Original Article

Meat and Components of Meat and the Risk of Bladder Cancer in the NIH-AARP Diet and Health Study

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BACKGROUND: Meat could be involved in bladder carcinogenesis via multiple potentially carcinogenic meat-related compounds related to cooking and processing, including nitrate, nitrite, heterocyclic amines (HCAs), and polycyclic aromatic hydrocarbons (PAHs). The authors comprehensively investigated the association between meat and meat components and bladder cancer. **METHODS:** During 7 years of follow-up, 854 transitional cell bladder-cancer cases were identified among 300,933 men and women who had completed a validated food-frequency questionnaire in the large prospective NIH-AARP Diet and Health Study. The authors estimated intake of nitrate and nitrite from processed meat and HCAs and PAHs from cooked meat by using quantitative databases of measured values. Total dietary nitrate and nitrite were calculated based on literature values. **RESULTS:** The hazard ratios (HR) and 95% confidence intervals (CI) for red meat (HR for fifth quintile compared with first quintile, 1.22; 95% CI, 0.96-1.54; $P_{trend} = .07$) and the HCA 2-amino-1 methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) (HR, 1.19; 95% CI, 0.95-1.48; $P_{trend} = .06$) conferred a borderline statistically significant increased risk of bladder cancer. Positive associations were observed in the top quintile for total dietary nitrite (HR, 1.28; 95% CI, 1.02-1.61; $P_{trend} = .06$) and nitrate plus nitrite intake from processed meat (HR, 1.29; 95% CI, 1.00-1.67; $P_{trend} = .11$). **CONCLUSIONS:** These findings provided modest support for an increased risk of bladder cancer with total dietary nitrite and nitrate plus nitrite from processed meat. Results also suggested a positive association between red meat and PhIP and bladder carcinogenesis. ***Cancer*** **2010;116:4345–53.**

KEYWORDS: diet, bladder cancer, meat, nitrate, nitrite.

Recognized risk factors for bladder cancer include smoking, as well as occupational or environmental exposure to aromatic amines, polycyclic aromatic hydrocarbons (PAHs), and arsenic.[1-3] However, these exposures only partly explain the etiology of bladder cancer. Because nutrients or their metabolites are excreted through the urinary tract, some dietary factors could be involved in carcinogenesis via contact with the bladder epithelium[2,4,5] or through systemic exposure.

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Meat is an important dietary component to consider in relation to bladder cancer, as it is a source of multiple potentially carcinogenic compounds resulting from cooking or processing. Evidence from prospective epidemiologic studies of meat is inconsistent, with some positive associations between certain meat types and bladder cancer[6,7] and other studies observing no association.[8-12] Comprehensive epidemiologic data on meat-related exposures potentially mechanistically involved in bladder carcinogenesis are lacking.

A key hypothesis for bladder carcinogenesis involves nitrate and nitrite, compounds added to processed meat for preservation and enhancement of color and flavor. Nitrate and nitrite are precursors to *N*-nitroso compounds (NOCs), which induce tumors in many organs, including the bladder, in multiple animal species.[13-16] In healthy individuals, NOCs can form endogenously from nitrite in the presence of amines, amides, and bacteria and may be excreted in the urine.[17-19] Additional NOC formation can also occur directly in the bladder when bacterial infection occurs. The source of nitrate and nitrite is important to consider because the primary sources of nitrate can be fruits and vegetables, which contain inhibitors of endogenous nitrosation.[20,21] There are few epidemiologic studies of dietary nitrate[19,22,23] and nitrite[23,24] and bladder cancer.

Given the role of aromatic amines and PAHs from occupational exposures in bladder cancer and the presence of these compounds in cigarette smoke, another important risk factor, heterocyclic amines (HCAs) and PAHs formed in meats prepared by high temperature cooking methods,[25-28] could be implicated in this malignancy. HCAs and PAHs are mutagenic and carcinogenic in animal studies,[29,30] and some HCAs induce bladder tumors specifically.[31-33] Two case-control studies of HCAs from meat in relation to bladder cancer have been null.[34,35]

We evaluated the role of meat, nitrate, nitrite, and meat mutagens in relation to transitional-cell bladder cancer in a large, prospective, cohort study by using a detailed meat questionnaire linked to a database of published values from the literature and quantitative databases of laboratory measures of meat samples.

