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Meat and Meat Mutagens and Risk of Prostate Cancer in the Agricultural Health Study

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Abstract

Meats cooked at high temperatures, such as pan-frying or grilling, are a source of carcinogenic heterocyclic amines and polycyclic aromatic hydrocarbons. We prospectively examined the association between meat types, meat cooking methods, meat doneness, and meat mutagens and the risk for prostate cancer in the Agricultural Health Study. We estimated relative risks (RR) and 95% confidence intervals (CI) for prostate cancer using Cox proportional hazards regression, using age as the underlying time metric and adjusting for state of residence, race, smoking status, and family history of prostate cancer. During 197,017 person years of follow-up, we observed 668 incident prostate cancer cases (613 of these were diagnosed after the first year of follow-up and 140 were advanced cases) among 23,080 men with complete dietary data. We found no association between meat type or specific cooking method and prostate cancer risk. However, intake of well or very well done total meat was associated with a 1.26-fold increased risk of incident prostate cancer (95% CI 1.02, 1.54) and a 1.97-fold increased risk (95% CI 1.26, 3.08) of advanced disease when the highest tertile was compared with the lowest. Risks for the two heterocyclic amines 2-amino-3,4,8-trimethylimidazo-[4,5-*f*]quinoxaline (DiMeIQx) and 2-amino-3,8-dimethylimidazo-[4,5-*b*] quinoxaline (MeIQx) were of borderline significance for incident disease, 1.24 (95% CI 0.96, 1.59) and 1.20 (95% CI 0.93, 1.55) respectively, when the highest quintile was compared with the lowest. In conclusion, well and very well done meat was associated with an increased risk for prostate cancer in this cohort.

Keywords

Epidemiology; meat intake; prostate cancer; heterocyclic amines; polycyclic aromatic hydrocarbons

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INTRODUCTION

Prostate cancer is the most common cancer in men in the United States (other than non-melanoma skin cancer), with an estimated 234,460 new cases and 27,350 deaths during 2006 (1). Variations in incidence and mortality rates among ethnically similar populations in different geographic locations have implicated environmental risk factors, such as diet (2,3). Some studies have observed an increased risk of prostate cancer with high meat intake, specifically red meat (4).

A potential mechanism linking meat to prostate cancer risk is related to the way in which various meats are cooked. Many meats are cooked at high temperatures by pan-frying, barbecuing or broiling, which results in the formation of carcinogenic heterocyclic amines (HCA's) and polycyclic aromatic hydrocarbons (PAH's). The HCA and PAH content of meat varies according to meat type, cooking method and doneness level, though most are generally formed in meats cooked well-done by high temperature cooking methods (5–8). One of the most abundant HCAs, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) has been found to increase mutation frequency and induces tumors in the rat prostate (9,10).

There is limited epidemiologic evidence regarding the impact of various meat mutagens on prostate cancer risk. Two small case-control studies found no association between PhIP or other major HCA's and prostate cancer (11,12); whereas a prospective study, with a larger sample size, found a significant 1.22-fold increased risk of prostate cancer for individuals in the highest quintile of PhIP intake (13). Only one previous epidemiologic study has evaluated the association between benzo(a)pyrene (BaP) from meat, a marker of PAH intake, and prostate cancer (13). In this study we investigate meat type, cooking method and doneness level as risk factors for prostate cancer in the Agricultural Health Study (AHS), a large cohort of licensed pesticide applicators in Iowa and North Carolina.

METHODS

Study Population

The AHS is a prospective cohort study that includes 57,311 licensed pesticide applicators from Iowa and North Carolina; a detailed description of this cohort has been described elsewhere (14). Briefly, applicators were recruited from December 1993 through December 1997 (Phase I of the study). Upon enrollment, participants completed an enrollment questionnaire; applicators completing the enrollment questionnaire were given a self-administered take-home questionnaire, which provided detailed pesticide exposure data, medical history, and included a section on meat cooking practices. This take-home questionnaire was completed by ~ 40% of the applicators and we have previously shown few important differences between those applicators who did or did not return the take-home questionnaire (15). This analysis excluded applicators who did not provide information on meat cooking practices (n=31,462), prevalent cancer cases (n=1,424) and females (n=1,345), resulting in 23,080 individuals available for analysis. Follow-up was censored at the time of death, movement out of the state or at December 31, 2003, whichever came first. Cohort members were linked to cancer registry files in Iowa and North Carolina for case identification and to the state death registries and the National Death Index to ascertain vital status. All participants provided informed consent, and the protocol was approved by the institutional review boards of the National Cancer Institute, Battelle (the North Carolina field station), the University of Iowa, and the AHS study coordinating center, Westat (Rockville, Maryland).

