Published in final edited form as:

Breast Cancer Res Treat. 2011 October ; 129(3): 919–928. doi:10.1007/s10549-011-1538-7.

Well-done meat intake and meat-derived mutagen exposures in relation to breast cancer risk: the Nashville Breast Health Study

Zhenming Fu¹, Sandra L. Deming¹, Alecia M. Fair^{1,2}, Martha J. Shrubsole¹, Debra M. Wujcik³, Xiao-Ou Shu¹, Mark Kelley⁴, and Wei Zheng¹

¹Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN

²Department of Surgery, Meharry Medical College, Nashville, TN

³Meharry/Vanderbilt Cancer Partnership, Nashville General Hospital, Nashville, TN

⁴Division of Surgical Oncology and Endocrine Surgery, Department of Surgery, Vanderbilt University School of Medicine, Nashville, TN

Abstract

Previous studies of the association of meat intake and meat-derived mutagen exposure with breast cancer risk have produced inconsistent results. We evaluated this association in a population-based case-control study of incident breast cancer conducted in Nashville, United States, including 2,386 breast cancer cases and 1,703 healthy women controls. Telephone interviews were conducted to obtain information related to meat intake including amount, cooking methods, and doneness levels, as well as other known or hypothesized risk factors for breast cancer. Unconditional logistic regression was used to derive odds ratios (ORs) after adjusting for potential confounders. High intake of red meat was associated with a significantly elevated risk of breast cancer (*P-trend* <0.001). The association was particularly strong for high intake of well-done red meat (*P-trend* <0.001), with an adjusted OR of 1.5 (95% CI = 1.3–1.9) for the highest versus the lowest quartile. Associations between red meat and breast cancer risk were slightly stronger for postmenopausal women than for premenopausal women. Meat-derived mutagens such as 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline and 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline, were significantly associated with increased breast cancer risk among postmenopausal women only (*P-trend* = 0.002 and 0.003, respectively). The results from this study provide strong support for the hypotheses that high red meat intake and meat-derived mutagen exposure may be associated with an increase in breast cancer risk.

Keywords

Dietary factors; nutrition epidemiology; meat-derived mutagens; heterocyclic amines breast cancer

Introduction

High meat intake has been suggested to be associated with an increased risk of breast cancer [1]. Results from previous studies, however, have been inconsistent. An early meta-analysis of 14 cohort studies and 31 case-control studies showed a positive association [2]. In

Corresponding author: Wei Zheng, M.D., Ph.D., Vanderbilt Epidemiology Center, 2525 West End Avenue, 8th floor, Suite 800, Nashville, TN 37203-1738, wei.zheng@vanderbilt.edu.

Disclosure of Potential Conflicts of Interest

None of the authors declare any conflicts of interest.

contrast, a pooled analysis of nine cohort studies did not find apparent evidence for an association [3]. Results from more recent studies are also conflicting [4–17]. While some studies support a positive association [4, 6–9, 11], others do not [10, 12–15, 17].

One possible explanation for this inconsistency is that the association between meat consumption and breast cancer may differ by meat type, meat cooking method, and meat doneness [18]. The amount of meat-derived mutagens, such as heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs), is related to the methods of cooking as well as cooking temperatures and duration. These mutagens have been shown to induce mammary gland tumors in animal models [19, 20]. Epidemiologic studies, however, have been inconsistent in the association between consumption of well-done meat intake (and thus exposure to meat-derived mutagens) and risk of breast cancer. Most previous studies did not comprehensively assess meat intake by cooking methods and doneness levels, and thus measurement error is a major concern. Herein, we describe findings from a large population-based case-control study conducted in Tennessee, USA, to evaluate the association of breast cancer risk with well-done meat intake and meat-derived mutagen exposures.

Subjects and Methods

Nashville Breast Health Study

NBHS is a population-based case-control study conducted between February 1, 2001 and December 31, 2008, in Nashville, Tennessee. Included in the study were 2,386 breast cancer cases and 1,703 healthy women controls. Eligible cases were women who were newly diagnosed with primary breast cancer (invasive ductal or ductal carcinoma *in situ*) between the ages of 25 and 75 years old. They had no prior history of cancer other than non-melanoma skin cancer. The majority of participants (92%) were residents of the Nashville eight-county metropolitan area. Eligibility criteria for study participation included a resident telephone, English speaking and capable of providing informed consent. Control subjects had virtually identical criteria to cases with the exception that they had no prior breast cancer diagnosis. Included in the study were 1,482 control subjects who were identified by random digit dialing (RDD) of households in the same eight counties including and surrounding the Nashville metro area. Controls identified using the RDD protocol accounted for 87% of all control subjects included in the study. The remaining controls for the study were identified mostly from women who received a screening mammography with a normal finding (equivalent to Breast Imaging Reporting and Data System 1 or 2 (BI-RADS 1 and 2) [21]. This study was approved by the Institutional Review Boards of Vanderbilt University, the Tennessee State Cancer Registry, and the local area hospitals where recruitment was conducted.

The NBHS is ongoing and additional cases and controls are being recruited. Current participation rates are approximately 62% for eligible cases and 71% for eligible controls. The main reasons non-participation for cases included poor health, participation refusal, inability to contact the patient, and for controls included visiting other cities, changes of address, loss of communication, and refusal to participate. A total of 4,117 subjects (2,409 cases and 1,708 controls) successfully completed a telephone interview, including the questionnaire for well-done meat intake. Among these, 3,620 participants completed a self-administered food frequency questionnaire (FFQ) developed for a similar southern United States population [22, 23].

