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Research Article

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Intake of Meat Mutagens and Risk of Prostate Cancer in a Cohort of U.S. Health Professionals

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Abstract

Background: Evidence relating heterocyclic aromatic amines (HCA), associated with high-temperature cooking methods, to prostate cancer risk is inconsistent.

Methods: In a large U.S. cohort study, intakes of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), and 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx) and a meat-derived mutagenicity (MDM) index were assessed using a cooking method questionnaire administered in 1996. Until 2010, 2,770 prostate cancer cases were observed among 26,030 participants.

Results: Intake of PhIP from red meat was statistically significantly associated with total prostate cancer risk (top vs. bottom quintile HR, 1.18; 95% confidence intervals; CI, 1.03–1.35), but not other HCAs (MeIQx, 1.12; 0.98–1.27, PhIP from white meat, 1.08; 0.95–1.22, DiMeIQx, 1.09; 0.97–1.21) or MDM (1.13; 1.00–1.28). For high-grade (Gleason sum 7 with pattern 4+3 and Gleason sum 8–10, $n = 483$ cases) and advanced cancers ($n = 281$), we only observed positive associations for PhIP from red meat (top vs. bottom quintile: high grade: HR, 1.44; 95% CI, 1.04–1.98, $P_{\text{trend}} = 0.03$; advanced: HR, 1.50; 95% CI, 0.99–2.26; $P_{\text{trend}} = 0.12$), but associations for advanced cancers did not reach statistical significance. Observed associations remained similar after adjustment for total, unprocessed, or processed red meat intake.

Conclusion: Observed positive associations between PhIP intake from red meat and prostate cancer, particularly high-grade and possibly also advanced prostate cancer, need to be confirmed in other studies.

Impact: Results do not provide strong evidence that HCAs increase risk of prostate cancers. *Cancer Epidemiol Biomarkers Prev; 24(10); 1557–63. ©2015 AACR.*

Introduction

Higher meat, especially red meat consumption has been suggested to be associated with higher risk of prostate cancer, but the evidence is not consistent (reviewed in ref. 1). One possible mechanism through which red meat may be involved in prostate carcinogenesis is the formation of mutagenic heterocyclic amines (HCA) in muscle meat when meats are cooked at high temperature and for long duration (e.g., grilling, barbecuing, frying, and broiling; ref. 2).

HCAs were first detected in the 1970s in smoke condensate of grilled fish and were shown to be mutagenic (3). HCAs are formed from precursors (creatine/creatinine, sugar, amino acids) found in the muscle of meat and fish cooked at temperatures exceeding 130°C (4, 5). HCA production and amount depend mainly on cooking method, temperature, and the type of meat or fish (4). Meat drippings and gravy made from these drippings contain considerable amounts of HCA (6). The most abundant HCAs in the human diet are 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx; ref. 7).

Thus far, five cohort studies have examined the association between HCA intake and prostate cancer risk. Some reported positive associations for red meat that was consumed mostly well done (8–10) or for HCA intake (10), in particular with advanced disease. Two found no associations (11, 12). In addition, three (13–15) of five (16, 17) case–control studies reported no clear associations between HCA intake and risk of prostate cancer.

The aim of this study was to examine in the PSA era the association of HCA intake and a meat-derived mutagenicity (MDM) index with risk of prostate cancer in a large prospective U.S. cohort study.

Materials and Methods

Study population

The Health Professionals Follow-up Study includes 51,529 dentists, veterinarians, pharmacists, optometrists, osteopathic physicians, and podiatrists who were between 40 and 75 years of age at enrollment in 1986. At baseline, all participants

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completed a 131-item semi-quantitative food-frequency questionnaire (FFQ) and provided information on age, race or ethnicity, weight, height, physical activity, cigarette smoking, alcohol consumption, and medical history. Every 2 years, questionnaires were mailed to collect updated information on exposure and new diagnoses; follow-up FFQs were mailed every 4 years. The validity and reproducibility of our FFQs have been documented previously (18). Deaths were reported by next-of-kin, the postal service, and searches of the National Death Index. The study was approved by the Human Subjects Committee at the Harvard School of Public Health (Boston, MA).

