Red Meat Intake and Risk of Breast Cancer Among Premenopausal Women

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Background: The association between red meat intake and breast cancer is unclear, but most studies have assessed diet in midlife or later. Although breast tumors differ clinically and biologically by hormone receptor status, few epidemiologic studies of diet have made this distinction.

Methods: Red meat intake and breast cancer risk were assessed among premenopausal women aged 26 to 46 years in the Nurses’ Health Study II. Red meat intake was assessed with a food frequency questionnaire administered in 1991, 1995, and 1999, with respondents followed up through 2003. Breast cancers were self-reported and confirmed by review of pathologic reports.

Results: During 12 years of follow-up of 90,659 premenopausal women, we documented 1021 cases of invasive breast carcinoma. Greater red meat intake was strongly related to elevated risk of breast cancers that were estrogen and progesterone receptor positive (ER+/PR+; n=512) but not to those that were estrogen and progesterone receptor negative (ER−/PR−; n=167). Compared with those eating 3 or fewer servings per week of red meat, the multivariate relative risks (95% confidence intervals) for ER+/PR+ breast cancer with increasing servings of red meat intake were 1.14 (0.90–1.45) for more than 3 to 5 or fewer servings per week, 1.42 (1.06–1.90) for more than 5 per week to 1 or fewer servings per day, 1.20 (0.89–1.63) for more than 1 to 1.5 or fewer servings per day, and 1.97 (1.35–2.88) for more than 1.5 servings per day (test for trend, P=.001). The corresponding relative risks for ER−/PR− breast cancer were 1.34 (0.89–2.00), 1.21 (0.73–2.00), 0.69 (0.39–1.23), and 0.89 (0.43–1.84) (test for trend, P=.28). Higher intakes of several individual red meat items were also strongly related to elevated risk of ER+/PR+ breast cancer.

Conclusion: Higher red meat intake may be a risk factor for ER+/PR+ breast cancer among premenopausal women.

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Red meat intake has been hypothesized to increase breast cancer risk. Both case-control1 and ecologic2 studies have supported a positive association, and an expert panel described red meat intake as a possible cause of breast cancer.3 Prospective cohort studies have been inconsistent,1,4 but few of these previous studies included many young, premenopausal women. Therefore, an association between red meat intake and premenopausal breast cancer risk could not be excluded.

Breast tumors are often characterized by hormone (estrogen and progesterone) receptor status. Although the incidence rates of hormone receptor–negative tumors have remained relatively constant, the incidence of hormone receptor–positive tumors has been increasing in the United States, especially among middle-aged women. On the basis of surveillance, Epidemiology, and End Results data, the incidence rate for estrogen receptor–positive (ER+) cancers increased from 65.2 (per 100,000 person-years) in 1992 to 75.1 (per 100,000 person-years) in 1998 among women diagnosed as having breast cancer in the 40- to 49-year age group.5 A similar increasing trend was found for progesterone receptor–positive (PR+) tumors. Epidemiologic studies6–12 examining hormonal risk factors and breast cancer by hormone receptor status have generally supported the hypothesis that receptor-positive tumors differ etiologically from receptor-negative tumors.

Dietary factors may also have different effects on the risk of breast cancers characterized by hormone receptor status. We are not aware of any studies evaluating the intake of red meat and breast cancer risk according to tumor hormone receptor status. Some components in red meat, including heterocyclic amines in...
cooked meat,\textsuperscript{13-15} heme iron,\textsuperscript{16,17} and exogenous hormone residues,\textsuperscript{18,19} are estrogenic and may influence breast cancer through hormone receptors. Thus, it is plausible that red meat intake is related to hormone receptor–positive breast cancer. Because several known risk factors, such as reproductive factors and ionizing radiation, operate primarily before middle age,\textsuperscript{20} diet in early adult life may have a stronger impact than that later in life. In a previous analysis of the Nurses’ Health Study II, we found that animal fat intake was associated with an increased risk of premenopausal breast cancer and also found some positive association with intake of red meat, one of the major food sources of animal fat intake.\textsuperscript{21} In this study, we added 4 additional years of follow-up with more than 300 additional breast cancer cases and examined intake of red meat and types of red meat in relation to the risk of breast cancer by hormone receptor status.

