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Red and processed meat, nitrite, and heme iron intakes and postmenopausal breast cancer risk in the NIH-AARP Diet and Health Study

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Abstract

Previous studies have shown inconsistent associations between red and processed meat intake and breast cancer risk. *N*-nitroso compounds and heme iron have been hypothesized as contributing factors. We followed 193,742 postmenopausal women in the NIH-AARP Diet and Health Study and identified 9,305 incident breast cancers (1995–2006). Dietary intake was assessed using a food frequency questionnaire at baseline. We adjusted daily intakes of meat, nitrite, and heme iron for energy intake using the nutrient density method. We estimated multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) by quintiles of dietary exposures for all breast cancer, by stage (*in-situ*, localized, regional/distant), and by estrogen/progesterone receptor (ER/PR) status using Cox proportional hazards regression. Total red meat intake was positively associated with risk of regional/distant cancer (*p*-trend=0.02). The risk was 25% higher in the highest vs. lowest intake quintile (95%CI=1.03–1.52). Higher processed red meat intake (Q5 vs. Q1) was associated with 27% higher risk of localized breast cancer (95%CI=1.01–1.27, *p*-trend=0.03) and a 19% higher risk of regional/distant cancer (95%CI=0.98–1.44, *p*-trend=0.10). In addition, higher nitrite intake from processed red meat was positively associated with localized cancer (HR for Q5 vs. Q1=1.23, 95%CI=1.09–1.39, *p*-trend<0.0001). Heme iron intake was positively associated with breast cancer risk overall and all cancer stages (*p*-trend=0.02–0.05). No heterogeneity was observed in risk associations by hormone receptor status. Our findings suggest that high consumption of red meat and processed meat may increase risk of postmenopausal breast cancer. Added nitrite and heme iron may partly contribute to these observed associations.

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Keywords

Red meat; processed meat; breast cancer; nitrite; heme iron

Introduction

Dietary intake has been extensively studied in relation to breast cancer, but only a few dietary risk factors (e.g., total fat and alcohol intakes) have been identified to date (1). Red meat and processed meat have been evaluated predominantly in relation to the risk of colorectal cancer in a number of epidemiologic studies with many showing a positive association (2). Their associations with breast cancer risk have been inconsistent. A recent meta-analysis found non-significant positive summary trends between red meat or processed meat intake and breast cancer risk, with significant statistical heterogeneity among studies (3). In addition, although breast cancer is a heterogeneous disease with different etiologies, few studies considered hormone receptor status and cancer stage in the analyses. Several meat-related factors, such as heterocyclic amines (4, 5), *N*-nitroso compounds (NOC) (6, 7), and heme iron (8, 9), have been hypothesized as contributing factors to associations between red and processed meat intakes and cancer risk. However, their relative contributions to breast cancer development are unclear.

Nitrate and nitrite are precursors of NOC (e.g., nitrosamines and nitrosamides), which are potent animal carcinogens and potential human carcinogens (10, 11). Nitrate is a natural component of foods, and is found mostly in plants, especially dark green vegetables; whereas, nitrite occurs at low levels in animal and plant sources. Nitrite and nitrate are also used as food additives to enhance the color and flavor of processed meats, which contain high amounts of amines, precursors of NOC. About five percent of ingested nitrate is reduced to nitrite by oral bacteria (12, 13). In the stomach and other parts of the gastrointestinal tract, nitrite reacts with amines and amides, which come predominantly from meat and fish, to form NOC. Nitrosamides directly alkylate DNA, but nitrosamines must be activated by specific cytochrome P450 enzymes to be carcinogenic (11, 14). These enzymes have been detected in many human tissues including the breast (15). However, to date, a limited number of epidemiologic studies have evaluated dietary nitrite intake and risk of breast cancer (16).

Certain micronutrients are known to influence endogenous NOC formation. Antioxidants, especially vitamin C, which are abundantly found in fruits and vegetables, inhibit NOC formation (17). In contrast, heme iron, mainly found in red meat, enhances endogenous NOC formation (8, 18). Inconsistent associations between heme iron intake and breast cancer risk have been found in a few prospective cohort studies including a previous analysis using a subset of the NIH-AARP Diet and Health Study participants (19–21). A recent meta-analysis of these prospective cohort studies showed no association between heme iron intake and breast cancer risk (22).

The aim of this study was to evaluate the association between meat intake and the risk of breast cancer overall, by cancer stage, and by hormone receptor status among postmenopausal women. We further investigated associations of nitrite intake from animal sources and processed meats and heme iron intake with the risk of breast cancer. In a previous analysis in the NIH-AARP Diet and Health Study, red and processed meat intakes were not associated with breast cancer risk (19). The previous analysis followed 120,755 women who provided meat preparation methods in the second questionnaire. In the current study, we followed the entire cohort of participants who provided meat intake data in the baseline questionnaire, resulting in a considerably larger number of breast cancer cases than the previous analysis.

Methods

Study Population

The NIH-AARP Diet and Health Study is a prospective cohort study designed to investigate diet, lifestyle factors, and health outcomes. The details of the study were previously described (23). Briefly, in 1995–1996, we mailed a baseline questionnaire to 3.5 million current members of the AARP aged 50–71 years who resided in six U.S. states (California, Florida, Pennsylvania, New Jersey, North Carolina, and Louisiana) and two U.S. metropolitan areas (Atlanta, Georgia and Detroit, Michigan). This baseline questionnaire assessed participants' demographics, anthropometrics (self-reported), lifestyle, usual dietary intake, familial history of cancer, and reproductive/gynecologic history. Of 617,119 individuals who returned the baseline questionnaire (18% response rate), 566,398 successfully completed the questionnaire and thus comprised the cohort. Within six months following the baseline questionnaire, a second questionnaire was mailed to these respondents to collect more detailed information on risk factors for cancer, such as previous mammographic screening (23). A total of 332,913 participants completed the second questionnaire (63% response rate). The NIH-AARP Diet and Health Study was approved by the Special Studies Institutional Review Board of the National Cancer Institute. Return of the questionnaire was considered to be informed consent.

