Breast Cancer Research and Treatment (2022) 193:187–201
https://doi.org/10.1007/s10549-022-06555-x

The association between meat and fish intake by preparation methods and breast cancer in the Carolina Breast Cancer Study (CBCS)

Omonefe O. Omofuma[1] · Susan E. Steck[1,3] · Andrew F. Olshan[2] · Melissa A. Troester[2]

Received: 15 November 2021 / Accepted: 22 February 2022 / Published online: 11 March 2022

Abstract

Purpose We examined the associations between intake of meat and fish by preparation methods and breast cancer in the Carolina Breast Cancer Study, a racially diverse population-based case–control study.

Methods African American (AA) and European American (EA) women aged 20–74 years with a first diagnosis of invasive or in situ breast cancers were frequency matched by race and age group to controls identified through the North Carolina Division of Motor Vehicles and Medicare lists [AA: 548 cases, 452 controls; EA: 858 cases, 748 controls]. Participants self-reported meat preparation methods and intake frequencies. Adjusted odds ratios (OR) and 95% confidence intervals (CIs) were calculated using multivariable logistic regression adjusted for age, race, alcohol intake, body mass index, family income, lactation, marital status, use of oral contraceptives, postmenopausal hormone use, smoking status, and offsets.

Results Positive associations with breast cancer were observed for intakes of grilled/barbecued hamburger (≥ once/week, OR: 1.28; 95% CI 1.01, 1.63), and pan-fried/oven-broiled beef steak (≥ once/week, OR: 1.36; 95% CI 1.08, 1.72). Inverse associations were observed for pan-fried fish (≥ once/week, OR: 0.77; 95% CI 0.60, 0.98), and for grilled/ barbecued pork chops (> 0 time/week OR: 0.81, 95% CI 0.68, 0.97). Associations tended to be stronger among EA women than among AA women.

Conclusion More frequent consumption of beef prepared with high temperature methods was associated with higher odds of breast cancer while more frequent consumption of pan-fried fish or grilled/barbecued pork chops was associated with lower odds of breast cancer.

Keywords Meat · Fish · Breast cancer · Case–control study · Diet

Abbreviations

AA	African American
BMI	Body mass index
CBCS	Carolina Breast Cancer Study
CI	Confidence interval
CIS	Carcinoma in situ
DAG	Directed acyclic graph
DCIS	Ductal carcinoma in situ
EA	European American
ER	Estrogen receptor
HCA	Heterocyclic amines
HER	Human epidermal growth factor receptor
IHC	Immunohistochemistry
LCIS	Lobular carcinoma in situ
NOCs	*N*-Nitroso compounds
OR	Odds ratio
PAH	Polycyclic aromatic hydrocarbons
PR	Progesterone receptor
PUFA	Polyunsaturated fatty acid

✉ Susan E. Steck
stecks@mailbox.sc.edu

[1] Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, SC, USA

[2] Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[3] Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, 915 Greene Street, Room 456, Discovery I Building, Columbia, SC 29208, USA

Introduction

Breast cancer is the most common cancer and the second leading cause of cancer deaths among women in the USA [1]. Several dietary factors including red meat are suggested to contribute to the risk of breast cancer [2, 3]. Processed meat may contain *N*-nitroso compounds (NOCs) and preparing meat at high temperatures such as grilling or pan-frying could increase the production of heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs), which are implicated in breast carcinogenesis [4–6]. In addition, advanced glycation end-products formed from non-enzymatic reaction of sugars and proteins or lipids have been implicated in oxidative stress and inflammation [7], and high levels have been detected in food cooked at high temperatures, particularly meat and animal products [8] and associated with increased risk of breast cancer [9, 10].

Several studies have reported an increase in breast cancer risk with high intake of red meat [3, 11, 12] especially meat prepared at high degrees of temperature or doneness [2, 13–15]. However, not all findings have been supportive [16, 17]. A meta-analysis from 2018 combining data from up to 15 prospective studies found increased breast cancer risk with consumption of processed meat and a nonsignificant increased risk for consumption of red meat [18]. AA women were reported to have higher total meat intake compared to EA women [19]. Studies on the association between red meat and poultry and breast cancer stratified by race have produced mixed results [20] but few studies have enrolled enough AA women to precisely examine these associations. Data on relationships by breast cancer hormone receptor status are also limited but some studies show differential associations by estrogen receptor (ER)/progesterone receptor (PR) status [20, 21]. In this study, we examined associations between meat and fish cooked by various preparation methods and risk of breast cancer among AA and EA North Carolina women enrolled in the population-based Carolina Breast Cancer Study (CBCS). We further examined whether associations differed by ER status and menopausal status.

Materials and methods

Study population

The CBCS is a population-based case–control study of women diagnosed with breast cancer across 24 counties in central and eastern North Carolina. Women aged between 20 and 74 years and diagnosed with invasive breast cancer between 1993 and 2001 were enrolled in Phases 1 & 2 and carcinoma in situ (CIS) cases diagnosed between 1996 and 2001 were enrolled in Phase 2 [22]. AAs and younger women aged less than 50 years were oversampled [23, 24]. Controls were selected from the North Carolina Division of Motor Vehicles (for women younger than 65 years) or selected from the US Health Care Financing Administration (for women aged 65 and older) and were approximately frequency matched to cases by age and race [22].

Data collection

Questionnaires were administered to subjects in the home by a trained registered nurse. The median time between diagnosis and data collection by the registered nurse for cases was 3 months (ranged between 1 and 19 months; 80% interviewed by 5 months). For controls, the median time from enrollment to interview was 2 months (ranged between 0 and 26 months; 80% interviewed by 5 months). The questionnaire requested information on demographic data, reproductive and family history of cancer and dietary practices. In the section on dietary intake, information was acquired on intake levels of eight meat and fish items five years prior to the interview.

Outcome assessment

Cases were identified using the North Carolina Central Cancer Registry's rapid case ascertainment system. Invasive and CIS cases were enrolled. CIS included women with ductal carcinoma in situ (DCIS), DCIS with micro invasion to a depth of 2 mm, lobular CIS (LCIS) and mixed DCIS and LCIS. Immunohistochemistry (IHC) profiles were created by microarray analysis and IHC staining for ER, human epidermal growth factor receptors-1 & -2 (HER2 and HER1), and cytokeratin 5/6 [25]. Cases were further classified based on ER status as ER-positive or ER-negative.

Exposure assessment

The categories of meat included chicken; hamburger; beef steak; pork chops; bacon; breakfast sausage; and hot dog or other sausage. Fish intake was queried as fish steak or fish prepared whole. Preparation methods included "pan-fried or oven-broiled" and "grilled or barbecued" for beef and pork products; and "pan-fried," "oven-broiled," and "grilled or barbecued" for chicken and fish. Frequency of intake by meat and fish type and preparation method were reported as times per week, month, or year and converted to times per week. Missing values for frequency of intake of meat and fish cooked were imputed based on the most frequent responses for observations with similar information on age group, race, and breast cancer status. Preference for level of

doneness on the inside of meat such as beef steak, roast, or hamburger was categorized as NA/rare to medium rare (red or dark pink); medium to medium well (light pink); or well-done (gray-brown with juice or dry) while doneness on the outside of meat was categorized as NA/not browned/lightly browned; well-browned; or heavily browned or charred.

Statistical analyses

There were 4333 women in the CBCS dataset, of which 4267 self-identified as AA or EA. Women enrolled in Phase 1 ($n = 1651$) did not complete the meat intake questionnaire and were excluded from analyses. Women in Phase 2 who were missing meat intake information ($n = 10$) also were excluded (Fig. 1). Multivariable logistic regression was used to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer by frequency of intake of meat and fish cooked by various preparation methods. Offset terms derived from sampling probabilities, age at enrollment (continuous), and self-identified race (AA or EA) were included for adjustment in all models. Important potential confounders identified through a literature review on risk factors for breast cancer included alcohol intake, reproductive factors (age at menarche, parity, lactation, use of oral contraceptives, post-menopausal status), post-menopausal hormone use, family history of breast cancer, current body mass index (BMI as weight(kg)/height(m)^2), BMI at 18 years, smoking, marital status, and family income. Because a complete dietary assessment method was not utilized in the study, we were unable to adjust for energy intake and considered BMI as a potential surrogate for energy intake in the multivariable analyses. Directed acyclic graphs (DAG) were used to identify a minimally sufficient set of confounders for adjustment which included alcohol intake (yes or no/missing); BMI, kg/m^2 [missing, underweight (< 18.5), normal weight (18.5–24.9), overweight (25–29.9) or obese (≥ 30)]; family income (missing, $<$ \$15,000, \$15,000–\$30,000, \$30,000–\$50,000 or $>$ \$50,000); ever lactated (yes or no); marital status (never married/lived as married/missing, married/living as married, widowed or separated/divorced/no longer living as married); ever used oral contraceptives (never/missing or ever); post-menopausal hormone use (never/missing, past, current); and smoking status (never, former, or current). Further adjustments were made for fruit and vegetable intake during the summer and winter seasons of the previous year [low (< 19 half cup-sized servings/week), moderate (19–31 half cup-sized servings/week), or high (≥ 32 half cup-sized servings/week)] and regular physical activity in the previous three months (yes or no).

