Research Article

Cancer Prevention Research

Association of Meat Intake and Meat-Derived Mutagen Exposure with the Risk of Colorectal Polyps by Histologic Type

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Abstract

The association of meat intake and meat-derived mutagens with colorectal tumor risk remains unclear. We evaluated this hypothesis in a large colonoscopy-based case–control study. Included in the study were 2,543 patients with polyp [(1,881 with adenomas and 622 with hyperplastic polyp (HPP)] and 3,764 polyp-free controls. Surveys obtained information about meat intake by cooking methods and doneness levels plus other suspected or known risk factors for colorectal tumors. Unconditional logistic regression was used to derive ORs after adjusting for potential confounders. High intake of red meat and processed meat ($P_{trend} < 0.05$), particularly red meat cooked using high-temperature cooking methods ($P_{trend} \leq 0.01$), was associated with an elevated risk for colorectal polyps. A significant positive association between exposures to meat-derived heterocyclic amines (HCA) and risk of polyps was found for both adenomas and HPPs. Furthermore, the positive association with red meat intake and HCA exposure was stronger for multiple adenomas than for single adenoma as well as for serrated than for nonserrated adenomas. This study supports a role for red meat and meat-derived mutagen exposure in the development of colorectal tumor. *Cancer Prev Res; 4(10); 1686–97. ©2011 AACR.*

Introduction

High meat intake has been shown to be associated with an increased risk of colorectal cancer (1, 2). Heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH) are mutagens found in meats cooked at high temperature (3–5), which may explain part of the positive association between meat intake and colorectal cancer risk. Colorectal adenomatous polyps are well-established precursors of colorectal cancer (6, 7). Hyperplastic polyps (HPP), on the other hand, have long been considered not to be cancer precursors. However, growing evidence suggests that some HPPs may have neoplasm potential through an alternate pathway from the adenoma–carcinoma pathway (8–13). Over the past 15 years, several studies have evaluated the association of meat intake with colorectal adenoma risk (14–22). However, only a few of these studies used a questionnaire designed to assess well-done meat intake and HCA exposures (14–17, 19–22), and most previous studies had a relatively small sample size (14–16, 18, 19, 21, 22). Furthermore, most studies did not address the association of meat intake with HPP risk or the association with adenomas by histologic types (14–19, 21, 22). Therefore, potential association of meat intake in the risk of colorectal polyps has not been investigated adequately.

In 2007, we reported that well-done meat intake and HCA exposure may be associated with an elevated risk of colorectal polyps in a colonoscopy-based case–control study including approximately 2,500 participants (20). Most of the associations evaluated in that article, however, were not statistically significant owing to a small sample size. Since then, we have recruited additional participants to bring the total sample size of the study to 6,307 subjects, making this study the largest investigation of its kind. In this report, we reevaluated the association of meat intake and meat-derived mutagen exposure in relation to colorectal polyps and further investigated this association by histology subtypes.

Materials and Methods

Study population

The Tennessee Colorectal Polyp Study is a colonoscopy-based case–control study conducted in Nashville, TN. Detailed methods used in this study have been described elsewhere (20, 23). Briefly, eligible participants aged 40 to 75 years were identified from patients scheduled for colonoscopy at an academic medical center (Vanderbilt

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Medical Center) and a Veterans Affairs medical center (Tennessee Valley Health System, Nashville, TN) between February 1, 2003, and March 26, 2010. Excluded from our study were participants who had genetic colorectal cancer syndromes (e.g., hereditary nonpolyposis colorectal cancer or familial adenomatous polyposis), a history of inflammatory bowel disease, adenomatous polyps, or any cancer other than nonmelanoma skin cancer. The study was approved by the Vanderbilt University Institutional Review Board, the Veterans' Affairs Institutional Review Board, and the Veterans' Affairs Research and Development Committee.

Among 10,074 eligible participants, 7,330 (72.8%) provided written informed consent and 6,638 participated in the study. Among them, 5,744 were recruited before colonoscopy and 894 were recruited after colonoscopy. All of them were successfully interviewed soon after colonoscopy. Among those interviewed, 6,331 (86.4% of responders) completed a telephone interview. There were 6,307 participants who completed a meat-intake questionnaire (including 5,449 recruited before and 858 recruited after colonoscopy), and 5,489 (74.9% of responders) completed a food frequency questionnaire (FFQ) developed (24) and validated (25) for a similar southern U.S. population. On the basis of the colonoscopy and pathologic findings, participants were assigned as patients with adenomas or HPP only. Eligible controls were participants who had received a complete colonoscopy reaching the cecum and had been found to be polyp-free. On the basis of the endoscopic report, advanced adenomas were defined as adenomas with a diameter of 1 cm or more, high-grade dysplasia, or tubulovillous or villous morphology.

Assessment of meat intake

Participants were asked to complete an interviewer-administered telephone interview to obtain information on medication use, demographics, medical history, and selected lifestyle factors, including questions on usual intake frequencies and the portion size of 11 meats in the previous year before interview. The meat questionnaire for our study was modified from those used in previous studies (26), including our previous studies for breast cancer (27) and colorectal polyps (20). Intake of meat includes hamburgers or cheeseburgers from fast food, hamburgers or cheeseburgers not from fast food, beef steaks, pork chops or ham steaks, bacon, sausage, hotdogs/franks, chicken, fish, meat gravies made with drippings, and short ribs or spareribs. Data were obtained about the intake frequency and usual portion size of each meat item, and for each meat item, except hamburgers or cheeseburgers from fast food and meat gravies made with drippings, the proportion of time was estimated for meat consumption by each of the cooking methods [oven-broiled or oven-baked, grilled or barbecued, pan fried, deep fried (for chicken and fish), and all other ways].