**METHODS**

**STUDY POPULATION**

The Nurses’ Health Study II is a prospective cohort study of 116,671 female registered nurses who were 25 to 42 years of age and living in 1 of 14 states in the United States when they responded in 1989 to a questionnaire regarding their medical histories and lifestyles. Follow-up questionnaires have been sent biennially to update information on risk factors and medical events. For the current analysis, we started follow-up on June 1, 1991, when diet was first measured. From the 97,807 women who returned the 1991 dietary questionnaire, we excluded women who had an implausible total energy intake (\(<800\) or \(>4200\) kcal/d) or who left more than 70 food items blank on the 1991 food frequency questionnaire (FFQ) (\(n=2361\)). We also excluded women who reported a diagnosis of cancer, except nonmelanoma skin cancer, before returning the 1991 questionnaire (\(n=1319\)). To limit the analysis to premenopausal women, we excluded postmenopausal women at baseline (\(n=3468\)) and censored women after they reached either natural or surgical (bilateral oophorectomy with or without hysterectomy) menopause during follow-up. Women who had undergone a hysterectomy but not a bilateral oophorectomy were also excluded at the time of surgery because their menopausal status was unknown. A total of 90,659 premenopausal women were included in the analysis at baseline. Among those who answered the FFQ in 1991, the response rate was higher than 90% through June 1, 2003, the end of the follow-up period. The study was approved by the Human Research Committees at the Harvard School of Public Health and Brigham and Women’s Hospital.

**DIETARY ASSESSMENT**

A semiquantitative FFQ with more than 130 food items was sent to women in 1991, 1995, and 1999 to assess usual dietary intake during the past year. Participants were asked how often, on average, they had consumed each type of food or beverage during the past year. The FFQ had 9 possible responses, ranging from never or less than once per month to 6 or more times per day. We examined intakes of total red meat and individual red meat items, including beef or lamb as a main dish; pork as a main dish; beef, pork, or lamb as a sandwich or mixed dish; hamburger; bacon; hot dogs; and other processed meats.

Because dietary intake may promote breast carcinogenesis during an extended period, we calculated cumulative averaged intakes of red meat using the 1991, 1993, and 1999 dietary data to best represent long-term intake for our primary analysis.\textsuperscript{21} Thus, 1991 intake was used for the 1991 to 1995 follow-up periods, the average of 1991 and 1995 intake was used for the 1995 to 1999 follow-up periods, and the average of all 3 was used for the 1999 to 2003 follow-up periods to maintain a strictly prospective analysis. Baseline and most recent intake were each examined alone in secondary analyses.

The reproducibility and validity of individual red meat items have also been evaluated in women 39 to 59 years of age in the Nurses’ Health Study.\textsuperscript{22} The correlation coefficients between diet records and the FFQ for intake of individual red meat items were mostly higher than 0.5 after correction for attenuation because of random error in diet records.

**DOCUMENTATION OF BREAST CANCER**

Biennial questionnaires mailed between 1993 and 2003 were used to identify newly diagnosed cases of breast cancer. Deaths were documented by responses to follow-up questionnaires by family members or the postal service and by a search of the National Death Index. When a case of breast cancer was reported, we asked the participant (or next of kin for those who had died) for confirmation of the diagnosis and for permission to seek relevant hospital records and pathologic reports. Pathologic reports confirmed 98% of the self-reported breast cancers. Information on ER and PR status was obtained from pathologic reports. Cases of carcinoma in situ were not included in analyses.