The results presented include analyses on the whole study population, as well as with stratification by race, menopausal status, invasiveness of disease, and fruit and vegetable intake. Interaction was assessed by including a cross-product of the meat or fish variable with race, menopausal status, or fruit and vegetable intake, and p-values for the multiplicative interaction term were reported. Multinomial logistic regression was used to calculate adjusted ORs for the associations of the frequency of intake of meat and fished cooked by various preparation methods and breast cancer by ER status. In sensitivity analyses, we mutually adjusted for other meat and fish intake variables for those dietary variables that showed

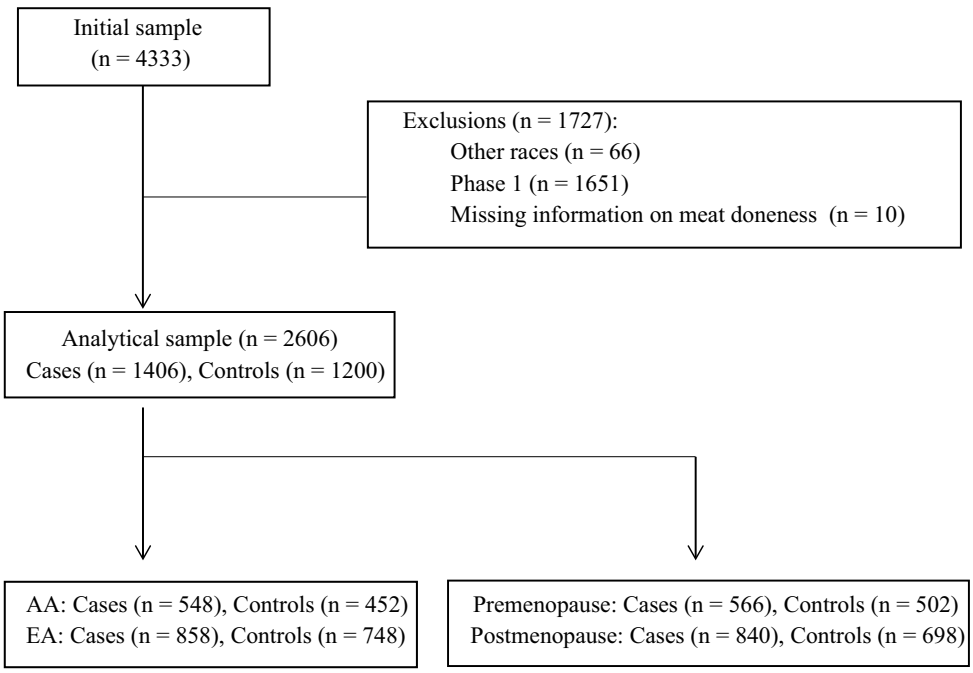

Fig. 1 Flow chart of study participants in the Carolina Breast Cancer Study

significant associations in the main analyses. All statistical analyses were performed using SAS version 9.4.

Results

The analytical sample consisted of 1406 cases and 1200 controls (Fig. 1). Women diagnosed with breast cancer appeared to have similar baseline and demographic characteristics with the control group (Table 1). Associations with breast cancer were estimated using the lowest level of no reported intake of each specific meat or fish as the referent. After adjusting for DAG-identified confounders, statistically significant ($p < 0.05$) positive associations were found for the intakes of grilled/barbecued hamburger (one or more times per week, OR: 1.28; 95% CI 1.01, 1.63) and pan-fried/oven-broiled beef steak (one or more times per week, OR: 1.36; 95% CI 1.08, 1.72) (Table 2). Significant inverse associations were observed for pan-fried fish consumed one or more times per week (OR: 0.77; 95% CI 0.60, 0.98) and grilled/barbecued pork chops consumed more than 0 times per week (OR: 0.81; 95% CI 0.68, 0.97) (Table 2). There were no statistically significant associations for the other types of meat and cooking methods. The associations did not change after further adjustment for physical activity or fruit and vegetable intake (data not shown).

In the race-stratified adjusted analyses, pan-fried fish (one or more times per week, OR: 0.63; 95% CI 0.44, 0.89), grilled/barbecued beef steak (less than once per week, OR: 0.76; 95% CI 0.59, 0.98), and grilled/barbecued pork chops (more than 0 times per week, OR: 0.75; 95% CI 0.60, 0.94) were inversely associated with breast cancer in EA women (Table 3). Also, among EA women, consuming oven-broiled chicken once per week was associated with reduced odds of breast cancer (OR: 0.64; 95% CI 0.44, 0.94), while there was no association for higher intake of greater than once per week (OR: 1.25; 95% CI 0.75, 2.09), compared to no intake. Some modestly elevated or reduced ORs were observed among AA women but were more imprecise. An increased odds for breast cancer was found for AA women who preferred meat (beef steak, roast, or hamburger) cooked medium to medium well on the inside (OR: 2.77, 95% CI 1.49, 5.14) and well done on the inside (OR: 1.78, 95% CI 1.03, 3.08), when compared to AA women with preference for rare to medium rare doneness. Overall, associations appeared to be stronger among EA women but showed inconsistency within beef and fish categories depending upon cooking method and all diet x race interaction p-values were ≥ 0.05.

Among post-menopausal women, grilled/barbecued hamburger intake (one or more times per week, OR: 1.46; 95% CI 1.05, 2.03) was positively associated with breast cancer in adjusted analyses (Table 4). Pan-fried/oven-broiled beef steak was positively associated with breast cancer among pre-menopausal women (one or more times per week, OR: 1.56; 95% CI 1.08, 2.26). In post-menopausal women, reduced odds of breast cancer were observed for intakes of pan-fried fish (one or more times per week, OR: 0.72; 95% CI 0.52, 0.98), grilled/barbecued fish (one or more times per week, OR: 0.52; 95% CI 0.27, 0.99; p for interaction by menopausal status = 0.01), and for grilled/barbecued pork chops (more than 0 times per week, OR: 0.71, 95% CI 0.56, 0.90). A significant interaction ($p = 0.02$) between menopausal status and pan-fried/oven-broiled hamburger was observed whereby associations were inverse for pre-menopausal women and positive for post-menopausal women.

When the outcome was restricted to invasive breast cancer (Supplemental Table 1), preference for meat cooked medium to medium well on the inside was associated with increased odds of invasive breast cancer (OR: 1.39; 95% CI 1.02, 1.89) as compared to preference for rare to medium rare doneness. The associations were also assessed by breast cancer ER status as shown in Supplemental Table 2. Compared to no reported meat intake, positive associations for intakes of pan-fried/oven-broiled beef steak were observed for both ER-positive breast cancer (one or more times per week, OR: 1.33; 95% CI 1.01, 1.76) and ER-negative breast cancer (OR: 1.46; 95% CI 1.07, 1.98). An inverse association was also observed for consuming pan-fried fish one or more times per week in ER-positive breast cancer (OR: 0.73; 95% CI 0.55, 0.98) and in ER-negative breast cancer (OR: 0.77; 95% CI 0.55, 1.08) though the confidence interval included the null value. Oven-broiled chicken (less than once per week, OR: 0.69; 95% CI 0.50, 0.94) and grilled/barbecued pork chops (more than 0 times per week, OR: 0.75, 95% CI 0.59, 0.95) were inversely associated with ER-negative breast cancer.

The results were unchanged in sensitivity analyses, additional adjustment was done for meat and fish preparation methods showing significant associations with breast cancer (Supplemental Table 3). The associations were also stratified by fruit and vegetable intake (Supplemental Table 4) and the positive associations observed for pan-fried/oven-broiled beef steak were stronger among low consumers of fruits and vegetables (OR: 1.63; 95% CI 1.10, 2.42) than among moderate or high consumers of fruits and vegetables. The inverse association observed for pan-fried fish was strongest among moderate consumers of fruits and vegetables (OR: 0.59; 95% CI 0.38, 0.91), though interaction p-values were all > 0.20.

