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Dietary trace element intake and liver cancer risk: results from two population-based cohorts in China

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Abstract

Dietary factors have been hypothesized to affect the risk of liver cancer via various mechanisms, but the influence has not been well studied and the evidence is conflicting. We investigated associations of dietary trace element intake, assessed through a validated food frequency questionnaire, with risk of liver cancer in two prospective cohort studies of 132,765 women (1997–2013) and men (2002–2013) in Shanghai, China. The associations were first evaluated in cohort studies and further assessed in a case-control study nested within these cohorts adjusting for hepatitis B virus infection. For cohort analyses, Cox proportional hazard models were used to estimate hazard ratios and 95% confidence intervals. For nested case-control analyses, conditional logistic regression was used to calculate odds ratios and 95% confidence intervals. After a median follow-up time of 15.2 years for the Shanghai Women's Health Study and 9.3 years for the Shanghai Men's Health Study, 192 women and 344 men developed liver cancer. Dietary intake of manganese was inversely associated with liver cancer risk (highest vs. lowest quintile, HR = 0.51, 95% CI: 0.35–0.73; $p_{\text{trend}} = 0.001$). Further adjustment for hepatitis B virus infection in the nested case-control study yielded a similar result (highest vs. lowest quintile, OR = 0.38, 95% CI: 0.21–0.69; $p_{\text{trend}} < 0.001$). No significant associations were found between dietary intake of selenium, iron, zinc, copper and liver cancer risk. The results suggest that higher intake of manganese may be associated with a lower risk of liver cancer in China.

Keywords

liver cancer; dietary trace elements; manganese; prospective cohort study; nested case-control study

Introduction

Liver cancer is the sixth most commonly diagnosed cancer^{1, 2}. Prognosis of liver cancer is poor with an overall 5-year survival rate of less than 15%, making it the second most common cause of cancer mortality worldwide^{2, 3}. An estimated 782,000 new cases and 745,000 deaths occurred worldwide in 2012⁴. Liver cancer is primarily associated with chronic hepatitis B virus (HBV) and chronic hepatitis C viruses (HCV) infection⁵. Other major risk factors include long-term exposure to aflatoxin, chronic alcoholism, body fatness, diabetes, use of oral contraceptives and nutritional deficiency^{6–8}.

A healthy diet provides not only adequate fat, carbohydrate and protein but also small amounts of micronutrients such as trace elements and vitamins. Some trace elements are essential for human life such as selenium (Se), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), chromium (Cr), cobalt (Co), iodine (I), and molybdenum (Mo)⁹. Unraveling of the new roles of some trace elements as antioxidants which protect the cellular damage to the basic constituents, i.e., lipids, protein and DNA from in situ generated reactive oxygen species (ROS) and oxygen free radicals (OFR) gives a new dimension to our understanding the mechanism of action of these essential trace elements¹⁰.

Among nutritional factors, higher red meat, saturated fat and lower vegetable, fish intake were found associated with higher risk of liver cancer^{11–14}. Dietary micronutrients such as vitamin A, C and E, Se, Fe, Zn, Mn and Cu are necessary to regulate the reactions that release OFR and modulate the host susceptibility or resistance to infectious pathogens, thus may reduce the liver cancer risk^{7, 15–18}.

In our previous study, we have found that high intake of vitamin E was related to lower risk of liver cancer in middle-aged or older people from China¹⁶. However, there were no prospective cohort studies evaluating the relationship between dietary trace elements and liver cancer risk. In this study, we examined whether the common dietary trace elements, such as Se, Fe, Zn, Mn and Cu, were related to liver cancer risk in two large, population-based, prospective cohorts of women and men from China.

Methods

Study population

The Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS) are both prospective, population-based cohort studies in Shanghai, China^{19, 20}. In brief, for the SWHS, 74,941 women aged 40 to 70 years in Shanghai were recruited from 1997 to 2000, with an overall study participation rate of 92.7%. For the SMHS, 61,480 men aged 40 to 74 years with no history of cancer were recruited in Shanghai from 2002 to 2006, with an overall study participation rate of 74.1%. Trained interviewers administered the baseline surveys and obtained anthropometric measurements of the participants. The two cohorts were approved by all relevant Institutional Review Boards and an informed consent was obtained from each participant.

We excluded participants from this analysis who (i) had a diagnosis of cancer before enrollment (1,598 women and 0 men), (ii) loss to follow-up shortly (only have baseline information) after enrollment (3 women and 6 men), (iii) died of cancers of unknown primary site or without diagnosis date (250 women and 202 men); (iv) were possible liver cancer cases waiting to be confirmed (0 women and 3 men); (v) extreme values for total energy intake (outside the range of 700–3,500 kcal/day for women and 800–4,000 kcal/day for men) (224 women and 253 men); (vi) missing data for any of the covariates of interest (69 women and 1,018 men). Given that participants diagnosed of carcinoma in situ of digestive organs may be liver cancer or not, we further excluded these participants to avoid misclassification (12 women and 18 men). After exclusions, 72,785 women and 59,980 men were included in current analysis.

