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Grilled Meat Consumption and PhIP-DNA Adducts in Prostate Carcinogenesis

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Abstract

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is the major heterocyclic amine generated from cooking meats at high temperatures, and dietary exposures have been shown to induce prostate cancer in rats. PhIP derives its carcinogenic potential through the formation of PhIP-DNA adducts. The purpose of this study was to examine whether self-reported consumption and preparation doneness of grilled meats were associated with PhIP-DNA adduct levels in prostate epithelial cells. The study population consisted of 268 African-American and Caucasian men who underwent radical prostatectomy for prostate cancer. PhIP-DNA adducts in tumor and adjacent nontumor cells were measured using immunohistochemical methods, and dietary meat intake information was based on food frequency questionnaires. Data were analyzed using multivariate linear regression models. After adjusting for age at prostatectomy and race, grilled meat consumption ($P = 0.002$) was significantly associated with higher adduct levels in tumor cells, but this association seemed to be primarily due to consumption of grilled red meats ($P = 0.001$) as opposed to grilled white meat consumption ($P = 0.15$). Among the specific food items, grilled hamburger consumption had the most significant association with adduct level in tumor cells ($P = 0.002$). Similar trends in positive associations with grilled meat consumption and adduct levels were observed in nontumor cells, but none of these associations reached statistical significance. Our results suggest that dietary interventions targeted at lower consumption of grilled red meats may reduce prostate cancer risk via the PhIP prostate carcinogenic pathway.

Introduction

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is the most abundant heterocyclic amine (HCA) formed during the cooking of meat (1) and is a potential dietary risk factor for prostate and other cancers. In rats, PhIP preferentially targets the colon and prostate in males, the mammary glands in females, and lymphoid cells in both males and females (2), whereas in mice, it induces lymphoma (3). Subsequent studies have firmly established that PhIP is a

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potent prostate carcinogen in rats (4,5). In humans, meat consumption assessed by food frequency questionnaires has been used as a possible surrogate for PhIP and other HCA intake. Studies have found that intake of grilled meat increased the risk of colorectal adenomas (6) and stomach cancer (7), intake of fried meat increased lung cancer risk (8), and higher estimated HCA intake increased breast cancer risk (9). A large prospective study of men enrolled in the Prostate, Lung, Colorectal, and Ovarian Screening Trial found that the highest quintile of dietary PhIP intake was associated with a 1.2-fold increased risk of prostate cancer (10). Overall, epidemiologic evidence for consumption of meat as a risk factor for prostate cancer risk is equivocal (11). In two of the more recent studies of prostate cancer and meat consumption conducted in the United States, increased meat consumption was positively associated with prostate cancer risk in African Americans, but not Caucasians (12,13). In the United States, African-American men have a 60% higher incidence of prostate cancer compared with whites (14). Coincidentally, mean dietary HCA intake is ~2- to 3-fold greater in African-American males than their white male counterparts (15).

Compared with the use of food frequency questionnaires to estimate HCA intake or urinary excretion to assess metabolism, PhIP-DNA adducts serve as a biomarker of a chemical-specific measure of individual biologically effective dose. PhIP is a promutagen that is efficiently metabolized into reactive species that are direct acting mutagens. Bioactivation of PhIP to carcinogenic species *in vivo* is initiated by N-oxidation of the compound, which is catalyzed by cytochrome P4501A2 (CYP1A2; ref. 16). Subsequent acetylation or sulfation of the *N*-hydroxy-PhIP catalyzed by *N*-acetyltransferases or sulfotransferases generate *N*-acetoxy- or *N*-sulfonyloxy-PhIP, electrophilic compounds that bind covalently to DNA to form PhIP adducts (17,18). The formation of PhIP-DNA adducts via nitrenium ion chemistry results in structural changes in the DNA and possibly mutations in genes controlling cell proliferation, thus leading to tumor formation (19). Human prostate cells metabolize PhIP to its mutagenic form (20,21) and form PhIP-DNA adducts after being exposed to PhIP *in vivo* (22-24).

