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

Stella Koutros,[1,3] Amanda J. Cross,[2] Dale P. Sandler,[4] Jane A. Hoppin,[4] Xiaomei Ma,[3] Tongzhang Zheng,[3] Michael C.R. Alavanja,[1] and Rashmi Sinha[2]

[1]Occupational and Environmental Epidemiology Branch and [2]Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Department of Health and Human Services, Rockville, Maryland; [3]Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, Connecticut; and [4]National Institute of Environmental Health Sciences, NIH, Department of Health and Human Services, Research Triangle Park, North Carolina

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 and 95% confidence intervals (95% 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 of advanced disease (95% CI, 1.26-3.08) 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 and 2-amino-3,8-dimethylimidazo-[4,5-*b*]quinoxaline 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. (Cancer Epidemiol Biomarkers Prev 2008;17(1):80–7)

Introduction

Prostate cancer is the most common cancer in men in the United States (other than nonmelanoma 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 and polycyclic aromatic hydrocarbons (PAH). The heterocyclic amine and PAH content of meat varies according to meat type, cooking method, and doneness level, although most are generally formed in meats cooked well done by high-temperature cooking methods (5-8). One of the most abundant heterocyclic amines, 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 effect of various meat mutagens on prostate cancer risk. Two small case-control studies found no association between PhIP or other major heterocyclic amines 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, a large cohort of licensed pesticide applicators in Iowa and North Carolina.

Materials and Methods

Study Population. The Agricultural Health Study 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

Received 4/30/07; revised 10/16/07; accepted 11/9/07.

Grant support: Intramural Research Program of the NIH (National Cancer Institute Division of Cancer Epidemiology and Genetics and National Institute of Environmental Health Sciences) and National Cancer Institute grant TU2 CA105666.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Stella Koutros, Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Boulevard, EPS 8111, Rockville, MD 20852. Phone: 301-594-6352; Fax: 301-402-1819. E-mail: koutross@mail.nih.gov

doi:10.1158/1055-9965.EPI-07-0392

Table 1. Selected characteristics by quintiles of red meat intake

Characteristic	Quintile of red meat intake				
	Q1	Q2	Q3	Q4	Q5
Participants (*n*)	4,551	4,607	4,742	4,509	4,671
Prostate cases (*n*)	158	159	133	113	105
Mean age (y)	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/ general educational development 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
<1 Drink/mo	15.4	15.4	14.7	15.4	14.5
1-4 Drinks/mo	23.9	29.2	27.2	30.0	28.8
2-4 Drinks/wk	10.2	13.8	15.0	15.3	18.0
Almost daily	3.5	4.4	5.7	5.0	6.7
Daily	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

NOTE: 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.

elsewhere (14). Briefly, applicators were recruited from December 1993 to 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 and medical history and included a section on meat cooking practices. This take-home questionnaire was completed by ~40% of the applicators and we have shown previously few important differences between those applicators who did and 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 Agricultural Health Study Coordinating Center, Westat.

Dietary Assessment. The dietary module in the phase I 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 12 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.

Table 2. RRs (95% CIs) for meat intakes and risk of prostate cancer

Variable	Median (g/d)	All cases (n = 668)*		Incident cases (n = 613)*		Advanced cases (n = 140)*	
		Cases (n)	RR (95% CI)	Cases (n)	RR (95% CI)	Cases (n)	RR (95% CI)
Total meat (g/d)							
Q1 (reference)	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 (reference)	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 (reference)	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/sausage (g/d)							
Q1 (reference)	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
Beef steaks (g/d)							
Q1 (reference)	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 steaks (g/d)							
Q1 (reference)	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
Hamburgers (g/d)							
Q1 (reference)	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

NOTE: All cases refer to total incident cases; incident cases refer to those diagnosed after 1 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).