Dietary assessment

Semi-quantitative food-frequency questionnaires (FFQs), designed to assess commonly consumed foods in urban Shanghai over the preceding year, were administered in face-to-face interviews. Similar FFQs were used in the SWHS and SMHS, which contained 77 and 81 food items, respectively. During the in-person interview, the participants were asked about the frequency (daily, weekly, monthly, yearly, or never) and quantity [liangs (50 g)] that they consumed each time over the past 12 months. Energy and nutrients intake were calculated on the basis of the 2002 Chinese Food Composition Table ²¹. The FFQs have been validated against multiple 24-h dietary recalls separately for women ²² and men ²³ and showed moderate validity and reproducibility; The correlation between FFQ and 24-h dietary recall were 0.50 for Se, 0.50 for Fe, 0.55 for Zn, 0.57 for Mn, 0.26 for Cu in the SWHS and 0.46 for Se, 0.50 for Fe, 0.51 for Zn, 0.55 for Mn, 0.36 for Cu in the SMHS.

Follow-up and Cancer Identification

Cohort members have been followed for cancer occurrence through in-person follow-up surveys taking place every 2–3 years and annual record linkage with databases of the population-based Shanghai Cancer Registry, Shanghai Vital Statistics Registry, and Shanghai Resident Registry. For the SWHS, the response rates for the first (2000–2002), second (2002–2004), third (2004–2007), and fourth (2008–2011) in-person follow-up surveys were 99.8%, 98.7%, 96.7%, and 92.3%, respectively. For the SMHS, the response rates for the first (2004–2008) and second (2008–2011) follow-up surveys were 97.6% and 93.7%.

All possible cancer diagnoses were verified through home visits and review of medical charts by a panel of clinical and pathological experts. Cancers were coded according to the International Classification of Disease, Ninth Revision (ICD-9). Liver cancer was defined as primary malignant tumor with an ICD-9 code of 155 ²⁴.

Nested case-control study subset

A nested case-control subset within the full cohort was conducted to investigate whether associations between dietary trace element intake and liver cancer risk were independent of chronic HBV infection. The design has been previously described in detail ²⁵. Cases of liver cancer were identified during the period between participants' recruitment and December

31, 2012, and ten controls were matched to the cases by gender, age at recruitment (± 2 years), date (± 30 days) of biospecimen collection, time (morning or afternoon) of blood sample collection, interval since last meal (less than 2 hours) and menopausal status (for women, yes or no). The plasma hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) were measured in 363 liver cancer cases and 3,511 matched controls.

Statistical analysis

We conducted analyses separately for women and men. For the baseline characteristics between the liver cancer cases and non-cases in women and men, continuous variables were compared using t test and categorical variables were compared using chi-square test and Cochran-Mantel-Haenszel chi-square test. Cox proportional hazards regression model was used to calculate hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) for each quintile of intake using the lowest quintile as the reference category. The entry time was the date at which the participant enrolled in the SWHS or SMHS and the exit time was the date when the participant developed incident liver cancer or was censored due to death, loss to follow-up, or end of study follow-up on December 31, 2013, using whichever censoring date occurred first.

The model was adjusted for age, education, income, smoking status (men only), alcohol consumption (men only), family history of liver cancer, body mass index (BMI), total physical activity, total energy intake, total vegetable intake, total fruit intake, total red meat intake, total egg intake, total fish intake, total soy intake, vitamin E intake, history of diabetes, history of viral hepatitis/chronic liver disease, history of cholelithiasis, cholecystectomy, menopausal status (women only), and ever had oral contraceptive (women only). In analyses of data from women and men combined, dietary intake variables were categorized on the basis of sex-specific quintile distributions. To evaluate linear trends, we entered the median level of dietary trace element by quintile into the model as a continuous variable. All estimates of dietary trace element intake in these analyses were energy adjusted using the residual method ²⁶. We assessed the effect modification of gender on a multiplicative scale by including interaction term between dietary trace elements and gender in Cox proportional hazards models. We have checked proportional hazards assumptions for all the covariates using the Schoenfeld residual method ²⁷ and found no evidence of violation of these assumptions. The interaction was not statistically significant; therefore we assumed that the proportional hazards assumption was not violated. The associations between dietary trace element intake and liver cancer risk stratified by history of hepatitis/chronic liver disease were conducted for subgroup analysis.

In the nested case-control study, conditional logistic models were used to calculate the odds ratio (OR) for liver cancer by quintiles of dietary trace elements. In addition to the aforementioned confounders, we also adjusted for HBsAg status (positive and negative), and HBeAg status (positive and negative) in the model.

Sensitivity analyses were conducted (i) excluding all participants with a follow-up time of <2 years to rule out possible reverse causation in the cohort studies; (ii) excluding all participants with diabetes at recruitment, owing to this disease may change an individual's

dietary pattern or other lifestyle factors; (iii) using the energy density method²⁶ to adjust for dietary trace elements re-analysing the full cohort and nested case-control samples.

Statistical analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC) and a two-sided *P* value of 0.05 was considered statistically significant.

Results

After a median follow-up time of 15.2 years for the SWHS and 9.3 years for the SMHS, 192 women and 344 men liver cancer cases were identified. The baseline characteristics of participants by liver cancer diagnosis status are shown in table 1. Compared to cohort members without liver cancer, patients with liver cancer were generally older, had lower family income, education levels and higher dietary soy intake. Liver cancer patients were more likely to have reported a history of liver cancer and a history of chronic diseases, including viral hepatitis/chronic liver disease, diabetes and cholelithiasis. Among women, liver cancer patients tended to have higher BMI status, lower intake of fruit, red meat, higher proportion of cholecystectomy and menopause, and lower dietary Se, Cu than other cohort participants. Among men, liver cancer patients tended to have lower dietary Mn intake.

