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ARTICLE

Association of Meat and Fat Intake With Liver Disease and Hepatocellular Carcinoma in the NIH-AARP Cohort

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Background Several plausible mechanisms, including fat, iron, heterocyclic amines, and *N*-nitroso compounds, link meat intake with chronic liver disease (CLD) and hepatocellular carcinoma (HCC). Few studies have investigated these associations.

Methods We prospectively examined the relationship between meat and associated exposures with CLD mortality (n = 551; not including HCC) and HCC incidence (n = 338) in 495 006 men and women of the National Institutes of Health–AARP Diet and Health Study. Hazard ratios (HRs) and 95% confidence intervals (CIs) for the fifth (Q5) vs the first (Q1) quintile were estimated from multivariable adjusted Cox proportional hazards regression models. All tests of statistical significance were two-sided.

Results We found inverse associations between white meat and risk of CLD (HR = 0.52, 95% CI = 0.39 to 0.70, 7.5 vs 18.2 cases per 100 000 person-years) and HCC (HR = 0.52, 95% CI = 0.36 to 0.77, 5.8 vs 14.3 cases per 100 000 person-years). Red meat was associated with higher risk of CLD (HR = 2.59, 95% CI = 1.86 to 3.61, 22.3 vs 6.2 cases per 100 000 person-years) and HCC (HR = 1.74, 95% CI = 1.16 to 2.61, 14.9 vs 5.7 cases per 100 000 person-years). Among fat types, results were strongest for saturated fat (for CLD, HR = 3.50, 95% CI = 2.48 to 4.96, 23.0 vs 6.5 cases per 100 000 person-years; for HCC, HR = 1.87, 95% CI = 1.23 to 2.85, 14.5 vs 6.3 cases per 100 000 person-years). After mutual adjustment, risk estimates persisted for saturated fat, red meat, and white meat. Heme iron, processed meat, nitrate, and nitrite were positively associated with CLD but not with HCC. Individual heterocyclic amines, 2-amino-3,4,8-trimethylimidazo[4,5,-*f*]quinoxaline (DiMeIQx), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP), were not associated with either outcome.

Conclusion Our results suggest that red meat and saturated fat may be associated with increased CLD and HCC risk, whereas white meat may be associated with reduced risk.

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Hepatocellular carcinoma (HCC) is the sixth most common incident cancer and third most common cause of cancer-related mortality worldwide (1). Strong risk factors for HCC have been identified, including exposure to aflatoxins, excess alcohol consumption, and chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) (2). Exposure to these risk factors can injure the liver and lead to chronic liver disease (CLD). Individuals with CLD are at substantial risk of dying from its complications and also are at high risk of developing HCC (3). A substantial proportion of HCC occurs in patients without exposure to aflatoxins, alcohol, HBV, or HCV, particularly in the United States and other Western countries (4,5), suggesting the importance of additional risk factors.

In contrast with many other cancers, relatively few studies have investigated the association of diet and HCC risk (6–13). Most existing studies have had a case–control design that assessed diet after HCC diagnosis, at which stage the health of individuals can be compromised, perhaps affecting the accuracy of dietary recall. Few studies have investigated the association of white meat intake with HCC risk and little or no information is available regarding the role of either red or white meat in CLD.

Recently, a positive association between red meat intake and liver cancer was found in the prospective National Institutes of Health (NIH)–AARP (formerly known as the American Association of Retired Persons) Diet and Health Study, as part of an analysis of all cancers (14). In this report, we investigate several mechanisms that may underlie this association, along with the possible role of white meat. Red meat is an important dietary source of saturated and monounsaturated fatty acids. Fatty acid deposition in the liver can lead to nonalcoholic fatty liver disease that may increase the risk of CLD and HCC (15). Alternatively, red meat

contains high amounts of bioavailable heme iron (16). Individuals with hemochromatosis, an iron overload disease, have substantially increased liver cancer risk (17), excess dietary iron contributes to risk of CLD and HCC in several parts of Africa (18–20), and phlebotomy and low iron diets are a treatment for chronic HCV (21) and thus might lower liver cancer risk. Whether moderate dietary iron intake plays a role in the development of CLD and HCC is unclear. Heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons, carcinogens produced during high-temperature cooking, and *N*-nitroso compounds formed from nitrate and nitrate in processed meats could also play a role. In primates and in other animals, high doses of HCAs or *N*-nitroso compounds cause liver tumors (22,23). We examined associations of meat and meat-related exposures with both CLD mortality and HCC incidence because consistent associations for both endpoints would suggest that these exposures affect liver disease progression.

Subjects and Methods

Study Population

The NIH-AARP Diet and Health Study has been described previously (24). In 1995–1996, a questionnaire was mailed to 3.5 million members of AARP, aged 50–71 years, who resided in eight US states (California, Florida, Georgia, Louisiana, Michigan, New Jersey, North Carolina, and Pennsylvania). Of those who returned the baseline questionnaire, 566 402 completed the survey satisfactorily and consented to be part of the study. Proxy respondents (15 760) and those who developed cancer (51 205) or died before their questionnaires were scanned (12) were excluded, as were respondents with total energy intake more than twice the interquartile range of log-transformed energy intake (4419). The resulting cohort included 495 006 participants: 295 332 men and 199 674 women. Information on meat cooking methods and doneness levels were available for 176 845 men and 126 327 women who satisfactorily completed an additional risk factor questionnaire in 1996–1997. The conduct of the NIH-AARP Diet and Health Study was reviewed and approved by the Special Studies Institutional Review Board of the US National Cancer Institute and all participants gave informed consent by virtue of completing and returning the questionnaire.

Cohort Follow-up

Addresses for members of the NIH-AARP cohort were updated annually by matching the cohort database to that of the National Change of Address database maintained by the US Postal Service, specific change of address requests from participants, updated addresses returned during yearly mailings, and the Maximum Change of Address database (Anchor Computer). We ascertained vital status, but not cause of death, by periodic linkage of the cohort to the Social Security Administration Death Master File, cancer registry linkage, questionnaire responses, and responses to other mailings.

Identification of Deaths Due To CLD

Cause of death was provided by linking cohort participants to the National Death Index Plus maintained by the National Center for Health Statistics. Underlying cause of death codes from death certificates were provided as *International Classification of Diseases* (*ICD*)*-9* and *ICD-10* codes. We used the same definition of CLD as the National Center for Health Statistics and thus classified participants who were given specific underlying cause of death codes for CLD, liver fibrosis and cirrhosis, alcoholic liver disease, and chronic hepatitis (*ICD-9*: 571.0, 571.2–571.6, 571.8, and 571.9; *ICD-10*: K70, K73, and K74) (25). Classification of CLD in the National Death Index was recently validated against the electronic medical records from members of the Kaiser Permanente Medical Care Program of Northern California and was found to have high specificity (89%) (26).

Identification of Incident Primary Hepatocellular Carcinomas

Because death certificates can erroneously miscode cancer metastatic to the liver as a primary liver tumor, we elected not to analyze liver cancer mortality. Instead, we analyzed incident HCC as determined by state cancer registries. The NIH-AARP cohort membership was linked to incident cancer information from the cancer registries of the eight baseline states together with Arizona, Nevada, and Texas. We estimated the sensitivity of this approach to be 90% and the specificity to be nearly 100% (27).

CONTEXT AND CAVEATS

Prior knowledge

Few studies have examined whether meat and fat intake are associated with chronic liver disease (CLD) and/or hepatocellular carcinoma.

Study design

Information concerning diet, CLD, and hepatocellular cancer incidence was collected for 495 006 men and women, aged 50–71 years, from the National Institutes of Health–AARP Diet and Health Study. All participants completed food-frequency questionnaires in 1995–1996, and some completed an additional food cooking questionnaire in 1996–1997. Incidence of hepatocellular carcinoma was collected from state cancer registries until the end of 2003, and incidence of CLD was determined from National Death Index records through the end of 2005. Participants were divided into quintiles on the basis of meat, fat, or other nutrient intakes, and hazard ratios for risk of CLD and hepatocellular carcinoma were estimated using Cox proportional hazards models.

