



Heterocyclic Amines, Meat Intake, and Association with Colon Cancer in a Population-based Study

L. M. Butler¹, R. Sinha², R. C. Millikan³, C. F. Martin^{3,4}, B. Newman⁵, M. D. Gammon³, A. S. Ammerman⁶, and R. S. Sandler^{3,4}

¹ Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

² Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD.

³ Department of Epidemiology, University of North Carolina, Chapel Hill, NC.

⁴ Department of Medicine, University of North Carolina, Chapel Hill, NC.

⁵ School of Public Health, Queensland University of Technology, Brisbane, Australia.

⁶ Department of Nutrition, University of North Carolina, Chapel Hill, NC.

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The authors examined the association between colon cancer and meat intake categorized by level of doneness, cooking method, and estimated levels of heterocyclic amines (HCAs), benzo[*a*]pyrene, and mutagenicity. Data were collected as part of a population-based, case-control study of colon cancer in North Carolina between 1996 and 2000 that included 701 African-American (274 cases, 427 controls) and 957 White (346 cases, 611 controls) participants. Odds ratios were calculated by using unconditional logistic regression, comparing the fifth to the first quintile levels of intake or exposure. Intake of red meat was positively associated with colon cancer (odds ratio (OR) = 2.0, 95% confidence interval (CI): 1.3, 3.2). Associations with meat intake by cooking method were strongest for pan-fried red meat (OR = 2.0, 95% CI: 1.4, 3.0). Associations with meat intake by doneness were strongest for well-/very well done red meat (OR = 1.7, 95% CI: 1.2, 2.5). The strongest association for individual HCAs was reported for 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) across all levels of exposure, with odds ratios of 1.8–2.0. Overall, sophisticated exposure measures were used to report modest, positive associations between red meat intake and colon cancer consistent with the hypothesis that HCAs may be among the etiologically relevant compounds in red meat.

amines; case-control studies; colorectal neoplasms; meat; polycyclic hydrocarbons, aromatic

Abbreviations: DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; HCA, heterocyclic amine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; PAH, polycyclic aromatic hydrocarbon; PhIP, 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine.

Initial evidence from migrant (1) and international ecologic studies (2, 3), followed by case-control studies (2, 4), suggested that dietary factors were important contributors to the etiology of colon cancer (5, 6). However, many of the associations have been either refuted or weakened by findings from prospective studies (7), for example, studies of dietary fat (8, 9), vegetable and fruit (10, 11), and fiber intake (12–15). One dietary exposure repeatedly associated with risk of colon cancer is meat intake, specifically red meat, which has been consistently reported in numerous prospective analyses (15–20). Some of the compounds

formed during the cooking of meat, such as the heterocyclic amines (HCAs), 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP), and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) (21), and the most common polycyclic aromatic hydrocarbon (PAH) compound, benzo[*a*]pyrene, have been identified as possible human carcinogens (21–26). HCAs are most concentrated in meat juices (27). The optimal conditions for HCA formation are longer cooking times (28), internal temperatures of between 150°C and 200°C (29), and greater external charring (30), typically achieved with cooking methods such as barbe-

Correspondence to Dr. Lesley M. Butler, Epidemiology Branch, National Institute of Environmental Health Sciences, P.O. Box 12233, Mail Drop A3-05, Research Triangle Park, NC 27709 (e-mail: butler3@niehs.nih.gov).

cuing, grilling, and pan-frying (31, 32). Meat cooked above a heat source, by using methods such as grilling or barbecuing, contains the highest levels of PAHs, because it is exposed to smoke formed from the pyrolysis of fatty juices that drip down onto the heat source (33, 34).

The epidemiologic evidence for an etiologic role of HCAs and PAHs in colon carcinogenesis is inconsistent, most likely because assessment of dietary exposure to these compounds has not been very precise or accurate. Biomarkers have been designed to measure HCA metabolic products in urine, but they assess dietary exposure only within the past 12 hours of consumption, which is not likely to be a good measure of usual intake (35, 36). To assess long-term exposure, epidemiologic studies have relied on surrogates of HCA and PAH exposure by collecting data on frequency of meat consumption by degree of doneness and charring as well as cooking method. These are important characteristics of meat intake because they represent potentially modifiable risk factors for colon cancer, but they may not be accurate measures of exposure across populations. Recently, more specific assessment of dietary exposure to carcinogens has been made possible by HCA and PAH measurements in meat cooked to varying degrees of doneness by different methods, then attributing these levels of exposure to persons on the basis of their reported dietary intakes (27, 36–38).

In this population-based, case-control study of African Americans and Whites in North Carolina, we investigated the association between colon cancer and meat intake by cooking method and doneness, and MeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx), PhIP, benzo[*a*]pyrene, and mutagenicity exposure from meat.

MATERIALS AND METHODS

Study population

Data were collected from participants in the North Carolina Colon Cancer Study. Cases and controls were selected from 33 counties in North Carolina through a randomized recruitment approach (39, 40), wherein race-, sex-, and age-specific incidence rates between 1991 and 1993 were used to calculate selection probabilities that would result in approximately equal numbers of African-American and White cases and a control group that was approximately frequency matched to cases by race, age, and sex.

Cases were selected through a rapid ascertainment system (41) established in conjunction with the North Carolina Central Cancer Registry. Cases were eligible if they were aged 40–84 years at first primary diagnosis of invasive adenocarcinoma of the colon and were diagnosed between July 1, 1996, and June 30, 2000. Diagnoses were confirmed by the study pathologist on the basis of review of pathology slides and relevant medical records. Written consent to examine tissue and records was obtained from participants. Controls were randomly selected from North Carolina Division of Motor Vehicle lists if they were less than 65 years of age or from the Center for Medicare and Medicaid Services list if they were aged 65 years or older. The study was approved by the Institutional Review Board at the University

of North Carolina School of Medicine and by equivalent committees at the collaborating hospitals.

