Heterocyclic Amine Content of Cooked Meat and Risk of Prostate Cancer

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Background: **Some epidemiologic studies have described positive associations between prostate cancer risk and meat consumption, but underlying mechanisms have not been identified. Heterocyclic amines are mutagens formed during the cooking of meat. Well-done meat has been associated with increased risks of colorectal and breast cancers in humans. This study examined associations between prostate cancer risk and 1) estimated daily intake of heterocyclic amines from cooked meat and 2) level of cooked-meat doneness.** ***Methods:*** **A population-based, case–control study involving 317 case patients with prostate cancer and 480 age-matched control subjects was carried out in Auckland, New Zealand. Levels of meat doneness and daily intake of heterocyclic amines were determined from self-reported dietary data and experimentally measured heterocyclic amine levels in locally sourced meat samples cooked under controlled conditions to varying degrees of doneness.** ***Results:*** **The heterocyclic amines found in the highest concentrations in meat samples were 2-amino-1,6-dimethylfuro[3,2-*e*]imidazo[4,5-*b*]pyridine (IFP) and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) from well-done chicken and pork and very well-done beefsteak. Meat doneness was weakly and inconsistently associated with prostate cancer risk for individual types of meat, but increased risk was observed for well-done beefsteak (relative risk = 1.68; 95% confidence interval = 1.02–2.77; two-sided *P* for trend = .03). A weak positive gradient of increased risk was associated with estimated daily exposure to IFP but not with the other major heterocyclic amines.** ***Conclusions:*** **Meat doneness and estimated intake of heterocyclic amines from cooked meat were not clearly associated with prostate cancer risk. [J Natl Cancer Inst 1999;91:2038–44]**

Although several studies *(1)* of prostate cancer risk have described positive associations with consumption of meat and saturated fat, the epidemiologic evidence has been inconsistent. No clear underlying biologic mechanisms involving dietary fat or other nutrient components of meat have been identified for carcinogenesis or progression of prostate cancer. Heterocyclic amines, which form from amino acid, creatine, and polysaccharide precursors during the high-temperature cooking of meat and fish, have been shown to be mutagenic in the Ames assay and carcinogenic in experimental animal studies *(2,3)*. There is limited epidemiologic evidence concerning the association between dietary heterocyclic amines and cancer risk. An increased risk of breast cancer has been reported with the consumption of well-done meat *(4)*. There have been similar reports from some studies of colorectal cancer *(5,6)* but not others *(7,8)*. A Swedish case–control study has reported that estimated dietary exposure to heterocyclic amines is not associated with the risk of cancers of the colon, rectum, kidney, or bladder *(9)*.

The heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) has been clearly demonstrated to act as a carcinogen in rat prostates, although only at relatively high doses *(10)*. However, association between dietary heterocyclic amine intake and prostate cancer risk in humans has not been reported because most epidemiologic studies of prostate cancer have not collected data concerning meat-cooking practices. This study was undertaken to estimate dietary exposure to heterocyclic amines and to assess prostate cancer risk in relation to meat-cooking practices.

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See "Notes" following "References."

Subjects and Methods

Case–Control Study Recruitment

The Auckland Prostate Study is a population-based, case–control study that was carried out in the greater metropolitan area of Auckland, New Zealand. The study population included all men aged 40–80 years who normally resided in the Auckland area during the 13-month study recruitment period from January 1996. Almost all of the men with newly diagnosed prostate cancer in this Auckland population age group attended urologists either in one public hospital-based clinic or in private clinics involving seven urologists. In this study, all of the patients who attended the public hospital urology clinic and all of the patients who attended five of seven private clinic urologists were eligible to participate. To improve response rates and to reduce biases of dietary assessment arising from knowledge of a recent cancer diagnosis, the majority of prostate cancer case patients (60%) were identified from a larger group of urology clinic attendees (referred for investigation of prostate-related symptoms and signs and/or elevated serum prostate-specific antigen levels) who were recruited before the completion of clinical investigations. The remainder of the prostate cancer case patients in the study population were identified retrospectively from histology reports but within 3 weeks of diagnosis. No eligible case patients died before the recruitment or data collection.