The content of PhIP in cooked meats varies by both the type of meat and its method of preparation (25,26), but the assessment of individual human exposure is very complex with estimated PhIP exposure levels varying by at least two orders of magnitude (27). Recent studies that link survey data to food databases of HCA content have estimated that pan frying and chicken are the cooking method and meat that comprise the primary source of dietary PhIP exposure in American men (15,28), but these studies rely on the linkage of two data sources with a large amount of variation. In addition, most studies have consistently found that grilling/barbecuing is the cooking method that generally produces the highest HCA content (25,28, 29). In any particular population, the relationship between dietary consumption of PhIP and its biologically effective dose will depend on local dietary habits and cooking methods, individual susceptibilities in PhIP metabolism, and the target organ of interest.

If PhIP is an important prostate cancer risk factor, then identification of dietary sources of PhIP correlated with a marker of its biologically effective dose, PhIP-DNA adducts, in the prostate will provide useful data for future dietary chemoprevention. In the present study, we tested for associations between PhIP-DNA adduct level in prostatic epithelial cells and known dietary sources of PhIP exposure, namely, consumption of grilled and overcooked meats.

Materials and Methods

Study Sample

The study population consisted of men who were part of the Henry Ford Health System (HFHS). The HFHS is composed of an 800-bed hospital in the city of Detroit, 3 smaller hospitals in surrounding suburbs, and 31 medical clinics located throughout the metropolitan Detroit area. Eligible cases used the HFHS as their primary source of health care, lived in the

study area at time of recruitment, had no other serious medical problems that would preclude participation, and had no previous history of prostate cancer. Potential cases were identified by HFHS pathology reports of primary adenocarcinoma of the prostate. Cases recruited for study were sent a letter introducing the study protocol, followed by a phone call from a study interviewer. Those who agreed to participate were asked to complete a two-part interviewer-administered risk factor questionnaire (the first part was conducted over the phone, and the second part was done in person) and donate a blood sample for DNA analysis. Race was self-reported by participants. All study protocols were approved by the Henry Ford Hospital Institutional Review Board.

Between July 1, 2001 and December 31, 2004, we attempted to enroll 863 men who had been diagnosed with prostate cancer within the last 2 years as part of a prostate cancer case-control study, and 668 agreed to participate (77%). During the course of enrollment, 8 cases were found ineligible, and 23 cases did not complete the study protocol, resulting in final study participation percentages of 74% (637/855). Of these 637 cases, 434 (68%) underwent radical prostatectomy. Cases undergoing prostatectomy were, on average, younger (61.0 years versus 65.1 years, $P < 0.001$) but did not differ by race (43.2% African American in both groups) or by Gleason score (biopsy Gleason ≥ 7: 43.1% versus 42.1%). The present study includes the first 268 prostatectomy patients who had tissue samples available for immunohistochemical studies of PhIP-DNA adduct determination. The demographic and clinical characteristics of the study population are shown in Table 1. Date of surgery and tumor grade were abstracted from the surgical pathology report.

Food Questionnaires

Dietary intake as well as food preparation method and doneness were ascertained using questions adapted from a validated questionnaire (30). Grilled meats were defined as meats cooked over charcoal or a hot gas flame. Meat servings and preparation doneness data were collected through the following questions. For determining grilled meat servings, the question of “in the summer months, did you eat meats cooked on an outdoor grill or barbecue” was asked. If the respondent answered yes for outdoor grilled meat intake, then the following questions of “in the summer months, how often did you eat the following grilled meats (steak or pork chops, hamburgers, hot dogs, chicken with skin, chicken without skin, fish)” were asked. For determining smoked meat servings, the questions of “how often did you eat smoked ham, turkey, or other smoked meats” and “how often did you eat smoked fish” were asked. Preparation doneness was determined for grilled meats in grilled steaks or chops, grilled hamburgers, and grilled hot dogs through the questions of “when you ate grilled (steak or pork chops, hamburgers, and hot dogs), how were they cooked?” The number of servings categories include “<1 per month,” “1 to 4 times per month,” “5 to 9 times per month,” “10 to 15 times per month,” and “>15 times per month.” The preparation doneness categories include “rare,” “medium,” “well done,” and “very well done.”