Contribution

Red meat and saturated fat intakes were associated with increased risk of CLD and hepatocellular carcinoma, whereas white meat intake was associated with less than average risks of both diseases. Meat processing and its heme iron, nitrate, and nitrite contents were associated with CLD but not with cancer.

Implication

Intake of red meat and saturated fats may increase hepatocellular cancer risk.

Limitations

The results are based on self-reported food intakes. They were stratified by alcohol intake and other possible confounders; however, data concerning hepatitis virus infections were unavailable.

From the Editors

HCC topography and morphology were determined using *International Classification of Diseases for Oncology* (*ICD-O*) version 3 (28). We restricted our definition of primary HCC (C22.0) to those with morphology codes of 8000, 8010, 8140, 8170, 8171, 8175, and 8190. As a sensitivity analysis, we further restricted classification of participants as having HCC to those who had specific morphology of HCC (8170–8175), but the results did not change (data not shown). Ten participants who were identified both as having a primary incident HCC by the state cancer registries and as dying from CLD by the National Death Index were classified in our study as having had incident HCC but not CLD.

Exposure Assessment

In the baseline questionnaire, participants were asked about demographics, health conditions, alcohol intake, tobacco smoking, and physical activity; it also included a food-frequency questionnaire (FFQ) of 124 items. For the FFQ, participants reported their typical frequency of intake in the last year using 10 categories ranging from never to two or more times per day for solid foods and also recorded three categories of typical portion size. The food items, portion sizes, and nutrient database (29) used data from the U.S. Department of Agriculture's 1994–1996 Continuing Survey of Food Intake by Individuals (30). The nutrient and food database used a recipe file to disaggregate food mixtures into their component ingredients and assign them to the appropriate food group. Meat variables included meat from individual items, such as hamburgers, and complex food mixtures including chili, lasagna, pizza, and stew. Red meat included beef and pork; white meat included chicken, turkey, and fish. Processed meat included bacon, cold cuts, ham, hot dogs, luncheon meats, and sausage from both red and white meat sources. Total fat, types of fat, dietary iron intake, and fruit and vegetable intake were also calculated from the baseline FFQ.

The FFQ was calibrated against two nonconsecutive 24-hour dietary recalls for a subset of 1953 participants (24,31). The energy-adjusted Pearson correlation coefficients for total fat, monounsaturated fat, polyunsaturated fat, saturated fat, iron, and red meat in this subset of the cohort were 0.72, 0.71, 0.53, 0.76, 0.59, and 0.62 for men and 0.62, 0.62, 0.56, 0.69, 0.56, and 0.70 for women, respectively.

The risk factor questionnaire included a meat cooking module that ascertained the participant's usual cooking method (pan-fried; grilled or barbecued; oven-broiled; and others such as sauteed, baked, or microwaved) and internal and external appearance that were categorized into three doneness levels (rare-medium, well done, and other) (32). The meat cooking module was previously calibrated in a US population (33). From participant responses, we used the CHARRED database to estimate daily intake of the HCA, 2-amino-3,4,8-trimethylimidazo[4,5,-*f*]quinoxaline (DiMeIQx), 2-amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP) (32).

Heme iron was also calculated from the risk factor questionnaire, using a database of measured heme values from meat samples cooked by different methods and doneness levels (unpublished data). Nitrate and nitrite were generated from the risk factor questionnaire using a separate database with measured values from 10 types of processed meat, constituting 90% of processed meat types consumed in the United States (unpublished data).

Statistical Analysis

All tests were two-sided and *P* values less than .05 were considered to denote statistical significance. For HCC, follow-up time extended from the date the questionnaire was returned to the time of diagnosis of HCC or end of follow-up (December 31, 2003), date of death, or the date moved out of registry ascertainment area, whichever came first. For CLD, follow-up time was from the date the questionnaire was returned until the date of death or end of follow-up (December 31, 2005), whichever came first. Longer follow-up was available for CLD than for HCC because of a more recent linkage to the death records. Absolute risks were standardized within 5-year age bands to the age distribution of the entire NIH-AARP cohort (34). Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using Cox proportional hazards regression (35), with person-years as the underlying time metric in SAS version 9.1. We tested the proportional hazards assumption by modeling interaction terms of time and continuous red meat, white meat, total fat, saturated fat, monounsaturated fat, and polyunsaturated fat intake and found no statistically significant deviations. Excluding the first 2, 3, 4, or 5 years of follow-up did not affect risk estimates; nor did excluding those with self-reported poor health at baseline (59 982) (data not shown).

We used the nutrient density method (36) of energy adjustment for the meat, fruit, vegetable, grain, and heme iron variables, including total energy from nonalcohol sources in the model and present results per 1000 kcal. Because excess alcohol intake is a known risk factor for HCC and CLD, we included alcohol intake as a separate covariate. We also used the residual method (36) for energy adjustment of red and white meat, and results were similar. Total iron intake included both dietary and supplemental sources; dietary iron intake was residually adjusted (36) on total energy from nonalcohol sources and added to iron from supplements.

Risk estimates for each type of meat were adjusted for the intake of other types of meat such that the meat variables in each model added up to total meat intake. For example, red meat was adjusted for white meat, and processed meat was adjusted for nonprocessed meat. We also examined models in which a particular meat type, such as red meat, was adjusted for total meat intake. In this model, risk estimates for red meat reflect both increasing consumption of the meat type and decreasing consumption of other meat types. Finally, we created a red and white meat joint-effects variable. For this variable, we created tertiles of red and white meat. From these tertiles, we created a new nine category variable containing the intersection of these two sets of tertiles. This new variable allowed us to compare the risk of participants in the highest tertile of red meat who were also in the lowest tertile of white meat with those participants in the lowest tertile of red meat who were also in the highest tertile of white meat consumption.

Risk estimates for fat intake are presented as the percentage of nonalcohol energy (a density model) (36) and include total energy from nonalcohol sources and alcohol intake in the model. As a sensitivity analysis, we also modeled fat intake using the residual method (36), whereby we modeled the residual regression of log-transformed fat intake on log-transformed nonalcohol energy and included nonalcohol energy and alcohol intake in the model. Results for both the density and the residual methods were equivalent. Risk estimates for sources of fat are adjusted for other

sources, that is, saturated fat from red meat was adjusted for saturated fat from nonred meat sources.

Intake quintiles were created based on the distribution of intake in the cohort. We tested for a linear trend across increasing categories using values for the median intake of each quintile. This trend variable was included in the Cox models as a continuous variable, and *P* values were obtained from the Wald test (37). For continuous estimates, intakes were scaled as follows: meat variables per 10 g, dietary iron per 10 mg, heme iron per 100 μg, MeIQx per 5 ng, DiMeIQx per 0.5 ng, PhIP per 25 ng, nitrate per 0.1 mg, and nitrite per 0.1 mg. Fat variables were per 5% of nonalcohol energy from that fat. The Spearman correlation coefficients between saturated fat and monounsaturated fat, polyunsaturated fat were 0.8 and 0.4, respectively; between monounsaturated fat and polyunsaturated fat were 0.7; between red meat and saturated fat, monounsaturated fat, polyunsaturated fat were 0.5, 0.6, and 0.2, respectively; between white meat and saturated fat, monounsaturated fat, polyunsaturated fat were −0.1, −0.05, and 0.1, respectively.