MATERIALS AND METHODS

Study population

From 1995 to 1996, the NIH-AARP Diet and Health Study enrolled men and women, aged 50 to 71 years, from 6 US states (California, Florida, Louisiana, New Jersey, North Carolina, Pennsylvania) and 2 metropolitan areas (Atlanta, Georgia; Detroit, Michigan). At baseline, participants completed a mailed self-administered questionnaire on demographic, lifestyle, and medical characteristics. Details of the study design have been described elsewhere.[36] The study was approved by the US National Cancer Institute Special Studies Institutional Review Board.

Dietary variables

At baseline, participants completed a 124-item food-frequency questionnaire (FFQ), based on the National Cancer Institute's Diet History Questionnaire (http://riskfactor.cancer.gov/DHQ/forms/files/shared/dhq1.2002.sample.pdf). Portion sizes and daily nutrient intake were calculated with the 1994-1996 US Department of Agriculture's Continuing Survey of Food Intake by Individuals.[37] The FFQ compared favorably to other FFQs[38] and was validated in a subset of this cohort against 2 nonconsecutive 24-hour dietary recalls.[36] Energy-adjusted correlation coefficients for red meat were 0.62 and 0.70 for men and women, respectively.[39] Approximately 6 months after baseline, participants completed a mailed risk-factor questionnaire (RFQ) with questions on meat cooking methods and doneness levels. The FFQ meat-cooking module has been compared by using multiple food diaries, and its ability to rank individuals according to HCA intake was acceptable.[40] Red meat included bacon, beef, cold cuts, ham, hamburger, hot dogs, liver, pork, sausage, and steak. White meat included all chicken and turkey meat products and fish. Processed meat included bacon, sausage, luncheon meats, ham, and hotdogs. Meat products from mixed dishes were included in the relevant meat groups.

Nitrate and nitrite intake from processed meats was calculated with a database of laboratory measured values of these compounds in 10 types of processed meats representing 90% of processed meats consumed by the US population.[41] For total dietary exposure, the published literature for nitrate and nitrite measurement data was reviewed, and a mean of the published values for individual foods was calculated and weighted by the sample size of the study. Food-specific nitrate and nitrite values were combined by using the same methodology applied to other nutrients.[38]

With meat cooking method (grilled, pan-fried, microwaved, and broiled) and doneness level (well-done/very well-done, and medium/rare) data and the Computerized Heterocyclic Amines Resource for Research in

Epidemiology of Disease ([CHARRED] http://charred.cancer.gov),[41] we estimated 3 HCAs: 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-phenylimidazo(4,5-*b*)pyridine (PhIP). With CHARRED, we also estimated benzo(*a*)pyrene (B[*a*]P), a marker of overall PAH exposure from meat, and total mutagenic activity, a measure incorporating mutagenicity of all meat-related mutagens.

Identification of cases and cohort follow-up

We identified incident transitional cell bladder cancers through probabilistic linkage with state cancer registries, 8 original states plus 3 additional states where participants commonly move (Texas, Arizona, Nevada). Cancer endpoints were defined by anatomic site and histologic code of the International Classification of Diseases for Oncology.[42] Cases included transitional cell bladder cancer with codes C67.0-C67.9, encompassing morphologies 8050, 8120-8122, and 8130.

Cohort members were followed for change of address by using the US Postal Service. Vital status was ascertained by annual linkage of the cohort to the US Social Security Administration Death Master File, follow-up searches of the National Death Index Plus for participants who matched to the Social Security Administration Death Master File, cancer registry linkage, questionnaire responses, and responses to other mailings. Follow-up for this analysis was from the date the RFQ was received until December 31, 2003, or when the participant moved out of 1 of the state cancer-registry areas, had a cancer diagnosis, or died, whichever came first. Overall, only 4% of participants were lost to follow-up as a result of moving, and these individuals had similar baseline characteristics to those for whom follow-up information was available.

