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A Large Prospective Study of Meat Consumption and Colorectal Cancer Risk: An Investigation of Potential Mechanisms Underlying this Association

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Abstract

Although the relation between red and processed meat intake and colorectal cancer has been reported in several epidemiologic studies, very few investigated the potential mechanisms. This study examined multiple potential mechanisms in a large U.S. prospective cohort with a detailed questionnaire on meat type and meat cooking methods linked to databases for estimating intake of mutagens formed in meats cooked at high temperatures (heterocyclic amines, polycyclic aromatic hydrocarbons), heme iron, nitrate, and nitrite. During 7 years of follow-up, 2,719 colorectal cancer cases were ascertained from a cohort of 300,948 men and women. The hazard ratios (HR) and 95% confidence intervals (95% CI) comparing the fifth to the first quintile for both red (HR, 1.24; 95% CI, 1.09–1.42; $P_{trend} < 0.001$) and processed meat (HR, 1.16; 95% CI, 1.01–1.32; $P_{trend} = 0.017$) intakes indicated an elevated risk for colorectal cancer. The potential mechanisms for this relation include heme iron (HR, 1.13; 95% CI, 0.99–1.29; $P_{trend} = 0.022$), nitrate from processed meats (HR, 1.16; 95% CI, 1.02–1.32; $P_{trend} = 0.001$), and heterocyclic amine intake [HR, 1.19; 95% CI, 1.05–1.34; $P_{trend} < 0.001$ for 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and HR, 1.17; 95% CI, 1.05–1.29; $P_{trend} < 0.001$ for 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx)]. In general, the elevated risks were higher for rectal cancer than for colon cancer, with the exception of MeIQx and DiMeIQx, which were only associated with colon cancer. In conclusion, we found a positive association for red and processed meat intake and colorectal cancer; heme iron, nitrate/nitrite, and heterocyclic amines from meat may explain these associations. *Cancer Res; 70(6); 2406–14. ©2010 AACR.*

Introduction

Although a recent consensus report concluded there was "convincing" evidence supporting a positive association between both red meat and processed meat intakes and colorectal cancer, it noted that there was inadequate evidence to implicate specific components of meat (1). There are very few epidemiologic studies that have comprehensively assessed potential mechanisms relating meat to carcinogenesis.

Meat is a key source of iron because this heme iron is more readily absorbed than iron from other sources. Epidemiologic studies of dietary iron intake and colorectal neoplasia are inconsistent; the World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) consensus report concluded that "the evidence was sparse, of poor quality, and inconsistent" (1). Iron can induce oxidative DNA damage (2, 3), and heme iron is associated with fecal water cytotoxicity (4, 5) and the promotion of colorectal cancer in rodents (6). Furthermore, heme iron intake increases endogenous formation of *N*-nitroso compounds (NOC; ref. 7), which are multisite carcinogens (8). In addition, NOCs can be formed exogenously in processed meats from nitrate and nitrite added during the processing procedure. Meat cooked well-done at high temperatures is also a source of heterocyclic amines (HCA; refs. 9–11) and polycyclic aromatic hydrocarbons (PAH; refs. 12, 13), which are known gastrointestinal carcinogens in animal models (12, 14).

The WCRF/AICR report noted that there was insufficient evidence to reach any consensus for nitrate, nitrite, HCAs, or PAHs as risk factors for colorectal cancer (1). To better understand the association between meat and colorectal cancer, we examined this relationship in a large prospective cohort and investigated components of meat, as well as risks by tumor subsite.

Materials and Methods

Study population. The NIH-AARP Diet and Health Study is a large prospective cohort of men and women, ages 50 to

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71 y, from six U.S. states (California, Florida, Louisiana, New Jersey, North Carolina, and Pennsylvania) and two metropolitan areas (Atlanta, Georgia and Detroit, Michigan). At baseline (1995–1996), a self-administered questionnaire regarding demographic and life-style characteristics was completed; further study details have previously been described (15). The Special Studies Institutional Review Board of the U.S. National Cancer Institute approved the study.

Dietary assessment. A 124-item food frequency questionnaire (FFQ) that compared favorably to other FFQs (16) and was calibrated within the study population against two nonconsecutive 24-h dietary recalls (15) was completed at baseline. Portion sizes and daily nutrient intakes were calculated from the 1994 to 1996 U.S. Department of Agriculture's Continuing Survey of Food Intake by Individuals (17). Approximately 6 mo after baseline, participants who did not have self-reported prostate, breast, or colon cancer at baseline were mailed the risk factor questionnaire (RFQ), which collected information on meat type, meat cooking methods, and doneness levels. The red meat variable contained all types of beef, pork, and lamb, including bacon, beef, cold cuts, ham, hamburger, hot dogs, liver, pork, sausage, and steak. White meat included chicken, turkey (poultry cold cuts, chicken mixtures, low-fat sausages, and low-fat hot dogs made from poultry), and fish. The processed meat variable included bacon, red meat sausage, poultry sausage, luncheon meats (red and white meat), cold cuts (red and white meat), ham, regular hotdogs, and low-fat hotdogs made from poultry. The meat variables also included meats added to complex food mixtures, such as pizza, chili, lasagna, and stew.

