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Meat consumption, heterocyclic amines, NAT2 and the risk of breast cancer

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Abstract

Meat consumption and heterocyclic amine (HCA) intake have been inconsistently associated with breast cancer risk in epidemiologic studies. Genetic variation in N-acetyltransferase2 (NAT2) has been suggested to modify the association of meat intake with breast cancer through its influence on metabolism of HCAs. We examined associations between meat intake, HCA exposure, acetylator genotype, and breast cancer risk in a case-control study of 2,686 case women and 3,508

controls. Women were asked to report their usual intake, cooking method, and preferred doneness of specific meats. We observed no association between total meat, red meat, or chicken with breast cancer risk. Women who consumed 5 or more servings of meat per week had no increased risk of breast cancer compared to women consuming fewer than 2 servings per week (OR = 0.99, 95% CI 0.84–1.15). No statistically significant associations with breast cancer were found for individual HCAs or for total estimated mutagenic activity of meat. Results varied modestly according to menopausal status. There were no statistically significant interactions with NAT2 genotype. Results do not support an important association of HCAs with breast cancer risk, although potential biases in case-control studies should be considered.

INTRODUCTION

In epidemiologic studies, regular consumption of well-cooked meat has been inconsistently associated with breast cancer risk. Several epidemiologic studies have shown an increased risk of breast cancer associated with meat consumption (1–11). In a meta-analysis based on 9 cohort and 22 case-control studies, consumption of meat was positively associated with the development of breast cancer: The summary relative risk for cohort studies was 1.32 (95% confidence interval [CI] = 1.12–1.56), and the summary relative risk for case-control studies was 1.13, comparing the highest with the lowest category of intake (4). However, a pooled analysis of 9 cohort studies showed no overall association (12). It has been suggested that the inconsistencies across the results of these studies may be attributed to the fact that the potential modifying effects of cooking method and doneness level were not evaluated in these analyses (6).

The carcinogenic effect of meat is thought to be mediated through the formation of heterocyclic amines (HCA), known human carcinogens. Cooking meat at high temperatures and for long durations greatly increases HCA content, and customary household methods of cooking meats are sufficient to create HCAs (13). The HCAs most commonly found in cooked meats include 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx) (13, 14). PhIP is the most abundant HCA in cooked meats (15, 16).

The carcinogenic properties of HCAs have been demonstrated in many animal studies. Rats treated with HCAs developed increased numbers of mammary tumors (13,17–20). In humans, the association between HCA exposure and carcinogenesis has been less consistent. HCAs require host-mediated metabolic activation before initiating DNA mutations and tumors in target organs (21). Human studies have shown that HCAs are absorbed into the body and can be metabolically activated (21–23).

In humans, 3 N-acetyltransferase (NAT) loci result in 2 expressed genes, NAT1 and NAT2, and a pseudogene, N-acetyltransferase pseudogene (NATP). NAT2 is an important enzyme in the biotransformation of aromatic and HCAs (24). The relationship between genotype and phenotype in NAT2 has been well studied (24); the effect of each single nucleotide polymorphism (SNP) on N-acetylation and O-acetylation was highly correlated for human NAT2 (24). Persons who possess two copies of the wild type allele are fast acetylators. An

inter-mediate acetylation phenotype was observed among those with only one copy of the wild type allele, whereas persons with two copies of the mutated allele are slow acetylators (25). Between 51 and 60% of Caucasians, and a lower proportion of African Americans, have slow acetylator genotypes for NAT2 (26). In a study of human mammary epithelial cell cultures exposed to the HCAs, DNA adducts were more often observed in cells from NAT2 fast acetylators than slow acetylators (27).

A number of studies have examined NAT2 genotype in relation to breast cancer risk with predominantly null results (10,21,28–32). A recent pooled analysis showed that NAT2 may be a genotype modifier between smoking and breast cancer (33).

Epidemiologic studies of meat cooking in relation to NAT2 acetylation status have also produced mixed results: one study (21) suggested that breast cancer risk associated with HCA exposure may be elevated among fast NAT2 acetylators, whereas the remaining studies have suggested no interaction (28–30).

Because the relationship between breast cancer, meat consumption, and NAT2 has been inconsistent, we examined these associations in a large, population-based, U.S. case-control study in which data on cooking practices were also collected.

