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Prediagnostic selenium status and hepatobiliary cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort[1,2]

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ABSTRACT

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

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

Design: We assessed prediagnostic selenium status by measuring serum concentrations of selenium and selenoprotein P (SePP; the major circulating selenium transfer protein) and examined the association with hepatocellular carcinoma (HCC; n = 121), gallbladder and biliary tract cancers (GBTCs; n = 100), and intrahepatic bile duct cancer (IHBC; n = 40) risk in a nested case-control design within the EPIC study. Selenium was measured by total reflection X-ray fluorescence, and SePP was determined by a colorimetric sandwich ELISA. Multivariable ORs and 95% CIs were calculated by using conditional logistic regression.

Results: HCC and GBTC cases, but not IHBC cases, showed significantly lower circulating selenium and SePP concentrations than their matched controls. Higher circulating selenium was associated with a significantly lower HCC risk (OR per 20-μg/L increase: 0.41; 95% CI: 0.23, 0.72) but not with the risk of GBTC or IHBC. Similarly, higher SePP concentrations were associated with lowered

HCC risk only in both the categorical and continuous analyses (HCC: *P*-trend ≤ 0.0001; OR per 1.5-mg/L increase: 0.37; 95% CI: 0.21, 0.63).

Conclusion: These findings from a large prospective cohort provide evidence that suboptimal selenium status in Europeans may be associated with an appreciably increased risk of HCC development. *Am J Clin Nutr* 2016;104:406–14.

Keywords: hepatocellular carcinoma, selenium, selenoprotein P, prospective cohort, liver cancer, hepatobiliary cancer, selenium status

INTRODUCTION

Worldwide, primary liver cancers [PLCs[48]; e.g., hepatocellular carcinomas (HCCs) 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 2 established risk factors: viral hepatitis B and C (HBV and HCV, respectively) 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 gallbladder and biliary tract cancers (GBTCs; tumors of the gallbladder and extrahepatic bile ducts) are anatomically related to PLCs and both of these cancer types are difficult to detect early, have poor prognoses, and have limited understood etiology (6, 7). Thus, a greater scientific understanding of the role of diet and lifestyle factors in the etiology of hepatobiliary cancers is important.

A growing body of experimental and observational evidence suggests that suboptimal intakes of the micronutrient selenium contribute to the development of several cancers (8). Selenium 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 epidemiologic studies suggest that implications for selenium intake in cancer risk are probably more apparent in populations with low selenium availability, such as many across Europe (13–15).

Absorbed selenium is primarily retained by the liver and recirculated as a constituent of selenoprotein P (SePP) (16). Selenium and SePP are the 2 major biomarkers of blood selenium status, whereas SePP also affects the expression of antioxidative selenoproteins (17–19). Selenium has been shown to play vital roles in multiple metabolic processes in the liver (20). Evidence from primary human hepatocytes and animal models implicate selenium in liver cancer development (21–24), whereas decreasing selenium concentrations in HCC tumor tissues were associated with progressive cancer grade (25). A Chinese prospective study also showed an inverse association between toenail selenium status and the risk of HCC mortality (26). Selenium deficiency has been observed in patients with alcoholic liver cirrhosis and primary biliary cirrhosis (27–29). Interestingly, low selenium 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 showed an inverse association between HCC development risk and plasma selenium concentrations (31).

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

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[2]Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[48]Abbreviations used: AFP, α-fetoprotein; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CRP, C-reactive protein; EPIC, European Prospective Investigation into Cancer and Nutrition; GBTC, gallbladder and biliary tract cancer; GGT, γ-glutamyltransferase; GPX3, glutathione peroxidase 3; HBV, hepatitis B virus; HCC, hepatocellular cancer; HCV, hepatitis C virus; ICD-O-2, *International Classification of Diseases for Oncology, Second Edition*; IHBC, intrahepatic bile duct cancer; PLC, primary liver cancer; SePP, selenoprotein P.

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has been described previously (32, 33). Briefly, between 1992 and 2000, >520,000 men and women, primarily aged 25–70 y, were recruited in 23 centers throughout 10 European countries (Denmark, France, Germany, Greece, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom). At recruitment, standardized dietary, lifestyle, and sociodemographic 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 (Lyon, France) in −196°C liquid nitrogen for all countries except for Denmark (−150°C, nitrogen vapor) and Sweden (−80°C, freezers). All of the cohort members provided written informed consent. Ethical approval for this study was obtained from the International Agency for Research on Cancer ethical review board (Lyon, France) and local participating centers.

