

BREAST CANCER RISK, MEAT CONSUMPTION AND N-ACETYLTRANSFERASE (NAT2) GENETIC POLYMORPHISMS

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Although inconsistencies exist, some studies have shown that meat consumption is associated with breast cancer risk. Several heterocyclic amines (HAs), formed in the cooking of meats, are mammary carcinogens in laboratory models. HAs are activated by polymorphic *N*-acetyltransferase (NAT2) and rapid NAT2 activity may increase risk associated with HAs. We investigated whether ingestion of meat, chicken and fish, as well as particular concentrated sources of HAs, was associated with breast cancer risk, and if NAT2 genotype modified risk. Caucasian women with incident breast cancer ($n = 740$) and community controls ($n = 810$) were interviewed and administered a food frequency questionnaire. A subset of these women ($n = 793$) provided a blood sample. Polymerase chain reaction and restriction fragment length polymorphism analyses were used to determine NAT2 genotype. Consumption of red meats, as well as an index of concentrated sources of HAs, was not associated with increased breast cancer risk, nor did risk vary by NAT2 genotype. In post-menopausal women, higher fish consumption was inversely associated with risk (odds ratio = 0.7; 95% confidence interval, 0.4–1.0); among pre-menopausal women, there was the suggestion of inverse associations between risk and pork and chicken intake. Our results suggest that consumption of meats and other concentrated sources of HAs is not associated with increased breast cancer risk. However, due to the strong biologic plausibility for a role of some HAs in mammary carcinogenesis, and the likely measurement error in evaluation of sources of HAs in this study, further studies of these possible relationships are warranted. *Int. J. Cancer* 75:825–830, 1998.

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The incidence of breast cancer varies widely by geographic region (Parkin and Muir, 1992), and it has been suggested that variability in diet, particularly intake of dietary fat and protein, may be related to this disparity in breast cancer rates (Prentice *et al.*, 1988), although the majority of epidemiologic studies have not supported this association (Hunter and Willett, 1996). Studies of the consumption of animal products, particularly meat, have also yielded inconsistent results, although a meta-analysis of 5 cohort and 12 case-control studies by Boyd *et al.*, (1993) did reveal a summary relative risk of 1.54 (95% CI 1.31–1.82) associated with consumption of red meat (Boyd *et al.*, 1993).

The assessment of meat as a risk factor for breast cancer has focused primarily on its role as a source of dietary fat or animal protein. However, in 3 studies it was found that consumption of meat, when controlling for total fat or protein, significantly increased breast cancer risk (Toniolo *et al.*, 1994, Ronco *et al.*, 1996, De Stefani *et al.*, 1997). It is possible that if meat consumption does play a role in breast cancer etiology, it may be due to its being a source of mutagens and/or carcinogens, such as heterocyclic amines, which are potent mammary mutagens and carcinogens in rodent models (Snyderwine, 1994). Disparate cooking methods in different populations or survey instruments inadequate to assess concentrated sources of heterocyclic amines (HAs) may be related to these inconsistencies in results across studies.

Metabolism of heterocyclic and aromatic amines varies among individuals, depending, in part, on polymorphisms in genes involved in xenobiotic metabolism, such as *N*-acetyltransferases NAT1 and NAT2 and cytochrome P4501A2 (CYP1A2) (Lang *et al.*, 1994). Several polymorphic sites have been identified at the NAT2 locus, and result in decreased *N*-acetyltransferase activity (Bell *et al.*, 1993). Slow NAT2 acetylation of aromatic amines is associated with increased risk for bladder cancer (Cartwright, 1984) and may increase post-menopausal breast cancer risk associated with cigarette smoking (Ambrosone *et al.*, 1996). HAs appear to be poor substrates for *N*-acetylation in the liver, however, and rapid *O*-acetylation of the activated metabolites by NAT2 in the target tissue appears to be associated with increased risk of colon cancer related to consumption of red meat (Welfare *et al.*, 1997; Lang *et al.*, 1994). If HAs are etiologic agents in human breast carcinogenesis, it is plausible that rapid activation by NAT2 would also be associated with increased breast cancer risk.