Table 2 presents associations between dietary trace elements and liver cancer by quintiles of trace element intake in cohort analyses. Dietary Mn was inversely associated with liver cancer risk. The liver cancer risk was reduced by 49% for the highest quintile compared with the lowest in the model with men and women combined. Compared with the first quintile, the HRs for liver cancer risk were 0.67 (95% CI 0.51–0.89), 0.70 (95% CI 0.53–0.93), 0.61 (95% CI 0.45–0.83), and 0.51 (95% CI 0.35–0.73) for the second through the fifth quintile, respectively, after adjustment for potential confounders ($P_{\text{trend}} = 0.001$). The inverse associations were consistently seen for both women and men, and the trend tests were statistically significant. The test for multiplicative interaction between sex and Mn intake in the combined models, however, indicated that sex did not modify the association between Mn intake and liver cancer risk ($P_{\text{interaction}} = 0.29$). There seems to be some weak evidence of an inverse association between iron intake and liver cancer in women (HR 5th quintile versus 1st quintile = 0.46; 95% CI 0.21–1.04, $P_{\text{trend}} = 0.05$). There were no significant associations between dietary intake of Se, Zn, Cu and liver cancer risk in the combined or separate models.

We also analyzed the associations stratified by history of hepatitis/chronic liver disease in table 3 because the status may have the potential to modify the association between diet, other lifestyle factors and liver cancer risk. The inverse association between dietary Mn intake and liver cancer risk was consistent among participants with and without history of hepatitis/chronic liver disease. And the test for multiplicative interaction was not statistically significant ($P_{\text{interaction}} = 0.58$).

The odds ratios for liver cancer in the nested case-control subset were shown in table 4. The odds ratio for liver cancer was 0.38 (95% CI 0.21–0.69) for the highest quintile compared with the lowest quintile of dietary Mn for men and women combined ($P_{\text{trend}} < 0.001$). In addition, a significant inverse trend ($P_{\text{trend}} = 0.007$) for liver cancer risk in relation to dietary

Fe intake (OR_{5th quintile versus 1st quintile} = 0.33; 95% CI 0.15–0.71) was observed primarily due to the inverse association observed for men, in contrast to the results in cohort analyses. No associations between dietary intake of Se, Zn, Cu and liver cancer risk were found.

The inverse association between dietary Mn intake and liver cancer risk remained after removal of participants with a follow-up time of <2 years (Supplementary Table 1) and in participants without diabetes at recruitment (Supplementary Table 2) in our cohort analyses. And the results remained similar using energy density method in cohort analysis and nested case-control analysis (results not shown).

Discussion

In these large, prospective studies of women and men in Shanghai, we found that higher intake of Mn was inversely associated with liver cancer risk. This result was confirmed in an analysis of the nested case-control study which also adjusted for HBV infection.

One biologically plausible explanation as to why dietary Mn intake is associated with liver cancer risk is its potential effect on antioxidants to protect the cellular damage. The liver has a relatively high concentration of Mn and Mn is primarily excreted into the bile²⁸. Three experimental studies have suggested that Mn is a cofactor of antioxidant enzymes including manganese superoxide dismutase (MnSOD) which is the fundamental mitochondrial enzyme that scavenges ROS^{29–31}. The activity of this enzyme could modulate the host susceptibility or resistance to infectious pathogens, protect the cellular damage, inhibit cell proliferation and contribute to apoptosis, thus to prevent liver cancer development^{15, 32–34}. Mn is often considered to be among the least toxic of the trace elements when administered orally in contrast to exposure to occupational exposures such as welding fume exposure^{35, 36}. Thus, reported cases of human toxicity caused by oral ingestion of large amounts of Mn are few⁹. Metabolic balance studies on normal subjects consuming widely differing diets have revealed positive balances when Mn intake ranged from 2.47 to 10.7 mg/d³⁵. High Mn intakes of 8–9 mg/d are safe, since there was no evidence of Mn toxicity in the individuals consuming these high-Mn diets³⁵. In our cohorts, the mean $\pm$ standard deviation of daily Mn intake was 5.9 $\pm$ 1.4 mg for females and 7.0 $\pm$ 1.8 mg for males, which were more than the Dietary Reference Intake (DRI) of The Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences (females, >30 y: 1.8 mg; males, >30 y: 2.3 mg)³⁷. The diets rich in Mn are whole cereals, nuts, legume seeds, and green leafy vegetables³⁸. Tea is exceptionally rich in Mn, one cup of tea contributing as much as 1.3 mg of Mn³⁵. Our findings are concordant with previous meta-analyses of vegetables, tea drinking in relation to liver cancer risk^{12, 39, 40}. Gender may impact on Mn retention in body and time of the male retention was longer than female⁴¹, which may be the reason that stronger inverse association was observed between dietary Mn and liver cancer risk in men than women in our study.

The DRI of Fe is 8 mg for adult men and postmenopausal women (>50 y) and 16 mg for premenopausal women³⁷. In our middle-age cohorts, increasing participant age was associated with lower dietary Fe intakes. For males, the mean Fe intake daily was 21.8 mg and the mean age was 55.3 y in the main cohort analyses, while in the nested case-control

studies the intake was 21.7 mg and the mean age was 59.5 y. The mean Fe intakes were very similar between the two studies, and both were much higher than the DRI. We observed a different association of Fe intake with liver cancer risk between cohort and nested case-control studies, however, it cannot be explained by comparing the average Fe intakes in our studies with the DRI.

