Research Article

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Meat Mutagens and Breast Cancer in Postmenopausal Women—A Cohort Analysis

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Abstract

Background: Mutagenic compounds produced when meats are cooked at high temperatures have been hypothesized to increase risk of breast cancer.

Methods: We examined the association between intakes of the heterocyclic amines (HCA) MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline), PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]), and meat-derived mutagenic (MDM) activity and risk of breast cancer using a cooking method questionnaire administered in 1996 in the Nurses' Health Study. Between 1996 and 2006, 2,317 breast cancer cases were diagnosed during 533,618 person-years.

Results: Higher intake of HCAs or MDM was not associated with elevated risk of breast cancer [multivariate relative risk and 95% confidence interval for the highest versus lowest quintile: MeIQx: 0.90 (0.79-1.03); PhIP: 0.92 (0.80-1.05); DiMeIQx: 0.92 (0.80-1.05); and MDM: 0.98 (0.85-1.12)]. HCA or MDM was not associated with estrogen receptor–positive/progesterone receptor–positive breast cancer risk either. There was some suggestion of a decreased risk of estrogen receptor–negative/progesterone receptor–negative breast cancer with higher intakes of MeIQx, DiMeIQx, and PhIP, but none of the associations were statistically significant. There was little evidence for an interaction between intake of cruciferous vegetables and HCA or MDM intake and risk of breast cancer.

Conclusion: Higher consumption of mutagens from meats cooked at higher temperature and longer duration was not associated with increased risk of postmenopausal breast cancer.

Impact: Overall prospective data including results from our study do not provide support for a substantial increase in risk of breast cancer with higher intake of HCAs. *Cancer Epidemiol Biomarkers Prev; 19(5); 1301–10.*

Introduction

Cooking meats at high temperatures and for long duration can result in the production of mutagenic compounds, such as heterocyclic amines (HCA; ref. 1). Findings from animal studies have long suggested a role of HCAs in breast carcinogenesis (2, 3), and there is some evidence from epidemiologic studies that higher intakes of well-done meats may raise breast cancer risk (4-6). However, only a limited number of epidemiologic studies, including three recent prospective studies, have examined the associations between HCAs and breast cancer risk (5, 7-13). In two prospective studies, no evidence for an association between HCA intake and risk of postmenopausal breast cancer was observed (10, 11), whereas another recent prospective study (13) reported a significant positive association between higher intake of MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline) and a marginally significant positive association between higher intake of DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]) and risk of postmenopausal breast cancer. Considering the paucity of prospective data on the association between HCAs and breast cancer, we examined the association between intakes of major HCAs, including MeIQx, PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine), DiMeIQx, and meat-derived mutagenic (MDM) activity and risk of breast cancer using a cooking method questionnaire administered in 1996 in the Nurses' Health Study (NHS), a large female cohort. The large number of cases allowed us to examine associations between HCAs and breast cancer risk by hormone receptor status, as some recent evidence suggests that certain risk factors for breast cancer, including red meat intake, may differ by hormone receptor status (14, 15). Only a limited number of epidemiologic studies have examined associations between

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HCA intake and risk of breast cancer separately by hormone receptor status (5, 11, 13).

Materials and Methods

Study population. More detailed information on the NHS cohort is provided elsewhere (16). In brief, the NHS cohort was founded in 1976 when 121,700 U.S. female nurses aged between 30 to 55 years were sent a questionnaire to obtain information on their lifestyle factors and medical histories. Follow-up questionnaires were sent every 2 years, and in 1980, 1984, 1986, and every 4 years thereafter, semiquantitative food frequency questionnaires (FFQ) were added to the follow-up questionnaires. In 1996, a cooking method questionnaire containing questions on cooking methods and doneness of several meats was added to the questionnaire. The study was approved by the Human Subjects Committee of the Brigham and Women's Hospital.

Cooking method questionnaire. All questions included in the 1996 cooking method questionnaire were based on results from a pilot study (17) designed to ascertain a set of questions that would best predict the intake of HCAs in the NHS. On this cooking method questionnaire, participants were asked about frequency of intake (i.e., never, <1/month, 1/month, 2-3/month, 1/week, 2-3/week, and 4+/week) and doneness (i.e., depending on type of meat: lightly browned, medium browned, well browned, and blackened/charred) of cooked meats and fish (i.e., pan-fried, broiled, and grilled chicken; broiled fish; roast beef; pan-fried steak; grilled or barbecued steak; and homemade beef gravy). In addition, participants were also asked whether they cooked and/or ate chicken with or without skin ("when you eat chicken, how often is it cooked with the skin on: always, most of the time, sometimes, and never," and "how often do you eat the skin: always, most of the time, sometimes, and never"; for more information, also refer to ref. 18). We estimated intake of pan-fried hamburgers by multiplying the frequency of intake of hamburger from the 1994 FFQ (also see below) with the proportion of participants from the NHS in the pilot study that had reported eating pan-fried hamburgers (i.e., 0.33; ref. 17), assuming the outside appearance to be medium brown (based on the median value for doneness obtained from the pilot study). Because only limited variability with regard to doneness levels of pan-fried bacon was observed in our pilot study, we estimated intake of pan-fried bacon using bacon intake from the 1994 FFQ and assumed bacon to be prepared at high degree of doneness (i.e., well-done; ref. 17).