A specifically developed database (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 heterocyclic amines: 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 (95% CI) describing the effect of meat, meat cooking methods, meat doneness, and meat mutagen exposure on prostate cancer risk. All analyses were done on three different groups: (*a*) all incident cases occurring after enrollment, (*b*) incident cases diagnosed after 1 year of follow-up, referred to as incident cases, and (*c*) 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

Table 3. RRs (95% CIs) for meat cooking methods and doneness levels and risk of prostate cancer

Variable	Median (g/d)	All cases (n = 668)*		Incident cases (n = 613)*		Advanced cases (n = 140)*	
		Cases (n)	RR (95% CI)	Cases (n)	RR (95% CI)	Cases (n)	RR (95% CI)
Cooking method							
Grilled meat (g/d)							
Q1 (reference)	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 (reference)	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 (reference)	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 (reference)	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
Well and very well done total meat (g/d)							
Q1 (reference)	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

NOTE: All cases refer to total incident cases; incident cases refer to those diagnosed after 1 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).

quintile as the reference 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 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 as 1 month, yes/no), history of diabetes (yes/no), leisure time physical activity (hours/week; none, <1, 1-2, 3-5, 6-10, >10), alcohol intake in the past 12 months (never, less than once a month, 1-3 times a month, once a week, 2-4 times a 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) linked previously to prostate cancer in subsets of applicators in the Agricultural Health Study: 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).

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,

Table 4. RRs (95% CIs) for meat mutagens and risk of prostate cancer

Variable	Median	All cases (n = 668)*		Incident cases (n = 613)*		Advanced cases (n = 140)*	
		Cases (n)	RR (95% CI)	Cases (n)	RR (95% CI)	Cases (n)	RR (95% CI)
PhIP (ng/d)							
Q1 (reference)	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 (reference)	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 (reference)	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 (reference)	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
Mutagenic activity (per 1,000 revertant colonies/d)							
Q1 (reference)	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

NOTE: All cases refer to total incident cases; incident cases refer to those diagnosed after 1 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).

0.80-1.35); RR, 1.06 (95% CI, 0.81-1.38); and 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/sausage, beef steaks, pork chops/ham steaks, and hamburgers. 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], incident cases [RR, 1.26 (95% CI, 1.02-1.54); P for trend = 0.03], and 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 heterocyclic amine 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] (Table 4).

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 heterocyclic amines, 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 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 has been consistently identified to be sources of PAHs, heterocyclic amines, 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 heterocyclic amine 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 heterocyclic amines, DiMeIQx and MeIQx, but these observations must be interpreted with caution because the biological effect of these compounds remains unclear. At high doses, PhIP has been shown 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 effect. In addition, few epidemiologic studies have evaluated these mutagens with consistent results; two previous case-control studies found no association for these heterocyclic amines (11, 12), whereas 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 shown the carcinogenicity of heterocyclic amines. 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) and PhIP-DNA adducts have been detected *in vivo* in human prostate cells (41, 42). Oral administration of MeIQx induces tumors in rodents at multiple tissue sites (43). The *N*-hydroxy metabolite of MeIQx leads to prostate hyperplasia in rats and induces MeIQx-DNA adduct formation in human prostate epithelial cells (40, 44). DiMeIQx is mutagenic in bacterial assays (45) but has not been extensively evaluated as an animal or human carcinogen due to its similar chemical structure as MeIQx.

Heterocyclic amines and PAHs 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 *P*450 family. *N*-oxidation of exocyclic amine groups results in the formation of carcinogenic *N*-hydroxy heterocyclic amine metabolites. For example, PhIP itself is not genotoxic, but its activation to *N*-hydroxy-PhIP catalyzed by CYP1A1 and CYP1B1 results in this highly genotoxic intermediate (46, 47). Incubation of prostate cells with BaP and *N*-hydroxy metabolites of PhIP and MeIQx has been shown to induce their corresponding DNA adducts in prostate tissues, lending further support to the role of meat mutagens in prostate carcinogenesis (40, 47, 48).

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 meat mutagen intermediates for future excretion. Various phase I and II enzymes are expressed in human prostate tissue, including NAT1 and NAT2 (40, 49) and GSTP1, GSTM2, and GSTM3 (48), supporting a role for these enzymes and meat mutagens in prostate carcinogenesis. The presence and active expression of these enzymes in the prostate and their documented role in activating and detoxifying meat mutagens suggest that genes coding for these particular enzymes may be important factors in prostate carcinogenesis. Lending further support to the role of phase I and II enzymes in mutagen metabolism are various studies of single nucleotide polymorphisms in these enzymes that are associated with increased prostate cancer risk (50-54).