Completed interviews were obtained from 701 African Americans (274 cases, 427 controls) and 957 Whites (346 cases, 611 controls). Of those who were eligible, cooperation rates, or percentage of persons interviewed, were 84 percent among cases (81 percent for African-American men, 78 percent for African-American women, 90 percent for White men, 88 percent for White women), and 62 percent among controls (57 percent for African-American men, 60 percent for African-American women, 70 percent for White men, 61 percent for White women).

Dietary assessment

Questionnaires were administered in person in the participants' homes by specially trained registered nurses. The questionnaire collected information on lifestyle factors such as diet, physical activity, and tobacco use as well as medical, family, and work histories and use of over-the-counter medications. A 150-item food frequency questionnaire (42) was used to measure usual dietary intake over the year prior to diagnosis for cases or the year prior to date of selection for controls.

The food frequency questionnaire had been previously modified to include 29 foods commonly consumed in North Carolina to better assess regional dietary practices, as part of a study conducted in eastern North Carolina among African Americans (43). A second modification to the food frequency questionnaire was included to assess individual exposure to dietary carcinogens and mutagenicity based on a questionnaire developed by Sinha and Rothman (36). Briefly, questions were added to assess 14 meat and fish items (i.e., hamburgers/cheeseburgers, beefsteaks, pork chops/ham steaks, bacon, sausage, hot dogs, fried chicken, chicken/turkey and fried fish/shellfish/fish sandwich) regarding frequency of intake, portion size (i.e., small, medium, or large), and cooking method. For each meat item, a participant reported the consumption frequency for each cooking method, which included pan-fried, grilled/barbecued, and oven-broiled for red meat (i.e., hamburger, steak, pork chop, sausage, and bacon); baked/roasted, stewed, oven-broiled, and grilled/barbecued for chicken/turkey; and deep fat fried/fast food and pan-fried for fried chicken. Multiple digitized color photographs were shown of each meat type (i.e., hamburger, steak, pork chop, bacon, and chicken/turkey) to facilitate reporting of cooking doneness. In the analyses, responses were categorized into rare/medium, well done, and very well done (or into a joint well-/very well done category).

Meat intake frequency data, cooking method, and level of doneness were used to estimate values of three HCAs (MeIQx, PhIP, and DiMeIQx), one PAH (benzo[*a*]pyrene), and mutagenic activity by using an exposure index described in detail previously (27, 31, 34, 36–38, 44, 45). In addition to meat intake by cooking method and doneness, the index also incorporated information relevant to compound and mutagenicity exposure, such as consumption of meat gravies, consumption of chicken with the skin, and use of fat from fried bacon in cooking.

Variable coding

All meat, HCA, PAH, and mutagenic activity variables were derived from food frequency questionnaire responses. These continuous variables (g/day) included total meat (sum of red meat, white meat, meat from spaghetti sauce, and beef stew), red meat (sum of hamburger, steak, pork chop, sausage, and bacon), white meat (sum of chicken/turkey, fried chicken, tuna, fried fish), and fried chicken.

Derived variables for doneness and cooking methods were created for total meat, red meat, white meat, and fried chicken in units of g/day. Derived variables for MeIQx, DiMeIQx, PhIP, and benzo[*a*]pyrene were created in units of ng/day, and the mutagenicity variable was created in units of revertant colonies/day. The meat-derived compound and mutagenicity variables were derived by multiplying grams of meat intake (stratified by type, doneness, and cooking method) by the compound concentration (ng/day) or activity (revertant colonies/day) measured in that meat type. All compounds and mutagenicity values were derived from both red and white meat sources, with one exception. Although the association between PhIP and colon cancer was the same whether PhIP exposure values were derived from combined (red + white) meat sources or red-meat-only sources, we presented values derived from red meat intake only, because the PhIP content of grilled chicken can be variable and can add to misclassification of HCA exposure (45). All exposure variables were categorized into quintiles based on the distributions among controls—overall controls, among African-American and White controls separately, and among male controls and female controls separately—depending on the analysis. If a continuous variable had more than 20 percent zero values, then quantiles were created by including all zero values in the reference group and categorizing the remaining values into quartiles.

For continuous covariates, tertile cutpoints were determined on the basis of the distributions among all controls. These covariates included fruit, vegetable, dietary fiber, total fat, dietary folate, and total energy intake; physical activity; height; weight; and body mass index (kg/m²). Fat intake was adjusted for total caloric intake by using the residual method (46) to provide a measure of fat intake uncorrelated with total energy intake (47). Because total energy intake was associated with colon cancer, it was included in the model along with the energy-adjusted fat intake variable, as recommended by Willett (47). Physical activity in the past year (“in the year before your illness” for cases) was measured in metabolic-equivalent task-hours per day for combined occupational, nonoccupational, and nonwork/weekend activities. Height and weight were measured during the in-person interview. Cigarette smoking was categorized according to duration (years of smoking), with never smokers defined as persons who smoked fewer than 100 cigarettes during their life.

It was determined that participants were above or below the poverty line, as reported from US Census data, on the basis of their self-reported annual income, after accounting for the number of adults and children in the household. Residential type was coded as “urban” if persons lived in a Metropolitan Statistical Area as determined by the 1997 US

Census Bureau list and “rural” if they did not. Participants reported their race as African American, White, or other. The small number of persons who reported “other races” ($n = 11$) were categorized as White. Additional covariates also assessed for confounding and effect modification included approximate 5-year age groups (≤ 45 , 46–50, ..., ≥ 76 years), education (less than high school graduate, high school graduate/some college, \geq college graduate), regular use of nonsteroidal anti-inflammatory drugs (≤ 15 , > 15 times/month), first-degree relative with colorectal cancer (yes, no), and alcohol consumption (ever, never).