Assessment of diet, meat, and HCA intake. The validity and reproducibility of the FFQs administered in the NHS cohort have been reported elsewhere (19, 20). Because we did not request information on frequency of total meat intake on the 1996 cooking questionnaires (i.e., total meat intake that does not take into account specific cooking methods), we estimated total red, white, and processed meat intake using information from the 1994 to 2002 FFQs and calculated a cumulative average intake of total red, white, and processed meats. Cumulative average intake of meats represents the average intake of meats from all available FFQs up to the start of each follow-up interval (21).

For participants who had reported frequency of cooked meat intake on the cooking method questionnaire but did not report on the outside appearance of meat, we imputed the median value for doneness (i.e., lightly brown for broiled fish and medium brown for the other cooked meat items). Participants who reported eating cooked chicken but who left the "cook/eat chicken with skin" sections blank were assigned to the "chicken not cooked with skin" category.

HCAs values were calculated using an online database, the "Charred Database" created by Sinha and colleagues from the National Cancer Institute, which provides users with data on HCA and MDM from measured meat sample extracts (18, 22, 23). Briefly, the mutagenic activity of meat samples was assessed by the Ames/Salmonella test (24, 25), and MeIQx, DiMeIQx, and PhIP were determined using a method previously reported by Gross and Gruter (26). Intake of MeIQx, PhIP, DiMeIQx, and MDM was calculated by multiplying the HCA or MDM values from the Charred Database (ng/g meat for HCA and revertant colonies/g meat for MDM) with standard portion sizes from the Charred Database and frequency of intake of the relevant cooked meat item obtained from the 1996 cooking questionnaire. HCA values for roast beef were 0 for all doneness categories, and HCA values for broiled fish were not available from the Charred Database (22) and thus did not contribute to our calculations for HCAs and MDM intake. More detailed information about HCA and MDM values used in this study can be found on the Web site for the Charred Database (18, 22).

Ascertainment of breast cancer cases. Whenever a participant reported a breast cancer diagnosis during the past 2 years on a biennial follow-up questionnaire, we contacted them and requested their consent to obtain and review their medical records pertaining to this specific diagnosis. Investigators reviewed those medical records to (*a*) confirm a breast cancer diagnosis and (*b*) extract information relevant to breast cancer, including histology of breast cancer. In addition, we were able to extract information on hormone receptor status [estrogen receptor (ER) and progesterone receptor (PR)] from the pathology reports. After exclusion of ineligible participants (for exclusion criteria, see below), a total of 2,317 breast cancer cases (diagnosed between 1996 and 2006) were included in our analysis. Of the 1,779 cases with information on hormone receptor status, 1,174 were ER^+/PR^+, 295 were ER^-/PR^-, 279 were ER^+/PR^-, and 31 were ER^-/PR^+. Breast cancer cases with a carcinoma *in situ* histology were excluded from our analyses.

Exclusion criteria. Participants were ineligible for this study if they (*a*) had reported a history of any cancer (except for nonmelanoma skin cancer) before 1996, (*b*) were

premenopausal in 1996 because we had few premenopausal cases, (*c*) had calculated energy intakes of <600 or >3,500 kcal/d or had left >70 food items on the FFQs blank, and (*d*) had left the entire cooking method section on the 1996 questionnaire blank. In addition, we also excluded participants for whom HCA/MDM could not be calculated (e.g., due to missing information on bacon or hamburger intake on the 1994 FFQ or had reported information on doneness of meat but not on frequency of cooked meat intake). Thus, our study population consisted of 54,440 women who were followed between 1996 and 2006 and contributed a total of 533,618 person-years of follow-up.

