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Cancer

Meat intake and risk of hepatocellular carcinoma in two large US prospective cohorts of women and men

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Abstract

Background: Epidemiological evidence on the associations between meat intake and risk of hepatocellular carcinoma (HCC) was limited and inconsistent.

Methods: We prospectively examined the association between consumption of meats and meat mutagens with HCC risk using data from the Nurses' Health Study and the Health Professionals Follow-up Study. Cox proportional-hazards regression models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) after adjusting for known liver-cancer risk factors.

Results: During up to 32 years of follow-up, we documented 163 incident HCC cases. The HRs of HCC for the highest vs the lowest tertile intake levels were 1.84 (95% CI: 1.16–2.92, $P_{trend} = 0.04$) for processed red meats and 0.61 (95% CI: 0.40–0.91, $P_{trend} = 0.02$) for total white meats. There was a null association between unprocessed red meats and HCC risk (HR = 1.06, 95% CI: 0.68–1.63, $P_{trend} = 0.85$). We found both poultry (HR = 0.60, 95% CI: 0.40–0.90, $P_{trend} = 0.01$) and fish (HR = 0.70, 95% CI: 0.47–1.05, $P_{trend} = 0.10$) were inversely associated with HCC risk. The HR for HCC risk was 0.79 (95% CI: 0.61–1.02) when

1 standard deviation of processed red meats was substituted with an equivalent amount of poultry or fish intake. We also found a suggestive positive association of intake of meat-derived mutagenicity or heterocyclic amines with risk of HCC.
Conclusions: Processed red meat intake might be associated with higher, whereas poultry or possibly fish intake might be associated with lower, risk of HCC. Replacing processed red meat with poultry or fish might be associated with reduced HCC risk.

Key words: Red meat, processed red meat, fish, poultry, hepatocellular carcinoma, cohort study

Key Messages

- Although meat intake has been suggested to play a role in human carcinogenesis, epidemiological studies evaluating the association between meat intake or meat mutagens and risk of hepatocellular carcinoma (HCC) are sparse.
- Our findings from two large US cohorts—the Nurses' Health Study and the Health Professionals Follow-up Study—showed that intake of processed red meats, possibly meat-derived mutagenicity or heterocyclic amines, but not unprocessed red meats, was associated with a higher risk of HCC, whereas intake of white meats, including both poultry and fish, was associated with a lower risk of HCC.
- Replacing processed red meat with poultry or fish was associated with lower HCC risk. There was a suggestive positive association between meat mutagens and HCC risk. We did not find any significant association between heme iron and nitrate intake and HCC risk.

Introduction

Primary liver cancer is the second leading cause of cancer-related death worldwide and the seventh leading cause in the USA.[1–3] The incidence rate of hepatocellular carcinoma (HCC), the most common (>80%) histological type of liver cancer, has tripled since the 1980s in the USA.[1,2] A fair amount of HCC (>35%) cannot be explained by currently known risk factors, including hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, excessive alcohol consumption and metabolic disorders,[4] indicating an important role of other risk factors, including diet.[5] However, epidemiological studies evaluating the association between diet and HCC are sparse.[6]

Recently, meat intake has been suggested to play a role in human carcinogenesis. In 2015, the International Agency for Research on Cancer classified consumption of red meats as 'probably carcinogenic to humans' (Group 2A), whereas consumption of processed red meats was classified as 'carcinogenic to humans' (Group 1).[7] In addition to high levels of saturated fat and heme iron found in meats, possible underlying mechanisms include the potentially carcinogenic chemicals N-nitroso compounds (NOCs) formed endogenously from nitrate or nitrite during meat processing or preservation and heterocyclic amines (HCAs) during meat cooking. By contrast, white meats (i.e. poultry and fish), particularly fish intake, has been shown to decrease cancer risk,[8–10] possibly due to long-chain omega-3 poly-unsaturated fatty acids (PUFAs) present in fish, particularly fatty fish. Despite these data, there has been a limited number of epidemiological studies on the association between meat intake or meat mutagens and risk of HCC.[9–12]

Therefore, we prospectively evaluated the associations between intakes of total meats, unprocessed red meats, processed red meats, as well as several major individual meat items (hot dogs, bacon, hamburger and others) and the risk of HCC. We also assessed the associations with heme iron, meat-derived mutagenicity (MDM) and HCAs including MeIQx (2-amino-3,8-dimethylimidazo [4,5,-f] quinoxaline), PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine) and DiMeIQx (2-amino-3,4,8-trimethylimidazo [4,5,-f]).