Assessment of meat intake

Participants were asked to complete an interviewer-administered telephone interview to obtain information regarding medication use, demographics, medical history, and selected lifestyle factors, including questions about usual intake frequency and portion size of 11 meats in the previous year before interview (for controls) or cancer diagnosis (for cases). Intake of meat includes hamburgers or cheeseburgers from fast food, hamburgers or cheeseburgers not from fast food, beef steaks, pork chops or ham steaks, bacon, sausage, hot dogs/franks, chicken, fish, meat gravies made with drippings, and short ribs or spareribs. Data were obtained regarding intake frequency and usual portion size of each meat item. For each meat item (except hamburgers or cheeseburgers from fast food and meat gravies made with drippings), the proportion of time the meat was prepared using each of the cooking methods was estimated for: oven-broiled or oven-baked, grilled or barbecued, pan fried, deep fried (for chicken and fish), and all other ways.

Participants were also asked to report their usual preference level of meat doneness over the previous year before interview (for controls) or cancer diagnosis (for cases) by using a series of color photographs of each meat item, labeled with a number between 1 and 3 to represent increasing levels of doneness. Photos were displayed for seven meat items: hamburger patties, beef steaks, pork chops, bacon, grilled chicken, pan fried chicken, and pan fried or grilled fish. For each meat item, participants were asked whether each of their cooked items usually looked less than one, about the same as one, about the same as two, about the same as three, or more than three. There were 5 categories in total, representing rare, medium or just done, well-done, very well-done, and extremely very well-done. All participants who completed questions on food doneness had the photograph booklet in front of them during the telephone interview. The questionnaire for our study was modified from those used in previous studies [11, 24], including our previous studies for breast cancer [25] and colorectal polyps [23].

Statistical Analysis

Meat was classified by type (red, chicken, fish, and processed). Intakes and exposure levels were categorized into 4 to 5 groups for statistical analysis. Red meat included hamburgers, cheeseburgers, beef steaks, pork chops, ham steaks, and ribs (short ribs or spareribs). Processed meat included bacon, sausage, and hotdogs/franks. Total meat included all red meat items, chicken, fish, processed meat, and meat gravies made with drippings. The software CHARRED, which was developed by the U.S. National Cancer Institute [24], was used to estimate exposure levels to meat-derived mutagens, including 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), benzo[*a*]pyrene, and the overall mutagenicity index, as measured by revertants colony. Average values of mutagen levels of pork chops and ham in the CHARRED database were used for the item “pork chops or ham steaks” in our questionnaire, and average values of broiled chicken and baked chicken in the database were used for the item “chicken cooked by oven broiled or oven baked” in our questionnaire. Sausages are typically cooked to well-done level, and thus mutagens for well-done sausages from the CHARRED database were used. No carcinogen information is available in the CHARRED database for hotdogs, fish, or short ribs. Therefore, these foods were not included in the calculation of HCAs and PAHs exposure level.

Generalized linear models and Mantel-Haenszel χ^2 tests were used to compare the distribution of demographic characteristics and known risk factors for breast cancer between case and control groups with adjustment for age when appropriate. Unconditional logistic regression models were used to estimate odds ratios (OR) and their 95% confidence

intervals (CI) for the association between exposures and breast cancer. The quartile or quintile distributions in the controls were used to determine the cut-off points for categorization of the exposure variables. Adjusted variables in multivariate analysis included age (<40, 40–49, 50–59, 60), menopausal status, educational attainment (high school or lower, college, above college), annual household income (U.S.\$ <20,000, 20,000–40,000, 40,001–60,000, >60,000), recruitment year (2001–2004, 2005–2006, after 2007), and known risk factors for breast cancer such as first-degree relative breast cancer history (yes/no), personal history of benign breast diseases, parity, age at first birth (years; continuous), hormone replacement therapy (yes/no), age at menarche, BMI [weight (kg)/height (m)²; continuous], regular alcohol intake (yes/no), regular exercise participation (yes/no), smoking status (never/ever), and total energy intake (kcal/day; continuous). Energy intake level for those who did not provide FFQ information ($n = 488$, 10.9% of total subjects) was imputed with age-specific (<40, 40–49, 50–59, 60) mean values. Sensitivity analyses were also performed for two conditions, excluding participants without FFQ information or excluding non-RDD controls ($n = 221$). Excluded from this study were 23 cases and 5 control subjects who did not provide completed data on all meat consumption (6 cases and 1 control), or those missed all or most of doneness levels for individual meats (23 cases and 5 control). Total consumption was estimated by summing across all levels of meat consumption. Missing doneness levels for individual meats (in a total of 25 subjects) were assigned for the meat-specific medium doneness level. The current analyses included 2,386 cases and 1,703 controls. P -values for trend tests were derived by entering categorical variables as ordinal variables in the models [26]. Heterogeneity tests were conducted in the polytomous model, testing the null hypothesis that the regression coefficient for each meat intake and meat-derived mutagen exposure was the same between premenopausal and premenopausal women. P -values of 0.05 (2-sided probability) were considered as being statistically significant. Statistical tests were based on two-sided probabilities using SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina).

Results

Distributions of demographic and selected breast cancer risk factors for cases and controls are presented in Table 1. Compared with controls, cases were more likely to be older and non-white because of imperfect frequency matching of these variables. Cases were less educated and from lower-income households. They were more likely to have a first-degree relative with breast cancer, a history of benign breast disease, be postmenopausal, have entered menopause at a later age, have a higher body mass index (BMI), and report less regular physical activity than controls ($P < 0.05$). Cases were less likely to use hormone replacement therapy, were younger at first live birth, were older at menarche, and were less likely to drink alcohol regularly. All of the above variables were considered potential confounders and adjusted for in the subsequent multivariate analyses. Cases and controls were similar in parity, number of live births, cigarette smoking, total fat intake, and total energy intake.

The median intake of meat and meat-derived mutagen exposure is presented in Table 2 by case-control status. Total meat intake was significantly higher among breast cancer cases compared to controls, primarily due to higher intake of red meat (18.9% median case/control difference, $P < 0.001$) and processed meat (15.2% median difference, $P = 0.006$) among cases. Cases also reported a higher intake of well-done meat, and this difference was largely due to a higher intake of well-done red meat (19.4% difference, $P < 0.001$). Cases and controls consumed a similar amount of chicken, well-done chicken, and fish. Cases also had higher exposure to meat-derived mutagens such as MeIQx and DiMeIQx, and total meat-derived mutagenicity than controls, but not to PhIP and benzo[*a*]pyrene.