In 1996, questions on meat preparation (cooking method and degree of doneness) were included on the biennial questionnaire. This analysis is based on the cohort of men who provided information on meat preparation in 1996 and who responded to the 1994 FFQ. Of these participants, we excluded all men with a cancer diagnosis except for nonmelanoma skin cancer before 1996 and men with missing information on meat preparation (no answer to any of the cooking method questions, missing frequency of meat intake of at least one cooked meat item, or no information on bacon consumption, which is needed to estimate HCA intake). The final cohort for this analysis included 26,030 men.

Case ascertainment

On the biennial follow-up questionnaires, men were asked to report whether they were diagnosed with prostate cancer, in the previous 2 years. Diagnosis was confirmed by review of medical records (>90% of the cases). For nonresponders, we used information from the National Death Index, postal service, and next-of-kin to determine whether a participant had died. After the review of death certificates, informed consent was obtained from next-of-kin of participants who died of prostate cancer to obtain medical records. Study investigators reviewed the medical records to confirm a prostate cancer diagnosis and prostate cancer deaths.

This analysis was based on 2,770 non-T1a prostate cancer cases ascertained after the date of return of the 1996 questionnaire through January 31, 2010. Of these cancer cases, 281 were classified as advanced or lethal [diagnosed at an advanced stage (T3b, T4, N1, and M1), developed metastases during follow-up, or died due to prostate cancer]. We confirmed 2,285 cases as organ-confined or having limited extraprostatic extension (T1b, T1c, T2, T3a, and N0 or Nx and M0), and the remainder could not be assigned a stage. We defined low-grade prostate cancer as patients having Gleason sum of 2–6 or 7 with a pattern of 3 plus 4 ($n = 1859$) and high-grade cases as those with Gleason sum 7 and pattern of 4 plus 3 and those with Gleason sum 8–10 ($n = 483$).

Assessment of meat consumption and cooking methods

Total, red (processed and unprocessed), and white meat intake was estimated from the 1994 FFQ. For each food item, a commonly used unit or serving size was specified. The participants were asked to indicate how often, on average, they consumed each food item, with nine possible response categories ranging from "never" to "six or more times per day." In 1996, participants completed a cooking methods questionnaire based on the results of a pilot study, which ascertained a group of cooking method questions that best predicted HCA intake in the HPFS (7). Information on cooking methods and typical outside appearance of pan-fried, broiled, and grilled or barbecued chicken, hamburger, and steak; fried, microwaved, and broiled bacon; fried sausage; roast beef; and homemade gravy were assessed (7). On the basis of the results of the pilot study, variation in doneness level of fried bacon was small. Therefore, fried bacon intake was based on the 1994 FFQ and assumed to be medium browned (7). The Charred Database (19) contains data on HCA and MDM concentration in various meat items depending on cooking method and outside appearance (20). The mutagenic activity of meat samples was assessed by the Ames/Salmonella test. The resulting MDM is a measure of the total mutagenic activity found in cooked meats, which integrates mutagenic activity from all classes of mutagens, including heterocyclic amines, but also benzo[a]pyrene or yet unidentified compounds, found in cooked meats (21).

A participant's HCA intake or MDM was calculated by multiplying the given consumption frequency of each food by its HCA content or MDM value for the specified cooking method and degree of browning derived from the Charred Database (20).