Statistical analysis

A total of 566,402 persons returned the baseline questionnaire (after excluding duplicates and subjects who died, moved before entry, or withdrew from the study) and of these, 337,074 returned the RFQ. We further excluded individuals who: died before the RFQ was received (n = 1619), moved out of the 8 study areas before returning the RFQ (n = 547), had a proxy complete either questionnaire (n = 10,383), had prevalent cancer (based on cancer registry or self-report) at RFQ entry (n = 18,844), had a death-only report for any cancer (n = 2246), or reported extreme total energy intake (n = 2483) defined as more than 2 interquartile ranges above the 75th or below the 25th percentile on the logarithmic scale. Our analytic cohort comprised 300,933 persons: 125,574 women and 175,359 men.

Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by using Cox proportional hazards regression with age as the underlying time metric. Diagnostic testing, using a time interaction model, for proportional hazards, indicated that assumptions were not violated. Dietary variables were energy adjusted by using the multivariate nutrient-density method; residual energy adjustment resulted in similar risk estimates.[43] Quantile cutpoints were based on intake in the analytic cohort; the lowest quantile served as the referent; although quintiles were used for most variables, tertiles were used for cooking methods and doneness levels because of a smaller range of intake. All models summed to total meat; for example, red meat and white meat were included in the same model as were meats cooked rare, medium, well, and very-well done plus those with no doneness information. Multivariate models were adjusted for the following characteristics that altered risk estimates by 10% or greater: age (continuous, years), sex, smoking (never, quit $\geq$10 years ago, quit 5-9 years ago, quit 1-4 years ago, quit $<$1 year ago or $\leq$20 cigarettes/day, 20-40 cigarettes/day, $>$40 cigarettes/day), and intake of fruit (continuous, cup equivalents/1000 kcal), vegetables (continuous, cup equivalents/1000 kcal), beverages (continuous, mL/day; sum of beer, coffee, juice, liquor, milk, soda, tea, and wine), and total energy (continuous, kcal/day). Adjustment for other possible confounding variables, including aspirin use, body mass index (BMI), ethnicity, history of diabetes, physical activity, and intake of dairy and vitamins C and E did not alter risk estimates. Tests for linear trend were based on quantile median values. *P*-values were 2-tailed, and analyses were conducted by using SAS Version 9 (SAS Institute, Cary, NC).

We assessed effect modification by sex, smoking, beverage intake, and vitamin C (dietary, supplemental, total) with cross-product terms in the multivariate models. To account for potential exposure to nitrate from drinking water, in sensitivity analyses, we excluded individuals whose enrollment address was in a census-tract area in which 50% of the area of the tract was estimated to have groundwater nitrate levels $\geq$10 mg/L nitrate-nitrogen (US Environmental Protection Agency Maximum Contaminant Level) as determined by a nationwide model incorporating information on land use, soil type, and other factors.[44]

Table 1. Means and Proportions of Baseline Characteristics by Red Meat Quintiles, g/1000 kcal, N=300,933

	Quintiles of Red Meat (Mean)				
Characteristics	**1 (8.9)**	**2 (20.8)**	**3 (30.8)**	**4 (42.3)**	**5 (66.5)**
Age, y, mean	63.1	63.1	63.0	62.8	62.2
Education, college graduate or post graduate, %	46.7	41.7	40.6	39.0	37.3
Race, %					
Non-Hispanic white	89.0	92.1	93.1	94.2	94.4
Non-Hispanic black	5.2	3.7	3.1	2.5	2.0
Hispanic	2.1	1.6	1.5	1.4	1.6
Asian	1.9	1.2	1.0	0.8	0.7
Pacific Islander, American Indian, Alaskan Native	1.8	1.4	1.3	1.2	1.4
Body mass index, kg/m^2, mean	25.6	26.5	27.0	27.4	28.2
Smoking history, %					
Never smoker	41.2	38.4	35.9	33.8	30.8
Former smoker	47.6	47.6	48.3	48.5	48.9
Current smoker or quit <1 y ago	7.7	10.7	12.7	14.4	17.1
Vigorous physical activity, >5 times per wk, %	27.7	21.3	18.6	17.1	15.8
Dietary variables, mean					
Total energy, kcal/d	1685	1741	1812	1879	1978
Beverages,[a] mL/d	1883	1967	1978	1960	1947
Fruit, cup equivalents/1000 kcal	1.7	1.3	1.1	1.0	0.8
Vegetables, cup equivalents/1000 kcal	1.3	1.1	1.1	1.1	1.0
Dietary vitamin E, mg/1000 kcal	5.6	5.1	5.0	4.9	4.7
Supplemental vitamin E, mg/d	97.4	79.7	70.5	63.4	56.9
Dietary vitamin C, mg/1000 kcal	120.6	100.0	89.8	80.9	69.6
Supplemental vitamin C, mg/d	428.6	339.0	297.9	263.8	243.9

[a] Sum of beer, coffee, juice, liquor, milk, soda, tea, and wine.