Discussion

In this racially diverse, population-based case–control study of women in central and eastern North Carolina, we examined the associations between various preparation methods of meat and fish and breast cancer. More frequent

Table 1 Baseline characteristics by case–control status ($n = 2606$)

	Controls $n = 1200$	Cases $n = 1406$
Age at diagnosis/enrollment, years, mean (SD)	53 (11)	53 (11.3)
Age at menarche, years, mean (SD)	13 (1.6)	12.6 (1.6)
Number of pregnancies, mean (SD)	3 (2.1)	3 (2.1)
	n (%)	*n* (%)
Race		
European American	748 (62.3)	858 (61.0)
African American	452 (37.7)	548 (39.0)
Family income		
Missing	83 (6.9)	103 (7.3)
< 15,000	280 (23.3)	298 (21.2)
15,000–30,000	263 (21.9)	312 (22.2)
30,000–50,000	379 (31.6)	421 (29.9)
> 50,000	195 (16.3)	272 (19.4)
Marital status		
Never married/lived as married/ missing	78 (6.5)	128 (9.1)
Married or living as married	768 (64.0)	822 (58.5)
Widowed	145 (12.1)	178 (12.7)
Separated, divorced or no longer living as married	209 (17.4)	278 (19.8)
Alcohol intake		
No/missing	428 (35.7)	463 (32.9)
Yes	772 (64.3)	943 (67.1)
Fruit and vegetable intake, (half cup-sized servings/week)		
< 19	400 (33.3)	515 (36.6)
19–31	407 (33.9)	419 (29.8)
≥ 32	393 (32.8)	472 (33.6)
Smoking status		
Never	647 (53.9)	746 (53.1)
Former	318 (26.5)	385 (27.4)
Current	235 (19.6)	275 (19.6)
Body mass index at 18 years, kg/m^2		
Missing	27 (2.3)	28 (2.0)
Underweight (< 18.5)	299 (24.9)	344 (24.5)
Normal weight (18.5–24.9)	742 (61.8)	904 (64.3)
Overweight (25–29.9)	97 (8.1)	86 (6.1)
Obese (≥ 30)	35 (2.9)	44 (3.1)
Body mass index, kg/m^2		
Missing	32 (2.7)	34 (2.4)
Underweight (< 18.5)	17 (1.4)	33 (2.4)
Normal weight (18.5–24.9)	332 (27.7)	441 (31.4)
Overweight (25–29.9)	352 (29.3)	394 (28.0)
Obese (≥ 30)	467 (38.9)	504 (35.9)
Physical activity		
No/missing	541 (45.1)	665 (47.3)
Yes	659 (54.9)	741 (52.7)
Menopausal status		
Premenopausal	502 (41.8)	566 (40.3)
Postmenopausal	698 (58.2)	840 (59.7)
First degree family history		

Table 1 (continued)

	n (%)	*n* (%)
No/missing	1054 (87.8)	1149 (81.7)
Yes	146 (12.2)	257 (18.3)
Ever lactated		
No	729 (60.8)	881 (62.7)
Yes	471 (39.3)	525 (37.3)
Ever use oral contraceptives		
Never/missing	407 (33.9)	485 (34.5)
Ever	793 (66.1)	921 (65.5)
Postmenopausal hormone use		
Never/missing	769 (64.1)	913 (64.9)
Past	130 (10.8)	149 (10.6)
Current	301 (25.1)	344 (24.5)

consumption of pan-fried/oven-broiled beef steak or grilled/barbecued hamburger was associated with higher odds of breast cancer. Increased odds of breast cancer were also observed with more frequent consumption of grilled/barbecued hamburger among post-menopausal women and pan-fried/oven-broiled beef steak among pre-menopausal women and among both ER-positive and ER-negative breast cancer subtypes. In contrast, more frequent consumption of pan-fried fish and weekly consumption of grilled/barbecued pork chops were associated with lower odds of breast cancer compared to less frequent consumption and the association was stronger among post-menopausal women. The positive association for pan-fried/oven-broiled beef steak and inverse association for grilled/barbecued pork chops were more pronounced among EA women than among AA women, while the positive association with grilled/barbecued hamburger and inverse association for pan-fried fish were present only among EA women and not among AA women.

The majority of studies that have examined total red meat intake in relation to overall breast cancer have found no or weak associations [21, 26–28] but a few found significant positive associations with red and processed meat intake [11, 26, 29, 30]. Many of these studies did not account for preparation methods and doneness levels when collecting data on meat intake. Some studies examining meat preparation methods found increased risk of breast cancer with increasing intake of red and processed meat cooked to well-done by methods that promote carcinogen formation [31–33]. Similarly, findings on consumption of meat cooked at high temperature were supported by some [2, 13, 21, 34] but not all studies [32, 35].

In our study, consumption of pan-fried fish once or more per week when compared to no consumption was inversely associated with breast cancer which was consistent with one prior study [36] though was not supported in a meta-analysis [37]. The inverse association may be attributed to the omega-3 polyunsaturated fatty acids (PUFA) contained in fish products which may have anti-inflammatory and other anti-cancer effects, and intake of PUFAs were associated with reduced breast cancer risk in the same meta-analysis [37].

The finding of an inverse association with grilled/barbecued pork chops was unexpected since pork is a red meat. In the South, the term "barbecue" often refers to a marinade-based slow-roasting method unlike the traditional grilling method that can result in charring of the meat surfaces. It has been shown that marinating meat with antioxidant rich marinades prior to grilling may reduce the formation of carcinogens [38, 39]. On the other hand, the use of marinades with a sugar rich base may increase the formation of carcinogens [40]. We hypothesize that women with higher consumption of barbecued pork may be consuming pork in place of other meats that have been cooked with methods that promote carcinogen formation, which may partially explain the inverse associations observed with the consumption of grilled/barbecued pork chops. It is also possible that certain meat products are more commonly prepared at home or served with different vegetables and thus, may be a marker for a healthier diet overall. We did not have data on these dietary patterns and preparation methods to assess this possible explanation.

Our results reported stronger associations among EA women with no statistically significant associations observed among AA women except for preference for medium or well-done meat on the inside. Similarly in previous studies, the risks in AA women were not statistically significant [20, 27] while positive associations were reported in EA women [20]. It is unclear why a differential association was observed between EA and AA women, though other factors may play a larger role in breast cancer risk among AA women such as socioeconomic inequalities and other patterns of risk factors affecting access to health care [41]. Differences in frequencies of polymorphisms

Table 2 Associations of meat and fish intake by preparation methods with breast cancer

	Controls (*n*/%)	Cases (*n*/%)	OR (95% CI)[a]	OR (95% CI)[b]
Chicken				
Pan-fried				
0 time per week	290 (24.2)	349 (24.8)	Ref	Ref
< 1 time per week	405 (33.8)	466 (33.1)	1.00 (0.80, 1.24)	1.02 (0.82, 1.27)
1 time per week	309 (25.8)	347 (24.7)	1.02 (0.80, 1.29)	1.08 (0.84, 1.38)
> 1 time per week	196 (16.3)	244 (17.4)	1.10 (0.83, 1.46)	1.13 (0.85, 1.52)
Oven-broiled				
0 time per week	779 (64.9)	950 (67.6)	Ref	Ref
< 1 time per week	220 (18.3)	236 (16.8)	0.85 (0.68, 1.06)	0.86 (0.69, 1.07)
1 time per week	124 (10.3)	132 (9.4)	0.84 (0.63, 1.10)	0.83 (0.63, 1.11)
> 1 time per week	77 (6.4)	88 (6.3)	0.97 (0.69, 1.35)	0.95 (0.68, 1.34)
Grilled/barbecued				
0 time per week	300 (25.0)	356 (25.3)	Ref	Ref
< 1 time per week	575 (47.9)	700 (49.8)	1.11 (0.90, 1.36)	1.13 (0.92, 1.39)
1 time per week	203 (16.9)	233 (16.6)	1.01 (0.78, 1.32)	1.04 (0.80, 1.36)
> 1 time per week	122 (10.2)	117 (8.3)	0.89 (0.64, 1.22)	0.92 (0.66, 1.27)
Fish steak or fish prepared whole				
Pan-fried				
0 time per week	427 (35.6)	513 (36.5)	Ref	Ref
< 1 time per week	411 (34.3)	526 (37.4)	1.03 (0.84, 1.25)	1.03 (0.85, 1.27)
≥ 1 time per week	362 (30.7)	367 (26.1)	0.75 (0.59, 0.95)	0.77 (0.60, 0.98)
Oven-broiled				
0 time per week	872 (72.7)	1003 (71.3)	Ref	Ref
< 1 time per week	242 (20.2)	300 (21.3)	1.09 (0.89, 1.34)	1.04 (0.84, 1.28)
≥ 1 time per week	86 (7.2)	103 (7.3)	1.18 (0.86, 1.63)	1.17 (0.85, 1.61)
Grilled/barbecued				
0 time per week	980 (81.7)	1129 (80.3)	Ref	Ref
< 1 time per week	178 (14.8)	231 (16.4)	1.16 (0.92, 1.47)	1.11 (0.88, 1.41)
≥ 1 time per week	42 (3.5)	46 (3.3)	0.99 (0.63, 1.56)	0.94 (0.59, 1.49)
Hamburger				
Pan-fried/oven-broiled				
0 time per week	417 (34.8)	488 (34.7)	Ref	Ref
≤ 0.5 time per week	306 (25.5)	364 (25.9)	0.95 (0.77, 1.18)	0.98 (0.79, 1.22)
≤ 1 time per week	343 (28.6)	420 (29.9)	1.02 (0.82, 1.26)	1.05 (0.85, 1.31)
> 1 time per week	134 (11.2)	134 (9.5)	0.83 (0.62, 1.12)	0.86 (0.64, 1.15)
Grilled/barbecued				
0 time per week	412 (34.33)	479 (34.07)	Ref	Ref
< 1 time per week	539 (44.92)	625 (44.45)	1.11 (0.92, 1.35)	1.18 (0.97, 1.43)
≥ 1 time per week	249 (20.75)	302 (21.48)	1.19 (0.94, 1.51)	1.28 (1.01, 1.63)
Beef steak				
Pan-fried/oven-broiled				
0 time per week	619 (51.6)	712 (50.6)	Ref	Ref
< 1 time per week	391 (32.6)	433 (30.8)	0.96 (0.80, 1.16)	0.96 (0.80, 1.16)
≥ 1 time per week	190 (15.8)	261 (18.6)	1.37 (1.09, 1.73)	1.36 (1.08, 1.72)
Grilled/barbecued				
0 time per week	451 (37.6)	592 (42.1)	Ref	Ref
< 1 time per week	558 (46.5)	584 (41.5)	0.84 (0.70, 1.01)	0.87 (0.72, 1.05)
≥ 1 time per week	191 (15.9)	230 (16.4)	0.94 (0.73, 1.21)	0.96 (0.74, 1.24)
Pork chops				
Pan-fried/oven-broiled				