Dietary Intake Assessment

Dietary intake was assessed at baseline using a self-administered food frequency questionnaire (FFQ), the NCI-Diet History Questionnaire (DHQ). Participants reported their intake frequency of 124 food items for the past 12 months. Intake frequency was listed as 10 categories ranging from "never" to "2+ times per day" for foods and "never" to "6+ times per day" for beverages. Each line item was accompanied by three portion size categories based on sex- and age group-specific portion sizes (24). The Pyramid Servings Database, based on the U.S. Department of Agriculture's 1994–1996 Continuing Survey of Food Intakes by Individuals (CSFII) database (25), was used to disaggregate component ingredients of mixed foods, such as vegetable soup, beef stew, salads, and sandwiches, and assign them to food groups (24). Use of multivitamin and selected individual dietary supplements was also assessed. Based on the FFQ data, we computed grams consumed per day for total meats as well as red (beef, pork) and white (poultry) meats overall and processed (ham, bacon, sausage, hot dogs, and cold cuts) and fresh (unprocessed) meats separately. The validity of the NCI-DHQ in the NIH-AARP Diet and Health Study population for the assessment of major macro- and micronutrients (26) and nitrate and nitrite (Inoue-Choi *et al.*, under review) was assessed using two 24-hr dietary recalls.

We developed a database of nitrate and nitrite contents of food items included in the DHQ by conducting a review of studies or reports published between 1967 and 2008, focusing mostly on US and Canadian foods. Nitrate and nitrite contents were assigned to individual foods as described elsewhere (27) (Inoue-Choi, *et al.*, under review). Briefly, we calculated means of published values weighted by the number of food samples analyzed and considering food preparation methods (e.g., raw, cooked and canned) when possible (28). Nitrate and nitrite contents of foods constituting FFQ line items were calculated by weighting the food-specific nitrate and nitrite values by age group- and sex-specific intake amounts from the CSFII. The values for mixed dishes were weighted averages of nitrate and nitrite concentrations in the foods based on standardized recipes in the CSFII. We estimated daily nitrite intake from all animal sources and from processed meats (total, red, and white meats). Daily heme iron intake was computed using a previously developed database based on measurements of heme iron content of a variety of fresh meats (e.g., steak, pork chop, chicken breast) and processed meats (e.g., bacon, sausage, hot dogs) (29).

Cohort Follow-up and Ascertainment of Incident Breast Cancer

Cohort participants were followed annually for address changes by matching the cohort database to the U.S. Postal Service's National Change of Address database. First primary breast cancers that occurred through December 2006 were identified via linkage to the eight cancer registries corresponding to cohort participants' state of residence at baseline as well as registries in three additional states (Arizona, Nevada, and Texas) to capture cancers occurring in participants who moved to these states during the follow-up. A previous study estimated that the cancer registry linkage identified about 90% of all incident cancers in this cohort (30). Breast cancer estrogen receptor (ER) and progesterone receptor (PR) status was reported by cancer registries except for the registry in Texas, and coded as described in the American Joint Committee on Cancer's Collaborative Staging Site-Specific Factors Manual. Histology was defined using International Classification of Diseases for Oncology (ICD-O) codes, 3rd edition (31). Cancer stage information was available from all 11 state registries. Vital status of study participants was identified by annual linkage with the Social Security Administration Death Master File, supplemented by National Death Index (NDI), cancer registry linkages, and responses to study mailings.

Analytic cohort

Among the 566,398 cohort participants who had satisfactorily completed the baseline questionnaire, we excluded individuals whose questionnaires were completed by proxy (n = 15,760), men (n = 325,171), women who reported previous cancer diagnosis except for non-melanoma skin cancer (n = 23,957), and those who moved out of the study area or died at or before processing of the baseline questionnaire (n = 27). We further excluded premenopausal women (n = 3,864), women with unknown menopause status (n = 2,123), women who had nonepithelial breast tumors (ICD-O histology code ≥ 8800; n = 19), and women who reported extreme values in total energy intake (Box-Cox transformed values more than two interquartile ranges from the median; n = 1,735). As a result, 193,742 postmenopausal women constituted the analytic cohort.

Statistical Analysis

Cox proportional hazards regression was used to estimate multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer in relation to quintiles of dietary exposures using person-years as the underlying time metric. Person-years of follow-up for each participant accrued from the date of return of the baseline questionnaire through the date of breast cancer diagnosis, death, the date of moving out of the cancer registry catchment area, or the end of follow-up (December 31, 2006), whichever came first.

Final regression models were adjusted for an *a priori* set of covariates including age at entry (continuous), race (White, Black, other, unknown), body mass index (BMI; kg/m^2, continuous), height (continuous), education level (< high school, high school, post high school, unknown), cigarette smoking (never, quit ≥ 5 years ago, quit 1–4 years ago, quit < 1 year ago, current smoking, unknown), alcohol intake (continuous), physical activity (weekly, 1–2 times/week, ≥ 3 times/week), familial history of breast cancer (no, yes, unknown), age at menarche (< 13, 13–14, ≥ 15, unknown), age at menopause (< 45, 45–49, 50–54, ≥ 55), age at first live birth (nulliparous, < 25, 25–29, ≥ 30), number of live births (0, 1–2, ≥ 3, unknown), hormone use (never, former, current, unknown), oral contraceptive use (never, ever, unknown), and number of previous breast biopsies (0, 1, 2, ≥ 3). These variables were used as covariates in the previous analysis of meat intake and breast cancer in a subset of the NIH-AARP Diet and Health Study (19). We additionally adjusted the analyses for total fat and fiber intakes (continuous) because they are risk factors for breast cancer (1) and were associated with breast cancer risk in our study population ($p < 0.10$). To adjust for energy intake, dietary intake variables were expressed in units per 1,000 kcal intake using the nutrient density method and risk models were additionally adjusted for total calorie intake. Models for meat intake were further adjusted for other meats, so that the sum of the meat variables in each model represented total meat consumption (32). Tests for linear trend across quintiles of meat groups were performed using the median values of each exposure category as an ordinal variable in the model. We tested heterogeneity of associations by cancer stage (*in-situ*, localized, regional/distant) and ER/PR status.