Participants also were asked to report their usual preference level of meat doneness over the previous year before interview by using a series of color photographs labeled with a number between 1 and 3 for each meat item representing increasing levels of doneness. Photographs for 7 meat items included hamburger patties, beef steaks, pork chops, bacon, grilled chicken, pan fried chicken, and pan fried or grilled fish. For each meat item, participants were asked whether the item usually looked less than 1, about the same as 1, about the same as 2, about the same as 3, or more than 3. A score of 0–5 represents 6 categories in total, representing rare (score 0), medium (score 1) or just done (score 2), well-done (score 3), very well-done (score 4), and extremely very well-done (score 5). The food photo booklet was typically given to subjects in-person on the day of colonoscopy. All participants who completed the questions about food doneness had the photograph booklet in front of them during the telephone interview.

Statistical analysis

Meats were classified by type (red, white, and processed) for statistical analysis. Red meat included hamburgers or cheeseburgers, beef steaks, pork chops or ham steaks, and ribs (short ribs or spareribs). Processed meat included bacon, sausage, and hotdogs/franks. White meat included chicken and fish. All meat included all meat items. The software CHARRED (http://www.charred.cancer.gov/) developed by the U.S. National Cancer Institute (26) was used to estimate exposure levels to meat-derived mutagens, including 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo-[4,5-*f*]-quinoxaline (DiMeIQx), 2-amino-I-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), benzo[α]pyrene, and the overall mutageneity index, as measured by revertants colony. We used the predicted overall mutageneity index, which integrates mutagenic capacity and amount for all meat-derived mutagens simultaneously. Average values of mutagen levels of pork chops and ham in the CHARRED database were used for the item "pork chops or ham steaks" in our questionnaire, and average values of broiled chicken and baked chicken in this database were used for the item "chicken cooked by oven-broiled or oven-baked" in our questionnaire. Sausages are typically cooked to the well-done level and thus mutagens for well-done sausages from the CHARRED database were used. No carcinogen information is available in the CHARRED database for hotdogs, fish, and short ribs. Therefore, these foods were not included in the calculation of HCA and PAH exposure levels.

Generalized linear models and Mantel–Haenszel χ^2 tests were used to compare the distribution of demographic characteristics and known risk factors for colorectal cancer across case and control groups, with adjustment for age and sex differences when appropriate. Unconditional polytomous logistic regression models were used to estimate ORs and their 95% CIs for the association between exposures and types of polyps or between exposures and number, size, and anatomic location of adenomas. Quartile cutoff points for meat or meat-derived mutagen exposures were based on intake for control subjects, with the lowest quartile as referent. A red meat to chicken intake ratio

was calculated on the basis of the ratio of the quartile of red meat intake to the quartile of chicken intake. Variables selected in multivariate analysis were either known risk factors or variables that showed significantly different distributions among comparison groups. Variables were adjusted for multivariate analysis including age, sex, race, study site, education, indication for colonoscopy, smoking status, alcohol consumption, body mass index (BMI), regular exercise participation, regular nonsteroidal anti-inflammatory drug (NSAID) use, total energy intake, and recruitment before or after colonoscopy. Regular cigarette smoking was defined as smoking at least 1 cigarette per day continuously for at least 3 months. Former smokers were regular smokers who had stopped smoking for at least 1 year before colonoscopy. Regular alcohol drinking was defined as consumption of 5 or more drinks per week continuously for 12 months. Regular NSAID users were defined as those who used the medications at least 3 days each week continuously for at least 1 year. Energy intake level for each subject was derived from a self-administered FFQ developed (20, 24) and validated (25) for a similar southern U.S. population. Excluded were participants with unreasonably high (>3,500 kcal for females and >4,200 kcal for males) or low (<600 kcal for females and <800 kcal for males) daily energy intake ($n = 24$). The current analyses included 1,881 adenomas cases, 622 cases with HPPs only, and 3,764 polyp-free controls. Of the 6,307 subjects included in the current analysis, 818 subjects did not provide FFQ information. For these individuals, energy intake level was assigned with age- (40–49, 50–59, 60–64, ≥65) and sex-specific mean values. Sensitivity analyses were carried out excluding participants without FFQ information. *P* values for trend tests were derived by entering categorical variables as continuous parameters in the models (28). To evaluate heterogeneity among the 2 polyp groups, we calculated the *P* values by case-case comparisons in polytomous logistic regression models. *P* values of 0.05 or less (2-sided probability) were considered as being statistically significant. All analyses were conducted using SAS statistical software (version 9.2; SAS Institute).

Table 1. Characteristics of study participants by comparison group, the Tennessee Colorectal Polyp Study, 2003–2010

Characteristic	Controls ($n = 3,764$)	Polyp groups: Adenomas ($n = 1,881$)	Polyp groups: HPP only ($n = 662$)	P^a
Study site, %				<0.001
Vanderbilt University	75.4	64.5	61.5	
Veterans Affairs	24.6	35.5	38.5	
Age, mean (SD), y	56.8 (7.7)	58.5 (7.3)	56.7 (7.0)	<0.001
Sex (female), %	45.1	27.8	33.5	<0.001
Indications for colonoscopy,[b] %				0.361
Screening	58.2	57.2	55.7	
Other	41.8	42.8	44.3	
Educational attainment,[b] %				<0.001
High school or less	24.0	29.6	30.8	
Some college	28.3	28.1	29.8	
College graduate	20.8	21.5	20.3	
Graduate or professional education	26.9	20.8	19.1	
Race (white), %	89.4	89.2	90.5	0.625
Enrolled before colonoscopy, %	93.4	72.7	85.2	<0.001
Colorectal cancer family history,[b] %	8.6	9.8	8.0	0.298
Regular cigarette smoking,[b] %	48.3	59.3	69.2	<0.001
Regular alcohol consumption,[b] %	42.4	45.5	48.5	0.001
BMI, mean,[c] kg/m^2	28.1	28.9	28.9	<0.001
Regularly exercised,[b] %	57.9	52.0	53.3	<0.001
Regular NSAID use[b]	59.8	53.8	59.2	0.002
Total energy intake, mean,[b] kcal/d	2,014.4	2,084.8	2,064.7	<0.001
Total folate intake, mean,[b] μg/d	508.0	532.7	501.0	0.075

[a]Derived from ANOVA for continuous variables and χ^2 test for categorical variables.
[b]Standardized by age (40–49, 50–59, 60–64, and ≥65 years old) and sex distribution of all study participants.
[c]Standardized by age distribution (40–49, 50–59, 60–64, and ≥65 years old) of all study participants.