METHODS

Study Population

The Collaborative Breast Cancer Study (CBCS) was a population-based, case-control study of breast cancer risk factors conducted in the states of Massachusetts, New Hampshire, and Wisconsin. Women of all races between the ages of 20 and 69 years were eligible. Enrollment began in 1997 and was completed in 2001. Cases were comprised of women with a recent incident diagnosis of invasive breast cancer identified through respective state cancer registries. Community controls were selected at random (within age strata) from lists of licensed drivers (women aged 64 and younger) and Medicare beneficiaries (women ages 65 and older). Eligibility was limited to women with a listed telephone number. To maintain comparability with controls, case women under the age of 65 were licensed drivers, whereas women of ages 65 or older were recipients of Medicare. Control women had no history of breast cancer. Of women eligible to participate in the study, approximately 80% of cases and 76% of controls completed the telephone interview. The present analysis is based on a subset of women (2,686 invasive breast cancer cases and 3,508 community controls) who participated in the study between 1997 and 1999 when information on meat consumption and meat cooking preferences was collected. A subset of this group provided DNA for NAT2 genotyping.

Data Collection

Participants completed a structured 30–40 min telephone interview that included questions on established and emerging risk factors for breast cancer including dietary sources of HCAs. All exposures were assessed prior to a reference date corresponding to the date of diagnosis in cases and a date randomly selected from among the diagnosis dates of recently interviewed cases for all controls. Relevant to this analysis, the interview included detailed

questions on meat consumption and cooking practices in the recent past (approximately 5 yr before diagnosis in the cases or a comparable time referent in the controls). Women were asked to report on typical servings per week of grilled hamburger, fried hamburger, broiled hamburger, grilled steak, fried steak, broiled steak, grilled chicken, fried chicken, and broiled chicken. These questions were followed for each meat by a question on the degree of browning (“was the outside usually lightly browned, medium browned, or blackened / charred?”) and for red meat the degree of doneness (“was it usually rare, medium, or well done?”). The section related to chicken consumption included a question on whether the chicken was cooked with the skin on or off and whether the skin of the chicken was consumed. Roast beef consumption was also ascertained. These questions were used to estimate quantities of HCAs consumed. This sequence of questions to assess meat consumption has been validated in another study (34). Because HCA activity may be modified by fruit and vegetable intake, detailed information was collected on carotenoid rich fruits and vegetables and cruciferous vegetables.

Assessment of HCA Consumption

Studies have shown that information on meat intake, serving size, doneness, and cooking method provide an adequate means for assessing individual exposure to dietary HCAs (35). The data collected on meat consumption in the interview was entered into the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) developed by Dr Rashmi Sinha at the National Cancer Institute to calculate the HCA intake for each participant (36–39). Because serving size information was not assessed, HCA levels were calculated assuming a medium portion size for women. A medium portion size was 84 g for hamburgers, 79 g for chicken, and 85 g for steak for middle-aged women. Among older women, a medium serving was 70 g for hamburgers, 64 g for chicken, and 84 g for steak. Based on the estimated serving size, type of meat, and degree of doneness, levels of PhIP, DiMeIQx, MeIQx, and total mutagenic activity were estimated. HCA levels in chicken also took into account whether the chicken was cooked with the skin on and if the skin was consumed. The database also includes a measure of total mutagenic activity (TMA) of foods. This measure was calculated using the Ames Salmonella test and is a measure of the total mutagenic ability of meat (the mutagenicity of the HCAs included in this analysis as well as other mutagens found in meat) (36).

NAT2 Genotyping

Beginning in February 1997, cheek swabbings were collected from women through the mail for planned studies of genetic susceptibility factors in breast cancer. For purposes of these analyses, a total of 324 case and 414 control women had DNA available and were interviewed in the era of the study that included assessment of dietary HCA exposures. Genotyping for NAT2 was carried out as previously described (32). All women were genotyped for 3 NAT2 slow acetylators alleles including NAT2*5A, NAT2*6A, and NAT2*7A (40). These 3 polymorphic sites predict 90 to 95% of the slow acetylation phenotype among Caucasians. Laboratory personnel were blinded to the case or control status of the samples. Participants were classified as rapid acetylators if they were homozygous wild type or heterozygous for any combination of the 3 slow acetylators alleles.