For females, the mean Fe intake daily was 18.6 mg (≤ 50 y, 35,774 participants: 49.2%) and 18.2 mg (>50 y, 37,011 participants: 50.8%) in cohort studies, and in the nested case-control analyses the intake was 18.1 mg (≤ 50 y, 307 participants: 22.3%) and 18.1 mg (>50 y, 1068 participants: 77.7%). Most participants in the nested case-control studies were postmenopausal women, and the daily iron intake were much more than the DRI for postmenopausal women (8 mg). So the protective effect for dietary Fe intake was weaker in the nested case-control analyses than cohort analyses for women.

Previously, two epidemiological studies, both using a case-control study design, have evaluated dietary trace elements and liver cancer risk^{42,43}. A hospital-based case-control study conducted in Greece, involving 97 hepatocellular carcinoma (HCC) cases and 128 controls reported a null association for dietary trace elements intake⁴². Another hospital-based case-control study conducted in Italy, including 185 HCC cases and 412 cancer-free control subjects, showed dietary Fe was significantly associated with HCC risk (OR = 3.00; 95% CI: 1.25–7.23) and no association between Zn and HCC risk⁴³. However, both of the two studies analyzing two kinds of trace elements were limited by small sample size and by their retrospective and hospital-based study designs. Due to the case-control nature of both of the studies, recall bias in reporting dietary intake may have occurred, especially for cases, since they may associate diet with their disease status. Similarly, cases may have recently changed their diet due to symptoms or treatment and therefore the FFQ results would not represent habitual intake.

As we know, micronutrients including vitamins and trace elements play a central part in metabolism and in the maintenance of tissue function. In our present study, we analyzed the association between five trace elements intake and liver cancer risk. To the best of our knowledge, this was the first prospective cohort study investigating the association between dietary Mn intake and liver cancer risk. Our study has a number of important strengths. First, the SWHS and SMHS are both rigorously designed cohort studies with high participation and retention rates. Second, all of the covariates used in analysis were assessed before the development of any cancer which decreases potential reverse bias. Third, we had a relatively large number of incident liver cancer cases in our analysis having sufficient power of test to find the association. The fourth, our nested case-control study incorporates both biomarkers of HBsAg and HBeAg into the analysis to minimize the possibility of hepatitis B virus infection affecting the association between dietary trace elements and the occurrence of liver cancer. Finally, the results from the various secondary analyses we conducted yielded similar results throughout which suggest that our findings are fairly robust.

Our study also has some limitations that should be considered in the interpretation of the results. First, we did not incorporate the multi-mineral supplement intake which may also be a source of the trace element because we had no questions about multi-mineral supplement

intake. Second, we used the FFQ data assessed only at the baseline and this may not have accounted for potential dietary changes during follow-up, including early pre-diagnostic period of the disease. Third, dietary trace elements were assessed using an FFQ which may not be accurate at estimating the actual amount of dietary intake. The correlation between the estimated micronutrients intake from an FFQ and a 24-h recall instrument was moderate in both study populations^{22, 23}. Fourth, the mutual effect between food groups and nutrition were complex and we did not test the interaction between diets and trace elements in our analysis. Finally, given the multiple comparisons carried out in the current study, a chance finding cannot be ruled out. However, the consistent pattern observed for dietary Mn intake in cohort, nested case-control studies and sensitivity analyses, argue against a chance finding.

In conclusion, in these two population-based cohort studies, we found that high intake of Mn was related to lower risk of liver cancer in middle-aged or older people in China. Moreover, our findings, if independently confirmed, would point to potential benefits from daily dietary trace elements for primary preventing liver cancer-one of the most lethal tumors in the world.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Se	selenium
Fe	iron
Zn	zinc
Mn	manganese
Cu	copper

ROS	reactive oxygen species
OFR	oxygen free radicals
OR	odd ratio
HR	hazard ratio
CI	confidence interval
HBV	hepatitis B virus
HCV	hepatitis C virus
HBsAg	hepatitis B surface antigen
HBeAg	hepatitis B e antigen
SWHS	the Shanghai Women's Health Study
SMHS	the Shanghai Men's Health Study
FFQs	food-frequency questionnaires
ICD-9	the international classification of disease, ninth revision
HCC	hepatocellular carcinoma
BMI	body mass index
MnSOD	manganese superoxide dismutase
DRI	dietary reference intake

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Novelty and Impact

Currently, the role of dietary trace element intake in the development of liver cancer is unclear. Our study investigated the association between dietary trace elements and liver cancer risk for the first time, using detailed data from two large population-based cohorts in China. It was found that higher dietary manganese intake was associated with decreased liver cancer risk in China. The result may have important implications for the primary prevention of liver cancer.