Dietary Assessment

The dietary module in the Phase 1 take-home questionnaire included questions on supplemental vitamin intake, meat intake, and meat cooking practices. The questions asked about the frequency of intake of hamburgers, beef-steaks, chicken, pork chops/ham steaks, and bacon/sausage in the last twelve months. Additional questions were asked on 'doneness' of hamburgers and beef steaks (rare, medium, well done, and very well done), and bacon/sausage (just until done, well-done, charred/blackened) and cooking methods (pan-fried, broiled, and grilled) for all meats. A specifically developed database (http://charred.cancer.gov) (16) was used to estimate daily intake of meat mutagens based on the responses from the cooking practices module; using this database we estimated intake of the following HCA's: PhIP, 2-amino-3,8-dimethylimidazo-[4,5-*b*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo-[4,5-*f*]quinoxaline (DiMeIQx) and the PAH BaP (6,7,10,17). This database also estimated overall mutagenic activity in meat, determined by the standard plate incorporation assay with *Salmonella typhimurium* strain TA98, measured as revertant colonies (18).

Data Analysis

Cox proportional hazards regression, with age as the underlying time metric, was used to estimate relative risks (RR) and 95% confidence intervals (CI) describing the effect of meat, meat cooking methods, meat doneness, and meat mutagen exposure on prostate cancer risk. All analyses were performed on three different groups: 1) all incident cases occurring after enrollment, 2) incident cases diagnosed after one year of follow-up, referred to as incident cases, and 3) advanced prostate cancer cases, defined as those classified as disease stage III or IV. RRs are presented within quintiles (where possible) of exposure using the first quintile as the referent category; in analyses for doneness we present the data within tertiles due to a smaller range of intake. Potential confounding variables investigated included: family history of prostate cancer (yes/no), education level (high school/General Educational Development (GED) or less, college or more), body mass index (weight (kg)/height $(m)^2$, <25, 25–29, ≥30), smoking status (never, former, current), regular use of aspirin or other nonsteroidal anti-inflammatory drugs (nearly every day for as long a month, yes/no), history of diabetes (yes/no), leisure time physical activity (hours/week, none, up to 1 hour, 1–2 hours, 3–5 hours. 6–10 hours, more than 10 hours), alcohol intake in the past 12 months (never, < once/month, 1–3 times/month, once/week, 2–4 times/week, almost every day and every day), supplemental vitamin E intake (ever/never), race (White, Black, American Indian or Alaskan Native, Asian or Pacific Islander, Other), state of residence (Iowa or North Carolina), and use of the following pesticides (ever/never use) previously linked to prostate cancer in subsets of applicators in the AHS: methyl bromide, chlorpyrifos, fonofos, permethrin, coumaphos, phorate, and butylate. For each model, a potential confounding variable was retained if the variable changed any of the RRs for meat-related variables by more than 10%. Tests for trend were calculated using the midpoint value of each exposure category where it was treated as a continuous response in regression models. All p-values are two sided. SAS statistical software was used for all analyses (SAS Institute, Inc., Cary, North Carolina).

RESULTS

During 197,017 person years of follow-up, 668 incident prostate cancer cases were observed (613 of these were diagnosed after the first year of follow-up and 140 of these were advanced cases with a disease stage of III or IV) among 23,080 men. Compared with men in the lowest quintile of red meat intake, men in the highest quintile tended to be younger and more likely to be White, to be obese, to have a family history of prostate cancer, to be a current smoker, and to consume alcohol more frequently (Table 1). Furthermore, those in the highest quintile of red meat intake were less educated, and less likely to take aspirin or vitamin E supplements.

There was no association between total meat intake and prostate cancer risk among all cases, incident cases, or advanced cases when the highest quintile of intake was compared with the lowest, RR=1.04 (95% CI 0.80, 1.35), RR=1.06 (95% CI 0.81, 1.38), RR=0.93 (95% CI 0.51, 1.70), respectively (Table 2). Similarly no association was observed for any of the following meat items: total meat, red meat, chicken, bacon or sausage, steak, pork chops/ham steaks, hamburger. Increased intake of grilled meat, pan-fried meat, or broiled meat was not associated with an increased risk of prostate cancer in any of the case definitions (Table 3). Well and very well done total meat was significantly associated with prostate cancer in all cases, RR=1.22 (95% CI 1.00, 1.49) p for trend=0.06, in incident cases, RR=1.26 (95% CI 1.02, 1.54) p for trend=0.03, and in advanced cases, RR=1.97 (95% CI 1.26, 3.08) p for trend=0.004, when the highest tertile was compared with the lowest (Table 3).