Women who were homozygous for all 3 of the slow acetylator alleles were classified as slow acetylators.

Analysis

Odds ratios (ORs) and 95% CIs were calculated to estimate the risk of breast cancer associated with meat consumption using logistic regression models. A total meat category was created by summing the number of servings of red meat and chicken together. Meat variables were analyzed both as quintiles and as servings per week. Quintiles and deciles were created using the data from the control population. Deciles of intake were analyzed in order to examine a greater range of intake. HCAs were modeled using both quintiles and deciles. Trends in meat consumption and HCA exposure were evaluated using ordinal values for each level of intake, with the term included as a continuous variable in the logistic regression model. Interaction between the NAT2 genotype and meat/HCAs intake was evaluated by including cross-product terms for meat/HCA (ordinal values) and NAT2 genotype (fast acetylator and/or slow acetylator) in the multivariate model. Model log likelihoods were then compared to assess improvements in goodness of fit. Age as a continuous variable and state of residence were included in all models. Multivariate models also included terms for age at menarche, parity, body mass index (BMI) (kg/m^2), smoking status and pack years of smoking, recent alcohol consumption (drinks per week), fruit and vegetable consumption (servings per week), use of multivitamins, and menopausal status as previously described (41). All analyses were conducted for all women and then separately for premenopausal and postmenopausal women.

RESULTS

The mean age of both the cases and the controls was 55 years. Table 1 shows the distribution of demographic characteristics and breast cancer risk factors in cases and controls. Cases and controls had similar levels of education. Approximately 40% of the women were premenopausal. Cases were more likely to be nulliparous and to have fewer children. A family history of breast cancer was more common among cases than controls as was a history of benign breast disease. A higher percentage of cases reported not having used multivitamins. Cases were also more likely to regularly drink alcohol and in premenopausal women, to have a lower body mass. The great majority of women ($\approx 97\%$) reported European ancestry.

Table 2 presents the results for meat consumption and breast cancer risk. There was no overall association of total meat intake with breast cancer risk. Adjusting for all covariates, ORs were comparable in women consuming 5+ vs. <2 servings/wk of any meat (OR = 0.99, 95% CI = 0.84–1.15). However, modest differences were observed according to menopausal status: A statistically significantly reduced breast cancer risk was observed for the highest level of consumption (5+ servings/wk) in premenopausal (OR = 0.74, 95% CI = 0.57–0.95), whereas a suggestion of an increase in risk was observed among postmenopausal women (OR = 1.19, 95% CI = 0.97–1.46); the test for interaction according to menopausal status was statistically significant for the highest quintile of meat intake (P for interaction = 0.005). There was no evidence of dose response with increasing servings per week in either

premenopausal ($P_{\text{trend}} = 0.11$) or postmenopausal ($P_{\text{trend}} = 0.38$) women. Considering red meat and chicken separately, an inverse trend was observed for servings/wk of chicken ($P_{\text{trend}} = 0.05$), although not red meat ($P_{\text{trend}} = 0.55$), in premenopausal women; no statistically significant associations were found in postmenopausal women according to type of meat consumed (Table 2).

Table 3 shows the results for breast cancer risk in relation to the most common individual HCAs present in cooked meat (PhIP, DiMeIQx, and MeIQx) and estimated total mutagenic activity. For all women combined, the data suggested no relationship between individual HCAs or TMA and breast cancer risk: the adjusted OR comparing the highest to lowest quintile for TMA was 1.01 (95% CI = 0.85–1.19), with similar null results obtained for individual HCAs. In premenopausal women, results suggested a statistically nonsignificant inverse association for PhIP and DiMeIQx, and TMA. In contrast, statistically nonsignificant positive associations were observed in the postmenopausal women. Tests for interaction according to menopausal status were statistically nonsignificant (data not shown). We examined results according to decile of consumption for each individual HCA, and also TMA, also with similar results (data not shown). For PhIP, we observed a statistically significant decrease in risk in premenopausal women (OR = 0.62, 95% CI = 0.41–0.93) and increase in risk in postmenopausal women (OR = 1.38, 95% CI = 1.01–1.90) comparing highest to lowest decile; however, tests for dose response across deciles were statistically nonsignificant in both groups. When we adjusted for total chicken intake, the protective association among premenopausal women for the highest decile was attenuated and no longer statistically significant (OR = 0.68, 95% CI = 0.40–1.15). Premenopausal women in the highest decile of DiMeIQx intake had a statistically significantly decreased risk when compared to the lowest decile (OR = 0.63, 95% CI = 0.41–0.98; $P_{\text{for trend}} = 0.24$); whereas there was no statistically significant association in postmenopausal women (OR = 1.09, 95% CI = 0.74–1.62; $P_{\text{for trend}} = 0.14$). All results were similar after adjustment for total meat intake (data not shown). All associations were consistent across strata of smoking (never, past, current), BMI (tertiles), and total servings of fruits and vegetables including cruciferous vegetables (tertiles; data not shown).