Statistical analyses

Adjusted odds ratios and 95 percent confidence intervals were calculated from unconditional logistic regression models (48). PROC LOGISTIC from the software package SAS (version 8.1; SAS Institute, Inc., Cary, North Carolina) was used with an option in the MODEL statement to incorporate offsets, which takes into account the selection probabilities by age, race, and sex. The offset term for each age-sex-race stratum was calculated from the selection probabilities used to identify eligible participants, as follows: $OFFSET = \ln[\text{Prob}(\text{Case})/\text{Prob}(\text{Control})]$ (40). Meat intake and meat-derived exposures were assessed by using categorical variables. First, odds ratios were calculated for all quintile meat intake variables (total meat, red meat, white meat, fried chicken), then separately by doneness and by cooking method with adjustment for age, race, and sex. Total meat intake was included in the models for red and white meat intake to isolate the estimated effects of the cooking method and doneness subgroups, independent of overall consumption levels. Odds ratios were also calculated for quintile variables of HCAs (MeIQx, DiMeIQx, and PhIP), PAH (benzo[*a*]pyrene), and mutagenic activity.

Adjusted models were created using forward and backward methods by including covariates in the model individually and jointly, then by evaluating the difference in the odds ratio for total meat, comparing the fifth with the first quintile of intake, between crude and adjusted models (49). If a 10 percent or greater change was observed in the odds ratio for total meat (fifth vs. first quintile), then the covariate was retained for all final adjusted models. Stratified analyses were used to assess possible heterogeneity of the odds ratios for total meat and colon cancer across levels of selected covariates (48).

RESULTS

Characteristics of the study population are presented, stratified by race, in table 1. The mean age of the North Carolina Colon Cancer Study population was 65 years. Among controls, there was a greater percentage of African Americans with a lower educational level compared with Whites, although statistically significant case-control differences were present among Whites only. There was a slightly greater percentage of ever smokers among White controls than among African-American controls. Statistically significant case-control differences were also found for smoking status and duration of smoking among Whites. In general,

TABLE 1. Characteristics of cases and controls in the North Carolina Colon Cancer Study, 1996–2000*

	African Americans				Chi-square <i>p</i> value	Whites				Chi-square <i>p</i> value
	Cases (<i>n</i> = 274)		Controls (<i>n</i> = 427)			Cases (<i>n</i> = 346)		Controls (<i>n</i> = 611)		
	No.	%	No.	%		No.	%	No.	%	
Age (years)										
40–49	37	13.5	28	6.5		26	7.5	35	5.7	
50–59	71	25.9	80	18.9		71	20.5	110	18.0	
60–69	90	32.9	136	31.4		119	34.4	205	33.6	
70–79	73	26.6	172	40.2		123	35.6	242	39.6	
≥80	3	1.1	11	3.0	<0.01	7	2.0	19	3.1	0.43
Sex: Female	143	52.2	243	56.9	0.22	155	44.8	281	46.0	0.72
Education										
Less than high school graduate	120	43.8	188	44.0		96	27.8	121	19.8	
High school graduate/some college	128	46.7	183	42.9		172	49.7	327	53.5	
College graduate	26	9.5	56	13.1	0.30	78	22.5	163	26.7	0.02
Cigarette smoking										
Never smoker	129	47.1	196	45.9		116	33.5	250	40.9	
Former smoker	88	32.1	142	33.3		182	52.6	263	43.0	
Current smoker for ≤35 years	30	10.8	41	9.6		21	6.1	34	5.6	
Current smoker for ≥36 years	26	10.9	47	11.0	0.85	27	7.8	63	10.3	0.03
Physical activity (average MET†-hours/day)										
≤32.2	79	30.4	171	40.7		80	23.5	159	26.4	
32.3–36.8	89	34.2	114	27.1		125	29.8	235	39.0	
≥36.9	92	35.4	135	32.1	0.02	136	32.4	209	34.7	0.26
Body mass index (kg/m ²)										
≤25.4	87	31.8	121	28.4		139	40.2	224	37.0	
25.5–29.8	88	32.1	121	28.4		111	32.1	224	37.0	
≥29.9	99	36.1	184	43.2	0.12	96	27.7	158	26.1	0.31
Dietary folate (μg/day)										
≤214.6	106	38.7	179	41.9		95	27.5	166	27.2	
214.7–311.3	88	32.1	134	31.4		128	37.0	211	34.5	
≥311.4	80	29.2	114	26.7	0.66	123	35.6	234	38.3	0.66
Dietary fiber (g/day)										
≤10.7	117	42.7	163	38.2		105	30.4	182	29.8	
10.8–15.3	84	30.7	154	36.1		129	37.3	191	31.3	
≥15.4	73	26.6	110	26.8	0.31	112	32.4	238	39.0	0.08
Dietary fat (g/day)										
≤57.1	80	29.2	152	35.6		94	27.2	190	31.1	
57.2–83.0	66	24.1	133	31.2		76	22.0	215	35.2	
≥83.1	128	46.7	142	33.2	<0.01	176	50.9	206	33.7	<0.01
Total energy (kcal/day)										
≤1,430.1	82	29.9	166	38.9		81	23.4	177	29.0	
1,430.2–1,982.9	65	24.3	127	29.7		101	29.2	220	36.0	
≥1,983.0	127	45.8	134	31.4	<0.01	164	47.4	214	35.0	<0.01

* Values for the following variables were missing: cigarette smoking (*n* = 3), physical activity (*n* = 34), body mass index (*n* = 6).

