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Pre-diagnostic selenium status and hepatobiliary cancer risk in the European Prospective Investigation into Cancer and Nutrition Cohort

David J. Hughes[#1], **Talita Duarte-Salles**[#2,3], **Sandra Hybsier**[4], **Antonia Trichopoulou**[5,6], **Magdalena Stepien**[2], **Krasimira Aleksandrova**[7], **Kim Overvad**[8], **Anne Tjønneland**[9], **Anja Olsen**[9], **Aurélie Affret**[10,11,12], **Guy Fagherazzi**[10,11,12], **Marie-Christine Boutron-Ruault**[10,11,12], **Verena Katzke**[13], **Rudolf Kaaks**[13], **Heiner Boeing**[7], **Christina Bamia**[5,6], **Pagona Lagiou**[5,6,14], **Eleni Peppa**[5], **Domenico Palli**[15], **Vittorio Krogh**[16], **Salvatore Panico**[17], **Rosario Tumino**[18], **Carlotta Sacerdote**[19], **H.B(as). Bueno-de-Mesquita**[20,21,22,23], **Petra H. Peeters**[24,25], **Dagrun Engeset**[26], **Elisabete Weiderpass**[27,28,29,30], **Cristina Lasheras**[31], **Antonio Agudo**[32], **Maria-José Sánchez**[33,34], **Carmen Navarro**[34,35,36], **Eva Ardanaz**[34,37,38], **Miren Dorronsoro**[39], **Oskar Hemmingsson**[40], **Nick Wareham**[41], **Kay-Tee Khaw**[42], **Kathryn Bradbury**[43], **Amanda J. Cross**[22], **Marc Gunter**[22], **Elio Riboli**[22], **Isabelle Romieu**[2], **Lutz Schomburg**[#4], and **Mazda Jenab**[#2]

[1]Department of Physiology & Centre for Systems Medicine, Royal College of Surgeons in Ireland, 31A York Street, Dublin 2, Ireland [2]Section of Nutrition and Metabolism, International Agency for Research on Cancer (IARC-WHO), Lyon, France [3]Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP Jordi Gol), Barcelona, Spain [4]Institut für Experimentelle Endokrinologie, Charité - Universitätsmedizin Berlin, Berlin, Germany [5]Hellenic Health Foundation, Athens, Greece [6]WHO Collaborating Center for Nutrition and Health, Unit of Nutritional Epidemiology and Nutrition in Public Health, Dept. of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Greece [7]Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany [8]Section for Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark [9]Danish Cancer Society Research Center, Copenhagen, Denmark [10]Inserm, CESP Centre for Research in Epidemiology and Population Health, U1018, Lifestyle, genes and health: integrative trans-generational epidemiology, F-94805, Villejuif, France [11]Univ Paris Sud, UMRS 1018, F-94805, Villejuif, France [12]Gustave Roussy, F-94805, Villejuif, France [13]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany [14]Department of Epidemiology, Harvard School of Public Health, Boston, USA [15]Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence, Italy [16]Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian, 1, 20133 Milano, Italy [17]Dipartimento di Medicina Clinica e

Corresponding Author: David J. Hughes, Department of Physiology & Centre for Systems Medicine, Royal College of Surgeons in Ireland, 31A York Street, Dublin 2, Ireland. Tel.:+353 (0) 1 402 8531, davidhughes@rcsi.ie.

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Chirurgia Federico II, Naples, Italy [18]Cancer Registry and Histopathology Unit, "Civic - M.P.Arezzo" Hospital, ASP Ragusa, Italy [19]Unit of Cancer Epidemiology, Citta' della Salute e della Scienza Hospital-University of Turin and Center for Cancer Prevention (CPO), Via Santena 7, 10126 Torino, Italy [20]Department for Determinants of Chronic Diseases (DCD), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands [21]Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, The Netherlands [22]Department of Epidemiology and Biostatistics, The School of Public Health, Imperial College London, London, United Kingdom [23]Department of Social & Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia [24]Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht [25]MRC-PHE Centre for Environment and Health, Department of Epidemiology and Biostatics, School of Public Health, Imperial College, London, United Kingdom [26]The Norwegian Scientific Committee for Food Safety (VKM), Oslo, Norway [27]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway [28]Department of Research, Cancer Registry of Norway, Oslo, Norway [29]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden [30]Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland [31]Oviedo University, Asturias [32]Unit of Nutrition, Environment and Cancer.Cancer Epidemiology Research Program. Catalan Institute of Oncology-IDIBELL. L'Hospitalet de Llobregat, Barcelona, Spain [33]Escuela Andaluza de Salud Pública. Instituto de Investigación Biosanitaria ibs.GRANADA. Hospitales Universitarios de Granada/ Universidad de Granada, Granada, Spain [34]CIBER de Epidemiología y Salud Pública (CIBERESP), Spain [35]Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Murcia, Spain [36]Department of Health and Social Sciences, Universidad de Murcia, Murcia, Spain [37]Navarra Public Health Institute, Pamplona, Spain [38]IdiSNA, Navarra Institute for Health Research, Pamplona, Spain [39]Public Health Direction and biodonostia-Ciberesp, Basque regional health department, San Sebastian. Spain [40]Department of Surgical and Perioperative Sciences, Umeå University, Umeå, Sweden [41]MRC Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom [42]Clinical Gerontology, School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom [43]Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford

[#] These authors contributed equally to this work.