Pathology

H&E-stained slides of study cases were reviewed by the study pathologist (A.T. Savera) to confirm the diagnosis and to identify a paraffin block with sufficient tumor and nontumor prostatic tissue staining. For each patient sample, a microtome was used to cut five consecutive sections (5 μmol/L thick) from the tissue block. One slide was H&E stained and examined by the study pathologist who circled two separate areas of tumor and normal cell populations to be used for adduct scoring.

Immunohistochemistry

Immunohistochemical studies were done as described by Takahashi et al. (31) and Zhu et al. (32). Sensitivity and specificity of the antibody were described previously (31). The sensitivity

was one to two adducts per 10^7 nucleotides, whereas the target of the anti–PhIP-DNA adduct antibody was PhIP-bound DNA rather than unbound PhIP or its metabolite (31). The paraffin-embedded sections were baked at 59°C for 1 h, deparaffinized in xylene, and rehydrated in serial alcohol. Endogenous peroxidase activity was blocked using 0.3% H_2O_2 in methanol for 20 min. After treatment using RNase and proteinase K, the sections were blocked using 3% bovine serum albumin and normal goat serum. The primary anti–PhIP-DNA adduct polyclonal antibody was provided by Dr. Shirai (Nagoya City University Medical School, Nagoya, Japan). The polyclonal antibody was incubated with the sections at 4°C overnight in a humid chamber at a dilution of 1:750. In addition, the biotinylated secondary antibody was incubated with the sections at room temperature for 30 min at a dilution of 1:200. The antibody complex was detected using an avidin-biotin-peroxidase complex solution and visualized using 3,3′-diaminobenzidine (Zymed Laboratories, Inc., San Francisco, CA). A negative control was included in each experiment by omitting the primary antibody. The staining specificity was confirmed using the primary antibody that had been pre-absorbed with 2 or 20 μg/mL DNA extract from MCF-7 cells treated with 150 μmol/L *N*-hydroxy-PhIP. A cytospin sample of MCF-7 cells without PhIP treatment was included in each batch of staining. Staining was measured by absorbance image analysis using a Cell Analysis System 200 microscope as described previously (33). Absorbance of light at a wavelength of 500 nmol/L was measured because methyl green does not absorb light at this wavelength, whereas diaminobenzidine does. For each prostate specimen, two technicians independently scored 50 epithelial cells (five fields with 10 cells per field scored) in the two areas (tumor and nontumor) circumscribed by the study pathologist. The final score was the mean of the two technicians' scores. Scored cells were selected to be representative, in terms of intensity, of the cells in the field. Staining intensity was represented by the absorbance value.

Statistical Analyses

Multivariate linear regression analyses were used to determine whether meat consumption was associated with PhIP-DNA adduct levels in nontumor and tumor prostatic epithelial cells. Potential batch effects in the PhIP-DNA adduct assay were taken into account by assaying a control slide with each experimental batch to compute a batch correction factor that was the difference between the adduct level of the control slide in a single batch and the mean adduct level of the control slides across all batches. The batch-adjusted adduct level was the crude adduct level minus the batch correction factor. This approach was used in our previous studies involving PAH-DNA (33) and PhIP-DNA[7] adducts. The distribution of adjusted adduct levels was found to be close to normal, and hence, no log transformation was necessary for that variable. Due to the low number of subjects who reported eating many servings of individual meats, as well as rare and very well-done meat, both number of servings and preparation doneness variables were dichotomized for multiple regression analyses. For variables of consumption, study subjects were grouped into consumers or nonconsumers. For variables of preparation doneness, subjects were grouped into "rare and medium" meat consumers or "well done and very well done" meat consumers. For the combined meat consumption variables, individual dichotomous meat consumption variables were scored as a "1" for those who consumed each meat and "0" for those who did not and summed across all meat categories. Total grilled white meat consumption included intake of grilled chicken with and without skin and fish. Total grilled red meat consumption included intake of grilled hamburger, hot dog, and steak/pork chop.