**STATISTICAL ANALYSIS**

Participants contributed person-time from the date of return of the 1991 questionnaire until the date of breast cancer diagnosis, death, menopause (natural or surgical), or June 1, 2003, whichever came first. Participants were divided into categories according to their red meat intake. Relative risks (RRs) of breast cancer were calculated as the incidence rate for a given category of red meat intake compared with the rate among participants in the lowest category of intake. We used Cox proportional hazards regression to account for potential effects of other risk factors for breast cancer.\textsuperscript{24} To control as finely as possible for confounding by age, calendar time, and any possible 2-way interactions between these 2 time scales, we stratified the analysis jointly by age in months at the start of each follow-up period and calendar year of the current questionnaire cycle. Multivariate models also simultaneously adjusted for tobacco use, body mass index (calculated as weight in kilograms divided by the square of height in meters), height, age at menarche, oral contraceptive use, family history of breast cancer, history of benign breast disease, parity and age at first birth, and intakes of calories and alcohol. All covariates except height, age at menarche, and family history of breast cancer were updated in each questionnaire cycle. The SAS PROC PHREG program\textsuperscript{25} was used for all analyses, and the Anderson-Gill data structure\textsuperscript{26} was used to handle time-varying covariates efficiently, with a new data record created for every questionnaire cycle at which a participant was at risk and covariates set to their values at the time the questionnaire was returned. For all RRs, 95% confidence intervals (CIs) were calculated. Tests for trend were conducted using the median value for each category of food or food group as a continuous variable. A test of the difference in the estimates of red meat intake (median values for each category as a continuous variable) for hormone receptor status was conducted by using the squared t statistic,\textsuperscript{27} which has a \(\chi^2\) distribution with 1 df. All \(P\) values were 2 sided.
During 12 years (862,486 person-years) of follow-up of 90,639 women through 2003, we documented 1,021 cases of invasive premenopausal breast carcinoma. The age range of the participants at baseline was 26 to 46 years (mean ± SD, 36.0 ± 4.5 years). We had information on ER/PR status for 779 cases (77%). Among them, 512 were ER+/PR+ and 167 were ER and PR negative (ER−/PR−). For analyses by ER/PR status, we did not report results for cancers with mixed ER/PR status because of small case numbers (ER−/PR− = 39 and ER+/PR− = 71).

Table 1 presents the distribution of risk factors for breast cancer by categories of red meat intake. Women with a higher intake of red meat were more likely to be current smokers, to have 3 or more children, and to have a higher body mass index and caloric intake but less likely to have a history of benign breast disease.

The highest intake of red meat was weakly and non-significantly associated with elevated risk of overall breast cancer (Table 2). However, when we divided cases according to ER and PR status, higher red meat intake was strongly related to an increased risk of ER+/PR+ breast cancers but not ER−/PR− cancers. Compared with those eating 3 or fewer servings of red meat per week, the multivariate RRs for ER+/PR+ breast cancer with increasing servings of red meat intake were 1.14 (95% CI, 0.90-1.45) for more than 3 to 5 or fewer servings per week, 1.42 (95% CI, 1.06-1.90) for more than 5 per week to 1 or fewer servings per day, 1.20 (95% CI, 0.89-1.63) for more than 1 to 1.5 or fewer servings per day, and 1.97 (95% CI, 1.35-2.88) for more than 1.5 servings per day (test for trend; P = .001). The corresponding RRs for ER−/PR− breast cancer were 1.34 (95% CI, 0.89-2.00), 1.21 (95% CI, 0.73-2.00), 0.69 (95% CI, 0.39-1.23), and 0.89 (95% CI, 0.43-1.84; test for trend, P = .28). The difference in results for ER+/PR+ vs ER−/PR− breast cancers was statistically significant (P = .01). The positive association between red meat intake and ER+/PR+ breast cancer was consistent when grams instead of serving sizes of red meat intake were calculated; multivariate RRs for increasing intake of red meat (<20, 20 to <40, 40 to <60, 60 to <80, 80 to <100, and ≥100 g/d) were 1.00, 1.17 (95% CI, 0.81-1.69), 1.37 (95% CI, 0.96-1.95), 1.31 (95% CI, 0.90-1.90), 1.52 (95% CI, 1.03-2.25), and 1.71 (95% CI, 1.17-2.48), respectively. The results were also similar when quintiles of red meat intake were examined; multivariate RRs for increasing quintiles of red meat intake were 1.00, 1.24 (95% CI, 0.93-1.66), 1.17 (95% CI, 0.87-1.58), 1.39 (95% CI, 1.03-1.88), and 1.61 (95% CI, 1.16-2.22). The association was not attenuated when we adjusted for other food groups, including fruits, vegetables, and dairy foods, and when breast cancer cases diagnosed within the first 2 years of follow-up were excluded (n = 481 after exclusion) (data not shown).

We also examined the individual red meat items in relation to overall breast cancer risk and by receptor status (Table 3). Although no strong associations were found for overall breast cancer risk, almost all of the individual red meat items had statistically significant positive trends of increasing ER+/PR+ breast cancer risk. The 2 items that were not statistically significant (beef, pork, or lamb as a sandwich or mixed dish and bacon) still suggested a positive association with ER+/PR+ breast cancer risk. Individual red meat items were not positively related to ER−/PR− cancers (Table 3).