All prostate cancer cases were confirmed by reference to histology reports. Before the study analysis, a cancer case was defined as "advanced" when it was a case with documented pathologic or radiologic evidence of tumor invasion beyond the prostate capsule or a tumor with a combined Gleason score of greater than or equal to 7. Only a small proportion of case patients (3%) presented with overt symptoms of advanced prostate cancer (bone pain and weight loss). Excluded from analysis were six patients whose diagnosis was made incidental to transurethral surgery, whose serum prostate-specific antigen was in the normal range, and whose tumor was localized with a combined Gleason score of less than or equal to 6. A total of 317 patients were included in the study.

Study control subjects were composed of men ages 40–80 years with no history of prostate cancer who were randomly selected from the general electoral rolls (these provide 95% coverage of the adult European men in the Auckland region). Control participants were matched to case patients during the study recruitment period by use of 10-year age groups and an approximate case : control ratio of 1 : 1.5. A total of 480 control subjects were included. Approval to carry out the study was obtained from the Northern Regional Health Authority Ethics Committee of New Zealand, and written informed consent was obtained from all participants.

Collection of Questionnaire Data

Study participants completed self-administered questionnaires covering personal, sociodemographic, anthropometric, medical, and lifestyle data and a validated 107-item food-frequency questionnaire *(11)* that collected data concerning usual food frequency and portion size (a photograph of a standard portion size was included for each food item) for food consumed at home or elsewhere over the preceding 12-month period. The questionnaire was modified to include questions on usual cooking methods for seven commonly consumed types of meat, including beefsteaks, lamb/mutton chops, small cuts of pork, minced beef, chicken, bacon, and sausages. Participants were asked to specify whether beefsteak was usually cooked "rare," "medium," or "well-done" and for the other meat types whether they were usually "cooked in liquid, microwaved or baked" or "fried, grilled, or barbecued." In the latter case, a further question determined whether the meat was cooked "medium or well-done" (lamb/mutton/pork), "lightly browned or well-browned" (chicken/minced beef/sausages), and "soft, crisp, or very crisp" (bacon). Men were instructed to consult with their wives or partners, where appropriate, concerning the use of meat-cooking methods.

Questionnaire data were provided by the majority of case patients before their cancer diagnosis and within 3 weeks of diagnosis in the remainder (retrospectively recruited case patients). Identical procedures were used for exposure data collection from case patients and control subjects. Research nurses visited all of the participants at home to obtain blood samples (not relevant to the current analyses) and to check the completeness of responses to the questionnaires that had been mailed to participants previously. Participants with missing responses were encouraged to complete the questionnaires (self-administered) at the time of the visit. Although the nurse interviewers were not blind to the case–control status of the participants, neither they nor the participants were aware of the specific hypotheses being tested.

Cooking and Estimation of Heterocyclic Amines in New Zealand Meat Samples

Meat samples were obtained from licensed retail outlets that are the major meat suppliers for New Zealand households. Where more than one source is commonly used, samples were obtained from several sources. Three samples of each meat type were cooked separately and pooled for analysis. The meat was cooked under controlled conditions in a research kitchen to a degree of doneness defined by consumer judgment and meat-industry charts, where possible. A meat probe was used to measure the internal temperature for each sample except bacon. All samples were cooked in a flat-bottomed frying pan at a constant temperature of 200 °C until the target temperature was reached.