Total iron was calculated as the sum of dietary iron and supplementary iron. Dietary iron included all dietary sources of iron, including cereals, vegetables and meat. We developed a new heme iron database based on measured values from meats cooked by different methods and to varying doneness levels, which we used in conjunction with the detailed meat cooking questionnaire to quantitatively assess heme iron intake (18). We estimated nitrate and nitrite intake from processed meats using a database containing measured values of nitrate and nitrite from 10 types of processed meats, which represent 90% of processed meats consumed in the United States (18). In an analysis of total dietary nitrate and nitrite, we estimated intake of these compounds by determining the content of foods that constituted the food item database from the literature, as described previously (19, 20). Meat cooking method (grilled/barbecued, pan-fried, microwaved, and broiled) and doneness level (well-done/very well-done and medium/rare) were used in conjunction with the CHARRED database[6] to estimate intake of several HCAs (18), including 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), as well as one PAH, benzo(*a*)pyrene [B(*a*)P], and mutagenic activity (a measure of total mutagenic potential incorporating all meat-related mutagens).

[6] http://charred.cancer.gov

Cohort follow-up and case ascertainment. Cohort members were followed for change of address using the U.S. Postal Service. We ascertained vital status through annual linkage of the cohort to the U.S. 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. We identified cancer cases through probabilistic linkage with state cancer registries. In addition to the eight original states from which the cohort recruited, our cancer registry ascertainment area was expanded to include Texas, Arizona, and Nevada, wherein participants have most commonly moved during follow-up. Approximately 4% of participants were lost to follow-up as a result of moving out of the 11 states. Colorectal cancer end points were defined by anatomic site and histologic code of the International Classification of Diseases for Oncology (ICD-0-3; ref. 21) and included codes C180-C189, C199, C209, and C260. We further classified cases as proximal colon (C180-184), distal colon (C185-187), and rectum (C199, C209). We included first primary diagnoses, and our analysis was based on adenocarcinomas; we excluded cases with unspecified histologies (n = 132), lymphomas (n = 19), sarcomas (n = 5), neuroendocrine tumors (n = 48), squamous cell tumors (n = 7), a large cell rhabdoid tumor (n = 1), a gastrinoma (n = 1), and a melanoma (n = 1). Follow-up for these analyses began on the date the RFQ was received until censoring at the end of 2003 or when the participant moved out of 1 of the 11 state cancer registry areas, had a cancer diagnosis, or died, whichever came first.

Statistical analysis. There were a total of 566,402 persons who returned the baseline questionnaire (after excluding duplicates and subjects who died or moved before entry or withdrew from the study) and 337,074 who returned the RFQ. For our study, we excluded individuals who had not completed the RFQ, who died before the RFQ was received (n = 1,619), who moved out of one of the original eight study areas before returning the RFQ (n = 547), whose baseline questionnaire or RFQ was filled in by someone else on their behalf (n = 10,383), who had prevalent cancer (as noted by cancer registry or self-report) at the time they completed the RFQ (n = 18,844), who had a death only report for any cancer (n = 2,246), who had zero person-years of follow-up (n = 4), and those with extreme daily total energy intake (n = 2,483), defined as more than two interquartile ranges above the 75th or below the 25th percentile on the logarithmic scale. After all exclusions, our analytic cohort consisted of 300,948 persons (175,369 men and 125,579 women).

Hazard ratios (HR) and 95% confidence intervals (95% CI) were estimated using Cox proportional hazards regression with person-years as the underlying time metric; analyses using age as the underlying time metric yielded almost identical HRs. The proportional hazard assumption was verified using a time interaction model. The models were constructed as addition models that summed to total meat; for example, red and white meat were included in the same model, as were processed and nonprocessed meat. The final multivariate models only contained variables that changed

Table 1. Selected means and proportions for characteristics of participants in the NIH-AARP Diet and Health Study by red meat quintile from the RFQ (n = 300,948)