Baseline characteristics of the Shanghai Women's and Men's Health Studies, comparing liver cancer patients with study participants who did not develop liver cancer (1997–2013, 2002–2013)

Characteristic ¹	Women			Men		
	Cases (n=192)	Non-cases (n=72,593)	P ²	Cases (n=344)	Non-cases (n=59,636)	P ²
Age at recruitment (years)	58.8±8.3	52.5±9.0	<0.001	58.6±9.9	55.3±9.7	<0.001
Body mass index (BMI) (kg/m ²)	25.0±3.8	24.0±3.4	<0.001	23.7±3.2	23.72±3.1	0.75
Total physical activity (MET hours/week)	101.7±46.7	106.6±45.1	0.13	61.7±37.5	59.6±34.1	0.26
Total energy intake (kcal/day)	1623±384	1675±392	0.07	1,896±510	1,910±473	0.59
Total vegetable intake (g/day) ³	292.0±149.5	289.0±154.1	0.78	321.8±165.3	336.2 ±177.5	0.13
Total fruit intake (g/day)	215.2±137.3	258.7±169.5	<0.001	158.1±123.0	147.5±120.2	0.10
Total red meat intake (g/day)	44.3±27.8	49.1±31.4	0.03	61.8±40.1	61.0±38.2	0.67
Total egg intake (g/day)	24.6±20.4	26.5±19.1	0.16	27.8±20.5	26.8±19.7	0.36
Total fish intake (g/day)	44.0±41.2	49.1±42.0	0.09	48.3±41.8	50.3±44.1	0.39
Total soy intake (g/day)	156.1±128.5	137.4±120.0	0.03	166.5±127.4	153.3±115.0	0.03
Vit E (mg/day)	12.7±4.3	13.1±4.8	0.29	14.8±5.7	14.6±5.4	0.38
Selenium (ug/d)	40.8±14.5	44.2±17.3	0.006	49.1±17.2	50.0±19.0	0.42
Iron (mg/d)	18.0±2.7	18.4±2.9	0.10	21.5±3.8	21.8±3.8	0.26
Manganese (mg/d)	5.8±0.6	5.8±0.6	0.68	6.7±0.8	6.8±0.7	<0.001
Zinc (mg/d)	10.4±1.0	10.5±1.1	0.15	12.2±1.3	12.3±1.4	0.12
Copper (mg/d)	2.1±0.3	2.1±0.4	0.03	2.3±0.3	2.3±0.4	0.64
Family income, per person per year (N, %)			0.02			0.04
Low	44 (22.9%)	11,648 (16.1%)		213 (61.9%)	32,926 (55.2%)	
Middle	74 (38.5%)	27,733 (38.2%)		106 (30.8%)	20,926 (35.1%)	
High	74 (38.5%)	33,212 (45.8%)		25 (7.3%)	5784 (9.7%)	
Education level (N, %)			<0.001			<0.001
Elementary school or less	81 (42.2%)	15,400 (21.2%)		42 (12.2%)	3,950 (6.6%)	
Middle school	46 (24.0%)	27,045 (37.3%)		121 (35.2%)	20,002 (33.5%)	
High school	48 (25.0%)	20,311 (28.0%)		122 (35.5%)	21,534 (36.1%)	
College or above	17 (8.9%)	9,837 (13.6%)		59 (17.2%)	14,150 (23.7%)	
Ever smoking (N, %)	7 (3.7%)	1,992 (2.7%)	0.45	242 (70.4%)	41,574 (69.7%)	0.80

Characteristic ¹	Women		Men		<i>P</i> ²
	Cases (n=192)	Non-cases (n=72,593)	Cases (n=344)	Non-cases (n=59,636)	
Ever alcohol drinker (N, %)	4 (2.1%)	1,414 (2.0%)	109 (31.7%)	19,793 (33.2%)	0.55
History of hepatitis/chronic liver disease (N, %)	39 (20.3%)	2,412 (3.3%)	146 (42.4%)	5,743 (9.6%)	<0.001
Family history of liver cancer (N, %)	18 (9.4%)	2,374 (3.3%)	37 (10.8%)	2,117 (3.6%)	<0.001
Ever had diabetes (N, %)	20 (10.4%)	3,103 (4.3%)	40 (11.6%)	3,714 (6.2%)	<0.001
Ever had cholelithiasis (N, %)	40 (20.8%)	8,054 (11.1%)	49 (14.2%)	4,447 (7.5%)	<0.001
Ever had cholecystectomy (N, %)	17 (8.9%)	3,113 (4.3%)	14 (4.1%)	1,629 (2.7%)	0.13
Menopausal status (N, %)	149 (77.6%)	35,374 (48.7%)			<0.001
Ever had oral contraceptive (N, %)	38 (19.8%)	14,841 (20.4%)			0.82

¹Continuous variables are presented as the mean $\pm$ the standard deviation.

²The general linear model was used to calculate *P* for continuous variables: age at recruitment, body mass index, total physical activity, total energy intake, total vegetable intake, total fruit intake, total red meat intake, total egg intake, total fish intake, total soy intake, vitamin E intake, selenium, iron, zinc, manganese, copper. Chi-square tests were used to calculate *P* for binary variables: ever smoking (yes or no); alcohol drinker (yes or no); history of viral hepatitis/chronic liver disease (yes or no); ever family history of liver cancer (yes or no); history of diabetes (yes or no); history of cholelithiasis (yes or no); cholecystectomy (yes or no), menopausal status (yes or no), and ever had oral contraceptive (yes or no). All statistical tests were two-sided. Cochran-Mantel-Haenszel statistics were used to calculate *P* for ordinal categorical variables: family income level (low income for <5000 yuan/year in the SWHS and <12 000 yuan/year in the SMHS; medium income for 5000 to <10 000 yuan/year in the SWHS and 12 000 to <24 000 yuan/year in the SMHS; and high income for >10 000 yuan/year in the SWHS and >24 000 yuan/year in the SMHS); education level (elementary school or less, middle school, high school, college or above).