Forty percent of the breast cancer cases ($N = 132$) and 47% of the controls ($N = 192$) in this analysis were classified as NAT2 rapid acetylators. NAT2 genotype was unrelated to breast cancer risk overall (OR for slow versus rapid acetylators = 1.28, 95% CI = 0.93–1.75). Table 4 presents results for NAT2 genotype according to meat and HCA consumption among postmenopausal women. ORs were consistent regardless of acetylation status; and tests for interaction between NAT2 and total meat, total mutagenic activity, or individual HCAs were all statistically nonsignificant. Similarly, no interactions were observed in the smaller subgroup of premenopausal women (data not shown).

DISCUSSION

In this analysis, we did not observe an association of breast cancer with consumption of all meat, red meat, or chicken. Like-wise, we detected no consistent associations for breast cancer and individual HCAs or the measure of total mutagenic ability of meat. The analysis was based on a large, population-based, case-control study and had power to detect modest

associations of meats and specific HCAs with breast cancer risk. Differential associations by menopausal status—with statistically significant inverse associations observed only in premenopausal women—were unexpected and could be due to chance or possibly selection bias in the data. Although based on limited data, there was no evidence that associations varied according to NAT2 genotype. Overall, findings in this study add little support to the hypothesis that exposure to HCAs through consumption of meat contributes appreciably to breast cancer risk.

The hypothesized carcinogenic effect of meat is believed to be mediated primarily through the formation of HCAs. Studies have shown that information on meat intake, serving size, doneness, and cooking method provide an adequate means for assessing individual exposure to dietary HCAs (35). The meat questions included in our interview focused on capturing the consumption of meats cooked using methods that result in high levels of HCAs (16,42). Grilled steak and chicken, to different degrees of doneness, were shown to be the primary sources of PhIP, whereas fried and grilled hamburgers are among the most common sources of MeIQx (42).

NAT2 rapid acetylator status has been hypothesized to increase the risk of cancer among those with a high exposure to HCAs (29,43). A study conducted by Zhu et al. (44) showed that women preferring well-done meat and had a rapid NAT2 genotype had higher levels of PhIP-DNA adducts in breast tissue than women with the slow acetylator genotype. Stone et al. (27) reported that primary cultures of human mammary epithelial of DNA adducts formed by O-acetylation of HCA compared to slow acetylator cells. In the present data, we found no association for all meat, PhIP, or total mutagenic activity with breast cancer regardless of NAT2 genotype. The current results suggest a slightly elevated risk among slow acetylators (OR = 1.35). Although based on limited data, our results do not support an association in humans predicted from experimental models and are consistent with most previous studies that have not supported an interaction of NAT2 with HCAs from meat consumption (28–30).

It is possible that other exposures may interact with HCAs in the development of cancer. Cigarette smoke contains aromatic arylamines and may contribute to HCA exposure (45). An earlier analysis conducted in the Collaborative Breast Cancer Study found no evidence for interaction between smoking and NAT2 genotype (32). Data were too sparse in the present analysis to examine whether smoking modified NAT2/HCA–breast cancer associations.