Abstract

Background—Selenium (Se) status is suboptimal in many Europeans, and may be a risk factor for development of various cancers including those of the liver and biliary tract.

Objective—We wished to examine if Se status in advance of cancer onset is associated with hepatobiliary cancers in the European Prospective Investigation into Cancer and Nutrition (EPIC) study.

Design—We assessed pre-diagnostic Se status by measuring serum levels of Se and Selenoprotein P (SePP; the major circulating Se transfer protein) and examined the association with hepatocellular carcinoma (HCC; N=121), gallbladder and biliary tract cancers (GBTC, N=100), and intrahepatic bile duct (IHBC; N=40) cancer risk in a nested case-control design

within the EPIC study. Se was measured by total reflection X-ray fluorescence and SePP by a colorimetric sandwich ELISA. Multivariable odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression.

Results—HCC and GBTC cases, but not IHBC, had significantly lower circulating Se and SePP levels than their matched controls. Higher circulating Se was associated with a significantly lower HCC risk (OR=0.41, 95%CI: 0.23, 0.72 per 20 μg/L increase) but not with risk of GBTC or IHBC. Similarly, higher SePP concentration was associated with lowered HCC risk only in both the categorical and continuous analyses (HCC: P_{trend}=<0.0001; OR=0.37, 95%CI: 0.21, 0.63 per 1.5 mg/L increase).

Conclusions—These findings from a large prospective cohort provide evidence that suboptimal Se status in Europeans may be associated with an appreciably increased risk of HCC development.

Keywords

hepatocellular carcinoma; selenium; selenoprotein P; prospective cohort

Introduction

Worldwide, primary liver cancers (PLC=hepatocellular carcinomas [HCC] and intrahepatic bile duct cancer [IHBC]) are the sixth most commonly diagnosed cancer group (1), and have the second highest cancer mortality rate(2). Geographic variation in PLC incidence rates reflects the prevalence of two established risk factors – viral hepatitis B/C (HBV/HCV) and aflatoxin exposure(3). However, current data show that PLC rates are rapidly increasing in traditionally lower-risk industrialized countries(4,5), likely due to Western lifestyle and dietary habits. The group of biliary tract cancers (GBTC; tumours of the gallbladder and extra-hepatic bile ducts) are anatomically related to PLC and both these cancer types are difficult to detect early, have poor prognoses and have limited understood aetiology(6,7). Thus, greater scientific understanding of the role of diet and lifestyle factors in the aetiology of hepatobiliary cancers is important.

A growing body of experimental and observational evidence suggests that suboptimal intakes of the micronutrient selenium (Se) contribute to the development of several cancers(8). Se is incorporated as the amino acid selenocysteine in selenoproteins which are thought to help prevent carcinogenesis largely due to the role of several of these proteins in cell protection from oxidative stress, redox control and the inflammatory response(8–12). Data from intervention trials and epidemiological studies suggest implications for Se intake in cancer risk are probably more apparent in populations with low Se availability, such as many across Europe(13–15).

Absorbed Se is primarily retained by the liver and re-circulated as a constituent of Selenoprotein P (SePP)(16). Se and SePP are the two major biomarkers of blood Se status, while SePP also affects the expression of anti-oxidative selenoproteins(17–19). Se has been shown to play vital roles in multiple metabolic processes in the liver(20). Evidence from primary human hepatocytes and animal models implicate Se in liver cancer development(21–24), while decreasing Se concentrations in HCC tumor tissues were

associated with progressive cancer grade (25). A Chinese prospective study also showed an inverse association between toenail Se status and risk of HCC mortality (26). Se deficiency has been observed in patients with alcoholic liver cirrhosis and primary biliary cirrhosis(27–29). Interestingly, low Se intake is thought to increase vulnerability to viral infections, which may be particularly important due to the marked link between hepatitis virus infection and liver cancer development(10–12,30). In support of this, findings from a cohort of chronic HBV and/or HCV carriers in Taiwan show an inverse association between HCC development risk and plasma Se levels(31).

However, to date there is no major epidemiologic evidence exploring the association of Se status with hepatobiliary cancers risk in European populations. In the present study, we hypothesized that a low Se status is associated with a higher risk of hepatobiliary cancer development. Thus, our aim was to assess the association between pre-diagnostic circulating levels of Se and SePP with HCC, GBTC, and IHBC risk in a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) study.

Methods

Study design and population

EPIC is a large prospective cohort study designed to investigate the association between diet, lifestyle and environmental factors and the incidence of cancers and other chronic diseases. Detailed information on the study design, rationale and methods of the EPIC study, including assessment of diet and lifestyle factors, has been described previously(32,33). Briefly, between 1992 and 2000 more than 520,000 men and women mostly aged 25-70 years were recruited in 23 centres throughout 10 European countries (Denmark/France/Germany/Greece/Italy/the Netherlands/Norway/Spain/Sweden/the United Kingdom). At recruitment, standardised dietary, lifestyle and socio-demographic questionnaires including information on physical activity, education, smoking and medical history; anthropometric data, and blood samples were collected from participants. Blood samples are stored at the International Agency for Research on Cancer (IARC, Lyon, France) in -196°C liquid nitrogen for all countries except Denmark (-150°C, nitrogen vapour) and Sweden (-80°C, freezers). All cohort members provided written informed consent. Ethical approval for this study was obtained from the IARC ethical review board (Lyon, France) and local participating centres.