All models were additionally adjusted for age (continuous) and categorical variables of sex; alcohol intake (none, >0 to 0.5, >0.5 to 1, >1 to 2, >2 to 4, and >4 drinks per day); body mass index (BMI, kg/m^2) (<18.5, 18.5 to <25, 25 to <30, 30 to 35, and ≥35); cigarette smoking (never–cigarette smokers, quit ≤1 pack per day, quit >1 pack per day, currently smoking ≤1 pack per day, and currently smoking >1 pack per day); education (less than high school education, completion of high school, some post-high school training, completion of college, and completion of graduate school); race and/or ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, and Asian/Pacific Islander/Native American); marital status (yes vs no); vigorous physical activity (never, rarely, one to three times per month, one to two times per week, three to four times per week, and five or more times per week); usual physical activity throughout the day (sit all day, sit much of the day, stand and/or walk often with no lifting, lift and/or carry light loads, and carry heavy loads); self-reported diabetes at baseline (yes vs no); and continuous measures for intakes of fruit, vegetables, and total energy from nonalcohol sources. Additional adjustment for grain or coffee intake did not alter risk estimates; therefore, these variables were not included in the final models. For the less than 4% of the cohort who had missing data for a particular covariate, a separate indicator variable for missing was included in the models. We examined risk estimates for red meat, white meat, and saturated fat by stratum of alcohol intake (drinker vs nondrinker), BMI (18.5–25 vs >25 kg/m^2), diabetes (yes vs no), iron supplements (user vs nonuser), and sex (male vs female). Formal interaction tests were performed by examining the Wald *P* value (38) for an interaction term between continuous meat or fat intake with the dichotomous stratification variable.

Hepatitis B and Hepatitis C Infection

We lacked HBV and HCV status for cohort participants. The association between meat intake and HBV and HCV in the US population was examined in National Health and Nutrition Examination Survey (NHANES) data collected between 1999 and 2004 by merging the NHANES 1999–2000, 2001–2002, and 2003–2004 datasets (39). NHANES is a cross-sectional survey designed to assess the health and nutritional status of the entire US population by enrolling a nationally representative sample of approximately 5000 participants per year. Recent dietary intake was assessed by a 24-hour dietary recall. Food items were then disaggregated into constituents by use of recipe files. Red meat included beef, game, lamb, pork, and veal, whereas white meat included chicken, fish, and turkey. We restricted our analyses to 12 178 adults (aged 21 years or older) with satisfactory 24-hour dietary recalls and data on HBV surface antigen, and HCV antibody seropositivity (anti-HCV). The association between red and white meat intake (continuous scale) with HBV and HCV status was assessed using logistic regression models adjusted for age (21 to <40, 40 to <60, 60 to <80, ≥80 years), sex, education (less than high school education, completion of high school, some post-high school training, and completion of college), income (<25 000, 25 000 to <45 000, 45 000 to <75 000, and ≥75 000 dollars per year), and race and/or ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other race including multiracial, and other Hispanic); we report the Wald *P* value (37) from this analysis.

Results

Among 495 006 participants, the median age was 62.6 years, 92.5% were non-Hispanic white, 40.3% were women, 20.3% completed graduate school, 63.0% were overweight or obese, and 9.0% had diabetes. Also, 14.3% were current smokers, 5% drank more than four alcoholic beverages per day, and 24.1% did not drink alcohol. Red and white meat intake differed in their association with these and other features of the cohort (Table 1). Consumption of red meat was associated with male sex and white non-Hispanic ethnicity, more frequent use of alcohol and cigarettes, less fruit and vegetable intake, fewer years of education, and less physical activity. White meat was associated with female sex, less frequent use of alcohol and cigarettes, more vegetable intake, and more years of education.

There were 551 deaths from CLD in 4 419 092 person-years of follow-up and 338 incident cases of HCC in 3 568 243 person-years. In fully adjusted models that included red meat intake, we found an inverse association between white meat consumption and both CLD mortality and HCC incidence (for CLD mortality, quintile 5 [Q5] vs quintile 1 [Q1], HR = 0.52, 95% CI = 0.39 to 0.70, 7.5 vs 18.2 cases per 100 000 person-years, $P_{trend} < .001$; for HCC incidence, Q5 vs Q1, HR = 0.52, 95% CI = 0.36 to 0.77, 5.8 vs 14.3 cases per 100 000 person-years, $P_{trend} < .001$) (Table 2). When white meat intake was additionally adjusted for total meat intake, as opposed to just red meat intake, the risk estimates for white meat were slightly stronger (for CLD, Q5 vs Q1, HR = 0.30, 95% CI = 0.21 to 0.43; and for HCC, Q5 vs Q1, HR = 0.43, 95% CI = 0.27 to 0.68).

Red meat intake was associated with increased CLD (Q5 vs Q1, HR = 2.59, 95% CI = 1.86 to 3.61, 22.3 vs 6.2 cases per 100 000 person-years; $P_{trend} < .001$) and HCC (Q5 vs Q1, HR = 1.74, 95% CI = 1.16 to 2.61, 14.9 vs 5.7 cases per 100 000 person-years; $P_{trend} = .024$) (Table 2). Adjusting red meat intake for total meat intake, as opposed to white meat intake, strengthened risk estimates for both CLD (Q5 vs Q1, HR = 4.00, 95% CI = 2.63 to 6.09) and HCC (Q5 vs Q1, HR = 2.85, 95% CI = 1.71 to 4.76). Because red

Table 1. Distribution of covariates by quintile of baseline red and white meat intake in the NIH-AARP Diet and Health Study (n = 495 006)*

	Red meat			White meat		
Variable	**Q1 (n = 99 001)**	**Q3 (n = 99 002)**	**Q5 (n = 99 001)**	**Q1 (n = 99 001)**	**Q3 (n = 99 002)**	**Q5 (n = 99 001)**
Age, median, y	63.0	62.8	61.7	63.2	62.7	61.8
Sex						
Male, No (%)	44483 (44.9)	58666 (59.3)	74660 (75.4)	61032 (61.7)	60343 (61.0)	54052 (54.6)
Married						
Yes, No (%)	56460 (57.7)	69547 (70.7)	76011 (77.4)	65436 (66.8)	69363 (70.5)	66315 (67.5)
Food intake						
Total energy from nonalcohol sources, median, kcal	1542	1654	1789	1725	1660	1571
Red meat, median, g/1000 kcal	10.0	32.2	64.8	28.6	35.0	28.6
White meat, median, g/1000 kcal	28.6	27.5	28.6	9.7	28.0	65.8
Processed meat, median, g/1000 kcal	2.5	8.1	15.6	5.8	8.4	8.6
Fruit, median, servings/1000 kcal	2.2	1.5	1.0	1.4	1.5	1.5
Vegetables, median, servings/1000 kcal	2.4	2.0	1.9	1.7	2.0	2.4
Education						
Completed graduate school, No (%)	24516 (25.6)	18819 (19.6)	16466 (17.1)	15113 (15.9)	19986 (20.8)	23147 (24.0)
Race and/or ethnicity						
Non-Hispanic white, No (%)	85868 (88.3)	91224 (93.2)	92502 (94.6)	90125 (92.6)	90913 (93.0)	88979 (91.1)
Non-Hispanic black, No (%)	6200 (6.4)	3464 (3.5)	2326 (2.4)	2987 (3.1)	3662 (3.7)	5056 (5.2)
Hispanic, No (%)	2428 (2.5)	1733 (1.8)	1822 (1.9)	2263 (2.3)	1630 (1.7)	1983 (2.0)
Asian/Pacific Islander/Native American, No (%)	2708 (2.8)	1429 (1.5)	1120 (1.2)	1919 (2.0)	1602 (1.6)	1663 (1.7)
Alcohol intake						
>4 drinks per day, No (%)	2121 (2.2)	4665 (4.7)	8384 (8.5)	5381 (5.5)	5020 (5.1)	4345 (4.4)
Smoking use						
Current smokers, No (%)	7946 (8.4)	13577 (14.2)	19289 (20.3)	17685 (18.7)	13260 (13.9)	10168 (10.7)
Self-reported Diabetes						
Yes, No (%)	6361 (6.4)	8490 (8.6)	12652 (12.8)	8399 (8.5)	8836 (8.9)	9951 (10.1)
Body mass index, median, kg/m^2	25.1	26.5	27.5	26.0	26.5	26.6
Vigorous physical activity						
≥5 times/wk, No (%)	26124 (26.8)	17754 (18.1)	14821 (15.1)	18966 (19.5)	18576 (18.9)	20421 (20.9)

* kcal = kilocalories; No = number; Q = quintile.

and white meat appeared to have opposing effects, we created a joint effects variable that combined tertiles of red and white meat intake. Relative to participants in the lowest tertile of red meat intake who were also in the highest tertile of white meat intake, those in the highest tertile of red meat who were also in the lowest tertile of white meat had increased risk of both CLD (HR = 2.55, 95% CI = 1.66 to 3.90, 23.2 vs 5.2 cases per 100 000 person-years) and HCC (HR = 1.67, 95% CI = 1.03 to 2.71, 16.9 vs 5.7 cases per 100 000 person-years).