Table 2 (continued)

	Controls (*n*/%)	Cases (*n*/%)	OR (95% CI)[a]	OR (95% CI)[b]
0 time per week	396 (33.0)	484 (34.4)	Ref	Ref
< 1 time per week	459 (38.3)	565 (40.2)	0.96 (0.79, 1.17)	0.96 (0.79, 1.17)
≥ 1 time per week	345 (28.8)	357 (25.4)	0.82 (0.66, 1.02)	0.84 (0.67, 1.05)
Grilled/barbecued				
0 time per week	745 (62.1)	937 (66.6)	Ref	Ref
> 0 time per week	455 (37.9)	469 (33.4)	0.80 (0.68, 0.96)	0.81 (0.68, 0.97)
Bacon				
Pan-fried/oven-broiled				
0 time per week	392 (32.7)	488 (34.7)	Ref	Ref
< 1 time per week	280 (23.3)	326 (23.2)	0.91 (0.73, 1.14)	0.92 (0.74, 1.15)
≥ 1 time per week	528 (44.0)	592 (42.1)	0.86 (0.71, 1.04)	0.87 (0.71, 1.06)
Breakfast sausage				
Pan-fried/oven-broiled				
0 time per week	504 (42.0)	595 (42.3)	Ref	Ref
< 1 time per week	304 (25.3)	367 (26.1)	1.02 (0.83, 1.25)	1.04 (0.84, 1.28)
≥ 1 time per week	392 (32.7)	444 (17.0)	0.90 (0.74, 1.10)	0.93 (0.76, 1.15)
Hot dog or other sausage				
Pan-fried/oven-broiled				
0 time per week	912 (76.0)	1081 (76.9)	Ref	Ref
> 0 time per week	288 (24.0)	325 (23.1)	0.96 (0.79, 1.17)	0.97 (0.80, 1.18)
Grilled/barbecued				
0 time per week	717 (59.8)	832 (59.2)	Ref	Ref
> 0 time per week	483 (40.3)	574 (40.8)	1.05 (0.89, 1.25)	1.08 (0.91, 1.28)
Doneness of meat[c] on the inside				
NA/rare to medium rare	222 (18.5)	252 (17.9)	Ref	Ref
Medium to medium well	387 (32.3)	477 (33.9)	1.11 (0.87, 1.41)	1.14 (0.90, 1.46)
Well done	591 (49.3)	677 (48.2)	0.96 (0.76, 1.22)	1.05 (0.82, 1.35)
Doneness of meat[c] on the outside				
NA/not browned/lightly browned	369 (30.8)	426 (30.3)	Ref	Ref
Well browned	698 (58.2)	849 (60.4)	1.05 (0.87, 1.26)	1.06 (0.88, 1.28)
Heavily browned or charred	133 (11.1)	131 (9.3)	0.80 (0.59, 1.08)	0.80 (0.59, 1.08)

[a]Adjusted for age at diagnosis/enrollment, race, and offsets

[b]Adjusted for age at diagnosis/enrollment, race, alcohol intake, BMI, family income, lactation, marital status, use of oral contraceptives, postmenopausal hormone use, smoking status, and offsets

[c]Meat includes beef steak, roast, or hamburger

in *NAT1, NAT2, GSTM1*, and *GSTT1* genes which encode enzymes involved in detoxification of HCAs may also play a role in the racial differences observed in the associations between meat and fish intake and breast cancer [20, 42]. For example, the stronger associations observed among EA women for grilled/barbecued hamburger or pan-fried/oven-broiled beef steak may be attributed to *GSTM1* deletions occurring more frequently in EA women compared to AA women, resulting in reduced ability to detoxify HCAs [43]. In the CBCS, smaller numbers of AA women and greater imprecision of effect estimates may also explain the differential effects observed.

Previous studies observed stronger positive associations for pan-fried meat intake and ER-positive/PR-negative breast tumors [21] and among post-menopausal women [13] consuming grilled/barbecued and smoked meat [15]. Our positive associations observed between breast cancer and red meat consumption were similar in ER-positive and ER-negative tumors and pre- and post-menopausal women. However, pan-fried fish and grilled/barbecued fish were inversely associated with breast cancer among post-menopausal women only.

Carcinogenic combustion byproducts formed during cooking are one plausible biological mechanism underlying

Table 3 Associations of meat and fish intake by preparation methods and breast cancer by race

	European American Women			African American Women			
	Controls (n/%)	Cases (*n*/%)	OR (95% CI)[a]	Controls (*n*/%)	Cases (*n*/%)	OR (95% CI)[a]	Interaction *p* value
	748/46.6	858/53.4		452/45.2	548/54.8		
Chicken							
Pan-fried							0.87
0 time per week	242 (32.4)	298 (34.7)	Ref	48 (10.6)	51 (9.3)	Ref	
< 1 time per week	313 (41.8)	350 (40.8)	0.97 (0.76, 1.25)	92 (20.4)	116 (21.2)	1.29 (0.77, 2.16)	
1 time per week	160 (21.4)	162 (18.9)	0.96 (0.70, 1.30)	149 (33.0)	185 (33.8)	1.40 (0.86, 2.26)	
> 1 time per week	33 (4.4)	48 (5.6)	1.56 (0.94, 2.61)	163 (36.1)	196 (35.8)	1.21 (0.75, 1.95)	
Oven-broiled							0.55
0 time per week	500 (66.8)	603 (70.3)	Ref	279 (61.7)	347 (63.3)	Ref	
< 1 time per week	144 919.3)	149 (17.4)	0.82 (0.62, 1.09)	76 (16.8)	87 (15.9)	0.97 (0.66, 1.41)	
1 time per week	73 (9.8)	62 (7.2)	0.64 (0.44, 0.94)	51 (11.3)	70 (12.8)	1.14 (0.75, 1.75)	
> 1 time per week	31 (4.1)	44 (5.1)	1.25 (0.75, 2.09)	46 (10.2)	44 (8.0)	0.82 (0.51, 1.30)	
Grilled/barbecued							0.99
0 time per week	164 (21.9)	194 (22.6)	Ref	136 (30.1)	162 (29.6)	Ref	
< 1 time per week	346 (46.3)	415 (48.4)	1.14 (0.86, 1.50)	229 (50.7)	285 (52.0)	1.12 (0.82, 1.53)	
1 time per week	160 (21.4)	168 (19.6)	0.94 (0.67, 1.32)	43 (9.5)	65 (11.9)	1.25 (0.77, 2.02)	
> 1 time per week	78 (10.4)	81 (9.4)	1.00 (0.66, 1.51)	44 (9.7)	36 (6.6)	0.71 (0.41, 1.23)	
Fish steak or fish prepared whole							
Pan-fried							0.32
0 time per week	363 (48.5)	447 (52.1)	Ref	64 (14.2)	66 (12.0)	Ref	
< 1 time per week	275 (36.8)	322 (37.5)	0.95 (0.75, 1.20)	136 (30.1)	204 (37.2)	1.43 (0.93, 2.22)	
≥ 1 time per week	110 (14.7)	89 (10.4)	0.63 (0.44, 0.89)	252 (55.8)	278 (50.7)	1.06 (0.70, 1.60)	
Oven-broiled							0.49
0 time per week	519 (69.4)	566 (66.0)	Ref	353 (78.1)	437 (79.7)	Ref	
< 1 time per week	164 (21.9)	224 (26.2)	1.19 (0.92, 1.55)	78 (17.3)	76 (13.9)	0.76 (0.52, 1.12)	
≥ 1 time per week	65 (8.7)	68 (7.9)	1.09 (0.74, 1.62)	21 (4.7)	35 (6.4)	1.55 (0.85, 2.81)	
Grilled/barbecued							0.8
0 time per week	563 (75.3)	630 (73.4)	Ref	417 (92.3)	499 (91.1)	Ref	
< 1 time per week	148 (19.8)	189 (22.0)	1.08 (0.82, 1.42)	30 (6.6)	42 (7.7)	1.09 (0.64, 1.85)	
≥ 1 time per week	37 (5.0)	39 (4.6)	0.86 (0.52, 1.43)	5 (1.1)	7 (1.3)	1.49 (0.44, 4.98)	
Pan-fried/oven-broiled							0.07
0 time per week	338 (45.2)	370 (43.1)	Ref	79 (17.5)	118 (21.5)	Ref	
≤ 0.5 time per week	186 (24.9)	219 (25.5)	1.05 (0.81, 1.38)	120 (26.6)	145 (26.5)	0.86 (0.58, 1.29)	
≤ 1 time per week	168 (22.5)	203 (23.7)	1.14 (0.86, 1.51)	175 (38.7)	217 (39.6)	0.91 (0.63, 1.32)	
> 1 time per week	56 (7.5)	66 (7.7)	1.21 (0.79, 1.85)	78 (17.3)	68 (12.4)	0.64 (0.40, 1.02)	
Grilled/barbecued							0.05
0 time per week	210 (28.1)	224 (26.1)	Ref	202 (44.7)	255 (46.5)	Ref	
< 1 time per week	362 (48.4)	413 (48.1)	1.28 (0.98, 1.68)	177 (39.2)	212 (38.7)	1.11 (0.83, 1.50)	
1 time per week	176 (23.5)	221 (25.8)	1.54 (1.13, 2.11)	73 (16.2)	81 (14.8)	0.89 (0.59. 1.35)	
Beef steak							
Pan-fried/oven-broiled							0.92
0 time per week	451 (60.3)	513 (59.8)	Ref	168 (37.2)	199 (36.3)	Ref	
< 1 time per week	210 (28.1)	225 (26.2)	0.96 (0.75, 1.23)	181 (40.0)	208 (38.0)	0.94 (0.69, 1.28)	
≥ 1 time per week	87 (11.6)	120 (14.0)	1.47 (1.06, 2.04)	103 (22.8)	141 (25.7)	1.24 (0.88, 1.76)	