Table 3 shows the association of red meat intake with breast cancer risk by cooking methods. Red meat intake from all cooking methods was positively associated with breast cancer risk ($P\text{-trend}<0.001$). Increased risk was primarily from consuming red meat cooked at high temperatures, i.e. grilled ($P\text{-trend}<0.001$), fried ($P\text{-trend}<0.001$), and to a less extent broiled red meat ($P\text{-trend}=0.028$). Overall, a high intake of red meat cooked at high temperatures was positively associated with an elevated risk of breast cancer ($P\text{-trend}<0.001$). No apparent association, however, was observed for red meat cooked by other methods which use lower cooking temperatures ($P\text{-trend}=0.429$). Table 4 presents results for the association of breast cancer risk with individual red meat items cooked well-done. Statistically significant associations were found for a high intake of non-fast food hamburger, pork chops, short ribs or spareribs, and bacon. No apparent associations were found for beef patties/steaks, sausages, and hotdogs or franks.

The association of breast cancer risk with exposure to meat-derived mutagens is presented in Table 5. High intake of both MeIQx ($P\text{-trend}=0.001$) and DiMeIQx ($P\text{-trend}=0.025$) were associated with an elevated risk of breast cancer. No significant associations were observed for breast cancer risk with PhIP, benzo[*a*]pyrene, and total meat-derived mutagenicity. Further analyses were performed by menopausal status (Table 6). With the exception of total meat, PhIP and benzo[*a*]pyrene, more significant associations with red meat and meat-derived mutagen exposure were found for postmenopausal breast cancer than for premenopausal breast cancer, although the heterogeneity test was not statistically significant, except for DiMeIQx ($P\text{-heterogeneity}=0.042$). The association with MeIQx and DiMeIQx exposure only was statistically significant in postmenopausal women. No significant heterogeneity in the association of breast cancer risk with meat intake and meat-derived mutagen exposure was observed across different breast cancer subtypes defined by hormonal receptors (estrogen and progesterone receptors) and HER2 status (data not shown).

Discussion

We observed positive associations of breast cancer risk with high intake of red meat, and well-done red meat, or high exposure to meat-derived mutagens (MeIQx and DiMeIQx). Some of the associations were found to be stronger for post-menopausal women than for premenopausal women. The elevated risk was primarily from consuming red meat cooked at high temperature. These results are consistent with the hypothesis that high intake of meat may be associated with an elevated risk of breast cancer, and this positive association may be mediated in part through exposure to meat-derived mutagens.

Epidemiologic studies examining meat intake in relation to breast cancer risk have been inconsistent. We found a clear positive association of breast cancer risk with red meat and also with red meat cooked at high temperature. This is in line with some [5, 18, 25, 27], but not all, previous studies [12, 28, 29]. Several recent studies did not support an association between red meat intake and overall breast cancer risk but suggested that red meat cooked at high temperatures may increase the risk of some breast cancers [5, 13, 15]. We have previously shown that women who consistently consumed very well done red meats had a 4.62 times higher risk (95% CI = 1.36–15.70) than that of women who consumed those meats rare or medium done [25]. Dietary intake of well-done meat and meat-derived HCAs are significantly correlated with DNA adducts in female breast tissue [30]. It is possible that high red meat intake increase the risk for breast cancer, at least in part, through meat-derived HCAs generated in high temperature cooking.

Intake levels of meat-derived mutagens may differ remarkably across countries. For a U.S. population, Layton *et al.* [31] calculated an average intake for HCAs of 26 ng/kg body

weight/day, whereas in European countries, the estimated intake levels were much lower, about 2.5 ng/kg body weight/day [32] to 5 ng/kg body weight/day [33]. A recent large study conducted in Europe found that the frequency of high-temperature cooking varies considerably between countries [15], which may explain some of the inconsistent findings from previous studies conducted in different populations. Since HCA levels are determined to a large extent by cooking methods and doneness levels, studies that did not assess cooking methods and doneness levels may suffer considerably from errors in estimating HCA exposure. Only a few epidemiologic studies have comprehensively assessed meat intake by cooking time and doneness level [1]. Many earlier studies included only a few items of meat cooked at high-temperature in the food frequency questionnaire. In a recent report from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) study, a large cohort study that comprehensively assessed meats and meat-derived mutagens, Ferrucci LM *et al* [11] reported that red meat and MeIQx were associated with the risk of postmenopausal invasive breast cancer, which is consistent with our findings.

We did not find PhIP to be associated with an elevated breast cancer risk. Previous studies that addressed meat-derived mutagens mainly focused on PhIP, because it is the most abundant heterocyclic amine in the U.S. diet [31]. In our current study, more than 60% of PhIP was derived from chicken, using estimates from the CHARRED database. As with several previous studies, we did not find any positive association between chicken intake and breast cancer risk [29, 34]. The lack of a positive association between dietary PhIP exposure and breast cancer risk is puzzling, and the reasons for this observation are unclear.

Most MeIQx and DiMeIQx were derived from red meat [31]. For example, more than 85% of MeIQx in the current study originated from red meat (data not shown). The exposure level to MeIQx through diet was considerably higher than DiMeIQx. In addition, MeIQx and DiMeIQx were approximately three- to seven-fold more potent than PhIP assessed by mean potencies from the given multipotent carcinogenicity on a body surface area basis [31], and were also more strongly associated with other cancers than was PhIP [35]. However, it was difficult to disentangle the individual effect of MeIQx and DiMeIQx since their exposure levels were highly correlated ($r=0.80$) and could not be adjusted in the same model.