Results

Distributions of demographic and other characteristics for 3 case groups and polyp-free controls are presented in Table 1. More controls than cases were recruited at the Vanderbilt Medical Center than the Veterans Affairs Medical Center. Compared with controls, cases with polyp were more likely to be male, smokers, regular alcohol consumers, have high BMI, and have lower educational attainment, less likely to use NSAIDs regularly, have a higher daily total energy intake, and recruited after colonoscopy. Although adenoma patients tended to be more likely to have a family history of colorectal cancer than controls, the difference was not statistically significant. In addition, adenoma cases were older than controls whereas HPP-only cases had a mean age similar to controls. Cases and controls were similar in race distribution and indication for colonoscopy. Daily intake level of folate was slightly higher in cases than in controls, and the statistical test was marginally significant.

Table 2 presents results for the association of polyp risk with the intake of individual meat items. Statistically significant associations were found for a high intake of almost all red meat items except for short ribs/spareribs. However, approximately 88% did not report consuming short ribs/spareribs. Significant association was also found for a high intake of hotdogs/franks ($P = 0.05$). No apparent associations were found for fish or chicken intake.

Table 3 presents the association of meat intake with the risk of adenomas, HPPs, and any polyps. High intake of all meat, red meat, and processed meat was statistically significantly associated with an elevated risk of polyp in all of these case groups. Chicken meat intake was not significantly associated with the risk of either polyp type. Elevated risk of polyps was also significantly associated with a high red meat to chicken meat intake ratio. The association between red meat intake and polyp risk remained essentially unchanged after additional adjustment for processed meat (Spearman $r = 0.50$ with red meat intake) and white meat intake (Spearman $r = 0.09$ with red meat intake). No significant heterogeneity between adenomas and HPPs was found in relation to meat intake. Exclusion of 858 participants recruited after colonoscopy and exclusion of 341 participants with a history of nonadenomatous polyps did not appreciably change the association. For example, the ORs and 95% CIs for red meat intake for all polyps risk are essentially the same whether those participants are excluded or included (data not shown).

Associations of polyp risk with red meat intake by cooking methods are presented in Table 4. High intake of red meat prepared by high-temperature cooking methods such as frying, grilling, and broiling was associated with an elevated risk of both adenomas and HPPs. Intake of red meat prepared by other methods (typically at a lower temperature), however, was not associated with polyp risk. Although high intake of chicken prepared using

Table 2. Association of selected meat item intake and polyp risk, the Tennessee Colorectal Polyp Study, 2003–2010

	Intake quartiles								
	Q1, low		Q2		Q3		Q4		
Meat items	*n*	OR (95% CI)[a]	*n*	OR (95% CI)[a]	*n*	OR (95% CI)[a]	*n*	OR (95% CI)[a]	P_{trend}
Fast food hamburgers	913/1,617	1.0 (reference)	624/923	1.1 (1.0–1.3)	378/535	1.1 (0.9–1.2)	628/689	1.2 (1.0–1.4)	0.028
Non–fast food hamburgers	1,177/2,056	1.0 (reference)	673/950	1.2 (1.1–1.4)	339/400	1.3 (1.1–1.5)	354/358	1.2 (1.0–1.5)	0.002
Beef patties/steaks	1,158/1,921	1.0 (reference)	512/833	1.0 (0.9–1.2)	518/628	1.2 (1.0–1.4)	355/382	1.3 (1.1–1.5)	0.002
Pork chops	1,241/2,069	1.0 (reference)	687/1,032	1.1 (1.0–1.3)	215/275	1.1 (0.9–1.4)	400/388	1.4 (1.2–1.6)	<0.001
Short ribs/spareribs	2,217/3,355	1.0 (reference)	176/249	1.0 (0.8–1.3)	N/A	N/A	150/160	1.1 (0.9–1.5)	0.291
Bacon	981/1,654	1.0 (reference)	502/788	1.0 (0.9–1.2)	456/628	1.1 (1.0–1.3)	604/694	1.1 (1.0–1.3)	0.048
Sausage	1,363/2,327	1.0 (reference)	276/426	1.1 (0.9–1.3)	466/610	1.1 (0.9–1.3)	438/401	1.3 (1.1–1.5)	0.006
Hotdogs/franks	1,560/2,554	1.0 (reference)	216/340	1.1 (0.9–1.3)	378/473	1.1 (0.9–1.3)	389/397	1.2 (1.0–1.4)	0.050
Fish	849/1,131	1.0 (reference)	599/961	1.0 (0.8–1.1)	583/793	1.1 (0.9–1.2)	512/879	0.9 (0.7–1.0)	0.156
Chicken	649/892	1.0 (reference)	608/975	1.0 (0.9–1.2)	786/1,266	1.0 (0.9–1.2)	428/631	1.0 (0.9–1.2)	0.809

Abbreviations: *n*, number of cases/controls; N/A, not available.

[a]Adjusted for age, sex, race, study sites, educational attainment, indications for colonoscopy, smoking, alcohol consumption, BMI, physical activity, regular NSAID use, total energy intake, and recruitment before or after colonoscopy.