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 for 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, Netherlands, Norway, Spain, Sweden, and 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, and Greece). For this study, the latest dates of complete information for cancer incidence and vital status ranged from December 2002 to December 2006 among different centers.

Case ascertainment

First incident HCC and IHBC were defined as codes C22.0 and C22.1, respectively, as per the *International Statistical Classification of Diseases, Injury and Causes of Death, 10th Revision*, and the *International Classification of Diseases for Oncology, Second Edition* (ICD-O-2). GBTCs included tumors in the gallbladder (C23.9), extrahepatic bile ducts (C24.0), ampulla of Vater (C24.1), and biliary tract (C24.8 and C24.9) with the morphology code ICD-O-2 8162/3. Cholangiocarcinoma was defined as a tumor 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 was 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 the availability of blood samples for selenium status analysis (see flowchart in **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 analyzed (261 case-control pairs), including 35 cholangiocarcinoma cases (intrahepatic = 29 and extrahepatic = 6) within the GBTC and IHBC groups. For selenium, 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 analyzed. For each case, a control was selected by incidence density sampling from all cohort members alive and free of cancer (except for nonmelanoma skin cancer) and matched by age at blood collection (±1 y), sex, study center, time of day (±3 h), and fasting status (<3, 3–6, and >6 h) at blood collection. Women were additionally matched by menopausal status (pre-, peri-, and postmenopausal) and hormone replacement therapy use at the time of blood collection (yes or 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), γ-glutamyltransferase (GGT), liver-specific alkaline phosphatase (AP), albumin, and total bilirubin] were available and measured as previously detailed elsewhere (35).

Serum selenium and SePP measurements

The case-control status was blinded. Concentrations of total selenium were measured by X-ray fluorescence as described previously (36). Briefly, 4 μL of serum sample was analyzed by using a bench-top total reflection X-ray fluorescence spectrometer (Picofox S2; Bruker Nano GmbH). For quantification of selenium, a certified reference gallium solution (1000 mg/L; Sigma) with a defined concentration was equally added to each sample. An internal serum standard was applied to each measurement. The interassay CV of this standard was a 10.0% relative SD within 48 analysis procedures. A colorimetric ELISA (Selenotest; ICI GmbH) was used to measure SePP concentrations from 5 μL of each serum sample in a 1:21 dilution according to the manufacturer's instructions. CVs were determined with 3 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 the 16 separate analyses needed to assay all samples and yielded CVs 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 by using a 4-parameter logistic function. The samples were measured in duplicate, and mean concentration values, SDs, and CVs were calculated.

Statistical analyses

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

Conditional logistic regression models were used to calculate ORs with 95% CIs and tests for trend for associations between circulating selenium and SePP in relation to the risk of HCC, GBTC, and IHBC, as well as specific subsites of the gallbladder, and cholangiocarcinoma (intra- and extrahepatic). Selenium and SePP concentrations were included in models as continuous (per 20 μg/L and 1.50 mg/L, respectively; ∼1 SD) and as categorical variables, with tertile cutoffs based on the distribution in all control subjects. Models were run separately for each cancer site by using the same categorical cutoffs for all tests.

TABLE 1
Selected baseline characteristics of incident liver cancer cases and their matched controls within the EPIC nested case-control study[1]

	HCC			GBTC			IHBC		
Baseline characteristics	Cases (n = 121)	Matched controls (n = 121)	P	Cases (n = 100)	Matched controls (n = 100)	P	Cases (n = 40)	Matched controls (n = 40)	P
Women, n (%)	39 (32.2)	39 (32.2)	—	62 (62.0)	62 (62.0)	—	17 (42.5)	17 (42.5)	—
Age at recruitment, y	60.1 ± 7.4[2]	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	6.0 ± 3.5	—		5.5 ± 3.5	—		5.9 ± 3.5	—	
BMI, kg/m^2	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	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
With diabetes,[3] n (%)	18 (14.9)	9 (7.4)	0.185	9 (9.0)	9 (9.0)	1.000	2 (5.0)	2 (5.0)	1.000
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,[4] n (%)			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	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)	
Liver function score,[5] n (%)			<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 (%)									
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
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									
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

(Continued)

TABLE 1 (*Continued*)