Statistical analysis

Intake of PhIP, MeIQx, DiMeIQx, and MDM index was categorized into quintiles based on the distribution in the analytic cohort. We calculated HRs and corresponding 95% confidence intervals (CI) using Cox proportional hazards regression to evaluate the association of HCA intake and MDM index with total and advanced or lethal prostate cancer. We observed no violation of the proportional hazards assumption. The models were adjusted for age, race/ethnicity, pack-years smoked in the past 10 years, family history of prostate cancer, updated history of diabetes mellitus, height, body mass index (BMI) at age 21, updated vigorous leisure-time physical activity, updated current aspirin use, cumulative average updated intake of tomato products and fish, and updated intake of energy, alcohol, vitamin E, α-linolenic acid, and total calcium. We decided on this set of confounders *a priori* based on the overall literature. Cumulative updated average intake represents the average intake of a food or nutrient from all available FFQs up to the start of each follow-up interval (22). To test whether associations between HCA intake or MDM index might be explained by meat intake *per se*, we ran models that additionally adjusted for (i) total red meat, (ii) unprocessed red meat [which includes regular hamburger; lean or extra-lean hamburger; beef, pork, or lamb as a sandwich or mixed dish (e.g., stew, casserole, lasagna, etc.); beef or lamb as a main dish (e.g., steak, roast, ham, etc.); pork as a main dish (e.g., ham or chops)], (iii) processed red meat salami, bologna, or other processed meat sandwiches; other processed meats [e.g., sausage, kielbasa, etc.); bacon; hot dogs], and (d) white meat (chicken and turkey). We also examined the association of total red meat, unprocessed red meat, processed red meat and white meat consumption in 1994 (closest date to HCA assessment in 1996) and the risk of prostate cancer. To test for trend, we entered the median of each quintile of HCA intake or MDM index as a single continuous variable into the model and used the Wald test to assess statistical significance of the coefficient. We ran stratified models to determine whether the associations of HCA intake or MDM index with prostate cancer varied by age (<60 vs. ≥60 years in 1996), current BMI, aspirin use (nonusers in 1996 vs. user; infrequent vs. frequent user; never user vs. user), consumption of tomato products (sum of tomato sauce and pizza; < vs. ≥2.0 servings/week), intake of vitamin E (< vs. ≥ median intake), and history of prostatitis. In a sensitivity analysis, we restricted the analysis to men who have had at least one PSA test until the end of the observation period in 2010. The presence of multiplicative interaction was assessed by including a cross-product term for these factors and HCA intake or MDM index in the regression model along with the main effect terms. The statistical significance of the coefficient for the cross-product term was evaluated by the Wald test. A P value of <0.05 was considered statistically significant.

Results

The analysis included 2,770 prostate cancer cases in 314,746 person-years of follow-up. Selected baseline characteristics of the study participants by highest and lowest category of PhIP, MeIQx, and DiMeIQx intake are shown in Table 1. Participants in the highest categories engaged less often in vigorous physical activity and were more likely to have smoked in the past 10 years. These participants also had a higher intake of red meat, but lower intake of vitamin E than participants in the lowest quintiles.

Intake of PhIP from red meat was statistically significantly associated with total prostate cancer risk (multivariable HR = 1.18; 95% CI, 1.03–1.35, top vs. bottom quintile; Table 2). However, intake of PhIP from white meat or intake of MeIQx, DiMeIQx, or the MDM index was not associated with total prostate cancer risk (Table 2). For high-grade cancers (Gleason sum 7 with pattern 4+3 and Gleason sum 8–10, $n = 483$ cases), we also observed an increased risk among participants with high intake of PhIP from red meat (HR = 1.44; 95% CI, 1.04–1.98, $P_{trend} = 0.03$), but we did not observe any significant associations with MDM index (HR = 1.05; 95% CI, 0.78–1.41, $P_{trend} = 0.54$), MeIQx, PhIP from white meat, or DiMeIQx.

For advanced cancers ($n = 281$), we only observed positive associations for PhIP from red meat (top vs. bottom quintile: HR = 1.50 (95% CI, 0.99–2.26; $P_{trend} = 0.12$), but associations for advanced cancers did not reach statistical significance (Table 2). HCA intake or MDM index was not statistically significantly associated with the risk of organ-confined or low-grade prostate cancer (data not shown).

Adding total red meat intake to the multivariable model did not materially change the associations between HCA intake or MDM index with total, advanced, and high-grade prostate (data not shown); results were also similar after adding unprocessed red meat or processed meat separately to the models (data not shown). Similarly, no major changes were observed when adding white meat to the model. In order to evaluate whether the effect on high-grade and advanced tumors was due to HCA or other carcinogens in cooked meat, we included both MDM and PhIP from red meat into the same model; but the risk estimates for PhIP from red meat were similar (top vs. bottom quintile, HR, 95% CI; high grade: PhIP from red meat 1.50, 1.06–2.14; MDM 0.90, 0.65–1.23; advanced: PhIP from red meat 1.45, 0.92–2.28; MDM 1.17, 0.76–1.81).

Generally, the associations of HCA intake or MDM index with total prostate cancer were not modified by age, BMI, aspirin use, vitamin E intake, or history of prostatitis (all $P_{interaction} > 0.05$; data not shown). However, we observed an increased risk of total prostate cancer associated with high HCA intake or MDM index among those with lower (but not higher) consumption of tomato products; however, interactions were not statistically significant, except for MDM ($P_{interaction} = 0.03$; Table 3). Excluding men who had never had a PSA test did not change our results appreciably (data not shown).