Table 3. Association of meat intake and polyp risk, the Tennessee Colorectal Polyp Study, 2003–2010

	Controls ($n = 3,764$)	Patient groups					
		Any polyps ($n = 2,543$)		Adenomas ($n = 1,881$)		HPP only ($n = 662$)	
Intake amount, g/d	(*n*)	*n*	OR (95% CI)[a]	*n*	OR (95% CI)[a]	*n*	OR (95% CI)[a]
All meat, g/d							
≤59.0	977	545	1.0 (reference)	427	1.0 (reference)	118	1.0 (reference)
59.1–90.9	929	496	1.0 (0.9–1.2)	352	0.9 (0.8–1.1)	144	1.3 (1.0–1.7)
91.0–138.6	929	669	1.2 (1.0–1.4)	484	1.1 (0.9–1.3)	185	1.5 (1.1–1.9)
≥138.7	929	833	1.3 (1.1–1.5)	618	1.2 (1.0–1.4)	215	1.5 (1.2–2.0)
	P_{trend}		<0.001		0.013		0.002
Red meat,[b] g/d							
≤9.5	1,118	540	1.0 (reference)	398	1.0 (reference)	142	1.0 (reference)
9.6–28.4	932	581	1.2 (1.0–1.4)	435	1.2 (1.0–1.5)	146	1.1 (0.9–1.5)
28.5–51.3	792	581	1.3 (1.1–1.5)	428	1.2 (1.0–1.5)	153	1.2 (0.9–1.6)
≥51.4	922	841	1.4 (1.2–1.6)	620	1.4 (1.2–1.6)	221	1.3 (1.0–1.7)
	P_{trend}		<0.001		<0.001		0.029
Chicken, g/d							
≤10.1	892	649	1.0 (reference)	478	1.0 (reference)	171	1.0 (reference)
10.2–40.3	975	680	1.0 (0.9–1.2)	515	1.1 (0.9–1.3)	165	0.9 (0.7–1.2)
40.4–50.0	1,266	786	1.0 (0.9–1.2)	571	1.0 (0.9–1.2)	215	1.0 (0.8–1.2)
≥50.1	631	428	1.0 (0.9–1.2)	317	1.1 (0.9–1.3)	111	1.0 (0.7–1.3)
	P_{trend}		0.809		0.812		0.962
Processed meat,[c] g/d							
0	1,103	567	1.0 (reference)	427	1.0 (reference)	140	1.0 (reference)
0.1–8.0	816	487	1.2 (1.0–1.4)	356	1.1 (0.9–1.4)	131	1.2 (0.9–1.6)
8.1–22.4	924	622	1.2 (1.0–1.4)	453	1.1 (0.9–1.3)	169	1.2 (1.0–1.6)
>22.5	921	867	1.3 (1.1–1.5)	645	1.3 (1.1–1.5)	222	1.4 (1.1–1.8)
	P_{trend}		0.003		0.010		0.024
Red meat[b] to chicken ratio							
0.25–0.67	1,142	548	1.0 (reference)	409	1.0 (reference)	139	1. 0 (reference)
0.68–1.00	1,267	779	1.1 (0.9–1.2)	572	1.0 (0.9–1.2)	207	1.2 (0.9–1.5)
1.01–1.50	545	451	1.3 (1.0–1.5)	331	1.3 (1.0–1.6)	120	1.4 (1.0–1.8)
>1.51	810	765	1.3 (1.1–1.6)	569	1.3 (1.1–1.6)	196	1.4 (1.1–1.8)
	P_{trend}		<0.001		0.001		0.010

[a]Adjusted for age, sex, race, study sites, educational attainment, and indications for colonoscopy, smoking, alcohol consumption, BMI, physical activity, regular NSAID use, total energy intake, and recruitment before or after colonoscopy.
[b]Includes hamburgers, cheeseburgers, beef patties/beef steaks, pork chops/ham steaks, and short ribs or spareribs.
[c]Includes bacon, sausage, and hotdogs/franks.

high-temperature cooking methods was associated with slightly increased risk of adenomas, the trend test was only marginally significant ($P = 0.075$). Again, no heterogeneity test was statistically significant between adenomas and HPPs in these associations (data not shown).

High intake of HCAs (MeIQx, PhIP, and DiMeIQx) was associated with an elevated risk of colorectal polyps, and all trend tests were statistically or marginally significant (Table 5). A positive association with benzo[α]pyrene exposure, however, was observed only for adenomas ($P_{trend} = 0.027$); no association was observed for HPPs ($P_{trend} = 0.434$). Mutageneity index, an aggregate measure of the effect from meat mutagens, was strongly, positively associated with the risk adenomas ($P_{trend} < 0.001$) and HPPs ($P_{trend} = 0.008$). Again, no heterogeneity was observed between these 2 polyp groups for association with any of the meat-derived mutagens. Again, exclusion of participants recruited after colonoscopy and participants with a history of nonadenomatous polyps did not change the association (data not shown).