Baseline characteristics	HCC			GBTC			IHBC		
	Cases (n = 121)	Matched controls (n = 121)	P	Cases (n = 100)	Matched controls (n = 100)	P	Cases (n = 40)	Matched controls (n = 40)	P
Baseline serum biomarkers[6]									
Selenium,[7] μg/L	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
SePP, 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

[1] P values were derived by using t tests (continuous variables) or chi-square tests (categorical variables). Missing values were not excluded from percentage calculations; thus, the sum of percentages across subgroups may not add up to 100%. EPIC, European Prospective Investigation into Cancer and Nutrition; GBTC, gallbladder and biliary tract cancer; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IHCB, intrahepatic bile duct cancer; MET, metabolic equivalent task; SePP, selenoprotein P.

[2] Mean ± SD (all such values).

[3] Self-reported data.

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

[5] Ranges from 0 to 6; the score was grouped in categories as follows: 0 or ≥1 abnormal liver function tests [alanine aminotransferase >55 U/L, aspartate aminotransferase >34 U/L, γ-glutamyltransferase (men) >64 U/L, γ-glutamyltransferase (women) >36 U/L, alkaline phosphatase >150 U/L, albumin <35 g/L, total bilirubin >20.5 μmol/L; based on the values provided by the laboratory].

[6] Values are geometric means; 5th, 95th percentiles in parentheses.

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

Tests for dose-response by linear trend were performed by assigning the median values of each tertile of selenium 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, or unknown), BMI (kg/m^2), smoking status (never, former, current, or not specified), level of education (none/primary school, technical school, secondary school, or university degree or higher), physical activity (combination of physical activity, cycling, and sport activities in metabolic equivalents), waist circumference (cm), total energy intake (kcal/d), and self-reported diabetes. Other factors (height, weight, waist-to-hip ratio, and dietary intakes of energy, fiber, tea, coffee, red and processed meats, fish and shellfish, and fruit and vegetables) were tested as potential confounders but were excluded from final models for parsimony, because they did not affect our estimates (change in estimate: <10%).

Interactions for potential biologically plausible effect-modifying variables (age at diagnosis, BMI, and self-reported diabetes) were tested by including interaction terms formed by the product of modifying variable categories and the value of categories of exposure of interest. To explore the main proposed underlying mechanism of selenium action (i.e., antioxidant defense), we also tested interactions with circulating CRP (a marker of chronic inflammation, which is likely to be heightened under oxidative stress) and smoking (because smokers are under oxidative stress and have higher antioxidant defense requirements). Because subjects with alcoholic liver cirrhosis have been observed to have lower selenium status (27, 28), we explored interactions with alcohol intake. The significance of interactions was assessed by using likelihood ratio tests on the basis of the models with and without the interaction terms. In sensitivity analyses, we excluded subjects for the following reasons: *1*) those with self-reported type 2 diabetes at baseline (yes or no), because of the potential for modifications in diet after diagnosis of this disease; *2*) those with hepatitis infection, because it is an established risk factor for liver cancers; and *3*) subjects with follow-up of <2 or <4 y 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, or ≥3 = possible injury), which summarizes the number of abnormal values for 6 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, and total bilirubin >20.5 μmol/L; cutoffs were provided by the laboratory and were based on assay specifications].

All of the statistical tests were 2-sided, and P values <0.05 were considered significant. Analyses were performed by using the Stata version 11 (StataCorp) statistical package.

RESULTS

Baseline characteristics of participants

The baseline characteristics of all study subjects are presented in **Table 1**. On average, HCC, GBTC, and IHBC cases were diagnosed 6.0, 5.5, and 5.9 y after blood collection, respectively. HCC cases were more likely to be current smokers, to be former

TABLE 2
ORs (95% CIs) for HCC and GBTCs by circulating selenium and SePP concentrations in the EPIC nested case-control study[1]

	HCC			GBTCs[2]		
	Cases/controls, *n*	Matching factors[3]	Multivariable adjusted[4]	Cases/controls, *n*	Matching factors[3]	Multivariable adjusted[4]
Selenium						
Tertile						
≤80.5 μg/L	60/37	Ref	Ref	47/34	Ref	Ref
80.6–94.4 μg/L	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 μg/L	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						
Tertile						
≤4.9 mg/L	64/31	Ref	Ref	40/31	Ref	Ref
5–6.3 mg/L	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 mg/L	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)

[1]EPIC, European Prospective Investigation into Cancer and Nutrition; GBTC, gallbladder and biliary tract cancer; HCC, hepatocellular carcinoma; Ref, reference; SePP, selenoprotein P.