Characteristics	Quintiles of red meat intake, g/1,000 kcal				
	Q1	Q2	Q3	Q4	Q5
Mean intake of red meat (g/1,000 kcal)	8.9	20.8	30.8	42.3	66.5
Gender (% male)	45.1	51.1	58.0	64.5	72.7
Age (y)	63.1	63.1	63.0	62.8	62.2
Education, college graduate or postgraduate (%)	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, Pacific Islander, American Indian, Alaskan Native	2.2	1.5	1.3	1.1	1.1
Family history of colorectal cancer (%)	10.0	10.1	10.1	9.5	9.3
BMI, kg/m^2	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	48.8
Current smoker or having quit <1 y ago	7.7	10.7	12.7	14.4	17.1
Vigorous physical activity, >5 times/wk (%)	27.7	21.3	18.6	17.1	15.8
Regular* use of NSAIDs (%)	63.3	66.7	68.0	68.2	67.9
Dietary variables (mean intake)					
Energy (kcal/d)	1685	1741	1812	1879	1978
Alcohol (g/d)	11.9	13.5	12.9	12.2	11.0
Calcium (mg/1,000 kcal)	501	467	435	405	361
Fiber (g/1,000 kcal)	13.8	11.5	10.7	10.0	9.1
Fruits (cup equivalents/1,000 kcal)	1.7	1.3	1.1	1.0	0.8
Vegetables (cup equivalents/1,000 kcal)	1.3	1.1	1.1	1.1	1.0

Abbreviation: NSAIDs; Nonsteroidal anti-inflammatory drugs.
*Defined as two to three times per month or more.

the HR by 10% or more or were established risk factors for colorectal cancer, and they included gender, education, body mass index (BMI), smoking, and intake of total energy, fiber, and dietary calcium. Dietary variables were adjusted for energy by the nutrient density method (22); using the residual energy adjustment method resulted in similar risk estimates (given in table footnote). The covariates that attenuated the risk estimates the most were BMI and intake of fiber and calcium. Multivariate HRs are reported within quintiles, using the lowest quintile as the reference category. Tests for linear trend were calculated using the median value of each quintile. All reported P values are two sided.

Interactions were evaluated by including cross-product terms in multivariate models. Furthermore, we conducted a lag analysis excluding the first 2 y of follow-up. To test for heterogeneity between the anatomic subsites, we calculated the weighted average of the two β coefficients from the Cox model, with weights being proportional to the inverse of the variances. We then calculated the following χ^2 statistic with one degree of freedom: $T = \sum_{i=1}^{2}(\hat{\beta}_i - \bar{\beta})^2/\sigma_i^2$ wherein $\hat{\beta}_i$ and σ_i^2 are the coefficient and its variance for each subsite, and $\bar{\beta}$ is the weighted average of the β coefficients. All statistical analyses were carried out using Statistical Analytic Systems software (SAS Institute Inc.).

Results

After up to 7.2 years of follow-up, we ascertained 2,719 incident colorectal cancer cases (1,806 male and 913 female cases), of which 1,995 were colon cancers (1,150 proximal, 787 distal colon, 58 lacked definitive site information) and 724 were rectal cancers. We had stage information on 81% of the cases; of these, 43% were stage 1, 16% were stage II, 26% were stage III, and 15% were stage IV at diagnosis. Individuals in the highest quintile of red meat intake were more likely to be non-Hispanic White, current smokers, and have a higher BMI compared with those in the lowest quintile; furthermore, they were less educated, less physically active, less likely to have a family history of colorectal cancer, and consumed less calcium, fiber, fruits, and vegetables (Table 1). The correlation between red meat intake and heme iron was high ($r_{Spearman}$ = 0.82), as was the correlation between processed meat and both nitrate ($r_{Spearman}$ = 0.93) and nitrite ($r_{Spearman}$ = 0.97) in meat.

Red meat and total processed meat (processed red and white meat) intake were both positively associated with colorectal cancer (HR for the fifth compared with the first quintile, 1.24; 95% CI, 1.09–1.42; P_{trend} < 0.001; HR, 1.16; 95% CI, 1.01–1.32; P_{trend} = 0.017, respectively; Table 2). Dividing red meat into processed red meat and nonprocessed red meat revealed similar risks (comparing the highest to the lowest quintiles: HR, 1.11; 95% CI, 0.96–1.28; P_{trend} = 0.083 for processed red meat; HR, 1.13; 95% CI, 0.98–1.30; P_{trend} = 0.002 for nonprocessed red meat). There was no evidence of an interaction by gender for either red ($P_{interaction}$ = 0.385) or processed meat ($P_{interaction}$ = 0.138). White meat was inversely associated with colorectal cancer (HR, 0.85; 95% CI, 0.76–0.97; P_{trend} = 0.017); this association was evident for chicken (HR, 0.85; 95% CI, 0.75–0.97; P_{trend} = 0.020), but not for turkey (HR, 1.02; 95% CI, 0.90–1.17; P_{trend} = 0.412) or fish intake (HR, 0.95; 95% CI, 0.84–1.08; P_{trend} = 0.903).