Methods

Study population

Participants in this study were based on two prospective US cohorts, including the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). The NHS was established in 1976, enrolling 121 700 US female registered nurses aged 30–55 years. The HPFS was established in 1986, enrolling 51 529 US male health

professionals (dentists, pharmacists, optometrists, osteopath physicians, podiatrists and veterinarians) aged 40–75 years. In each cohort, participants have returned questionnaires biennially with over 90% follow-up to provide information on demographics, lifestyle factors and medical history. In this analysis, we excluded individuals who had missing values in unprocessed red meats, processed red meats and white meats or had prior history of any cancer except for non-melanoma skin cancer at baseline. After these exclusions, a total of 92 389 women and 50 468 men were included in the final analysis. The Institutional Review Board at the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health approved this study.

Dietary assessments

A validated semi-quantitative food-frequency questionnaire (FFQ) was sent in 1980, 1984, 1986 and every 4 years thereafter in the NHS. Likewise, dietary information was collected in 1986 and every 4 years thereafter using similar FFQs in the HPFS. There were nine possible intake-frequency responses, ranging from 'never' to 'more than 6 times a day'. Consistently with previous studies from the same cohorts,[13–15] unprocessed red meat included hamburger, beef, pork or lamb as a sandwich or mixed dish; beef, pork or lamb as a main dish; and liver. Processed red meats included beef or pork hot dogs; bacon and salami, bologna or other processed red meat sandwiches; and other processed red meats such as sausage, kielbasa, etc. Total red meat consumption was derived by summing consumption of unprocessed and processed red meats. Total poultry consumption included chicken or turkey with or without skin, chicken or turkey hot dogs and chicken or turkey sandwiches. Total fish intake included dark meat fish, canned tuna fish, breaded fish cakes, pieces or fish sticks and other fish. Other dietary intake information for total energy, alcohol and coffee were also available. The reproducibility and validity of the FFQs used in these cohorts have been reported elsewhere.[16–19] Specifically, the correlation coefficients were mostly higher than 0.5 for individual red meat items after correction for attenuation due to random within-person variation in dietary records in NHS[17] and also higher than 0.5 for red and processed red meats in HPFS.[18,19]

In 1996, the cooking-method questionnaire was designed to estimate the intake of HCAs in the cohorts. The questions were based on results from a pilot study identifying specific questions related to cooking methods that would best predict HCA intake in the cohorts.[20] The doneness was generally categorized as lightly browned, medium browned, well browned and blackened/charred, depending on the type of meats.[21] Intakes of HCAs were calculated from the data provided on the 1996 cooking-method questionnaire and dietary data in 1994 using the CHARRED Database.[22,23] The mutagenic activity of meat samples was assessed by the Ames/Salmonella test.[24,25] Intakes of MDM and HCAs including MeIQx, PhIP and DiMeIQx were derived using a method described in detail elsewhere.[21]

Ascertainment of HCC

In each cohort, participants were asked for written permission to obtain their medical records and pathological reports if they reported liver cancer on biennial questionnaires. Considering potential unreported cancer deaths, we further searched state vital-statistics records, the National Death Index.[26] For all deaths attributable to liver cancer, we requested permission from next of kin to review medical and pathological records. All possible cancer cases were further confirmed by a study physician who was blinded to exposure data and extracted information from the medical or pathological reports regarding the histological subtypes of the cancer (e.g. HCC vs intrahepatic cholangiocarcinoma), the presence of underlying cirrhosis diagnosed by histopathology or by appropriate cross-sectional imaging and the presence of HBV or HCV infections. Additional data on HBV/HCV infection status were also available from a nested case–control study of HCC in the NHS/HPFS, which were derived from laboratory blood tests.[27]