Follow-up for Cancer Incidence and Mortality

Vital status follow-up (98.5% complete) was collected by record linkage with regional and/or national mortality registries in all countries except Germany and Greece, where follow-up is based on active follow-up through study subjects or their next-of-kin. Cancer incidence was determined through record linkage with population-based regional cancer registries (Denmark/Italy/the Netherlands/Norway/Spain/Sweden/the United Kingdom) or via 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). For this study, the latest date of complete information for

cancer incidence and vital status ranged from December 2002 to December 2006 among different centres.

Case Ascertainment

First incident HCC and IHBC were defined as C22.0 and C22.1, respectively, as per the 10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death (ICD-10) and the 2nd edition of the International Classification of Diseases for Oncology (ICD-O-2). Gallbladder and biliary tract cancers (GBTC) included tumours in the gallbladder (C23.9), extrahepatic bile ducts (C24.0), Ampulla of Vater (C24.1), and biliary tract (C24.8 and C24.9) with morphology code ICD-O-2 “8162/3”. Cholangiocarcinoma was defined as tumors in the intra/extrahepatic bile ducts (morphology code ICD-O-2 "8160/3"). For each identified case, the histology and the methods used to diagnose the cancer were reviewed to exclude metastatic cases or other types of liver cancers.

Nested Case-Control Study Design

The design of the nested case-control study has been previously described in detail(34). The sample size for the present analysis (261 cases, 261 controls) was based on cases identified between recruitment into the cohort until 2006 and availability of blood samples for Se status analysis (see the flow chart in the Supplemental Figure 1). For SePP, all of the available 121 HCC, 100 GBTC (gallbladder = 44, Ampulla of Vater = 19, and biliary tract = 37), and 40 IHBC cases were analysed (261 case-control pairs), including 35 cholangiocarcinoma cases (intrahepatic = 29 and extrahepatic = 6) within the GBTC and IHBC groups. For Se, fewer cases were available due to insufficient volume of blood sample or failed laboratory assay (106 HCC, 96 GBTC, and 36 IHBC cases included and 27 cases excluded) so that 238 case-control pairs were successfully analysed. For each case, one control was selected by incidence density sampling from all cohort members alive and free of cancer (except non-melanoma skin cancer), and matched by age at blood collection (±1 year), sex, study center, time of day (±3 hours) and fasting status (<3,3-6,and >6 hours) at blood collection. Women were additionally matched by menopausal status (pre-, peri-, and postmenopausal), and hormone replacement therapy use at time of blood collection (yes/no).

Existing data for HBV and HCV seropositivity, as well as α-fetoprotein (AFP), c-reactive protein [CRP] and markers of liver injury (alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma-glutamyltransferase [GGT], liver-specific alkaline phosphatase [AP], albumin, total bilirubin) were available and measured as previously detailed elsewhere(35).

Serum Se and SePP measurements

The case–control status was blinded. Concentrations of total Se were measured by X-ray fluorescence as described previously(36). Briefly, 4 μL of serum sample was analysed using a bench-top total reflection X-ray fluorescence (TXRF) spectrometer (PicofoxTM S2, Bruker Nano GmbH, Berlin, Germany). For quantification of Se a certified reference gallium solution (1,000 mg/L, Sigma, Taufkirchen, Germany) with a defined concentration was equally added to each sample. An internal serum standard was applied to each measurement. The inter-assay coefficient of variation (CV) of this standard was a 10.0%

relative standard deviation within 48 analysis tests. A colorimetric enzyme-linked immunoassay (SelenotestTM, ICI GmbH, Berlin, Germany) was used to measure SePP levels from 5 μL of each serum sample in a 1:21 dilution according to the manufacturer's instructions. The CVs were determined with three controls covering the upper, middle and lower part of the assay's working range (13.5-484.8 μg/L). These controls were included in 16 assay procedures, and yielded a CV of 4.1%, 6.7% and 11.4% for controls 1 (SePP: 18.2 μg/L), 2 (SePP: 79.0 μg/L) and 3 (SePP: 292.9 μg/L), respectively. The evaluation was performed with GraphPad Prism 6.01 (La Jolla, CA) using a four parameter logistic function. The samples were measured in duplicate and the mean concentration values, standard deviation (SD) and coefficient of variation were calculated.

Statistical analyses

Generalized linear models (GLM) (values were natural logarithm transformed to approximate a normal distribution) were used to examine geometric mean differences in Se and SePP concentrations among the controls by baseline characteristics, with adjustment for country and sex. P-values for tests of trend (for ordinal variables) or of heterogeneity were reported.

Conditional logistic regression models were used to calculate the odds ratio (OR) with 95% confidence interval (CI) and tests for trend for associations between circulating Se and SePP in relation to risk of HCC, GBTC, and IHBC, as well as specific sub-sites of the gallbladder, and cholangiocarcinoma (intra- and extra-hepatic). Se and SePP concentrations were included in models as continuous (per 20 μg/L and 1.50 mg/L, respectively; approximately one SD) and as categorical variables, with tertile cut-points based on the distribution in all control subjects. Models were run separately for each cancer site using the same categorical cut-points for all tests. Tests for dose-response by linear trend were performed by assigning the median values of each tertile of Se and SePP.