RESULTS

During 1,922,817 person-years of follow-up, we identified 854 transitional cell bladder cancers (720 men, 134 women). Individuals consuming the most red meat were younger, less educated, less physically active, and had lower dietary intake of fruits, vegetable, and vitamins C and E than those consuming the least red meat (Table 1). Those in the highest quintile of red meat consumption compared with those in the lowest quintile were more likely to be non-Hispanic white, current smokers, to have a higher BMI, and to have higher intake of beverages and total energy.

We observed a borderline, statistically significant, increased risk of bladder cancer for those in the highest versus the lowest quintile of red meat consumption (HR, 1.22; 95% CI, 0.96-1.54; $P_{trend} = .07$) but no association with white meat or processed meat (Table 2). The red meat association was driven by processed red meats (HR for fifth compared with first quintile, 1.30; 95% CI, 1.00-1.69; $P_{trend} = .17$; data not shown) rather than unprocessed red meats (HR, 1.08; 95% CI, 0.84-1.38; $P_{trend} = .22$; data not shown). There were no associations with beef, bacon, hamburger, sausage, or steak; however, we did observe a positive nonlinear association for red meat cold cuts (HR for fifth compared with first quintile, 1.42; 95% CI, 1.10-1.84; $P_{trend} = .18$; data not shown). Analyses using residual energy adjustment (g/day) resulted in a similar association for red meat (HR, 1.17; 95% CI, 0.93-1.47; $P_{trend} = .10$) and a slightly stronger association for processed meat (HR, 1.14; 95% CI, 0.91-1.43; $P_{trend} = .06$). Including squamous-cell carcinomas, adenocarcinomas, and not-otherwise-specified carcinomas of the bladder (an additional 113 cases) did not alter risk estimates. There was also no evidence of effect modification for the meat exposures by sex, smoking, or beverage intake (data not shown).

We saw no clear association for total dietary nitrate (HR, 0.80; 95% CI, 0.58-1.10; $P_{trend} = .28$). However, total dietary nitrite was positively associated with bladder cancer in the top quintile (HR, 1.28; 95% CI, 1.02-1.61), although the P for linear trend was only borderline statistically significant ($P_{trend} = .06$) (Table 3). There was a suggestive positive association between measured values of nitrate from processed meat and bladder cancer, but this

Table 2. Distribution and HRs with 95% CIs for Bladder Cancer Risk Within Quintiles of Meat, g/1000 kcal

Characteristic	Q1	Q2	Q3	Q4	Q5	P_{trend}[a]
Red meat						
Cases	134	150	174	170	226	
Median	9.5	20.9	30.7	42.1	61.6	
HR (95% CI)	1.00	0.99 (0.78-1.25)	1.05 (0.83-1.33)	0.97 (0.77-1.23)	1.22 (0.96-1.54)	.07
White meat						
Cases	191	194	167	152	150	
Median	9.5	18.6	27.5	39.5	64.2	
HR (95% CI)	1.00	1.09 (0.89-1.33)	0.99 (0.80-1.23)	0.98 (0.79-1.22)	1.09 (0.87-1.36)	.68
Processed meat						
Cases	117	150	169	218	200	
Median	1.6	4.3	7.4	12.1	22.3	
HR (95% CI)	1.00	1.09 (0.85-1.39)	1.10 (0.86-1.41)	1.28 (1.01-1.62)	1.10 (0.86-1.40)	.55

Q indicates quintile; HR, hazard ratio; CI, confidence interval.
Hazard ratios (HR) were adjusted for age (continuous, years), sex, smoking (never, quit ≥10 years ago, quit 5-9 years ago, quit 1-4 years ago, quit <1 year ago, or ≤20 cigarettes/day, 20-40 cigarettes/day, >40 cigarettes/day), and intakes of fruit (continuous, cup equivalents/1000 kcal), vegetables (continuous, cup equivalents/1000 kcal), beverages (continuous, mL/day; sum of beer, coffee, juice, liquor, milk, soda, tea and wine), and total energy (continuous, kcal/day).
[a] P_{trend} based on quintile medians.