The identification of heterocyclic amines for quantification in cooked meat samples was based on an analysis of U.S. restaurant foods *(12)* and studies of new mutagens showing 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), 2-amino-1,6-dimethylfuro[3,2-*e*]imidazo[4,5-*b*]pyridine (IFP), and PhIP to be commonly found *(13)*. Chemicals and solvents were high-performance liquid chromatography (HPLC) or analytic grade. Heterocyclic amines (MeIQx, PhIP, and DiMeIQx,) were purchased from Toronto Research Chemicals (Downsview, ON, Canada) and quantified by measurement of molar extinction coefficients as described previously *(14)*. Since a synthetic standard is not available, IFP was isolated from a heated mixture of creatine, glucose, and glutamic acid, and its exact structure was determined by nuclear magnetic resonance spectroscopy (Knize MG: unpublished data). IFP was quantified by use of the molar extinction coefficient of PhIP, another imidazopyridine. Amounts were quantified by HPLC according to the solid-phase extraction procedures of Gross and Grüter *(15)* as described previously *(14)*. Samples were analyzed in duplicate. An aliquot was spiked with a mixture of heterocyclic amines to determine extraction recovery. Results are corrected for recoveries determined from spiked samples, which varied depending on the meat matrix: 31%–68% for PhIP, 74%–97% for MeIQx, 69%–90% for DiMeIQx, and 14%–58% for IFP.

Statistical Analysis

Participants were assigned to one of three categories of meat doneness (defined before study analyses) for each of the seven meat types: low (cooked rare or baked/cooked in liquid/microwaved or self-reported nonconsumption of the meat), medium (fried/grilled/barbecued to a "medium" or "lightly browned" state), and high (fried/grilled/barbecued to a "well-done" or "well-browned" state). For assessment of the combined effect of doneness of meat across all seven meat types, participants were assigned a score of 1, 2, or 3 corresponding to their reported consumption of each meat type in the three categories (*above*), and these scores were summed to give meat-doneness scores for each participant that could range from 7 to 21. Age-adjusted and multivariate relative risks for prostate cancer were calculated by use of an unconditional logistic regression model *(16)*, comparing the high and medium levels of doneness-exposure categories with the low (reference) category.

Daily heterocyclic amine exposure was estimated for the four major amines, including MeIQx, DiMeIQx, IFP, and PhIP, and for total heterocyclic amines (the sum of these four). Heterocyclic amine concentrations (ng/g of meat) were assigned to the high-, medium-, and low-doneness "usual" meat-cooking method, based on data from the laboratory analysis of locally sourced cooked-meat samples. When meat was reported to be baked, cooked in liquid, microwaved, or not consumed, heterocyclic amine content was assigned a value of zero. Heterocyclic amine exposure (ng/day) was calculated as the product of the self-reported daily meat consumption (g/day) and the estimated heterocyclic amine concentration for usual meat-cooking method (ng/g), summed across the seven meat types. Categories of exposure for estimated daily heterocyclic amine intake were defined by quartiles, based on the distribution in the control group. Relative risks for prostate cancer were calculated for quartile categories, with the reference group composing the lowest quartile.

Since control subjects were matched to case patients by age group during recruitment, age-adjusted relative risks were calculated for all analyses. Age was included as a continuous variable in regression models, but other covariates were included as categoric terms in multivariate analyses. Potentially confounding nutrients [derived from the food-frequency questionnaire data and the New Zealand

food composition tables *(17)*] were defined by quartile categories, based on the distribution in the control group. Socioeconomic status was defined by the participant's usual current or former occupation (if retired), according to the modified Elley–Irving Classification *(18)* that has been widely used in New Zealand population research. Energy adjustment was carried out by including categoric terms for total energy consumption (derived from the food-frequency questionnaire) in the logistic regression model. A test for overall trend across categories was carried out by including ordinal terms for each category of intake (1, 2, 3. . .) as continuous variables in a logistic regression model with covariates. All *P* values reported are two-sided.

RESULTS

Participants in the Auckland Prostate Study included a total of 317 prostate cancer case patients (77% response rate) and 480 control subjects (71% response rate). The case patients included 192 men with advanced disease. The study population was composed of men predominantly of European descent (96%), with only a small proportion from Maori (2%), Pacific Island (1%), and other ethnic groups (1%). Table 1 describes sociodemographic and other characteristics of study case patients and control subjects that were considered to be potentially confounding variables in the current analyses. Because of the age-matched recruitment process, there was little difference in age distribution of case patients and control subjects; however, case patients were of lower socioeconomic status and less likely to take nonsteroidal anti-inflammatory drugs on a regular basis. Median intakes of energy (kJ/day) and fat (g/day) were similar in the two groups. Case patients reported a higher median intake of meat, but age-adjusted and multivariate analyses showed no association between prostate cancer risk and increasing quartiles of meat consumption, for either aggregated or individual meat types (data not presented).