Because red meat is an important source of dietary fat, we investigated whether the inclusion of fat in the multivariate models attenuated risk estimates for the association of red meat intake with CLD or HCC. Addition of total fat to the models attenuated risk estimates modestly (for CLD, Q5 vs Q1, HR = 1.96, 95% CI = 1.38 to 2.79; for HCC, HR = 1.64, 95% CI = 1.06 to 2.53) (Table 2). When we examined specific fat types, saturated fat caused the greatest attenuation of the red meat associations (Table 2). Addition of monounsaturated fat also attenuated estimates, whereas the addition of polyunsaturated fat did not alter risk estimates (data not shown). In contrast to red meat, hazard ratios for white meat were unaffected by adjustment for total fat (data not shown) or saturated fat intake (for CLD, Q5 vs Q1, HR = 0.57, 95% CI = 0.43 to 0.77; for HCC, Q5 vs Q1, HR = 0.54, 95% CI = 0.37 to 0.79).

Total fat intake was positively associated with CLD (Q5 vs Q1, HR = 2.91, 95% CI = 2.07 to 4.06, 21.5 vs 7.6 cases per 100 000 person-years; $P_{trend} < .001$) and had a borderline statistically significant higher risk of HCC (Q5 vs Q1, HR = 1.46, 95% CI = 0.98 to 2.19, 13.6 vs 7.5 cases per 100 000 person-years; $P_{trend} = .045$) (Table 2). These risk estimates were attenuated after additional adjustment for red and white meat intake (for CLD, HR = 2.29, 95% CI = 1.60 to 3.28; for HCC, HR = 1.24, 95% CI = 0.81 to 1.91). When we investigated the types of fat, the association of greatest magnitude was for saturated fat (for CLD, Q5 vs Q1, HR = 3.50, 95% CI = 2.48 to 4.96, 23.0 vs 6.5 cases per 100 000 person-years; for HCC, Q5 vs Q1, HR = 1.87, 95% CI = 1.23 to 2.85, 14.5 vs 6.3 cases per 100 000 person-years). Monounsaturated fat, but not polyunsaturated fat, was also associated with CLD (Q5 vs Q1, HR = 2.24, 95% CI = 1.61 to 3.12, 19.3 vs 8.3 cases per 100 000 person-years). Risk estimates for monounsaturated fat were elevated for HCC but were not statistically significant (Q5 vs Q1, HR = 1.34,

Table 2. Multivariable hazard ratios (HRs) for baseline red meat, white meat, and fat intake with chronic liver disease (CLD) mortality (n = 551) and hepatocellular carcinoma incidence (HCC; n = 338)*

	Continuous†	Q1		Q2		Q3		Q4		Q5		
Association	**HR‡ (95% CI)**	**No.**	**HR‡**	**No.**	**HR‡ (95% CI)**	**No.**	**HR‡ (95% CI)**	**No.**	**HR‡(95% CI)**	**No.**	**HR‡ (95% CI)**	**P_{trend}§**
White meat, range, g/1000 kcal		0–14.5			14.5–23.2		23.2–33.5		33.5–49.7		> 49.7	
CLD	0.91 (0.87 to 0.95)	164	1.00	130	0.83 (0.66 to 1.05)	108	0.72 (0.56 to 0.92)	83	0.58 (0.44 to 0.76)	66	0.52 (0.39 to 0.70)	<.001
HCC	0.92 (0.87 to 0.97)	101	1.00	77	0.83 (0.62 to 1.12)	71	0.82 (0.60 to 1.11)	50	0.61 (0.43 to 0.87)	39	0.52 (0.36 to 0.77)	<.001
Red meat, range, g/1000 kcal		0–16.4			16.4–27.0		27.0–37.7		37.7–52.2		> 52.2	
CLD	1.12 (1.08 to 1.16)	57	1.00	89	1.52 (1.08 to 2.13)	97	1.63 (1.16 to 2.29)	122	1.93 (1.38 to 2.70)	186	2.59 (1.86 to 3.61)	<.001
With Total fat	1.08 (1.04 to 1.12)		1.00		1.42 (1.01 to 2.01)		1.44 (1.01 to 2.04)		1.60 (1.12 to 2.27)		1.96 (1.38 to 2.79)	<.001
With Saturated fat	1.08 (1.04 to 1.12)		1.00		1.40 (0.99 to 1.97)		1.40 (0.98 to 1.99)		1.55 (1.09 to 2.21)		1.91 (1.34 to 2.72)	<.001
HCC	1.04 (0.99 to 1.09)	41	1.00	63	1.45 (0.97 to 2.17)	68	1.47 (0.98 to 2.21)	69	1.40 (0.93 to 2.11)	97	1.74 (1.16 to 2.61)	.024
With Total fat	1.02 (0.97 to 1.08)		1.00		1.45 (0.97 to 2.18)		1.45 (0.96 to 2.21)		1.36 (0.88 to 2.10)		1.64 (1.06 to 2.53)	.089
With Saturated fat	1.02 (0.97 to 1.07)		1.00		1.39 (0.93 to 2.09)		1.38 (0.91 to 2.10)		1.30 (0.84 to 2.00)		1.58 (1.03 to 2.43)	.10
Total fat, range, % of total energy		1.17–24.3			24.3–29.0		29.0–33.0		33.0–37.4		>37.4	
CLD	1.23 (1.16 to 1.32)	70	1.00	75	1.25 (0.89 to 1.76)	97	1.67 (1.19 to 2.32)	121	2.07 (1.48 to 2.89)	188	2.91 (2.08 to 4.07)	<.001
With Red and white meat	1.17 (1.09 to 1.26)		1.00		1.15 (0.82 to 1.63)		1.45 (1.03 to 2.05)		1.71 (1.20 to 2.43)		2.29 (1.60 to 3.28)	<.001
HCC	1.10 (1.01 to 1.20)	54	1.00	57	1.05 (0.72 to 1.55)	64	1.14 (0.77 to 1.68)	70	1.19 (0.80 to 1.77)	93	1.46 (0.98 to 2.19)	.045
With Red and white meat	1.06 (0.97 to 1.16)		1.00		0.99 (0.67 to 1.46)		1.03 (0.69 to 1.54)		1.05 (0.69 to 1.59)		1.24 (0.81 to 1.91)	.26
Saturated fat, range, % of total energy		0.3–7.1			7.1–8.7		8.7–10.2		10.2–12.0		>12.0	
CLD	1.80 (1.56 to 2.09)	60	1.00	73	1.41 (0.99 to 2.01)	99	1.95 (1.38 to 2.76)	119	2.32 (1.63 to 3.28)	200	3.50 (2.48 to 4.96)	<.001
With Red and white meat	1.58 (1.35 to 1.86)		1.00		1.27 (0.88 to 1.83)		1.65 (1.15 to 2.37)		1.84 (1.27 to 2.66)		2.65 (1.83 to 3.84)	<.001
HCC	1.38 (1.12 to 1.69)	45	1.00	66	1.47 (0.99 to 2.17)	63	1.35 (0.89 to 2.03)	66	1.34 (0.88 to 2.04)	98	1.87 (1.23 to 2.85)	.009
With Red and white meat	1.24 (1.00 to 1.55)		1.00		1.35 (0.90 to 2.03)		1.19 (0.77 to 1.83)		1.13 (0.73 to 1.78)		1.51 (0.96 to 2.38)	.14
Monounsaturated fat, range, % of total energy		0.4–8.9			8.9–10.9		10.9–12.5		12.5–14.3		>14.3	
CLD	1.55 (1.32 to 1.81)	76	1.00	73	1.08 (0.77 to 1.50)	101	1.53 (1.11 to 2.11)	132	1.94 (1.40 to 2.68)	169	2.24 (1.61 to 3.12)	<.001
With Red and white meat	1.33 (1.12 to 1.59)		1.00		0.97 (0.69 to 1.37)		1.28 (0.91 to 1.81)		1.54 (1.09 to 2.18)		1.68 (1.17 to 2.41)	<.001
HCC	1.18 (0.97 to 1.45)	50	1.00	53	1.05 (0.70 to 1.56)	71	1.35 (0.91 to 1.99)	79	1.40 (0.94 to 2.10)	85	1.34 (0.88 to 2.05)	.096
With Red and white meat	1.08 (0.86 to 1.35)		1.00		0.98 (0.65 to 1.48)		1.22 (0.81 to 1.84)		1.23 (0.80 to 1.89)		1.13 (0.72 to 1.79)	.47
Polyunsaturated fat, range, % of total energy		0.3–5.3			5.3–6.4		6.4–7.4		7.4–8.8		>8.8	
CLD	1.20 (0.99 to 1.44)	103		105	1.13 (0.86 to 1.50)	108	1.19 (0.90 to 1.58)	117	1.27 (0.96 to 1.68)	118	1.22 (0.92 to 1.62)	.16
With Red and white meat	1.18 (0.97 to 1.42)		1.00		1.11 (0.84 to 1.47)		1.16 (0.87 to 1.54)		1.23 (0.92 to 1.63)		1.18 (0.88 to 1.57)	.25
HCC	1.06 (0.83 to 1.37)	69	1.00	62	0.93 (0.66 to 1.32)	68	1.02 (0.72 to 1.44)	68	1.02 (0.71 to 1.45)	71	1.06 (0.74 to 1.51)	.63
With Red and white meat	1.06 (0.82 to 1.37)		1.00		0.93 (0.65 to 1.32)		1.02 (0.72 to 1.45)		1.02 (0.71 to 1.46)		1.06 (0.74 to 1.51)	.62