To account for the effect of mammographic screening on the association between meat intake and breast cancer risk, we additionally adjusted the analyses for history of at least one mammogram in the past three years – yes (n = 108,876; 56%), no (n = 15,297; 8%), and unknown (n = 69,569; 36%). We assigned “unknown” history of mammograms to women who left this question blank (n = 1,288) or did not complete the second questionnaire (n = 68,281). Compared with women who provided information on previous mammograms, those who did not provide such information were more likely to report younger age, current smoking, less physical activity, higher BMI, lower education, lower alcohol intake, a larger number of live births, earlier menopause, never use of hormones, fair or poor perceived general health, and higher fat (total and saturated) and lower fiber intakes ($p < 0.05$). We further performed the analyses restricting to the 108,876 women who reported at least one mammographic screening in the last three years.

We also evaluated the associations of nitrite intake from all animal sources and from processed meats separately and heme iron intake with breast cancer risk using the aforementioned analytic methods. In risk models of nitrite intake, we adjusted for or stratified by total vitamin C and heme iron intakes (median intake as a cut-point for stratification), because they are known to affect endogenous NOC formation. In addition, as a post hoc analysis to understand the observed associations by cancer stage, we evaluated associations between meat group, nitrite, and heme iron intakes and breast cancer risk by histological type including ductal (n = 6,265), lobular (n = 959), mixed (n = 692) and other (n = 1,389). Statistical analyses were performed using SAS version 9.3 (Cary, NC). Probability (p) values < 0.05 (two-sided) were considered statistically significant.

Results

The mean (standard deviation; SD) age at baseline was 62.0 (5.3) years. A total of 9,305 women developed breast cancer during an average follow-up of 9.4 years. Among these breast cancers, cancer stage was ascertained for 6,866 cases (74%) including 1,795 *in-situ* and 5,071 invasive cases (3,625 localized and 1,446 regional or distant cancers). Hormone receptor status was available for 4,628 cases (50% of cases in states where ER/PR status data were available) including 3,140 ER+/PR+, 678 ER+/PR−, 68 ER−/PR+, and 742 ER −/PR−.

The mean (SD) intake levels were 102.7 (71.6) g/d for total meat, 47.1 (40.6) g/d for red meat, and 13.2 (15.9) g/d for processed meat. On average, processed red meat intake [mean (SD) = 10.8 (13.0) g/d] constituted about 82% of total processed meat intake. Mean (SD) processed white meat intake was 3.6 (7.8) g/d, which constituted only 6% of total white meat intake [55.6 (50.5) g/d]. Higher total red meat and total processed meat intakes were associated with higher BMI, lower education level, current smoking, lower physical activity, oral contraceptive use, higher number of live births (red meat only), younger age at first live birth, earlier menopause, and never use of hormones ($p < 0.0001$) (Table 1). Women who reported higher red meat intake or higher processed meat intake were less likely to have had a mammographic screening in the past three years as well as previous breast biopsies ($p < 0.0001$). Higher total red and total processed meat intakes were both associated with higher intakes of total calories, total fat, saturated fat, and heme iron intakes and lower alcohol, vegetable, fruit, and total vitamin C and E intakes ($p < 0.0001$).

Associations between meat intakes and risk of breast cancer, overall and according to cancer stage and hormone receptor status, are shown in Table 2. Total meat, total processed meat, total red meat, fresh red meat, and total white meat intakes were not associated with overall breast cancer risk. Higher processed red meat intake was associated with higher overall risk of breast cancer ($HR_{Q5\ vs\ Q1}$ = 1.09, 95%CI = 1.01 – 1.17, p-trend = 0.05). When evaluating the risk by cancer stage, there was a positive association between total processed meat intake and risk of localized cancer (p-trend = 0.03). The risk was 1.14 times higher in the fourth (95%CI = 1.02 – 1.28) and fifth (95%CI = 1.01 – 1.27) quintiles of total processed meat intake compared with the risk in the lowest quintile. Total red meat intake was associated with higher risk of regional/distant cancer (p-trend = 0.02) with 1.21 (95%CI = 1.01 – 1.46), 1.26 (95%CI = 1.05 – 1.52) and 1.25 (95%CI = 1.03 – 1.52) times higher risk in the third, fourth, and fifth quintiles, respectively compared with the risk in the lowest quintile. A similar positive association was observed between fresh red meat intake and regional/distant cancer (p-trend = 0.02). Processed red meat intake was positively associated with localized breast cancer (p-trend < 0.0001). The risk was significantly higher in the fourth (HR = 1.25, 95%CI = 1.11 – 1.40) and fifth (HR = 1.27, 95%CI = 1.13 – 1.44) quintile of processed red meat intake compared with the lowest quintile. A similar yet statistically non-significant positive trend was observed between processed red meat intake and regional/distant cancer ($HR_{Q5\ vs\ Q1}$ = 1.19, 95%CI = 0.98 – 1.44, p-trend = 0.10). Total and processed white meat intakes were not associated with breast cancer risk overall and by cancer stage. Nonetheless, heterogeneity of risk associations with any of the meat group intakes by cancer stage did not reach statistical significance. None of the meat group intakes was associated with breast cancer risk by hormone receptor status. Additional adjustment of the analyses for previous mammogram status did not change the results (data not shown).

When restricting the analyses to the 108,879 women who reported at least one mammographic screening during the past three years (88% of the 124,173 women who provided previous mammographic screening information), the associations observed in the overall analyses remained unchanged (Supplemental Table S1). Risks of regional/distant cancer in the highest quintile of total red meat intake and fresh red meat intake were 1.32 (95%CI = 1.01 – 1.72) and 1.21 (95%CI = 0.94 – 1.56) times, respectively, higher than the risk in the lowest quintiles with a marginally non-significant positive trend across intake quintiles (p-trend = 0.09 for both). Higher processed red meat intake was associated with higher risk of localized cancer (p-trend = 0.0007) and regional/distant cancer (p-trend = 0.20) with 1.35 (95%CI = 1.16 – 1.59) and 1.31 (95%CI = 1.00 – 1.71) times, respectively, higher risk in the highest intake quintile compared with the risk in the lowest quintile.