Table 3 (continued)

	European American Women			African American Women			
	Controls (n/%)	Cases (*n*/%)	OR (95% CI)[a]	Controls (*n*/%)	Cases (*n*/%)	OR (95% CI)[a]	Interaction *p* value
	748/46.6	858/53.4		452/45.2	548/54.8		
Grilled/barbecued							0.47
0 time per week	198 (26.5)	270 (31.5)	Ref	253 (56.0)	322 (58.7)	Ref	
< 1 time per week	392 (52.4)	404 (47.1)	0.76 (0.59, 0.98)	166 (36.7)	180 (32.9)	0.97 (0.72, 1.30)	
≥ 1 time per week	158 (21.1)	184 (21.5)	0.85 (0.62, 1.15)	33 (7.3)	46 (8.4)	1.14 (0.68, 1.91)	
Pork chops							
Pan-fried/oven-broiled							0.3
0 time per week	309 (41.3)	377 (43.9)	Ref	87 (19.3)	107 (19.5)	Ref	
< 1 time per week	275 (36.8)	334 (38.9)	0.98 (0.77, 1.24)	184 (40.7)	231 (42.2)	0.97 (0.67, 1.40)	
≥ 1 time per week	164 (21.9)	147 (17.1)	0.74 (0.55, 1.00)	181 (40.0)	210 (38.3)	0.93 (0.64, 1.35)	
Grilled/barbecued							0.27
0 time per week	430 (57.5)	546 (63.6)	Ref	315 (69.7)	391 (71.4)	Ref	
> 0 time per week	318 (42.5)	312 (36.4)	0.75 (0.60, 0.94)	137 (30.3)	157 (28.7)	0.90 (0.67, 1.21)	
Bacon							
Pan-fried/oven-broiled							0.09
0 time per week	295 (39.4)	369 (43.0)	Ref	97 (21.5)	119 (21.7)	Ref	
< 1 time per week	180 (24.1)	221 (25.8)	0.94 (0.72, 1.24)	100 (22.1)	105 (19.2)	0.90 (0.60, 1.36)	
≥ 1 time per week	273 (36.5)	268 (31.2)	0.77 (0.60, 0.99)	255 (56.4)	324 (59.1)	1.03 (0.73, 1.45)	
Breakfast sausage							
Pan-fried/oven-broiled							0.96
0 time per week	389 (52.0)	449 (52.3)	Ref	115 (25.4)	146 (26.6)	Ref	
< 1 time per week	201 (26.9)	246 (28.7)	1.06 (0.83, 1.37)	103 (22.8)	121 (22.1)	0.93 (0.63, 1.36)	
≥ 1 time per week	158 (21.1)	163 (19.0)	0.94 (0.70, 1.26)	234 (51.8)	281 (51.3)	0.89 (0.65, 1.23)	
Hot dog or other sausage							
Pan-fried/oven-broiled							0.49
0 time per week	552 (73.8)	634 (73.9)	Ref	360 (79.7)	447 (81.6)	Ref	
> 0 time per week	196 (26.2)	224 (26.1)	1.05 (0.82, 1.34)	92 (20.4)	101 (18.4)	0.89 (0.63, 1.25)	
Grilled/barbecued							0.89
0 time per week	456 (61.0)	518 (60.4)	Ref	261 (57.7)	314 (57.3)	Ref	
> 0 time per week	292 (39.0)	340 (39.6)	1.06 (0.85, 1.32)	191 (42.3)	234 (42.7)	1.15 (0.87, 1.53)	
Doneness of meat[b] on the inside							
NA/rare to medium rare	184 (24.6)	223 (26.0)	Ref	38 (8.4)	29 (5.3)	Ref	0.58
Medium to medium well	323 (43.2)	362 (42.2)	0.94 (0.72, 1.23)	64 (14.2)	115 (21.0)	2.77 (1.49, 5.14)	
Well done	241 (32.2)	273 (31.8)	0.99 (0.74, 1.35)	350 (44.4)	404 (73.7)	1.78 (1.03, 3.08)	
Doneness of meat[b] on the outside							
NA/not browned/lightly browned	299 (40.0)	335 (39.0)	Ref	70 (15.5)	91 (16.6)	Ref	0.79
Well browned	379 (50.7)	452 (52.7)	1.08 (0.86, 1.35)	319 (70.6)	397 (72.4)	1.01 (0.70, 1.47)	
Heavily browned or charred	70 (9.3)	71 (8.3)	0.80 (0.54, 1.18)	63 (13.9)	60 (11.0)	0.78 (0.47, 1.31)	

[a]Adjusted for age at diagnosis/enrollment, alcohol intake, BMI, family income, lactation, marital status, use of oral contraceptives, postmenopausal hormone use, smoking status, and offsets

[b]Meat includes beef steak, roast, or hamburger

these results. HCAs and PAHs are two classes of carcinogens that are formed inside and on the surface of meat when cooked at high temperatures for long periods of time and have been shown in experimental studies to be implicated in DNA damage, promoting the development of mammary cancers [5]. The levels of HCAs formed vary by the preparation

Table 4 Associations of meat and fish intake by preparation methods and breast cancer among pre-menopausal and post-menopausal women