* CI = confidence interval; No. = number; Q = quintile.

† Risk estimates for meat intake are per 10 g/1000 kcal; risk estimates for fat intake are per 5% increase in the percentage of total energy from each fat.

‡ Risk estimates are adjusted for age (continuous), sex (male vs female), alcohol (none, >0 to 0.5, >0.5 to 1, >1 to 2, >2 to 4, and >4 drinks per day), body mass index (kg/m^2; <18.5, 18.5 to <25, 25 to <30, 30 to 35, and ≥35), cigarette smoking (never–cigarette smokers, quit ≤ 1 pack per day, quit >1 pack per day, currently smoking ≤1 pack per day, and currently smoking >1 pack per day), diabetes (yes vs no), education (less than high school education, completion of high school, some post-high school training, completion of college, and completion of graduate school), fruit intake (continuous), vegetable intake (continuous), marital status (yes vs no), race and/or ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, and Asian/Pacific Islander/Native American), total energy from nonalcohol sources (continuous), usual physical activity throughout the day (sit all day, sit much of the day, stand and/or walk often with no lifting, lift and/or carry light loads, and carry heavy loads), and vigorous physical activity (never, rarely, one to three times per month, one to two times per week, three to four times per week, and five or more times per week). Red meat and white meat intake are mutually adjusted for quintiles of the other.

§ Two-sided Wald χ^2 test of linear trend across categories, using the median intake for each quintile.

95% CI = 0.88 to 2.05, 12.4 vs 7.0 cases per 100 000 person-years). In a model that included saturated, monounsaturated, and polyunsaturated fat, statistically significant risk estimates persisted for saturated but not monounsaturated fat. When examined by source, monounsaturated, polyunsaturated, and saturated fat from red meat were associated with both CLD and HCC (Figure 1). From other dietary sources (nonred meat), however, only saturated fat was statistically significantly associated with both CLD (HR per 5% increase in energy from fat = 1.58, 95% CI = 1.34 to 1.86) and HCC (HR = 1.32, 95% CI = 1.05 to 1.65). Monounsaturated fat from nonmeat sources was associated with CLD (HR = 1.24, 95% CI = 1.03 to 1.49) but not with HCC (HR = 1.07, 95% CI = 0.84 to 1.35). Saturated fat from dairy products was associated with increased risk of both CLD (HR = 1.95, 95% CI = 1.46 to 2.59, 17.5 vs 10.5 cases per 100 000 person-years; $P_{trend} < .001$) and HCC (HR = 1.57, 95% CI = 1.08 to 2.27, 12.7 vs 8.5 cases per 100 000 person-years; $P_{trend} = .022$). Among participants in the first quintile of red meat intake, saturated fat from dairy products (Q5 vs Q1) was associated with increased risk of both CLD (HR = 2.19, 95% CI = 1.03 to 4.63) and HCC (HR = 2.99, 95% CI = 1.08 to 8.26).

Red meat is also an important source of heme iron. Total iron intake including iron from meat, other dietary sources, and supplements was inversely associated with CLD (Q5 vs Q1, HR = 0.62, 95% CI = 0.46 to 0.83, 7.3 vs 16.3 cases per 100 000 person-years; $P_{trend} = .013$) but had no association with HCC (P_{trend} across increasing categories = .76) (Table 3). Heme iron, data available for 303 172 participants who completed the risk factor questionnaire, was associated with higher risk of CLD (Q5 vs Q1, HR = 1.77, 95% CI = 1.15 to 2.72, 17.8 vs 6.4 cases per 100 000 person-years; $P_{trend} = .016$) but not of HCC (Q5 vs Q1, HR = 1.06, 95% CI = 0.64 to 1.77, 12.2 vs 6.9 cases per 100 000 person-years; $P_{trend} = .69$).

For the participants who completed the risk factor questionnaire, we also examined meat cooking methods and doneness levels. No associations were observed with cooking methods and doneness levels (data not shown), with the exception of pan frying. Modeled as tertiles due to low intake range, the hazard ratios for pan frying from fully adjusted models (tertile 3 vs tertile 1) were 1.74 (95% CI = 1.28 to 2.35, 17.2 vs 7.7 cases per 100 000 person-years; $P_{trend} < .001$) for CLD and 1.68 (95% CI = 1.17 to 2.43, 14.5 vs 7.0 cases per 100,000 person-years; $P_{trend} = .003$) for HCC. We found no association with the meat mutagens DiMeIQx, or MeIQx, or PhIP (Table 3). We did observe an association with processed meat for CLD (Q5 vs Q1, HR = 1.41, 95% CI = 1.04 to 1.90, 16.4 vs 8.2 cases per 100 000 person-years; $P_{trend} = .013$) but not HCC risk (Q5 vs Q1, HR = 1.17, 95% CI = 0.79 to 1.72, 11.6 vs 6.4 cases per 100 000 person-years). Intakes of nitrate and nitrite