Strengths of this analysis include the comprehensive assessments of meat intake and meat-derived mutagen. Similarly to other case-control studies, recall bias could be a concern. However, there is no reason to speculate that breast cancer cases would recall intake of well-done meats substantially differently from controls. Measurement error in dietary assessment is a concern, which typically results in attenuation of risks. Although all major known risk factors for breast cancer were adjusted for, it is possible that some residual confounding effect may remain. The response rate is not optimal, although it is comparable with most other case-control studies conducted during a similar time frame [36, 37]. The specific associations with intake of meat using high temperature methods but not with intake of meat cooked using low temperature methods cannot be explained entirely by possible selection bias.

In summary, this large study provides strong evidence for a positive association between red meat intake and meat-derived mutagen exposure with breast cancer risk. The results from our study provide justification to conduct cohort studies to elucidate these associations further and evaluate potential interactions of genetic factors with dietary exposure to meat-derived mutagens in the etiology of breast cancer. With few modifiable risk factors for breast cancer and consistent harmful effects for red meat seen for colorectal cancer [38], cardiovascular diseases [39] and type 2 diabetes [40], it is prudent to suggest women reduce their intake of well-done red meat.

Acknowledgments

Grant sponsor: National Cancer Institute, USA; Grant number: R01CA100374

This work was supported by research grant (R01CA100374) from the US National Cancer Institute. The authors would like to thank the study participants and research staff of The Nashville Breast Health Study (NBHS) for their support of this research, Dr. Rashmi Sinha for providing food photos to assess meat doneness levels, and Mary Jo Daly for technical assistance in the preparation of this manuscript. Survey for this study was conducted by the Biospecimen and Survey Shared Resource, which is supported in part by P30CA68485.

References

1. Zheng W, Lee SA. Well-done meat intake, heterocyclic amine exposure, and cancer risk. *Nutrition and Cancer*. 2009; 61:437–46. [PubMed: 19838915]
2. Boyd NF, Stone J, Vogt KN, Connelly BS, Martin LJ, Minkin S. Dietary fat and breast cancer risk revisited: a meta-analysis of the published literature. *Br J Cancer*. 2003; 89:1672–85. [PubMed: 14583769]
3. Missmer SA, Smith-Warner SA, Spiegelman D, Yaun S-S, Adami H-O, Beeson WL, van den Brandt PA, Fraser GE, Freudenheim JL, Goldbohm RA, Graham S, Kushi LH, et al. Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. *Int J Epidemiol*. 2002; 31:78–85. [PubMed: 11914299]
4. Cho E, Chen WY, Hunter DJ, Stampfer MJ, Colditz GA, Hankinson SE, Willett WC. Red Meat Intake and Risk of Breast Cancer Among Premenopausal Women. *Arch Intern Med*. 2006; 166:2253–9. [PubMed: 17101944]
5. Steck SE, Gaudet MM, Eng SM, Britton JA, Teitelbaum SL, Neugut AI, Santella RM, Gammon MD. Cooked meat and risk of breast cancer--lifetime versus recent dietary intake. *Epidemiology*. 2007; 18:373–82. [PubMed: 17435448]
6. Taylor EF, Burley VJ, Greenwood DC, Cade JE. Meat consumption and risk of breast cancer in the UK Women's Cohort Study. *Br J Cancer*. 2007; 96:1139–46. [PubMed: 17406351]
7. Egeberg R, Olsen A, Autrup H, Christensen J, Stripp C, Tetens I, Overvad K, Tjønneland A. Meat consumption, N-acetyl transferase 1 and 2 polymorphism and risk of breast cancer in Danish postmenopausal women. *European Journal of Cancer Prevention*. 2008; 17:39–47. [PubMed: 18090909]
8. Hu J, La Vecchia C, DesMeules M, Negri E, Mery L. Canadian Cancer Registries Epidemiology Research Group. Meat and Fish Consumption and Cancer in Canada. *Nutrition and Cancer*. 2008; 60:313–24. [PubMed: 18444165]
9. Linos E, Willett WC, Cho E, Colditz G, Frazier LA. Red Meat Consumption during Adolescence among Premenopausal Women and Risk of Breast Cancer. *Cancer Epidemiology Biomarkers & Prevention*. 2008; 17:2146–51.
10. Sonestedt E, Ericson U, Gullberg B, Skog K, Olsson H, Wirfalt E. Do both heterocyclic amines and omega-6 polyunsaturated fatty acids contribute to the incidence of breast cancer in postmenopausal women of the Malmö diet and cancer cohort? *International Journal of Cancer*. 2008; 123:1637–43.
11. Ferrucci LM, Cross AJ, Graubard BI, Brinton LA, McCarty CA, Ziegler RG, Ma X, Mayne ST, Sinha R. Intake of meat, meat mutagens, and iron and the risk of breast cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Br J Cancer*. 2009; 101:178–84. [PubMed: 19513076]
12. Kabat GC, Cross AJ, Park Y, Schatzkin A, Hollenbeck AR, Rohan TE, Sinha R. Meat intake and meat preparation in relation to risk of postmenopausal breast cancer in the NIH-AARP diet and health study. *International Journal of Cancer*. 2009; 124:2430–5.
13. Larsson SC, Bergkvist L, Wolk A. Long-term meat intake and risk of breast cancer by oestrogen and progesterone receptor status in a cohort of Swedish women. *European Journal of Cancer*. 2009; 45:3042–6. [PubMed: 19464165]
14. Mignone LI, Giovannucci E, Newcomb PA, Titus-Ernstoff L, Trentham-Dietz A, Hampton JM, Orav EJ, Willett WC, Egan KM. Meat consumption, heterocyclic amines, NAT2, and the risk of breast cancer. *Nutr Cancer*. 2009; 61:36–46. [PubMed: 19116874]