Statistical analyses

Study participants contributed person-time beginning from the return of the first FFQs (1980 for the NHS and 1986 for the HPFS) to the date of diagnosis of HCC, date of death, loss to follow-up or the end of the follow-up (June 2012 for NHS or January 2012 for HPFS), whichever came first. We calculated the cumulative average meat intake by averaging the meat intake over time from 1980 in the NHS and 1986 in the HPFS to the current questionnaire cycle and updating data when information on meat intake was updated. Similarly, the cumulative average intake of other dietary variables and other covariates, when appropriate, was created to best reflect long-term food intake and lifestyle, and to minimize within-person variation.[28]

A time-varying Cox proportional-hazards regression model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between red, processed and white meats and risk of HCC. This Cox model was stratified simultaneously by age and year of questionnaire return, allowing the finest possible control

of confounding for age and secular trends.[29] To maximize the statistical power, we combined results from the two cohorts because we did not detect any significant heterogeneity between the two cohorts (all $P > 0.05$ for heterogeneity tests of meat intake by sex). We have adjusted for cohort (gender), age, race, physical-activity level, body mass index (BMI), smoking, type 2 diabetes, regular aspirin use, alcohol intake and total calorie intake (see Table 1 and Table 2 footnote). We found no violation of proportional-hazards assumption after testing an interaction term between meat intake and follow-up time (all $P > 0.05$ for all tests). Meat intake was divided into tertiles, with the lowest tertile as the reference. Poultry and fish were combined as white meats and the association of total white meats with HCC risk was then analysed. Consistently with a previous study from the same cohorts,[30] the trend tests were conducted using the median of each category as a continuous variable. To facilitate the translation to dietary recommendations regarding meat intake, we estimated the associations of substituting 1 standard deviation (SD) of poultry or fish for 1 SD of red meat with HCC risk by including both as continuous variables in the same multivariate model. The difference in their beta coefficients, as well as their variances and covariance, were used to estimate the relative risk and 95% CI for the substitution associations.[31]

We also conducted several secondary analyses (see the 'Methods' section). All statistical tests were two-sided and performed using SAS version 9.4 (SAS Institute Inc, Cary, NC).

Results

Participants with higher unprocessed red meat and processed red meat intake were more likely to have a lower physical-activity level, smoke cigarettes, have a lower intake of multivitamin, folate, vitamin D and fibre, and have a higher total fat intake (Table 1). Conversely, these trends were reversed among participants with high total white meat intake. Participants with higher meat intake, regardless of meat types, seemed to have higher prevalence of type 2 diabetes, were more likely to use aspirin and consumed more total calories. Similar patterns were observed in both women (Supplementary Table 1, available as Supplementary data at *IJE* online) and men (Supplementary Table 2, available as Supplementary data at *IJE* online).

During up to 32 years of follow-up, 163 incident HCC cases were documented (87 women and 76 men). We found that the higher intake of processed red meats was significantly associated with an 84% increased risk of HCC (comparing the highest to lowest tertile intake, HR = 1.84, 95% CI: 1.16–2.92, $P_{trend} = 0.04$, Table 2), whereas a higher intake of total white meats was associated with a 39% lower risk of HCC (for the same comparison, HR = 0.61, 95% CI: 0.40–0.91, $P_{trend} = 0.02$). When examining white meat intake by type with HCC risk, we observed an inverse association of poultry (HR = 0.60, 95% CI: 0.40–0.90, $P_{trend} = 0.01$) and a suggestive inverse association of fish (HR = 0.70, 95% CI: 0.47–1.05, $P_{trend} = 0.10$). Non-significant positive association was observed for unprocessed red meats and HCC risk (HR = 1.06, 95% CI: 0.68–1.63, $P_{trend} = 0.85$). The substitution of poultry or fish for 1 SD of processed red meat intake was associated with a decrease in risk of HCC (HR = 0.79, 95% CI: 0.61–1.02). Risk estimates in Table 2 did not substantially vary when dietary intakes of unprocessed red meats, processed red meats and white meats were mutually adjusted in multivariable models (data not shown). These results did not materially change after excluding HCC cases ($n = 26$) with HBV or HCV infection (data not shown). After separate examination of the associations between meats and HCC in each cohort, the results were consistent with the pooled analysis (Supplementary Table 2, available as Supplementary data at *IJE* online). After further adjustment for Western dietary pattern, the results were very similar to those shown in the primary analysis.