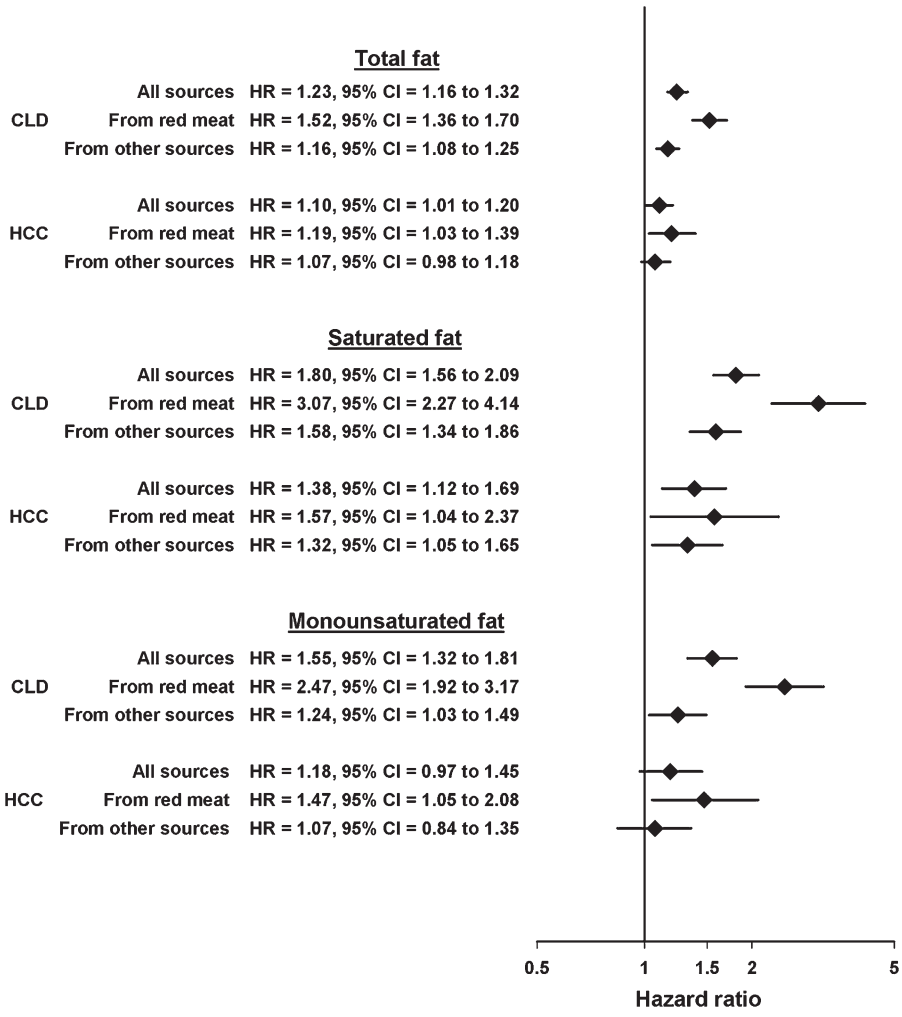

Figure 1. Risk estimates for a 5% increase in the proportion of total energy from each fat type and source with chronic liver disease (CLD) mortality (n = 551) and hepatocellular carcinoma (HCC) incidence (n = 338). Risk estimates are adjusted for age, sex, total energy, body mass index, education, ethnicity, alcohol, cigarette smoking, diabetes, physical activity, and fruit and vegetable intake. Intake of red meat and nonred meat sources were adjusted for each other. The 10%–90% range for the proportion of total energy from each fat type in the cohort was 21%–41% for total fat, 6%–13% for saturated fat, and 8%–16% for monounsaturated fat.

Table 3. Multivariable hazard ratios (HRs) for dietary iron, heme iron, meat mutagens, nitrate, and nitrite with chronic liver disease (CLD) mortality (n = 551) and hepatocellular carcinoma incidence (HCC; n = 338)*

	Continuous†	Q1		Q2		Q3		Q4		Q5		
Association	**HR‡ (95% CI)**	**No.**	**HR‡**	**No.**	**HR‡ (95% CI)**	**No.**	**HR‡ (95% CI)**	**No.**	**HR‡ (95% CI)**	**No.**	**HR‡ (95% CI)**	P_{trend}§
Total iron intake,‖ range, mg/d		1.7–12.6		12.6–16.3		16.3–27.9		27.9–32.3		>32.3		
CLD	0.92 (0.84 to 1.00)	147	1.00	109	0.87 (0.68 to 1.12)	112	0.91 (0.71 to 1.17)	113	0.90 (0.70 to 1.16)	70	0.62 (0.46 to 0.83)	.013
HCC	0.99 (0.89 to 1.10)	85	1.00	67	0.84 (0.60 to 1.16)	60	0.80 (0.57 to 1.12)	58	0.80 (0.57 to 1.12)	68	0.95 (0.68 to 1.32)	.76
Heme iron intake, range, µg/1000 kcal		0–55.7		55.7–103.0		103.0–156.9		156.9–238.2		>238.2		
CLD	1.11 (1.04 to 1.19)	34	1.00	48	1.31 (0.84 to 2.05)	63	1.61 (1.05 to 2.47)	63	1.53 (0.99 to 2.36)	87	1.77 (1.15 to 2.72)	.016
HCC	1.00 (0.90 to 1.11)	28	1.00	39	1.26 (0.77 to 2.07)	44	1.31 (0.80 to 2.13)	40	1.10 (0.66 to 1.82)	44	1.06 (0.64 to 1.77)	.69
DiMeIQx,¶ range, ng/1000 kcal		0		0–0.2		0.2–0.5		0.5–1.2		>1.2		
CLD	0.98 (0.94 to 1.03)	113	1.00	41	0.88 (0.61 to 1.26)	50	1.06 (0.76 to 1.48)	41	0.84 (0.59 to 1.21)	50	0.99 (0.71 to 1.39)	.97
HCC	0.96 (0.90 to 1.02)	68	1.00	39	1.25 (0.84 to 1.86)	29	0.89 (0.57 to 1.37)	26	0.75 (0.48 to 1.19)	33	0.89 (0.58 to 1.35)	.31
MeIQx, range, ng/1000 kcal		0–1.4		1.4–3.8		3.8–7.6		7.6–15.3		>15.3		
CLD	1.00 (0.97 to 1.03)	45	1.00	48	1.03 (0.69 to 1.55)	70	1.44 (0.98 to 2.10)	67	1.34 (0.91 to 1.96)	65	1.17 (0.79 to 1.74)	.69
HCC	0.98 (0.94 to 1.03)	30	1.00	35	1.13 (0.69 to 1.85)	37	1.11 (0.68 to 1.81)	46	1.28 (0.80 to 2.04)	47	1.11 (0.68 to 1.81)	.80
PhIP, range, ng/1000 kcal		0–6.1		6.1–17.4		17.4–35.8		35.8–75.8		>75.8		
CLD	0.98 (0.94 to 1.02)	47	1.00	63	1.21 (0.83 to 1.78)	56	1.03 (0.70 to 1.53)	66	1.20 (0.82 to 1.76)	63	1.14 (0.77 to 1.69)	.73
HCC	0.98 (0.94 to 1.03)	34	1.00	47	1.22 (0.78 to 1.91)	37	0.91 (0.56 to 1.46)	38	0.91 (0.57 to 1.47)	39	0.94 (0.59 to 1.50)	.50
Nitrate, range, mg/1000 kcal		0–0.05		0.05–0.09		0.09–0.14		0.14–0.22		>0.22		
CLD	1.11 (1.04 to 1.20)	36	1.00	50	1.26 (0.82 to 1.95)	59	1.40 (0.91 to 2.14)	59	1.27 (0.83 to 1.97)	91	1.66 (1.09 to 2.53)	.024
HCC	1.02 (0.93 to 1.13)	27	1.00	33	1.10 (0.65 to 1.84)	39	1.18 (0.71 to 1.96)	45	1.21 (0.73 to 1.99)	51	1.11 (0.67 to 1.84)	.81
Nitrite, range, mg/1000 kcal		0–0.02		0.02–0.05		0.05–0.08		0.08–0.14		>0.14		
CLD	1.12 (1.02 to 1.23)	38	1.00	51	1.19 (0.78 to 1.82)	57	1.26 (0.83 to 1.92)	68	1.45 (0.96 to 2.20)	81	1.56 (1.04 to 2.35)	.026
HCC	0.90 (0.77 to 1.06)	26	1.00	38	1.31 (0.79 to 2.16)	51	1.61 (0.99 to 2.61)	43	1.23 (0.74 to 2.03)	37	0.93 (0.55 to 1.57)	.15

* CI = confidence interval; No. = number; Q = quintile.