³All dietary variables were adjusted for energy by residual method.

Table 2
Hazard ratios for liver cancer by quintiles of dietary trace elements in the Shanghai Women’s and Men’s Health Studies (1997–2013, 2002–2013)

Dietary trace elements intake and quintile ^f				Women		Men		Women and Men		<i>P</i> _{interaction}
				Range of intake	Cases (n=192)/Person-years	HR (95% CI) ^g	Range of intake	Cases (n=344)/Person-years	HR (95% CI) ^h	
Selenium				ug/d						0.64
	1			<31.77	52/212,564	1.00 (reference)	<36.24	71/109,747	1.00 (reference)	
	2			31.77–38.29	44/213,438	0.92 (0.58,1.46)	36.24–43.44	65/109,494	0.87 (0.61,1.26)	
	3			38.29–44.79	37/214,743	0.87 (0.50,1.52)	43.44–50.47	71/109,692	1.00 (0.68,1.49)	
	4			44.79–54.52	34/216,018	0.88 (0.46,1.69)	50.47–61.14	75/110,901	1.07 (0.69,1.68)	
	5			54.52	25/216,848	0.70 (0.26,1.90)	61.14	62/113,139	0.95 (0.51,1.76)	
<i>P</i> _{trend}						0.53			0.91	0.75
Iron				mg/d						0.84
	1			<16.19	45/214,421	1.00 (reference)	<18.88	75/109,712	1.00 (reference)	
	2			16.19–17.47	43/214,743	0.90 (0.57,1.42)	18.88–20.53	76/109,736	0.90 (0.63,1.27)	
	3			17.47–18.66	37/214,934	0.75 (0.44,1.25)	20.53–22.08	58/110,022	0.70 (0.47,1.04)	
	4			18.66–20.32	37/215,016	0.68 (0.38,1.22)	22.08–24.26	69/110,639	0.79 (0.50,1.22)	
	5			20.32	30/214,497	0.46 (0.21,1.04)	24.26	66/112,864	0.74 (0.41,1.33)	
<i>P</i> _{trend}						0.05			0.31	0.08
Zinc				mg/d						0.38
	1			<9.71	42/214,250	1.00 (reference)	<11.25	78/109,380	1.00 (reference)	
	2			9.71–10.2	48/214,257	1.16 (0.72,1.85)	11.25–11.84	61/109,692	0.79 (0.56,1.13)	
	3			10.2–10.65	31/214,436	0.78 (0.44,1.39)	11.84–12.4	67/110,124	0.84 (0.58,1.23)	
	4			10.65–11.27	37/215,219	1.06 (0.56,2.00)	12.4–13.18	69/110,811	0.92 (0.60,1.41)	
	5			11.27	34/215,449	1.14 (0.51,2.56)	13.18	69/112,965	0.93 (0.54,1.60)	
<i>P</i> _{trend}						0.83			0.98	0.94
Manganese				mg/d						0.29
	1			<5.3	32/216,836	1.00 (reference)	<6.27	95/111,272	1.00 (reference)	
	2			5.3–5.63	38/215,787	0.87 (0.53,1.43)	6.27–6.63	60/110,449	0.60 (0.42,0.84)	
	3			5.63–5.89	39/214,711	0.74 (0.44,1.24)	6.63–6.94	73/109,871	0.69 (0.49,0.97)	
	4			5.89–6.21	44/213,810	0.72 (0.41,1.24)	6.94–7.31	61/109,845	0.57 (0.39,0.83)	
	5			6.21	39/212,467	0.54 (0.28,1.05)	7.31	55/111,535	0.51 (0.32,0.79)	

Dietary trace elements intake and quintile ¹									
	Women			Men			Women and Men		
	Range of intake	Cases (n=192)/Person-years	HR (95% CI) ²	Range of intake	Cases (n=344)/Person-years	HR (95% CI) ³	Cases (n=536)/Person-year	HR (95% CI) ⁴	P _{interaction}
P _{trend}			0.06			0.003		0.001	
Copper	mg/d			mg/d					0.23
1	<1.84	41/214,707	1.00 (reference)	<2.05	70/109,615	1.00 (reference)	111/324,323	1.00 (reference)	
2	1.84–2	48/213,983	1.19 (0.74,1.92)	2.05–2.2	70/109,125	0.99 (0.69,1.41)	118/323,108	1.04 (0.79,1.37)	
3	2–2.16	36/214,688	0.96 (0.55,1.68)	2.2–2.35	50/110,070	0.66 (0.44,1.00)	86/324,759	0.74 (0.53,1.01)	
4	2.16–2.36	39/215,046	1.12 (0.60,2.08)	2.35–2.56	82/110,896	1.15 (0.76,1.73)	121/325,942	1.07 (0.77,1.49)	
5	2.36	28/215,187	0.85 (0.38,1.93)	2.56	72/113,266	0.99 (0.60,1.66)	100/328,453	0.89 (0.58,1.36)	
P _{trend}			0.67			0.71		0.75	

¹Energy adjusted using the residual method. In analyses of data from women and men combined, dietary intake variables were categorized on the basis of sex-specific quintile distributions.