	Pre-menopausal women ($n=1068$)			Post-menopausal women ($n=1538$)			
	Controls ($n=502$)	Cases ($n=566$)	OR (95% CI)[a]	Controls (698)	Cases (840)	OR (95% CI)[a]	Interaction p value
Chicken							
Pan-fried							0.82
0 time per week	134 (26.7)	148 (26.2)	Ref	156 (22.4)	201 (23.9)	Ref	
< 1 time per week	151 (30.1)	184 (32.5)	1.19 (0.85, 1.69)	254 (36.4)	282 (33.6)	0.93 (0.69, 1.25)	
1 time per week	130 (25.9)	130 (23.0)	1.17 (0.79, 1.74)	179 (25.6)	217 (25.8)	1.03 (0.75, 1.43)	
> 1 time per week	87 (17.3)	104 (18.4)	1.22 (0.77, 1.92)	109 (15.6)	140 (16.7)	1.05 (0.71, 1.55)	
Oven-broiled							0.88
0 time per week	320 (63.8)	370 (65.4)	Ref	459 (65.8)	580 (69.1)	Ref	
< 1 time per week	97 (19.3)	106 (18.7)	0.89 (0.63, 1.25)	123 (17.6)	130 (15.5)	0.83 (0.61, 1.11)	
1 time per week	51 (10.2)	55 (9.7)	0.97 (0.63, 1.51)	73 (10.5)	77 (9.2)	0.76 (0.52, 1.10)	
> 1 time per week	34 (6.8)	35 (6.2)	0.81 (0.48, 1.38)	43 (6.2)	53 (6.3)	1.03 (0.66, 1.62)	
Grilled/barbecued							0.89
0 time per week	85 (16.9)	91 (16.1)	Ref	215 (30.8)	265 (31.6)	Ref	
< 1 time per week	256 (51.0)	299 (52.8)	1.08 (0.75, 1.56)	319 (45.7)	401 (47.7)	1.16 (0.90, 1.50)	
1 time per week	95 (18.9)	105 (18.6)	1.00 (0.64, 1.56)	108 (15.5)	128 (15.2)	1.07 (0.76, 1.51)	
> 1 time per week	66 (13.2)	71 (12.5)	0.99 (0.61, 1.61)	56 (8.0)	46 (5.5)	0.77 (0.48, 1.23)	
Fish steak or fish prepared whole							
Pan-fried							0.49
0 time per week	208 (41.4)	226 (39.9)	Ref	219 (31.4)	287 (34.2)	Ref	
< 1 time per week	159 (31.7)	211 (37.3)	1.27 (0.93, 1.73)	252 (36.1)	315 (37.5)	0.91 (0.69, 1.19)	
≥ 1 time per week	135 (26.9)	129 (22.8)	0.83 (0.56, 1.22)	227 (32.5)	238 (28.3)	0.72 (0.52, 0.98)	
Oven-broiled							0.94
0 time per week	368 (73.3)	406 (71.7)	Ref	504 (72.2)	597 (71.1)	Ref	
< 1 time per week	109 (21.7)	124 (21.9)	0.94 (0.68, 1.29)	133 (19.1)	176 (21.0)	1.12 (0.85, 1.48)	
≥ 1 time per week	25 (5.0)	36 (6.4)	1.45 (0.82, 2.54)	61 (8.7)	67 (8.0)	1.06 (0.71, 1.58)	
Grilled/barbecued							0.01
0 time per week	408 (81.3)	426 (75.3)	Ref	572 (82.0)	703 (83.7)	Ref	
< 1 time per week	80 (15.9)	113 (20.0)	1.34 (0.93, 1.92)	98 (14.0)	118 (14.1)	0.95 (0.69, 1.31)	
≥ 1 time per week	14 (2.8)	27 (4.8)	1.93 (0.95, 3.90)	28 (4.0)	19 (2.3)	0.52 (0.27, 0.99)	
Hamburger							
Pan-fried/oven-broiled							0.02
0 time per week	171 (34.1)	222 (39.2)	Ref	246 (35.2)	266 (31.7)	Ref	
≤ 0.5 time per week	118 (23.5)	126 (22.3)	0.76 (0.53, 1.08)	188 (26.9)	238 (28.3)	1.19 (0.90, 1.58)	
≤ 1 time per week	144 (28.7)	156 (27.6)	0.83 (0.59, 1.16)	199 (28.5)	264 (31.4)	1.30 (0.98, 1.72)	
> 1 time per week	69 (13.8)	62 (11.0)	0.62 (0.40, 0.96)	65 (9.3)	72 (8.6)	1.21 (0.80, 1.83)	
Grilled/barbecued							0.33
0 time per week	124 (24.7)	140 (24.7)	Ref	288 (41.3)	339 (40.4)	Ref	
< 1 time per week	249 (49.6)	273 (48.2)	1.03 (0.74, 1.42)	290 (41.6)	352 (41.9)	1.23 (0.96, 1.59)	
≥ 1 time per week	129 (25.7)	153 (27.0)	1.10 (0.76, 1.60)	120 (17.2)	149 (17.7)	1.46 (1.05, 2.03)	
Beef steak							
Pan-fried/oven-broiled							0.32
0 time per week	273 (54.4)	293 (51.8)	Ref	346 (49.6)	419 (49.9)	Ref	
< 1 time per week	154 (30.7)	169 (29.9)	1.12 (0.83, 1.51)	237 (34.0)	264 (31.4)	0.86 (0.67, 1.10)	
≥ 1 time per week	75 (14.9)	104 (18.4)	1.56 (1.08, 2.26)	115 (16.5)	157 (18.7)	1.25 (0.92, 1.69)	

Table 4 (continued)

	Pre-menopausal women ($n=1068$)			Post-menopausal women ($n=1538$)			
	Controls ($n=502$)	Cases ($n=566$)	OR (95% CI)[a]	Controls (698)	Cases (840)	OR (95% CI)[a]	Interaction p value
Grilled/barbecued							0.87
0 time per week	171 (34.1)	208 (36.8)	Ref	280 (40.1)	384 (45.7)	Ref	
< 1 time per week	255 (50.8)	265 (46.8)	0.88 (0.65, 1.18)	303 (43.4)	319 (38.0)	0.84 (0.65, 1.07)	
≥ 1 time per week	76 (15.1)	93 (16.4)	0.90 (0.60, 1.36)	115 (16.5)	137 (16.3)	0.99 (0.71, 1.38)	
Pork chops							
Pan-fried/oven-broiled							0.38
0 time per week	164 (32.7)	200 (35.3)	Ref	232 (33.2)	284 (33.8)	Ref	
< 1 time per week	194 (38.7)	228 (40.3)	0.90 (0.66, 1.23)	265 (38.0)	337 (40.1)	0.99 (0.76, 1.29)	
≥ 1 time per week	144 (28.7)	138 (24.4)	0.71 (0.49, 1.02)	201 (28.8)	219 (26.1)	0.93 (0.69, 1.24)	
Grilled/barbecued							0.08
0 time per week	295 (58.8)	332 (58.7)	Ref	450 (64.5)	605 (72.0)	Ref	
> 0 time per week	207 (41.2)	234 (41.3)	0.96 (0.74, 1.26)	248 (35.5)	235 (28.0)	0.71 (0.56, 0.90)	
Bacon							
Pan-fried/oven-broiled							0.4
0 time per week	158 (31.5)	191 (33.8)	Ref	234 (33.5)	297 (35.4)	Ref	
< 1 time per week	121 (24.1)	157 (27.7)	1.08 (0.77, 1.51)	159 (22.8)	169 (20.1)	0.81 (0.60, 1.09)	
≥ 1 time per week	223 (44.4)	218 (38.5)	0.80 (0.58, 1.10)	305 (43.7)	374 (44.5)	0.92 (0.71, 1.19)	
Breakfast sausage							
Pan-fried/oven-broiled							0.9
0 time per week	210 (41.8)	233 (41.2)	Ref	294 (42.1)	362 (43.1)	Ref	
< 1 time per week	134 (26.7)	171 (30.2)	1.21 (0.88, 1.66)	170 (24.4)	196 (23.3)	0.91 (0.69, 1.20)	
≥ 1 time per week	158 (31.5)	162 (28.6)	0.94 (0.67, 1.33)	234 (33.5)	282 (33.6)	0.94 (0.72, 1.23)	
Hot dog or other sausage							
Pan-fried/oven-broiled							0.31
0 time per week	386 (76.9)	428 (75.6)	Ref	526 (75.4)	653 (77.7)	Ref	
> 0 time per week	116 (23.1)	138 (24.4)	1.09 (0.81, 1.48)	172)24.6)	187 (22.3)	0.90 (0.70, 1.17)	
Grilled/barbecued							0.59
0 time per week	260 (51.8)	275 (48.6)	Ref	457 (65.5)	557 (66.3)	Ref	
> 0 time per week	242 (48.2)	291 (51.4)	1.20 (0.93, 1.56)	241 (34.5)	283 (33.7)	1.01 (0.80, 1.27)	
Doneness of meat[b] on the inside							
NA/rare to medium rare	94 (18.7)	114 (20.1)	Ref	128 (18.3)	138 (16.4)	Ref	0.99
Medium to medium well	167 (33.3)	186 (32.9)	1.02 (0.70, 1.47)	220 (31.5)	291 (34.6)	1.26 (0.91, 1.73)	
Well done	241 (48.0)	266 (47.0)	1.02 (0.69, 1.51)	350 (50.1)	411 (48.9)	1.09 (0.78, 1.52)	
Doneness of meat[b] on the outside							
NA/not browned/lightly browned	135 (26.9)	162 (28.6)	Ref	234 (33.5)	264 (31.4)	Ref	0.6
Well browned	310 (61.8)	349 (61.7)	0.97 (0.71, 1.32)	388 (55.6)	500 (59.5)	1.14 (0.90, 1.46)	
Heavily browned or charred	57 (11.4)	55 (9.7)	0.81 (0.50, 1.31)	76 (10.9)	76 (9.1)	0.77 (0.52, 1.14)	

[a]Adjusted for age at diagnosis/enrollment, race, alcohol intake, marital status, family income, smoking status, BMI, lactation, use of oral contraceptives, and offsets

[b]Meat includes beef steak, roast, or hamburger

method, temperature, preparation time, and type of meat prepared [44]. In particular, pan-frying and grilling are methods that create high levels of HCAs in red meat and the formation of HCAs increases with higher temperatures [44, 45]. PAHs are formed when the smoke produced from drippings of meat prepared on open flame, adhere to meat surfaces [46]. NOCs are carcinogens formed in processed meat and are also produced endogenously through the nitrosylation of heme found in red meat [47–49]. We found the strongest positive associations in women reporting high intakes of red meat cooked by pan-frying, oven-broiling or grilled/barbecued, supporting our hypothesis that HCAs and PAHs may play a role in the etiology of human breast cancer. Though a meta-analysis showed increased breast cancer risk with high consumption of processed meat [18], we did not find an association with individual processed meat products (bacon, breakfast sausage, or hot dog). Other plausible mechanisms, most notably that distinct dietary patterns may be associated with intake of specific types of meat, could account for the observed associations.