Fruits and vegetables are believed to reduce the risk of cancer through several potential anticarcinogenic mechanisms. Cruciferous vegetables (broccoli, cauliflower, Brussels sprouts, etc.) have been shown to inactivate the mutagenic ability of HCAs (46). A small feeding study suggested that consuming cruciferous vegetables increases metabolism of HCAs from food (47), consistent with the ability of these foods to induce specific xenobiotic metabolizing enzymes (47–50). We examined the association between HCA levels and breast cancer according to tertiles of consumption of cruciferous vegetables (data not shown). However, associations for HCA consumption were null regardless of cruciferous vegetable intake in both premenopausal and postmenopausal women.

As in all case-control studies of dietary exposure and cancer risk, the present results may reflect various selection biases. The study had a relatively high participation rate, with 80% of the eligible cases and 76% of the controls completing the interview. Nevertheless, selection bias on factors related to diet could have affected the current results. A lower proportion of women provided a DNA sample (buccal response rate: 60% of the cases and 50% of the eligible controls). Null result for the interaction between NAT2 and HCAs has a greater potential to reflect selection bias, although we note that the proportion of slow acetylators among the controls (53%) is very similar to that reported for Caucasians in other populations (51,52). The current results are also consistent with the prospective Nurses' Health Study that also found no interaction of NAT2 and meat intake with breast cancer risk (28). Because the genotyping analysis was conducted only on a subset of the population, we were powered to only look at strong interactions and may have missed moderate-sized associations.

Recall bias may occur in case-control studies. In this analysis, we asked women to report their usual consumption of meat 5 yr prior to their diagnosis in the cases or a comparable time referent in controls. Recall bias might have occurred if cases or controls differentially recalled their meat cooking practices. The hypothesis that overcooking meat is a risk factor for breast cancer was not well known at the time this study was conducted. This suggests that differential reporting of meat consumption is unlikely to have materially affected our results. It is possible that women were aware of the health hazards of consuming undercooked meat and were more likely to report consuming well-cooked meat. This would result in an underestimation of the role HCA exposure plays in the development of breast cancer.

Random misclassification would have biased results toward the null. Women were asked to report their preference for meat doneness without the aid of photographic standards. As persons may have had different perceptions of rare and well-done cooking, some blending of the doneness categories may have occurred in our study. An association between well-done meat and breast cancer was found in a previous case-control study that used photographic aids (7). Furthermore, we did not ask if the women marinated or precooked meat in the microwave prior to cooking; both practices have been shown to reduce the HCA content of foods (53). Random misclassification from these sources would have tended to weaken associations. In addition, because of the telephone interview format, not all foods that are known sources of HCAs could be included in the survey. Rather, we inquired only about major contributors to total HCA based on an analysis of data from the Nurses' Health Study (54). Our food list would have underestimated total HCAs in the participants' diet, with some attenuation in the range of intake; this would have reduced power to detect associations at the extreme end of HCA exposure. Finally, although the food frequency questionnaire is currently the best method to measure HCA intake in large epidemiologic studies, no study has validated whether reported consumption of well-cooked meats is predictive of biochemical measures of HCA exposure; thus, it is not established how well the questionnaire captures these exposures. In one study, the correlation between the food frequency questionnaire and diet records was high for the consumption of different types of meat but relatively low for PhIP and MeIQx intake (results for other HCAs were not reported) (55). This result implies that HCA-disease associations may be difficult to detect

in studies based on the current questionnaire. A future questionnaire that collects information on a larger variety of meats and cooking techniques may better assess HCA intake.

In summary, the current results are consistent with some though not all previous studies that have failed to support a role of HCAs in breast cancer development. However, limitations in dietary case-control studies including selection and recall bias should be considered when evaluating the current results. Further, prospective analyses using improved questionnaire methods for evaluating HCA exposure may help to resolve whether meat-derived HCAs make any detectable contribution to breast cancer risk.