Total meat-derived mutagens (HR = 1.49, 95% CI: 0.89–2.50) as well as individual HCAs including MeIQx (HR = 1.30, 95% CI: 0.78–2.17), PhIP (HR = 1.24, 95% CI: 0.73–2.08) and DiMeIQx (HR = 1.05, 95% CI: 0.62–1.75) were suggestively positively associated with risk of HCC among US adults (Table 3). Intake of nitrate, total iron or heme iron seemed not to be associated with HCC risk (Supplementary Table 3, available as Supplementary data at *IJE* online).

In exploratory subgroup analyses, there appeared to be no interactions with age, BMI, physical activity, smoking, alcohol drinking, type 2 diabetes and aspirin use either for unprocessed red and processed red meat (Supplementary Table 4, available as Supplementary data at *IJE* online) or for white meat intake (Supplementary Table 5, available as Supplementary data at *IJE* online). We did not detect any differential associations (all $P_{herterogenity\ by\ cirrhosis\ status} > 0.07$) of processed red meats or poultry intake with risk of HCC subtypes by history of cirrhosis (i.e. cirrhotic vs non-cirrhotic HCC). There were no correlations between each meat intake and HBV/HCV infection status among participants (105 HCC cases and 78 non-cases) with available data on HBV/HCV in our cohorts.

Discussion

In this large prospective cohort study, we found that intake of processed red meats, but not unprocessed red meats,

Table 1. Age-standardized characteristics of participants according to tertiles of red and white meat intake in the Nurses' Health Study and Health Professionals Follow-up Study[a]

	Processed red meat			Unprocessed red meat			White meat		
	Tertile 1	Tertile 2	Tertile 3	Tertile 1	Tertile 2	Tertile 3	Tertile 1	Tertile 2	Tertile 3
Age (year)[b]	62.9 (11.3)	61.7 (11.4)	61.2 (11.4)	62.9 (11.6)	62.0 (11.4)	61.0 (11.2)	62.0 (11.7)	61.8 (11.4)	62.0 (11.1)
Whites, %	96.5	97.2	97.0	95.9	97.3	97.4	97.6	97.2	95.9
Body mass index, kg/m^2	24.6 (3.8)	25.3 (4.0)	25.9 (4.6)	24.8 (3.9)	25.3 (4.1)	25.7 (4.5)	24.9 (4.1)	25.2 (4.1)	25.7 (4.3)
Physical activity, METS-hours/week	23.6 (24.8)	20.7 (22.3)	19.5 (22.0)	23.4 (25.2)	20.6 (21.9)	19.8 (22.1)	18.5 (21.8)	20.8 (22.1)	24.1 (24.8)
Type 2 diabetes, %	3.6	5.0	6.9	3.9	5.1	6.4	4.5	5.1	5.8
Regular aspirin use,[c] %	38.1	40.0	41.0	38.2	40.4	40.4	37.8	40.1	41.2
Past smoking, %	39.9	40.2	38.4	40.9	40.2	37.3	35.6	40.0	42.5
Current smoking, %	9.6	11.7	14.8	11.0	12.3	13.1	14.9	12.0	9.6
Multivitamin use, %	45.8	44.3	41.1	45.0	45.3	40.8	38.5	45.2	47.2
Post-menopausal status, %	82.4	82.5	81.8	82.1	82.4	82.4	81.9	82.4	82.6
Post-menopausal hormone use, %	48.3	49.1	45.7	46.7	48.9	47.5	44.6	48.9	49.5
Poultry, servings/week	2.8 (2.1)	2.1 (1.4)	2.1 (2.1)	2.1 (2.1)	2.1 (1.4)	2.8 (2.1)	1.4 (0.7)	2.1 (0.7)	3.5 (2.1)
Fish, servings/week	0.3 (0.3)	0.3 (0.2)	0.2 (0.2)	0.3 (0.3)	0.3 (0.2)	0.2 (0.2)	0.1 (0.1)	0.2 (0.1)	0.5 (0.3)
Fruits, servings/day	2.5 (1.5)	2.2 (1.3)	2.2 (1.4)	2.4 (1.5)	2.3 (1.3)	2.3 (1.4)	1.9 (1.3)	2.3 (1.3)	2.8 (1.6)
Vegetables, servings/day	3.0 (1.7)	2.8 (1.4)	2.9 (1.6)	2.8 (1.7)	2.8 (1.4)	3.1 (1.7)	2.2 (1.2)	2.8 (1.3)	3.6 (1.9)
Alcohol, g/day	6.6 (9.9)	7.6 (11.0)	8.2 (12.2)	6.7 (10.0)	7.7 (11.1)	7.9 (12.0)	7.2 (11.7)	7.7 (11.1)	7.5 (10.5)
Total folate intake, µg/day	508 (262)	457 (220)	420 (205)	512 (267)	457 (219)	416 (198)	424 (233)	459 (223)	499 (237)
Total vitamin D intake (IU/day)	428 (276)	375 (226)	340 (207)	436 (281)	375 (224)	332 (198)	342 (236)	371 (224)	427 (251)
Total fat, g/day	59.4 (12.2)	64.3 (11.0)	68.3 (11.2)	58.4 (11.5)	64.3 (10.8)	69.1 (11.3)	67.1 (12.7)	64.2 (11.4)	61.0 (11.3)
Total fibre, g/day	21.0 (6.8)	18.5 (5.2)	17.1 (4.7)	20.9 (6.9)	18.5 (5.2)	17.3 (4.7)	17.4 (5.8)	18.7 (5.4)	20.5 (5.9)
Total calorie intake, kcal/day	1580 (451)	1728 (461)	1980 (517)	1501 (432)	1726 (423)	2054 (496)	1628 (480)	1763 (478)	1900 (518)