In joint analysis, we observed that men with both low tomato consumption and high MDM had a 22% (95% CI, 2%–47%) higher risk of developing prostate cancer when compared with those with high tomato consumption and low MDM (data not shown).

Discussion

In this large prospective cohort study conducted in the PSA era, we did not observe statistically significant associations between HCA intake and risk of prostate cancer. However, we observed positive associations between PhIP intake from red meat and risk of total, high-grade and advanced prostate cancer. Also, we noted that men with low tomato product consumption had an increased

Table 1. Baseline (1996) age-standardized characteristics by lowest and highest quintiles of HCA intake and MDM index, Health Professionals Follow-up Study

	Total MeIQx (ng/d)		Total DiMeIQx (ng/d)		Total PhIP (ng/d)		MDM index (revertant colonies/d)	
Quintile (median intake)	**Q1 (1.6)**	**Q5 (37.8)**	**Q1 (0)**	**Q5 (4.0)**	**Q1 (13.2)**	**Q5 (218.1)**	**Q1 (693)**	**Q5 (8233)**
N	5,035	5,202	8,208	5,320	4,890	5,412	4,925	5,312
Age in 1996 (mean, SD)	61.4 (8.3)	59.7 (7.9)	61.7 (8.3)	58.5 (7.4)	62.8 (8.5)	58.2 (7.3)	62.0 (8.4)	58.8 (7.6)
Caucasian (%)	90	93	92	93	91	93	91	93
Smoked in past 10 y (%)	12	18	15	17	14	16	13	17
Family history of prostate cancer (%)	12	15	13	15	13	14	12	14
History of diabetes (up to 1996; %)	3	5	4	4	3	4	3	5
Current use of aspirin in 1996 (%)	70	76	72	76	70	76	70	76
Screening PSA test by 2006 (%)	96	93	95	95	94	95	95	95
Body height (inches), mean (SD)	70.0 (2.6)	70.5 (2.5)	70.2 (2.7)	70.4 (2.5)	70.2 (2.6)	70.3 (2.5)	70.1 (2.7)	70.4 (2.6)
BMI at age 21 (kg/m^2), mean (SD)	23.0 (2.6)	23.0 (2.7)	23.0 (2.6)	23.1 (2.7)	22.9 (2.6)	23.1 (2.6)	23.0 (2.7)	23.0 (2.7)
BMI in 1996 (kg/m^2), mean (SD)	25.0 (3.0)	26.5 (3.5)	25.6 (3.3)	26.1 (3.4)	25.1 (3.2)	26.3 (3.3)	25.1 (3.2)	26.2 (3.5)
Vigorous MET-h/wk, mean (SD)	20.7 (29.6)	11.9 (23.3)	16.8 (28.1)	14.9 (24.3)	17.8 (29.1)	15.1 (25.7)	18.5 (29.2)	14.3 (24.5)
Daily intake in 1994, mean (SD)								
Alcohol intake (drinks/w)	4.57 (6.00)	6.23 (7.71)	5.25 (6.67)	6.27 (7.27)	4.50 (6.27)	6.91 (7.78)	4.77 (6.28)	6.27 (7.52)
Tomato products (servings/w)	1.55 (1.29)	1.73 (1.29)	1.59 (1.26)	1.75 (1.26)	1.55 (1.29)	1.75 (1.27)	1.53 (1.25)	1.75 (1.31)
Red meat (servings/w)	3.65 (3.19)	10.9 (5.38)	5.98 (4.52)	8.11 (5.23)	4.88 (4.13)	8.47 (5.19)	4.46 (3.63)	8.98 (5.36)
Fish (servings/w)	2.57 (1.91)	1.78 (1.34)	2.32 (1.80)	2.21 (1.65)	2.12 (1.71)	2.32 (1.66)	2.19 (1.73)	2.22 (1.67)
White meat (servings/w)	2.68 (1.68)	2.66 (1.59)	2.69 (1.66)	2.96 (1.58)	2.25 (1.53)	3.21 (1.67)	2.28 (1.52)	3.13 (1.66)
Calcium (mg/d)	981 (441)	843 (343)	943 (423)	875 (361)	990 (448)	847 (355)	972 (441)	864 (358)
Vitamin E (mg/d)	108 (129)	64.5 (97.3)	93.6 (122)	77.1 (109)	97.6 (126)	78.9 (111)	97.5 (126)	76.2 (107)
Alpha-linolenic acid (mg/d)	1,129 (359)	1,155 (361)	1,118 (348)	1,133 (369)	1,139 (359)	1,137 (344)	1,134 (364)	1,148 (347)