For all analyses, both crude and multivariable models were run. Crude models included matching factors; multivariable models were additionally adjusted for *a priori* selected confounders including baseline alcohol intake at recruitment (g/d), pattern of lifetime alcohol intake (never drinkers, former drinkers, drinkers only at recruitment, always drinkers, unknown), body mass index (BMI; kg/m2), smoking status (never, former, current, and not specified), level of education (none/primary school, technical school, secondary school, university degree or higher), physical activity (combination of physical activity, cycling and sport activities in metabolic equivalents [METs]), waist circumference (cm), total energy intake (kcal/day), and self-reported diabetes. Other factors (height, weight, waist-to-hip ratio, and dietary intake of energy, fibre, tea, coffee, red and processed meats, fish and shellfish, fruits and vegetables) were tested as potential confounders, but were excluded from final models for parsimony, as they did not affect our estimates (change-in-estimate <10%).

Interactions for potential biologically plausible effect modifying variables (age at diagnosis, BMI, self-reported diabetes) was tested by including interaction terms formed by the product of modifying variable categories and the value of categories of exposure of interest. In order to explore the main proposed underlying mechanism of Se action (i.e. antioxidant defence),

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we also tested interactions with circulating CRP (a marker of chronic inflammation, likely to be heightened under oxidative stress) and smoking (since smokers are under oxidative stress and have higher antioxidant defence requirements). Since subjects with alcoholic liver cirrhosis have been observed to have lower Se status(27,28), we explored interactions with alcohol intake. The statistical significance of interactions was assessed using likelihood ratio tests based on the models with and without the interaction terms. In sensitivity analyses, we excluded subjects with (i) self-reported type 2 diabetes at baseline (yes/no), because of the potential for modifications in diet after diagnosis of this disease, (ii) hepatitis infection, since it is an established risk factor for liver cancers, and (iii) subjects with follow-up of <2 or <4 years after blood collection to exclude possible reverse causation. Additional analyses were performed including adjustment for an ad hoc liver function score (range from 0 to 6; categorized as 0=no liver injury, 1-2=possible minor injury, ≥3=possible injury), which summarizes the number of abnormal values for six liver function tests (ALT>55 U/L, AST>34 U/L, GGT men>64 U/L, GGT women>36 U/L, AP>150 U/L, albumin<35 g/L, total bilirubin>20.5 μmol/L; cut-points were provided by the laboratory and were based on assay specifications).

All statistical tests were two-sided, and p-values<0.05 were considered statistically significant. Analyses were performed using Stata version 11 (StataCorp, College Station, Texas) statistical package.

Results

Baseline Characteristics of Participants

The baseline characteristics of all study subjects are presented in Table 1. HCC, GBTC, and IHBC cases were diagnosed, on average, 6.0, 5.5, and 5.9 years after blood collection, respectively. HCC cases were more likely to be current smokers, to be former alcohol drinkers, to have higher waist circumference, to have chronic HBV and/or HCV infection and liver enzyme abnormalities, and to have lower intakes of fruits and vegetables compared to their matched controls. For GBTC and IHBC, none of the variables in Table 1 were significantly different between cases and controls. Serum concentrations of SePP and Se showed a strong, significant correlation among cases and controls (r =0.62; P=<0.001). Geometric means of serum Se were significantly lower in HCC and GBTC cases vs. their respective matched controls (71.3 vs. 85.2 μg/L; P=<0.001, and 82.1 vs. 85.9 μg/L; P=0.041, respectively), while no significant differences were observed for IHBC. Concentrations of SePP were lower in HCC cases vs. controls (geometric means were 4.3 vs. 5.4 mg/L; P=<0.001, respectively), and did not differ significantly among GBTC or IHBC cases and controls.

HCC

The associations between serum Se and SePP concentrations with HCC risk are shown in Table 2. A higher Se concentration was statistically significantly associated with lower HCC risk (multivariable $OR_{T3\ vs.\ T1}$=0.18, 95%CI: 0.05, 0.66, P_{trend}=0.016; OR=0.41, 95%CI: 0.23, 0.72 per 20 μg/L increase in Se concentration). Similarly, SePP levels were highly significantly associated with lower HCC risk (multivariable $OR_{T3\ vs.\ T1}$=0.09, 95%CI: 0.03,

0.32, P_{trend}=<0.0001; OR=0.37, 95%CI: 0.21, 0.63 per 1.5 mg/L increase in SePP concentration).

GBTC

The associations between serum Se and SePP concentrations with GBTC risk are also shown in Table 2. Higher serum Se levels were not associated with a statistically significant lower risk of GBTC (multivariable $OR_{T3\ vs.\ T1}$=0.37, 95%CI: 0.13, 1.03, P_{trend}=0.055; OR=0.74, 95%CI: 0.47, 1.18 per 20 μg/L increase in Se concentration), although the dose response estimate was close to significance (P_{trend}=0.055) and was significant when analyzed by matching factors only (P_{trend}=0.022). Higher SePP levels were significantly associated with lower GBTC risk (multivariable $OR_{T3\ vs.\ T1}$=0.27, 95%CI: 0.09, 0.78, P_{trend}=0.016). However, the association between SePP levels and GBTC risk was not significant when SePP was analyzed as a continuous variable (multivariable OR=0.79, 95%CI: 0.51, 1.21 per 1.5 mg/L increase in SePP concentration).