Associations of red meat intake, meat-derived mutagens, and overall meat mutageneity with selected adenoma characteristics are summarized in Table 6. In general, high intake of red meat and high exposure to meat-derived mutagens were associated with an elevated risk of adenomas regardless of stage (advanced or nonadvanced),

Table 4. Association[a] of meat cooking methods and polyp risk, the Tennessee Colorectal Polyp Study, 2003–2010

	Intake quartiles[b]								
	Q1, low		Q2		Q3		Q4, high		
Cooking methods	*n*[c]	OR (95% CI)	*n*[c]	OR (95% CI)	*n*[c]	OR (95% CI)	*n*[c]	OR (95% CI)	P_{trend}
Adenomas ($n = 1{,}881$)									
Red meat									
High temperature[d]	353/1,009	1.0 (reference)	404/931	1.1 (0.9–1.3)	468/906	1.3 (1.0–1.5)	656/918	1.4 (1.2–1.7)	<0.001
Other methods	942/2,026	1.0 (reference)	281/628	1.0 (0.8–1.1)	288/550	1.0 (0.9–1.2)	370/560	1.2 (1.0–14)	0.107
Chicken									
High temperature[d]	475/964	1.0 (reference)	652/1,302	1.1 (0.9–1.3)	405/843	1.1 (0.9–1.3)	349/655	1.2 (1.0–14)	0.075
Other methods	564/988	1.0 (reference)	516/1,041	0.9 (0.8–1.1)	429/958	0.9 (0.8–11)	372/777	0.9 0.7–1.1)	0.189
HPP ($n = 662$)									
Red meat									
High temperature[d]	119/1,009	1.0 (reference)	140/931	1.2 (0.9–1.5)	174/906	1.4 (1.0–18)	229/918	1.4 (1.1–19)	0.007
Other methods	351/2,026	1.0 (reference)	108/628	1.0 (0.8–1.3)	80/550	0.8 (0.6–1.0)	123/560	1.0 (0.8–1.3)	0.605
Chicken									
High temperature[d]	169/964	1.0 (reference)	218/1,302	1.0 (0.8–1.2)	146/843	1.0 (0.8–1.2)	129/655	1.2 (0.9–1.5)	0.304
Other methods	205/988	1.0 (reference)	169/1,041	0.8 (0.6–1.0)	157/958	0.9 (0.7–11)	131/777	0.9 (0.7–11)	0.303

Abbreviation: *n*, number of cases/controls.

[a]Adjusted for age, sex, study sites, educational attainment, indications for colonoscopy, cigarette smoking, alcohol consumption, BMI, physical activity, regular NSAID use, total energy intake, and recruitment before or after colonoscopy.

[b]Quartiled by intake amount (g/d) distribution for relevant cooking methods in controls.

[c]Counts may not sum to the total because of missing data.

[d]Including grilled, pan/deep-fried, and broiled.

multiplicity (single or multiple), morphology (serrated or nonserrated), side (left, right, or both sides of the colon; data not shown), and location (colon or rectum; data not shown). The association for all meat intake and mutageneity index was stronger for multiple adenomas than for a single adenoma and for serrated adenomas than for nonserrated adenomas, and the test for heterogeneity was statistically or marginally significant. Results for adenomas of both sides were similar to that of multiple adenomas, and the results for large or small adenomas were similar to advanced or nonadvanced adenomas, respectively.

Table 7 shows the risk of polyps by joint distributions of red meat intake and meat-derived overall mutageneity with cigarette smoking status, using never smokers who consumed the lowest amount of red meat or experienced lowest exposure level to meat-derived mutagens as the reference group. Both cigarette smoking and high red meat intake/high meat-derived mutagen exposure were related to increased risk of colorectal polyps. Overall, current smokers who also are exposed to a high level of red meat or meat-derived mutagens had the highest risk of colorectal polyps, and the association was particularly strong for HPPs. None of the tests for a multiplicative interaction between cigarette smoking and meat intake or meat-derived mutagen exposure, however, were statistically significant.

Discussion

In this large colonoscopy-based case–control study, we provided strong evidence that high intake of meat, particularly red meat cooked at a high temperature, and high exposure to meat-derived mutagens are associated with the risk for both adenomas and HPPs. To our knowledge, this is the largest colonoscopy-based study that evaluated the hypothesis related to meat intake and meat-derived mutagen exposure by polyp histology types. The results from this study provide strong support for an etiologic role of red meat and meat-derived mutagens in the pathogenesis of colorectal polyps.

Several previous studies have provided some support for a possible association between the risk of colorectal adenomas and high intake of red meat or high exposure to meat-derived mutagens (14–22). The results from these studies, however, were inconsistent. For example, 2 of the studies reported that well-done red meat intake and meat-derived mutagen exposure were associated with an increased risk of nonadvanced and single distal colorectal adenoma (17) or small and distal colon adenoma (22). In other studies, however, this association was more evident for clinically significant adenomas such as advanced adenomas (19) or large adenomas (14, 16). Furthermore, results within the same studies were also

Table 5. Association of meat-derived mutagen exposure and risk of polyps, the Tennessee Colorectal Polyp Study, 2003–2010