Table 3. Distribution and HRs with 95% CIs for Bladder Cancer Risk Within Quintiles of Nitrate and Nitrite (mg/1000 kcal)

Characteristic	Q1	Q2	Q3	Q4	Q5	P_{trend}[a]
Dietary nitrate[b]						
Cases	236	185	150	145	138	
Median	19.7	30.4	41.5	58.0	95.4	
HR (95% CI)	1.00	0.86 (0.71-1.06)	0.76 (0.60-0.95)	0.77 (0.60-0.99)	0.80 (0.58-1.10)	.28
Plant sources						
Cases	237	184	148	149	136	
Median	17.0	27.6	38.6	55.1	92.6	
HR (95% CI)	1.00	0.85 (0.69-1.03)	0.73 (0.55-0.91)	0.77 (0.60-0.99)	0.77 (0.56-1.06)	.21
Animal sources						
Cases	175	162	190	185	142	
Median	1.5	2.2	2.7	3.3	4.3	
HR (95% CI)	1.00	0.96 (0.77-1.19)	1.13 (0.92-1.39)	1.17 (0.94-1.44)	1.03 (0.82-1.29)	.44
Dietary nitrite[b]						
Cases	176	181	164	161	172	
Median	0.46	0.57	0.65	0.74	0.91	
HR (95% CI)	1.00	1.17 (0.90-1.45)	1.10 (0.89-1.37)	1.14 (0.91-1.44)	1.28 (1.02-1.61)	.06
Plant sources						
Cases	215	175	159	155	150	
Median	0.25	0.35	0.42	0.51	0.69	
HR (95% CI)	1.00	0.97 (0.79-1.19)	0.97 (0.78-1.21)	1.05 (0.84-1.33)	1.16 (0.90-1.50)	.18
Animal sources						
Cases	150	132	187	178	207	
Median	0.10	0.15	0.20	0.25	0.36	
HR (95% CI)	1.00	0.85 (0.67-1.07)	1.15 (0.92-1.43)	1.04 (0.83-1.31)	1.09 (0.87-1.36)	.21
Nitrate from processed meat[c]						
Cases	126	140	173	187	228	
Median	0.02	0.07	0.11	0.17	0.29	
HR (95% CI)	1.00	0.97 (0.76-1.24)	1.09 (0.87-1.38)	1.07 (0.85-1.36)	1.20 (0.95-1.51)	.06
Nitrite from processed meat[c]						
Cases	119	158	163	227	187	
Median	0.01	0.03	0.06	0.10	0.19	
HR (95% CI)	1.00	1.15 (0.90-1.46)	1.08 (0.85-1.37)	1.39 (1.11-1.74)	1.07 (0.85-1.36)	.79
Nitrate and nitrite from processed meat[c]						
Cases	109	147	173	191	234	
Median	0.06	0.16	0.29	0.50	0.95	
HR* (95% CI)	1.00	1.19 (0.92-1.53)	1.15 (0.90-1.48)	1.21 (0.94-1.55)	1.29 (1.00-1.67)	.11

Hazard ratios (HR) were adjusted for age (continuous, years), sex, smoking (never, quit ≥10 years ago, quit 5-9 years ago, quit 1-4 years ago, quit <1 year ago, or ≤20 cigarettes/day, 20-40 cigarettes/day, >40 cigarettes/day), and intakes of fruit (continuous, cup equivalents/1000 kcal), vegetables (continuous, cup equivalents/1000 kcal), beverages (continuous, mL/day; sum of beer, coffee, juice, liquor, milk, soda, tea and wine), and total energy (continuous, kcal/day).
[a] P_{trend} based on quintile medians.
[b] Literature values.
[c] Measured values from meat samples.