Statistical analysis. For every participant, we calculated person-years of follow-up from the date of return of the 1996 follow-up questionnaire to the end of our follow-up period (June 2006), death, date of breast cancer, or other cancer diagnosis (except for nonmelanoma skin cancer), whichever occurred first. Intake of HCA and MDM as well as red meat, white meat, and processed meat intake were divided into quintiles. To assess associations between quintiles of meat mutagen intake, quintiles/categories of total and cooked meat intake and risk of breast cancer, we used a Cox proportional hazards model stratified by age and calendar year. The multivariate models were also adjusted for known and suspected risk factors for breast cancer [i.e., smoking status (never, past, current 1-14 cigarettes/day, current 15-24 cigarettes/day, current ≥25 cigarettes/day), body mass index (BMI; kg/m^2; <25, 25-<30, ≥30), height (inches; <63, 63-<64, 64-<66, ≥66), physical activity (hours per week; <1, 1-<2, 2-<4, 4-<7, ≥7), age at menarche (years; ≤12, 13, ≥14), family history of breast cancer (yes, no), history of benign breast disease (yes, no), parity and age at first birth (nulliparous, 1-2 children and age at first birth <25 y, 1-2 children and age at first birth 25-<30 y, 1-2 children and age at first birth ≥30 y, 3-4 children and age at first birth <25 y, 3-4 children and age at first birth 25-<30 y, 3-4 children and age at first birth ≥30 y, 5-8 children and age at first birth <25 y, 5-8 children and age at first birth 25-<30 y), postmenopausal hormone use (never, current, past), weight change (1996-2006; kg; <−2, −2 to +2, +2.1 to +5, +5.1 to +10, +10.1 to +20, +20.1 to +25, ≥+25.1 kg), and total energy and alcohol intake (continuous)]. Because results obtained from the age-adjusted models were similar to those from the multivariate models, only multivariate relative risks (RR) are presented.

Trend test was calculated by adding the median of each quintile or category of the exposure variable as a continuous variable to the model. Associations were also examined separately by hormone receptor status (i.e., ER$^+$/PR$^+$ and ER$^-$/PR$^-$ breast cancers) and after stratification by age (<65, ≥65 y), BMI (<25, ≥25 kg/m^2), smoking status (never, past, current), and postmenopausal hormone use (never, current, past). As there is some evidence that the mutagenic effects of HCAs may be modified by certain compounds found in fruits and vegetables such as chlorophyllin and isothiocyanates (27-29), associations were also examined after stratification by intakes of cruciferous vegetables and total fruits and vegetables (<5.25 servings/day, ≥5.25 servings/day). To obtain *P* values for interaction, we added a cross-term product consisting of the meat mutagen (medians of quintiles) and the exposure variable (as a binary variable) to the multivariate model and did a Wald test. All *P* values were two-sided.

Results

Baseline characteristics pertaining to intake of meat mutagens (lowest and highest quintile) are shown in Table 1. Participants with higher intake of meat mutagens were more likely to have a higher BMI as well as higher intake of calories, fat, alcohol, white meat, processed meat, and red meat. Furthermore, women with higher MeIQx intake seemed to be less active than those with lower MeIQx intake. Participants with high and low meat mutagen intake did not seem to differ with regard to most reproductive factors, except that a slightly higher percentage of women with higher meat mutagen intake had at least three children.

The amount of HCAs or MDM consumed by each participant varied according to the specific type of meat as well as the cooking method and the outside appearance of the cooked meat (Table 2). The top three contributors to PhIP intake were grilled chicken, grilled steak, and broiled chicken. Pan-fried hamburgers, pan-fried bacon, and pan-fried steak contributed most to MeIQx intake, and grilled chicken, pan-fried hamburgers, and pan-fried bacon contributed most to DiMeIQx intake. On the other hand, broiled and grilled chicken as well as grilled steak contributed most to MDM intake. Correlations between each individual HCAs were r = 0.60 (MeIQx versus PhIP), r = 0.83 (DiMeIQx versus MeIQx), and r = 0.68 (DiMeIQx versus PhIP).

Higher intakes of red, white, and processed meats, HCAs, and MDM were not associated with increased risk of total breast cancer [multivariate RR and 95% confidence interval (95% CI) for the highest versus lowest quintile: MeIQx: 0.90 (0.79-1.03); PhIP: 0.92 (0.80-1.05); DiMeIQx: 0.92 (0.80-1.05); and MDM: 0.98 (0.85-1.12); Table 3]. When we examined associations between meat mutagens and breast cancer separately by hormone receptor status (Table 4), intakes of HCAs and MDM were not associated with risk of ER$^+$/PR$^+$ breast cancer. There was some suggestion of a lower risk of ER$^-$/PR$^-$ breast cancer with higher intakes of MeIQx, DiMeIQx, and PhIP, but none of the associations were statistically significant [RR and 95% CI for the highest versus lowest quintile: MeIQx: 0.79 (0.54-1.15), P_{trend} = 0.23; PhIP: 0.73 (0.50-1.08), P_{trend} = 0.12; DiMeIQx: RR = 0.74 (0.50-1.10), P_{trend} = 0.06]. MDM intake was not associated with risk of ER$^-$/PR$^-$ breast cancer. Intakes of total red, white, and processed meat were not significantly associated with risk of breast cancer regardless of hormone receptor status.