Hepatobiliary cancer subtypes

The associations between serum Se and SePP concentrations with risk of other hepatobiliary cancer subtypes (IHBC, gallbladder, and cholangiocarcinoma cancers) are shown in Table 3 (note that gallbladder cancers were also included in the GBTC category and cholangiocarcinoma cancers in the GBTC and IHBC groups). Due to the modest numbers of case-control pairs for these sites, we only analyzed the Se status markers for the continuous model. Although all point estimates indicated a lowered risk for all these cancers with increases in Se and SePP concentrations, none were statistically significant after multivariable adjustment.

Sensitivity analyses and Effect Modifications

The results did not change substantially after exclusion of participants that self-reported type 2 diabetes at baseline or cases diagnosed during the first 2 or 4 years of follow-up, as well as after additional adjustment for liver function score (results not shown). Among hepatitis free HCC cases (n_{cases}=52), the association with Se was not statistically significant (multivariable OR for 20 μg/L=0.52, 95%CI: 0.26, 1.05), while the association with SePP remained statistically significant after exclusion of HBV/HCV positive cases (multivariable OR for 1.5 mg/L= 0.51, 95%CI: 0.26, 0.99). However, we did not observe statistically significant effect modification by hepatitis infection status (*P* for interaction for Se and SePP were 0.425 and 0.854, respectively). We observed a significant interaction between BMI and SePP concentrations on HCC risk (*P* for interaction in crude and multivariable models were 0.036 and 0.006, respectively). The association between Se and SePP and HCC risk was stronger among overweight and obese than in normal weight participants. We did not observe any statistically significant effect modifications for other factors such as smoking, CRP, alcohol intake or self-reported diabetes; also, no interactions were observed for other cancer subtypes (all *P* for interaction>0.05).

Discussion

This study presents the largest prospective examination of the association of serum Se status biomarkers (serum Se levels and SePP protein concentrations) with risk of HCC and GBTC in European populations. Our findings indicate that higher levels of Se were significantly associated with a lower HCC risk but were not associated with GBTC risk. Higher concentrations of SePP, a functional biomarker of Se status, were significantly associated with a lower risk of HCC and GBTC, although the latter association was only seen for the categorical analysis. Analyses of distinct hepatobiliary cancer subtypes (IHBC, gallbladder, and cholangiocarcinoma) showed no significant associations with Se status levels, although we had limited power for these analyses. Overall, the results suggest that in areas of marginally low Se status, such as the populations examined here from Western Europe(19), Se intake and/or status may be important factors in the development of HCC and GBTC.

Optimal Se intake should ensure a circulating Se level of at least 124μg/L to fully express SePP and Glutathione Peroxidase 3 (GPX3) selenoproteins(17,18,37,38). In this study, the correlation between Se and SePP levels was relatively high (r=0.62; P=<0.001), reflecting that most subjects had suboptimal Se levels to fully saturate SePP (and GPX3)**,** and very similar to our previous analysis of the same Se status markers in a separate study on colorectal cancer, also nested within EPIC(15). This provides further evidence of the marginally low Se status in many Western European populations(19). Attenuated expression of SePP and dysregulation of the expression of other selenoproteins resulting from suboptimal Se availability affects responses to important carcinogenic processes like oxidative stress(9,12) and this may underline the association of these Se status markers with liver cancer.

For both HCC and GBTC, the point estimates comparing the highest and lowest tertiles of circulating Se and SePP concentrations show strong inverse associations (as does the continuous estimate for HCC). Although a preventative effect of Se against these cancers is in line with our hypothesis, the surprising strength of the observed association requires further confirmation. Nevertheless, there are several lines of evidence to support a strong preventative effect of higher Se status levels against hepatobiliary cancers. Mouse and rat models in particular have indicated a central role of the liver for Se metabolism(39–43). Data from SePP knockout mice suggest that healthy hepatocytes are the major cell type to contribute to circulating SePP levels(42) and these cells are sensitive to oxidative and inflammatory stress(43) and to hypoxia(44). Together, these studies indicate that even a minor dysfunction of hepatocytes may reduce serum Se and SePP concentrations (through lower circulating SePP levels). This suggests a potential mechanism of liver carcinogenesis whereby the dysregulation of SePP expression and secretion due to impaired Se organification (i.e., weakened conversion of dietary Se into selenoproteins such as SePP by sub-functional or de-differentiated hepatocytes) contributes to oxidative stress damage in hepatocytes. In this scenario, SePP may be an early and sensitive indicator of hepatocyte-related liver health. This may also explain why we observe weaker associations for GBTC compared to HCC and no signification associations for the other liver-related cancer sites that we investigated (IHBC, gallbladder, and cholangiocarcinoma cancers).

However, it remains possible that the strong estimates provided by this study may reflect, at least in part, that these Se markers are acting as biomarkers of liver disease, as seen for example in cirrhosis studies (27–29), and that this inadequate liver functioning may lead to cancer. In this regard, it is notable that in a prospective study of men with chronic hepatitis infection a reduction in HCC risk was associated with higher plasma Se levels(31). Our stratified analyses provide support for these Se measures as biomarkers of both general liver damage and liver cancer risk. After excluding hepatitis positive cases, the association of HCC remained statistically significant with SePP but not Se. Among groups with no marked liver damage, the association of HCC with Se or SePP was not significant, while for those with clear liver damage scores the association of decreased HCC risk was significant for higher SePP levels only. However, we did not observe statistically significant effect modifications by either hepatitis infection status or liver damage scores (results not shown), which may be due to low power for these analyses.