Values were means (SD) or percentages and were standardized to the age distribution of the study population. Values of polytomous variables may not sum to 100% due to rounding.

[a]Weighted by person-years.

[b]Value was not age-adjusted.

[c]Regular aspirin use was defined as the consumption of aspirin at least two times per week. Non-regular use was defined otherwise.

Table 2. Meat intake and risk of hepatocellular carcinoma in the Nurses' Health Study and Health Professionals Follow-up Study

	Tertiles, HR (95% CI)			P_{trend}
	Tertile 1	Tertile 2	Tertile 3	
Processed red meat				
Number of cases	29	65	69	
Age-adjusted model	1 (Reference)	2.29 (1.47–3.55)	2.50 (1.61–3.86)	0.0002
Multivariable-adjusted model[a]	1 (Reference)	1.97 (1.26–3.08)	1.84 (1.16–2.92)	0.04
Unprocessed red meat				
Number of cases	45	58	60	
Age-adjusted model	1 (Reference)	1.31 (0.89–1.94)	1.49 (1.01–2.20)	0.05
Multivariable-adjusted model[a]	1 (Reference)	1.12 (0.75–1.68)	1.06 (0.68–1.63)	0.85
White meat				
Number of cases	63	56	44	
Age-adjusted model	1 (Reference)	0.86 (0.60–1.24)	0.71 (0.48–1.05)	0.08
Multivariable-adjusted model[a]	1 (Reference)	0.81 (0.56–1.18)	0.61 (0.40–0.91)	0.02
Poultry				
Number of cases	66	55	42	
Age-adjusted model	1 (Reference)	0.77 (0.54–1.11)	0.70 (0.47–1.03)	0.06
Multivariable-adjusted model[a]	1 (Reference)	0.73 (0.50–1.05)	0.60 (0.40–0.90)	0.01
Fish				
Number of cases	56	61	46	
Age-adjusted model	1 (Reference)	1.02 (0.71–1.47)	0.79 (0.53–1.17)	0.24
Multivariable-adjusted model[a]	1 (Reference)	0.94 (0.65–1.36)	0.70 (0.47–1.05)	0.10

HR, hazard ratio; CI, confidence interval.

[a]Adjusted for age (in months), sex (women vs men), race (White vs non-White), physical-activity level (<3, 3 to <27, ≥27 METS-hours/week), body mass index (BMI, <25, 25 to <27.5, 27.5 to <30, ≥30 kg/m^2), smoking (0, 0 to <10, ≥10 pack-years), type 2 diabetes (yes vs no), regular aspirin use (yes vs no), alcohol intake (<5, 5 to <15, ≥15 g/day) and total calorie intake (tertiles).