The purpose of our analyses was 3-fold: 1) we sought to evaluate relationships between breast cancer risk and consumption of meats, poultry and fish in pre- and post-menopausal women; 2) we were interested in determining whether risk associated with meat consumption could be related to dietary HAs, as measured by consumption of products known to be concentrated sources of these carcinogens; and 3) we wanted to determine whether polymorphisms in NAT2 might modify the association between breast cancer risk and consumption of sources of heterocyclic amines.

MATERIAL AND METHODS

Study population

This study population and research methodology have been described in detail previously (Freudenheim *et al.*, 1996; Ambrosone *et al.*, 1996; Graham *et al.*, 1991). Briefly, cases were women diagnosed with incident, primary, histologically confirmed breast cancer, identified from all the major hospitals in Erie and Niagara counties; included were women ranging in age from 40 to 85.

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Women under age 50 were considered post-menopausal if they had ceased menstruation because of natural menopause, bilateral oophorectomy, or irradiation to the ovaries; all others were considered pre-menopausal. Women 50 years of age and over were considered post-menopausal if they were no longer menstruating. Cases were interviewed, on average, within 2 months of diagnosis. Controls under 65 years of age were randomly selected from the New York State Motor Vehicle Registry, and those 65 and over were identified from Health Care Finance Administration lists. Of pre-menopausal women contacted, 66% of eligible cases ($n = 301$) and 62% of eligible controls ($n = 316$) participated, and of post-menopausal women, 54% of cases ($n = 439$) and 44% of controls ($n = 494$) participated. Controls were frequency-matched to cases by age and county of residence. The protocol for the study was reviewed by the Institutional Review Board of the State University of New York at Buffalo and each participating hospital, and informed consent was received from all participants. Cases and controls were interviewed in person by trained interviewers, with an in-depth food frequency questionnaire regarding usual diet 2 years prior to the interview, including frequency of consumption and usual portion size of over 300 specific foods. Reproductive, medical and family histories were obtained, as well as lifetime tobacco and alcohol histories. Of the women interviewed, approximately 45% of pre-menopausal and 63% of post-menopausal women agreed to have blood drawn for research purposes.

Analytic methodology

An extensive food frequency questionnaire was administered, assessing usual intake 2 years prior to the interview. Using food models, women were questioned about usual dietary intake 2 years prior to the interview, including quantity and frequency of intake, seasonal intake and food preparation. Grams of meats per day were computed by multiplying frequency of consumption by portion size, estimated by food models. Participants were asked about portion size and frequency of consumption of steak, round steak, hamburger patties, ground beef, other beef, including roasts and stews, veal, lamb and beef liver. From this information, usual grams of consumption of each item were calculated and items were grouped to create a beef index. A pork index was based on queries regarding intake of pork roast, chops and spareribs. A processed meats index, including ham, hot dogs, sausages, bacon and cold cuts was also assessed. A poultry index included chicken and turkey. The fish index included fresh or frozen fish, canned fish, shrimp and other shellfish. In addition to frequency of consumption and usual portion size of various types of meat, women were also asked how frequently they used gravy made from pan drippings or fried foods in bacon grease. We also evaluated associations between risk and grams consumed per month of bacon, breakfast sausages and gravy made from pan drippings, all concentrated sources of HAs, particularly PhIP (Murray *et al.*, 1993). Data were not available on how well done the meat consumed was cooked, which is another indicator of exposure to HAs.

Risks for pre- and post-menopausal women were examined separately, based on variability in some risk factors and the possibility that breast cancer may be different diseases in the 2 groups. Furthermore, mean levels of intake of certain meats varied significantly between the 2 groups. Quartiles of intake of types of meats were based on approximately uniform distribution in controls. Odds ratios (OR) with 95% confidence intervals (CI) were calculated by unconditional logistic regression for each category of the risk variables, with the lowest intake quartile as the referent category. The p values for trend were the levels of significance of the beta coefficient for each independent variable as a continuous variable in the logistic regression model with the relevant adjusting variables. ORs were adjusted for putative breast cancer risk factors including age, education, body mass index (BMI), age at menarche, age at first pregnancy, family history of breast cancer and age at menopause for post-menopausal women. BMI was computed as $\text{weight}(\text{kg})/\text{height}(\text{m})^2$, where weight was as reported for 2 years

prior to the interview, and family history was defined as the presence of breast cancer in a mother and/or sister. Because there may be a tendency for fish and poultry eaters also to consume more fruits and vegetables, and because some components of fruits and vegetables, which were associated with reduced risk in these data (Freudenheim *et al.*, 1996), may reduce mutagenic activity, an additional model was employed, adjusting for total fruit and vegetable consumption. To evaluate variable risk in relation to consumption of sources of HAs, cases and controls were stratified by acetylator status and the relationship between breast cancer risk and these foods was assessed within rapid and slow acetylator groups. Sample size for these latter determinations was restricted to those who provided a blood sample and for whom NAT2 data were available. This included 118 and 114 pre-menopausal cases and controls, and 185 and 213 post-menopausal cases and controls.