Table 1. Baseline characteristics in the NHS study population by lowest and highest quintiles of HCA and MDM activity intake

	PhIP (ng/d)		MeIQx (ng/d)		DiMeIQx (ng/d)		MDM activity (revertant colonies/day)	
	Q1 (14.3)	Q5 (201)	Q1 (3.4)	Q5 (39.1)	Q1 (0.27)	Q5 (5.5)	Q1 (834)	Q5 (7372)
Mean age (y)	64.7	61.3	63.4	62.8	64.0	62.3	64.2	62.1
Mean pack-years smoking among smokers	24.0	25.9	22.1	29.0	22.9	27.6	23.5	26.8
Mean age at first birth	22.8	22.8	23.0	22.8	23.0	22.9	22.9	22.8
Age at menarche <12 y (%)	49.1	49.2	50.0	47.8	50.0	48.4	49.4	49.0
Parity, ≥3 children (%)	58.5	61.8	57.5	62.7	57.1	62.7	58.6	61.1
Height (in)	64.5	64.5	64.5	64.5	64.5	64.5	64.5	64.5
BMI (kg/m^2)	25.7	27.1	25.3	27.4	25.5	27.2	25.5	27.1
Physical activity (MET)	18.8	18.3	20.9	15.9	20.1	17.0	19.2	17.8
Family history of breast cancer in mother/sisters (%)	14.8	14.2	15.1	14.0	15.0	14.4	15.0	14.4
History of benign breast disease (%)	31.4	30.7	33.2	29.3	32.7	29.0	32.1	30.1
Mean daily intake (94 FFQ)*								
Calories (kcal)	1,602	1,813	1,526	1,911	1,541	1,858	1,557	1,845
Total fat (g)	48.4	62.0	42.4	71.5	44.0	67.0	46.3	64.4
Total iron (mg)	20.5	20.7	20.8	20.6	20.6	20.4	20.1	20.8
Folate (μg)	488	485	505	466	501	472	486	483
Alcohol (g)	3.8	6.1	4.2	5.4	4.2	5.5	4.3	5.3
Processed meat (serving/day)	0.07	0.10	0.04	0.14	0.05	0.12	0.06	0.11
Chicken + turkey (serving/day)	0.32	0.44	0.36	0.38	0.36	0.40	0.31	0.43
Red meat (serving/day)	0.11	0.18	0.05	0.27	0.06	0.24	0.08	0.21

NOTE: Standardized for age in 1996 except for age and age at first birth: Q1, lowest quintile; Q5, highest quintile. Number in parenthesis denote median value in each quintile (ng/d for HCAs and revertant colonies/day for MDM activity). If not noted otherwise, numbers denote mean values.

Abbreviation: MET, metabolic equivalent hours per week.

*Mean daily intakes of nutrients are energy adjusted.

We also investigated whether intakes of cooked meats that contributed to intake of HCAs (from the 1996 cooking method questionnaire) were associated with total breast cancer risk. In general, intakes of cooked meats and gravy (pan-fried, grilled chicken, broiled fish, roast beef, pan-fried steak, grilled or barbecued steak, and homemade beef gravy) did not seem to be associated with total breast cancer (data not shown). However, there was some evidence for a slightly and marginally increased risk of total breast cancer with higher intake of broiled chicken [highest versus lowest category: RR = 1.13 (95% CI = 0.97-1.31); P_{trend} = 0.05] and some evidence for a nonsignificant inverse association between higher intake of barbequed/grilled steak and breast cancer [highest versus lowest category: RR = 0.84 (95% CI = 0.69-1.02); P_{trend} = 0.12].