Nitrite intakes from all animal sources, total processed meats, and processed red or white meats were not associated with overall risk of breast cancer (Table 3). Although we did not observe significant heterogeneity in associations with any of nitrite or heme iron intakes by cancer stage, higher nitrite intake from total processed meats was associated only with higher risk of localized cancer (p-trend = 0.01). An even stronger positive association with localized cancer was observed for nitrite intake from processed red meat (p-trend < 0.0001). Compared with the lowest quintile, the risk was 1.20 and 1.23 times higher in the fourth (95%CI = 1.07 – 1.35) and fifth (95%CI = 1.09 – 1.39) quintile of nitrite intake from processed red meat. These associations did not differ by low or high heme iron or total vitamin C intakes (data not shown). Higher heme iron intake was associated with higher risk of overall risk of breast cancer (p-trend = 0.02). Compared with the risk in the lowest quintile, women in the fourth and fifth quintiles of heme iron intake were at 1.12 (95%CI = 1.05 – 1.20) and 1.11 (95%CI = 1.03 – 1.19) times higher overall risk of breast cancer. Similar positive associations were observed with all cancer stages (p-trend = 0.04 – 0.05). Nitrite and heme iron intakes were not associated with breast cancer risk by hormone receptor status. Limiting the analyses to invasive cancers did not change any of the observed associations of meat group, nitrite, or heme iron intakes (data not shown).

Discussion

In this large prospective cohort study of postmenopausal female AARP members, we found that higher total and fresh red meat intakes were associated with regional/distant breast cancer. Processed red meat intake was positively associated with risk of localized breast cancer and marginally associated with regional/distant cancer. We also observed that higher nitrite intake from processed red meat was associated with higher risk of localized breast cancer, and higher heme iron intake was associated with increased risk of breast cancer, regardless of cancer stage. Previous epidemiologic studies evaluating the association between meat intake and breast cancer risk have shown conflicting findings. In a meta-analysis of the previous pooling project of eight cohort studies (33) and additional nine studies published between 2004 and 2009, there were positive non-statistically significant summary associations between red or processed meat consumptions and breast cancer risk among postmenopausal women (3). However, heterogeneities in study designs across studies included in this meta-analysis, such as dietary assessment methods, and variable definitions and categorizations, were evident. For example, meat consumption is accompanied by fat intake, especially saturated fat, and high fat intake is a suggestive risk factor for postmenopausal breast cancer as determined by a comprehensive expert literature review by the World Cancer Research Fund/American Institute for Cancer Research (1). However, many studies included in this meta-analysis did not consider fat intake in the analyses. In the NIH-AARP study population, total fat intake had a positive association (34) and dietary fiber intake had an inverse association (35) with risk of breast cancer. In our analysis, in which we included both total fat and dietary fiber intakes as covariates, total meat intake was not associated with overall breast cancer risk. However, when we removed these variables, we observed a positive association between total meat intake and overall breast cancer risk (p-trend = 0.01). Similarly, additional adjustment for total fat and dietary fiber intakes attenuated the positive association between processed red meat intake and overall breast cancer risk (p-trend = 0.01 vs. 0.05). Two of the four prospective studies among postmenopausal women (19, 21, 36, 37) included in the aforementioned meta-analysis of meat intake and breast cancer risk (3) adjusted the analysis for fat intake. One study that considered fat intake in the analysis found positive associations between total, red, and processed meat intakes and breast cancer risk, but other three studies did not find associations. Thus, adjustment for fat intake may not explain inconsistent results among the previous studies. None of the studies included in this meta-analysis adjusted the analyses for fiber intake.

Breast cancer is a heterogeneous disease with differing etiologies. Yet, the association between meat intake and breast cancer risk by hormone receptor status has been evaluated in only two cohort studies – among Swedish women and among African American women in the United States – and neither found associations (38, 39). Our finding of no association between meat group intakes and breast cancer risk by ER/PR status are in agreement with these previous findings. To our knowledge, no previous epidemiologic study considered cancer stage in the analysis of meat intake and breast cancer risk. In the current study, we found that higher total red meat intake was associated with higher risk of regional/distant cancer. These associations may have reflected the positive association between heme iron intake and breast cancer risk. Higher total processed meat intake was associated with higher risk of localized cancer but not regional/distant cancer, which may be partly due to the nitrite content of processed meat. The association between higher processed red meat intake and risk of localized as well as regional/distant cancers may be explained by both added nitrite and heme iron in processed meats. Ductal cancers are more prevalent in localized cancer, and lobular cancers are more prevalent in regional/distant cancer (Supplemental Table S2); however, we did not see clear patterns in risk associations between meat intake groups, nitrite, or heme iron intake and histologic types of breast cancer (Supplemental Table S3). Future studies are warranted to better understand etiologic mechanisms behind the link between red and processed meat intakes and risk of localized or regional/distant breast cancer.

Endogenously produced NOC due to high nitrite intake from processed meat is one possible biologic mechanism behind the association between red or processed meat intake and breast cancer risk (40, 41). Heme iron, which is found at high levels in red meat, also enhances endogenous NOC formation and has been implicated in the etiology of breast cancer (8, 42, 43). Processed red meats are rich in added nitrite/nitrate, amines, and heme iron. In our study, nitrite intake from processed red meat and heme iron intake were moderately correlated ($r = 0.57$). The combination of higher intake of nitrate/nitrite with an amine source and heme iron has been shown to increase the endogenous formation of NOC in animal and human biomonitoring studies (18, 40). Another possible explanation of the association between red and processed meat intake and breast cancer risk is that hormonal steroids, such as 17β-estradiol (E2), for growth stimulation in beef cattle may play a role in the etiology of breast cancer. A study in Japan, where exogenous hormone use in animal husbandry is uncommon, reported that beef imported from the United States contained about 600 times and 10 times higher median concentrations of E2 and estrone (E1), respectively, compared with levels in Japanese beef (44). Higher consumption of estrogen-rich beef due to hormone application might facilitate estrogen accumulation in the body and thus affect women’s risk for breast cancer.