NAT2 genotyping

Blood specimens were collected, serum was separated and blood clots were stored at -70°C . Methods for DNA extraction from clots and determination of NAT2 genotype have been described previously (Ambrosone *et al.*, 1996). Briefly, DNA was extracted and amplified by polymerase chain reaction (PCR) in the presence of primers specific for NAT2 (Bell *et al.*, 1993). An aliquot (18 μl) was then subjected to restriction fragment length polymorphism (RFLP) analysis for the C⁴⁸¹T (KpnI; New England Biolabs, Beverly, MA), G⁵⁹⁰A (TaqI, New England Biolabs) and the G⁸⁵⁷A (BamHI, New England Biolabs) polymorphisms. Individuals were classified as genotypically determined rapid acetylator (carrying 0 or 1 slow acetylator mutation) or slow acetylator (individuals with 2 slow acetylator mutations) (Lin *et al.*, 1993; De Stefani *et al.*, 1994). Assays were performed in duplicate and were interpreted by 2 reviewers who were blinded to case-control status.

RESULTS

Table I shows reported mean values of consumption of various meats for all pre- and post-menopausal women. Pre-menopausal controls consumed significantly more pork and fish than cases. In the interpretation of these reports, it is important to note that the diet assessment instrument used is a well-established tool for qualitative assessment of intake and that quantitative assessment may be less accurate. There were no significant differences in means for any of the variables tested among post-menopausal women. Associations between breast cancer risk and quartiles of consumption of various meats for pre- and post-menopausal women are shown in Tables II and III. Total calories consumed were not related to breast cancer risk in these data, and the addition of this variable to the model did not significantly alter estimates of risk. Models adjusted for cigarette smoking, found to increase risk among post-menopausal women with slow NAT2 genotype in these data, also did not differ significantly from unadjusted. For pre-menopausal women, there was no increased risk associated with consumption of beef, processed meats, pork, chicken or fish (Table II). In fact, there were inverse associations between breast cancer risk and consumption of pork, chicken and fish, although of borderline significance. However, the association between fish and chicken consumption and breast cancer risk was weaker after adjustment for fruit and vegetables.

Among post-menopausal women, there was no increase in breast cancer risk associated with higher consumption of beef, pork or processed meats (Table III). Both chicken and fish consumption were inversely associated with risk of post-menopausal breast cancer (4th quartile ORs and 95% CIs, respectively, 0.7, 0.5–1.0, and 0.6, 0.4–0.9). These relationships remained when adjustment was made for total fruit and vegetable consumption.

Tables IV and V present analyses for the subset of women who provided blood specimens. When associations were assessed within categories of rapid and slow acetylators, there were no clear

TABLE I – CHARACTERISTICS OF CASES AND CONTROLS: WESTERN NEW YORK DIET STUDY, 1986–1991¹

	Pre-menopausal		Post-menopausal	
	Cases (n = 301)	Controls (n = 316)	Cases (n = 439)	Controls (n = 494)
Age (years)	46 (4)	46 (4)	64 (8)	63 (8)
Education (years)	14 (3)	14 (3)	12 (3)	12 (3)
Beef intake (g/day)	60 (47)	60 (39)	52 (44)	51 (37)
Pork intake (g/day)	12 (9) ²	14 (11) ²	11 (10)	11 (10)
Processed meat intake ³ (g/day)	39 (41)	37 (31)	31 (30)	31 (31)
Poultry intake (g/day)	31 (22)	34 (24)	23 (17)	25 (21)
Fish intake (g/day)	27 (15) ²	32 (28) ²	25 (20)	28 (22)
Ratio of red meat/poultry and fish	1.5 (1.3)	1.5 (1.3)	2.8 (4.5)	2.4 (2.3)

¹Values are expressed as mean (standard deviation).—² $p < 0.01$, Student's *t*-test for difference between means of cases and controls.—³Includes bacon, breakfast sausages, ham, hot dogs, bologna and other cold cuts.