One limitation of the study is that a complete dietary assessment method was not utilized so we did not have information on total energy intake or on other aspects of the diet besides fruits and vegetables to adjust for potential confounding. It is possible that the relationship between intake of pan-fried or oven-broiled beef steak and breast cancer could be confounded by total energy intake given that obesity, as a surrogate for high energy intake, is an established risk factor for post-menopausal breast cancer and may also be correlated with meat intake. Though we queried about several types of meats, cooking methods, and doneness preferences, a validated method such as that developed by Sinha et al. [50] which utilizes color photographs of meats at varying levels of doneness was not utilized in this study. Future studies of cooked meat and cancer may benefit from using the color photographs to more accurately estimate exposure to cooked meat carcinogens. Given the retrospective nature of the case–control study design, recall bias is also a possibility in this study. The diagnosis of disease may cause breast cancer cases to recall cooked meat intake differently than controls, resulting in differential misclassification of the exposure which could bias the effect estimates. A strength of our study is the oversampling of AA women and women younger than 50 years to allow for examination of associations between cooked meat and breast cancer among different racial groups and by menopausal status. However, some subgroup analyses (e.g., by race and ER status) were limited by sample size resulting in imprecision.

In conclusion, we found a modest increased risk of breast cancer in women consuming beef at least once per week prepared in ways that enhance carcinogen formation compared to non-consumers, and a reduced risk of breast cancer for higher intake of fish or barbecued pork. The results build upon the evidence for a role of meat and fish intake in the etiology of breast cancer and are consistent with dietary guidelines aimed at reducing fried or broiled red meat intake and increasing fish intake for cancer prevention.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10549-022-06555-x.

Acknowledgements We are grateful to CBCS participants and study staff.

Author contributions All authors contributed to the study conception and design. Data analysis was performed by Omonefe Omofuma. The first draft of the manuscript was written by Omonefe Omofuma and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by a Susan G. Komen Graduate Training in Disparities Research Grant (GTDR17500160; SES, OOO) and a Cancer Prevention Research Fellowship from the American Society of Preventive Oncology and the Prevent Cancer Foundation (SES); This research was supported by a grant from UNC Lineberger Comprehensive Cancer Center, which is funded by the University Cancer Research Fund of North Carolina, the Susan G Komen Foundation, the National Cancer Institute of the National Institutes of Health (P01CA151135), and the National Cancer Institute Specialized Program of Research Excellence (SPORE) in Breast Cancer (NIH/NCI P50-CA58223). This research recruited participants and/or obtained data with the assistance of Rapid Case Ascertainment, a collaboration between the North Carolina Central Cancer Registry and UNC Lineberger. RCA is supported by a grant from the National Cancer Institute of the National Institutes of Health (P30CA016086). We are grateful to CBCS participants and study staff.

Data availability Data from the Carolina Breast Cancer Study can be requested from study investigators at the following: https://unclineberger.org/cbcs/for-researchers/data-sharing/.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Institutional Review Board at the University of North Carolina at Chapel Hill (UNC IRB#92-0410). The current analyses of deidentified existing data were reviewed by the University of South Carolina Institutional Review Board and declared not human subjects research (#PR00074227).

Consent to participate Informed consent was obtained from all individual participants included in the study.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A (2021) Cancer statistics. CA Cancer J Clin 71(1):7–33. https://doi.org/10.3322/caac.21654

2. Boldo E, Castello A, Aragones N, Amiano P, Perez-Gomez B, Castano-Vinyals G, Martin V, Guevara M, Urtiaga C, Dierssen-Sotos T, Fernandez-Tardon G, Moreno V, Solans M, Peiro R, Capelo R, Gomez-Acebo I, Castilla J, Molina AJ, Castells X, Altzibar JM, Lope V, Kogevinas M, Romieu I, Pollan M (2018) Meat intake, methods and degrees of cooking and breast cancer risk in the MCC-Spain study. Maturitas 110:62–70. https://doi.org/10.1016/j.maturitas.2018.01.020
3. Wu J, Zeng R, Huang J, Li X, Zhang J, Ho JC, Zheng Y (2016) Dietary protein sources and Incidence of Breast Cancer: A Dose-Response Meta-Analysis of Prospective Studies. Nutrients. https://doi.org/10.3390/nu8110730
4. International Agency for Research on Cancer (2018) Red meat and processed meat. For more information contact publications@iarc.fr. International Agency for Research on Cancer, Lyon
5. Papaioannou MD, Koufaris C, Gooderham NJ (2014) The cooked meat-derived mammary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) elicits estrogenic-like microRNA responses in breast cancer cells. Toxicol Lett 229(1):9–16. https://doi.org/10.1016/j.toxlet.2014.05.021
6. White AJ, Bradshaw PT, Herring AH, Teitelbaum SL, Beyea J, Stellman SD, Steck SE, Mordukhovich I, Eng SM, Engel LS, Conway K, Hatch M, Neugut AI, Santella RM, Gammon MD (2016) Exposure to multiple sources of polycyclic aromatic hydrocarbons and breast cancer incidence. Environ Int 89–90:185–192. https://doi.org/10.1016/j.envint.2016.02.009
7. Clarke RE, Dordevic AL, Tan SM, Ryan L, Coughlan MT (2016) Dietary Advanced Glycation End Products and Risk Factors for Chronic Disease: A Systematic Review of Randomised Controlled Trials. Nutrients 8(3):125. https://doi.org/10.3390/nu8030125
8. Uribarri J, Cai W, Sandu O, Peppa M, Goldberg T, Vlassara H (2005) Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects. Ann N Y Acad Sci 1043:461–466. https://doi.org/10.1196/annals.1333.052
9. Omofuma OO, Turner DP, Peterson LL, Merchant AT, Zhang J, Steck SE (2020) Dietary Advanced Glycation End-products (AGE) and Risk of Breast Cancer in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO). Cancer Prev Res (Phila) 13(7):601–610. https://doi.org/10.1158/1940-6207.CAPR-19-0457
10. Peterson LL, Park S, Park Y, Colditz GA, Anbardar N, Turner DP (2020) Dietary advanced glycation end products and the risk of postmenopausal breast cancer in the National Institutes of Health-AARP Diet and Health Study. Cancer 126(11):2648–2657. https://doi.org/10.1002/cncr.32798
11. Inoue-Choi M, Sinha R, Gierach GL, Ward MH (2016) Red and processed meat, nitrite, and heme iron intakes and postmenopausal breast cancer risk in the NIH-AARP Diet and Health Study. Int J Cancer 138(7):1609–1618. https://doi.org/10.1002/ijc.29901
12. Lo JJ, Park YM, Sinha R, Sandler DP (2020) Association between meat consumption and risk of breast cancer: Findings from the Sister Study. Int J Cancer 146(8):2156–2165. https://doi.org/10.1002/ijc.32547
13. Fu Z, Deming SL, Fair AM, Shrubsole MJ, Wujcik DM, Shu XO, Kelley M, Zheng W (2011) Well-done meat intake and meat-derived mutagen exposures in relation to breast cancer risk: the Nashville Breast Health Study. Breast Cancer Res Treat 129(3):919–928. https://doi.org/10.1007/s10549-011-1538-7
14. Guo J, Wei W, Zhan L (2015) Red and processed meat intake and risk of breast cancer: a meta-analysis of prospective studies. Breast Cancer Res Treat 151(1):191–198. https://doi.org/10.1007/s10549-015-3380-9
15. Steck SE, Gaudet MM, Eng SM, Britton JA, Teitelbaum SL, Neugut AI, Santella RM, Gammon MD (2007) Cooked meat and risk of breast cancer–lifetime versus recent dietary intake. Epidemiology 18(3):373–382. https://doi.org/10.1097/01.ede.0000259968.11151.06
16. Wu K, Sinha R, Holmes MD, Giovannucci E, Willett W, Cho E (2010) Meat mutagens and breast cancer in postmenopausal women–a cohort analysis. Cancer Epidemiol Biomark Prev 19(5):1301–1310. https://doi.org/10.1158/1055-9965.epi-10-0002
17. Alexander DD, Morimoto LM, Mink PJ, Cushing CA (2010) A review and meta-analysis of red and processed meat consumption and breast cancer. Nutr Res Rev 23(2):349–365. https://doi.org/10.1017/s0954422410000235
18. Farvid MS, Stern MC, Norat T, Sasazuki S, Vineis P, Weijenberg MP, Wolk A, Wu K, Stewart BW, Cho E (2018) Consumption of red and processed meat and breast cancer incidence: a systematic review and meta-analysis of prospective studies. Int J Cancer 143(11):2787–2799. https://doi.org/10.1002/ijc.31848
19. Wang Y, Beydoun MA, Caballero B, Gary TL, Lawrence R (2010) Trends and correlates in meat consumption patterns in the US adult population. Public Health Nutr 13(9):1333–1345. https://doi.org/10.1017/s1368980010000224
20. Chandran U, Zirpoli G, Ciupak G, McCann SE, Gong Z, Pawlish K, Lin Y, Demissie K, Ambrosone CB, Bandera EV (2013) Racial disparities in red meat and poultry intake and breast cancer risk. Cancer Causes Control 24(12):2217–2229. https://doi.org/10.1007/s10552-013-0299-5
21. Larsson SC, Bergkvist L, Wolk A (2009) Long-term meat intake and risk of breast cancer by oestrogen and progesterone receptor status in a cohort of Swedish women. Eur J Cancer (Oxf, Engl 1990) 45(17):3042–3046. https://doi.org/10.1016/j.ejca.2009.04.035
22. Newman B, Moorman PG, Millikan R, Qaqish BF, Geradts J, Aldrich TE, Liu ET (1995) The Carolina Breast Cancer Study: integrating population-based epidemiology and molecular biology. Breast Cancer Res Treat 35(1):51–60
23. Weinberg CR, Wacholder S (1990) The design and analysis of case-control studies with biased sampling. Biometrics 46(4):963–975
24. Weinberg CR, Sandler DP (1991) Randomized recruitment in case-control studies. Am J Epidemiol 134(4):421–432
25. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC (2006) Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 295(21):2492–2502. https://doi.org/10.1001/jama.295.21.2492
26. Anderson JJ, Darwis NDM, Mackay DF, Celis-Morales CA, Lyall DM, Sattar N, Gill JMR, Pell JP (2018) Red and processed meat consumption and breast cancer: UK Biobank cohort study and meta-analysis. Eur J Cancer (Oxf, Engl 1990) 90:73–82. https://doi.org/10.1016/j.ejca.2017.11.022
27. Genkinger JM, Makambi KH, Palmer JR, Rosenberg L, Adams-Campbell LL (2013) Consumption of dairy and meat in relation to breast cancer risk in the Black Women's Health Study. Cancer Causes Control (CCC) 24(4):675–684. https://doi.org/10.1007/s10552-013-0146-8
28. Holmes MD, Colditz GA, Hunter DJ, Hankinson SE, Rosner B, Speizer FE, Willett WC (2003) Meat, fish and egg intake and risk of breast cancer. Int J Cancer 104(2):221–227. https://doi.org/10.1002/ijc.10910
29. Diallo A, Deschasaux M, Latino-Martel P, Hercberg S, Galan P, Fassier P, Alles B, Gueraud F, Pierre FH, Touvier M (2018) Red and processed meat intake and cancer risk: results from the prospective NutriNet-Sante cohort study. Int J Cancer 142(2):230–237. https://doi.org/10.1002/ijc.31046
30. Han MA, Zeraatkar D, Guyatt GH, Vernooij RWM, El Dib R, Zhang Y, Algarni A, Leung G, Storman D, Valli C, Rabassa M, Rehman N, Parvizian MK, Zworth M, Bartoszko JJ, Lopes LC,