With further investigation by location, risks were elevated, although not all reached statistical significance, for both colon and rectal cancer for red meat (HR, 1.21; 95% CI, 1.03–1.41; P_{trend} < 0.001; HR, 1.35; 95% CI, 1.03–1.76; P_{trend} = 0.024, respectively) and processed meat (HR, 1.11; 95% CI, 0.95–1.29; P_{trend} = 0.057; HR, 1.30; 95% CI, 1.00–1.68; P_{trend} = 0.145, respectively; Table 2); although the risks were slightly higher for rectal cancer, there was no evidence of subsite heterogeneity for either red ($P_{heterogeneity}$ = 0.485) or processed meat ($P_{heterogeneity}$ = 0.320). Within the colon, the risks for proximal or distal tumors were not statistically significantly

Table 2. Meat intake and colorectal cancer in the NIH-AARP Diet and Health Study (*n* = 300,948)

	Colorectal cancer (*n* = 2,719)		Colon cancer (*n* = 1,995)		Rectal cancer (*n* = 724)	
	Cases	HR* (95% CI)	Cases	HR* (95% CI)	Cases	HR* (95% CI)
Red meat† (median, g/1,000 kcal)						
Q1 (9.5)	451	1.0	340	1.0	111	1.0
Q2 (20.9)	484	1.00 (0.87–1.14)	345	0.94 (0.81–1.09)	139	1.18 (0.91–1.52)
Q3 (30.7)	502	0.99 (0.87–1.13)	367	0.96 (0.82–1.12)	135	1.09 (0.84–1.42)
Q4 (42.1)	614	1.18 (1.03–1.34)	457	1.16 (1.00–1.36)	157	1.21 (0.93–1.58)
Q5 (61.6)	668	1.24 (1.09–1.42)	486	1.21 (1.03–1.41)	182	1.35 (1.03–1.76)
P_{trend}		<0.001		<0.001		0.024
White meat (median, g/1,000 kcal)						
Q1 (9.6)	605	1.0	454	1.0	151	1.0
Q2 (18.6)	562	0.93 (0.83–1.05)	414	0.91 (0.79–1.04)	148	1.00 (0.80–1.26)
Q3 (27.5)	563	0.95 (0.84–1.06)	395	0.88 (0.76–1.00)	168	1.17 (0.94–1.46)
Q4 (39.5)	523	0.91 (0.81–1.03)	392	0.90 (0.78–1.03)	131	0.95 (0.75–1.21)
Q5 (64.2)	466	0.85 (0.76–0.97)	340	0.81 (0.71–0.94)	126	0.98 (0.77–1.25)
P_{trend}		0.017		0.012		0.639
Processed meat‡ (median, g/1,000 kcal)						
Q1 (1.6)	440	1.0	334	1.0	106	1.0
Q2 (4.3)	496	1.04 (0.91–1.18)	357	0.98 (0.84–1.14)	139	1.22 (0.94–1.58)
Q3 (7.4)	538	1.07 (0.94–1.23)	393	1.03 (0.89–1.20)	145	1.20 (0.93–1.56)
Q4 (12.1)	612	1.16 (1.02–1.32)	453	1.14 (0.98–1.32)	159	1.24 (0.95–1.61)
Q5 (22.3)	633	1.16 (1.01–1.32)	458	1.11 (0.95–1.29)	175	1.30 (1.00–1.68)
P_{trend}		0.017		0.057		0.145

*Adjusted for gender, education (high school or less/unknown, post-high school/some college, college/postgraduate), BMI (<18.5, ≥18.5 to <25, ≥25 to <30, ≥30 to <35, ≥35, unknown), smoking (never smoker, former smoker who quit ≥10 y ago, former smoker who quit 1–9 y ago, current/those who quit <1 y ago, missing), and intake of total energy (kcal/d), fiber (g/1,000 kcal), and dietary calcium (mg/1,000 kcal). Red meat and white meat were mutually adjusted for each other. Processed meat was adjusted for nonprocessed meat.

†Red meat energy adjusted by the residual method; highest (median: 104.1 g/d) versus lowest (median: 15.7 g/d) quintile: HR for colorectal cancer = 1.21 (95% CI, 1.07–1.38); HR for colon cancer = 1.16 (95% CI, 1.00–1.35); HR for rectal cancer = 1.36 (95% CI, 1.06–1.75); on the continuous scale per 100 g/d increment: HR for colorectal cancer = 1.23 (95% CI, 1.10–1.36); HR for colon cancer = 1.20 (95% CI, 1.05–1.36); HR for rectal cancer = 1.31 (95% CI, 1.07–1.61).