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, heterocyclic amines and PAHs, and 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 nonrespondents 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 affects the formation of heterocyclic amines and PAHs, 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 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 Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease 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, however, did a sensitivity analysis on the subgroup of subjects who also completed a full food frequency questionnaire developed and validated by the National Cancer Institute (56, 57) during phase II of the study, before a diagnosis of prostate cancer, to estimate the effect of total energy adjustment. Energy adjustment was implemented by including total energy in multivariate models and by the multivariate nutrient density method (58). 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 heterocyclic amine exposure may alter prostate cancer risk, although this was less clear. Because individual heterocyclic amines or PAHs 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 effect of meat intake and risk for prostate cancer.

Acknowledgments

We thank the participants in the Agricultural Health Study for their contributions in support of this research.

References

1. American Cancer Society. What are the key statistics about prostate cancer? Available from: http://www.cancer.org/docroot/home/index.asp. Accessed 2006 Aug 1.
2. Maskarinec G, Noh JJ. The effect of migration on cancer incidence among Japanese in Hawaii. Ethn Dis 2004;14:431–9.
3. Marugame T, Katanoda K. International comparisons of cumulative risk of breast and prostate cancer, from cancer incidence in five continents. Vol. VIII. Jpn J Clin Oncol 2006;36:399–400.
4. Kolonel LN. Fat, meat, and prostate cancer. Epidemiol Rev 2001;23:72–81.
5. Skog K. Cooking procedures and food mutagens: a literature review. Food Chem Toxicol 1993;31:655–75.
6. Sinha R, Knize MG, Salmon CP, et al. Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. Food Chem Toxicol 1998;36:289–97.
7. Sinha R, Rothman N, Salmon CP, et al. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. Food Chem Toxicol 1998;36:279–87.
8. Sinha R, Rothman N, Brown ED, et al. High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine (PhIP) occur in chicken but are dependent on the cooking method. Cancer Res 1995;55:4516–9.
9. Shirai T, Sano M, Tamano S, et al. The prostate: a target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) derived from cooked foods. Cancer Res 1997;57:195–8.
10. Stuart GR, Holcroft J, de Boer JG, Glickman BW. Prostate mutations in rats induced by the suspected human carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. Cancer Res 2000;60:266–8.
11. Norrish AE, Ferguson LR, Knize MG, Felton JS, Sharpe SJ, Jackson RT. Heterocyclic amine content of cooked meat and risk of prostate cancer. J Natl Cancer Inst 1999;91:2038–44.
12. Rovito PM, Morse PD, Spinek K, et al. Heterocyclic amines and genotype of *N*-acetyltransferases as risk factors for prostate cancer. Prostate Cancer Prostatic Dis 2005;8:69–74.