† MET, metabolic-equivalent task.

for dietary factors, controls consumed similar levels of folate, similar levels of fiber, less fat, and less energy compared with cases, regardless of race.

Patterns of meat intake by doneness, cooking method, meat-derived HCA and PAH compounds, and mutagenicity exposure, stratified by race, are shown in table 2. Among

controls, differences by race included greater intakes of pan-fried red meat, well-/very well done red meat, white meat, and pan-fried chicken and higher MeIQx, DiMeIQx, and mutagenicity exposure among African Americans, and greater grilled/barbecued red meat intake and benzo[*a*]pyrene exposure among Whites. Categories of meat

TABLE 2. Means and standard deviations of meat intake, meat-derived heterocyclic amine and polycyclic aromatic hydrocarbon compounds, and mutagenicity exposure for cases and controls, North Carolina Colon Cancer Study, 1996–2000

	African Americans					Whites					<i>t</i> -test <i>p</i> value*
	Cases (<i>n</i> = 274)		Controls (<i>n</i> = 427)		<i>t</i> -test <i>p</i> value	Cases (<i>n</i> = 346)		Controls (<i>n</i> = 611)		<i>t</i> -test <i>p</i> value	
	Mean	SD†	Mean	SD		Mean	SD	Mean	SD		
Meat (g/day)											
Total meat	131.7	78.7	114.4	68.0	<0.01	116.0	76.4	103.1	55.5	<0.01	<0.01
Red meat‡	45.6	36.5	34.7	28.9	<0.01	43.5	38.2	33.1	25.9	<0.01	0.33
By cooking method											
Pan-fried	25.1	25.9	17.8	20.5	<0.01	20.5	25.0	13.0	16.1	<0.01	<0.01
Baked	1.4	4.0	1.1	2.8	0.42	1.7	4.6	1.9	4.7	0.63	<0.01
Grilled/barbecued	6.9	14.9	5.3	11.1	0.10	14.4	19.1	12.8	17.4	0.18	<0.01
By doneness											
Well/very well done	39.1	31.5	30.4	27.7	<0.01	32.6	33.9	24.2	22.7	<0.01	<0.01
White meat‡	51.1	37.4	47.4	35.1	0.20	35.9	27.1	38.2	28.3	0.23	<0.01
Chicken/turkey by cooking method											
Pan-fried	8.4	14.4	5.7	11.9	0.01	2.8	7.1	2.1	5.3	0.11	<0.01
Chicken/turkey by doneness											
Very well done	7.4	22.3	5.7	13.5	0.22	4.8	13.4	4.2	12.3	0.48	0.06
Meat-derived compounds (ng/day)											
MeIQx†	79.2	70.8	62.1	65.4	<0.01	70.6	78.1	51.0	55.3	<0.01	<0.01
DiMeIQx†	5.6	5.6	4.7	6.0	0.05	4.9	6.3	3.8	4.8	<0.01	<0.01
PhIP†	93.2	110.1	95.5	170.6	0.84	114.0	168.7	86.7	134.2	0.01	0.35
Benzo[a]pyrene	22.5	42.5	16.7	31.0	0.04	41.9	59.4	35.4	52.6	0.08	<0.01
Meat-derived mutagenicity (revertant colonies × 10 ³)	10.3	9.8	9.6	14.3	0.50	10.0	12.9	7.5	8.8	<0.01	<0.01

* The *t*-test statistic compares mean values between African-American and White controls.

† SD, standard deviation; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; PhIP, 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine.

‡ All red meat intake by cooking method (i.e., pan-fried, grilled/barbecued, and oven-broiled) adds up to total red meat, and all red meat intake by doneness level (i.e., rare/medium, well-/very well done) adds up to total red meat. All chicken/turkey intake by cooking method (i.e., baked/roasted, stewed, deep fat fried/fast food, pan-fried, grilled/barbecued, and oven-broiled) adds up to total chicken/turkey, and all chicken/turkey intake by doneness level (i.e., rare/medium and well-/very well done) adds up to total chicken/turkey. Chicken/turkey and fish intake add up to total white meat.

intake that were higher among cases compared with controls were similar for African Americans and Whites, with the exception of pan-fried chicken, with no difference found among Whites. Significantly higher levels of meat-derived HCA and PAH compounds and mutagenicity exposures among cases were observed more consistently among Whites. Among controls, regardless of race, the strongest correlations for DiMeIQx, MeIQx, PhIP, and mutagenicity were with well-/very well done red meat; the strongest correlation for benzo[a]pyrene was with grilled/barbecued red meat (table 3).

Multivariable-adjusted odds ratios for colon cancer were calculated by type of meat, cooking method, and doneness level (table 4). In general, no differences were found by race or sex (data not shown), so groups were combined for greater precision. There was no association with total meat; for red meat, however, the odds ratio increased with greater intake, resulting in a modest positive association when the highest was compared with the lowest levels of intake. Dose-response trends were similar for consumption of well-/very well done red meat but not for rare/medium-red meat. Regarding cooking method, consumption of only pan-fried

red meat had a modest positive association with colon cancer; there was no association with baked, broiled, or grilled/barbecued red meat. A weak inverse association was observed between white meat consumption and colon cancer when we compared the highest with the lowest levels of consumption. This association was attenuated when doneness level was considered (data not shown). However, consumption of pan-fried chicken had a weak positive association with colon cancer. With regard to white meat intake by cooking method, weak inverse associations were observed for broiled and grilled/barbecued chicken, and no association was seen with baked chicken, stewed chicken (data not shown), or fried fish.