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Table 2. Association of heterocyclic amine intake and MDM index with prostate cancer, Health Professionals Follow-up Study 1996–2010

	Total prostate cancer ($n = 2,770$)				High-grade prostate cancer ($n = 483$)[a]				Advanced or lethal prostate cancer ($n = 281$)[b]			
	N	HR[c]	HR[d]	95% CI	*N*	HR[c]	HR[d]	95% CI	*N*	HR[c]	HR[d]	95% CI
MeIQx (ng/d)												
<3.6	531	1.00	1.00	ref.	92	1.00	1.00	ref.	60	1.00	1.00	ref.
3.7–<8.0	546	1.03	1.04	0.92–1.17	97	1.12	1.12	0.83–1.49	45	0.85	0.83	0.56–1.24
8.1–<14.1	581	1.08	1.12	0.99–1.27	102	1.19	1.17	0.87–1.57	66	1.24	1.24	0.86–1.79
14.1–<25.0	554	1.10	1.08	0.95–1.22	94	1.11	1.09	0.80–1.48	54	1.03	1.00	0.67–1.47
≥25.0	558	1.11	1.12	0.98–1.27	98	1.17	1.15	0.84–1.56	56	1.11	1.08	0.72–1.60
P_{trend}		0.11	0.07			0.35	0.49			0.38	0.52	
PhIP (ng/d)												
<25.6	523	1.00	1.00	ref.	104	1.00	1.00	ref.	66	1.00	1.00	ref.
25.6–<51.4	571	1.08	1.08	0.96–1.22	103	1.02	1.00	0.76–1.33	51	0.84	0.84	0.58–1.22
51.4–<89.4	553	1.04	1.02	0.90–1.15	80	0.80	0.77	0.57–1.04	63	1.06	1.06	0.74–1.51
89.4–<152.9	555	1.06	1.06	0.94–1.20	92	0.96	0.95	0.71–1.27	52	0.97	0.98	0.67–1.43
≥152.9	568	1.09	1.08	0.95–1.22	104	1.13	1.08	0.81–1.44	49	1.00	1.01	0.68–1.49
P_{trend}		0.29	0.35			0.60	0.79			0.77	0.73	
PhIP from red meat (ng/d)												
<0.7	441	1.00	1.00	ref.	50	1.00	1.00	ref.	51	1.00	1.00	ref.
0.7–<9.5	597	1.05	1.05	0.92–1.19	100	1.02	1.02	0.75–1.37	63	1.05	1.05	0.71–1.54
9.5–<18.4	571	1.11	1.11	0.97–1.26	90	1.04	1.05	0.77–1.44	52	0.99	1.00	0.67–1.50
18.4–<45.0	638	1.05	1.04	0.91–1.18	107	1.07	1.06	0.78–1.45	56	0.98	0.98	0.65–1.47
≥45.0	523	1.18	1.18	1.03–1.35	106	1.47	1.44	1.04–1.98	59	1.48	1.50	0.99–2.26
P_{trend}		0.03	0.05			0.02	0.03			0.12	0.12	
PhIP from white meat (ng/d)												
<14.4	510	1.00	1.00	ref.	94	1.00	1.00	ref.	60	1.00	1.00	ref.
14.4–<29.9	578	1.09	1.08	0.96–1.22	107	1.11	1.09	0.82–1.45	65	1.16	1.18	0.82–1.69
29.9–<56.3	580	1.10	1.10	0.97–1.24	96	1.04	1.03	0.77–1.37	51	1.00	1.00	0.68–1.47
56.3–<110.4	547	1.05	1.05	0.92–1.18	98	1.06	1.06	0.79–1.42	59	1.10	1.12	0.77–1.63
≥110.4	555	1.07	1.07	0.94–1.21	88	1.02	0.99	0.73–1.34	46	0.99	1.02	0.68–1.53
P_{trend}		0.49	0.51			0.98	0.87			0.92	0.99	
DiMeIQx (ng/d)												
<0.10	869	1.00	1.00	ref.	164	1.00	1.00	ref.	86	1.00	1.00	ref.
0.10–<0.20	272	1.15	1.14	0.99–1.31	49	1.14	1.13	0.82–1.56	34	1.64	1.67	1.11–2.51
0.20–<0.87	539	1.12	1.11	1.00–1.24	85	0.95	0.94	0.72–1.23	48	1.10	1.10	0.77–1.58
0.87–<1.90	528	1.00	1.00	0.89–1.11	91	0.92	0.90	0.69–1.16	60	1.28	1.29	0.92–1.81
≥1.90	562	1.09	1.09	0.97–1.21	94	1.02	1.00	0.77–1.30	53	1.26	1.28	0.90–1.83
P_{trend}		0.32	0.37			0.74	0.61			0.23	0.21	
MDM index (revertant colonies/d)												
<1,222	520	1.00	1.00	ref.	98	1.00	1.00	ref.	54	1.00	1.00	ref.
1,222–<2,256	575	1.09	1.09	0.96–1.23	102	1.04	1.01	0.76–1.34	64	1.32	1.31	0.90–1.90
2,256–< 3,551	531	1.02	1.03	0.91–1.16	80	0.85	0.83	0.61–1.13	50	1.04	1.03	0.69–1.53
3,551–< 5,663	567	1.10	1.11	0.98–1.25	105	1.14	1.12	0.84–1.49	57	1.25	1.25	0.85–1.84
≥5,663	577	1.12	1.13	1.00–1.28	98	1.10	1.05	0.78–1.41	56	1.32	1.30	0.88–1.93
P_{trend}		0.08	0.07			0.38	0.54			0.25	0.30	