[2]GBTCs included tumors in the gallbladder, extrahepatic bile ducts, ampulla of Vater, and biliary tract.

[3]Estimated by conditional logistic regression conditioned on the matching factors.

[4]Additionally adjusted for BMI (kg/m^2, continuous), waist circumference (cm, continuous), baseline alcohol intake (g/d, continuous), physical activity (metabolic equivalent tasks, continuous), smoking status (never, former, current, or unknown), education (none/primary, technical/professional, secondary, or university or higher), alcohol intake pattern (never drinkers, former drinkers, drinkers only at recruitment, or always drinkers), self-reported diabetes, and total energy intake (kcal/d).

alcohol drinkers, to have a greater waist circumference, to have chronic HBV and/or HCV infection and liver enzyme abnormalities, and to have lower intakes of fruit and vegetables than 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 selenium showed a strong, significant correlation among cases and controls ($r = 0.62$, $P \leq 0.001$). Geometric means of serum selenium were significantly lower in HCC and GBTC cases than in their respective matched controls [71.3 compared with 85.2 μg/L ($P \leq 0.001$) and 82.1 compared with 85.9 μg/L ($P = 0.041$), respectively], whereas no significant differences were observed for IHBC. Concentrations of SePP were lower in HCC cases than in controls (geometric mean: 4.3 and 5.4 mg/L, respectively; $P \leq 0.001$) and did not differ significantly between GBTC or IHBC cases and controls.

HCC

The associations between serum selenium and SePP concentrations with HCC risk are shown in **Table 2**. A higher selenium concentration was significantly associated with lower HCC risk. Comparing tertile 3 with tertile 1, the multivariable OR ($OR_{T3\ vs.\ T1}$) was 0.18 (95% CI: 0.05, 0.66), *P*-trend = 0.016; per 20-μg/L increase in selenium concentration, the OR was 0.41 (95% CI: 0.23, 0.72). Similarly, SePP concentrations 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; per 1.5-mg/L increase in SePP concentration, OR: 0.37 (95% CI: 0.21, 0.63)].

GBTC

The associations between serum selenium and SePP concentrations with GBTC risk are also shown in Table 2. Higher serum selenium concentrations were not associated with a statistically significantly lower risk of GBTC (multivariable $OR_{T3\ vs.\ T1}$: 0.37; 95% CI: 0.13, 1.03; *P*-trend = 0.055; per 20-μg/L increase in selenium concentration, OR: 0.74; 95% CI: 0.47, 1.18), 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 concentrations 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 concentrations and GBTC risk was not significant when SePP was analyzed as a continuous variable (per 1.5-mg/L increase in SePP concentration, multivariable OR: 0.79; 95% CI: 0.51, 1.21).

Hepatobiliary cancer subtypes

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

Sensitivity analyses and effect modifications

The results did not change substantially after the exclusion of participants who self-reported type 2 diabetes at baseline or cases diagnosed during the first 2 or 4 y of follow-up, as well as after

TABLE 3
ORs (95% CIs) for IHBC, gallbladder cancer, and cholangiocarcinoma by circulating selenium and SePP concentrations in the EPIC nested case-control study[1]

Cancer site	Cases/ controls, *n*	Matching factors[2]	Multivariable adjusted[3]
Selenium, per 20 μg/L			
IHBC	36/36	0.70 (0.39, 1.25)	0.42 (0.15, 1.20)
Gallbladder[4]	41/41	0.50 (0.26, 0.97)	0.55 (0.22, 1.37)
Cholangiocarcinoma[5]	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[4]	44/44	0.69 (0.37, 1.26)	0.33 (0.08, 1.35)
Cholangiocarcinoma[5]	35/35	0.74 (0.45, 1.22)	0.51 (0.23, 1.22)

[1]EPIC, European Prospective Investigation into Cancer and Nutrition; GBTC, gallbladder and biliary tract cancer; IHBC, intrahepatic bile duct cancer; SePP, selenoprotein P.

[2]Estimated by conditional logistic regression conditioned on the matching factors.