Multivariable-adjusted odds ratios for colon cancer were calculated for meat-derived HCA and PAH compounds and mutagenicity exposure, stratified by race (table 5). For individual HCAs, DiMeIQx was independently associated with colon cancer, whereas other HCAs were not. There was no evidence of a dose-response relation; rather, persons who consumed the lowest levels appeared to be at the lowest risk for colon cancer. A weak positive association was found for higher levels of mutagenicity, but a monotonic relation was

TABLE 3. Spearman rank correlation coefficients between meat intake and meat-derived compounds and mutagenicity among controls, North Carolina Colon Cancer Study, 1996–2000

	African Americans					Whites				
	Meat-derived compounds				Meat-derived mutagenicity	Meat-derived compounds				Meat-derived mutagenicity
	DiMeIQx†	MeIQx†	PhIP†	BaP†		DiMeIQx	MeIQx	PhIP	BaP	
Meat										
Total meat	0.49*	0.62*	0.44*	0.41*	0.60*	0.40*	0.53*	0.44*	0.44*	0.55*
Red meat‡	0.55*	0.69*	0.54*	0.41*	0.63*	0.48*	0.65*	0.54*	0.42*	0.57*
By cooking method										
Pan-fried	0.52*	0.73*	0.39*	0.33*	0.62*	0.58*	0.75*	0.39*	0.04	0.57*
Baked	0.0	−0.03	0.05	0.0	0.01	0.03	0.04	0.02	0.07	0.04
Grilled/ barbecued	0.22*	0.26*	0.34*	0.65*	0.27*	0.26*	0.32*	0.53*	0.83*	0.36*
By doneness										
Well/very well done	0.64*	0.83*	0.65*	0.50*	0.77*	0.65*	0.84*	0.62*	0.33*	0.70*
White meat‡	0.22*	0.25*	0.12**	0.25*	0.30*	0.13*	0.13*	0.11**	0.27*	0.28*
Chicken/turkey by cooking method										
Pan-fried	0.24*	0.27*	0.12**	0.25*	0.31*	0.26*	0.28*	0.16*	0.16*	0.25*
Chicken/turkey by doneness										
Very well done	0.21*	0.13**	0.18*	0.09	0.13**	0.18*	0.21*	0.13*	0.02	0.15*

* $p < 0.01$; ** $p < 0.05$.

† DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; HCA, heterocyclic amine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; PhIP, 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine; BaP, benzo[a]pyrene.

‡ All red meat intake by cooking method (i.e., pan-fried, grilled/barbecued, and oven-broiled) adds up to total red meat, and all red meat intake by doneness level (i.e., rare/medium, well-/very well done) adds up to total red meat. All chicken/turkey intake by cooking method (i.e., baked/roasted, stewed, deep fat fried/fast food, pan-fried, grilled/barbecued, and oven-broiled) adds up to total chicken/turkey, and all chicken/turkey intake by doneness level (i.e., rare/medium and well-/very well done) adds up to total chicken/turkey. Chicken/turkey and fish intake add up to total white meat.

not observed. Associations with benzo[a]pyrene, stratified by race, were imprecise, but stronger effects were seen among African Americans (odds ratio = 1.7, 95 percent confidence interval: 0.9, 3.2) than among Whites (odds ratio = 0.9, 95 percent confidence interval: 0.6, 1.5). There were no differences between men and women regarding meat-derived HCA and PAH compounds or mutagenicity exposure and colon cancer.

DISCUSSION

We investigated the association between meat intake and colon cancer in a population-based, case-control study in North Carolina. The study included information on type of meat; doneness; cooking method; MeIQx, DiMeIQx, PhIP, and benzo[a]pyrene exposure; and meat-derived mutagenicity. Moderate positive associations with increasing intake were observed for red meat, specifically for well-/very well done and pan-fried red meat and, to a lesser extent, pan-fried chicken. Of the meat-derived compounds, DiMeIQx was most strongly associated with colon cancer after adjustment for all other HCAs and dietary covariates.

Cooking method and doneness clearly affect the mutagen and carcinogen levels of meat (27, 32, 37, 38, 45). Our data support this observation, with a stronger association evident with increased consumption of well-/very well done red meat compared with rare/medium-done red meat. Similar results have been found in some studies of colon cancer (50, 51) and adenomas (52), but not all (53, 54). A possible explanation for the inconsistent findings is that these studies,

with one exception (52), did not incorporate meat doneness photographs in their exposure assessment. The importance of using photographs of the level of doneness of meat to reduce misclassification of exposure was illustrated in a study by Keating et al. (55). Meat samples cooked at home by study participants were photographed, then sent in for HCA quantification. After comparing the categorization of doneness levels by self-report versus an independent assessment of the photographs, the authors concluded that showing meat doneness photographs provided a person with a less subjective definition of meat doneness, which resulted in better classification into more representative categories based on HCA levels. Our data may therefore have been less subject to misclassification of exposure than those in previous studies, since we used doneness photographs for five meats in conjunction with frequency of meat intake and cooking methods to estimate HCA exposure.

For cooking methods assessed, we reported the strongest association for pan-fried red meat, less so for chicken. Of the few studies that have evaluated meat consumption by cooking method, one case-control study of colorectal adenomas reported a positive association with pan-fried red meat (52), but no association was reported in other case-control (51, 56) or prospective (57, 58) studies of colon cancer. Both prospective studies were conducted in Finnish populations, in which fried meat is typically cooked at low temperatures and eaten as mixed dishes (58). Frying temperature may be relevant, as indicated from findings of a case-control study in which high- versus medium-/low-temperature frying was modestly associated with colon cancer (51).