To determine whether our findings were specific to red meat consumption prepared on the grill during the summer, we also examined whether PhIP-DNA adduct level was associated with

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season. Date of prostatectomy was grouped into one of the four different seasons, and an ANOVA was done to test for heterogeneity of mean adduct levels across the four groups.

All models adjusted for age at prostatectomy and race (African American or Caucasian). Associations between meat consumption variables and PhIP-DNA adduct levels were examined separately for nontumor and tumor prostatic epithelial cells.

Results

PhIP-DNA adduct level was found to be significantly higher in nontumor cells (mean absorbance, 0.17) compared with that in tumor cells (mean absorbance, 0.10; $P < 0.0001$). Race and age at prostatectomy were not significantly associated with adduct level in either nontumor or tumor cells. Race was significantly associated with steak consumption, hamburger consumption, chicken with skin consumption, and chicken without skin consumption.

PhIP-DNA adduct levels across different meat consumption categories are shown in Table 2. With the exception of grilled fish, those who consumed grilled meats had higher mean PhIP-DNA adduct levels in both nontumor and tumor cells than those who did not. Those who consumed three different specific red meats had a mean nontumor cell adduct level of 0.177 ± 0.038 absorbance, compared with 0.163 ± 0.048 absorbance ($P = 0.057$) for those who consumed no red meat at all, whereas those who consumed three different specific red meats have a mean tumor cell adduct level of 0.113 ± 0.025 absorbance, compared with 0.0988 ± 0.025 absorbance for those who consumed no red meat at all ($P = 0.001$). In contrast, higher PhIP-DNA adduct levels were not observed across the four levels of doneness for the three food groups for which this meat preparation question was asked (Table 3).

After adjusting for age at prostatectomy and race, total meat consumption ($\beta = 0.002$, $P = 0.002$) and total grilled red meat consumption ($\beta = 0.005$, $P = 0.001$) were found to be significantly associated with adduct level in tumor cells (Table 4). Total grilled white meat consumption was not significantly associated with adduct level in either tissue type.

In analyses involving specific grilled red meats (Table 4), the association between grilled hamburger consumption and PhIP-DNA adduct level was marginally significant in nontumor cells ($\beta = 0.010$, $P = 0.077$), but was significant in tumor cells ($\beta = 0.011$, $P = 0.002$). Grilled steak/pork chop ($\beta = 0.008$, $P = 0.020$) and grilled hot dog consumption ($\beta = 0.009$, $P = 0.009$) were also significantly associated with adduct level in tumor cells. Other specific meat items in which consumption was associated with increased adduct level include grilled chicken with skin consumption ($\beta = 0.008$, $P = 0.019$) in tumor cells.

Next, we ran a series of multivariate models that included covariates for all six of the specific meat consumption categories to adjust for interdependence among the six categories. Because no significant associations were found between the different food doneness categories and adduct level, we chose not to include any variables for doneness in our multivariate models. First, we ran two saturated models for adduct levels of nontumor and tumor cells, forcing all six meat category variables into the model as well as covariates adjusting for race and age at prostatectomy. In general, effect estimates for all food variables tended to decrease both in magnitude and statistical significance in the saturated models, with none of the specific grilled red meat consumption categories significantly associated with adduct level in either nontumor or tumor cells (Table 5). We then used backward elimination regression to obtain best-fitting models of specific meat consumption categories for adduct levels after adjusting for age at prostatectomy and race. For nontumor cells, only grilled hot dog consumption was retained in the model ($\beta = 0.010$, $P = 0.076$), whereas for tumor cells, only grilled hamburger consumption was retained ($\beta = 0.011$, $P = 0.002$).