13. Cross AJ, Peters U, Kirsh VA, et al. A prospective study of meat and meat mutagens and prostate cancer risk. Cancer Res 2005;65:11779–84.
14. Alavanja MC, Sandler DP, McMaster SB, et al. The Agricultural Health Study. Environ Health Perspect 1996;104:362–9.
15. Tarone RE, Alavanja MC, Zahm SH, et al. The Agricultural Health Study: factors affecting completion and return of self-administered questionnaires in a large prospective cohort study of pesticide applicators. Am J Ind Med 1997;31:233–42.
16. Sinha R, Cross A, Curtin J, et al. Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. Mol Nutr Food Res 2005;49:648–55.
17. Knize MG, Sinha R, Rothman N, et al. Heterocyclic amine content in fast-food meat products. Food Chem Toxicol 1995;33:545–51.
18. Ames BN, Mccann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat Res 1975;31:347–64.
19. Gann PH, Hennekens CH, Sacks FM, Grodstein F, Giovannucci EL, Stampfer MJ. Prospective study of plasma fatty acids and risk of prostate cancer. J Natl Cancer Inst 1994;86:281–6.
20. Giovannucci E, Rimm EB, Colditz GA, et al. A prospective study of dietary fat and risk of prostate cancer. J Natl Cancer Inst 1993;85:1571–9.
21. Le Marchand L, Kolonel LN, Wilkens LR, Myers BC, Hirohata T. Animal fat consumption and prostate cancer: a prospective study in Hawaii. Epidemiology 1994;5:276–82.
22. Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer 1989;64:598–604.
23. Snowdon DA, Phillips RL, Choi W. Diet, obesity, and risk of fatal prostate cancer. Am J Epidemiol 1984;120:244–50.
24. Veierod MB, Laake P, Thelle DS. Dietary fat intake and risk of prostate cancer: a prospective study of 25,708 Norwegian men. Int J Cancer 1997;73:634–8.
25. Michaud DS, Augustsson K, Rimm EB, Stampfer MJ, Willet WC, Giovannucci E. A prospective study on intake of animal products and risk of prostate cancer. Cancer Causes Control 2001;12:557–67.
26. Nowell S, Ratnasinghe DL, Ambrosone CB, et al. Association of SULT1A1 phenotype and genotype with prostate cancer risk in African-Americans and Caucasians. Cancer Epidemiol Biomarkers Prev 2004;13:270–6.
27. Kazerouni N, Sinha R, Hsu CH, Greenberg A, Rothman N. Analysis of 200 food items for benzo[*a*]pyrene and estimation of its intake in an epidemiologic study. Food Chem Toxicol 2001;39:423–36.
28. Felton JS, Knize MG. Occurrence, identification, and bacterial mutagenicity of heterocyclic amines in cooked food. Mutat Res 1991;259:205–17.
29. Culp SJ, Gaylor DW, Sheldon WG, Goldstein LS, Beland FA. A comparison of the tumors induced by coal tar and benzo[*a*]pyrene in a 2-year bioassay. Carcinogenesis 1998;19:117–24.
30. Weyand EH, Chen YC, Wu Y, Koganti A, Dunsford HA, Rodriguez LV. Differences in the tumorigenic activity of a pure hydrocarbon and a complex mixture following ingestion: benzo[*a*]-]pyrene vs manufactured gas plant residue. Chem Res Toxicol 1995;8:949–54.
31. Lee BM, Shim GA. Dietary exposure estimation of benzo[*a*]pyrene and cancer risk assessment. J Toxicol Environ Health A 2007;70:1391–4.
32. Melikian AA, Djordjevic MV, Chen S, Richie J, Stellman SD. Effect of delivered dosage of cigarette smoke toxins on the levels of urinary biomarkers of exposure. Cancer Epidemiol Biomarkers Prev 2007;16:1408–15.