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TABLE 1

Descriptive characteristics of invasive breast cancer cases and controls^a

Characteristic	Cases		Controls		P Value ^b
	N	%	N	%	
Age (yr)					
<50	923	34.4	1,131	32.2	0.002
50–59	936	34.9	1,378	39.3	
>59	827	30.8	999	28.5	
Race					
Caucasian	2,602	96.9	3,349	95.5	0.005
Other	69	2.6	135	3.8	
Education (yr)					
<12	156	5.8	243	6.9	0.20
12	1,073	40.0	1,381	39.4	
>12	1,448	53.9	1,877	53.5	
Body mass index (kg/m ²)					
Premenopausal women					
<24	521	48.3	563	41.7	0.001
24–27	310	28.8	402	29.8	
>27	247	22.9	386	28.6	
Postmenopausal women					
<24	485	32.4	697	34.9	0.32
24–27	506	33.8	654	32.7	
>27	505	33.8	649	32.5	
Age at menarche (yr)					
<12	576	21.4	716	20.4	0.21
12–14	1,794	66.8	2,325	66.3	
>14	291	10.8	426	12.1	
Parity					
Nulliparous	381	14.2	405	11.5	0.001
1–2	1,172	43.6	1,384	39.5	

Characteristic	Cases		Controls		P Value ^b
	N	%	N	%	
>2	1,129	42.0	1,713	48.8	
Menopausal status					
Premenopausal	1,066	41.2	1,346	39.9	0.32
Postmenopausal	1,519	58.8	2,023	60.1	
Family history of breast cancer					
No	2,138	79.6	3,053	87.0	0.001
Yes	533	19.8	435	12.4	
History of benign breast disease					
No	1,846	68.7	2,670	76.1	0.001
Yes	808	30.1	810	23.1	
Recent alcohol consumption (drinks/wk)					
0	466	17.4	632	18.0	0.03
<2	1,172	43.7	1,606	45.8	
2-4	364	13.6	490	14.0	
4+	682	25.4	776	22.1	
Smoking					
Never smokers	1,277	47.7	1,617	46.3	0.44
Past smoker	843	31.5	1,110	31.8	
Current smoker	556	20.8	766	21.9	
Total fruit and vegetable intake (servings/day)					
<2	592	22.0	783	22.3	0.80
2-<4	1,487	55.4	1,913	54.5	
4+	607	22.6	812	23.1	
Multivitamin use					
Yes	1,040	39.5	1,444	42.1	0.04
No	1,593	60.5	1,986	57.9	

^aCases *N* = 2,686; controls *N* = 3,508. Column totals are unequal due to missing data.

^bChi-square *P* value comparing distributions in cases to controls.

Meat intake and the risk of breast cancer^a

Intake	All Women						Premenopausal				Postmenopausal			
	Cases	Controls	OR (95% CI) ^b	OR (95% CI) ^c	Cases	Controls	OR (95% CI) ^c	Cases	Controls	OR (95% CI) ^c	Cases	Controls	OR (95% CI) ^c	
Total meat														
<2 servings/wk	769	1,005	1.0 (ref)	1.0 (ref)	323	370	1.0 (ref)	420	609	1.0 (ref)				
2–<3 servings/wk	593	720	1.06 (0.92–1.23)	1.09 (0.94–1.26)	222	274	0.93 (0.73–1.19)	346	414	1.23 (1.01–1.50)				
3–<4 servings/wk	511	657	1.00 (0.86–1.16)	1.04 (0.89–1.22)	199	265	0.92 (0.72–1.18)	289	365	1.18 (0.96–1.45)				
4–<5 servings/wk	297	401	0.94 (0.79–1.12)	0.99 (0.82–1.19)	137	142	1.15 (0.86–1.53)	152	238	0.95 (0.74–1.22)				
5+ servings/wk	516	725	0.90 (0.78–1.05)	0.99 (0.84–1.15)	185	295	0.74 (0.57–0.95)	312	397	1.19 (0.97–1.46)				
<i>P</i> for trend			0.09	0.50			0.11			0.38				
Red meat														
<2 servings/wk	1,215	1,632	1.0 (ref)	1.0 (ref)	520	660	1.0 (ref)	647	912	1.0 (ref)				
2–<3 servings/wk	647	807	1.05 (0.92–1.19)	1.06 (0.93–1.21)	242	291	1.04 (0.84–1.29)	380	483	1.07 (0.90–1.28)				
3–<4 servings/wk	394	482	1.05 (0.90–1.23)	1.11 (0.95–1.30)	156	177	1.16 (0.90–1.50)	223	283	1.11 (0.90–1.37)				
4–<5 servings/wk	195	248	1.01 (0.83–1.24)	1.10 (0.89–1.35)	66	91	0.98 (0.69–1.39)	123	144	1.24 (0.94–1.62)				
5+ servings/wk	235	339	0.87 (0.73–1.05)	0.98 (0.81–1.18)	82	127	0.82 (0.60–1.13)	146	201	1.02 (0.80–1.31)				
<i>P</i> for trend			0.38	0.91			0.55			0.35				
Chicken														
<1 serving/wk	1,630	2,060	1.0 (ref)	1.0 (ref)	618	731	1.0 (ref)	953	1,266	1.0 (ref)				
1–<2 servings/wk	608	786	0.99 (0.87–1.12)	1.00 (0.88–1.14)	249	312	0.97 (0.79–1.19)	337	441	1.05 (0.89–1.25)				
2–<3 servings/wk	275	375	0.98 (0.83–1.12)	0.99 (0.83–1.17)	125	166	0.90 (0.69–1.17)	138	185	1.09 (0.85–1.39)				
3–<4 servings/wk	97	174	0.74 (0.57–0.96)	0.79 (0.61–1.03)	43	83	0.72 (0.48–1.07)	49	78	0.94 (0.64–1.37)				
4+ servings/wk	76	113	0.89 (0.66–1.21)	0.88 (0.65–1.21)	31	54	0.67 (0.42–1.08)	42	53	1.11 (0.72–1.71)				
<i>P</i> for trend			0.09	0.18			0.05			0.65				