† Total iron intake was assessed on the baseline questionnaire (551 CLD cases and 338 HCC cases). Heme iron, DiMeIQx, MeIQx, PhIP, nitrate, and nitrite were assessed on the risk factor questionnaire (295 CLD cases and 195 HCC cases). Total iron intake is per unit of 10 mg, heme iron per 100 µg, MeIQx per 5 ng, DiMeIQx per 0.5 ng, PhIP per 25 ng, nitrate per 0.1 mg, and nitrate per 0.1 mg. Heme iron, MeIQx, DiMeIQx, PhiP, nitrate, and nitrite were assed on the follow-up questionnaire. DiMeIQx = 2-amino-3,4,8-trimethylimidazo[4,5,-f]quinoxaline; MeiQx = 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline; PhIP = 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine.

‡ Risk estimates are adjusted for age (continuous), sex (male vs female), alcohol (none, >0 to 0.5, >0.5 to 1, >1 to 2, >2 to 4, and >4 drinks per day), body mass index (kg/m^2; <18.5, 18.5 to <25, 25 to <30, 30 to 35, and ≥35), cigarette smoking (never–cigarette smokers, quit ≤ 1 pack per day, quit >1 pack per day, currently smoking ≤1 pack per day, and currently smoking >1 pack per day), diabetes (yes vs no), education (less than high school education, completion of high school, some post-high school training, completion of college, and completion of graduate school), fruit intake (continuous), vegetable intake (continuous), marital status (yes vs no), race and/or ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, and Asian/Pacific Islander/Native American), total energy from nonalcohol sources (continuous), usual physical activity throughout the day (sit all day, sit much of the day, stand and/or walk often with no lifting, lift and/or carry light loads, and carry heavy loads), and vigorous physical activity (never, rarely, one to three times per month, one to two times per week, three to four times per week, five or more times per week).

§ Two-sided Wald χ^2 test of linear trend across categories, using the median intake for each quintile.

‖ Total iron intake includes iron from diet and supplements and was assessed on the baseline questionnaire.

¶ Because more than 25% of the cohort had zero intake of DiMeIQx, nonconsumers were placed in category 1, remaining participants were partitioned into quartiles (categories 2–5).

Table 4. Multivariable hazard ratios (HRs) for baseline red meat and white meat intake with chronic liver disease (CLD) mortality (n = 551) and hepatocellular carcinoma incidence (HCC; n = 338) by stratum of alcohol, body mass index, diabetes, and sex*

	CLD			HCC		
Stratification	**No.**	**HR† (95% CI)**	**$P_{interaction}$‡**	**No.**	**HR† (95% CI)**	**$P_{interaction}$‡**
Red meat						
All	551	1.12 (1.08 to 1.16)		338	1.04 (0.99 to 1.09)	
Alcohol drinker						
No	143	1.08 (1.00 to 1.15)	.039	129	1.03 (0.95 to 1.11)	.23
Yes	408	1.14 (1.09 to 1.18)		209	1.04 (0.98 to 1.11)	
Body mass index, kg/m^2						
18.5–25	187	1.16 (1.10 to 1.23)	.014	77	1.08 (0.98 to 1.19)	.24
>25	338	1.10 (1.06 to 1.15)		247	1.02 (0.97 to 1.08)	
Diabetes						
No	439	1.13 (1.09 to 1.18)	.025	254	1.06 (1.00 to 1.12)	.25
Yes	112	1.07 (0.99 to 1.15)		84	0.97 (0.89 to 1.07)	
Sex						
Male	387	1.12 (1.08 to 1.17)	.76	278	1.02 (0.97 to 1.08)	.19
Female	164	1.12 (1.04 to 1.20)		60	1.12 (1.00 to 1.25)	
White meat						
All	551	0.91 (0.87 to 0.95)		338	0.92 (0.87 to 0.97)	
Alcohol drinker						
No	143	0.92 (0.85 to 1.00)	.62	129	0.89 (0.81 to 0.98)	.53
Yes	408	0.90 (0.86 to 0.95)		209	0.94 (0.87 to 1.00)	
Body mass index, kg/m^2						
18.5–25	187	0.96 (0.89 to 1.02)	.021	77	0.94 (0.84 to 1.05)	.16
>25	338	0.89 (0.84 to 0.94)		247	0.91 (0.85 to 0.97)	
Diabetes						
No	439	0.91 (0.87 to 0.96)	.70	254	0.94 (0.88 to 0.99)	.26
Yes	112	0.89 (0.81 to 0.98)		84	0.87 (0.77 to 0.97)	
Sex						
Male	387	0.93 (0.88 to 0.98)	.48	278	0.91 (0.85 to 0.96)	.25
Female	164	0.87 (0.81 to 0.95)		60	0.95 (0.85 to 1.06)	

* CI = confidence interval; No = number.

† Risk estimates for meat intake are per 10 g/1000 kcal and are adjusted for age (continuous), sex (male vs female), alcohol (none, >0 to 0.5, >0.5 to 1, >1 to 2, >2 to 4, and >4 drinks per day), body mass index (kg/m^2; <18.5, 18.5 to <25, 25 to <30, 30 to 35, and ≥35), cigarette smoking (never–cigarette smokers, quit ≤ 1 pack per day, quit >1 pack per day, currently smoking ≤1 pack per day, and currently smoking >1 pack per day), diabetes (yes vs no), education (less than high school education, completion of high school, some post-high school training, completion of college, and completion of graduate school), fruit intake (continuous), vegetable intake (continuous), marital status (yes vs no), race and/or ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, and Asian/Pacific Islander/Native American), total energy from nonalcohol sources (continuous), usual physical activity throughout the day (sit all day, sit much of the day, stand and/or walk often with no lifting, lift and/or carry light loads, and carry heavy loads), and vigorous physical activity (never, rarely, one to three times per month, one to two times per week, three to four times per week, five or more times per week). Red meat and white meat intake are mutually adjusted for quintiles of the other.

‡ Two-sided Wald χ^2 test for the interaction term of continuous red or white meat, 10 g per 1000 kcal, with each marked dichotomous stratifying variable.

were associated with CLD (for nitrate, Q5 vs Q1, HR = 1.66, 95% CI = 1.09 to 2.53, 17.6 vs 6.8 cases per 100 000 person-years; for nitrite, Q5 vs Q1, HR = 1.56, 95% CI = 1.04 to 2.35, 15.6 vs 7.2 cases per 100 000 person-years) but not with HCC (for nitrate, Q5 vs Q1, HR = 1.11, 95% CI = 0.67 to 1.84, 12.7 vs 6.6 cases per 100 000 person-years; for nitrite, Q5 vs Q1, HR = 0.93, 95% CI = 0.55 to 1.57, 9.2 vs 6.4 cases per 100 000 person-years).

Risk estimates by stratum of alcohol drinking, BMI, diabetes, and sex, appeared to be generally similar to each other (Table 4). However, for CLD, but not HCC, we found statistically significant qualitative interactions between red meat and alcohol drinking, BMI, and diabetes and a statistically significant qualitative interaction between white meat and stratum of BMI. Yet, all estimates from stratified models were in the same direction as the unstratified estimates and the 95% confidence intervals for each stratum overlapped each other and those for the unstratified estimates. We found no evidence for effect modification for either CLD or HCC with saturated fat ($P_{interaction}$ > .29 for all; data not shown). Risk estimates for red meat, white meat, and saturated fat also did not vary among users and nonusers of iron supplements ($P_{interaction}$ > .12 for all; data not shown).

HBV and HCV are strong risk factors for CLD and HCC; yet, we lacked information about these potential confounders in our cohort. Therefore, we examined the association of red and white meat intake with HBV and HCV status using participants of NHANES enrolled from 1999 to 2004. Among 12 178 NHANES adults with available data on diet and viral hepatitis, 251 individuals were anti-HCV(+) and 48 individuals were HBV surface antigen (+). In NHANES, we found no association between red or white meat intake with HBV or HCV status (P > .3 for all).

Discussion

In this large prospective cohort study, red meat intake was associated with higher risk of CLD mortality and HCC incidence, whereas white meat was inversely associated with both endpoints.