Sit D, Bala MM, Alonso-Coello P, Johnston BC (2019) Reduction of red and processed meat intake and cancer mortality and incidence: a systematic review and meta-analysis of cohort studies. Ann Intern Med 171(10):711–720. https://doi.org/10.7326/M19-0699
31. Dai Q, Shu XO, Jin F, Gao YT, Ruan ZX, Zheng W (2002) Consumption of animal foods, cooking methods, and risk of breast cancer. Cancer Epidemiol Biomark Prev 11(9):801–808
32. Delfino RJ, Sinha R, Smith C, West J, White E, Lin HJ, Liao SY, Gim JS, Ma HL, Butler J, Anton-Culver H (2000) Breast cancer, heterocyclic aromatic amines from meat and N-acetyltransferase 2 genotype. Carcinogenesis 21(4):607–615. https://doi.org/10.1093/carcin/21.4.607
33. Zheng W, Gustafson DR, Sinha R, Cerhan JR, Moore D, Hong CP, Anderson KE, Kushi LH, Sellers TA, Folsom AR (1998) Well-done meat intake and the risk of breast cancer. J Natl Cancer Inst 90(22):1724–1729. https://doi.org/10.1093/jnci/90.22.1724
34. Pala V, Krogh V, Berrino F, Sieri S, Grioni S, Tjonneland A, Olsen A, Jakobsen MU, Overvad K, Clavel-Chapelon F, Boutron-Ruault MC, Romieu I, Linseisen J, Rohrmann S, Boeing H, Steffen A, Trichopoulou A, Benetou V, Naska A, Vineis P, Tumino R, Panico S, Masala G, Agnoli C, Engeset D, Skeie G, Lund E, Ardanaz E, Navarro C, Sanchez MJ, Amiano P, Svatetz CA, Rodriguez L, Wirfalt E, Manjer J, Lenner P, Hallmans G, Peeters PH, van Gils CH, Bueno-de-Mesquita HB, van Duijnhoven FJ, Key TJ, Spencer E, Bingham S, Khaw KT, Ferrari P, Byrnes G, Rinaldi S, Norat T, Michaud DS, Riboli E (2009) Meat, eggs, dairy products, and risk of breast cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Am J Clin Nutr 90(3):602–612. https://doi.org/10.3945/ajcn.2008.27173
35. Gertig DM, Hankinson SE, Hough H, Spiegelman D, Colditz GA, Willett WC, Kelsey KT, Hunter DJ (1999) N-acetyl transferase 2 genotypes, meat intake and breast cancer risk. Int J Cancer 80(1):13–17
36. Kim J, Lim SY, Shin A, Sung MK, Ro J, Kang HS, Lee KS, Kim SW, Lee ES (2009) Fatty fish and fish omega-3 fatty acid intakes decrease the breast cancer risk: a case-control study. BMC Cancer 9:216. https://doi.org/10.1186/1471-2407-9-216
37. Zheng JS, Hu XJ, Zhao YM, Yang J, Li D (2013) Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: meta-analysis of data from 21 independent prospective cohort studies. BMJ (Clin Res Ed) 346:f3706. https://doi.org/10.1136/bmj.f3706
38. Smith JS, Ameri F, Gadgil P (2008) Effect of marinades on the formation of heterocyclic amines in grilled beef steaks. J Food Sci 73(6):T100-105
39. Viegas O, Yebra-Pimentel I, Martinez-Carballo E, Simal-Gandara J, Ferreira IM (2014) Effect of beer marinades on formation of polycyclic aromatic hydrocarbons in charcoal-grilled pork. J Agric Food Chem 62(12):2638–2643. https://doi.org/10.1021/jf404966w
40. Nerurkar PV, Le Marchand L, Cooney RV (1999) Effects of marinating with Asian marinades or western barbecue sauce on PhIP and MeIQx formation in barbecued beef. Nutr Cancer 34(2):147–152. https://doi.org/10.1207/s15327914nc3402_4
41. Newman LA, Kaljee LM (2017) Health disparities and triple-negative breast cancer in African American women: a review. JAMA Surg 152(5):485–493. https://doi.org/10.1001/jamasurg.2017.0005
42. Rebbeck TR (1997) Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomark Prev 6(9):733–743
43. Cotton SC, Sharp L, Little J, Brockton N (2000) Glutathione S-transferase polymorphisms and colorectal cancer: a HuGE review. Am J Epidemiol 151(1):7–32. https://doi.org/10.1093/oxfordjournals.aje.a010124
44. Sinha R, Rothman N, Salmon CP, Knize MG, Brown ED, Swanson CA, Rhodes D, Rossi S, Felton JS, Levander OA (1998) Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. Food Chem Toxicol 36(4):279–287
45. Skog K, Steineck G, Augustsson K, Jagerstad M (1995) Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. Carcinogenesis 16(4):861–867. https://doi.org/10.1093/carcin/16.4.861
46. Larsson BK (1986) Formation of polycyclic aromatic hydrocarbons during the smoking and grilling of food. Prog Clin Biol Res 206:169–180
47. Cross AJ, Pollock JR, Bingham SA (2003) Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Res 63(10):2358–2360
48. Joosen AM, Kuhnle GG, Aspinall SM, Barrow TM, Lecommandeur E, Azqueta A, Collins AR, Bingham SA (2009) Effect of processed and red meat on endogenous nitrosation and DNA damage. Carcinogenesis 30(8):1402–1407. https://doi.org/10.1093/carcin/bgp130
49. Kuhnle GG, Story GW, Reda T, Mani AR, Moore KP, Lunn JC, Bingham SA (2007) Diet-induced endogenous formation of nitroso compounds in the GI tract. Free Radic Biol Med 43(7):1040–1047. https://doi.org/10.1016/j.freeradbiomed.2007.03.011
50. Sinha R, Rothman N (1997) Exposure assessment of heterocyclic amines (HCAs) in epidemiologic studies. Mutat Res 376(1–2):195–202