[3]Additionally adjusted for BMI (kg/m^2, continuous), waist circumference (cm, continuous), baseline alcohol intake (g/d, continuous), physical activity (metabolic equivalent tasks, continuous), smoking status (never, former, current, or unknown), education (none/primary, technical/professional, secondary, or university or higher), alcohol intake pattern (never drinkers, former drinkers, drinkers only at recruitment, or always drinkers), self-reported diabetes, and total energy intake (kcal/d).

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

[5]Cholangiocarcinomas were also included in the GBTC (see Table 2) and IHBC groupings.

additional adjustment for liver function score (results not shown). Among hepatitis-free HCC cases (n = 52), the association with selenium was not significant (multivariable OR for 20 μg/L: 0.52; 95% CI: 0.26, 1.05), whereas the association with SePP remained significant after the exclusion of HBV- or HCV-positive cases (multivariable OR for 1.5 mg/L: 0.51; 95% CI: 0.26, 0.99). However, we did not observe a significant effect modification by hepatitis infection status (P-interaction for selenium and SePP: 0.425 and 0.854, respectively). We observed a significant interaction between BMI and SePP concentrations on HCC risk (P-interaction in crude and multivariable models: 0.036 and 0.006, respectively). The association between selenium and SePP and HCC risk was stronger among overweight and obese than in normal-weight participants. We did not observe any significant effect modifications for other factors such as smoking, CRP concentration, alcohol intake, or self-reported diabetes; in addition, no interactions were observed for other cancer subtypes (all P-interaction > 0.05).

DISCUSSION

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

Optimal selenium intake should ensure a circulating selenium concentration of ≥124 μg/L to fully express SePP and glutathione peroxidase 3 (GPX3) selenoproteins (17, 18, 37, 38). In this study, the correlation between selenium and SePP concentrations was relatively high (r = 0.62, $P \leq 0.001$), which reflected that most subjects had suboptimal selenium concentrations to fully saturate SePP (and GPX3), and was very similar to our previous analysis of the same selenium status markers in a separate study on colorectal cancer, also nested within EPIC (15). This provides further evidence for the marginally low selenium status in many Western European populations (19). Attenuated expression of SePP and dysregulation of the expression of other selenoproteins resulting from suboptimal selenium availability affect responses to important carcinogenic processes such as oxidative stress (9, 12), and this may underline the association of these selenium status markers with liver cancer.

For both HCC and GBTC, the point estimates comparing the highest and lowest tertiles of circulating selenium and SePP concentrations showed strong inverse associations (as did the continuous estimate for HCC). Although a preventive effect of selenium 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 preventive effect of higher selenium concentrations against hepatobiliary cancers. Mouse and rat models, in particular, have indicated a central role of the liver for selenium metabolism (39–43). Data from SePP knockout mice suggest that healthy hepatocytes are the major cell type to contribute to circulating SePP concentrations (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 selenium and SePP concentrations (through lower circulating SePP concentrations). This suggests a potential mechanism of liver carcinogenesis whereby the dysregulation of SePP expression and secretion due to impaired selenium organification (i.e., weakened conversion of dietary selenium into selenoproteins such as SePP by subfunctional 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 observed weaker associations for GBTC than HCC and no significant associations for the other liver-related cancer sites that we investigated (IHBC, gallbladder cancer, and cholangiocarcinomas).

However, it remains possible that the strong estimates provided by this study may reflect, at least in part, that these selenium markers are acting as biomarkers of liver disease—for example, as seen 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 in men with chronic hepatitis infection a reduction in HCC risk was associated with higher plasma selenium concentrations (31). Our stratified analyses

provide support for these selenium measures as biomarkers of both general liver damage and liver cancer risk. After the exclusion of hepatitis-positive cases, the association of HCC remained significant with SePP but not selenium. Among groups with no marked liver damage, the association of HCC with selenium or SePP was not significant, whereas for those with clear liver damage scores the association of decreased HCC risk was significant for higher SePP concentrations only. However, we did not observe 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 selenium such as selenomethionine, the major source of selenium in the human diet, and selenium selenite may differentially contribute to the amount of biologically usable selenium for hepatocyte metabolism (45). Such factors, along with baseline selenium concentrations, may partly explain the varying success of intervention trials of selenium supplements to prevent cancer (14, 15). A national program of adding selenium to fertilizers in Finland indicated that selenium status can be safely increased on a population-wide basis in low-selenium areas (46). Although a recent analysis on incidence rates of major cancers (although not including liver cancer) did not show an obvious impact of this program, the lack of an adequate nonsupplemented control group is a major problem in assessing these data (46). Interestingly, intervention trials in China that used 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 intervention study that used different selenium forms (48), suggest that as cirrhosis increases the liver is less able to adequately metabolize selenium from selenomethionine sources. Possibly, then, further selenium deficiency caused by cirrhosis may predispose patients (especially those with already suboptimal selenium 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 selenium and SePP observed in our study. Thus, perhaps for subjects with pre-existing liver disease, a lower selenium intake, especially from sources with selenomethione, will not adequately contribute to the functional selenium availability. These individuals are thus more likely to suffer selenium 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 concentrations only (i.e., this may reflect inadequate metabolism of selenomethionine to SePP, compounded for those who also have lower baseline selenium concentrations). However, we have no data on these different sources of selenium (e.g., use of supplements containing selenite) to adequately investigate these hypotheses.