We did not observe a significant association between prostate cancer and any of the mutagens evaluated or mutagenic activity, although risks for DiMeIQx and MeIQx were of borderline significance, RR=1.24 (95% CI 0.96, 1.59) and RR=1.20 (95% CI 0.93, 1.55) respectively, among incident cases when the highest quintile was compared with the lowest. Additional adjustment of these two HCA models for PhIP slightly increased the estimates, for DiMeIQx, RR=1.28 (95% CI 0.97, 1.68) p for trend=0.09 and for MeIQx, RR=1.25 (95% CI 0.94, 1.66) p for trend=0.13.

DISCUSSION

In this prospective study, we found significant positive associations for well and very well done total meat intake and risk of prostate cancer in all case groups examined. We also observed suggestive evidence that two HCA's, DiMeIQx and MeIQx, also elevated the risk of prostate cancer among all cases, especially those with incident disease.

Several previous cohort studies have supported an association between meat and/or certain meat items and prostate cancer, although not all findings were statistically significant (19–24). However, two recent cohort studies with larger numbers of cases (n = 1,897 and n = 1,338) have reported no association between total or red meat intake and the risk of incident or advanced disease (13,25). Our findings are consistent with these studies as we did not observe an association between total or red meat, intake (or other specific types of meat) and prostate cancer.

Despite a lack of association for meat type, we did find that meat doneness level was positively associated with prostate cancer risk; in particular, intake of well and very well done meat was associated with a 22% increased risk of all prostate cancer, 26% increased risk of incident disease, and 97% increased risk of advanced prostate cancer. These findings are consistent with previous reports that have evaluated meat doneness and risk of prostate cancer. Two case-control studies have reported significantly elevated risks for prostate cancer for those in the highest categories of consumption, one reported a 1.7-fold increased risk associated with well done beef steak intake (11) and another reported a 1.7-fold increased risk in the top tertile of well done meat intake (26). Additionally, one large cohort study found a 42% significantly increased risk of prostate cancer when the highest tertile of very well-done meat intake was compared with the lowest (13). Cooking meat at high temperatures and increased duration of cooking have been consistently identified to be sources of PAH's, HCA's, and other mutagens and could explain the observed increase risk (6,7,27).

Although the increased risk associated with well and very well done meat may be a surrogate for HCA and PAH exposure, we did not observe any significantly increased risks for prostate cancer for the mutagens estimated in this analysis. An elevated but nonsignificant association was observed for two HCA's, DiMeIQx and MeIQx but these observations must be interpreted

with caution because the biological impact of these compounds remains unclear. At high doses, PhIP has been demonstrated to act as a prostate carcinogen in rodent models (9) but DiMeIQx and MeIQx are thought to be more potent mutagens (28) than PhIP so it is difficult to determine which might have more biological impact. In addition, few epidemiologic studies have evaluated these mutagens with consistent results; two previous case-control studies found no association for these HCA's (11,12), while one large study found a significant elevated risk for those in the highest category of PhIP intake, but not DiMeIQx or MeIQx (13). In agreement with the previously reported cohort study (13) we did not find any association between BaP and prostate cancer. BaP is highly toxic, however, and evidence from animal studies consistently shows a positive association between BaP and tumors at several anatomic sites (29,30). There are many sources of exposure to BaP, including tobacco smoke, pollution and other dietary sources (31–33). Studies of BaP from other sources, such as tobacco smoke and occupational exposures, have found positive associations with prostate cancer risk (34–37). It is also possible that some other compounds that we did not estimate in this study may have contributed to the observed increase in the risk of prostate cancer for those in the highest tertile of well and very well done meat.

Many animal and human experimental studies have demonstrated the carcinogenicity of HCA's. There are several lines of evidence to suggest that PhIP specifically may be a prostate carcinogen. In animal models, PhIP increases mutation frequency (10) and tumor incidence (9). Furthermore, *in vitro* work with human prostate cells has shown that PhIP increases genotoxicity and DNA adduct levels (38–40). Oral administration of another HCA, MeIQx, induces tumors in rodents at multiple tissue sites (41). The N-hydroxy metabolite of MeIQx leads to prostate hyperplasia in rats and induces MeIQx-DNA adduct formation in human prostate epithelial cells (40,42). DiMeIQx is mutagenic in bacterial assays (43), but has not been extensively evaluated as an animal or human carcinogen due to its similar chemical structure as MeIQx.