Mean daily consumption of the individual types of meat among the control group included the following: steak (24 g), minced beef (22 g), sausages (16 g), lamb/mutton (11 g), chicken (11 g), pork (7 g), and bacon (4 g). Concentrations of the major heterocyclic amines in cooked-meat samples increased with the degree of doneness (Table 2). The highest levels were observed for PhIP and IFP in well-done chicken and pork and in very well-done beefsteak. Table 3 shows age-adjusted and multivariate relative risks for prostate cancer according to the self-reported level of meat doneness. Data are presented for each individual meat type as well as for participants' composite meat-doneness score. Prostate cancer risk was positively associated with the doneness of beefsteak. An inverse association was found for sausages, and no significant association was observed for the other meats or for the composite meat-doneness score. Only minimal differences in the relative risks were observed when the reference category was restricted to meat nonconsumers or when meat nonconsumers were excluded from the reference category. Analyses that considered as the exposure the usual cooking method (fried/grilled/barbecued versus baked/microwaved/cooked in liquid, not including level of doneness) resulted in similar risk estimates (data not presented). Estimated daily heterocyclic amine intake was not clearly associated with prostate cancer risk, for either total or individual heterocyclic amines (Table 4), although a weak gradient of increasing risk was observed over increasing quartiles of exposure to IFP (not statistically significant). There was little difference in the risks of prostate cancer associated with meat-cooking practices or estimated heterocyclic amine intake for case patients with advanced cancer compared with case patients with all stages of cancer or for case patients who provided completed questionnaires before or after knowledge of their cancer diagnosis.

Table 1. Characteristics of total prostate cancer case patients and control subjects, Auckland Prostate Study, 1996–1997

Characteristic	Control subjects (n = 480)	Case patients* (n = 317)
Mean age, y (standard deviation)	69.1 (7.4)	68.2 (7.1)
Positive family history of prostate cancer (%)	15 (3)	26 (8)
Socioeconomic status—No. in upper socioeconomic status group (%)	210 (44)	97 (31)
Total nonsteroidal anti-inflammatory drug use† (%)	175 (36)	104 (33)
Total energy intake, kJ/day‡	8860 (6843, 10 292)	8854 (7013, 10 420)
Total fat, g/day‡	67.6 (52.4, 87.9)	67.4 (53.5, 88.1)
Total saturated fat, g/day‡	29.0 (21.3, 39.7)	28.3 (21.0, 38.4)
Total polyunsaturated fat, g/day‡	8.5 (6.2, 11.5)	8.9 (6.4, 12.4)
Total monounsaturated fat, g/day‡	21.8 (16.7, 27.8)	21.4 (16.8, 27.9)
Total meat, g/day‡,§	141.3 (101.7, 206.1)	153.2 (101.6, 210.1)
Red meat, g/day‡,‖	98.3 (65.5, 147.7)	103.7 (63.2, 156.7)

*Total case patients do not include six men whose cancer diagnosis was made incidentally following transurethral surgery, where their serum prostate-specific antigen was in the normal range and the tumor was assessed to be localized with a combined Gleason score ≤6.

†Regular use of aspirin or other nonsteroidal anti-inflammatory drugs.

‡Median (25th, 75th percentile).

§Total meat includes beefsteak, lamb/mutton, pork, bacon, sausages, chicken, and processed meats.

‖Red meat includes beefsteak, lamb/mutton, and pork.

DISCUSSION

The self-reported degree of doneness for the cooking of seven commonly consumed meat types and estimated daily intake of four major heterocyclic amines were neither strongly nor consistently associated with the risk of prostate cancer. Although weak positive gradients of increased risk were observed for the doneness of beefsteak and for the estimated daily intake of IFP, similar patterns were not observed for other types of meat or heterocyclic amines.