Our analysis comparing PhIP-DNA adduct levels by the season in which the prostatectomy was done showed that there was no significant seasonal variations for either nontumor or tumor cells.

Discussion

Our study results are novel in linking dietary PhIP exposure with a biologically effective dose biomarker, PhIP-DNA adducts, in the prostate. Although we could not examine whether higher PhIP-DNA adduct levels increased prostate cancer risk, a recent prospective human study found that the highest quintile of dietary PhIP intake was associated with a 1.2-fold increased risk of prostate cancer (10). Although several previous studies have examined biomarkers of PhIP exposure such as urine metabolites (34-36) and hair (37), only a few studies have attempted to correlate a biologically effective measure of PhIP exposure, PhIP-DNA adduct levels, in humans with self-reported exposure histories (32,38). Only a few studies have assayed for PhIP-DNA adducts in the tissues taken from the target organ in which the cancer occurred (32,39). In a breast cancer study, no direct correlation between different types of meat consumption and PhIP-DNA adducts levels was found, but a suggestive interaction between *N*-acetyltransferase genotype and well-done meat consumption was reported (32). In a pancreatic cancer study, PhIP-DNA adducts were detected in human pancreatic tissue samples obtained from patients with unknown exposure to HCA (39). In the present study, we examined self-reported grilled meat consumption as a potential dietary source of PhIP exposure and found that grilled red meat and total meat consumption were significantly associated with adduct levels in prostatic tumor cells.

In general, we found the strongest associations between specific types and amount of grilled meat consumption and PhIP-DNA adduct level in tumor cells. Differences in the cellular microenvironment of tumor and nontumor cells, such as aberrant methylation, may lead to decreased activity of enzymes involved in the detoxification of PhIP in tumor cells, which in turn could result in a stronger correlation between reported eating habits and a tissue-based biomarker of PhIP in tumor cells. For example, silencing of GSTP1 through hyper-methylation has been observed in prostate tumor cells but not normal cells (40), and *in vitro* studies have shown that GSTP1 expression is correlated with PhIP-DNA adduct levels in the prostate (21). In terms of consumption, PhIP-DNA adduct level was linearly associated with total grilled red meat consumption in both nontumor and tumor cells. This finding was consistent with previous animal studies, which found a dose-response relationship between PhIP intake and PhIP-DNA adduct formation (41,42). It was also consistent with epidemiologic studies involving humans, which showed associations between red meat intake and increased risk of colorectal adenomas (43), lung (44), stomach (7), and breast cancers (9,45). Our food questionnaire was limited to only several types of grilled meats, but given our results, it would be interesting to examine whether consumption of other grilled meats, such as grilled bacon or grilled sausage, is associated with PhIP-DNA adduct level in prostate cells. The main limitation of assessing the contributions of the consumption of specific meats on adduct levels using the saturated model is that these consumption categories are highly correlated to each other. In our study, specific red meat items that were significantly associated with PhIP-DNA adduct level in tumor cells when they were analyzed separately lost their significance when put together in the same multivariate model.

We did not find any association between preparation doneness and adduct level. Recall error and exposure misclassification may have contributed to our lack of finding an association between PhIP-DNA adduct level and meat doneness. Because the hamburgers, steaks, and hot dogs in this study were all grilled outdoors, there was a lack of a controlled standard for doneness levels. It was likely that subjects simply ascribed their preference for meat doneness in restaurants to the doneness of outdoor grilled meats, although the restaurant preference may

not reflect the actual doneness level of the outdoor grilled meats according to a fixed standard, because no definitions or pictures of the four doneness levels were presented to the subjects. This notion is supported by a previous doneness exposure indicator study, which found that HCA levels in home-cooked meat samples were significantly different when samples were visually classified for doneness, but not when self-reported doneness preference was used to classify doneness (46). Dietary PhIP intake is thought to be underestimated by food frequency questionnaires primarily because of the difficulty in accurately assessing cooking methods that produce high PhIP levels (47). Furthermore, whereas studies of meat samples show that HCA levels vary significantly by doneness level (15,25,29), the majority of the variation in dietary PhIP intake assessed by food frequency questionnaires is generally accounted for by the type of food and, secondarily, the cooking method (28,48). This likely explains why a previous study of dietary HCA intake and prostate cancer risk found an association between consumption of well-done beefsteak and prostate cancer, but failed to find any overall association between meat doneness and prostate cancer risk (49).