HCA's and PAH's require metabolic activation to carcinogenic intermediates, which is dependent on particular xenobiotic metabolizing enzymes. Several phase I enzymes act to activate carcinogens and these include members of the cytochrome P450 family. Phase II enzymes such as sulfotransferases, N-acetyltransferases, UDP-glucuronosyltransferases, and glutathione S-transferases can catalyze conjugation reactions to form detoxification products, or further metabolize other reactive intermediates for future excretion. Single nucleotide polymorphisms in genes that code for phase I and II enzymes involved in the metabolism of HCA's and PAH's have been described (44,45) and may cause decreased or increased enzyme expression or complete absence of the enzyme, resulting in differential mutagen metabolism and thus differential cancer risk (26,46).

The strengths of our study include a relatively large sample size, the ability to assess the intake of different meat types, cooking methods, doneness levels, HCA's and PAH's, as well as the ability to control for a wide set of potential confounders, including exposures specific to farming populations. The prospective design of this study allowed us to evaluate incident disease (diagnosed after the first year of follow-up) separate from all cases combined as latent disease may alter dietary choices and reporting. Furthermore, the percentage of recruitment and follow-up of participants was high with 82% of eligible participants enrolling and fewer than 2% lost to follow-up. Although not all of the take-home questionnaires were returned, the measured differences between respondents and non-respondents were small and were unlikely to be influential here (15).

This study also has certain limitations. The questionnaire used in this analysis is being enhanced in Phase II of the study to include fish, hotdog intake, and additional cooking methods, and other sources of carcinogenic compounds in meat. Furthermore, it is also important to note that

marinating meat and flipping of hamburgers, which impacts the formation of HCA's and PAH's, was not considered here. Despite convincing evidence from animal models, human metabolism studies, and molecular epidemiology studies, there could be various reasons for the lack of association with PhIP in this analysis. The results from this study may be true but it may also be due to inaccurate estimates of PhIP intake. The meat items and preparation methods in the questionnaire needed to estimate PhIP intake in this population may not be complete. Another important aspect could be that the CHARRED database may be missing some important sources of PhIP. There are also issues of measurement error that are common to dietary studies based on questionnaire data, which typically attenuate results.

We were also not able to adjust for total energy intake in this analysis. We did, however, perform a sensitivity analysis on the subgroup of subjects who also completed a full food frequency questionnaire (developed and validated by the National Cancer Institute (47,48) during Phase II of the study, before a diagnosis of prostate cancer, to estimate the impact of total energy adjustment. Energy adjustment was implemented by including total energy in multivariate models and by the multivariate nutrient density method (49). Results from these analyses, in greater than two thirds of our study population (N=15,659), found that adjustment resulted in negligible differences in risk estimates and thus we conclude do not significantly alter our findings.

In summary, this study supports the hypothesis that well done meat intake may contribute to an increase in the risk for prostate cancer. It also suggests that HCA exposure may alter prostate cancer risk, although this was less clear. Because individual HCA's or PAH's in cooked meat may be highly correlated with the presence of other similar compounds not measured here, further studies are needed to tease out the impact of meat intake and risk for prostate cancer.

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Abbreviations

PAH	Polycyclic aromatic hydrocarbon
HCA	Heterocyclic Amine
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
MeIQx	2-amino-3,8-dimethylimidazo-[4,5-*b*]quinoxaline
DiMeIQx	2-amino-3,4,8-trimethylimidazo-[4,5-*f*]quinoxaline
BaP	benzo(a)pyrene
RR	Relative Risk
CI	Confidence Interval
AHS	Agricultural Health Study

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Table 1

Selected Characteristics by Quintiles of Red Meat Intake[*†]