Among the sex, lifestyle, dietary, and disease variables adjusted for in our analyses, mean selenium concentrations differed by HBV and/or HCV infection and diabetes status, whereas mean SePP concentrations differed by sex, diabetes, and fish and red meat intake (results not shown), which is in line with previous studies (19, 20, 31, 49). There were no significant multiplicative interactions, except for the interaction between BMI and SePP for HCC. Stratified analysis showed that 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's strengths include the measurement of the 2 most meaningful biomarkers of selenium status—i.e., total selenium 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 prediagnostic blood samples. The main limitations are the single-time-point blood measurement per subject and the relatively short follow-up time ($\sim$6 y). However, the exclusion of cases with $<$2 y of follow-up did not appreciably alter the findings. There was limited power to assess the association of selenium status with hepatobiliary cancer types because of low study numbers, including IHBC and 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 that indicate a strong association between high selenium status and a lower risk of HCC. Randomized controlled trials in populations in which selenium status is suboptimal (e.g., Western Europe) are needed to test whether increasing selenium intake may reduce the risk of hepatobiliary cancers, especially for those at high risk (e.g., HBV or HCV positive) for HCC and GBTC.

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REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. GLOBOCAN 2012 [Internet]. Version 1.0. Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. Lyon (France): International Agency for Research on Cancer; 2013. [cited 2015 Apr 8]. Available from: http://globocan.iarc.fr.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69–90.
3. Gomaa A-I. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. World J Gastroenterol 2008;14:4300.
4. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol 2009;27:1485–91.
5. Center MM, Jemal A. International trends in liver cancer incidence rates. Cancer Epidemiol Biomarkers Prev 2011;20:2362–8.
6. Seyama Y, Makuuchi M. Current surgical treatment for bile duct cancer. World J Gastroenterol 2007;13:1505–15.
7. Augustine MM, Fong Y. Epidemiology and risk factors of biliary tract and primary liver tumors. Surg Oncol Clin N Am 2014;23:171–88.
8. Méplan C, Hesketh J. Selenium and cancer: a story that should not be forgotten-insights from genomics. Cancer Treat Res 2014;159:145–66.
9. Labunskyy VM, Hatfield DL, Gladyshev VN. Selenoproteins: molecular pathways and physiological roles. Physiol Rev 2014;94:739–77.
10. Fairweather-Tait SJ, Bao Y, Broadley MR, Collings R, Ford D, Hesketh JE, Hurst R. Selenium in human health and disease. Antioxid Redox Signal 2011;14:1337–83.
11. Rayman MP. Selenoproteins and human health: insights from epidemiological data. Biochim Biophys Acta 2009;1790:1533–40.