²Results were adjusted for age at recruitment, body mass index (BMI), total physical activity, total energy intake, total vegetable intake, total fruit intake, total red meat intake, total egg intake, total fish intake, total soy intake, vitamin E intake, income, education, family history of liver cancer (yes or no), history of viral hepatitis/chronic liver disease (yes or no), history of diabetes (yes or no), history of cholelithiasis (yes or no), history of cholecystectomy (yes or no), menopausal status (yes or no) and ever had oral contraceptive (yes or no).

³Results were adjusted for age at recruitment, body mass index (BMI), total physical activity, total energy intake, total vegetable intake, total fruit intake, total red meat intake, total egg intake, total fish intake, total soy intake, vitamin E intake, income, education, smoking history (ever/never), alcohol consumption (ever/never), family history of liver cancer (yes or no), history of viral hepatitis/chronic liver disease (yes or no), history of diabetes (yes or no), history of cholelithiasis (yes or no) and history of cholecystectomy (yes or no).

⁴Results were adjusted for sex, age at recruitment, body mass index (BMI), total physical activity, total energy intake, total vegetable intake, total fruit intake, total red meat intake, total egg intake, total fish intake, total soy intake, vitamin E intake, income, education, smoking history (ever/never), alcohol consumption (ever/never), family history of liver cancer (yes or no), history of viral hepatitis/chronic liver disease (yes or no), history of diabetes (yes or no), history of cholelithiasis (yes or no) and history of cholecystectomy (yes or no).

Table 3

Hazard ratios for liver cancer by quintiles of dietary trace elements in the Shanghai Women's (1997–2013) and Men's (2002–2013) Health Studies, stratified by self-reported history of hepatitis/chronic liver disease at baseline

Dietary trace elements intake and quintile ^a	Participants without history of hepatitis/chronic liver disease		Participants with history of hepatitis/chronic liver disease		<i>P</i> _{interaction}
	Cases (n=351)	HR (95% CI) ²	Cases (n=185)	HR (95% CI) ²	
Selenium					
1	89	1.00 (reference)	34	1.00 (reference)	0.27
2	77	0.90(0.64,1.26)	32	0.89(0.53,1.49)	
3	67	0.84(0.57,1.23)	41	1.24(0.72,2.13)	
4	62	0.84(0.54,1.32)	47	1.37(0.75,2.50)	
5	56	0.83(0.45,1.55)	31	0.94(0.39,2.26)	
<i>P</i> _{trend}		0.56		0.77	
Iron					
1	84	1.00 (reference)	36	1.00 (reference)	0.95
2	78	0.88(0.63,1.22)	41	0.93(0.57,1.51)	
3	59	0.64(0.43,0.94)	36	0.87(0.51,1.51)	
4	68	0.70(0.46,1.08)	38	0.82(0.44,1.52)	
5	62	0.57(0.32,1.01)	34	0.74(0.33,1.68)	
<i>P</i> _{trend}		0.09		0.45	
Zinc					
1	85	1.00 (reference)	35	1.00 (reference)	0.69
2	72	0.86(0.61,1.20)	37	1.08(0.66,1.75)	
3	57	0.69(0.47,1.02)	41	1.05(0.62,1.76)	
4	70	0.89(0.59,1.34)	36	0.99(0.55,1.78)	
5	67	0.88(0.51,1.51)	36	0.95(0.45,2.00)	
<i>P</i> _{trend}	.	0.93		0.86	
Manganese					
1	76	1.00 (reference)	51	1.00 (reference)	0.58
2	63	0.66(0.47,0.93)	35	0.71(0.45,1.12)	
3	76	0.70(0.49,0.99)	36	0.71(0.44,1.14)	
4	74	0.60(0.42,0.87)	31	0.61(0.37,1.02)	

Dietary trace elements intake and quintile ^{1/}		Participants without history of hepatitis/chronic liver disease		Participants with history of hepatitis/chronic liver disease		<i>P</i> _{interaction}
		Cases (n=351)	HR (95% CI) ²	Cases (n=185)	HR (95% CI) ²	
5		62	0.44(0.28,0.69)	32	0.65(0.36,1.18)	
<i>P</i> _{trend}			0.001		0.09	
Copper						0.96
1		76	1.00 (reference)	35	1.00 (reference)	
2		81	1.06(0.76,1.49)	37	1.00(0.61,1.64)	
3		55	0.74(0.50,1.11)	31	0.73(0.42,1.27)	
4		76	1.05(0.70,1.59)	45	1.12(0.64,1.98)	
5		63	0.95(0.56,1.61)	37	0.80(0.39,1.64)	
<i>P</i> _{trend}			0.93		0.67	

^{1/} Energy adjusted using the residual method. In analyses of data from women and men combined, dietary intake variables were categorized on the basis of sex-specific quintile distributions.

^{2/} Results were adjusted for sex, age at recruitment, body mass index (BMI), total physical activity, total energy intake, total vegetable intake, total fruit intake, total red meat intake, total egg intake, total fish intake, total soy intake, vitamin E intake, income, education, smoking history (ever/never), alcohol consumption (ever/never), family history of liver cancer (yes or no), history of diabetes (yes or no), history of cholelithiasis (yes or no) and history of cholecystectomy (yes or no).