One of the strengths of the present study is the large number of incident breast cancers accrued. In the previous analysis in the NIH-AARP Diet and Health Study, red and processed meat intakes were not associated with breast cancer risk (19). This previous analysis was performed in the subset of women who provided meat preparation methods in the second questionnaire, whereas the current study was performed within the entire baseline cohort. Therefore, our study included a considerably larger number of breast cancer cases than the number in the previous analysis, which enabled us to consider cancer stage and hormone receptor status in the analysis, although this information was not available for all incident breast cancers. We also expanded the analyses to nitrite and heme iron intakes from red/processed meats to evaluate their relative contributions to the association between meat intake and breast cancer risk. However, because of the large number of analyses for multiple dietary exposures and stratifications, statistically significant associations may have been found by chance. Although our analyses were based on the *a priori* hypotheses (except for post hoc stratification by histologic type), our findings need to be interpreted with cautions. The use of validated FFQ to estimate dietary nitrite intake from specific food groups is also a strength. Our estimates of nitrite intake were based on the database that was developed from the published literature on nitrite levels in foods (27). We also compared this estimated nitrite intake from processed meats with the intake estimate based on nitrite levels measured in a selection of U.S. processed meats (4, 45). Intakes using both estimates were highly correlated (r = 0.99) (4, 45). However, measurement error in dietary intake assessments based on FFQs is well described (46). Yet, due to the prospective study design, misclassification of dietary intake would be expected to be nondifferential and thus could have attenuated associations. Lastly, we did not include nitrate in drinking water in the analysis because we did not have individual drinking water source information. However, when we excluded women (2.6%) who lived in regions where exposure to high nitrate concentrations (maximum contaminant level = 10 mg/L as nitrate-nitrogen or greater) in ground water was highly probable based on the US Geological Survey model (47), our findings did not change.

In summary, higher intake of processed red meat was associated with higher overall risk of postmenopausal breast cancer in this large prospective cohort study. Red meat intake was positively associated with risk of regional/distant cancer and processed red meat intake was positively associated with localized cancer and marginally associated with regional/distant cancer. These associations may be partly due to the observed positive association between nitrite intake from processed red meat and risk of localized cancer and the positive association between heme iron intake and breast cancer regardless of cancer stage. These findings should be replicated in future studies considering cancer stage and receptor type.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Novelty and Impact

Associations between red and processed meat intakes and breast cancer have been inconclusive with few studies considering whether risk associations differ by cancer stage and hormone receptor status. We found that higher red meat intake was associated with regional/distant breast cancer. Higher processed red meat intake was also associated with localized cancer. Heme iron and nitrite may partly contribute to these observed associations. No heterogeneity was observed in risk associations by hormone receptor status.

Table 1

Distribution of demographic, lifestyle, reproductive, medical and dietary factors across quintiles of red and processed meat intake among 193,742 postmenopausal women. NIH-AARP Diet and Health Study

	All	Red meat intake[a] quintiles (Q)					Processed meat intake[a] quintiles (Q)				
		Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
Age, mean (years)[b]	62.0	62.3	62.3	62.2	61.9	61.5	62.2	62.0	61.9	61.9	62.1
Race, non-Hispanic Black (%)[b]	5.7	8.2	6.3	5.4	4.3	4.1	5.7	4.9	5.1	5.7	7.0
BMI, mean (kg/m^2)[b]	26.8	25.4	26.4	26.8	27.4	28.2	25.4	26.4	27.0	27.5	28.0
Height, mean (cm)[b]	163.2	163.1	163.3	163.3	163.3	163.2	163.1	163.3	163.3	163.4	163.2
Education, > high school (%)[b]	67.1	74.9	69.4	66.2	64.2	60.9	74.1	70.2	66.9	64.4	59.9
Smoking, current (%)[b]	14.8	8.6	12.2	14.7	17.3	21.1	9.8	13.4	45.5	16.7	18.5
Physical activity, ≥3 times/wk (%)[b]	41.7	54.2	45.4	40.6	36.7	31.4	52.2	43.5	39.5	37.4	35.7
Familial history of breast cancer (%)[c]	12.9	13.0	13.0	13.3	12.9	12.5	13.0	13.2	12.7	12.9	12.8
Oral contraceptive use, ever (%)[b]	39.4	37.9	38.7	39.2	40.2	41.0	38.5	40.4	40.4	40.1	37.7
Age at menarche, ≥13 y (%)[c]	51.1	51.2	51.4	51.1	51.7	50.1	51.2	51.3	51.4	51.0	50.5
Parity, nulliparous (%)[b]	15.0	16.1	14.3	13.7	13.7	13.3	15.4	14.0	13.8	13.4	14.6
Number of live births, ≥3 (%)[b]	49.1	44.6	49.0	50.2	50.6	51.2	45.7	49.3	50.2	50.7	49.7
Age at first live birth, ≥30 y (%)[b]	5.8	6.5	5.9	5.8	5.7	5.2	6.7	5.8	5.5	5.7	5.5
Age at menopause, ≥50 y (%)[b]	40.0	43.0	41.1	40.0	38.9	36.9	42.7	40.9	39.3	39.1	38.0
Ever hormone use (%)[b]	54.7	55.5	56.0	55.3	54.7	52.0	55.7	57.5	56.0	54.1	50.2
Previous mammogram (%)[b,d]	87.7	89.1	89.0	88.3	87.0	84.9	88.3	88.9	88.0	87.0	86.2
Previous breast biopsies (%)[b]	24.1	24.4	24.4	24.2	24.0	23.6	24.3	24.2	24.1	24.3	23.7
Dietary intake (mean)											
Red meat (g/1,000 kcal)[b]	29.5	7.4	17.6	26.4	36.8	59.3	14.6	23.9	29.9	35.5	43.7
Processed meat (g/1,000 kcal)[b]	8.3	3.5	5.6	7.6	10.0	14.7	1.1	3.3	5.8	9.5	21.7
Total calorie (kcal)[b]	1,570	1,529	1,531	1,555	1,599	1,638	1,542	1,521	1,549	1,599	1,641
Vegetables (g/1000 kcal)[b]	188.0	227.4	190.1	180.5	175.5	168.5	227.0	190.6	181.1	175.7	167.6