Characteristic	Quintile of Red Meat Intake				
	1	2	3	4	5
Participants (n)	4,551	4,607	4,742	4,509	4,671
Prostate Cases (n)	158	159	133	113	105
Age (mean yrs)	52.4	48.8	47.5	47.1	45.6
State of Residence (%)					
Iowa	53.6	66.3	67.3	75.7	80.2
North Carolina	46.4	33.7	32.7	24.3	19.8
Family History of prostate cancer (%)					
No	82.5	84.4	83.8	84.2	84.0
Yes	7.5	8.0	9.0	9.2	9.5
Race (%)					
White	94.5	96.6	96.8	97.2	97.7
Black	2.0	1.2	1.1	0.7	0.8
Other[‡]	0.6	0.5	0.2	0.3	0.3
Body Mass Index, kg/m^2 (%)					
<25	28.9	24.4	22.9	20.9	20.7
25–29	42.8	45.7	44.5	44.3	43.1
30+	15.4	18.6	20.7	23.3	24.3
Education (%)					
High School/GED or less	50.5	52.3	53.6	54.2	56.0
More than High School	44.1	42.9	41.8	41.9	40.0
Smoking Status (%)					
Never	53.2	50.4	51.3	53.0	54.1
Former	29.2	30.2	29.1	28.4	28.3
Current	11.0	13.8	14.4	13.6	13.5
Current Alcohol Intake (%)					
Never	40.0	31.7	31.2	28.2	26.6
< Once/month	15.4	15.4	14.7	15.4	14.5
1–4 Drinks/month	23.9	29.2	27.2	30.0	28.8

Characteristic	Quintile of Red Meat Intake				
	1	2	3	4	5
2–4 Drinks/week	10.2	13.8	15.0	15.3	18.0
Almost Everyday	3.5	4.4	5.7	5.0	6.7
Everyday	0.8	1.1	1.0	1.6	1.6
Leisure Time Physical Activity, (h/wk) (%)					
None	22.4	21.7	23.5	25.0	25.2
≤2	35.3	38.1	38.4	37.2	35.7
3–5	19.7	20.0	18.0	18.1	17.3
≥6	20.4	18.7	18.7	18.2	20.4
Aspirin Use (%)					
No	70.5	73.2	74.3	74.8	73.9
Yes	26.9	24.7	23.5	23.5	24.2
Supplemental Vitamin E (%)					
No	82.0	84.4	85.8	87.8	88.8
Yes	18.0	15.6	14.2	12.2	11.2

*All values (except age) are adjusted for age.

†Percent may not sum to 100 due to rounding and/or missing values.

‡Other includes Asian or Pacific Islander, American Indian or Alaskan Native, and other.

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Table 2

Relative Risks and 95% CIs for meat intakes and risk of prostate cancer

		All Cases (n=668)*		Incident Cases (n=613)*		Advanced Cases (n=140)*	
Variable	**Median (g/d)**	**Cases (n)**	**RR (95% CI)**	**Cases (n)**	**RR (95%) CI**	**Cases (n)**	**RR (95%) CI**
Total Meat (g/d)							
Q1(ref)	33.8	153	1.00	141	1.00	26	1.00
Q2	55.3	144	1.16 (0.92, 1.46)	127	1.12 (0.88, 1.42)	28	1.27 (0.72, 2.17)
Q3	74.9	145	1.19 (0.95, 1.50)	135	1.22 (0.96, 1.54)	36	1.56 (0.94, 2.60)
Q4	97.9	124	1.11 (0.87, 1.41)	116	1.14 (0.88, 1.46)	31	1.38 (0.81, 2.35)
Q5	140.7	102	1.04 (0.80, 1.35)	94	1.06 (0.81, 1.38)	19	0.93 (0.51, 1.70)
p for trend			0.93		0.71		0.80
Red Meat (g/d)							
Q1(ref)	23.2	158	1.00	145	1.00	28	1.00
Q2	42.5	159	1.30 (1.04, 1.62)	143	1.28 (1.15, 1.62)	30	1.21 (0.72, 2.05)
Q3	60.9	133	1.15 (0.91, 1.46)	121	1.15 (0.90, 1.48)	33	1.31 (0.78, 2.21)
Q4	81.6	113	1.09 (0.85, 1.40)	109	1.16 (0.90, 1.50)	28	1.20 (0.70, 2.06)
Q5	122.3	105	1.10 (0.85, 1.43)	95	1.11 (0.84, 1.46)	21	0.89 (0.50, 1.60)
p for trend			0.92		0.76		0.59
Chicken (g/d)							
Q1(ref)	2.8	162	1.00	150	1.00	31	1.00
Q2	10.3	164	0.95 (0.77, 1.19)	152	0.95 (0.76, 1.20)	40	1.27 (0.79, 2.03)
Q3	12.0	121	1.28 (1.00, 1.62)	108	1.24 (0.96, 1.59)	31	1.92 (1.15, 3.21)
Q4	24.0	154	1.14 (0.91, 1.43)	142	1.14 (0.90, 1.44)	22	1.02 (0.59, 1.78)
Q5	42.0	67	1.04 (0.78, 1.39)	61	1.02 (0.76, 1.39)	16	1.65 (0.90, 3.04)
p for trend			0.49		0.57		0.36
Bacon and Sausage (g/d)							
Q1(ref)	0.0	217	1.00	202	1.00	58	1.00
Q2	2.7	127	1.00 (0.80, 1.26)	118	0.99 (0.79, 1.25)	28	0.91 (0.57, 1.44)
Q3	4.7	112	0.98 (0.78, 1.25)	104	0.96 (0.75, 1.23)	22	0.74 (0.45, 1.24)
Q4	9.4	72	0.97 (0.73, 1.27)	64	0.90 (0.67, 1.20)	11	0.55 (0.28, 1.07)
Q5	17.2	140	0.98 (0.78, 1.24)	125	0.90 (0.70, 1.15)	21	0.69 (0.40, 1.18)
p for trend			0.83		0.33		0.11