As there is some evidence that the mutagenic effects of HCAs may be modified by certain compounds found in fruits and vegetables such as chlorophyllin and isothiocyanates (27-29), we examined associations after stratification by intake of cruciferous vegetables. However, we found little evidence for interaction between intake of cruciferous vegetables and HCA or MDM intake and risk of total breast cancer (data not shown). When we examined associations between meat mutagens and breast cancer risk by fruit and vegetable intake (Table 5), there was some suggestion of an inverse association between MDM intake and total breast cancer among those with high fruit/vegetable intake, whereas participants in the low fruit/vegetable category seemed to have a slightly increased risk of total breast cancer; however, none of the observed associations reached statistical significance ($P_{interaction}$ = 0.07; Table 5). We also examined associations after stratification by smoking status (never, past, current) and postmenopausal hormone use (never, past, current). Greater intake of MeIQx but not PhIP, DiMeIQx, or MDM intake was associated with a significantly decreased risk of total breast cancer among current smokers and current postmenopausal hormone users (highest versus lowest quintile: current smokers:

RR, 0.59; 95% CI, 0.36-0.99), current hormone users (RR, 0.79; 95% CI, 0.64-0.97), whereas no associations were seen for past/never smokers or past/never hormone users (data not shown). Associations between HCAs and MDM and total breast cancer were not modified by age, BMI, or physical activity. Furthermore, associations between HCA/MDM intake and total breast cancer were similar to those reported in Table 3 when cases diagnosed within the first 2 years of follow-up were excluded.

Discussion

Results from this large prospective cohort study do not support a positive association between higher intakes of meat mutagens and risk of breast cancer among postmenopausal women. There was some suggestion of a lower risk of ER^-/PR^- breast cancer with higher intakes of HCAs, but none of the associations were statistically significant. Data from animal studies have long suggested an enhancing role of HCAs in mammary carcinogenesis (2, 3). In addition to their mutagenic and carcinogenic properties (30), recent animal data also suggest that PhIP, the most abundant HCA in human diet, may also possess estrogenic activity (31) and can promote the secretion of prolactin (32). Endogenous hormones, especially estrogen and progesterone, and more recently also prolactin have been hypothesized to play a role in breast carcinogenesis, and findings from both epidemiologic as well as animal studies have provided compelling support for this hypothesis (33).

However, only a limited number of epidemiologic studies, including three recent prospective studies, have examined the associations between HCA intake and breast cancer risk (5, 7-13).

Consistent with our results, two of the three recent prospective studies have found no evidence for an association between HCA intake and risk of postmenopausal breast cancer (10, 11). The American Association of Retired Persons cohort using the Charred Database (11) included 3,818 breast cancer with 8 years of follow-up. No associations between red, white, processed meat intake, as well as meat cooked at high temperature, HCA, and total mutagenic activity and risk of breast cancer were found. Results were similar after examining associations separately by hormone receptor status or by fruit and vegetable intake. No evidence for an association between HCAs and risk of breast cancer was found in a Swedish study, including 430 cases with a mean follow-up of 10.4 years. That study used a HCA database developed in Sweden (10). Contrary to the two studies and our study, another recent study (13) using data from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial found some evidence for a statistically significant increased risk with higher intake of red meat and MeIQx intake and some suggestion of a nonsignificantly increased risk with higher DiMeIQx intake (13). In addition, the positive associations between red meat and breast cancer were more pronounced for ER^+/PR^+ breast cancer. This study also used the Charred Database (22) to calculate HCA intake.

Findings from case-control studies are inconsistent, and contrary to the hypothesized mechanisms, some case-control studies even observed inverse associations between breast cancer and some individual HCAs. For example, one study with 114 cases with benign breast

Table 2. Mean intake of HCA and MDM by meat type in women in the NHS

	PhIP		MeIQx		DiMeIQx		MDM activity	
	Mean (SD), ng/d	Percentage (%)	Mean (SD), ng/d	Percentage (%)	Mean (SD), ng/d	Percentage (%)	Mean (SD), revertants/day	Percentage (%)
All sources	92.1 (94.5)	100	18.5 (19.3)	100	2.4 (2.7)	100	3,604 (3,355)	100
Pan-fried chicken	6.1 (14.7)	6.6	0.8 (1.9)	4.5	0.02 (0.10)	1.0	473 (1,170)	13.0
Broiled chicken	17.8 (30.7)	19.3	0.4 (0.7)	1.9	0.003 (0.11)	0.1	764 (1,347)	21.2
Grilled chicken	37.0 (62.5)	40.2	1.0 (2.0)	5.5	0.84 (1.74)	34.4	687 (1,096)	19.1
Grilled steak	21.2 (37.8)	23.0	2.6 (4.2)	13.9	0	0	476 (990)	13.2
Pan-fried steak	5.0 (17.3)	5.4	3.7 (10.9)	19.8	0.32 (1.14)	12.9	372 (1,746)	10.3
Homemade beef gravy	0.84 (3.4)	0.9	2.0 (5.8)	10.8	0.22 (0.90)	9.0	214 (668)	5.9
Pan-fried bacon	4.2 (8.6)	4.6	3.8 (7.7)	20.5	0.44 (0.90)	18.1	234 (477)	6.5
Pan-fried hamburger	0	0	4.3 (4.2)	23.0	0.60 (0.59)	24.5	385 (381)	10.7