	All	Red meat intake[a] quintiles (Q)					Processed meat intake[a] quintiles (Q)				
		Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
Fruits (g/1000 kcal)[b]	234.3	317.0	263.5	230.7	198.3	162.2	298.4	249.6	225.4	207.1	191.1
Total fat (g/1000kcal)[b]	33.3	27.0	31.0	33.5	35.9	39.2	28.4	31.9	34.0	35.5	36.9
Saturated fat (g/1000kcal)[b]	10.3	8.0	9.5	10.4	11.2	12.4	8.6	9.9	10.6	11.0	11.4
Alcohol (g/d)[b]	5.8	5.5	6.3	6.3	5.9	5.2	5.9	6.5	6.2	5.7	4.9
Total vitamin C (mg/d)[b,e]	488.7	638.5	522.5	471.5	426.2	384.9	615.4	506.8	462.9	436.9	421.7
Total vitamin E (mg/d)[b,e]	86.4	107.8	91.2	84.5	77.8	71.1	104.0	89.5	82.9	78.8	77.1
Heme iron (mg/1000kcal)[b]	161.6	61.6	103.4	141.9	192.2	308.7	84.9	127.1	157.3	188.7	249.8

[a]Intake density (g/1,000 kcal)

[b]$p < 0.05$ by quintiles of total red meat and total processed meat intakes

[c]$p < 0.05$ by quintiles of total red meat intake

[d]At least one mammographic screening in the past three years. Information was collected in the second questionnaire installed within six months following the baseline questionnaire.

[e]Intake from diet and dietary supplements

Table 2

Multivariate hazard ratios (95% confidence intervals)[a] of the association between quintiles of selected meat group intakes and breast cancer risk according to stage and ER/PR status, NIH-AARP Diet and Health Study

	Quintiles (Q) of meat group intake[b]					*p*-trend	*p*-hetero[c]
	Q1	Q2	Q3	Q4	Q5		
Total meat							
Median (g/1,000kcal)	28.1	46.3	61.1	77.7	106.9		
Total No. of cases	1,740	1,874	1,880	1,943	1,859		
All breast cancer	1.00	1.05 (0.98 – 1.12)	1.06 (0.99 – 1.13)	**1.09 (1.02 – 1.17)**	1.06 (0.99 – 1.13)	0.10	
In-situ	1.00	1.05 (0.90 – 1.22)	1.15 (0.99 – 1.34)	**1.20 (1.03 – 1.40)**	1.06 (0.90 – 1.24)	0.30	
Localized	1.00	1.08 (0.97 – 1.20)	**1.14 (1.03 – 1.27)**	1.07 (0.96 – 1.19)	1.09 (0.97 – 1.22)	0.30	
Regional/distant	1.00	1.07 (0.90 – 1.28)	1.12 (0.94 – 1.33)	1.09 (0.92 – 1.30)	1.15 (0.96 – 1.37)	0.16	0.74
ER+/PR+	1.00	1.05 (0.94 – 1.18)	1.06 (0.94 – 1.19)	**1.14 (1.02 – 1.28)**	1.00 (0.99 – 1.13)	0.83	
ER−/PR−	1.00	0.86 (0.68 – 1.09)	0.99 (0.79 – 1.25)	0.94 (0.74 – 1.19)	0.87 (0.68 – 1.11)	0.42	0.35
Total processed meat							
Median (g/1,000kcal)	1.1	3.3	5.7	9.4	18.4		
Total No. of cases	1,819	1,838	1,822	1,943	1,883		
All breast cancer	1.00	0.99 (0.93 – 1.06)	0.98 (0.92 – 1.05)	1.05 (0.98 – 1.13)	1.04 (0.97 – 1.12)	0.09	
In-situ	1.00	0.97 (0.83 – 1.13)	**1.21 (1.04 – 1.42)**	1.03 (0.88 – 1.21)	1.08 (0.92 – 1.27)	0.46	
Localized	1.00	1.07 (0.96 – 1.19)	1.05 (0.94 – 1.17)	**1.14 (1.02 – 1.28)**	**1.14 (1.01 – 1.27)**	**0.03**	
Regional/distant	1.00	0.98 (0.82 – 1.17)	0.98 (0.82 – 1.18)	1.15 (0.97 – 1.38)	1.10 (0.92 – 1.32)	0.10	0.74
ER+/PR+	1.00	0.94 (0.84 – 1.05)	0.90 (0.80 – 1.01)	0.98 (0.87 – 1.10)	0.96 (0.85 – 1.09)	0.94	
ER−/PR−	1.00	1.01 (0.80 – 1.28)	0.89 (0.70 – 1.14)	0.84 (0.66 – 1.08)	1.01 (0.79 – 1.29)	0.91	0.99
Total red meat							
Median (g/1,000kcal)	7.8	17.6	26.4	36.6	54.7		
Total No. of cases	1,758	1,865	1,926	1,939	1,817		
All breast cancer	1.00	1.04 (0.97 – 1.11)	1.06 (0.99 – 1.14)	**1.10 (1.02 – 1.18)**	1.04 (0.97 – 1.13)	0.27	
In-situ	1.00	1.10 (0.94 – 1.28)	1.07 (0.91 – 1.26)	**1.21 (1.02 – 1.42)**	1.04 (0.87 – 1.24)	0.63	
Localized	1.00	1.06 (0.96 – 1.19)	**1.13 (1.01 – 1.26)**	**1.17 (1.04 – 1.31)**	1.04 (0.92 – 1.18)	0.53	
Regional/distant	1.00	1.08 (0.90 – 1.30)	**1.21 (1.01 – 1.46)**	**1.26 (1.05 – 1.52)**	**1.25 (1.03 – 1.52)**	**0.02**	0.20
ER+/PR+	1.00	1.04 (0.93 – 1.17)	1.10 (0.98 – 1.24)	1.09 (0.97 – 1.23)	0.97 (0.85 – 1.11)	0.59	