	Controls ($n = 3,764$)	Polyp groups					
		Any polyps ($n = 2,543$)		Adenomas ($n = 1,881$)		HPP only ($n = 662$)	
Exposure amount	***n***	***n***	**OR (95% CI)[a]**	***n***	**OR (95% CI)[a]**	***n***	**OR (95% CI)[a]**
MeIQx, ng/d							
≤12.2	1,041	499	1.0 (reference)	370	1.0 (reference)	129	1.0 (reference)
12.3–32.9	906	552	1.2 (1.0–1.4)	410	1.2 (1.0–1.4)	142	1.2 (0.9–1.5)
33.0–70.0	909	645	1.3 (1.1–1.5)	468	1.3 (1.1–1.5)	177	1.3 (1.0–1.7)
≥70.1	908	847	1.4 (1.2–1.6)	633	1.4 (1.2–1.7)	214	1.3 (1.0–1.7)
P_{trend}			<0.001		<0.001		0.022
PhIP, ng/d							
≤73.3	1,038	571	1.0 (reference)	426	1.0 (reference)	145	1.0 (reference)
79.4–169.3	908	554	1.1 (0.9–1.3)	412	1.1 (0.9–1.3)	142	1.1 (0.8–1.4)
169.4–339.3	909	691	1.3 (1.1–1.5)	502	1.3 (1.1–1.6)	189	1.3 (1.0–1.7)
≥339.4	909	727	1.3 (1.1–1.5)	541	1.3 (1.1–1.5)	186	1.2 (0.9–1.6)
P_{trend}			<0.001		0.001		0.053
DiMeIQx, ng/d							
≤0.82	1,039	577	1.0 (reference)	433	1.0 (reference)	144	1.0 (reference)
0.83–2.74	909	560	1.1 (0.9–1.3)	413	1.1 (0.9–1.3)	147	1.0 (0.8–1.3)
2.75–5.95	908	597	1.1 (0.9–1.3)	432	1.1 (0.9–1.3)	165	1.1 (0.9–1.5)
≥5.96	908	809	1.3 (1.1–1.5)	603	1.3 (1.1–1.6)	206	1.3 (1.0–1.6)
P_{trend}			<0.001		0.001		0.034
Benzo[α]pyrene, ng/d							
≤8.93	1,038	551	1.0 (reference)	411	1.0 (reference)	140	1.0 (reference)
8.94–31.9	909	633	1.1 (1.0–1.3)	462	1.1 (0.9–1.3)	171	1.2 (0.9–1.6)
32.0–79.8	909	645	1.2 (1.0–1.4)	481	1.2 (1.0–1.4)	164	1.2 (0.9–1.5)
≥79.9	908	714	1.2 (1.0–1.4)	527	1.2 (1.0–1.5)	187	1.2 (0.9–1.5)
P_{trend}			0.026		0.027		0.434
Mutageneity index							
≤2,556	1,038	520	1.0 (reference)	404	1.0 (reference)	125	1.0 (reference)
2,557–5,851	908	518	1.1 (0.9–1.2)	414	1.2 (1.0–1.4)	168	1.5 (1.1–1.9)
5,852–11,020	909	668	1.4 (1.2–1.6)	495	1.4 (1.2–1.6)	167	1.4 (1.1–1.8)
≥11,021	909	837	1.3 (1.1–1.6)	568	1.3 (1.1–1.6)	202	1.5 (1.2–1.9)
P_{trend}			<0.001		<0.001		0.008

[a]Adjusted for age, sex, race, study sites, educational attainment, indications for colonoscopy, smoking, alcohol consumption, BMI, physical activity, regular NSAID use, total energy intake, and recruitment before or after colonoscopy.

mixed. For example, the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial, the largest sigmoidoscopy-based study (17), suggested stronger associations between well-done red meat intake and meat-related mutagens with single and nonadvanced adenoma. It also found a positive association of red meat with known doneness level in relation to advanced but not nonadvanced adenomas. Similarly, in an recent European cohort study (22), the association between total meat consumed that is strongly/extremely browned was observed in large but not in small adenomas whereas a high consumption of total meat consumed prepared by hazardous cooking methods (fried, broiled, or grilled/barbecued) was only related to the risk of small but not large adenomas.

Previous studies on the associations of polyp risk with specific meat-derived mutagens also yielded conflicting results (14–22). For instance, Sinha and colleagues found in a small study (15) that only MeIQx was associated with increased risk of adenomas, and in the PLCO study (17) MeIQx, PhIP, and benzo[α]pyrene were all associated with increased risk for nonadvanced distal adenomas. Wu and colleagues (18) reported only total meat-derived mutageneity, but not individual HCAs (PhIP, MeIQx, and DiMeIQx), was associated with increased risk for distal adenomas, whereas Sinha and colleagues (15) reported

Table 6. Association of meat and mutagen intake with adenoma risk by stage and multiplicity, the Tennessee Colorectal Polyp Study, 2003–2010

		Stage of adenomas				Multiplicity of adenomas				Morphology of adenomas			
	Controls ($n = 3,764$)	Nonadvanced ($n = 1,406$)		Advanced ($n = 458$)		Single ($n = 1,070$)		Multiple ($n = 458$)		Nonserrated ($n = 1,715$)		Serrated ($n = 166$)	
Intake amounts	(n)	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]
All meat, g/d													
≤59.0	977	321	1.0 (reference)	101	1.0 (reference)	267	1.0 (reference)	112	1.0 (reference)	400	1.0 (reference)	27	1.0 (reference)
59.1–90.9	929	264	0.9 (0.7–1.1)	87	1.1 (0.8–1.5)	211	0.9 (0.7–1.1)	96	1.0 (0.7–1.3)	323	0.9 (0.8–1.1)	29	1.3 (0.7–2.2)
91.0–138.6	929	371	1.1 (0.9–1.3)	112	1.1 (0.8–1.5)	279	1.0 (0.8–1.3)	152	1.2 (0.9–1.7)	433	1.0 (0.9–1.2)	51	1.8 (1.1–3.1)
≥138.7	929	450	1.2 (1.0–1.4)	158	1.4 (1.1–1.9)	313	1.0 (0.8–1.3)	208	1.5 (1.2–2.0)	559	1.2 (1.0–1.4)	59	2.0 (1.2–3.4)
Trend test			$P = 0.035$		$P = 0.027$		$P = 0.473$		$P < 0.001$		$P = 0.049$		$P = 0.003$
Heterogeneity test[b,d]				$P = 0.544$				$P = 0.024$				$P = 0.018$	
Red meat, g/d[c]													
≤9.5	1,118	307	1.0 (reference)	88	1.0 (reference)	264	1.0 (reference)	94	1.0 (reference)	365	1.0 (reference)	33	1.0 (reference)
9.6–28.4	932	316	1.2 (1.0–1.4)	116	1.5 (1.1–2.1)	261	1.2 (0.9–1.4)	125	1.5 (1.1–2.0)	399	1.2 (1.0–1.5)	36	1.2 (0.7–1.9)
28.5–51.3	792	328	1.2 (1.0–1.5)	98	1.3 (0.9–1.8)	232	1.1 (0.9–1.3)	132	1.6 (1.2–2.1)	388	1.2 (1.0–1.5)	40	1.5 (0.9–2.4)
≥51.4	922	455	1.3 (1.1–1.6)	156	1.5 (1.1–2.1)	313	1.1 (0.9–1.4)	217	1.9 (1.4–2.5)	563	1.4 (1.1–1.6)	57	1.6 (1.0–2.6)
Trend test			$P = 0.003$		$P = 0.027$		$P = 0.342$		$P < 0.001$		$P = 0.002$		$P = 0.039$
Heterogeneity test[c,d]				$P = 0.651$				$P < 0.001$				$P = 0.317$	
Mutageneity index													
≤2,556	1,038	285	1.0 (reference)	95	1.0 (reference)	241	1.0 (reference)	99	1.0 (reference)	351	1.0 (reference)	32	1.0 (reference)
2,557–5,851	908	293	1.1 (0.9–1.3)	91	1.1 (0.8–1.5)	237	1.1 (0.9–1.3)	107	1.1 (0.8–1.5)	362	1.1 (0.9–1.3)	26	0.9 (0.5–1.5)
5,852–11,020	909	371	1.4 (1.2–1.7)	109	1.3 (0.9–1.7)	268	1.3 (1.0–1.6)	150	1.6 (1.2–2.1)	443	1.4 (1.1–1.7)	43	1.4 (0.9–2.4)
≥111,021	909	457	1.4 (1.1–1.6)	163	1.5 (1.1–2.0)	324	1.3 (1.0–1.5)	212	1.7 (1.2–2.2)	559	1.3 (1.1–1.6)	65	1.9 (1.2–3.0)
Trend test			$P < 0.001$		$P = 0.004$		$P = 0.018$		$P < 0.001$		$P < 0.001$		$P = 0.001$
Heterogeneity test[c,d]				$P = 0.578$				$P = 0.036$				$P = 0.077$	