Table 3. RR of total breast cancer by quintiles of HCA, MDM, and meat intake in women in the NHS (1996-2006)

	Quintile HCA intake (ng/d)/mutagenic activity (revertants/day)					
	1	2	3	4	5	P_{trend}
Total breast cancer						
Total red meat (1994-2002 FFQ)						
Cases/person-years	545/120,309	368/85,940	484/115,049	472/107,388	448/104,932	
Multivariate RR*	1	0.94 (0.82-1.08)	0.94 (0.83-1.06)	0.99 (0.87-1.13)	0.95 (0.83-1.09)	0.70
Total white meat (1994-2002 FFQ)						
Cases/person-years	597/133,118	330/74,700	484/109,534	474/113,775	432/102,491	
Multivariate RR*	1	1.04 (0.90-1.19)	0.99 (0.87-1.12)	0.93 (0.82-1.05)	0.96 (0.84-1.10)	0.25
Processed meat (1994-2002 FFQ)						
Cases/person-years	478/102,900	474/106,129	424/101,376	451/113,297	490/108,916	
Multivariate RR*	1	0.99 (0.87-1.12)	0.94 (0.82-1.07)	0.88 (0.77-1.01)	0.98 (0.86-1.12)	0.74
Total MeIQx						
Cases/person-years	491/106,844	469/106,911	443/106,717	462/106,677	452/106,469	
Multivariate RR*	1	0.95 (0.83-1.08)	0.90 (0.79-1.02)	0.94 (0.82-1.07)	0.90 (0.79-1.03)	0.23
Total PhIP						
Cases/person-years	482/106,864	475/106,622	459/106,735	475/106,712	426/106,686	
Multivariate RR*	1	1.00 (0.88-1.14)	0.97 (0.85-1.10)	1.00 (0.88-1.14)	0.92 (0.80-1.05)	0.20
Total DiMeIQx						
Cases/person-years	479/107,220	487/106,420	457/106,712	453/106,331	441/106,935	
Multivariate RR*	1	1.01 (0.89-1.14)	0.97 (0.85-1.10)	0.95 (0.83-1.09)	0.92 (0.80-1.05)	0.16
MDM activity						
Cases/person-years	459/106,823	494/106,745	460/106,719	457/106,649	447/106,682	
Multivariate RR*	1	1.08 (0.95-1.23)	1.01 (0.88-1.15)	1.01 (0.88-1.15)	0.98 (0.85-1.12)	0.38

NOTE: Numbers in parenthesis next to RR are 95% CIs.

*Multivariate models adjusted for age in months (continuous), smoking status (never, past, current 1-14 cigarettes/day, current 15-24 cigarettes/day, current ≥25 cigarettes/day), BMI (kg/m^2: <25, 25-<30, ≥30), height (inches; <63, 63-<64, 64-<66, ≥66), physical activity (hours per week; <1, 1-<2, 2-<4, 4-<7, ≥7), age at menarche (years; ≤12, 13, ≥14), family history of breast cancer (yes, no), history of benign breast disease (yes, no), parity and age at first birth (nulliparous, 1-2 children and age at first birth <25 y, 1-2 children and age at first birth 25-<30 y, 1-2 children and age at first birth ≥30 y, 3-4 children and age at first birth <25 y, 3-4 children and age at first birth 25-<30 y, 3-4 children and age at first birth ≥30 y, 5-8 children and age at first birth <25 y, 5-8 children and age at first birth 25-<30 y), postmenopausal hormone use (never, current, past), weight change (1996-2006; <–2 kg; –2 to +2, +2.1 to +5, +5.1 to +10, +10.1 to +20, +20.1 to +25, ≥+25.1), and total calorie and alcohol intake (continuous).

disease found inverse association between higher PhIP intake, and risk of breast cancer, which the authors attributed to higher white meat intake, which was also inversely associated with breast cancer risk (8). In that study MeIQx or DiMeIQx intake was not associated with breast cancer risk (8). On the contrary, in another case-control study of 273 cases from the Iowa Women's Health Study, a large cohort of postmenopausal women, PhIP, but not MeIQx and DiMeIQx, was associated with increased risk of breast cancer (9). In another study by Steck et al. (5), recent HCA intake was not associated with risk of postmenopausal breast cancer, but higher intake of MeIQx and DiMeIQx was associated with an ~40% decreased risk of premenopausal breast cancer. In addition, higher lifetime intakes of grilled and smoked meats were also associated with increased risk of postmenopausal breast cancer especially among those with low fruit and vegetable intake but were not associated with premenopausal breast cancer risk. In summary, whereas case-control studies show inconsistent results (5, 8, 9), two of the three prospective studies conducted thus far have found no evidence for an association between HCA and/or MDM intake and breast cancer (10, 11). In the third prospective study, there was some evidence for a statistically significant increased risk with higher intake of MeIQx intake and some suggestion of a nonsignificantly increased risk with higher DiMeIQx intake (13).