Odds ratios for liver cancer by quintiles of dietary trace elements in a nested case-control study of the Shanghai Women's and Men's Health Studies (1997–2012, 2002–2012)

Table 4

Dietary trace elements intake and quintile ^J	Women ²			Men ²			Women and Men ²		
	Case (n=129)	Control (n=1,246)	OR (95% CI) ³	Case (n=234)	Control (n=2,265)	OR (95% CI) ⁴	Case (n=363)	Control (n=3,511)	OR (95% CI) ⁴
Selenium									
1	28	249	1.00 (reference)	50	453	1.00 (reference)	78	702	1.00 (reference)
2	25	249	1.13(0.54,2.33)	38	453	0.87(0.47,1.62)	63	702	0.93(0.59,1.47)
3	25	249	1.35(0.60,3.04)	52	453	1.11(0.57,2.16)	77	702	1.19(0.72,1.95)
4	28	250	1.71(0.68,4.30)	51	453	1.16(0.57,2.39)	79	703	1.29(0.75,2.23)
5	23	249	1.18(0.31,4.53)	43	453	0.90(0.34,2.37)	66	702	0.97(0.45,2.07)
<i>P</i> _{trend}			0.63			0.99			0.78
Iron									
1	29	249	1.00 (reference)	53	453	1.00 (reference)	82	702	1.00 (reference)
2	29	250	1.21(0.60,2.44)	59	453	0.96(0.53,1.73)	88	703	1.07(0.69,1.66)
3	20	249	0.65(0.28,1.52)	42	453	0.50(0.25,1.01)	62	702	0.57(0.34,0.97)
4	29	248	1.26(0.51,3.12)	43	453	0.48(0.22,1.05)	72	701	0.71(0.40,1.26)
5	22	250	0.53(0.15,1.79)	37	453	0.26(0.09,0.77)	59	703	0.33(0.15,0.71)
<i>P</i> _{trend}			0.32			0.007			0.007
Zinc									
1	26	250	1.00 (reference)	55	453	1.00 (reference)	81	703	1.00 (reference)
2	28	248	1.55(0.75,3.20)	42	453	0.94(0.51,1.73)	70	701	1.12(0.71,1.77)
3	19	250	1.36(0.60,3.11)	47	453	1.05(0.53,2.07)	66	703	1.09(0.66,1.81)
4	24	248	1.82(0.74,4.46)	40	453	1.02(0.48,2.14)	64	701	1.15(0.66,2.01)
5	32	250	2.04(0.66,6.30)	50	453	1.78(0.68,4.62)	82	703	1.55(0.77,3.12)
<i>P</i> _{trend}			0.23			0.27			0.30
Manganese									
1	28	250	1.00 (reference)	69	453	1.00 (reference)	97	703	1.00 (reference)
2	31	248	0.90(0.45,1.80)	48	453	0.64(0.36,1.16)	79	701	0.71(0.46,1.09)
3	25	250	0.55(0.26,1.17)	42	453	0.60(0.31,1.15)	67	703	0.57(0.35,0.92)

Dietary trace elements intake and quintile ¹	Women ²			Men ²			Women and Men ²		
	Case (n=129)	Control (n=1,246)	OR (95% CI) ³	Case (n=234)	Control (n=2,265)	OR (95% CI) ⁴	Case (n=363)	Control (n=3,511)	OR (95% CI) ⁴
4	23	249	0.61(0.28,1.35)	39	453	0.38(0.19,0.77)	62	702	0.47(0.28,0.78)
5	22	249	0.43(0.17,1.07)	36	453	0.32(0.14,0.73)	58	702	0.38(0.21,0.69)
<i>P</i> _{trend}			0.05			0.003			<0.001
Copper									
1	26	250	1.00 (reference)	52	453	1.00 (reference)	78	703	1.00 (reference)
2	34	248	1.61(0.80,3.23)	45	453	0.80(0.43,1.49)	79	701	1.05(0.67,1.65)
3	21	250	1.01(0.44,2.34)	44	453	0.75(0.39,1.44)	65	703	0.78(0.47,1.29)
4	29	248	1.29(0.53,3.13)	46	453	0.65(0.31,1.35)	75	701	0.85(0.49,1.46)
5	19	250	0.83(0.25,2.69)	47	453	0.82(0.34,2.01)	66	703	0.79(0.40,1.57)
<i>P</i> _{trend}			0.66			0.63			0.44

¹Energy adjusted using the residual method. In analyses of data from women and men combined, dietary intake variables were categorized on the basis of sex-specific quintile distributions.

²Matched by gender, age at recruitment (± 2 years), date (± 30 days) of biospecimen collection, time (morning or afternoon) of blood sample collection, interval since last meal (less than 2h) and menopausal status (for women, yes or no).

³Results were adjusted for body mass index (BMI), total physical activity, total energy intake, total fruit intake, total red meat intake, total egg intake, total fish intake, total soy intake, vitamin E intake, income, education, family history of liver cancer (yes or no), history of viral hepatitis/chronic liver disease (yes or no), history of diabetes (yes or no), history of cholelithiasis (yes or no), ever had oral contraceptive (yes or no), HBsAg (positive and negative), and HBeAg (positive and negative).

⁴Results were adjusted for body mass index (BMI), total physical activity, total energy intake, total vegetable intake, total fruit intake, total red meat intake, total egg intake, total fish intake, total soy intake, vitamin E intake, income, education, smoking history (ever/never), alcohol consumption (ever/never), family history of liver cancer (yes or no), history of viral hepatitis/chronic liver disease (yes or no), history of diabetes (yes or no), history of cholelithiasis (yes or no), history of cholecystectomy (yes or no), HBsAg (positive and negative), and HBeAg (positive and negative).