TABLE II – MEAT CONSUMPTION (g/day) AND PREMENOPAUSAL BREAST CANCER RISK: WESTERN NEW YORK DIET STUDY, 1986–1991

Quartiles	Case (number)	Control (number)	OR (CI) ¹	OR (CI) ²
Beef (g/day)				
<33	74	82	1.0	1.0
33–51	85	77	1.3 (0.8–2.0)	1.3 (0.8–2.1)
51–78	68	78	1.0 (0.6–1.5)	1.0 (0.6–1.6)
>78	74	79	1.1 (0.7–1.7)	1.2 (0.8–1.9)
			p (trend) = 0.76	p (trend) = 0.3
Pork (g/day)				
<6	92	82	1.0	1.0
6–10	70	79	0.8 (0.5–1.2)	0.8 (0.5–1.2)
10–20	91	82	1.0 (0.6–1.5)	1.0 (0.6–1.5)
>20	48	73	0.6 (0.4–1.0)	0.6 (0.4–1.0)
			p (trend) = 0.02	p (trend) = 0.05
Processed meats (g/day) ³				
<14	65	80	1.0	1.0
14–29	94	79	1.6 (1.0–2.5)	1.5 (1.0–2.4)
29–48	60	78	1.0 (0.6–1.6)	1.0 (0.6–1.6)
>48	82	79	1.4 (0.8–2.2)	1.4 (0.9–2.3)
			p (trend) = 0.3	p (trend) = 0.09
Poultry (g/day)				
<19	95	79	1.0	1.0
19–28	66	79	0.7 (0.4–1.1)	0.7 (0.4–1.1)
28–43	84	79	0.8 (0.5–1.3)	0.9 (0.6–1.4)
>43	56	79	0.6 (0.4–0.9)	0.7 (0.4–1.1)
			p (trend) = 0.2	p (trend) = 0.6
Fish (g/day)				
<15	83	80	1.0	1.0
15–26	85	79	1.1 (0.7–1.7)	1.1 (0.7–1.8)
26–38	71	75	0.9 (0.6–1.5)	1.0 (0.6–1.6)
>38	62	82	0.8 (0.5–1.2)	0.9 (0.6–1.5)
			p (trend) = 0.03	p (trend) = 0.2
Ratio of red meat to chicken and fish				
<0.7	71	80	1.0	1.0
0.7–1.2	70	85	0.9 (0.6–1.4)	0.9 (0.5–1.4)
1.2–1.8	72	76	1.1 (0.7–1.7)	1.0 (0.6–1.7)
>1.8	88	75	1.4 (0.9–2.2)	1.3 (0.8–2.0)
			p (trend) = 0.5	p (trend) = 0.8

¹Odds ratios and 95% confidence intervals calculated by logistic regression, adjusted for age, education, age at menarche, age at first pregnancy, body mass index, family history of breast cancer.—²Adjusted for the variables listed above, and total fruits and vegetables.—³Includes bacon, breakfast sausages, ham, hot dogs, bologna and other cold cuts.

associations between risk and consumption of beef, pork, chicken, fish or processed meats among pre- or postmenopausal women by genotype (data not shown). Evaluation of risk associated with consumption of foods that are concentrated sources of heterocyclic amines (bacon, gravy, breakfast sausages) also revealed no clear or significant associations, when groups were evaluated all together, or when stratified by *NAT2* genotype. Associations with risk were also evaluated by frequency of consumption of various meats that were fried or grilled, but no effect was observed (data not shown).

DISCUSSION

In this case-control study of diet and breast cancer, we found that, in general, consumption of meats was not associated with increased breast cancer risk for pre- or post-menopausal women. Increased intake of fresh, frozen or canned fish, as well as poultry, appeared to be associated with decreased risk among post-menopausal women. Among pre-menopausal women, there was a suggestion of a slight inverse association with pork consumption.