We observed a statistically significant positive association with fat intake for both endpoints, with the highest risks observed for saturated fat. Mutual adjustment for saturated fat and red meat intake attenuated both risk estimates; yet, each remained statistically significant, suggesting overlapping, yet independent effects.

Few previous data are available for white meat consumption. Data from one recent case–control study from Italy showed an inverse association between white meat and liver cancer (13). Results from a Japanese cohort study also suggested an inverse association but were not adjusted for age or any other potential confounders (9). In the current study, we observed evidence for an inverse association for each type of white meat, including chicken, turkey, and fish. The observed inverse association does not simply reflect the absence of red meat; after energy adjustment, the Spearman correlation coefficient between red and white meat was low (−0.05), all risk estimates were adjusted for red meat intake, and the associations for red and white meat appeared independent in our analysis. The mechanism by which white meat intake might lead to reduced risk, however, is unclear.

Only two previous prospective studies investigated the association between red meat intake and liver cancer. One study of daily beef, pork, and poultry intake (8) observed a statistically significant positive association in an age- and sex-adjusted model (relative risk = 1.22, 90% CI = 1.04 to 1.43). A second study (9) examined both red and white meat separately but presented imprecise risk estimates that were not adjusted for age or other risk factors. Results from several previous cases–control studies were mixed (6,7,10–13). Most studies were limited by a narrow intake range and several did not distinguish between red and white meat. Previous studies have not examined the association between red meat intake and CLD mortality.

Red meat is an important source of dietary fat. Fat intake might play a role in insulin resistance (40), which may be associated with liver disease and liver cancer (41). Additional adjustment for total fat, monounsaturated fat, or saturated fat attenuated the risk estimates for red meat. We also observed evidence for associations between total fat, monounsaturated fat, and saturated fat with both CLD and HCC. It is difficult to distinguish the effect of monounsaturated fat and saturated fat due to their high correlation (Spearman coefficient = 0.8). Nevertheless, after mutually adjusting for all fat types, a statistically significant association remained for saturated fat but not for monounsaturated fat. In addition, risk estimates from nonred meat sources and HCC were statistically significant for saturated fat but not for monounsaturated fat. Saturated fat from dairy products was also independently associated with CLD and HCC risk. Only three previous studies have investigated the association of fat intake with CLD or liver cancer. In one study from Italy (42), an inverse association was observed with polyunsaturated fat and no association was observed with monounsaturated or saturated fat. Results from a Greek case–control study (7) showed no association with any fat type. A third study of NHANES I data (43) observed a non-statistically significant yet elevated risk estimate (HR = 1.24, 95% CI = 0.7 to 2.1) for the highest vs lowest category of saturated fat intake with a combined endpoint of incident liver cirrhosis and liver cancer, though case numbers were low. In our study, risk estimates for red meat remained statistically significant after adjustment for fat intake, suggesting additional aspects of meat, in addition to fat, may play a role in CLD and HCC.

Red meat may also be associated with liver cancer through an effect of iron. We observed an inverse association between total iron intake (dietary plus supplemental) and CLD but no association with HCC. Of the foods contributing to total iron in our study, 16% was from red meat, 50% was from grain, and 15% from vegetables. Grains and vegetables contain many other components, in addition to iron, that may be associated with the risk of CLD. Red meat contains heme iron, which is more readably absorbed than nonheme iron (16). In our study, heme iron was associated with higher risk of CLD but not of HCC. Whether this difference reflects distinct etiologies between endpoints or chance remains to be determined. One previous Italian case–control study observed a statistically significant positive association between dietary iron intake and HCC risk but did not investigate heme iron (42).

Very high doses of HCAs cause liver tumors in primates (22). Even though we found an association with pan-fried meats, we observed no association between well-done or grilled/barbecued cooking methods or the individual HCAs, DiMeIQx, MeIQx, and PhIP with either endpoint, perhaps due to lower concentrations of HCAs in cooked food than used in these intervention studies. These results suggest that HCAs are not the mechanism explaining the observed association with red meat in our study.

N-nitroso compounds have also been shown to cause cirrhosis and liver tumors in animals (23,44). We observed a positive association of processed meat, an important source of *N*-nitroso compounds, with CLD but not with HCC. It is not clear if this difference between endpoints reflects chance or a true difference in etiology. Similar to the results for processed meat, intake of nitrate and nitrite was also associated with CLD but not with HCC. Few previous studies have investigated the association with processed meat, and results have been mixed in existing studies (9,13); no studies have investigated the association of nitrate and nitrate intake.

It is possible that associations for meat and fat could reflect confounding by alcohol, diabetes, or adiposity, even though we adjusted for these risk factors in the multivariate models. Risk estimates appeared similar to those overall in never drinkers, among those with a BMI in the healthy range (18.5–25 kg/m^2) and in those without diabetes. Residual confounding by these risk factors, therefore, does not appear to have affected our results. Red meat, white meat, and saturated fat are also associated with other dietary components that could affect the risk of CLD and HCC. In our study, risk estimates persisted after adjustment for other dietary and lifestyle components, including intake of fruit, vegetables, grain, and coffee; education; and marital status. Yet, as an observational study, it is not possible to determine for certain whether meat and fat or another dietary or nondietary factor associated with these dietary components is responsible for the observed association.

Alternatively, individuals with preexisting liver disease may alter their diet due to poor health or physician recommendation, affecting risk estimates. Restricting our analyses to cases that occurred after the first 5 years of follow-up or excluding individuals with poor self-reported health at baseline did not affect risk estimates, suggesting that associations for meat and fat intake are not because individuals with CLD or a prediagnosed liver cancer

at baseline changed their diets after diagnosis. Furthermore, similar associations for red meat, white meat, and saturated fat with both CLD and HCC suggest that these exposures may affect the progression of liver disease, as opposed to HCC or liver decompensation occurring in individuals with advanced liver disease.

Our study had several notable strengths. Dietary components were comprehensively assessed, and the large size of our study allowed for a wide range of reported intake. In addition, the inclusion of a detailed meat cooking and doneness questionnaire allowed us to explore potential mechanisms of action. The prospective design of our study limited the possibility of recall and selection bias as diet was assessed before cancer diagnosis or death from CLD. Information was also available for a wide variety of potential confounders, including age, sex, alcohol use, cigarette use, BMI, diabetes, and physical activity.

Limitations include lack of information on HBV and HCV infection status. As HBV and HCV are major risk factors for HCC and CLD, failure to adjust for them could have a large effect on the results if meat intake were related to viral infection status. Because few data are available on the relationship between meat intake and viral status, we investigated this association in the NHANES 99/00, 01/02, and 03/04 datasets (39). In NHANES adults, neither red meat nor white meat intake was associated with HBV or HCV status. These results from NHANES suggest that viral status may not be a confounder in our NIH-AARP analysis. One previous Italian case–control study examined the association between meat intake and liver cancer and had data on HBV and HCV status (13). In models adjusted for HBV, HCV, and other covariates, an inverse association was observed with white meat and a suggestive, but not statistically significant, positive association with red meat. Though the authors did not present estimates for red meat that were stratified by viral status, the association for white meat was similar in those infected with HBV and HCV. Nevertheless, future studies are needed to determine whether the associations observed in this study are independent of HBV or HCV infection. In addition to the lack of information on HBV and HCV, our study assessed diet at a single time-point and used an FFQ, which is subject to measurement error (45). We also lacked data on preexisting liver disease at baseline but were able to perform lag analysis, excluding outcomes in the first 5 years of follow-up, and results did not change. Finally, perhaps due to the AARP membership or the response rate to our questionnaire, the NIH-AARP population was more likely to be non-Hispanic white, have attended college or graduate school, and to have healthier habits (such as not smoking) than the US population. Therefore, our results should be replicated in other populations.

In summary, we observed a positive association for red meat and an inverse association for white meat with CLD mortality and HCC incidence. Processed meat was associated with CLD but not with HCC. Statistically significant positive associations were observed for saturated fat from both red meat and other sources, suggesting that the association for red meat may be due in part to saturated fat.

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