^a Abbreviations are as follows: OR, odds ratio; CI, confidence interval; ref, reference.^b Adjusted for age and state of residence.^c Adjusted for age, state of residence, body mass index, education, alcohol intake, age at menarche, menopausal status (only in analysis of all women), age at first birth, family history of breast cancer, history of benign breast disease, parity, postmenopausal hormone use, multivitamin use, total fruits and vegetables intake, and smoking (smoking status and pack years).

TABLE 3

Consumption of heterocyclic amines and breast cancer risk^a

	All Women						Premenopausal				Postmenopausal			
	Cases	Controls	OR (95% CI) ^b	OR (95% CI) ^c	Cases	Controls	OR (95% CI) ^c	Cases	Controls	OR (95% CI) ^c	Cases	Controls	OR (95% CI) ^c	
PhIP														
Quintile 1	532	701	1.00 (ref)	1.00 (ref)	191	211	1.00 (ref)	321	469	1.00 (ref)				
Quintile 2	598	702	1.13 (0.97–1.33)	1.14 (0.97–1.34)	223	269	0.97 (0.73–1.27)	360	414	1.27 (1.03–1.56)				
Quintile 3	510	684	0.99 (0.84–1.16)	1.05 (0.88–1.25)	203	261	0.99 (0.75–1.30)	290	395	1.12 (0.90–1.39)				
Quintile 4	555	719	1.04 (0.88–1.22)	1.10 (0.91–1.32)	242	290	1.05 (0.80–1.38)	286	396	1.11 (0.89–1.38)				
Quintile 5	491	702	0.96 (0.81–1.13)	1.04 (0.84–1.29)	207	315	0.87 (0.63–1.09)	262	349	1.21 (0.97–1.52)				
<i>P</i> for trend			0.33	0.83			0.38			0.31				
DiMeIQx														
Quintile 1	581	772	1.00 (ref)	1.00 (ref)	235	269	1.00 (ref)	326	472	1.00 (ref)				
Quintile 2	503	631	1.02 (0.87–1.20)	1.05 (0.89–1.23)	203	225	1.03 (0.78–1.35)	278	383	1.03 (0.83–1.28)				
Quintile 3	555	690	1.03 (0.88–1.21)	1.06 (0.91–1.25)	207	270	0.91 (0.70–1.19)	326	398	1.17 (0.94–1.44)				
Quintile 4	543	708	0.99 (0.85–1.16)	1.03 (0.88–1.21)	217	283	0.92 (0.71–1.20)	308	399	1.12 (0.91–1.39)				
Quintile 5	504	707	0.94 (0.81–1.11)	1.01 (0.85–1.18)	204	299	0.85 (0.66–1.11)	281	371	1.18 (0.95–1.48)				
<i>P</i> for trend			0.44	0.89			0.22			0.07				
MeIQx														
Quintile 1	527	697	1.00 (ref)	1.00 (ref)	221	263	1.00 (ref)	287	412	1.00 (ref)				
Quintile 2	542	707	1.01 (0.86–1.19)	1.04 (0.88–1.22)	230	294	0.98 (0.75–1.26)	285	389	1.06 (0.85–1.33)				
Quintile 3	539	701	0.97 (0.83–1.14)	1.02 (0.87–1.21)	202	264	0.96 (0.73–1.25)	323	408	1.11 (0.89–1.39)				
Quintile 4	548	702	0.98 (0.83–1.15)	1.05 (0.89–1.24)	207	270	0.95 (0.72–1.24)	316	394	1.19 (0.95–1.49)				
Quintile 5	530	701	0.94 (0.80–1.10)	1.06 (0.89–1.25)	206	255	1.04 (0.79–1.37)	308	420	1.11 (0.90–1.40)				
<i>P</i> for trend			0.37	0.53			0.94			0.20				
Total mutagenic activity														
Quintile 1	547	701	1.00 (ref)	1.00 (ref)	215	246	1.00 (ref)	315	435	1.00 (ref)				
Quintile 2	530	702	0.95 (0.81–1.12)	0.98 (0.83–1.16)	200	265	0.91 (0.69–1.19)	309	417	1.04 (0.84–1.28)				
Quintile 3	511	702	0.91 (0.78–1.07)	0.97 (0.82–1.14)	201	279	0.90 (0.69–1.18)	287	397	1.03 (0.83–1.29)				
Quintile 4	580	702	1.02 (0.87–1.20)	1.08 (0.92–1.28)	250	275	1.09 (0.83–1.42)	309	390	1.14 (0.91–1.41)				
Quintile 5	518	701	0.91 (0.77–1.07)	1.01 (0.85–1.19)	200	281	0.88 (0.67–1.16)	299	384	1.17 (0.93–1.46)				