‡Processed meat energy adjusted by the residual method; highest (median: 38.0 g/d) versus lowest (median: 2.7 g/d) quintile: HR for colorectal cancer = 1.15 (95% CI, 1.01–1.31); HR for colon cancer = 1.13 (95% CI, 0.98–1.32); HR for rectal cancer = 1.20 (95% CI, 0.93–1.55); on the continuous scale per 100 g/d increment: HR for colorectal cancer = 1.19 (95% CI, 0.96–1.48); HR for colon cancer = 1.13 (95% CI, 0.88–1.45); HR for rectal cancer = 1.38 (95% CI, 0.93–2.05).

Table 3. Meat-related compounds and colorectal cancer in the NIH-AARP Diet and Health Study (n = 300,948)

	Colorectal cancer (n = 2,719)		Colon cancer (n = 1,995)		Rectal cancer (n = 724)	
	Cases	HR* (95% CI)	Cases	HR* (95% CI)	Cases	HR* (95% CI)
Total† iron (median, mg/d)						
Q1 (10.8)	646	1.0	483	1.0	163	1.0
Q2 (14.8)	578	0.91 (0.81–1.02)	425	0.89 (0.78–1.02)	153	0.96 (0.77–1.21)
Q3 (21.5)	539	0.88 (0.78–0.99)	387	0.85 (0.74–0.97)	152	0.99 (0.79–1.24)
Q4 (30.6)	496	0.81 (0.72–0.91)	368	0.80 (0.70–0.92)	128	0.85 (0.67–1.07)
Q5 (36.1)	460	0.75 (0.66–0.86)	332	0.73 (0.62–0.84)	128	0.84 (0.65–1.08)
P_{trend}		<0.001		<0.001		0.070
Dietary iron (median, mg/1,000 kcal)						
Q1 (5.9)	677	1.0	483	1.0	194	1.0
Q2 (7.2)	537	0.82 (0.73–0.92)	397	0.84 (0.73–0.96)	140	0.76 (0.60–0.95)
Q3 (8.2)	518	0.80 (0.70–0.90)	390	0.84 (0.72–0.96)	128	0.70 (0.55–0.88)
Q4 (9.3)	509	0.79 (0.69–0.90)	374	0.81 (0.70–0.94)	135	0.73 (0.57–0.93)
Q5 (11.4)	478	0.75 (0.65–0.87)	351	0.78 (0.66–0.92)	127	0.68 (0.52–0.90)
P_{trend}		<0.001		0.009		0.017
Heme iron (median, μg/1,000 kcal)						
Q1 (48.1)	468	1.0	347	1.0	121	1.0
Q2 (100.9)	508	1.00 (0.88–1.13)	378	0.99 (0.86–1.15)	130	1.01 (0.79–1.30)
Q3 (150.3)	538	1.02 (0.90–1.16)	397	1.01 (0.87–1.17)	141	1.06 (0.82–1.36)
Q4 (212.6)	577	1.07 (0.94–1.21)	421	1.04 (0.90–1.21)	156	1.13 (0.88–1.45)
Q5 (335.8)	628	1.13 (0.99–1.29)	452	1.10 (0.94–1.28)	176	1.24 (0.96–1.60)
P_{trend}		0.022		0.138		0.049
Nitrate from processed meats (median, μg/1,000 kcal)						
Q1 (23.9)	451	1.0	341	1.0	110	1.0
Q2 (65.3)	470	0.96 (0.85–1.10)	344	0.93 (0.80–1.08)	126	1.08 (0.83–1.40)
Q3 (109.6)	530	1.04 (0.91–1.18)	386	0.99 (0.86–1.16)	144	1.18 (0.91–1.52)
Q4 (169.2)	609	1.13 (1.00–1.29)	439	1.08 (0.93–1.25)	170	1.31 (1.01–1.68)
Q5 (289.2)	659	1.16 (1.02–1.32)	485	1.13 (0.97–1.32)	174	1.26 (0.97–1.63)
P_{trend}		0.001		0.009		0.066
Nitrite from processed meats (median, μg/1,000 kcal)						
Q1 (11.9)	457	1.0	344	1.0	113	1.0
Q2 (33.7)	488	0.99 (0.87–1.12)	359	0.96 (0.83–1.12)	129	1.07 (0.83–1.38)
Q3 (59.7)	554	1.07 (0.94–1.21)	397	1.01 (0.88–1.18)	157	1.23 (0.96–1.58)
Q4 (99.9)	603	1.12 (0.98–1.27)	441	1.09 (0.94–1.26)	162	1.21 (0.94–1.55)
Q5 (194.1)	617	1.11 (0.97–1.25)	454	1.09 (0.94–1.26)	163	1.16 (0.90–1.50)
P_{trend}		0.055		0.089		0.369
PhIP (median, ng/1,000 kcal)						
Q1 (2.1)	512	1.0	382	1.0	130	1.0
Q2 (10.9)	498	0.90 (0.79–1.02)	357	0.87 (0.75–1.00)	141	1.00 (0.78–1.27)
Q3 (24.7)	560	0.99 (0.87–1.12)	402	0.96 (0.83–1.10)	158	1.08 (0.85–1.36)
Q4 (49.4)	591	1.04 (0.92–1.17)	434	1.03 (0.90–1.19)	157	1.06 (0.83–1.34)
Q5 (123.6)	558	0.99 (0.87–1.12)	420	1.01 (0.87–1.16)	138	0.94 (0.73–1.20)
P_{trend}		0.507		0.212		0.440
MeIQx (median, ng/1,000 kcal)						
Q1 (0.5)	482	1.0	346	1.0	136	1.0
Q2 (2.4)	506	1.01 (0.89–1.15)	370	1.03 (0.89–1.20)	136	0.96 (0.76–1.22)
Q3 (5.3)	512	0.99 (0.88–1.13)	365	0.99 (0.85–1.15)	147	1.00 (0.79–1.27)
Q4 (10.3)	571	1.08 (0.95–1.22)	427	1.13 (0.98–1.31)	144	0.94 (0.74–1.20)
Q5 (24.4)	648	1.19 (1.05–1.34)	487	1.26 (1.09–1.45)	161	1.01 (0.79–1.28)
P_{trend}		<0.001		<0.001		0.852