15. Pala V, Krogh V, Berrino F, Sieri S, Grioni S, Tjonneland A, Olsen A, Jakobsen MU, Overvad K, Clavel-Chapelon F, Boutron-Ruault M-C, Romieu I, et al. Meat, eggs, dairy products, and risk of breast cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Am J Clin Nutr*. 2009; 90:602–12. [PubMed: 19491385]
16. Rabstein S, Bruning T, Harth V, Fischer HP, Haas S, Weiss T, Spickenheuer A, Piel C, Justenhoven C, Illig T, Vollmert C, Baisch C, et al. N-acetyltransferase 2, exposure to aromatic and heterocyclic amines, and receptor-defined breast cancer. *Eur J Cancer Prev*. 2009; 19:100–9. [PubMed: 19996973]
17. Wu K, Sinha R, Holmes MD, Giovannucci E, Willett W, Cho E. Meat Mutagens and Breast Cancer in Postmenopausal Women--A Cohort Analysis. *Cancer Epidemiology Biomarkers & Prevention*. 2010; 19:1301–10.
18. Dai Q, Shu X-O, Jin F, Gao Y-T, Ruan Z-X, Zheng W. Consumption of Animal Foods, Cooking Methods, and Risk of Breast Cancer. *Cancer Epidemiology Biomarkers & Prevention*. 2002; 11:801–8.
19. Knize MG, Felton JS. Formation and Human Risk of Carcinogenic Heterocyclic Amines Formed from Natural Precursors in Meat. *Nutrition Reviews*. 2005; 63:158–65. [PubMed: 15971410]
20. Felton JS, Knize MG, Wu RW, Colvin ME, Hatch FT, Malfatti MA. Mutagenic potency of food-derived heterocyclic amines. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2007; 616:90–4.
21. American College of Radiology. Breast imaging reporting and data system: BI-RADS Atlas. 4. Reston, VA: American College of Radiology; 2003.
22. Buchowski MS, Schlundt DG, Hargreaves MK, Hankin JH, Signorello LB, Blot WJ. Development of a culturally sensitive food frequency questionnaire for use in the Southern Community Cohort Study. *Cell Mol Biol (Noisy-le-grand)*. 2003; 49:1295–304. [PubMed: 14984001]
23. Shin A, Shrubsole MJ, Ness RM, Wu H, Sinha R, Smalley WE, Shyr Y, Zheng W. Meat and meat-mutagen intake, doneness preference and the risk of colorectal polyps: the Tennessee Colorectal Polyp Study. *Int J Cancer*. 2007; 121:136–42. [PubMed: 17354224]
24. Sinha R, Cross A, Curtin J, Zimmerman T, McNutt S, Risch A, Holden J. Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. *Mol Nutr Food Res*. 2005; 49:648–55. [PubMed: 15986387]
25. Zheng W, Gustafson D, Sinha R, Cerhan J, Moore D, Hong C, Anderson K, Kushi L, Sellers T, Folsom A. Well-done meat intake and the risk of breast cancer. *J Natl Cancer Inst*. 1998; 90:1724–9. [PubMed: 9827527]
26. Madure M, Greenland S. Tests for Trend and Dose Response: Misinterpretations and Alternatives. *Am J Epidemiol*. 1992; 135:96–104. [PubMed: 1736665]
27. De Stefani E, Ronco A, Mendilaharsu M, Guidobono M, Deneo-Pellegrini H. Meat intake, heterocyclic amines, and risk of breast cancer: a case-control study in Uruguay. *Cancer Epidemiology Biomarkers & Prevention*. 1997; 6:573–81.
28. Gertig DM, Hankinson SE, Hough H, Spiegelman D, Colditz GA, Willett WC, Kelsey KT, Hunter DJ. N-acetyl transferase 2 genotypes, meat intake and breast cancer risk. *Int J Cancer*. 1999; 80:13–7. [PubMed: 9935222]
29. Delfino RJ, Sinha R, Smith C, West J, White E, Lin HJ, Liao S-Y, Gim JSY, Ma HL, Butler J, Anton-Culver H. Breast cancer, heterocyclic aromatic amines from meat and N-acetyltransferase 2 genotype. *Carcinogenesis*. 2000; 21:607–15. [PubMed: 10753193]
30. Rohrmann S, Lukas Jung S-U, Linseisen J, Pfau W. Dietary intake of meat and meat-derived heterocyclic aromatic amines and their correlation with DNA adducts in female breast tissue. *Mutagenesis*. 2009; 24:127–32. [PubMed: 18980957]
31. Layton DW, Bogen KT, Knize MG, Hatch FT, Johnson VM, Felton JS. Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. *Carcinogenesis*. 1995; 16:39–52. [PubMed: 7834804]
32. Augustsson K, Skog K, Jagerstad M, Steineck G. Assessment of the human exposure to heterocyclic amines. *Carcinogenesis*. 1997; 18:1931–5. [PubMed: 9364002]
33. Zimmerli B, Rhyn P, Zoller O, Schlatter J. Occurrence of heterocyclic aromatic amines in the Swiss diet: analytical method, exposure estimation and risk assessment. *Food Additives &*

- Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment. 2001; 18:533–51.
34. Ronco AL, De Stefani E, Fabra A. White meat intake and the risk of breast cancer: a case-control study in Montevideo, Uruguay. *Nutr Res*. 2003; 23:151–62.
 35. Sinha R. An epidemiologic approach to studying heterocyclic amines. *Mutation Research/ Fundamental and Molecular Mechanisms of Mutagenesis*. 2002; 506–507:197–204.
 36. Moorman PG, Newman B, Millikan RC, Tse C-KJ, Sandler DP. Participation Rates in a Case-Control Study: The Impact of Age, Race, and Race of Interviewer. *Annals of Epidemiology*. 1999; 9:188–95. [PubMed: 10192651]
 37. Teschke K, Marino S, Chu R, Tsui JK, Harris MA, Marion SA. Public opinions about participating in health research. *Can J Public Health*. 2010; 101:159–64. [PubMed: 20524383]
 38. Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: Dose-response meta-analysis of epidemiological studies. *Int J Cancer*. 2002; 98:241–56. [PubMed: 11857415]
 39. Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. *Am J Clin Nutr*. 1999; 70:1001–8. [PubMed: 10584044]
 40. Fung TT, Schulze M, Manson JE, Willett WC, Hu FB. Dietary Patterns, Meat Intake, and the Risk of Type 2 Diabetes in Women. *Arch Intern Med*. 2004; 164:2235–40. [PubMed: 15534160]

Table 1

Comparison of study participants by demographic and selected breast cancer risk factors, the Nashville Breast Health Study (NBHS).