Table 3. Meat mutagens and risk of hepatocellular carcinoma in the Nurses' Health Study and Health Professionals Follow-up Study

	Tertiles, HR (95% CI)			P_{trend}
	Tertile 1	Tertile 2	Tertile 3	
MDM*				
Number of cases	24	30	45	
Age-adjusted model	1 (Reference)	1.37 (0.80–2.35)	2.12 (1.29–3.50)	0.02
Multivariable-adjusted model[a]	1 (Reference)	1.10 (0.64–1.91)	1.49 (0.89–2.50)	0.20
MeIQx				
Number of cases	25	29	45	
Age-adjusted model	1 (Reference)	1.22 (0.71–2.10)	1.89 (1.15–3.08)	0.15
Multivariable-adjusted model[a]	1 (Reference)	0.99 (0.57–1.70)	1.30 (0.78–2.17)	0.58
PhIP				
Number of cases	27	35	37	
Age-adjusted model	1 (Reference)	1.42 (0.86–2.36)	1.66 (1.01–2.75)	0.19
Multivariable-adjusted model[a]	1 (Reference)	1.19 (0.71–1.99)	1.24 (0.73–2.08)	0.70
DiMeIQx				
Number of cases	28	37	34	
Age-adjusted model	1 (Reference)	1.41 (0.86–2.31)	1.38 (0.84–2.29)	0.82
Multivariable-adjusted model[a]	1 (Reference)	1.21 (0.73–1.99)	1.05 (0.62–1.75)	0.47

HR, hazard ratio; CI, confidence interval; MDM, meat-derived mutagenicity; MeIQx, 2-amino-3,8-dimethylimidazo [4,5,-f] quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine; DiMeIQx, 2-amino-3,4,8-trimethylimidazo [4,5,-f].

*MDM and heterocyclic amine intakes were calculated from the data provided on the 1996 cooking-method questionnaire and dietary data in 1994 using the CHARRED Database.

[a]Adjusted for age (in months), gender (women vs men), race (White vs non-White), physical-activity level (<3, 3 to <27, ≥27 METS-hours/week), body mass index (BMI, <25, 25 to <27.5, 27.5 to <30, ≥30 kg/m^2), smoking (0, 0 to <10, ≥10 pack-years), type 2 diabetes (yes vs no), regular aspirin use (yes vs no), alcohol intake (<5, 5 to <15, ≥15 g/day) and total calorie intake (tertiles).

was associated with a higher risk of HCC, whereas intake of white meats, including both poultry and fish, was associated with a lower risk of HCC. Replacing processed red meat with poultry or fish was associated with lower HCC risk. There was a suggestive positive association between meat mutagens and HCC risk. We did not find any significant association between heme iron and nitrate and HCC risk.

There have been few cohort studies of the association between red meat intake and HCC risk.[10,11,32] Two other cohort studies have adjusted for age and other lifestyle and dietary factors.[10,11] One from the National Institutes of Health (NIH)-AARP (formerly known as the American Association of Retired Persons) showed a suggestive positive association between unprocessed red meat intake and HCC risk.[11] Another study—the European Prospective Investigation into Cancer and Nutrition (EPIC) study—did not observe an association between unprocessed red meat intake and HCC. Consistently with the EPIC study[10] and a recent meta-analysis,[9] the current study showed no association between unprocessed red meat intake and HCC risk and a positive association with processed red meat. However, a study from the Japanese Ministry of Education (JACC) cohort showed no significant association between beef or pork intake and HCC mortality without adjustment for any risk factors.[32] The lack of adjustments for HCC risk factors and different study populations (Japanese vs Americans or Europeans) may partly explain the inconsistent findings between the JACC and other cohort studies. Several case–control studies[12,33–37] have investigated the intake of unprocessed red meats in relation to HCC risk, with mixed results. The retrospective design, however, may hamper their conclusions,[12,33–37] because the meat intake as well as other dietary data were collected after cancer diagnosis and patients may have changed their dietary habits due to disease.

Experimental studies showed that dietary heme iron overload may lead to hepatocyte injury and death.[38] Heme iron may also act as a pro-oxidant and catalyse lipid peroxidation causing DNA damage in tissues.[39] In addition, heme iron has been shown to induce endogenous formation of NOCs.[40] Meats, especially red meats, are the main source of heme iron and may thereby influence HCC risk via the possible effect of iron. However, we did not observe a significant association with HCC either for heme iron or for total dietary iron intake. Similarly, results from the NIH-AARP cohort[11]—the only study investigating the association of heme iron with HCC risk—also showed a null association.