[a]Gleason 7 4+3 pattern and 8–10.

[b]T3b, T4, N1, M1 at diagnosis or had metastases during follow-up or died due to prostate cancer.

[c]Adjusted for age.

[d]Adjusted for age, race/ethnicity, pack-years smoked in the past 10 years, family history of prostate cancer, updated history of diabetes mellitus, height, BMI at age 21, updated vigorous leisure-time physical activity, updated current aspirin use, cumulative average updated intake of tomato products and fish, and updated intake of energy, alcohol, vitamin E, α-linolenic acid, and total calcium.

risk of total prostate cancer associated with intake of some HCAs or the MDM index, whereas no increased risks were observed among men with high tomato product consumption.

Heterocyclic amines have consistently been shown to be mutagenic in mutagenicity assays and carcinogenic in animal models (23, 24). PhIP contributes most to HCA intake in the United States, whereas DiMeIQx intake is comparably low, but is the most mutagenic HCA of the three examined HCAs (25). Epidemiologic evidence for an association between HCA intake and prostate cancer, however, is limited. In the most recent study, conducted among participants of the California Collaborative Prostate Cancer Study (16), high consumption of red meat cooked at high temperature and well-done red meat were positively associated with risk of advanced, but not overall prostate cancer. Intake of PhIP was also positively, although not statistically significantly, related with advanced prostate cancer. This result is similar to what has been reported in the NIH–AARP cohort (8). High intake of very well-done meat and of PhIP was associated with an increased risk of prostate cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (10). In the Agricultural Health Study (9), consumption of well or very well-done meat was related to an increased risk of total and in particular advanced prostate cancer, and there were nonstatistically significant positive associations with DiMeIQx and MeIQx intakes. In the Multiethnic cohort (11) and the EPIC–Heidelberg cohort (12), no associations of HCA intake or type of meat cooking or degree of doneness with risk of prostate cancer overall or with advanced disease were observed. In two case–control studies, HCA intake was not clearly associated with prostate cancer risk (13–15), although another study by John and

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Table 3. Association of heterocyclic amine intake and MDM index with total prostate cancer by consumption of tomato products, Health Professionals Follow-up Study 1996–2010