Chemical forms of dietary Se such as selenomethionine, the major source of Se in the human diet, and Se selenite may differentially contribute to the amount of biologically usable Se for hepatocyte metabolism(45). Such factors, along with baseline Se status levels, may partly explain varying successes of intervention trials of Se supplements to prevent cancer(14,15). A national program of adding Se to fertilizers in Finland has indicated that Se status can be safely increased on a population-wide basis in low Se areas(46). While a recent analysis on incidence rates of major cancers (though not including liver cancer) hasn’t shown an obvious impact of this program, the lack of an adequate non-supplemented control group is a major problem in assessing these data(46). Interestingly, intervention trials in China using selenized table salt or selenized yeast showed significant reductions in PLC incidence in the supplemented groups(47). Studies by Burk et al(27,48), including a recent fascinating intervention study using different Se forms, suggests that as cirrhosis increases the liver is less able to adequately metabolize Se from selenomethionine sources. Possibly, then, further Se deficiency caused by cirrhosis may predispose patients (especially those with already suboptimal Se status) to HCC. This is an intriguing area of future investigation, and may partly explain the large effect sizes and differences in the results for Se and SePP observed in our study. Thus, perhaps for subjects with pre-existing liver disease, a lower Se intake especially from sources with selenomethione will not adequately contribute to the functional Se availability. These individuals are thus more likely to suffer Se deficiency which may further add to potential liver cancer progression. This also may explain our observation that in subjects with clear liver damage scores the association of decreased HCC risk was significant for higher SePP levels only, i.e., this may reflect inadequate metabolism of selenomethionine to SePP, compounded for those also having lower baseline Se levels. However, we have no data on these different sources of Se (such as use of supplements containing selenite) to adequately investigate these hypotheses.

Among the sex, lifestyle, dietary, and disease variables adjusted for in our analyses, mean Se levels differed by HBV and/or HCV infection and diabetes status while mean SePP levels differed by sex, diabetes, and fish and red meat intake (results not shown), in line with previous studies(19,20,31,49). There were no statistically significant multiplicative interactions, except for the interaction between BMI and SePP for HCC. Stratified analysis showed associations for SePP and HCC were stronger among overweight and obese

participants (results not shown), possibly reflecting an influence of obesity on attenuating SePP expression and its regulation as a gluconeogenic enzyme(19,50).

The study strengths include measurement of the two most meaningful biomarkers of Se status, i.e., total Se and SePP serum concentrations(19) in an appreciable sample size within a large, prospective study with extensive data on lifestyle and other dietary factors, liver function markers, and pre-diagnostic bloods. The main limitations are the single time-point blood measure per subject and the relatively short follow-up time (~6 years). However, exclusion of cases with less than two years of follow-up did not appreciably alter the findings. There was limited power to assess the association of Se status with hepatobiliary cancer types with low study numbers, including IHBC, gallbladder, and cholangiocarcinoma cancers. 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.

In conclusion, the present study provides significant prospective data indicating a strong association between high Se status and a lower risk of HCC. Randomised controlled trials in populations where Se status is suboptimal (e.g. Western Europe) are needed to test whether increasing Se intake may reduce the risk of hepatobiliary cancers, especially for those at high risk (e.g. HBV/HCV positive) for HCC and GBTC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AFP	α-fetoprotein

GBTC	gallbladder and biliary tract cancers
BMI	Body mass index
CI	confidence interval
CRP	c-reactive protein
EPIC	European Prospective Investigation into Cancer and Nutrition
HBV	hepatitis B virus
HCC	hepatocellular cancer
HCV	hepatitis C virus
IARC	International Agency for Research on Cancer
IHBC	intrahepatic bile duct cancer
OR	Odds Ratio
PLC	primary liver cancers
Se	Selenium
SePP	Selenoprotein P

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Table 1

Selected baseline characteristics of incident liver cancer cases and their matched controls within the EPIC nested case-control study.