(*Continued on the following page*)

Table 3. Meat-related compounds and colorectal cancer in the NIH-AARP Diet and Health Study (n = 300,948) (Cont'd)

	Colorectal cancer (n = 2,719)		Colon cancer (n = 1,995)		Rectal cancer (n = 724)	
	Cases	HR* (95% CI)	Cases	HR* (95% CI)	Cases	HR* (95% CI)
DiMeIQx (median, ng/1,000 kcal)						
Q1 (0.0)	922	1.0	665	1.0	257	1.0
Q2 (0.04)	105	1.02 (0.84–1.25)	77	1.05 (0.83–1.33)	28	0.95 (0.64–1.40)
Q3 (0.19)	496	0.96 (0.86–1.07)	365	0.98 (0.86–1.11)	131	0.91 (0.73–1.12)
Q4 (0.58)	567	1.06 (0.95–1.18)	412	1.07 (0.95–1.21)	155	1.03 (0.84–1.25)
Q5 (1.74)	629	1.17 (1.05–1.29)	476	1.23 (1.10–1.39)	153	1.00 (0.81–1.22)
P_{trend}		<0.001		<0.001		0.806
B(a)P (median, ng/1,000 kcal)						
Q1 (0.21)	551	1.0	401	1.0	150	1.0
Q2 (1.50)	583	1.02 (0.91–1.14)	427	1.03 (0.90–1.18)	156	1.00 (0.80–1.25)
Q3 (6.17)	531	0.96 (0.85–1.08)	397	0.99 (0.86–1.14)	134	0.88 (0.70–1.12)
Q4 (16.83)	491	0.86 (0.76–0.97)	363	0.88 (0.76–1.01)	128	0.81 (0.64–1.02)
Q5 (43.97)	563	0.96 (0.85–1.08)	407	0.96 (0.83–1.11)	156	0.95 (0.75–1.19)
P_{trend}		0.291		0.322		0.694
Mutagenic activity (median, revertant colonies/1,000 kcal)						
Q1 (165)	480	1.0	349	1.0	131	1.0
Q2 (601)	511	0.98 (0.86–1.11)	366	0.97 (0.83–1.12)	145	1.01 (0.79–1.28)
Q3 (1152)	556	1.04 (0.92–1.18)	404	1.05 (0.91–1.21)	152	1.02 (0.80–1.30)
Q4 (2042)	563	1.05 (0.93–1.19)	420	1.09 (0.94–1.26)	143	0.95 (0.74–1.21)
Q5 (4349)	609	1.14 (1.01–1.29)	456	1.19 (1.03–1.38)	153	1.01 (0.79–1.29)
P_{trend}		0.010		0.002		0.967

*Adjusted for gender, education (high school or less/unknown, post-high school/some college, college/postgraduate), BMI (<18.5, ≥18.5 to <25, ≥25 to <30, ≥30 to <35, ≥35, unknown), smoking (never smoker, former smoker who quit ≥10 y ago, former smoker who quit 1–9 y ago, current/those who quit <1 y ago, missing), and intake of total energy (kcal/d), fiber (g/1,000 kcal), and dietary calcium (mg/1,000 kcal).