[a]Counts may not sum to the total because of missing data.

[b]Adjusted for age, sex, race, study sites, educational attainment, indications for colonoscopy, smoking, alcohol consumption, BMI, physical activity, regular NSAID use, total energy intake, and recruitment before or after colonoscopy.

[c]Includes hamburgers, cheeseburgers, beef patties/beef steaks/pork chops or ham steaks/short ribs or spareribs.

[d]Tests for heterogeneity are case-only comparisons.

Table 7. Joint association of red meat intake, meat-derived mutagen exposure, and smoking status with polyp risk, the Tennessee Colorectal Polyp Study, 2003–2010

	OR (95% CI)[a] for polyps								
	All polyps (n = 2,543)			All adenomas (n = 1,881)			HPP (n = 662)		
Intake tertiles	**Never smoker**	**Former smoker**	**Current smoker**	**Never smoker**	**Former smoker**	**Current smoker**	**Never smoker**	**Former smoker**	**Current smoker**
Cases/controls, n[b]	906/2,005	938/1,282	695/472	709/2,005	686/1,282	484/472	197/2,005	252/1,282	211/472
Red meat,[c] g/d									
<22.7	1.0 (reference)	1.4 (1.1–1.7)	3.1 (2.3–4.3)	1.0 (reference)	1.4 (1.1–1.7)	2.8 (1.9–4.0)	1.0 (reference)	1.5 (1.0–2.2)	3.9 (2.4–6.3)
22.7–61.9	1.2 (1.0–1.5)	1.7 (1.4–2.1)	3.6 (2.8–4.7)	1.2 (1.0–1.5)	1.6 (1.2–2.0)	3.0 (2.3–4.1)	1.1 (0.8–1.6)	2.2 (1.5–3.1)	5.1 (3.5–7.6)
>61.9	1.3 (1.1–1.6)	1.9 (1.5–2.3)	3.7 (2.9–4.7)	1.4 (1.1–1.7)	1.7 (1.3–2.2)	3.2 (2.5–4.2)	1.1 (0.8–1.7)	2.4 (1.7–3.4)	5.0 (3.4–7.3)
P for interaction		0.890			0.680			0.622	
Mutageneity index									
<4,789.3	1.0 (reference)	1.3 (1.1–1.6)	2.9 (2.2–3.8)	1.0 (reference)	1.2 (1.0–1.6)	2.4 (1.7–3.3)	1.0 (reference)	1.6 (1.1–2.3)	4.3 (2.8–6.6)
4,789.3–10,737.7	1.2 (1.0–1.5)	1.7 (1.4–2.2)	3.6 (2.7–4.7)	1.3 (1.0–1.6)	1.6 (1.3–2.1)	3.1 (2.3–4.2)	1.0 (0.7–1.4)	2.2 (1.5–3.2)	5.0 (3.3–7.6)
>10,737.7	1.2 (1.0–1.5)	1.8 (1.4–2.2)	3.9 (3.0–5.0)	1.2 (1.0–1.6)	1.7 (1.3–2.1)	3.5 (2.6–4.6)	1.3 (0.9–1.9)	2.1 (1.5–3.1)	5.0 (3.4–7.4)
P for interaction		0.481			0.333			0.825	

[a]Adjusted for age, sex, race, study sites, educational attainment, indications for colonoscopy, alcohol consumption, BMI, physical activity, regular NSAID use, total energy intake, and recruitment before or after colonoscopy.

[b]Counts may not sum to the total because of missing data.

[c]Includes hamburgers, cheeseburgers, beef patties/beef steaks/pork chops, or ham steaks/short ribs or spareribs.

that MeIQx and total meat-derived mutageneity remained positively associated with risk of adenomas even after adjustment for red meat or well-done red meat. In contrast, Gunter and colleagues (16) identified only benzo[α]pyrene, but not any individual HCAs, as being related to colorectal adenoma risk. Dietary benzo[α]pyrene was also associated with colorectal adenoma risk in another study (29).