Baseline characteristics	Hepatocellular carcinoma (HCC)			Biliary tract cancer (GBTC)			Intrahepatic bile duct cancer (IHBC)		
	Cases (n=121)	Matched controls (n=121)	p-value	Cases (n=100)	Matched controls (n=100)	p-value	Cases (n=40)	Matched controls (n=40)	p-value
Women, *N*(%)	39 (32.2)	39 (32.2)	-	62 (62.0)	62 (62.0)	-	17 (42.5)	17 (42.5)	-
Age at recruitment (y), mean (SD)	60.1 (7.4)	60.1 (7.4)	-	58.4 (7.9)	58.4 (7.9)	-	61.3 (7.6)	61.3 (7.6)	-
Years between blood collection and diagnosis, mean (SD)	6.0 (3.5)	-		5.5 (3.5)	-		5.9 (3.5)	-	
BMI (kg/m^2), mean (SD)	28.2 (4.4)	27.4 (4.3)	0.093	27.0 (4.2)	26.9 (4.0)	0.382	27.8 (4.3)	27.4 (4.1)	0.370
Waist circumference (cm), mean (SD)	97.1 (13.9)	93.1 (12.0)	0.009	89.5 (14.2)	88.8 (12.0)	0.362	92.9 (12.8)	91.7 (10.3)	0.328
No. with diabetes, *N*(%) [a]	18 (14.9)	9 (7.4)	0.185	9 (9.0)	9 (9.0)	1.000	2 (5.0)	2 (5.0)	1.000
No. with hypertension *N* (%)	49 (40.5)	34 (28.1)	0.126	34 (34.0)	26 (26.0)	0.222	15 (37.5)	9 (22.5)	0.341
Smoking status, *N*(%)			0.021			0.385			0.592
Never smoker	38 (31.4)	57 (47.1)		53 (53.0)	46 (46.0)		18 (45.0)	20 (50.0)	
Former smoker	38 (31.4)	39 (32.2)		22 (22.0)	32 (32.0)		12 (30.0)	8 (20.0)	
Current smoker	44 (36.4)	24 (19.8)		24 (24.0)	20 (20.0)		9 (22.5)	9 (22.5)	
Alcohol lifetime pattern of intake, *N*(%) [b]			0.001			0.373			0.138
Never drinkers	9 (7.4)	13 (10.7)		7 (7.0)	9 (9.0)		3 (7.5)	4 (10.0)	
Former drinkers	21 (17.4)	4 (3.3)		10 (10.0)	5 (5.0)		6 (15.0)	1 (2.5)	
Drinkers only at recruitment/always drinkers	91 (75.2)	104 (86.0)		83 (83.0)	86 (86.0)		31 (77.5)	35 (87.5)	
Physical activity (METS), mean (SD)	83.6 (54.6)	86.5 (50.1)	0.337	90.2 (55.1)	91.9 (51.0)	0.407	74.5 (33.4)	80.5 (40.7)	0.238
Education, *N*(%)			0.749			0.954			0.983
None / primary	63 (52.1)	60 (49.6)		49 (49.0)	46 (46.0)		20 (50.0)	18 (45.0)	
Technical / professional	32 (26.5)	27 (22.3)		20 (20.0)	23 (23.0)		9 (22.5)	9 (22.5)	
Secondary	6 (5.0)	10 (8.3)		14 (14.0)	13 (13.0)		3 (7.5)	4 (10.0)	
University or higher	18 (14.9)	21 (17.4)		16 (16.0)	16 (16.0)		5 (12.5)	5 (12.5)	

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Baseline characteristics	Hepatocellular carcinoma (HCC)			Biliary tract cancer (GBTC)			Intrahepatic bile duct cancer (IHBC)		
	Cases (n=121)	Matched controls (n=121)	p-value	Cases (n=100)	Matched controls (n=100)	p-value	Cases (n=40)	Matched controls (n=40)	p-value
Liver function score, *N* (%) [c]			<0.001			0.542			0.130
0	25 (20.7)	69 (57.0)		68 (68.0)	62 (62.0)		17 (42.5)	24 (60.0)	
≥1	57 (47.1)	14 (11.6)		11 (11.0)	16 (16.0)		12 (30.0)	5 (12.5)	
Missing	39 (32.2)	38 (31.4)		21 (21.0)	22 (22.0)		11 (27.5)	11 (27.5)	
Hepatitis status, *N* (%)									
Hepatitis B virus (HBV) positive	16 (13.2)	4 (3.3)	0.004	2 (2.0)	6 (6.0)	0.142	2 (5.0)	0 (0.0)	0.150
Hepatitis C virus (HCV) positive	17 (14.0)	3 (2.5)	0.001	2 (2.0)	1 (1.0)	0.567	0 (0.0)	1 (2.5)	0.313
HBV or HCV positive	31 (25.6)	6 (5.0)	<0.001	4 (4.0)	7 (7.0)	0.337	2 (5.0)	1 (2.5)	0.554
Baseline dietary intakes (g/d), mean (SD)									
Alcohol	21.5 (35.5)	15.5 (19.7)	0.050	11.0 (15.5)	12.2 (15.8)	0.289	13.7 (20.9)	13.8 (16.8)	0.494
Fish and shellfish	29.6 (26.0)	35.4 (43.0)	0.102	30.9 (37.5)	37.1 (35.2)	0.115	33.1 (19.1)	29.1 (20.8)	0.190
Fruit and vegetables	432.0 (284.4)	497.9 (295.7)	0.039	459.5 (273.8)	475.3 (247.7)	0.335	426.6 (276.8)	443.4 (219.8)	0.380
Red and processed meat	104.9 (63.4)	109.0 (63.7)	0.306	92.1 (48.9)	101.7 (52.2)	0.092	104.0 (60.3)	113.8 (54.3)	0.225
Baseline serum biomarkers, geometric mean (5^{th}, 95^{th} percentile)									
Selenium, µg/L [d]	71.3 (41.3, 105.9)	85.2 (55.3, 117.5)	<0.001	82.1 (59.7, 108.7)	85.9 (62.4, 121.0)	0.041	82.3 (62.9, 135, 8)	87.5 (58.2, 118.7)	0.115
Selenoprotein P, mg/L	4.3 (2.0, 7.0)	5.4 (2.9, 7.9)	<0.001	5.1 (3.0, 7.8)	5.3 (3.2, 7.4)	0.271	5.1 (3.3, 7.8)	5.5 (3.4, 8.9)	0.096

EPIC, European Prospective Investigation into Cancer and Nutrition; BMI, body mass index; SD, standard deviation;
p-values from t-tests (continuous variables) or chi-square test (categorical variables). Missing values were not excluded from percentage calculations, thus the sum of percents across sub-groups may not add up to 100%.