TABLE 4. Odds ratios and 95 percent confidence intervals for colon cancer and meat intake by quintiles of intake, doneness, and cooking method, North Carolina Colon Cancer Study, 1996–2000

	Cases (n = 620)	Controls (n = 1,038)	OR*,†	95% CI*	OR‡	95% CI
Total meat (g/day)						
≤56.8§	97	206	1.0		1.0	
56.9–84.7	121	208	1.2	0.9, 1.7	1.1	0.8, 1.5
84.8–111.5	113	208	1.1	0.8, 1.5	0.9	0.6, 1.3
111.6–150.0	121	209	1.1	0.8, 1.5	0.8	0.6, 1.3
>150.0	168	207	1.4	1.0, 2.0	0.9	0.6, 1.4
Red meat (g/day)						
≤11.8§	97	207	1.0		1.0	
11.9–22.4	90	207	1.0	0.7, 1.4	0.9	0.6, 1.3
22.5–33.6	99	208	1.1	0.8, 1.6	1.0	0.7, 1.5
33.7–51.8	138	209	1.7	1.2, 2.5	1.5	1.0, 2.2
>51.8	196	207	2.5	1.6, 3.8	2.0	1.3, 3.2
Rare/medium-done red meat (g/day)						
0.0§	384	620	1.0		1.0	
0.1–8.1	65	104	1.1	0.8, 1.5	1.1	0.8, 1.5
8.2–13.3	38	105	0.6	0.4, 0.9	0.6	0.4, 0.9
13.4–22.7	40	104	0.6	0.4, 0.9	0.6	0.4, 0.9
>22.7	93	105	1.2	0.9, 1.7	1.2	0.9, 1.7
Well-/very well done red meat (g/day)						
≤5.9§	95	208	1.0		1.0	
6.0–16.1	100	207	1.1	0.8, 1.5	1.0	0.7, 1.4
16.2–25.7	98	207	1.1	0.8, 1.6	1.0	0.7, 1.4
25.8–42.7	135	209	1.5	1.1, 2.2	1.3	0.9, 1.9
>42.7	192	207	2.1	1.4, 3.1	1.7	1.2, 2.5
Baked red meat (g/day)						
0.0§	476	791	1.0		1.0	
0.1–2.9	38	53	1.3	0.8, 2.0	1.4	0.9, 2.1
3.0–5.0	40	69	1.0	0.6, 1.5	1.0	0.6, 1.5
5.1–7.7	22	60	0.6	0.3, 1.0	0.6	0.3, 1.0
>7.7	44	65	1.1	0.7, 1.6	1.1	0.7, 1.7
Pan-fried red meat (g/day)						
0.0§	81	198	1.0		1.0	
0.01–5.3	96	210	1.1	0.8, 1.6	1.1	0.8, 1.6
5.4–12.7	111	209	1.4	1.0, 2.0	1.3	0.9, 1.9
12.8–25.2	133	210	1.7	1.2, 2.4	1.5	1.0, 2.2
>25.2	199	211	2.5	1.7, 3.5	2.0	1.4, 3.0
Broiled red meat (g/day)						
0.0§	388	682	1.0		1.0	
0.1–4.9	54	89	1.1	0.8, 1.7	1.1	0.8, 1.6
5.0–9.9	55	88	1.1	0.8, 1.6	1.2	0.8, 1.7
10.0–16.5	55	87	1.1	0.7, 1.5	1.1	0.7, 1.6
>16.5	68	92	1.2	0.9, 1.7	1.3	0.9, 1.9

Table continues

It has also been suggested that grilling or barbecuing beef, compared with other methods, results in the highest HCA levels (52), but we did not find a corresponding association.

An explanation for inconsistencies in studies of cooking methods may relate to differences in cooking practices by country and/or region. For example, in North Carolina,

TABLE 4. Continued

	Cases (n = 620)	Controls (n = 1,038)	OR*,†	95% CI*	OR‡	95% CI
Grilled/barbecued red meat (g/day)						
0.0§	268	461	1.0		1.0	
0.1–5.7	80	145	0.9	0.7, 1.3	0.9	0.6, 1.2
5.8–12.5	85	144	0.9	0.7, 1.3	0.9	0.7, 1.3
12.6–22.7	90	145	1.0	0.7, 1.3	1.0	0.7, 1.3
>22.7	97	143	0.9	0.7, 1.3	0.9	0.6, 1.3
White meat (g/day)						
≤17.8§	117	207	1.0		1.0	
17.9–29.1	120	207	0.9	0.6, 1.3	0.9	0.7, 1.3
29.2–40.4	131	208	0.9	0.6, 1.3	1.0	0.7, 1.4
40.5–62.6	137	209	0.9	0.6, 1.2	1.0	0.7, 1.4
>62.6	115	207	0.6	0.4, 0.9	0.7	0.4, 1.0
Baked chicken						
0.0§	211	354	1.0		1.0	
0.1–5.1	112	165	1.2	0.9, 1.6	1.2	0.9, 1.6
5.2–12.3	102	159	1.1	0.8, 1.5	1.1	0.8, 1.5
12.4–23.9	107	178	1.0	0.8, 1.3	1.1	0.8, 1.5
>23.9	88	182	0.7	0.5, 1.0	0.9	0.6, 1.2
Pan-fried chicken (g/day)						
0.0§	375	693	1.0		1.0	
0.1–3.6	41	78	1.0	0.7, 1.5	1.0	0.6, 1.4
3.7–7.3	60	87	1.2	0.8, 1.7	1.1	0.7, 1.6
7.4–12.7	52	80	1.2	0.8, 1.8	1.1	0.8, 1.6
>12.7	92	100	1.5	1.1, 2.1	1.4	1.0, 2.0
Broiled chicken (g/day)						
0.0§	559	926	1.0		1.0	
0.1–4.6	16	22	1.3	0.7, 2.6	1.4	0.7, 2.8
4.7–11.9	17	34	0.9	0.5, 1.6	0.9	0.5, 1.6
12.0–20.6	17	28	0.9	0.5, 1.7	0.9	0.5, 1.8
>20.6	11	28	0.6	0.3, 1.2	0.7	0.3, 1.4
Grilled/barbecued chicken (g/day)						
0.0§	489	809	1.0		1.0	
0.1–4.6	22	46	0.8	0.5, 1.3	0.7	0.4, 1.2
4.7–12.0	46	68	1.1	0.7, 1.6	1.1	0.7, 1.6
12.1–13.7	29	53	0.8	0.5, 1.3	0.8	0.5, 1.4
>13.7	34	62	0.8	0.5, 1.2	0.8	0.5, 1.3
Fried fish (g/day)						
0.0§	161	319	1.0		1.0	
0.1–3.5	81	160	1.0	0.7, 1.4	1.0	0.7, 1.3
3.6–7.0	132	189	1.3	1.0, 1.8	1.4	1.0, 1.9
7.1–12.1	132	208	1.2	0.9, 1.6	1.2	0.9, 1.7
>12.1	114	162	1.2	0.9, 1.7	1.2	0.9, 1.7

