)	38/82	> 5.04	0.68 (0.38, 1.23)
P_{trend}[a]			*0.31*			*0.17*
CRC mortality						
Age Adjusted[b]						
Quintile 1	54/137	≤ 3.51	1.00 (ref.)	29/67	≤ 3.51	1.00 (ref.)
Quintile 2	40/126	3.51 - 4.03	0.80 (0.51, 1.26)	25/77	3.51 - 4.03	0.95 (0.51, 1.77)
Quintile 3	41/118	4.03 - 4.48	0.85 (0.54, 1.32)	26/86	4.03 - 4.48	0.80 (0.43, 1.49)
Quintile 4	42/129	4.48 - 5.04	0.80 (0.51, 1.25)	25/73	4.48 - 5.04	0.95 (0.51, 1.75)
Quintile 5	46/121	> 5.04	0.83 (0.54, 1.29)	34/82	> 5.04	0.92 (0.51, 1.65)
P_{trend}[a]			*0.45*			*0.84*
Multivariable Adjusted[c]						
Quintile 1	54/137	≤ 3.51	1.00 (ref.)	29/67	≤ 3.51	1.00 (ref.)
Quintile 2	40/126	3.51 - 4.03	0.94 (0.57, 1.56)	25/77	3.51 - 4.03	1.08 (0.54, 2.13)
Quintile 3	41/118	4.03 - 4.48	0.80 (0.49, 1.32)	26/86	4.03 - 4.48	0.93 (0.47, 1.85)
Quintile 4	42/129	4.48 - 5.04	0.85 (0.52, 1.41)	25/73	4.48 - 5.04	0.91 (0.45, 1.83)
Quintile 5	46/121	> 5.04	0.85 (0.52, 1.40)	34/82	> 5.04	0.99 (0.51, 1.91)
P_{trend}[a]			*0.50*			*0.89*

[a]P_{trend} was calculated using the median value of each Se or SePP quintile as a continuous variable, adjusted for variables in the corresponding models.
[b]adjusted for age and stratified on country
[c]adjusted for age, sex, stage, grade, smoking status, BMI, site of primary tumor, year of diagnosis, sex-specific physical activity, diabetes and alcohol, and stratified on country