A number of epidemiologic studies (but not all) have shown positive associations between the consumption of meat and the risk of prostate cancer, particularly advanced-stage disease *(1)*, but we are not aware of epidemiologic studies of prostate cancer that have specifically examined the hypotheses concerning meat-cooking practices or doneness. Previous epidemiologic studies of cancer risk generally have not attempted to directly estimate dietary exposure to heterocyclic amines based on analysis of meat samples obtained from local sources and cooked under controlled conditions to states of doneness that are considered typical in the study population. In our study, higher quantities of heterocyclic amines were found in meat samples cooked to a well-done or very well-done state, particularly IFP and PhIP from chicken, beefsteak, and pork. The heterocyclic amine levels ranged from undetectable levels to 28.6 ng/g for the well-done chicken sample and are similar to those found in foods cooked in Sweden *(19)* or foods cooked in restaurants in the United States *(12)*. This considerable variation in intake with meat

Table 2. Heterocyclic amine concentrations in New Zealand meats, by degree of doneness*

Meat type	Doneness (internal temperature)	Heterocyclic amine concentration, ng/g ± standard deviation			
		MeIQx	DiMeIQx	IFP	PhIP
Beefsteak, 1.5 cm thick	Fried—medium-rare (51 °C), internal pink/red	0	0.06 ± 0.002	0	0.29 ± 0.14
	Fried—well-done (63 °C), internal color almost lost	0.25 ± 0.29	0.07 ± 0.02	0	0.73 ± 0.02
	Fried—very well-done (74 °C), internal light brown	3.80 ± 0.26	0.80 ± 0.13	4.22 ± 0.65	7.33 ± 0.11
Lamb/mutton chops†	Fried—medium (75 °C)	0.4	0	Not measured†	0
	Fried—well-done (85 °C)	1.0	0	Not measured†	2.4
Pork steak, 2 cm thick	Fried—medium (63 °C)	0.25 ± 0.09	0.10 ± 0.04	0	0.37 ± 0.06
	Fried—well-done (83 °C)	2.22 ± 0.05	0.95 ± 0.01	3.97 ± 0.13	7.82 ± 1.13
Minced beef patty, 2 cm thick	Fried—medium (51 °C)	0.29 ± 0.07	0.03 ± 0.01	0	0
	Fried—well-done (58 °C)	1.12 ± 0.21	0.29 ± 0.08	0.80 ± 0.04	3.96 ± 0.13
Chicken, 2.5 cm thick, skin removed	Fried—lightly browned (63 °C)	0.11 ± 0.04	0	0	0.20 ± 0.005
	Fried—well-done (79 °C)	2.27 ± 0.14	2.26 ± 0.05	6.50 ± 1.21	17.54 ± 0.17
Sausage, precooked, 2 cm thick	Fried—lightly browned (42 °C)	0.36 ± 0.10	0	0	0
	Fried—well-browned (70 °C)	0.07 ± 0.08	0	0	0.61 ± 0.06
Bacon, middle	Fried—lightly cooked	0.22 ± 0.01	0	0	0.11 ± 0.002
	Fried—well-cooked	3.79 ± 0.44	0	1.06 ± 0.03	1.93 ± 0.37

*MeIQx = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx = 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; IFP = 2-amino,1,6-dimethylfuro[3,2-*e*]imidazo[4,5-*b*]pyridine; and PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

†Heterocyclic amine composition data for lamb/mutton samples were taken from an earlier analysis *(27)* that was unable to quantify IFP.

types and preparations establishes the potential importance of heterocyclic amine quantification in epidemiologic studies.