		All Cases (n=668)*		Incident Cases (n=613)*		Advanced Cases (n=140)*	
Variable	**Median (g/d)**	**Cases (n)**	**RR (95% CI)**	**Cases (n)**	**RR (95%) CI**	**Cases (n)**	**RR (95%) CI**
Beef Steak (g/d)							
Q1(ref)	4.2	178	1.00	163	1.00	33	1.00
Q2	10.5	176	1.11 (0.90, 1.38)	161	1.11 (0.89, 1.39)	35	1.17 (0.72, 1.91)
Q3	18.0	179	1.00 (0.80, 1.26)	161	1.00 (0.78, 1.26)	41	1.16 (0.70, 1.92)
Q4	36.0	95	1.08 (0.82, 1.42)	90	1.12 (0.84, 1.49)	23	1.20 (0.67, 2.15)
Q5	63.0	40	1.03 (0.71, 1.49)	38	1.06 (0.73, 1.56)	8	0.87 (0.38, 1.99)
p for trend			0.90		0.67		0.84
Pork Chops/Ham Steak (g/d)							
Q1(ref)	3.3	173	1.00	155	1.00	35	1.00
Q2	8.3	168	0.96 (0.77, 1.20)	155	1.00 (0.80, 1.27)	34	0.89 (0.54, 1.45)
Q3	14.3	197	0.99 (0.79, 1.24)	183	1.06 (0.83, 1.35)	41	0.88 (0.54, 1.46)
Q4	16.0	11	1.03 (0.55, 1.95)	10	1.05 (0.55, 2.04)	2	0.45 (0.10, 1.91)
Q5	28.6	119	1.00 (0.76, 1.29)	110	1.05 (0.79, 1.38)	28	1.08 (0.62, 1.89)
p for trend			0.98		0.70		0.72
Hamburger (g/d)							
Q1(ref)	8.3	224	1.00	207	1.00	35	1.00
Q2	14.3	150	1.06 (0.85, 1.32)	135	1.04 (0.88, 1.31)	30	1.15 (0.69, 1.93)
Q3	28.6	148	1.01 (0.81, 1.29)	133	1.00 (0.78, 1.28)	44	1.42 (0.87, 2.33)
Q4	50.0	112	1.08 (0.84, 1.42)	105	1.12 (0.85, 1.46)	23	1.01 (0.57, 1.81)
Q5	78.6	34	1.06 (0.72, 1.57)	33	1.13 (0.76, 1.69)	8	1.08 (0.48, 2.44)
p for trend			0.70		0.41		0.94

Abbreviations: Relative Risk (RR); CI (Confidence Interval).

All cases refer to total incident cases; Incident cases refer to those diagnosed after one year of follow-up; Advanced cases defined as those classified as disease stage III or IV

*Adjusted for Age, state of residence (Iowa or North Carolina), Race (White, Black, Other, and Missing), Family History of Prostate Cancer (Yes/No), and Smoking Status (Never, Former, Current, and Missing).