TABLE III – MEAT CONSUMPTION (g/day) AND POST-MENOPAUSAL BREAST CANCER RISK: WESTERN NEW YORK DIET STUDY, 1986–1991

Quartiles	Case (number)	Control (number)	OR (CI) ¹	OR (CI) ²
Beef (g/day)				
<28	113	123	1.0	1.0
28–45	132	121	1.1 (0.8–1.7)	1.2 (0.8–1.7)
45–62	78	122	0.7 (0.5–1.0)	0.7 (0.5–1.0)
>62	116	128	0.9 (0.6–1.3)	1.0 (0.7–1.4)
			<i>p</i> (trend) = 0.5	<i>p</i> (trend) = 0.3
Pork (g/day)				
<4	96	98	1.0	1.0
4–8	118	137	0.9 (0.6–1.3)	0.9 (0.6–1.3)
8–15	128	133	1.0 (0.7–1.4)	1.0 (0.7–1.4)
>15	97	126	0.7 (0.5–1.1)	0.8 (0.5–1.2)
			<i>p</i> (trend) = 0.3	<i>p</i> (trend) = 0.5
Processed meats (g/day) ³				
<11	101	122	1.0	1.0
11–22	117	126	1.1 (0.8–1.6)	1.1 (0.8–1.6)
22–40	112	124	1.1 (0.7–1.6)	1.1 (0.8–1.6)
>40	109	122	1.0 (0.7–1.5)	1.1 (0.7–1.6)
			<i>p</i> (trend) = 0.9	<i>p</i> (trend) = 0.5
Poultry (g/day)				
<12	126	120	1.0	1.0
12–19	119	125	0.8 (0.6–1.2)	0.8 (0.6–1.2)
19–30	80	122	0.5 (0.4–0.8)	0.5 (0.4–0.8)
>30	114	127	0.7 (0.5–1.0)	0.8 (0.5–1.1)
			<i>p</i> (trend) = 0.01	<i>p</i> (trend) = 0.04
Fish (g/day)				
<13	129	124	1.0	1.0
13–23	117	131	0.9 (0.6–1.3)	0.9 (0.7–1.3)
23–38	112	120	0.8 (0.6–1.2)	0.9 (0.6–1.3)
>38	81	119	0.6 (0.4–0.9)	0.7 (0.4–1.0)
			<i>p</i> (trend) = 0.03	<i>p</i> (trend) = 0.2
Ratio of red meat to chicken and fish				
<1.2	107	130	1.0	1.0
1.2–1.9	94	125	0.9 (0.6–1.4)	0.9 (0.6–1.3)
1.9–2.8	94	109	1.0 (0.7–1.5)	1.0 (0.7–1.4)
>2.8	144	130	1.4 (1.0–2.1)	1.3 (0.9–1.9)
			<i>p</i> (trend) = 0.1	<i>p</i> (trend) = 0.1

¹Odds ratios and 95% confidence intervals calculated by logistic regression, adjusted for age, education, age at menarche, age at first pregnancy, age at menopause, body mass index, family history of breast cancer. ²Adjusted for the variables listed above, and total fruits and vegetables. ³Includes bacon, breakfast sausages, ham, hot dogs, bologna and other cold cuts.

TABLE IV – CONSUMPTION OF CONCENTRATED SOURCES OF HETEROCYCLIC AMINES (BACON, BREAKFAST SAUSAGE, GRAVY) AND BREAST CANCER RISK

Sources of heterocyclic amines (g/month)	Pre-menopausal			Post-menopausal		
	Cases	Controls	OR (CI) ¹	Cases	Controls	OR (CI) ¹
All women with genetic data						
<58	25	28	1.0	45	53	1.0
58–149	26	31	0.8 (0.4–1.9)	39	55	0.8 (0.4–1.4)
149–464	45	27	2.0 (0.9–4.3)	43	59	0.7 (0.4–1.3)
>464	22	28	0.9 (0.4–2.1)	58	46	1.4 (0.8–2.5)
NAT2 rapid						
<58	11	9	1.0	25	31	1.0
58–149	9	13	0.8 (0.2–3.1)	20	31	0.8 (0.3–1.9)
149–464	21	11	2.7 (0.7–9.9)	29	29	0.3 (0.1–0.9)
>464	10	16	0.9 (0.2–3.4)	31	22	1.0 (0.4–2.6)
NAT2 slow						
<58	14	19	1.0	20	22	1.0
58–149	17	18	0.9 (0.3–2.8)	19	24	0.9 (0.4–2.0)
149–464	24	16	1.8 (0.6–5.4)	14	30	1.2 (0.5–2.6)
>464	12	12	1.2 (0.3–3.9)	27	24	1.9 (0.9–4.3)

¹Odds ratios and 95% confidence intervals calculated by logistic regression, adjusted for age, education, age at menarche, age at first pregnancy, body mass index, family history of breast cancer, fruit and vegetable consumption and age at menopause for post-menopausal women.