The opposite patterns of prostate cancer risk observed in our study for sausages and beefsteak are apparently contradictory and may suggest that these findings have arisen by chance or as a result of uncontrolled confounding or other biases. However, the cooking of sausages may be less relevant to the hypotheses tested than for other meat types. Only relatively small quantities of heterocyclic amines were derived from sausages cooked under controlled conditions, and unlike the other meat types, heterocyclic amine levels were not consistently associated with the degree of doneness. On the contrary, well-done beefsteak was a marked source of heterocyclic amines, including PhIP and IFP, and greater variability and accuracy in the level of doneness are likely to be self-reported for beefsteak than for the other meat types in the New Zealand diet. Standard advice on cooking practices in New Zealand recommends that sheep-, pig-, and poultry-derived meat products be cooked to a relatively well-done state to kill bacteria. Furthermore, the possible increases in risk associated with well-done beefsteak and chicken are consistent with known prostate carcinogenicity of PhIP demonstrated in a rodent model *(10)*. In our study, the strongest monotonic increase in prostate cancer risk was observed for IFP, a recently identified heterocyclic amine with a similar structure to PhIP, which has been shown to have even greater mutagenic potential in the Ames test *(20)*.

There are limited data from previous population-based studies of cancer and heterocyclic amine intake with which to compare the levels of daily intake of heterocyclic amines estimated for our study population. The median daily intake for total heterocyclic amines in our study population was 146 ng, similar to the mean intake of 160 ng estimated for an elderly Swedish population *(21)* and greater than the median estimate for a Swedish case–control study population *(9)*, which was reported as 77 ng (this study also reported no significant increases in cancer risk associated with heterocyclic amine exposures). However, these intakes are much lower than earlier estimates of exposure for human populations *(3)* and considerably less than doses used in animal studies that have demonstrated a carcinogenic effect for heterocyclic amines *(2,22)*. A relatively low daily heterocyclic amine exposure in our study population may provide an explanation for the inconsistent associations observed with prostate cancer risk.

Our study has a number of limitations. Although initial response rates of 71% for control subjects and 77% for case patients are typical of those achieved by population-based, case–control studies, incomplete response introduces the possibility of selection biases because we are unable to characterize the initial nonresponder groups. The case patients attending two of seven private urologists and excluded from the study represent only 9% of the total number of patients and were expected to be representative of private cases overall. While their exclusion may contribute to differences in the socioeconomic status of case patients and control subjects, all analyses were adjusted for this variable.

Misclassification of self-reported dietary intake is a traditional concern with food-frequency questionnaires. Furthermore, it was not possible to determine the validity and reliability of our questionnaire with respect to self-reported meat-cooking practices and doneness. Differential recall of meat-cooking methods with respect to case–control status may be less likely for our study because the majority of participants were recruited before diagnosis of their cancer. Imprecision of food-composition data and inappropriate application of such data to dietary analyses comprise additional sources of nondifferential misclassification of dietary heterocyclic amine exposure. These errors may have further contributed to underestimation of an association with prostate cancer risk. No allowance was made for intake of heterocyclic amines from cooked fish (heterocyclic amine composition of locally sourced cooked fish was unavailable), but intake from this source was relatively low compared with that of other meat types among the predominantly European study population (median total daily intake of lean fish in the study population was <10 g).

Table 3. Relative risks (RRs) of prostate cancer and doneness of cooked meat, Auckland Prostate Study, 1996–1997