Characteristics	Cases (N=2,386)	Controls (N=1,703)	P-value ^a
Demographic factors			
Age (year, mean $\pm$ SD)	54.4 $\pm$ 10.4	52.0 $\pm$ 10.8	<0.001
Postmenopausal (n, %)	1578 (66.2)	958 (56.3)	<0.001
Race/ethnicity, white (n, %)	1,906 (80.0)	1,536 (90.2)	<0.001
Education attainment			<0.001
High school or below	868 (36.4)	540 (31.7)	
College	1111 (46.6)	899 (52.8)	
College above	407 (17.0)	264 (15.5)	
Family income per annum US\$ (n, %)			<0.001
<20,000	377 (15.8)	143 (8.4)	
20,000–40,000	500 (21.0)	327 (19.2)	
40,001–60,000	445 (18.7)	342 (20.1)	
>60,000	1064 (44.5)	891 (52.3)	
Known risk factors			
First degree relatives with breast cancer (n, %)	475 (19.9)	228 (13.4)	<0.001
Prior history of benign breast diseases (n, %)	1,064 (44.6)	557 (32.7)	<0.001
Hormone replacement therapy (n, %) ^c	1067 (67.6)	701 (73.3)	0.003
Age at menarche (y, mean $\pm$ SD)	12.8 $\pm$ 3.6	12.6 $\pm$ 3.3	<0.001
Nulliparous (n, %)	375 (15.7)	266 (15.6)	0.933
Number of live births (mean $\pm$ SD) ^b	2.4 $\pm$ 1.3	2.3 $\pm$ 1.2	0.283
Age at first live birth (mean $\pm$ SD, years) ^b	23.8 $\pm$ 5.5	24.4 $\pm$ 5.4	0.001
Age at menopause (mean $\pm$ SD, years) ^c	45.8 $\pm$ 8.7	44.8 $\pm$ 8.7	0.004
Body mass index in all women, (kg/m ²)	27.8 $\pm$ 6.3	27.2 $\pm$ 6.4	0.004
Body mass index in postmenopausal women, (kg/m ²)	28.2 $\pm$ 6.2	27.8 $\pm$ 6.2	0.003
Regular exerciser (n, %)	1210 (50.7)	981 (57.6)	<0.001
Regular alcohol drinker (n, %)	433 (18.2)	355 (20.9)	0.031
Selected other factors			
Regular cigarette smoker (n, %)	989 (41.5)	710 (41.7)	0.877
Total energy intake (kcal/day) ^d	1491.7 (1246.3, 1774.3)	1483.2 (1181.4, 1843.3)	0.893
Total fat intake (g/day) ^d	52.6 (43.6, 65.5)	53.6 (41.5, 68.5)	0.207

^aDerived from t-test for continuous variables and chi-square test for categorical variables

^bAmong parous women

^cAmong menopausal women

^dMedian (25th, 75th percentile) is presented.

Table 2

Comparison of meat intake and meat-derived mutagen exposure levels between breast cancer cases and controls, the Nashville Breast Health Study (NBHS)

	Median (25th, 75th)		% difference ^a	<i>p</i> ^b
	Cases (N=2,386)	Controls (N=1,703)		
Type of meat (g/day)				
Total meat	84.3 (55.4, 122.7)	81.0 (51.8, 116.1)	4.1	<0.001
Red meat ^c	37.8 (18.3, 64.3)	31.8 (13.2, 56.8)	18.9	<0.001
Chicken	40.5 (16.2, 40.5)	40.5 (16.2, 40.5)	0	0.556
Fish	9.5 (0, 16.2)	9.5 (0, 16.2)	0	0.600
Processed meat ^d	7.6 (1.9, 20.0)	6.6 (0, 16.2)	15.2	0.006
All red and processed meat ^e	46.9 (23.6,84.6)	38.9 (16.2,71.8)	20.6	<0.001
Well-done all meat	45.5 (23.7, 74.3)	42.5 (23, 70.5)	7.1	0.004
Well-done red meat	26.5 (11.8, 49.2)	22.2 (9.0, 42.2)	19.4	<0.001
Well-done chicken	10.1 (4.1, 20.3)	10.1 (4.7, 20.3)	0	0.566
Well-done processed meat	6.5 (1.9, 16)	5.7 (1.9, 13.2)	14.0	0.080
Mutagens from meat				
MeIQx (ng/day)	33.7 (13.8, 63.8)	27.5 (10.8, 56)	22.5	<0.001
PhIP (ng/day)	167.6 (74.1, 305.5)	162.4 (76.5, 317.6)	3.2	0.731
DiMeIQx (ng/day)	2.9 (1.0, 6.0)	2.5 (0.9, 5.4)	16.0	0.049
Mutagenicity (revertants	7214.1 (3875.8, 12173.2)	7039 (3593.5, 11603.8)	2.5	0.024
Benzo[]pyrene (ng/day)	25.6 (9.1, 57.6)	27.1 (8.7, 65.7)	-5.5	0.268

^aExpressed as (median cases-median controls)/median controls.

^bP values (two sided) were from Wilcoxon rank-sum tests.

^cIncludes hamburgers, cheeseburgers, beef patties/beef steaks/pork chops or hamsteaks and ribs (short ribs or spareribs).