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All Women					
Premenopausal			Postmenopausal		
Cases	Controls	OR (95% CI) ^b	Cases	Controls	OR (95% CI) ^c
<i>P</i> for trend					
		0.45			0.51
					0.92
					0.12

^a Abbreviations are as follows: OR, odds ratio; CI, confidence interval; PHP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; ref, reference; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline^a; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline.

^b Adjusted for age and state of residence.

^c Adjusted for age, state of residence, body mass index, education, alcohol intake, age at menarche, menopausal status (only in analysis for all women), age at first birth, family history of breast cancer, history of benign breast disease, parity, postmenopausal hormone use, multivitamin use, total fruits and vegetables intake, and smoking (smoking status and pack years).

TABLE 4

Meat consumption, heterocyclic amine intake, and breast cancer risk according to NAT2 acetylator status (postmenopausal women only)^a

	Rapid Acetylators			Slow Acetylators		
	Cases (77)	Controls (117)	OR (95% CI) ^b	Cases (120)	Controls (135)	OR (95% CI) ^b
All meat						
Low ^d	40	53	1.0 (ref)	63	63	1.34 (0.78–2.30)
High	37	64	0.80 (0.45–1.43)	57	72	1.12 (0.65–1.93)
<i>P</i> value interaction between NAT2 and all meat intake = 0.76						
PhIP						
Low	42	59	1.0 (ref)	57	65	1.22 (0.71–2.08)
High	35	58	0.85 (0.47–1.52)	63	70	1.32 (0.78–2.24)
<i>P</i> value interaction between NAT2 and PhIP exposure = 0.75						
Total mutagenic activity						
Low	44	57	1.0 (ref)	64	59	1.44 (0.84–2.45)
High	33	60	0.77 (0.43–1.38)	56	76	1.02 (0.60–1.75)
<i>P</i> value interaction between NAT2 and total mutagenic activity = 0.50						

^aAbbreviations are as follows: NAT2, N-acetyltransferase2; OR, odds ratio; CI, confidence interval; ref, reference; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

^bAdjusted for age and state of residence

^cAdjusted for age, state of residence, BMI, education, alcohol intake, age at menarche, age at first birth, family history of breast cancer, history of benign breast disease (BBD), parity, multivitamin use, postmenopausal hormone use, fruit and vegetable intake, and smoking (smoking status and pack years)

^dHigh and low categories were determined by the mean of the intake.