Meat type	Usual meat cooking method and doneness, RR (95% confidence interval)			Two-sided *P* for trend
	Never eaten or other cooking methods*	Fried/grilled/barbequed, medium or lightly browned	Fried/grilled/barbequed, well-done or well-browned	
Beefsteak				
Case patients : control subjects	31 : 69	163 : 260	123 : 151	
Age-adjusted RR	1.00	1.40 (0.88–2.24)	1.85 (1.14–3.02)	.008
Multivariate RR†	1.00	1.36 (0.84–2.18)	1.68 (1.02–2.77)	.03
Advanced case patients RR‡	1.00	1.38 (0.78–2.42)	1.56 (0.86–2.81)	.16
Lamb/mutton chops				
Case patients : control subjects	129 : 200	74 : 121	114 : 159	
Age-adjusted RR	1.00	0.92 (0.64–1.33)	1.09 (0.79–1.52)	.62
Multivariate RR†	1.00	0.97 (0.67–1.41)	1.07 (0.77–1.50)	.68
Advanced case patients RR‡	1.00	0.93 (0.60–1.44)	0.93 (0.63–1.39)	.72
Pork				
Case patients : control subjects	162 : 241	38 : 72	117 : 167	
Age-adjusted RR	1.00	0.75 (0.48–1.17)	1.02 (0.75–1.39)	.96
Multivariate RR†	1.00	0.78 (0.50–1.23)	1.02 (0.74–1.40)	.97
Advanced case patients RR‡	1.00	0.67 (0.38–1.18)	1.08 (0.74–1.56)	.76
Minced beef				
Case patients : control subjects	28 : 39	222 : 331	67 : 110	
Age-adjusted RR	1.00	0.92 (0.55–1.54)	0.82 (0.46–1.46)	.44
Multivariate RR†	1.00	0.90 (0.54–1.52)	0.79 (0.44–1.41)	.36
Advanced case patients RR‡	1.00	0.83 (0.45–1.52)	0.71 (0.36–1.39)	.30
Chicken				
Case patients : control subjects	233 : 368	19 : 37	65 : 75	
Age-adjusted RR	1.00	0.76 (0.42–1.36)	1.34 (0.92–1.94)	.19
Multivariate RR†	1.00	0.78 (0.43–1.40)	1.33 (0.91–1.94)	.21
Advanced case patients RR‡	1.00	0.76 (0.37–1.55)	1.30 (0.83–2.02)	.35
Bacon				
Case patients : control subjects	88 : 130	66 : 91	163 : 259	
Age-adjusted RR	1.00	1.06 (0.70–1.60)	0.93 (0.66–1.29)	.59
Multivariate RR†	1.00	1.07 (0.70–1.63)	0.91 (0.65–1.28)	.52
Advanced case patients RR‡	1.00	1.15 (0.69–1.90)	1.01 (0.67–1.52)	.96
Sausage				
Case patients : control subjects	92 : 113	81 : 114	144 : 253	
Age-adjusted RR	1.00	0.84 (0.56–1.25)	0.67 (0.48–0.95)	.02
Multivariate RR†	1.00	0.90 (0.60–1.35)	0.70 (0.49–0.99)	.04
Advanced case patients RR‡	1.00	0.86 (0.54–1.39)	0.65 (0.43–0.98)	.03
	Meat-doneness score§			
	Low (score, 7–13)	Medium (score, 14–16)	High (score, 17–21)	
All meat types				
Case patients : control subjects	135 : 195	80 : 105	102 : 180	
Age-adjusted RR	1.00	1.08 (0.75–1.56)	0.79 (0.57–1.10)	.18
Multivariate RR†	1.00	1.07 (0.74–1.56)	0.77 (0.55–1.08)	.14
Advanced case patients RR‡	1.00	1.14 (0.74–1.77)	0.81 (0.54–1.21)	.31

*"Other cooking methods" include baked, cooked in liquid, and microwaved. This category includes beefsteak fried/grilled/barbequed to a rare state.

†Multivariate regression model for total prostate cancer case patients included terms for age, socioeconomic status, total nonsteroidal anti-inflammatory drugs, and total energy intake.

‡Multivariate regression model for advanced prostate cancer case patients included terms for age, socioeconomic status, total nonsteroidal anti-inflammatory drugs, and total energy intake.

§Meat-doneness score based on scores of 1, 2, and 3 assigned to low, medium, and high categories of doneness for each of the seven meat types and summed for all meat types.

The availability of comprehensive food, nutrient, and other data allowed investigation of the potentially confounding effects of dietary and nondietary exposures in our analyses. However, bias arising from unrecognized confounding remains a concern in any observational study design. The uncertain nature of the mechanisms by which dietary factors such as heterocyclic amines may influence prostate cancer biology raises questions concerning the appropriateness of the period of exposure measurement (12 months before diagnosis) used in our case–control study design. In addition, we were not able to consider a number of factors that may modify the carcinogenic effect of heterocyclic amines, including the fat, water, or iron content of meat *(23)*, the use of precooking treatments *(24)*, and the presence of host factors such as the individual acetylator phenotype *(25,26)*.