Contrary to the hypothesized mechanisms, we found some suggestion of a lower risk of ER$^-$/PR$^-$ breast cancer with higher intakes of DiMeIQx and PhIP, but none of the associations were statistically significant. Reasons for these findings are unclear. Besides chance, one possible reason could be that higher HCA intake may be a marker

Table 4. RR of breast cancer by combinations of receptor status and quintiles of HCA, MDM, and meat intake in women in the NHS (1996-2006)

	Quintile HCA intake (ng/d)/mutagenic activity (revertants/day)					
	1	2	3	4	5	P_{trend}
ER^+/PR^+						
Total red meat (1994-2002 FFQ)						
Cases	281	174	244	237	238	
Multivariate RR	1	0.89 (0.73-1.08)	0.91 (0.76-1.08)	0.92 (0.77-1.10)	0.91 (0.75-1.10)	0.46
Total white meat (1994-2002 FFQ)						
Cases	295	168	256	233	222	
Multivariate RR	1	1.22 (1.01-1.49)	1.08 (0.91-1.28)	0.90 (0.76-1.08)	0.97 (0.81-1.17)	0.15
Processed meat (1994-2002 FFQ)						
Cases/person-years	237	239	194	237	267	
Multivariate RR*	1	1.03 (0.86-1.24)	0.90 (0.74-1.09)	0.94 (0.78-1.13)	1.06 (0.87-1.28)	0.54
Total MeIQx						
Cases	242	219	226	244	243	
Multivariate RR*	1	0.88 (0.73-1.06)	0.89 (0.74-1.08)	0.97 (0.81-1.17)	0.93 (0.77-1.12)	0.88
Total PhIP						
Cases	247	223	228	252	224	
Multivariate RR*	1	0.90 (0.75-1.08)	0.92 (0.76-1.10)	1.01 (0.84-1.21)	0.89 (0.74-1.08)	0.58
Total DiMeIQx						
Cases	242	211	234	238	249	
Multivariate RR*	1	0.84 (0.70-1.01)	0.94 (0.79-1.13)	0.96 (0.80-1.15)	0.98 (0.82-1.18)	0.56
MDM activity						
Cases	221	235	244	247	227	
Multivariate RR*	1	1.05 (0.87-1.26)	1.09 (0.91-1.31)	1.10 (0.91-1.33)	0.99 (0.82-1.20)	0.77
ER^-/PR^-						
Total red meat (1994-2002 FFQ)						
Cases	68	46	62	57	62	
Multivariate RR	1	0.99 (0.68-1.45)	1.00 (0.71-1.43)	1.02 (0.71-1.47)	1.05 (0.72-1.53)	0.79
Total white meat (1994-2002 FFQ)						
Cases	85	31	59	66	54	
Multivariate RR	1	0.77 (0.50-1.17)	0.93 (0.66-1.31)	0.86 (0.62-1.20)	0.88 (0.61-1.26)	0.53
Processed meat (1994-2002 FFQ)						
Cases	65	56	60	52	62	
Multivariate RR*	1	0.86 (0.60-1.24)	0.99 (0.69-1.42)	0.77 (0.53-1.12)	0.90 (0.61-1.31)	0.61
Total MeIQx						
Cases	67	63	56	54	55	
Multivariate RR*	1	0.91 (0.64-1.29)	0.83 (0.58-1.19)	0.78 (0.54-1.13)	0.79 (0.54-1.15)	0.23
Total PhIP						
Cases	69	68	51	61	46	
Multivariate RR*	1	1.02 (0.73-1.43)	0.76 (0.52-1.09)	0.94 (0.66-1.34)	0.73 (0.50-1.08)	0.12
Total DiMeIQx						
Cases	62	67	63	58	45	
Multivariate RR*	1	1.06 (0.75-1.51)	1.01 (0.71-1.45)	0.92 (0.64-1.33)	0.74 (0.50-1.10)	0.06
MDM activity						
Cases	62	61	60	55	57	
Multivariate RR*	1	0.97 (0.68-1.39)	0.96 (0.67-1.38)	0.91 (0.63-1.32)	0.93 (0.64-1.36)	0.70

NOTE: Numbers in parenthesis next to RR are 95% CIs.
*Multivariate models adjusted for same covariates as denoted in Table 3.