12. Bellinger FP, Raman AV, Reeves MA, Berry MJ. Regulation and function of selenoproteins in human disease. Biochem J 2009;422:11–22.
13. Méplan C, Rohrmann S, Steinbrecher A, Schomburg L, Jansen E, Linseisen J, Hesketh J. Polymorphisms in thioredoxin reductase and selenoprotein K genes and selenium status modulate risk of prostate cancer. PLoS One 2012;7:e48709.
14. Steinbrenner H, Speckmann B, Sies H. Toward understanding success and failures in the use of selenium for cancer prevention. Antioxid Redox Signal 2013;19:181–91.
15. Hughes DJ, Fedirko V, Jenab M, Schomburg L, Méplan C, Freisling H, Bueno-de-Mesquita HB, Hybsier S, Becker NP, Czuban M, et al. Selenium status is associated with colorectal cancer risk in the European prospective investigation of cancer and nutrition cohort. Int J Cancer 2015;136:1149–61.
16. Burk RF, Hill KE. Regulation of selenium metabolism and transport. Annu Rev Nutr 2015;35:109.
17. Ashton K, Hooper L, Harvey LJ, Hurst R, Casgrain A, Fairweather-Tait SJ. Methods of assessment of selenium status in humans: a systematic review. Am J Clin Nutr 2009;89(Suppl):2025S–39S.
18. Hurst R, Armah CN, Dainty JR, Hart DJ, Teucher B, Goldson AJ, Broadley MR, Motley AK, Fairweather-Tait SJ. Establishing optimal selenium status: results of a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 2010;91:923–31.
19. Combs GF. Biomarkers of selenium status. Nutrients 2015;7:2209–36.
20. Guo CH. Status of essential trace minerals and oxidative stress in viral hepatitis C patients with nonalcoholic fatty liver disease. Int J Med Sci 2013;10:730–7.
21. Kasaikina MV, Turanov AA, Avanesov A, Schweizer U, Seeher S, Bronson RT, Novoselov SN, Carlson BA, Hatfield DL, Gladyshev VN. Contrasting roles of dietary selenium and selenoproteins in chemically induced hepatocarcinogenesis. Carcinogenesis 2013;34:1089–95.
22. Liu JG, Zhao HJ, Liu YJ, Liu Y, Wang XL. Effect of two selenium sources on hepatocarcinogenesis and several angiogenic cytokines in diethylnitrosamine-induced hepatocarcinoma rats. J Trace Elem Med Biol 2012;26:255–61.
23. Moustafa ME, Carlson BA, Anver MR, Bobe G, Zhong N, Ward JM, Perella CM, Hoffmann VJ, Rogers K, Combs GF, et al. Selenium and selenoprotein deficiencies induce widespread pyogranuloma formation in mice, while high levels of dietary selenium decrease liver tumor size driven by TGFα. PLoS ONE 2013;8:e57389.
24. Rohr-Udilova N, Sieghart W, Eferl R, Stoiber D, Björkhem-Bergman L, Eriksson LC, Stolze K, Hayden H, Keppler B, Sagmeister S, et al. Antagonistic effects of selenium and lipid peroxides on growth control in early hepatocellular carcinoma. Hepatology 2012;55:1112–21.
25. Di Stasio M, Volpe MG, Colonna G, Nazzaro M, Polimeno M, Scala S, Castello G, Costantini S. A possible predictive marker of progression for hepatocellular carcinoma. Oncol Lett 2011;2:1247–51.
26. Sakoda LC, Graubard BI, Evans AA, London WT, Lin W-Y, Shen F-M, McGlynn KA. Toenail selenium and risk of hepatocellular carcinoma mortality in Haimen City, China. Int J Cancer 2005;115:618–24.
27. Burk RF, Early DS, Hill KE, Palmer IS, Boeglin ME. Plasma selenium in patients with cirrhosis. Hepatology 1998;27:794–8.
28. Nangliya V, Sharma A, Yadav D, Sunder S, Nijhawan S, Mishra S. Study of trace elements in liver cirrhosis patients and their role in prognosis of disease. Biol Trace Elem Res 2015;165:35–40.
29. McClain CJ, Marsano L, Burk RF, Bacon B. Trace metals in liver disease. Semin Liver Dis 1991;11:321–39.
30. Hesketh J. Nutrigenomics and selenium: gene expression patterns, physiological targets, and genetics. Annu Rev Nutr 2008;28:157–77.
31. Yu MW, Horng IS, Hsu KH, Chiang YC, Liaw YF, Chen CJ. Plasma selenium levels and risk of hepatocellular carcinoma among men with chronic hepatitis virus infection. Am J Epidemiol 1999;150:367–74.
32. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondière UR, Hémon B, Casagrande C, Vignat J, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
33. Riboli E, Kaaks R. The EPIC project: rationale and study design. Int J Epidemiol 1997;26(Suppl 1):S6–14.