In studying associations between dietary sources of heterocyclic amines and breast cancer risk, we had extensive data regarding portion size and method of cooking for a number of meats.

However, no data were available on how well-done the meat was cooked. Because a major determinant of HAs appears to be how well the meat is cooked (Sinha *et al.* 1995), it is possible that our

TABLE V – FREQUENCY OF CONSUMPTION OF GRAVY MADE FROM PAN DRIPPINGS AND FOODS FRIED IN BACON GREASE

	Pre-menopausal			Post-menopausal		
	Cases	Controls	OR (CI) ¹	Cases	Controls	OR (CI) ¹
Frequency of gravy consumption						
All women with genetic data						
Never	10	10	1.0	23	34	1.0
<once/month	27	35	1.3 (0.4–3.6)	40	52	1.0 (0.5–2.1)
1–3 times/month	41	25	0.7 (0.4–1.6)	49	60	1.1 (0.5–2.1)
Once/week–daily	40	44	1.9 (0.9–3.9)	73	67	1.6 (0.8–3.0)
NAT2 rapid						
Never	5	2	1.0	9	12	1.0
<once/month	11	14	3.8 (0.5–27.1)	21	28	0.9 (0.3–2.7)
1–3 times/month	19	9	0.7 (0.2–2.2)	19	26	0.8 (0.3–2.6)
Once/week–daily	16	24	2.6 (0.8–8.7)	31	34	1.0 (0.3–3.0)
NAT2 slow						
Never	5	8	1.0	14	22	1.0
<once/month	16	21	0.6 (0.2–2.5)	19	24	1.0 (4–2.7)
1–3 times/month	22	16	0.7 (0.2–1.8)	30	34	1.3 (0.5–3.0)
Once/week–daily	24	20	1.2 (0.4–3.1)	42	33	2.1 (0.9–5.0)
Frequency of consumption of foods fried in bacon fat						
All women with genetic data						
Never	89	83	1.0	131	158	1.0
<once/month	16	17	0.8 (0.4–1.8)	22	28	0.9 (0.5–1.7)
1–3 times/month	6	7	0.7 (0.2–2.3)	20	16	1.6 (0.8–3.4)
Once/week–daily	7	7	1.2 (0.4–3.9)	12	9	1.8 (0.7–4.5)
NAT2 rapid						
Never	38	31	1.0	58	75	1.0
<once/month	7	11	0.6 (0.2–1.8)	9	14	0.6 (0.2–1.6)
1–3 times/month	2	3	0.7 (0.1–4.9)	10	8	1.9 (0.6–5.5)
Once/week–daily	4	4	1.1 (0.2–5.4)	3	2	1.7 (0.2–13.7)
NAT2 slow						
Never	51	52	1.0	73	83	1.0
<once/month	9	6	1.4 (0.4–4.8)	13	14	1.1 (0.5–2.6)
1–3 times/month	4	4	0.9 (0.2–4.0)	10	8	1.5 (0.5–4.3)
Once/week–daily	3	3	1.3 (0.2–7.6)	9	7	1.6 (0.5–4.7)

¹Odds ratios and 95% confidence intervals calculated by logistic regression, adjusted for age, education, age at menarche, age at first pregnancy, body mass index, family history of breast cancer, fruit and vegetable consumption and age at menopause for post-menopausal women.

measurement of sources of HAs by grams of meats consumed was too crude to assess dietary intake of HAs accurately. However, bacon, breakfast sausages, and gravy made from pan drippings are documented sources of HAs, and these foods were also not associated with breast cancer risk.