Table 5. RR of total breast cancer by HCA and MDM intake and fruit and vegetable intake in women in the NHS (1996-2006)

	Low fruit/vegetable (<5.25 serving/day)	High fruit/vegetable (≥5.25 serving/day)	$P_{interaction}$
Total MeIQx			
Cases (lowest/highest quintile)	167/199	257/189	
Multivariate RR*	1.08 (0.87-1.35)	0.81 (0.66-1.00)	0.12
Total PhIP			
Cases (lowest/highest quintile)	181/153	244/197	
Multivariate RR*	1.03 (0.82-1.29)	0.77 (0.63-0.93)	0.45
Total DiMeIQx			
Cases (lowest/highest quintile)	176/173	235/200	
Multivariate RR*	0.95 (0.76-1.18)	0.92 (0.76-1.12)	0.86
MDM activity			
Cases (lowest/highest quintile)	180/177	224/205	
Multivariate RR*	1.16 (0.94-1.44)	0.85 (0.70-1.03)	0.07

NOTE: Numbers in parenthesis next to RR are 95% CIs.
*Multivariate models adjusted for same covariates as denoted in Table 3.

for higher intake of white meat, as PhIP levels in cooked chicken are quite high (www.charred.cancer.gov); however, in our study, neither overall white meat intake nor PhIP from white meat was associated with ER^-/PR^- breast cancer.

One limitation of our study is that we did not examine genetic polymorphisms of enzymes involved in metabolism of HCAs. HCAs are not mutagenic compounds per se but need to undergo metabolization to exert their potential mutagenic effects. On the other hand, HCAs can also be deactivated via detoxification pathways (34, 35). Xenobiotic metabolic enzymes involved in activation or deactivation of HCAs include cytochrome P450, *N*-acetyltransferase (NAT), sulfotransferase, and glutathione *S*-transferase (34, 35). Fruits and vegetables containing isothiocyanates, which can induce phase I and II metabolism enzymes, may also affect metabolism of HCAs and modify the effect of HCAs (28, 29). In human feeding studies, substantial interindividual variation with regard to urinary excretion of metabolites of MeIQx and PhIP has been observed, indicating considerable interindividual variation with regard to the metabolism of HCAs (36, 37). Intra-individual variation with regard to genetic polymorphisms of xenobiotic metabolic enzymes may in part explain the lack of positive associations between HCA and MDM intake and risk of breast cancer in our study. However, thus far, findings from epidemiologic studies that have assessed possible interactions between NAT1 and NAT2 genotypes and well-done meats and/or HCA intake with regard to breast cancer risk are inconsistent (8, 12, 38, 39).

There are also some other limitations inherent to our study design. First, we cannot rule out misclassification of exposure, as we do not know how well HCA intake estimated from our cooking method questionnaires correlates with true exposure of these mutagens at the breast tissue levels. In addition, estimation of HCA intake was based on a limited number of cooking method questions. However, the aforementioned set of questions was developed based on findings from a previous pilot study conducted to establish the group of questions that could optimally predict the intake of HCAs in the NHS (17). Furthermore, we did not take into account possible modifications to cooking methods that may affect the actual amount of HCAs ingested. For example, flipping meat during the cooking process (40), microwaving (41), or marinating (42) meat before cooking have been shown to modify the amount of HCAs produced. Secondly, exposure was assessed at one time point only (i.e., in 1996), which may not reflect changes in cooking methods over time. Thirdly, participants in this study were only followed for up to 10 years, which may not be long enough to see an effect of HCAs on breast cancer risk. Fourthly, we did not examine premenopausal breast cancer as an outcome. In a recent study using data from the NHS II, a large cohort of younger female nurses residing in the United States, higher red meat intake was significantly associated with elevated risk of ER^+/PR^+ premenopausal breast cancer (14). These findings suggest that red meat intake as a risk factor may differ by either menopausal and/or hormone receptor status or exposure earlier in life may be the more relevant exposure. Supporting the latter hypothesis are studies that have shown that breast cancer initiation may occur earlier in life (43-45). In a recent study from the NHS II, higher red meat intake during adolescence estimated using information from a validated high school FFQ was significantly associated with higher risk of premenopausal

breast cancer, and results were similar after adjusting for meat intake during adulthood (46). Therefore, assessment of HCA intake in later stages of adulthood may not represent the relevant exposure, which may in part explain the null associations between HCA intake and postmenopausal breast cancer observed in our and two other prospective studies (10, 11).

In conclusion, in this large cohort study of postmenopausal women, higher consumption of mutagens from meats cooked at higher temperature and longer duration was not associated with increased breast cancer risk. Our findings about HCAs and ER^-/PR^- breast cancer warrant further evaluation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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