The questionnaire data we collected in the present study did not allow us to examine year-round dietary intake of individual meat items nor all the various methods of food preparation. In fact, the associations we found with PhIP-DNA adduct level in the prostate were specific to meat consumption during the summer months. However, consumption of grilled red meat and hamburger in the summer months could also reflect consumption of these foods at different times of the year and cooked by different methods. Because PhIP-DNA adducts are gradually repaired in cells following their formation (50), we tested whether the season of surgery affected adduct level after controlling for intake level of various meats, but found no significant association between the date of prostate surgery and adduct level. This suggests that grilled red meat consumption did not increase significantly in our sample during the summer months, and that PhIP-DNA adduct level in the prostate is associated more with meat consumption rather than the specific method of cooking.

In summary, we have shown that in men with prostate cancer, consumption of certain types of meat with known high PhIP content is directly related to PhIP-DNA adduct level in tumor and nontumor prostate cells. These results may have important implications with regard to preventive strategies in prostate cancer. Although epidemiologic studies showing a direct link between PhIP-DNA adduct level and increased prostate cancer risk are still lacking, strong evidence exists from animal studies (4,5,51-53) that PhIP is involved in prostate carcinogenesis. Our results suggest that grilled red meat consumption is an important factor to consider in the study of the PhIP prostate carcinogenic pathway in humans.

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Table 1

Characteristics of study population (N = 268)

Characteristic	Mean ± SD
Age at prostatectomy (y)	61.3 ± 6.9
Prostate specific antigen at diagnosis (ng/mL)	7.0 ± 6.3
Race	Number (%)
Caucasian	163 (60.8)
African American	105 (39.2)
Total Gleason grade	
5	1 (0.4)
6	85 (32.7)
7	128 (49.2)
8	29 (11.2)
9	17 (6.5)

Table 2

Mean PhIP-DNA adduct level in prostate nontumor and tumor cells of prostate cancer cases across meat consumption categories (N = 268)

Specific meats	Nontumor					Tumor				
	Consumers		Nonconsumers			Consumers		Nonconsumers		
	n	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	*P* value	*n*	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	*P* value
Grilled steak/ pork Chop	144	0.171 ± 0.039	124	0.165 ± 0.049	0.28	144	0.108 ± 0.025	124	0.101 ± 0.029	0.036
Grilled hamburger	124	0.173 ± 0.042	144	0.165 ± 0.046	0.14	124	0.109 ± 0.029	144	0.101 ± 0.025	0.01
Grilled hot dog	84	0.175 ± 0.041	184	0.165 ± 0.045	0.076	84	0.111 ± 0.025	184	0.102 ± 0.028	0.008
Grilled chicken with skin	91	0.173 ± 0.039	177	0.166 ± 0.046	0.22	91	0.111 ± 0.026	177	0.102 ± 0.027	0.012
Grilled chicken without skin	99	0.172 ± 0.043	169	0.166 ± 0.045	0.27	99	0.106 ± 0.028	169	0.105 ± 0.027	0.76
Grilled fish	52	0.167 ± 0.042	216	0.169 ± 0.045	0.83	52	0.104 ± 0.024	216	0.105 ± 0.028	0.75

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Table 3

Mean PhIP-DNA adduct level in prostate nontumor and tumor cells of prostate cancer cases across meat preparation categories