We had hypothesized that consumption of all sources of HAs, including fish, chicken and pork, could be related to breast cancer risk. Reasons for the slight inverse associations between pork (pre-menopausal women) and chicken (post-menopausal women) are unknown, although there is the possibility that they are due to chance, or to biased reports. However, the finding of reduced risk with fish consumption among post-menopausal women is supported by some human and animal data. Few epidemiologic studies have investigated the association of breast cancer risk with fish consumption. Some case-control studies did find that fish consumption, particularly poached fish, was associated with decreased risk (Hirose *et al.*, 1995; Landa *et al.*, 1994; Vatten *et al.*, 1990; De Stefani *et al.*, 1997), and ecologic studies show that populations with high fish consumption have lower breast cancer rates (Caygill *et al.*, 1996; Kaizer *et al.*, 1989; Lund and Bonna, 1993). Additionally, laboratory studies in rodent models and with human mammary epithelial cells have shown that dietary omega-3 fatty acids, found in fish oil, suppress growth of carcinomas (Rose and Connolly, 1993). Fish that is pan-fried or broiled may be a source of HAs, however, which may counteract some of the anticarcinogenic effects that fish oil may have. Further investigations of breast cancer risk and fish consumption, particularly by method of cooking, may elucidate these issues.

The observation of a stronger association between risk and fish consumption among post-menopausal in relation to pre-menopausal breast cancer is consistent with other findings of differences in risk associated with some factors, such as body mass, among pre- and postmenopausal women. We have found that among women with slow *NAT2* genotype, cigarette smoking was a risk factor for post-, but not premenopausal breast cancer. In light of the evidence that pre-menopausal and post-menopausal breast cancer may have different etiologies, (Velentgas and Daling, 1994; Janerich and Hoff, 1982; de Waard, 1979), this heterogeneity is plausible. The disparity in results in these analyses by menopausal status may reflect different etiologic pathways associated with menopausal status.

Our study may have been hampered by biases common to case-control studies, particularly those involving selection, dietary recall and measurement. Regarding selection bias, most case non-participation was due to physicians' refusals to allow contact with their patients (72%). Among post-menopausal women, non-participants were, on average, about 3 years older than participants. Thus, the most ill patients may not have been included, limiting generalizability. Among controls, a sample refusing interview ($n = 117$) was compared with a sample of participants ($n = 372$) in a telephone interview prior to data collection. No differences in reported meat, vegetable or fruit consumption were found. Thus, non-response among controls is unlikely to be related to dietary exposure.

For many cancers, illness may have caused changes in dietary habits, possibly influencing memory of past eating habits. Thus,

recall bias may affect observed associations between dietary intake and cancer risk, although evidence for this bias is not consistent. With breast cancer, though, the growing tumor is often asymptomatic until diagnosis; it probably does not affect appetite. Questions in our study were focused on intake in the year 2 years before the interview. Regarding measurement error, clearly, the use of a food frequency questionnaire to assess macro- and micronutrients may result in misclassification of nutrient intake. Nonetheless, it is likely that the instrument enables us to rank order subjects and identify at least strong relationships. However, this questionnaire was not designed to estimate dietary intake of heterocyclic amines, and as such, allows only use of surrogates for evaluation of associations between probable HA consumption and risk, which certainly include measurement error.

It is also becoming clear that metabolic pathways are extremely complex, involving a number of Phase I and Phase II enzymes. It is possible that effects of NAT2 may only impact on risk if CYP1A2 phenotype is also rapid, that is, rapid activation at both junctures in the metabolic pathway. This phenomenon was observed by Lang *et al.*, (1994) in a study of colon cancer, where risk was highest for those with rapid NAT2 and rapid CYP1A2 phenotypes. Lack of

data on CYP1A2 may, therefore, also be related to the lack of association between meats, NAT2, and breast cancer risk.

A final caution regarding our findings is related to the size of the study group. In the overall assessment of meat and fish consumption on risk, we have adequate power to detect an effect. However, these findings may be affected by numerous sources of bias. In the analyses stratified by acetylator status, in which one would expect the bias to be non-differential and thus less of a problem, numbers are quite small. For some risk estimates, confidence intervals are wide and estimates of risk unstable. Thus, our findings must be viewed as tentative, and further studies of consumption of dietary heterocyclic amines, using a validated questionnaire for their assessment, are warranted, particularly in light of the laboratory data suggesting their association with mammary carcinogenesis.

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