HCAs are produced when meats react with amino acids, sugars and creatine at high temperatures during cooking and concentrations could increase with higher temperature and longer cooking time.[20] PhIP is the most abundant HCA in the human diet, followed by MeIQx and DiMeIQx,[21] and DiMeIQx is the most mutagenic HCA of the three examined HCAs.[41] Previous studies have reported that MDMs and HCAs are potential carcinogens for multiple organs, but its effects on hepatocytes is still unclear. Some animal studies showed meat mutagens including HCAs may cause liver tumours in mice.[42,43] In line with animal experiments, we found a suggestive positive association between intakes of total meat-derived mutagens and individual HCAs including DiMeIQx, MeIQx and PhIP and HCC risk. However, the only previous cohort study examining HCA intake in relation to HCC risk generally found a null association.[11] Pooled analyses across cohorts would be useful to further evaluate these associations given the rare nature of HCC outcome in the USA.

We observed a significant positive association between processed red meat intake and HCC risk, although the current existing three prospective studies[10,11,32] and a meta-analysis[9] suggested no statistically significant association. The possible reason for the inconsistent results is that, unlike other studies, our study used time-varying cumulative averaged dietary data, which consider changes in diet and risk factors during follow-up, whereas previous cohort studies only investigated meat intake at baseline in relation to HCC risk. Besides nitrate, nitrite and other added preservatives, processed red meats are an important source of exogenously derived NOCs, which may have carcinogenic potential.[44] However, there is a null association between dietary nitrate and HCC risk in our study, which was consistent with the results from the NIH-AARP cohort.[11]

We showed an inverse association between white meat (both fish and poultry) intake and risk of developing HCC. Compared with red meats, white meats have less saturated fat and heme iron, and are rich in PUFA. A large number of laboratory studies indicated that n-3 PUFA possesses anti-inflammatory activity by inhibiting interleukin-1 and tumour necrosis factor synthesis,[45,46] which can contribute to HCC prevention, given that chronic inflammation plays a central role in HCC development. N-3 PUFA might exert anticancer effects also through their ability to induce apoptosis and modulate cell cycle and eicosanoid production.[47] In particular, n-3 PUFAs have been shown to inhibit HCC growth *in vitro* through the blockage of β-catenin and cyclooxygenase-2.[48] Furthermore, n-3 PUFA supplementation can improve hepatic steatosis and insulin sensitivity, and reduce inflammation among patients with NAFLD.[49–51] In line with this evidence, our study and other observational studies[10,11] and the meta-analyses[8,9] suggested a protective effect of white meats or fish intake against HCC development.

Strengths of the current study include the prospective design, repeated measurements of meat intake, high follow-up rates and validated HCC outcome. In addition, accounting for the repeated dietary assessments and other covariates during the follow-up periods may strengthen the association.

Our study has several limitations. First, there could be a misclassification in dietary data, as in any observational study, although FFQs used in the cohorts have shown good reliability and validity for measuring meat intake as well as other dietary factors.[16–19] Second, we did not have data on chronic HBV/HCV infections in the entire cohorts. However, among a subset of participants for whom we have such data, HBV/HCV infection status showed no correlations with unprocessed red meats, processed red meats and white meats including poultry and fish. Moreover, results were very similar when we excluded the HCC cases with known chronic HBV/HCV infections. Taken together, our results were less likely to be substantially confounded by HBV/HCV infections. Last, all participants in our cohorts are health professionals, and most participants in our cohorts are Caucasians of European origin, which may limit the generalizability of our results to other racial/ethnical populations and require further investigation.

In summary, although chance findings cannot be totally ruled out due to the relatively limited number of HCC cases in these cohorts, we found that processed red meat intake might be associated with higher risk of HCC, whereas white meats, including poultry, intake might be associated with a lower risk of HCC. Substitution of poultry or fish for processed red meat intake may be associated with reduced risk of HCC among US adults. Additional prospective studies that carefully consider HBV/HCV infection are needed to replicate our findings in other racial/ethnical populations, ideally with pooling analyses across cohorts, given the rare nature of the disease.

Supplementary Data

Supplementary data are available at *IJE* online.

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