†Dietary iron residually energy adjusted plus iron from supplements.

different for either red meat (HR, 1.15; 95% CI, 0.94–1.41; P_{trend} = 0.024; HR, 1.29; 95% CI, 1.00–1.66; P_{trend} = 0.018, respectively; $P_{heterogeneity}$ = 0.432) or processed meat (HR, 1.09; 95% CI, 0.89–1.33; P_{trend} = 0.245; HR, 1.10; 95% CI, 0.86–1.41; P_{trend} = 0.363, respectively; $P_{heterogeneity}$ = 0.497; data not shown). In a lag analysis excluding the first 2 years of follow-up (n = 1,941 colorectal cancer cases), the findings for both red and processed meat remained (HR, 1.21; 95% CI, 1.03–1.42; P_{trend} = 0.001; HR, 1.19; 95% CI, 1.02–1.39; P_{trend} = 0.013, respectively; data not shown).

Using the detailed meat questionnaire, we examined specific components of meat in relation to colorectal cancer (Table 3). Interestingly, total iron intake and dietary iron were both inversely associated with colorectal cancer (HR, 0.75; 95% CI, 0.66–0.86; P_{trend} < 0.001; HR, 0.75; 95% CI, 0.65–0.87; P_{trend} < 0.001, respectively), although the more bioavailable heme iron was positively associated (HR, 1.13; 95% CI, 0.99–1.29; P_{trend} = 0.022). Although nitrate intake from processed meats was positively associated with this malignancy (HR, 1.16; 95% CI, 1.02–1.32; P_{trend} = 0.001), the association for nitrite did not quite reach statistical significance (HR, 1.11; 95% CI, 0.97–1.25; P_{trend} = 0.055). When we examined the highest compared with the lowest quintile of combined nitrate and nitrite intake (data not shown), there was an elevated risk for colorectal cancer (HR, 1.14; 95% CI, 1.00–1.30; P_{trend} = 0.019). An analysis of total dietary exposure revealed an inverse association in the highest quintile of dietary nitrate (HR, 0.82; 95% CI, 0.71–0.95; P_{trend} = 0.111) but null findings for total nitrite (HR, 1.05; 95% CI, 0.92–1.21; P_{trend} = 0.316) and colorectal cancer (data not shown). The findings for total dietary nitrate are likely due to the largest dietary sources of nitrate in our population, which include several fruits and vegetables, such as spinach, broccoli, potatoes, and bananas.

Individuals in the highest compared with the lowest quintile of MeIQx and DiMeIQx had an elevated risk of colorectal cancer (HR, 1.19; 95% CI, 1.05–1.34; P_{trend} < 0.001; HR, 1.17; 95% CI, 1.05–1.29; P_{trend} < 0.001, respectively; Table 3). Neither PhIP nor B(a)P were associated with colorectal cancer; nevertheless, those in the highest quintile of mutagenic activity (a marker of all meat mutagens) had an elevated risk (HR, 1.14; 95% CI, 1.01–1.29; P_{trend} = 0.010). In subsite analyses, the risk estimates for colon and rectal cancers were similar for most of the meat-related exposures, except for MeIQx,

DiMeIQx, and mutagenic activity, which were only associated with colon cancer (HR, 1.26; 95% CI, 1.09–1.45; P_{trend} < 0.001; HR, 1.23; 95% CI, 1.10–1.39; P_{trend} < 0.001; HR, 1.19; 95% CI, 1.03–1.38; P_{trend} = 0.002, respectively; Table 3).

Discussion

In this large cohort, both red and processed meat intakes were positively associated with colorectal cancer. Our data suggest that these associations could be related to heme iron, nitrate, and the HCAs MeIQx and DiMeIQx formed in meats cooked at high temperatures.

The findings for red and processed meat from this study are in agreement with a recent and large summary of the epidemiologic literature (1); however, very few studies have investigated the various components of meat that may explain these associations. In contrast to red meat, white meat is not associated with an elevated risk of colorectal cancer; one of the main differences between red and white meat is the iron content. The contrasting findings in this study for total iron and dietary iron compared with heme iron from meat highlight the importance of distinguishing between heme iron, which is from meat, and non-heme iron, which is mainly from fortified cereals, fruit juice, and bread. Thus far, the newly developed heme iron database has only been used in one small screening study of colorectal adenoma, in which there was an elevated risk (odds ratio in the top compared with the bottom quartile of intake, 1.50; 95% CI, 0.83–2.73), although it was not statistically significant, possibly due to a small number of cases (n = 158; ref. 23). Other studies that have investigated heme iron may not be comparable because they estimated heme iron as a percentage of total iron from meat by using a standard percentage (40%; ref. 24) or by applying a percentage according to the animal the meat was derived from, for example, beef (65%), pork (39%), or chicken/fish (26%; refs. 24–26); none of these previous studies found an overall association between heme iron intake and colorectal cancer.