34. Trichopoulos D, Bamia C, Lagiou P, Fedirko V, Trepo E, Jenab M, Pischon T, Nöthlings U, Overved K, Tjønneland A, et al. Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested case-control study. J Natl Cancer Inst 2011;103:1686–95.
35. Fedirko V, Lukanova A, Bamia C, Trichopolou A, Trepo E, Nöthlings U, Schlesinger S, Aleksandrova K, Boffetta P, Tjønneland A, et al. Glycemic index, glycemic load, dietary carbohydrate, and dietary fiber intake and risk of liver and biliary tract cancers in Western Europeans. Ann Oncol 2013;24:543–53.
36. Hoeflich J, Hollenbach B, Behrends T, Hoeg A, Stosnach H, Schomburg L. The choice of biomarkers determines the selenium status in young German vegans and vegetarians. Br J Nutr 2010;104:1601–4.
37. Combs GF, Jackson MI, Watts JC, Johnson LK, Zeng H, Idso J, Schomburg L, Hoeg A, Hoefig CS, Chiang EC, et al. Differential responses to selenomethionine supplementation by sex and genotype in healthy adults. Br J Nutr 2012;107:1514–25.
38. Xia Y, Hill KE, Li P, Xu J, Zhou D, Motley AK, Wang L, Byrne DW, Burk RF. Optimization of selenoprotein P and other plasma selenium biomarkers for the assessment of the selenium nutritional requirement: a placebo-controlled, double-blind study of selenomethionine supplementation in selenium-deficient Chinese subjects. Am J Clin Nutr 2010;92:525–31.
39. Geillinger KE, Rathmann D, Köhrle J, Fiamoncini J, Daniel H, Kipp AP. Hepatic metabolite profiles in mice with a suboptimal selenium status. J Nutr Biochem 2014;25:914–22.
40. Hill KE, Wu S, Motley AK, Stevenson TD, Winfrey VP, Capecchi MR, Atkins JF, Burk RF. Production of selenoprotein P (Sepp1) by hepatocytes is central to selenium homeostasis. J Biol Chem 2012;287:40414–24.
41. Nogales F, Ojeda ML, Fenutría M, Murillo ML, Carreras O. Role of selenium and glutathione peroxidase on development, growth, and oxidative balance in rat offspring. Reproduction 2013;146:659–67.
42. Renko K, Werner M, Renner-Müller I, Cooper TG, Yeung CH, Hollenbach B, Scharpf M, Köhrle J, Schomburg L, Schweizer U. Hepatic selenoprotein P (SePP) expression restores selenium transport and prevents infertility and motor-incoordination in Sepp-knockout mice. Biochem J 2008;409:741–9.
43. Stoedter M, Renko K, Hög A, Schomburg L. Selenium controls the sex-specific immune response and selenoprotein expression during the acute-phase response in mice. Biochem J 2010;429:43–51.
44. Becker N-P, Martitz J, Renko K, Stoedter M, Hybsier S, Cramer T, Schomburg L. Hypoxia reduces and redirects selenoprotein biosynthesis. Metallomics 2014;6:1079–86.
45. Hoefig CS, Renko K, Köhrle J, Birringer M, Schomburg L. Comparison of different selenocompounds with respect to nutritional value vs. toxicity using liver cells in culture. J Nutr Biochem 2011;22:945–55.
46. Alfthan G, Eurola M, Ekholm P, Venäläinen ER, Root T, Korkalainen K, Hartikainen H, Salminen P, Hietaniemi V, Aspila P, et al; Selenium Working Group. Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: from deficiency to optimal selenium status of the population. J Trace Elem Med Biol 2015;31:142–7.
47. Yu SY, Zhu YJ, Li WG. Protective role of selenium against hepatitis B virus and primary liver cancer in Qidong. Biol Trace Elem Res 1997;56:117–24.
48. Burk RF, Hill KE, Motley AK, Byrne DW, Norsworthy BK. Selenium deficiency occurs in some patients with moderate-to-severe cirrhosis and can be corrected by administration of selenate but not selenomethionine: a randomized controlled trial. Am J Clin Nutr 2015;102:1126–33.
49. Bettinger D, Schultheiss M, Hennecke N, Panther E, Knüppel E, Blum HE, Thimme R, Spangenberg HC. Selenium levels in patients with hepatitis C virus-related chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma: a pilot study. Hepatology 2013;57:2543–4.
50. Speckmann B, Walter PL, Alili L, Reinehr R, Sies H, Klotz L-O, Steinbrenner H. Selenoprotein P expression is controlled through interaction of the coactivator PGC-1alpha with FoxO1a and hepatocyte nuclear factor 4alpha transcription factors. Hepatology 2008;48:1998–2006.