Table 3

Relative Risks and 95% CIs for meat cooking methods and doneness levels and risk of prostate cancer

		All Cases (n=668)*		Incident Cases (n=613)*		Advanced Cases (n=140)*	
Variable	**Median (g/d)**	**Cases (n)**	**RR (95% CI)**	**Cases (n)**	**RR (95%) CI**	**Cases (n)**	**RR (95%) CI**
Cooking Method							
Grilled Meat (g/d)							
Q1(ref)	0.0	205	1.00	188	1.00	44	1.00
Q2	10.7	147	0.94 (0.75, 1.16)	136	0.95 (0.76, 1.19)	28	0.87 (0.53, 1.41)
Q3	24.8	121	0.83 (0.66, 1.05)	109	0.83 (0.65, 1.05)	18	0.59 (0.34, 1.03)
Q4	42.3	86	0.91 (0.70, 1.17)	77	0.90 (0.68, 1.18)	21	0.94 (0.56, 1.61)
Q5	73.3	109	1.12 (0.87, 1.43)	103	1.18 (0.91, 1.53)	29	1.27 (0.76, 2.10)
p for trend			0.43		0.27		0.28
Pan-fried Meat (g/d)							
Q1(ref)	1.0	152	1.00	142	1.00	44	1.00
Q2	10.2	124	0.94 (0.74, 1.20)	110	0.90 (0.70, 1.15)	22	0.63 (0.38, 1.06)
Q3	21.9	121	0.93 (0.73, 1.18)	113	0.93 (0.72, 1.19)	18	0.52 (0.30, 0.91)
Q4	38.5	140	1.04 (0.82, 1.31)	125	1.00 (0.78, 1.27)	26	0.72 (0.44, 1.19)
Q5	72.6	131	1.00 (0.78, 1.27)	123	1.00 (0.78, 1.29)	30	0.79 (0.49, 1.27)
p for trend			0.74		0.63		0.73
Broiled Meat (g/d)							
Q1(ref)	0.00	135	1.00	125	1.00	40	1.00
Q2	0.04	164	1.08 (0.86, 1.36)	151	1.07 (0.85, 1.36)	32	0.75 (0.47, 1.20)
Q3	0.14	86	1.01 (0.76, 1.34)	78	0.97 (0.73, 1.30)	12	0.53 (0.28, 1.03)
Q4	4.22	141	1.26 (0.99, 1.60)	125	1.18 (0.92, 1.53)	20	0.68 (0.40, 1.17)
Q5	23.43	142	1.14 (0.90, 1.44)	134	1.16 (0.91, 1.48)	36	0.95 (0.61, 1.49)
p for trend			0.40		0.26		0.38
Doneness Level							
Rare or Medium Total Meat (g/d)							
Q1(ref)	0.0	256	1.00	239	1.00	48	1.00
Q2	18.0	226	1.07 (0.89, 1.30)	205	1.06 (0.87, 1.29)	52	1.47 (0.95, 2.16)
Q3	63.0	186	1.05 (0.85, 1.29)	169	1.04 (0.84, 1.29)	40	1.19 (0.75, 1.88)
p for trend			0.78		0.80		0.71

		All Cases (n=668)*		Incident Cases (n=613)*		Advanced Cases (n=140)*	
Variable	**Median (g/d)**	**Cases (n)**	**RR (95% CI)**	**Cases (n)**	**RR (95%) CI**	**Cases (n)**	**RR (95%) CI**
Well and Very Well Done Total Meat (g/d)							
Q1(ref)	18.0	204	1.00	187	1.00	35	1.00
Q2	40.6	235	1.14 (0.94, 1.38)	212	1.12 (0.92, 1.37)	51	1.63 (1.06, 2.52)
Q3	80.3	229	1.22 (1.00, 1.49)	214	1.26 (1.02, 1.54)	54	1.97 (1.26, 3.08)
p for trend			0.06		0.03		0.004

Abbreviations: Relative Risk (RR); CI (Confidence Interval).

All cases refer to total incident cases; Incident cases refer to those diagnosed after one year of follow-up; Advanced cases defined as those classified as disease stage III or IV

*Adjusted for Age, state of residence (Iowa or North Carolina), Race (White, Black, Other, and Missing), Family History of Prostate Cancer (Yes/No), and Smoking Status (Never, Former, Current, and Missing).