[a] Self-reported data.

[b] No information on past alcohol consumption was available for the following EPIC centers: Naples, Bilthoven, Umeå, Malmö, and Norway.

[c] Ranges from 0 to 6; the score was grouped in categories as 0, ≥1 abnormal liver function tests (ALT>55 U/L, AST>34 U/L, GGT men >64 U/L, GGT women > 36 U/L, AP > 150 U/L, albumin < 35 g/L, total bilirubin > 20.5 µmol/L; based on the values provided by the laboratory).

[d] Available for 106 HCC, 96 GBTC, and 36 IHBC cases and their matched controls.

Table 2

ORs and 95% confidence intervals (95% CI) for hepatocellular carcinoma (HCC) and gallbladder and biliary tract cancers (GBTC) by circulating Se and SePP levels in the EPIC nested case-control study.

	Hepatocellular carcinoma (HCC)			Gallbladder & biliary tract cancers (GBTC)[a]		
	No. of ca/co	Matching factors[b] OR (95% CI)	Multivariable adjusted[c] OR (95% CI)	No. of ca/co	Matching factors[b] OR (95% CI)	Multivariable adjusted[c] OR (95% CI)
Se, μg/L						
Tertiles						
<80.5	60/37	ref.	ref.	47/34	ref.	ref.
80.6-94.4	34/30	0.62 (0.32, 1.21)	0.88 (0.35, 2.21)	32/33	0.69 (0.35, 1.36)	0.63 (0.29, 1.37)
>94.5	12/39	0.16 (0.07, 0.40)	0.18 (0.05, 0.66)	17/29	0.38 (0.17, 0.87)	0.37 (0.13, 1.03)
p-trend		<0.001	0.016		0.022	0.055
Per 20 μg/L	106/106	0.40 (0.27, 0.60)	0.41 (0.23, 0.72)	96/96	0.74 (0.51, 1.07)	0.74 (0.47, 1.18)
SePP, mg/L						
Tertiles						
<4.9	64/31	ref.	ref.	40/31	ref.	ref.
5-6.3	42/43	0.41 (0.21, 0.82)	0.43 (0.18, 0.99)	39/36	0.67 (0.31, 1.46)	0.46 (0.18, 1.17)
>6.4	15/47	0.11 (0.04, 0.28)	0.09 (0.03, 0.32)	21/33	0.39 (0.16, 0.94)	0.27 (0.09, 0.78)
p-trend		<0.0001	<0.0001		0.033	0.016
Per 1.5 mg/L	121/121	0.40 (0.27, 0.59)	0.37 (0.21, 0.63)	100/100	0.87 (0.60, 1.26)	0.79 (0.51, 1.21)

Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; CI, confidence interval; OR, incidence rate ratio; Se, selenium; SePP, selenoprotein P.

[a] Gallbladder and biliary tract cancers (GBTC) included tumours in the gallbladder, extrahepatic bile ducts, ampulla of Vater, and biliary tract.

[b] ORs and 95% CI estimated by conditional logistic regression conditioned on the matching factors.

[c] Additionally adjusted for body mass index (kg/m2, continuous), waist circumference (cm, continuous), baseline alcohol intake (g/d, continuous), physical activity (METs, continuous), smoking status (never, former, current, unknown), education (none/primary, technical/professional, secondary, university or higher), alcohol intake pattern (never drinkers, former drinkers, drinkers only at recruitment, always drinkers), self-reported diabetes, and total energy intake (kcal/d).

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Table 3

ORs and 95% confidence intervals (95% CI) for intrahepatic bile duct (IHBC), gallbladder, cholangiocarcinoma cancers by circulating Se and SePP levels in the EPIC nested case-control study.

Cancer site	No. of ca/co	Matching factors[a] OR (95% CI)	Multivariable adjusted[b] OR (95% CI)
Se, per 20 μg/L			
IHBC	36/36	0.70 (0.39, 1.25)	0.42 (0.15, 1.20)
Gallbladder[c]	41/41	0.50 (0.26, 0.97)	0.55 (0.22, 1.37)
Cholangiocarcinoma[d]	31/31	0.67 (0.37, 1.23)	0.34 (0.10, 1.08)
SePP, per 1.5 mg/L			
IHBC	40/40	0.71 (0.43, 1.16)	0.51 (0.21, 1.23)
Gallbladder	44/44	0.69 (0.37, 1.26)	0.33 (0.08, 1.35)
Cholangiocarcinoma	35/35	0.74 (0.45, 1.22)	0.51 (0.23, 1.22)

Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; CI, confidence interval; IHBC, intrahepatic bile duct; OR, incidence rate ratio; Se, selenium; SePP, selenoprotein P.

[a] ORs and 95% CI estimated by conditional logistic regression conditioned on the matching factors.

[b] Additionally adjusted for body mass index (kg/m2, continuous), waist circumference (cm, continuous), baseline alcohol intake (g/d, continuous), physical activity (METs, continuous), smoking status (never, former, current, unknown), education (none/primary, technical/professional, secondary, university or higher), alcohol intake pattern (never drinkers, former drinkers, drinkers only at recruitment, always drinkers), self-reported diabetes, and total energy intake (kcal/d).

[c] Gallbladder cancers were also included in the GBTC grouping (see table 2).

[d] Cholangiocarcinoma cancers were also included in the GBTC (see table 2) and IHBC groupings.