^dIncludes bacon/sausage/hotdogs or franks.

^eIncludes hamburgers, cheeseburgers, beef patties/beef steaks/pork chops or hamsteaks/bacon/sausage/hotdogs or franks, and short ribs or spareribs.

Table 3
Association of breast cancer risk with red meat by cooking methods, the Nashville Breast Health Study (NBHS)

Cooking methods	Intake quartiles								P_trend
	Q1 (Low)		Q2		Q3		Q4		
	n ^a	OR(95% CI) ^b	n ^a	OR(95% CI) ^b	n ^a	OR(95% CI) ^b	n ^a	OR(95% CI) ^b	
All cooking methods	460/435	1.0 (reference)	543/415	1.2 (1.0–1.5)	660/428	1.4 (1.2–1.7)	723/425	1.5 (1.2–1.8)	<0.001
High temperature cooking methods ^c	628/564	1.0 (reference)	768/533	1.2 (1.0–1.5)	639/415	1.4 (1.1–1.7)	351/191	1.5 (1.3–1.9)	<0.001
Grilled	509/426	1.0 (reference)	614/425	1.2 (1.0–1.5)	557/424	1.2 (1.0–1.4)	706/428	1.6 (1.3–1.9)	<0.001
Fried	766/672	1.0 (reference)	399/337	1.0 (0.9–1.3)	579/326	1.5 (1.3–1.8)	642/368	1.3 (1.1–1.6)	<0.001
Broiled	1082/857	1.0 (reference)	226/174	1.1 (0.9–1.4)	472/325	1.2 (1.0–1.4)	606/347	1.2 (1.0–1.4)	0.028
Other methods	1189/898	1.0 (reference)	140/88	1.3 (0.9–1.7)	460/341	1.0 (0.8–1.2)	597/376	1.1 (0.9–1.3)	0.429

^a n: Number of cases/controls.

^b Adjusted for age group, ethnicity, educational attainment, family income, first degree relative breast cancer history, personal history of benign breast disease, hormone replacement therapy, age at menarche, menopause status, ever have live birth, BMI, regular physical exercise, regular alcohol consumption, and study period.

^c Including grilled, pan-/deep-fried, and broiled.

Table 4

Association for breast cancer risk with major well-done red/processed meat items, the Nashville Breast Health Study (NBHS).

Well-done meat items ^a	Intake quartiles								P_trend
	Q1 (Low)		Q2		Q3		Q4		
	n ^b	OR(95% CI) ^c	n ^b	OR(95% CI) ^c	n ^b	OR(95% CI) ^c	n ^b	OR(95% CI) ^c	
Non-fast food hamburgers	1429/1085	1.0 (reference)	617/433	1.2 (1.1–1.4)	340/185	1.5 (1.2–1.8)	N/A ^d	N/A ^d	<0.001
Beef patties/steaks	1682/1160	1.0 (reference)	376/294	1.0 (0.9–1.2)	159/107	1.2 (0.9–1.5)	169/142	1.0 (0.8–1.3)	0.614
Pork chops	1480/1129	1.0 (reference)	296/189	1.2 (0.9–1.4)	365/258	1.1 (0.9–1.3)	245/127	1.4 (1.1–1.8)	0.007
Short ribs/spare ribs	2130/1586	1.0 (reference)	117/63	1.1 (0.8–1.6)	93/38	1.5 (1.0–2.2)	46/16	1.6 (0.9–3.0)	0.011
Bacon	1335/1041	1.0 (reference)	312/214	1.1 (0.9–1.4)	360/210	1.4 (1.2–1.7)	379/238	1.2 (1.0–1.4)	0.006
Sausage	1670/1253	1.0 (reference)	313/208	1.1 (0.9–1.3)	253/155	1.1 (0.9–1.4)	150/87	1.0 (0.7–1.3)	0.612
Hotdog/franks	1864/1353	1.0 (reference)	192/123	1.2 (0.9–1.5)	174/124	1.1 (0.8–1.4)	156/103	1.0 (0.8–1.3)	0.633

^aIncluding grilled, pan-/deep-fried, and broiled meats.

^bn: Number of cases/controls.

^cAdjusted for age group, ethnicity, educational attainment, family income and additionally for first degree relative breast cancer, benign breast disease, hormone replacement therapy, age at menarche, menopause status, ever have live birth, BMI, regular physical exercise, regular alcohol consumption, and study period.

^dN/A: not available.

Table 5

Association between breast cancer risk and meat-derived mutagen exposure by quintiles, the Nashville Breast Health Study (NBHS).

Meat-derived mutagens	Exposure quintiles					P_trend
	Q1 (Low)	Q2	Q3	Q4	Q5	
MeIQx						
No. cases/controls	426/363	426/336	439/335	538/335	557/334	
OR (95% CI) ^a	1.0 (reference)	1.2 (0.9–1.4)	1.2 (1.0–1.5)	1.4 (1.1–1.7)	1.3 (1.1–1.6)	0.001
PhIP						
No. cases/controls	542/363	408/336	494/335	489/335	453/334	
OR (95% CI) ^a	1.0 (reference)	0.9 (0.7–1.1)	1.0 (0.9–1.3)	1.0 (0.8–1.2)	0.9 (0.7–1.1)	0.603
DiMeIQx						
No. cases/controls	482/366	440/339	430/329	503/335	531/334	
OR (95% CI) ^a	1.0 (reference)	1.0 (0.8–1.3)	1.1 (0.9–1.3)	1.2 (1.0–1.5)	1.2 (1.0–1.5)	0.025
Benzo[a]pyrene						
No. cases/controls	497/364	503/335	513/335	497/335	376/334	
OR (95% CI) ^a	1.0 (reference)	1.2 (0.9–1.4)	1.2 (1.0–1.5)	1.2 (1.0–1.5)	1.0 (0.8–1.2)	0.898
Mutagenicity						
No. cases/controls	480/364	481/335	477/334	445/336	503/334	
OR (95% CI) ^a	1.0 (reference)	1.2 (0.9–1.4)	1.2 (1.0–1.4)	1.1 (0.9–1.3)	1.1 (0.9–1.4)	0.416

^aOdds ratio was adjusted for age group, ethnicity, educational attainment, family income, first degree relative breast cancer history, personal history of benign breast disease, hormone replacement therapy, age at menarche, menopause status, ever have live birth, BMI, regular physical exercise, regular alcohol consumption, and study period.