* OR, odds ratio; CI, confidence interval.

† The total meat model was adjusted for age, race, sex, and offsets. Remaining meat models were adjusted for total meat, age, race, sex, and offsets.

‡ The total meat model was adjusted for age, race, sex, energy-adjusted fat intake, energy intake, fiber intake, and offsets. Remaining meat models were adjusted for total meat intake in addition to the covariates listed previously.

§ Referent.

TABLE 5. Odds ratios and 95 percent confidence intervals for colon cancer and meat-derived compounds and mutagenicity, by quintile, among African Americans and Whites, North Carolina Colon Cancer Study, 1996–2000

	Total					African Americans					Whites				
	Cases (n = 620)	Controls (n = 1,038)	Quintile median*	OR†,‡	95% CI†	Cases (n = 274)	Controls (n = 427)	Quintile median*	OR‡	95% CI	Cases (n = 346)	Controls (n = 611)	Quintile Median*	OR‡	95% CI
MeIQx† (ng/day)															
Q1§	80	207	4.3	1.0		32	84	4.1	1.0		46	121	4.0	1.0	
Q2	101	207	18.9	0.8	0.6, 1.3	52	86	22.7	1.1	0.6, 2.1	55	123	16.5	0.9	0.5, 1.6
Q3	110	206	36.6	0.8	0.5, 1.3	51	85	43.4	0.8	0.4, 1.8	49	123	33.7	0.8	0.4, 1.5
Q4	148	210	66.1	1.0	0.6, 1.6	51	86	71.1	0.7	0.3, 1.5	102	121	59.9	1.6	0.8, 3.2
Q5	181	208	124.2	1.1	0.6, 2.0	88	86	140.2	1.2	0.5, 3.2	94	123	114.7	1.2	0.5, 2.7
DiMeIQx† (ng/day)															
Q1§	64	207	0.0	1.0		26	85	0.0	1.0		39	121	0.0	1.0	
Q2	114	208	1.0	1.8	1.2, 2.7	52	86	1.0	1.8	0.9, 3.5	71	123	0.9	1.7	1.0, 2.9
Q3	126	208	2.4	2.0	1.3, 3.1	51	85	2.2	1.7	0.8, 3.6	66	123	2.4	1.6	0.9, 2.9
Q4	149	208	4.6	2.0	1.2, 3.2	69	86	4.6	2.4	1.1, 5.3	79	122	4.6	1.3	0.7, 2.4
Q5	167	207	10.3	1.8	1.1, 3.1	76	85	10.9	2.1	0.9, 5.1	91	122	9.9	1.3	0.7, 2.6
PhIP† (ng/day)															
Q1§	92	207	0.0	1.0		33	85	0.0	1.0		56	121	0.1	1.0	
Q2	104	208	16.9	0.9	0.6, 1.5	45	85	10.2	1.1	0.6, 2.1	59	122	20.4	0.9	0.6, 1.5
Q3	122	207	44.9	1.0	0.7, 1.5	66	85	38.0	1.6	0.9, 3.1	59	123	47.7	0.8	0.5, 1.3
Q4	142	209	90.9	1.0	0.7, 1.5	69	87	96.6	1.4	0.7, 2.7	66	125	84.2	0.8	0.4, 1.3
Q5	160	207	218.5	0.9	0.6, 1.5	61	85	222.6	0.9	0.4, 1.9	106	120	210.7	1.1	0.7, 2.0
BaP† (ng/day)															
Q1§	84	207	0.5	1.0		28	85	0.5	1.0		60	122	0.5	1.0	
Q2	128	208	2.1	1.3	0.9, 1.8	56	86	1.5	2.1	1.2, 3.6	63	122	2.6	0.9	0.6, 1.4
Q3	139	208	6.9	1.2	0.9, 1.8	47	85	4.3	1.4	0.8, 2.5	81	123	16.9	1.1	0.7, 1.7
Q4	130	207	27.8	1.2	0.8, 1.7	64	86	10.5	1.6	0.9, 2.9	56	122	39.8	0.7	0.5, 1.2
Q5	139	208	78.2	1.2	0.8, 1.7	79	85	60.9	2.0	1.1, 3.6	86	122	87.9	1.1	0.7, 1.7
Mutagenicity (revertants × 10³)															
Q1§	81	207	0.8	1.0		31	85	0.7	1.0		50	122	0.8	1.0	
Q2	91	208	2.9	1.0	0.7, 1.5	42	86	2.8	1.4	0.8, 2.6	49	122	2.9	0.8	0.5, 1.4
Q3	127	208	5.2	1.3	0.9, 1.9	53	85	5.6	1.5	0.9, 2.7	73	123	5.0	1.2	0.8, 1.9
Q4	160	207	9.2	1.6	1.1, 2.2	76	85	9.6	2.1	1.2, 3.7	89	121	8.9	1.4	0.9, 2.2
Q5	161	208	17.6	1.4	1.0, 2.0	73	86	17.5	1.7	0.9, 3.1	85	123	17.6	1.2	0.8, 2.0

* Median value calculated for each quintile (Q) on the basis of the distribution among all controls, African-American controls, and White controls.