In conclusion, we have found no strong or consistent evidence for an increased risk of prostate cancer associated with the intake of heterocyclic amines from well-done meat. However, possible increases in risk associated with well-done beefsteak and dietary in-

Table 4. Relative risks (RRs) of prostate cancer and estimated daily exposure to heterocyclic amines from cooked meat, Auckland Prostate Study, 1996–1997*

Heterocyclic amine	Quartile of daily heterocyclic amine intake from cooked meat, RR (95% confidence interval)				Two-sided *P* for trend
	Q1	Q2	Q3	Q4, high	
Total heterocyclic amines†,‡					
ng/day	<56.8	56.8–146.1	>146.1–420.9	>420.9	
Age-adjusted RR	1.00	0.88 (0.58–1.33)	1.00 (0.67–1.50)	1.19 (0.80–1.77)	.30
Multivariate RR§	1.00	0.88 (0.58–1.35)	0.98 (0.65–1.48)	1.09 (0.72–1.65)	.57
Advanced case patients RR‖	1.00	1.04 (0.64–1.71)	0.96 (0.58–1.57)	1.12 (0.69–1.84)	.74
MeIQx					
ng/day	<19.9	19.9–41.4	>41.4–95.2	>95.2	
Age-adjusted RR	1.00	0.85 (0.56–1.35)	1.12 (0.75–1.67)	1.10 (0.73–1.64)	.39
Multivariate RR§	1.00	0.83 (0.54–1.28)	1.10 (0.73–1.66)	0.97 (0.63–1.49)	.77
Advanced case patients RR‖	1.00	0.74 (0.45–1.25)	1.14 (0.71–1.84)	0.90 (0.54–1.50)	.85
DiMeIQx					
ng/day	<2.6	2.6–7.2	>7.2–22.0	>22.0	
Age-adjusted RR	1.00	0.97 (0.64–1.46)	0.94 (0.62–1.41)	1.33 (0.90–1.97)	.17
Multivariate RR§	1.00	1.00 (0.66–1.52)	0.92 (0.60–1.41)	1.24 (0.82–1.87)	.36
Advanced case patients RR‖	1.00	0.95 (0.58–1.55)	0.82 (0.50–1.35)	1.11 (0.69–1.78)	.80
IFP‡					
ng/day	<1.6	1.6–18.9	>18.9–84.5	>84.5	
Age-adjusted RR	1.00	1.13 (0.74–1.72)	1.30 (0.86–1.95)	1.44 (0.96–2.16)	.06
Multivariate RR§	1.00	1.14 (0.75–1.75)	1.31 (0.86–1.99)	1.32 (0.87–2.00)	.16
Advanced case patients RR‖	1.00	1.20 (0.73–1.99)	1.24 (0.75–2.04)	1.34 (0.82–2.20)	.26
PhIP					
ng/day	<28.4	28.4–79.1	>79.1–223.8	>223.8	
Age-adjusted RR	1.00	0.88 (0.58–1.33)	1.00 (0.67–1.49)	1.15 (0.78–1.71)	.39
Multivariate RR§	1.00	0.87 (0.57–1.33)	0.97 (0.64–1.47)	1.05 (0.70–1.59)	.69
Advanced case patients RR‖	1.00	0.95 (0.58–1.55)	0.90 (0.55–1.47)	1.03 (0.63–1.69)	.94

*MeIQx = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx = 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; IFP = 2-amino-1,6-dimethylfuro-[3,2-*e*]imidazo[4,5-*b*]pyridine, and PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

†Total heterocyclic amines = MeIQx + DiMeIQx + IFP + PhIP.

‡Totals do not include IFP from lamb/mutton.

§Multivariate regression model for total case patients included terms for age, socioeconomic status, total nonsteroidal anti-inflammatory drugs, and total energy intake.

‖Multivariate regression model for advanced prostate cancer case patients included terms for age, socioeconomic status, total nonsteroidal anti-inflammatory drugs, and total energy intake.

take of the heterocyclic amine IFP deserve further investigation by future studies.

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NOTES

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