	Total prostate cancer		
	Lower consumption of tomato products (<2 portions/wk; 1,607 cases) HR[a] (95% CI)	Higher consumption of tomato products (≥2 portions/wk; 1,163 cases) HR[a] (95% CI)	p_i[b]
MeIQx (ng/d)			
<3.6	1.00 (ref.)	1.00 (ref.)	
3.7–<8.0	1.08 (0.92–1.26)	0.98 (0.80–1.19)	
8.1–<14.1	1.19 (1.02–1.40)	1.01 (0.83–1.23)	
14.1–<25.0	1.14 (0.97–1.34)	0.98 (0.80–1.20)	
≥25.0	1.15 (0.97–1.37)	1.07 (0.87–1.31)	
P_{trend}	0.08	0.55	0.27
PhIP (ng/d)			
<25.6	1.00 (ref.)	1.00 (ref.)	
25.6–<51.4	1.04 (0.89–1.21)	1.15 (0.94–1.41)	
51.4–<89.4	1.07 (0.92–1.26)	0.95 (0.77–1.17)	
89.4–<152.9	1.09 (0.93–1.28)	1.01 (0.82–1.23)	
≥152.9	1.14 (0.96–1.34)	1.02 (0.84–1.25)	
P_{trend}	0.10	0.63	0.12
PhIP from red meat (ng/d)			
<0.7	1.00 (ref.)	1.00 (ref.)	
0.7–<9.5	1.08 (0.92–1.27)	1.00 (0.81–1.23)	
9.5–<18.4	1.15 (0.97–1.35)	1.06 (0.86–1.30)	
18.4–<45.0	1.10 (0.93–1.30)	0.95 (0.78–1.17)	
≥45.0	1.27 (1.06–1.52)	1.05 (0.85–1.31)	
P_{trend}	0.02	0.87	0.12
PhIP from white meat (ng/d)			
<14.4	1.00 (ref.)	1.00 (ref.)	
14.4–<29.9	1.09 (0.93–1.27)	1.08 (0.88–1.32)	
29.9–<56.3	1.11 (0.95–1.30)	1.09 (0.89–1.33)	
56.3–<110.4	1.10 (0.93–1.28)	0.95 (0.77–1.16)	
≥110.4	1.15 (0.98–1.36)	0.99 (0.81–1.21)	
P_{trend}	0.12	0.42	0.29
DiMeIQx (ng/d)			
<0.10	1.00 (ref.)	1.00 (ref.)	
0.10–<0.20	1.11 (0.93–1.33)	1.21 (0.97–1.51)	
0.20–<0.87	1.12 (0.97–1.30)	1.09 (0.92–1.30)	
0.87–<1.90	1.04 (0.90–1.20)	0.91 (0.76–1.09)	
≥1.90	1.15 (1.00–1.34)	0.98 (0.83–1.16)	
P_{trend}	0.11	0.33	0.27
MDM index (revertant colonies/d)			
<1,222	1.00 (ref.)	1.00 (ref.)	
1,222–<2,256	1.12 (0.96–1.30)	1.04 (0.85–1.27)	
2,256–<3,551	0.98 (0.83–1.15)	1.09 (0.89–1.33)	
3,551–<5,663	1.13 (0.96–1.33)	1.10 (0.90–1.33)	
≥5,663	1.25 (1.06–1.47)	1.01 (0.83–1.24)	
P_{trend}	0.02	0.81	0.01

[a]Adjusted for age, race/ethnicity, pack-years smoked in the past ten years, family history of prostate cancer, updated history of diabetes mellitus, height, BMI at age 21, updated vigorous leisure-time physical activity, updated current aspirin use, cumulative average updated intake of fish, and updated intake of energy, alcohol, vitamin E, α-linolenic acid, and total calcium.

[b]$p_i = P_{interaction}$.

colleagues (13) reported a positive association of well-done and grilled red meat consumption with risk of advanced prostate cancer. A fourth case–control study including 470 cases of aggressive prostate cancer-related high intake of well-done meat and intake of MeIQx and DiMeIQx to an increased risk (17).

It is of interest to note that we observed associations between PhIP intake from red but not from white meat with risk of high-grade prostate cancer, although PhIP intake from white meat is twice as high as the intake from red meat. In addition, associations for PhIP from red meat remained similar after we adjusted for total, unprocessed, and processed meat intake separately. This argues against a causal association between PhIP intake (or HCA intake in general) and prostate cancer risk, and suggest the possibility that not HCA themselves but other mutagenic compounds that arise from cooking of red meat may also be a risk factor for prostate cancer. MDM integrates mutagenic activity of different compounds in cooked meats such as heterocyclic amines or benzo[a]pyrene, but also yet unidentified compounds (21). However, when we examined associations between PhIP from red meat and MDM and high-grade or advanced cancers in the same model estimates for PhIP from red meat were similar to estimates without MDM in the model.