Meat category	Doneness level								*P* value
	Rare		Medium		Well done		Very well done		
	n	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	
Nontumor									
Grilled steak/pork chop (*n* = 143)	7	0.161 ± 0.035	73	0.172 ± 0.041	58	0.173 ± 0.039	5	0.157 ± 0.027	0.81
Grilled hamburger (*n* = 124)	3	0.149 ± 0.018	40	0.174 ± 0.039	75	0.173 ± 0.044	6	0.167 ± 0.046	0.78
Grilled hot dog (*n* = 84)	3	0.157 ± 0.0088	12	0.191 ± 0.048	58	0.173 ± 0.039	11	0.177 ± 0.043	0.46
Tumor									
Grilled steak/pork chop (*n* = 143)	7	0.109 ± 0.020	73	0.109 ± 0.025	58	0.109 ± 0.026	5	0.0934 ± 0.024	0.55
Grilled hamburger (*n* = 124)	3	0.0916 ± 0.0095	40	0.113 ± 0.027	75	0.109 ± 0.030	6	0.105 ± 0.037	0.64
Grilled hot dog (*n* = 84)	3	0.104 ± 0.020	12	0.121 ± 0.027	58	0.109 ± 0.024	11	0.114 ± 0.027	0.49

Table 4

Association between meat consumption and PhIP-DNA adduct level, adjusted for age at prostatectomy and race (N = 268)

Food category	Nontumor		Tumor	
	***β* (95% CI)**	***P* value**	***β* (95% CI)**	***P* value**
Combined meats*				
Grilled red meat	0.004 (-9×10^{-5}, 0.009)	0.055	0.005 (0.002, 0,007)	0.001
Grilled white meat	0.004 (−0.002, 0.009)	0.224	0.003 (−0.001, 0.006)	0.146
All meat†	0.002 (-2×10^{-4}, 0.005)	0.072	0.002 (0.001, 0.004)	0.002
Specific meats‡				
Grilled steak/pork chop	0.007 (−0.004, 0.017)	0.225	0.008 (0.001, 0.014)	0.020
Grilled hamburger	0.010 (−0.001, 0.021)	0.077	0.011 (0.004, 0.018)	0.002
Grilled hot dog	0.010 (−0.001, 0.022)	0.076	0.009 (0.002, 0.016)	0.009
Grilled chicken with skin	0.007 (−0.005, 0.018)	0.241	0.008 (0.001, 0.015)	0.019
Grilled chicken without skin	0.008 (−0.004, 0.019)	0.181	0.002 (−0.005, 0.009)	0.510
Grilled fish	−0.001 (−0.015, 0.012)	0.877	−0.001 (−0.009, 0.008)	0.856

*β estimate represents increment of adduct level increase associated with consumption of each additional meat item in this category.

†Grilled meats and smoked meats.

‡β estimate represents increment of adduct level increase associated with consumption of meat item.

Table 5

Multivariate modeling of consumption of specific meats and PhIP-DNA adduct level, adjusted for age at prostatectomy and race (N = 268)

Food category	Nontumor		Tumor	
	β^* (95% CI)	*P* value	β (95% CI)	*P* value
Grilled steak/pork chop	−0.001 (−0.016, 0.014)	0.876	0.001 (−0.008, 0.010)	0.763
Grilled hamburger	0.005 (−0.011, 0.021)	0.547	0.008 (−0.002, 0.018)	0.102
Grilled hot dog	0.007 (−0.007, 0.022)	0.311	0.004 (−0.004, 0.013)	0.334
Grilled chicken with skin	0.003 (−0.011, 0.017)	0.701	0.004 (−0.005, 0.012)	0.380
Grilled chicken without skin	0.006 (−0.007, 0.019)	0.377	−0.002 (−0.010, 0.006)	0.659
Grilled fish	−0.007 (−0.021, 0.008)	0.378	−0.005 (−0.014, 0.004)	0.276

* β estimate represents increment of adduct level increase associated with consumption of meat item.