	Quintiles (Q) of meat group intake[b]					*p*-trend	*p*-hetero[c]
	Q1	Q2	Q3	Q4	Q5		
ER−/PR−	1.00	1.07 (0.85 – 1.35)	0.85 (0.66 – 1.09)	0.89 (0.69 – 1.15)	0.95 (0.73 – 1.24)	0.47	0.42
Fresh red meat (unprocessed)							
Median (g/1,000kcal)	5.5	13.0	19.7	28.0	43.4		
Total No. of cases	1,795	1,820	1,922	1,859	1,910		
All breast cancer	1.00	1.00 (0.93 – 1.07)	1.02 (0.95 – 1.09)	1.07 (1.00 – 1.15)	1.03 (0.96 – 1.11)	0.19	
In-situ	1.00	0.99 (0.85 – 1.15)	1.02 (0.87 – 1.19)	1.08 (0.92 – 1.26)	1.01 (0.85 – 1.20)	0.67	
Localized	1.00	1.07 (0.96 – 1.19)	1.04 (0.93 – 1.17)	1.12 (1.00 – 1.25)	1.01 (0.89 – 1.14)	0.99	
Regional/distant	1.00	0.99 (0.82 – 1.18)	1.15 (0.96 – 1.37)	**1.23 (1.03 – 1.48)**	1.16 (0.96 – 1.41)	**0.02**	0.48
ER+/ER+	1.00	1.05 (0.94 – 1.18)	1.06 (0.94 – 1.20)	1.13 (1.00 – 1.27)	1.04 (0.92 – 1.19)	0.50	
ER−/PR−	1.00	1.04 (0.82 – 1.31)	0.90 (0.70 – 1.14)	0.82 (0.63 – 1.06)	1.03 (0.80 – 1.34)	0.93	0.19
Processed red meat							
Median (g/1,000kcal)	1.1	2.8	4.8	7.6	14.5		
Total No. of cases	1,759	1,922	1,807	1,929	1,888		
All breast cancer	1.00	1.07 (1.00 – 1.14)	1.01 (0.94 – 1.09)	**1.10 (1.02 – 1.18)**	**1.09 (1.01 – 1.17)**	**0.05**	
In-situ	1.00	0.98 (0.84 – 1.15)	1.13 (0.97 – 1.33)	1.14 (0.97 – 1.34)	1.07 (0.90 – 1.28)	0.41	
Localized	1.00	1.11 (0.99 – 1.24)	1.09 (0.97 – 1.22)	**1.25 (1.11 – 1.40)**	**1.27 (1.13 – 1.44)**	**<0.0001**	
Regional/distant	1.00	1.10 (0.92 – 1.31)	1.07 (0.89 – 1.29)	1.14 (0.95 – 1.38)	1.19 (0.98 – 1.44)	0.10	0.28
ER+/ER+	1.00	1.06 (0.94 – 1.18)	0.97 (0.86 – 1.10)	1.03 (0.91 – 1.17)	0.99 (0.87 – 1.13)	0.67	
ER−/PR−	1.00	1.15 (0.92 – 1.44)	0.84 (0.66 – 1.08)	0.83 (0.64 – 1.08)	0.83 (0.64 – 1.09)	0.05	0.28
Total white meat							
Median (g/1,000kcal)	9.9	19.5	29.2	42.2	69.1		
Total No. of cases	1,794	1,820	1,922	1,859	1,910		
All breast cancer	1.00	0.98 (0.92 – 1.05)	1.04 (0.98 – 1.11)	1.00 (0.93 – 1.06)	1.03 (0.97 – 1.11)	0.27	
In-situ	1.00	1.03 (0.88 – 1.20)	1.16 (1.00 – 1.35)	1.07 (0.92 – 1.25)	1.11 (0.96 – 1.30)	0.21	
Localized	1.00	1.06 (0.95 – 1.18)	1.09 (0.98 – 1.21)	1.02 (0.92 – 1.14)	1.07 (0.96 – 1.19)	0.45	
Regional/distant	1.00	0.94 (0.80 – 1.12)	0.92 (0.78 – 1.09)	1.01 (0.85 – 1.19)	1.02 (0.86 – 1.20)	0.49	0.88
ER+/PR+	1.00	1.01 (0.90 – 1.13)	1.03 (0.92 – 1.15)	0.99 (0.88 – 1.11)	1.02 (0.91 – 1.15)	0.79	
ER−/PR−	1.00	0.83 (0.65 – 1.06)	0.95 (0.75 – 1.20)	1.05 (0.83 – 1.31)	0.90 (0.71 – 1.14)	0.93	0.61

[a]Adjusted for age (continuous), race (White, Black, other), BMI (continuous), height (continuous), education level (< high school, high school, > high school), cigarette smoking (never, quit ≥ 5 y ago, quit 1–4 y ago, quit < 1 y ago, current smoking), alcohol intake (continuous), physical activity (low, moderate, high), familial history of breast cancer (yes/no), age at menarche (< 13, 13–14, ≥15), age at menopause (< 45, 45–49, 50–54, ≥55), age at first live birth (nulliparous, < 25, 25–29, ≥30), number of live births (nulliparous, 1–2, ≥3), hormone use (never, former, current), oral contraceptive use (never,

ever), numbers of previous breast biopsy (0, 1, 2, ≥3), total calorie intake (continuous), total fat intake (continuous), fiber intake (continuous), and intake of other types of meat (white, red, processed, processed red, processed white, unprocessed, unprocessed red, unprocessed white meats, if applicable).

[b] Intake density (g/1,000 kcal)

[c] *p* values for heterogeneity of the associations among cancer stages (*in situ*, localized, and regional/distant) and among ER/PR status (ER+/PR+, ER+/PR−, ER−/PR+, and ER−/PR−) using medians of quintile intakes as continuous variables.

Table 3

Multivariate hazard ratios (95% confidence intervals)[a] for the association between intake levels of nitrite and heme iron and breast cancer risk according to stage and ER/PR status, NIH-AARP Diet and Health Study