Intake levels of meat-derived mutagens may differ remarkably across countries. For a U.S. population, Layton and colleagues (3) calculated an average HCA intake of 26 ng/kg body weight per day, whereas in European countries, the estimated intake levels were much lower, about 2.5 ng/kg body weight per day (30) to 5 ng/kg body weight per day (31). A recent large study conducted in Europe found that the frequency of high-temperature cooking varies considerably between countries (32), which may explain some of the inconsistent findings from previous studies conducted in different populations. Because HCA levels are determined to a large extent by cooking methods and doneness levels, studies that did not assess cooking methods and doneness levels may suffer considerably from errors in estimating HCA exposure.

In our study, red meat, meat-derived mutageneity, and all 3 HCAs (PhIP, MeIQx, and DiMeIQx) were associated with the risk for all polyps, including adenomas and HPPs. High dietary benzo[α]pyrene exposure also showed a statistically significant dose–response relationship with adenomas. We did not observed heterogeneity between adenomas and HPPs in relation to all meat or HCA variables; it is possible that red meat (especially well-done red meat), processed meat, and all meat-derived mutagens were related to risk of colorectal neoplasia to various degrees. It may be difficult to disentangle the effect of specific meat-derived mutagens, as the exposure levels of these mutagens were highly correlated (Spearman $r = 0.55$–0.96) and cannot be included in the model simultaneously. Moreover, consistent with a previous study (3), most MeIQx and DiMeIQx were derived from red meat. For example, 90.7% of MeIQx and 76.5% of DiMeIQx in the current study originated from red meat and they were highly correlated with red meat intake (Spearman $r = 0.79$ and 0.56, respectively). Nevertheless, consistent with most of the previous studies conducted in this area, our data suggested that red meat or well-done meat (16, 17, 19, 21, 22), processed meat (19, 22), meat-derived mutagens such as MeIQx (15, 19), DiMeIQx (19, 21), PhIP (17, 18, 22), and benzo[α]pyrene (16, 17, 29), as well as meat-derived total mutagenic activity (15, 18) may be related to the risk of colorectal polyps and that the associations with red meat intake and HCA exposure were more strongly associated with multiple adenomas (19).

Many previous studies used sigmoidoscopy to identify cases and controls. Because a sigmoidoscope cannot assess the proximal colon, patients with polyps only in the right side of the colon were classified as polyp-free controls, resulting in considerable misclassification errors. In addition, the sample sizes in some previous studies were small (14–16, 18, 19, 21, 22), and, similar to our previous report (20), many associations also were not statistically significant. Furthermore, because few studies have used a questionnaire specifically designed to assess meat intake by cooking methods and doneness levels, previous studies may be prone to significant measurement errors. In addition, most previous studies only evaluated colorectal adenomas without addressing HPPs. Although HPPs generally have not been regarded as precancerous in the past, recent studies have suggested that some HPPs may develop into cancer via serrated or microsatellite instability (MSI) pathways (10, 33). This pathway differs from the common adenoma–carcinoma sequence, which is mostly through a microsatellite stable (MSS) pathway (34). About 10% to 15% of colorectal cancers show MSI and are characterized by defective nucleotide mismatch repair (35). In addition, epidemiologic studies have shown that the risk factor profiles for both MSI and MSS tumors are different (36–42).

Cigarette smoking consistently has been associated with increased risk for HPPs, including in a previous report from this study (23). Cigarette smoking and high meat intake or meat-derived mutagen exposure each were related to increased risk for all 3 colorectal polyp groups, although tests for multiplicative interaction were not significant. A recent meta-analysis has provided strong evidence that cigarette smoking is associated with increased risk for adenomas as well (43), suggesting that cigarette smoking may be involved in the colorectal adenoma–carcinoma sequence (43, 44). In our study, we found that cigarette smoking was much more strongly associated with the risk of developing HPPs. Several studies have shown that cigarette smoking was more strongly associated with colorectal cancer characterized by MSI and somatic *BRAF* mutation, both hallmarks of serrated hyperplastic neoplasia, than cancer without these characteristics (36, 45, 46).

As with any case–control study, the possibility of selection and recall biases may be a concern in this study. Colonoscopy was used to classify the case–control status, minimizing misclassification error from incomplete examination of the entire colon. Most study participants (86.4%) were recruited prior to colonoscopy. Although a higher proportion of cases than controls were recruited after colonoscopy, exclusion of all participants recruited after colonoscopy (611 cases and 247 controls) did not appreciably change the association. Furthermore, all of these polyps were benign lesions and thus lifestyle changes or recall of meat intakes following polyp diagnosis is unlikely to be substantial. We used a questionnaire that was designed specifically to assess meat intake by cooking methods and doneness levels; our detailed exposure assessment reduced measurement errors. Nevertheless, measurement errors are unavoidable in nutritional epidemiologic studies; it is possible that measurement errors may be more substantial in estimating

HCA exposure than red meat intake, which might explain in part a stronger association of polyp risk with red meat intake than with HCA exposure. Although 818 subjects did not complete FFQ and thus did not provide their total energy intake level, the distribution by study groups for those 818 individuals was not significantly different from those 5,849 who completed FFQ ($P = 0.097$). Excluding participants without FFQ information did not materially alter the results reported in this article. Exposure levels of meat and meat-derived mutagens were correlated; therefore, it was difficult to disentangle the effect of specific meat-derived mutagens.

In summary, our study, the largest colonoscopy-based study conducted to date, provides strong evidence for a positive association of high red meat intake and meat-derived mutagen exposure with the risk for all colorectal polyps, particularly multiple adenomas. Studies to evaluate the interaction of HCA exposure and carcinogen-metabolizing enzyme polymorphisms may be helpful to further clarify the role of these meat-derived mutagens in the development of colorectal tumors.

Disclosure of Potential Conflicts of Interest

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. No potential conflicts of interest were disclosed.

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