Table 4. Distribution and HRs with 95% CIs for Bladder Cancer Risk Within Quintiles of Meat Mutagens

Characteristic	Q1	Q2	Q3	Q4	Q5	P_{trend}[a]
DiMeIQx						
Cases	304	39	156	160	195	
Median (ng/1000 kcal)	0.0	0.1	0.2	0.6	1.7	
HR (95% CI)	1.00	1.12 (0.80-1.57)	0.92 (0.76-1.12)	0.89 (0.73-1.07)	1.08 (0.90-1.30)	.31
MeIQx						
Cases	169	169	145	179	192	
Median. ng/1000 kcal	0.5	2.4	5.3	10.3	24.4	
HR (95% CI)	1.00	0.94 (0.76-1.16)	0.76 (0.61-0.95)	0.91 (0.73-1.12)	0.93 (0.75-1.15)	.95
PhIP						
Cases	137	173	163	183	198	
Median, ng/1000 kcal	2.1	10.9	24.7	49.4	123.6	
HR (95% CI)	1.00	1.07 (0.85-1.34)	0.94 (0.75-1.19)	1.05 (0.84-1.31)	1.19 (0.95-1.48)	.06
B[*a*]P						
Cases	174	182	145	163	190	
Median (ng/1000 kcal)	0.2	1.5	6.2	16.8	44.0	
HR (95% CI)	1.00	1.00 (0.81-1.23)	0.83 (0.66-1.03)	0.86 (0.69-1.07)	0.95 (0.77-1.17)	.84
Mutagenic activity						
Cases	138	186	159	188	183	
Median, revertant colonies/1000 kcal	165	601	1152	2042	4349	
HR (95% CI)	1.00	1.14 (0.92-1.43)	0.93 (0.74-1.18)	1.10 (0.88-1.38)	1.09 (0.87-1.37)	.55

Hazard ratios (HR) were adjusted for age (continuous, years), sex, smoking (never, quit ≥10 years ago, quit 5-9 years ago, quit 1-4 years ago, quit <1 year ago, or ≤20 cigarettes/day, 20-40 cigarettes/day, >40 cigarettes/day), and intakes of fruit (continuous, cup equivalents/1000 kcal), vegetables (continuous, cup equivalents/1000 kcal), beverages (continuous, mL/day; sum of beer, coffee, juice, liquor, milk, soda, tea and wine), and total energy (continuous, kcal/day).

[a] P_{trend} based on quintile medians.

association failed to reach statistical significance in the highest quintile (HR, 1.20; 95% CI, 0.95-1.51; P_{trend} = .06). There was no clear association with measured values of nitrite from processed meat and bladder cancer (HR, 1.07; 95% CI, 0.85-1.36; P_{trend} = .79). However, we observed a borderline statistically significant association for combined nitrate and nitrite from processed meat among those in the top quintile (HR, 1.29; 95% CI, 1.00-1.67; P_{trend} = .11).

There was no evidence of effect modification for the nitrate and nitrite exposures by sex, beverage intake, smoking, or vitamin C intake (data not shown). In addition, excluding individuals who may have had substantial exposure to nitrate from drinking water (n = 7085) because of residence in an area with high-nitrate groundwater levels did not alter our risk estimates (data not shown).

DiMeIQx, MeIQx, B[*a*]P, and total mutagenic activity were not associated with bladder cancer, but there was a suggestive increased risk with PhIP (HR, 1.19; 95% CI, 0.95-1.48; P_{trend} = .06; Table 4). There was no association between bladder cancer and grilled (top vs bottom tertile HR, 0.97; 95% CI, 0.82-1.15; P_{trend} = .50), panfried (HR, 1.02; 95% CI, 0.86-1.22; P_{trend} = .79), or well/very-well done meat (HR, 1.03; 95% CI, 0.86-1.23; P_{trend} = .33; data not shown).

DISCUSSION

In this large prospective cohort, we found an increased risk of bladder cancer among those in the top quintile of total dietary nitrite and nitrate plus nitrite intake from processed meat. There were also suggestive positive associations for this malignancy with increasing intake of red meat and PhIP.