† OR, odds ratio; CI, confidence interval; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; PhIP, 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine; BaP, benzo[a]pyrene.

‡ The heterocyclic amine model was adjusted for all heterocyclic amines (DiMeIQx, PhIP, MeIQx), age, race, sex, energy-adjusted fat intake, energy intake, fiber intake, and offsets. The BaP and mutagenicity models were adjusted for age, race, sex, energy-adjusted fat intake, energy intake, fiber intake, and offsets.

§ Referent.

barbecuing is a slow-roasting method with a vinegar-based marinade, not synonymous with the high-temperature grilling that results in charred meat surfaces termed “barbecued” in other regions. It is possible that these methods are still important in terms of quantifying HCA and PAH exposure, because the lack of association with grilled/barbecued meat in this study may be due to the fact that we asked about grilling and barbecuing methods together in one question.

From meat-derived HCA estimates, we reported the strongest association between DiMeIQx and colon cancer. Possible HCA exposure misclassification may have resulted from not accounting for other sources of exposure variability, such as the addition of marinades (59) or the use of

the microwave to thaw meat (60), which reduce HCA formation. Other possible contributions to variability of HCA formation are the different cuts of meat consumed (55) and/or cooking technique, such as how often the meat is flipped when pan-frying. These sources of HCA variability were not measured in our study and may lead to nondifferential misclassification of HCA exposure yielding biased estimates in either direction because more than two exposure categories were compared (61). However, the major sources of exposure variability were measured (e.g., doneness and cooking method), making it unlikely that the association with DiMeIQx or the lack of associations with MeIQx and PhIP were due to misclassification.

We know of only one previous study of colon cancer that also used estimates of individual-level exposure to dietary HCAs (62). In this population-based, case-control study by Augustsson et al., contrary to expectation, controls consumed slightly higher levels of HCAs (77 ng/day) than cases did (66 ng/day) (62). This finding resulted in moderate inverse associations for HCA-specific risk estimates for MeIQx, PhIP, and DiMeIQx (62). The meat consumption habits of this Swedish population have been described and indicate that estimates were generally lower than in other populations (63, 64). Even though levels of exposure were somewhat lower, these findings lend support to an alternative hypothesis that compounds other than HCAs from meat sources, such as PAHs, or *N*-nitroso compounds, from endogenous (65) or processed meat sources, may be relevant to colon carcinogenesis (16, 18–20, 66).

A strength of the present study is inclusion of an ethnically diverse population; this study is one of the largest to date of colon cancer among African Americans, who have a higher colorectal cancer incidence and mortality than Whites in the United States (67, 68). Compared with Whites in this control population, African Americans consumed higher levels of white meat, pan-fried meat, and well-/very well done red meat and had higher meat-derived MeIQx and DiMeIQx exposures and mutagenicity. It is probable that if the estimated PhIP exposure had included sources from chicken, the levels of exposure would also have been statistically significantly higher among African Americans. Most notable is the lack of differences in the associations between categories of meat intake and colon cancer by race. The only difference in association with colon cancer by race was observed for the specific meat-derived PAH compound, benzo[*a*]pyrene, with stronger associations found among African Americans. These estimates were imprecise; therefore, observed differences may have been due to chance. However, if these differences are real, then identifying cooking methods or doneness preference with a cultural and/or ethnic basis may be relevant to decreasing exposures to specific PAH compounds.

Potential sources of bias in this study include differential recall. It is plausible that cases may recall usual diet differently from controls because of the impact of disease on dietary habits, which could result in odds ratios biased toward or away from the null value (61). Selection bias represents another potential source of error, as indicated by an overall response rate (number interviewed/number eligible) of 61 percent, with a 16 percent greater response among cases than controls. However, the cooperation rates for our study were similar to those for other population-based studies. There may have been differential selection based on relevant covariates or factors associated with the main hypothesis that could have biased these findings. Two previously conducted population-based, case-control studies of breast cancer assessed differences between respondents and nonrespondents by administering a condensed version of the questionnaire to nonrespondents (69, 70). These studies suggest that differences in educational level, race, or dietary factors between respondent and nonrespondent cases and controls are unlikely to be an important source of bias, but we cannot exclude this possibility in our study with certainty.

In summary, we reported moderate, dose-dependent associations between colon cancer and red meat intake, in particular for well-/very well done red meat and pan-fried red meat. Our data suggest that HCAs, such as DiMeIQx, may be among the etiologically relevant compounds in cooked meat. Although we found no association with the PAH benzo[*a*]pyrene, this compound may still have etiologic relevance for colon cancer, but perhaps only at higher levels of exposure from such nondietary sources as cigarette smoke. Identification of cooking method and doneness combinations correlated with HCA and/or PAH levels could be useful in educating the meat-eating public on “less risky” meat preparation methods such as marinating, precooking in the microwave, and flipping meat frequently while grilling. A very specific recommendation for changes in meat preparation may be more successful and offer a greater public health benefit for reducing colon cancer incidence than recommendations for general reduction of meat in the diet. Future studies of gene-environment interactions between carcinogen metabolism loci and these compounds may also be useful to further define the etiology of specific compounds in meat and their association with colon cancer (71, 72).

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