In the rat, PhIP induced mutations in the ventral prostate, which was associated with an infiltrate of inflammatory cells (26). Similarly, PhIP induced prostate cancer in a CYP1A-humanized mice model, which is preceded by inflammatory proliferative epithelial lesions (27). In our analysis, we did not observe evidence for effect modification of the association between HCA intake or MDM index and prostate cancer risk by use of aspirin, intake of dietary vitamin E, or BMI, all of which are factors that influence inflammatory processes. We did, however, observe that associations were modified by consumption of tomato products. Lycopene is a potent antioxidant found in tomatoes (28), but the mechanism by which lycopene may modify the association of HCA intake and prostate cancer is currently unclear. Animal studies did not provide clear evidence that administration of lycopene reduced formation of HCA-DNA adducts, reduced oxidative stress induced by HCA administration, or influenced levels of phase II enzymes (29, 30). However, lycopene may affect cell cycle, apoptosis, inflammation, and angiogenesis by a variety of pathways (31, 32). To the best of our knowledge, this is the first study to observe a modifying effect of tomato intake on risk of prostate cancer and, thus, our results need to be confirmed by other studies. However, we cannot exclude the possibility that our results are due to chance and, thus, warrant confirmation from other studies.

We observed positive associations of PhIP intake from red meat with total, high-grade and advanced prostate cancer; even though associations for advanced cancers did not reach statistical significance. However, the number of advanced or lethal cases in our analytical cohort was small because we began follow-up in 1996 after the completion of the questionnaire, that is, in the PSA era; about 95% of men included in our analysis had reported at least one PSA between 1996 until the end of the follow-up period. Other studies that had reported associations of HCA intake with advanced prostate cancer (9, 13, 16, 17) did not separately evaluate high-grade disease.

We assessed meat cooking habits in 1996 and used that information throughout follow-up. It is possible that participants might have changed their meat preparation habits or overall meat intake during follow-up, which may result in another source of measurement error. For example, red meat consumption decreased in the HPFS over time. In 2004, we assessed meat cooking methods a second time in the HPFS cohort, and correlations between calculated HCA intake in 1996 and 2004 were $r = 0.31$, 0.40, and 0.23 (all $P < 0.0001$) for PhIP, MeIQx, and DiMeIQx, respectively. HCA intake estimations suffer from imprecision. Assessing a person's dietary intake by means of a FFQ is prone to recall bias due to over- or underestimation of dietary intake by the study participant leading to misclassification with respect to dietary intake. We estimated HCA intake and MDM

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index based on participants' responses to questions on the cooking method of specific meat items. Doneness levels specified by participants are proxies of the actual intake of heterocyclic amines, which varies by duration of cooking, temperatures used (33), type of oil used (34, 35), handling of the meat before and during the cooking process (36) as well as of the cooking method itself (37) such that cooking short time by high temperature and long time by lower temperature can result in the same degree of browning but not in the same degree of doneness. In addition, the use of limited data on the HCA content in differently prepared meats for the computation of HCA intake is another major shortcoming of this approach to quantify intake (38). However, the cooking questions used in this study are based on a pilot study (7) that determined which set of questions best predicted HCA intake in our cohorts. Also, because data on cooking methods were obtained before cancer diagnosis, possible measurement errors due to the aforementioned factors should be nondifferential, which tends to attenuate associations.

In conclusion, our results do not provide strong evidence that HCAs in general increase risk of prostate cancers. Observed associations between PhIP intake from red meat and prostate cancer, particularly high-grade and possibly also advanced prostate cancer need to be confirmed in other studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: W.C. Willett, E.A. Platz, K. Wu
Development of methodology: R. Sinha, E.L. Giovannucci
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W.C. Willett, E.L. Giovannucci, E.A. Platz
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Rohrmann, K. Nimptsch, R. Sinha, W.C. Willett, E.L. Giovannucci, E.A. Platz, K. Wu
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Study supervision: E.L. Giovannucci, E.A. Platz, K. Wu

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