Few prospective studies have found a positive association between meat consumption and bladder cancer. One cohort observed an increased risk with beef and pork,[6] and another analysis of 2 cohorts observed a positive association between bacon and bladder cancer.[7] A recent study also observed reduced risk of bladder cancer for vegetarians compared with those who ate meat.[45] However, several other prospective investigations of meat and bladder cancer,[8,10-12] including an analysis of baseline dietary data in the full NIH-AARP cohort,[9] were null. In addition, a nested case-control study in the European

Prospective Investigation into Cancer and Nutrition found a positive association between meat and bladder cancer limited to only individuals with a rapid N-acetyltransferase 2 genotype.[12] Data from case-control studies are similarly inconsistent, with some positive associations for red meat or individual meat items,[24,46-51] and several null findings.[35,52-56]

We saw no clear association between total processed meat consumption and bladder cancer, yet by separating red meat into processed and unprocessed and examining individual processed meat items, we observed positive associations with red processed meat and red meat coldcuts and bladder cancer. Other evidence for a positive association between processed meats and bladder cancer comes from a Hawaiian case-control study for bacon, ham, and sausage (limited to Japanese men, not Caucasians or Japanese women)[24] and a case-control study in Uruguay for salted meats.[49] Bacon has also been associated with an increased risk in 2 cohort studies.[7]

Although we saw no association with intake of processed meat, there was evidence of an elevated risk of bladder cancer with nitrate plus nitrite from processed meats. In addition, by estimating total dietary exposure to nitrate and nitrite from values in the literature, we observed a statistically significant increased risk with dietary nitrite. Our positive findings for nitrate plus nitrite from processed meat support the hypothesis of NOCs' involvement in bladder carcinogenesis, as processed meat also provides amines and amides necessary for the endogenous formation of NOCs. Our laboratory-measured values of nitrate and nitrite from processed meat represent more recent levels of these additives,[41] as the amount of added nitrate and nitrite was reduced in recent decades. When we estimated nitrate and nitrite from processed meat based on literature values from the 1970s, we observed similar associations, with a stronger positive association for nitrite (data not shown).

Three studies of dietary nitrate in relation to bladder cancer have been null.[19,22,23] One case-control study found a positive association for dietary nitrite and nitrosamines and bladder cancer for Japanese men only[24]; however, another case-control study in Iowa observed no association for dietary nitrite.[23] The suggestive inverse association with dietary nitrate in our population supports that the vast majority of this compound is coming from fruits and vegetables, which are potential protective factors against bladder cancer[5] and contain vitamins[20,21] and polyphenols[57,58] that can inhibit the formation of NOCs. We hypothesized that inhibitory action by vitamin C may modify risk, but we saw no evidence of an interaction, perhaps because of this population's relatively high fruit and vegetable intake.

We observed a possible increased risk of bladder cancer with PhIP, the most abundant HCA in cooked meat, but no clear associations with other HCAs. Two case-control studies that have investigated HCAs from meat and bladder cancer were null.[34,35] It is possible that the positive association with PhIP was due to chance; however, it should be noted that the major sources of the 3 HCAs varied. Well-done barbecued hamburgers were the largest source of MeIQx (36%) and DiMeIQx (50%), whereas the largest source of PhIP was medium-done barbecued steak (20%). Our results for B[*a*]P and total mutagenic activity were null, and we are not aware of other studies to which we can compare these findings. In addition, we saw no evidence of an association between meat cooking methods or doneness levels and bladder cancer.

Our study had several strengths including its large size, high follow-up rate, and detailed questionnaire on meat cooking and doneness to assess multiple components of meat. By using a quantitative database for processed meat and values from the literature for all food items, we were able to examine different dietary sources of nitrate and nitrite. We were also able to assess a wide range of potential confounding variables, including fine control for smoking. However, we lacked information on urination frequency and bladder infections and had only limited data on beverage intake (no data on water intake), yet epidemiologic evidence on total fluid intake in relation to bladder cancer is inconsistent. In addition, despite no individual-level data of nitrate exposure from drinking water, a subanalysis excluding those with potential high exposure did not alter our risk estimates.

This study provides limited evidence for a role of total dietary nitrite and nitrate plus nitrite from processed meat in bladder carcinogenesis. To better understand these associations, future analyses should continue to focus on the different dietary sources of these compounds. Additional research is needed to confirm our findings of a possible increased risk of bladder cancer with intake of red meat and especially for PhIP, as prospective investigations of meat-related mutagens and this malignancy are lacking.

CONFLICT OF INTEREST DISCLOSURES

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