33. Pratt GC, Palmer K, Wu CY, Oliaei F, Hollerbach C, Fenske MJ. An assessment of air toxics in Minnesota. Environ Health Perspect 2000; 108:815–25.
34. Boers D, Zeegers MP, Swaen GM, Kant I, van den Brandt PA. The influence of occupational exposure to pesticides, polycyclic aromatic hydrocarbons, diesel exhaust, metal dust, metal fumes, and mineral oil on prostate cancer: a prospective cohort study. Occup Environ Med 2005;62:531–7.
35. Giovannucci E, Rimm EB, Ascherio A, et al. Smoking and risk of total and fatal prostate cancer in United States health professionals. Cancer Epidemiol Biomarkers Prev 1999;8:277–82.
36. Krishnadasan A, Kennedy N, Zhao Y, Morgenstern H, Ritz B. Nested case-control study of occupational chemical exposures and prostate cancer in aerospace and radiation workers. Am J Ind Med 2007;50: 383–90.
37. Nock NL, Tang D, Rundle A, et al. Associations between smoking, polymorphisms in polycyclic aromatic hydrocarbon (PAH) metabolism and conjugation genes and PAH-DNA adducts in prostate tumors differ by race. Cancer Epidemiol Biomarkers Prev 2007;16: 1236–45.
38. Cui L, Takahashi S, Tada M, et al. Immunohistochemical detection of carcinogen-DNA adducts in normal human prostate tissues transplanted into the subcutis of athymic nude mice: results with 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 3,2′-dimethyl-4-aminobiphenyl (DMAB) and relation to cytochrome *P*450s and *N*-acetyltransferase activity. Jpn J Cancer Res 2000;91:52–8.
39. Martin FL, Cole KJ, Muir GH, et al. Primary cultures of prostate cells and their ability to activate carcinogens. Prostate Cancer Prostatic Dis 2002;5:96–104.
40. Wang CY, biec-Rychter M, Schut HA, et al. *N*-acetyltransferase expression and DNA binding of *N*-hydroxyheterocyclic amines in human prostate epithelium. Carcinogenesis 1999;20:1591–5.
41. Tang D, Liu JJ, Bock CH, et al. Racial differences in clinical and pathological associations with PhIP-DNA adducts in prostate. Int J Cancer 2007;121:1319–24.
42. Tang D, Liu JJ, Rundle A, et al. Grilled meat consumption and PhIP-DNA adducts in prostate carcinogenesis. Cancer Epidemiol Biomarkers Prev 2007;16:803–8.
43. U.S. Department of Health and Human Services PHSNTP: report on carcinogens. 11th ed. 2005.
44. Archer CL, Morse P, Jones RF, Shirai T, Haas GP, Wang CY. Carcinogenicity of the *N*-hydroxy derivative of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, 2-amino-3, 8-dimethyl-imidazo[4,5-*f*]quinoxaline and 3, 2′-dimethyl-4-aminobiphenyl in the rat. Cancer Lett 2000;155:55–60.
45. Poirier LA, Weisburger EK. Selection of carcinogens and related compounds tested for mutagenic activity. J Natl Cancer Inst 1979;62: 833–40.
46. Turesky RJ. The role of genetic polymorphisms in metabolism of carcinogenic heterocyclic aromatic amines. Curr Drug Metab 2004;5: 169–80.
47. Williams JA, Martin FL, Muir GH, Hewer A, Grover PL, Phillips DH. Metabolic activation of carcinogens and expression of various cytochromes *P*450 in human prostate tissue. Carcinogenesis 2000; 21:1683–9.
48. Di Paolo OA, Teitel CH, Nowell S, Coles BF, Kadlubar FF. Expression of cytochromes *P*450 and glutathione *S*-transferases in human prostate, and the potential for activation of heterocyclic amine carcinogens via acetyl-CoA-, PAPS- and ATP-dependent pathways. Int J Cancer 2005;117:8–13.
49. Agundez JA, Martinez C, Olivera M, et al. Expression in human prostate of drug- and carcinogen-metabolizing enzymes: association with prostate cancer risk. Br J Cancer 1998;78:1361–7.
50. Agalliu I, Lin DW, Salinas CA, Feng Z, Stanford JL. Polymorphisms in the glutathione *S*-transferase M1, T1, and P1 genes and prostate cancer prognosis. Prostate 2006;66:1535–41.
51. Aktas D, Hascicek M, Sozen S, Ozen H, Tuncbilek E. CYP1A1 and GSTM1 polymorphic genotypes in patients with prostate cancer in a Turkish population. Cancer Genet Cytogenet 2004;154:81–5.
52. Chang BL, Zheng SL, Isaacs SD, et al. Polymorphisms in the CYP1A1 gene are associated with prostate cancer risk. Int J Cancer 2003;106: 375–8.
53. Nock NL, Liu X, Cicek MS, et al. Polymorphisms in polycyclic aromatic hydrocarbon metabolism and conjugation genes, interactions with smoking and prostate cancer risk. Cancer Epidemiol Biomarkers Prev 2006;15:756–61.
54. Silig Y, Pinarbasi H, Gunes S, Ayan S, Bagci H, Cetinkaya O. Polymorphisms of CYP1A1, GSTM1, GSTT1, and prostate cancer risk in Turkish population. Cancer Invest 2006;24:41–5.
55. Lodovici M, Luceri C, Guglielmi F, et al. Benzo(*a*)pyrene diolepoxide (BPDE)-DNA adduct levels in leukocytes of smokers in relation to polymorphism of CYP1A1, GSTM1, GSTP1, GSTT1, and mEH. Cancer Epidemiol Biomarkers Prev 2004;13:1342–8.
56. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. Am J Epidemiol 2001;154:1089–99.
57. Thompson FE, Subar AF, Brown CC, et al. Cognitive research enhances accuracy of food frequency questionnaire reports: results of an experimental validation study. J Am Diet Assoc 2002;102: 212–25.
58. Willet WC. Nutritional epidemiology. 2nd ed. New York (NY): Oxford University Press; 1998. p. 293–6.

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

Stella Koutros, Amanda J. Cross, Dale P. Sandler, et al.

Cancer Epidemiol Biomarkers Prev 2008;17:80-87.