	Quintiles (Q) of nitrite/heme iron intake[b]					*p*-trend	*p*-hetero[c]
	Q1	Q2	Q3	Q4	Q5		
Nitrite							
From animal sources							
Median (mg/1,000kcal)	0.09	0.14	0.19	0.24	0.34		
Total No. of cases	1,785	1,912	1,825	1,945	1,938		
Breast cancer	1.00	1.01 (0.95 – 1.08)	1.00 (0.93 – 1.07)	0.98 (0.91 – 1.05)	0.99 (0.92 – 1.05)	0.39	
In-situ	1.00	0.99 (0.85 – 1.15)	1.00 (0.86 – 1.17)	1.08 (0.93 – 1.26)	0.99 (0.84 – 1.16)	0.89	0.56
Localized	1.00	1.10 (0.98 – 1.22)	1.10 (0.98 – 1.22)	1.12 (1.00 – 1.26)	1.10 (0.98 – 1.23)	0.20	
Regional/distant	1.00	1.19 (1.00 – 1.41)	1.02 (0.86 – 1.22)	1.13 (0.95 – 1.35)	0.98 (0.82 – 1.18)	0.41	
ER+/PR+	1.00	1.03 (0.92 – 1.15)	0.91 (0.81 – 1.02)	1.03 (0.92 – 1.16)	0.96 (0.85 – 1.08)	0.55	0.90
ER−/PR−	1.00	1.14 (0.90 – 1.43)	0.80 (0.62 – 1.03)	1.15 (0.91 – 1.45)	0.97 (0.76 – 1.25)	0.86	
From processed meat							
Median (mg/1,000kcal)	0.01	0.03	0.05	0.07	0.15		
Total No. of cases	1,800	1,838	1,818	2,011	1,838		
Breast cancer	1.00	0.99 (0.92 – 1.06)	0.99 (0.92 – 1.05)	0.98 (0.92 – 1.05)	0.98 (0.91 – 1.06)	0.71	
In-situ	1.00	1.09 (0.94 – 1.28)	**1.25 (1.07 – 1.47)**	**1.26 (1.07 – 1.49)**	1.02 (0.85 – 1.22)	0.59	0.30
Localized	1.00	1.05 (0.94 – 1.17)	1.05 (0.94 – 1.18)	**1.21 (1.08 – 1.35)**	**1.16 (1.02 – 1.31)**	**0.01**	
Regional/distant	1.00	0.89 (0.75 – 1.06)	0.99 (0.83 – 1.18)	1.05 (0.88 – 1.26)	1.03 (0.85 – 1.24)	0.35	
ER+/PR+	1.00	0.94 (0.84 – 1.06)	0.91 (0.81 – 1.02)	1.05 (0.93 – 1.18)	0.91 (0.80 – 1.03)	0.42	0.27
ER−/PR−	1.00	1.03 (0.82 – 1.30)	0.89 (0.70 – 1.13)	0.91 (0.71 – 1.16)	0.86 (0.66 – 1.11)	0.17	
From processed red meat							
Median (mg/1,000kcal)	0.005	0.02	0.04	0.07	0.13		
Total No. of cases	1,791	1,873	1,804	1,963	1,874		
Breast cancer	1.00	1.01 (0.95 – 1.09)	1.03 (0.97 – 1.11)	1.03 (0.96 – 1.10)	0.99 (0.92 – 1.06)	0.96	
In-situ	1.00	1.03 (0.89 – 1.20)	1.09 (0.93 – 1.28)	**1.19 (1.01 – 1.40)**	0.98 (0.82 – 1.17)	0.64	0.14
Localized	1.00	1.06 (0.95 – 1.18)	1.07 (0.96 – 1.20)	**1.20 (1.07 – 1.35)**	**1.23 (1.09 – 1.39)**	**<0.0001**	
Regional/distant	1.00	0.99 (0.83 – 1.18)	1.03 (0.86 – 1.23)	1.02 (0.85 – 1.23)	1.07 (0.88 – 1.30)	0.41	

	Quintiles (Q) of nitrite/heme iron intake[b]					*p*-trend	*p*-hetero[c]
	Q1	Q2	Q3	Q4	Q5		
ER+/ER+	1.00	1.03 (0.92 – 1.15)	0.96 (0.85 – 1.08)	1.07 (0.95 – 1.21)	0.95 (0.83 – 1.07)	0.36	0.40
ER−/PR−	1.00	1.12 (0.89 – 1.40)	0.93 (0.73 – 1.19)	0.87 (0.67 – 1.12)	0.92 (0.71 – 1.20)	0.27	
Heme iron							
Median (mg/1,000kcal)	44.2	93.4	138.4	194.5	303.5		
Total No. of cases	1,709	1,896	1,868	1,948	1,884		
Breast cancer	1.00	**1.08 (1.01 – 1.16)**	**1.07 (1.00 – 1.15)**	**1.12 (1.05 – 1.20)**	**1.11 (1.03 – 1.19)**	**0.02**	
In-situ	1.00	1.15 (0.99 – 1.34)	1.09 (0.93 – 1.27)	1.15 (0.98 – 1.35)	**1.22 (1.03 – 1.44)**	**0.05**	0.56
Localized	1.00	1.10 (0.98 – 1.22)	**1.15 (1.03 – 1.29)**	**1.15 (1.02 – 1.28)**	**1.16 (1.03 – 1.30)**	**0.04**	
Regional/distant	1.00	**1.23 (1.03 – 1.47)**	1.15 (0.96 – 1.38)	**1.28 (1.07 – 1.54)**	**1.27 (1.05 – 1.53)**	**0.04**	
ER+/PR+	1.00	**1.16 (1.03 – 1.30)**	1.10 (0.97 – 1.24)	**1.20 (1.06 – 1.35)**	1.07 (0.94 – 1.21)	0.63	0.44
ER−/PR−	1.00	1.00 (0.79 – 1.27)	0.89 (0.69 – 1.14)	0.93 (0.73 – 1.20)	1.06 (0.83 – 1.37)	0.58	

[a] Adjusted for age (continuous), race (White, Black, other), BMI (continuous), height (continuous), education level (< high school, high school, > high school), cigarette smoking (never, quit ≥ 5 y ago, quit 1–4 y ago, quit < 1 y ago, current smoking), alcohol intake (continuous), physical activity (low, moderate, high), familial history of breast cancer (yes/no), age at menarche (< 13, 13–14, ≥15), age at menopause (< 45, 45–49, 50–54, ≥55), age at first live birth (nulliparous, < 25, 25–29, ≥30), number of live births (nulliparous, 1–2, ≥3), hormone use (never, former, current), oral contraceptive use (never, ever), numbers of previous breast biopsy (0, 1, 2, ≥3), total calorie intake (continuous), total fat (continuous) and fiber (continuous) intake.

[b] Intake density (mg/1,000 kcal)

[c] *p* values for heterogeneity of the associations among cancer stages (*in situ*, localized, and regional/distant) and among ER/PR status (ER+/PR+, ER+/PR−, ER−/PR+, and ER−/PR−) using medians of quintile intakes as continuous variables.