Table 4

Relative Risks and 95% CIs for meat mutagens and risk of prostate cancer

		All Cases (n=668)*		Incident Cases (n=613)*		Advanced Cases (n=140)*	
Variable	**Median**	**Cases (n)**	**RR (95% CI)**	**Cases (n)**	**RR (95%) CI**	**Cases (n)**	**RR (95%) CI**
PhIP (ng/d)							
Q1(ref)	19.8	158	1.00	145	1.00	34	1.00
Q2	49.5	141	1.15 (0.91, 1.44)	130	1.16 (0.91, 1.47)	27	0.99 (0.60, 1.65)
Q3	84.8	125	1.01 (0.80, 1.28)	112	0.99 (0.78, 1.27)	25	0.96 (0.57, 1.61)
Q4	140.6	120	1.02 (0.81, 1.30)	111	1.04 (0.81, 1.33)	22	0.88 (0.51, 1.50)
Q5	281.3	124	1.04 (0.82, 1.32)	115	1.06 (0.83, 1.35)	32	1.23 (0.76, 2.01)
p for trend			0.91		0.96		0.36
MeIQx (ng/d)							
Q1(ref)	12.3	138	1.00	124	1.00	33	1.00
Q2	30.2	130	1.06 (0.84, 1.35)	117	1.07 (0.83, 1.37)	22	0.76 (0.44, 1.30)
Q3	50.8	138	1.19 (0.94, 1.51)	127	1.23 (0.96, 1.57)	27	0.96 (0.58, 1.60)
Q4	80.7	133	1.14 (0.90, 1.45)	125	1.20 (0.94, 1.54)	29	1.00 (0.60, 1.65)
Q5	148.2	129	1.15 (0.90, 1.47)	120	1.20 (0.93, 1.55)	29	0.92 (0.55, 1.52)
p for trend			0.29		0.16		0.94
DiMeIQx (ng/d)							
Q1(ref)	0.1	140	1.00	127	1.00	37	1.00
Q2	1.9	155	1.16 (0.92, 1.45)	141	1.16 (0.91, 1.48)	26	0.73 (0.44, 1.20)
Q3	3.6	113	1.10 (0.86, 1.41)	102	1.10 (0.85, 1.43)	13	0.48 (0.25, 0.90)
Q4	5.8	127	1.17 (0.92, 1.50)	118	1.21 (0.94, 1.56)	35	1.14 (0.71, 1.81)
Q5	10.9	133	1.19 (0.93, 1.51)	125	1.24 (0.96, 1.59)	29	0.85 (0.52, 1.39)
p for trend			0.23		0.12		0.87
BaP (ng/d)							
Q1(ref)	0.9	184	1.00	170	1.00	42	1.00
Q2	3.6	138	0.79 (0.64, 0.99)	123	0.77 (0.61, 0.97)	31	0.79 (0.49, 1.25)
Q3	25.0	116	0.81 (0.64, 1.02)	108	0.82 (0.64, 1.04)	24	0.74 (0.45, 1.22)
Q4	59.0	122	0.99 (0.79, 1.25)	109	0.96 (0.76, 1.23)	21	0.80 (0.47, 1.35)
Q5	124.2	108	0.91 (0.71, 1.16)	103	0.95 (0.74, 1.22)	22	0.84 (0.50, 1.42)
p for trend			0.69		0.43		0.78

		All Cases (n=668)*		Incident Cases (n=613)*		Advanced Cases (n=140)*	
Variable	**Median**	**Cases (n)**	**RR (95% CI)**	**Cases (n)**	**RR (95%) CI**	**Cases (n)**	**RR (95%) CI**
Mutagenic Activity (per 1,000 revertant colonies/d)							
Q1(ref)	1.9	154	1.00	139	1.00	34	1.00
Q2	4.1	134	1.04 (0.83, 1.32)	122	1.06 (0.83, 1.35)	23	0.80 (0.47, 1.37)
Q3	6.5	132	1.10 (0.87, 1.39)	119	1.11 (0.87, 1.42)	22	0.80 (0.46, 1.37)
Q4	10.0	126	1.10 (0.87, 1.40)	118	1.15 (0.90, 1.48)	32	1.21 (0.74, 1.97)
Q5	17.8	122	1.06 (0.83, 1.35)	115	1.11 (0.87, 1.43)	29	0.98 (0.60, 1.62)
p for trend			0.68		0.39		0.59

Abbreviations: Relative Risk (RR); CI (Confidence Interval).

All cases refer to total incident cases; Incident cases refer to those diagnosed after one year of follow-up; Advanced cases defined as those classified as disease stage III or IV

*Adjusted for Age, state of residence (Iowa or North Carolina), Race (White, Black, Other, and Missing), Family History of Prostate Cancer (Yes/No), and Smoking Status (Never, Former, Current, and Missing).