Whereas heme iron is thought to catalyze endogenous formation of NOCs (7), nitrate, and nitrite, which are added to processed meats, also contribute to exogenous formation of these compounds within the meat, although this reaction is minimized by the addition of ascorbic acid. Processed meat is typically the predominant source of human exposure to nitrite, but generally not the largest source of nitrate, which can also be reduced to nitrite by bacteria in the oral cavity and gastrointestinal tract. Nevertheless, processed meat contains all the necessary precursors for NOC formation, including nitrosating agents (derived from nitrite), as well as nitrosatable substrates in the form of amines and amides. In agreement with this hypothesis, we observed elevated risks for colorectal cancer for those in the highest quintile of nitrate intake from processed meats and a suggestive association with nitrite. The sources of nitrate and nitrite in processed meat in this population varied slightly. The largest source of nitrate from meat was red meat cold cuts (24%), hotdogs (22%), and bacon (19%); although the highest contributor to nitrite intake was also red meat cold cuts (39%), the second and third largest sources were poultry cold cuts (26%) and ham (24%). Other epidemiologic data on these exposures in relation to colorectal neoplasia is limited, but nitrate and nitrite intake from animal sources (27), processed meat (28), as well as individual NOCs (29) have been positively associated with colorectal neoplasia.

In addition to this NOC-related mechanism, meat is a source of potentially carcinogenic HCAs and PAHs, formed in meats cooked at high temperatures (9–11, 14). We observed a positive association for MeIQx, DiMeIQx, and mutagenic activity in relation to colorectal cancer, but not for PhIP or B(a)P. Examining the contributing variables to intake of each of these HCAs, we noted that the largest source of MeIQx (36%) and DiMeIQx (50%) was well-done barbecued hamburgers, whereas the largest source of PhIP (20%) was medium-done barbecued steak. Data regarding the role of HCAs in colorectal neoplasia are unclear, as other studies have found a positive association for MeIQx, but not other HCAs (23, 30). Additionally, some studies have reported B(a)P intake increases the risk of colorectal adenoma (31, 32).

HCAs, PAHs, and NOCs are activated and detoxified by phase I and phase II xenobiotic metabolizing enzymes; however, in epidemiologic studies, the evidence for an interaction between meat and meat-mutagen intake, expression of these enzymes, and colorectal neoplasia is inconsistent. Phenotyping studies have found associations between proxies of HCA intake (well-done meat) and higher activity of both phases I and II enzymes (33, 34). Furthermore, some genotyping studies have reported that the association between HCA intake and colorectal cancer risk differs according to phase II enzyme activity (35–37); however, a recent study was null (38). Furthermore, interactions between processed meat intake, phase I enzymes, and colorectal adenoma have been identified (39), but a study that estimated nitrate and nitrite intake from processed meat found that the relation of these compounds with colorectal adenoma was not modified by variation in phase I enzyme activity (28). The inconsistencies in these genetic studies may be due to inadequate statistical power to investigate interactions.

Based on our subsite analyses, the meat exposures seemed to be more strongly associated with distal tumors (distal colon or rectum), except for MeIQx and DiMeIQx, which increased the risk of colon, but not rectal, cancer. These data suggest that the various meat components may be acting at different locations within the colorectum. It is speculated that risk factors for colon and rectal cancer may vary due to subsite differences in, for example, rates of metabolism, fermentation, and transit time, as well as expression of enzymes and differences in morphology (40). Previous studies have also reported similar subsite differences, including that NOCs increase rectal cancer specifically (39), whereas HCAs increase the risk of colon, but not rectal, neoplasia (32).

This study had several strengths, including a wide range of meat intake and the administration of a detailed meat questionnaire enabling the investigation of multiple components of meat. Furthermore, the questionnaire was completed before diagnoses, which limited recall bias and reverse

causation. The limitations of this study include the possibility of some degree of measurement error, as is the case with any observational study; however, we attempted to minimize this error by adjusting our models for total energy intake (41). In the analyses of nitrate intake, we were unable to assess exposure from drinking water. We must also note that the heme iron database is still limited and, therefore, likely underestimates total heme iron intake. Lastly, it is possible that some residual confounding may remain.

In summary, red meat and processed meat were positively associated with colorectal cancer. Our analysis indicates that potential mechanisms underlying these associations include heme iron, nitrate/nitrite, and HCAs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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