Association of breast cancer risk in relation to meat intake and meat-derived mutagen exposure by menopausal status^a, the Nashville Breast Health Study (NBHS).

Table 6

	Intake quartiles				<i>P</i> _trend
	Q1 (Low)	Q2	Q3	Q4	
Total meat					
Pre-menopause					
No. cases/controls	230/243	226/220	209/183	142/97	0.109
OR(95% CI) ^b	1.0 (reference)	1.1 (0.8–1.5)	1.2 (0.9–1.6)	1.3 (0.9–1.8)	
Post-menopause					
No. cases/controls	504/337	457/258	391/223	226/140	0.667
OR(95% CI) ^b	1.0 (reference)	1.2 (0.9–1.5)	1.2 (1.0–1.6)	0.9 (0.7–1.3)	
<i>P</i> for heterogeneity= 0.711					
Red meat					
Pre-menopause					
No. cases/controls	212/240	263/260	208/154	124/89	0.031
OR(95% CI) ^b	1.0 (reference)	1.2 (0.9–1.5)	1.4 (1.1–2.0)	1.3 (0.9–2.0)	
Post-menopause					
No. cases/controls	427/350	521/303	406/209	224/96	<0.001
OR(95% CI) ^b	1.0 (reference)	1.4 (1.1–1.7)	1.5 (1.2–1.9)	1.7 (1.3–2.4)	
<i>P</i> for heterogeneity = 0.539					
Well-done red meat					
Pre-menopause					
No. cases/controls	189/220	250/234	234/198	134/91	0.017
OR(95% CI) ^b	1.0 (reference)	1.3 (1.0–1.7)	1.4 (1.0–1.9)	1.5 (1.1–2.2)	
Post-menopause					
No. cases/controls	438/342	518/299	405/217	217/100	<0.001
OR(95% CI) ^b	1.0 (reference)	1.4 (1.1–1.8)	1.5 (1.2–2.0)	1.7 (1.2–2.3)	
<i>P</i> for heterogeneity = 0.499					
MeIQx					

	Intake quartiles				<i>P</i> _trend
	Q1 (Low)	Q2	Q3	Q4	
Pre-menopause					
No. cases/controls	224/229	245/226	224/176	114/112	0.784
OR(95% CI) ^b	1.0 (reference)	1.1 (0.8–1.5)	1.3 (1.0–1.8)	0.8 (0.5–1.1)	
Post-menopause					
No. cases/controls	456/335	412/269	404/206	306/148	0.002
OR(95% CI) ^b	1.0 (reference)	1.2 (1.0–1.5)	1.4 (1.1–1.8)	1.4 (1.1–1.9)	
<i>P</i> for heterogeneity = 0.051					
PhIP					
Pre-menopause					
No. cases/controls	197/185	211/200	211/201	188/157	0.968
OR(95% CI) ^b	1.0 (reference)	1.0 (0.8–1.4)	1.0 (0.8–1.4)	1.0 (0.7–1.4)	
Post-menopause					
No. cases/controls	486/290	420/265	399/222	273/181	0.165
OR(95% CI) ^b	1.0 (reference)	0.9 (0.8–1.2)	1.0 (0.8–1.3)	0.8 (0.6–1.0)	
<i>P</i> for heterogeneity = 0.433					
DiMeIQx					
Pre-menopause					
No. cases/controls	201/187	220/203	225/207	161/146	0.778
OR(95% CI) ^b	1.0 (reference)	1.0 (0.7–1.3)	1.0 (0.7–1.4)	0.9 (0.7–1.3)	
Post-menopause					
No. cases/controls	420/295	412/281	406/219	340/163	0.003
OR(95% CI) ^b	1.0 (reference)	1.0 (0.8–1.3)	1.3 (1.0–1.7)	1.4 (1.1–1.8)	
<i>P</i> for heterogeneity = 0.042					
Benzol pyrene					
Pre-menopause					
No. cases/controls	182/178	229/205	239/214	157/146	0.503
OR(95% CI) ^b	1.0 (reference)	1.1 (0.8–1.5)	1.1 (0.8–1.5)	1.1 (0.8–1.6)	
Post-menopause					
No. cases/controls	520/331	482/238	377/225	199/164	0.674

	Intake quartiles				P_trend
	Q1 (Low)	Q2	Q3	Q4	
OR(95% CI) ^b	1.0 (reference)	1.3 (1.0–1.6)	1.2 (1.0–1.6)	0.8 (0.6–1.1)	
P for heterogeneity = 0.135					
Mutagenicity					
Pre-menopause					
No. cases/controls	218/194	214/196	215/209	160/144	0.556
OR(95% CI) ^b	1.0 (reference)	0.9 (0.7–1.3)	1.0 (0.7–1.3)	0.9 (0.6–1.2)	
Post-menopause					
No. cases/controls	466/317	410/248	406/239	296/154	0.087
OR(95% CI) ^b	1.0 (reference)	1.2 (0.9–1.5)	1.1 (0.9–1.4)	1.3 (1.0–1.7)	
P for heterogeneity= 0.110					

^aThree participants with unknown menopausal status were excluded. Thus, 4,086 subjects were included.

^bAdjusted for age group, ethnicity, educational attainment, family income, total energy intake, first degree relative breast cancer history, personal history of benign breast disease, hormone replacement therapy, age at menarche, have live birth, BMI, regular physical exercise, regular alcohol consumption, and study period.