The association between red meat intake and ER+/PR+ breast cancer risk was consistent when we examined baseline intake (intake from the 1991 FFQ) and most recent (updated) intake (ie, intake from the 1995 FFQ for cases diagnosed between 1995 and 1999), although the cumulative averaged intake showed the strongest re-

Table 1. Age-Standardized Distribution of Potential Risk Factors for Breast Cancer According to Red Meat Intake in 1991 in Women 26 to 46 Years of Age at Baseline*

<table>
<thead>
<tr>
<th>Variable</th>
<th>≤3/wk (n = 26787)</th>
<th>&gt;3 to ≤5/wk (n = 27253)</th>
<th>&gt;5/wk to =1/d (n = 11185)</th>
<th>&gt;1 to ≤1.5/d (n = 16777)</th>
<th>&gt;1.5 per d (n = 8649)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>BMI</td>
<td>23</td>
<td>24</td>
<td>25</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Height, in</td>
<td>64.9</td>
<td>64.9</td>
<td>64.9</td>
<td>64.9</td>
<td>64.9</td>
</tr>
<tr>
<td>Age at first birth among parous women, y</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Alcohol intake, g/d</td>
<td>3.2</td>
<td>3.2</td>
<td>3.1</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Caloric intake, kcal/d</td>
<td>1524</td>
<td>1696</td>
<td>1862</td>
<td>2032</td>
<td>2359</td>
</tr>
</tbody>
</table>

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

*Except for the data on mean age, all data shown are standardized to the age distributions of the cohort in 1991.
In this prospective study of premenopausal women, we found that red meat intake was strongly associated with an elevated risk of hormone receptor–positive breast cancer but not hormone receptor–negative cancers. Positive associations existed across most of the individual red meat items.

Previous epidemiologic data on red meat intake and breast cancer risk have been inconclusive. A meta-analysis of case-control and cohort studies reported an increased risk of breast cancer with a higher intake of meat, which included both red and white meat and did not differentiate by menopausal status. However, a pooled analysis of 8 prospective studies found no association between red meat intake and overall breast cancer risk (n = 7379 breast cancer cases). The association did not differ by menopausal status; the multivariate RR for breast cancer for a 100-g/d increment in red meat intake was 0.97 (95% CI, 0.79-1.20) among premenopausal women. None of the studies examined red meat intake and breast cancer by hormone receptor status, and most of these studies were heavily weighted by results for older, postmenopausal women. One case-control study23 that included a large number of relatively young women found a positive association between intake of high-fat meat during adolescence and breast cancer risk.

Because the origins of premenopausal and postmenopausal breast cancer vary in many respects, the relationship (Table 4). The associations between red meat intake and breast cancer risk were similar among cases with information on hormone receptor status and those without the information (data not shown).

<table>
<thead>
<tr>
<th>Variable</th>
<th>≤3/wk</th>
<th>&gt;3 to ≤5/wk</th>
<th>&gt;5/wk to ≤1/d</th>
<th>&gt;1 to ≤1.5/d</th>
<th>&gt;1.5/d</th>
<th>P Value for Trend*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total breast cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>276</td>
<td>336</td>
<td>157</td>
<td>165</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>0.99 (0.85-1.16)</td>
<td>1.04 (0.86-1.27)</td>
<td>0.90 (0.74-1.09)</td>
<td>1.16 (0.91-1.48)</td>
<td>.70</td>
</tr>
<tr>
<td>Multivariate†</td>
<td>1.00</td>
<td>1.03 (0.87-1.21)</td>
<td>1.10 (0.90-1.35)</td>
<td>0.96 (0.78-1.19)</td>
<td>1.27 (0.96-1.67)</td>
<td>.28</td>
</tr>
<tr>
<td>ER+/PR+ cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>121</td>
<td>165</td>
<td>88</td>
<td>86</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>1.09 (0.86-1.38)</td>
<td>1.32 (1.00-1.73)</td>
<td>1.07 (0.81-1.41)</td>
<td>1.63 (1.18-2.26)</td>
<td>.01</td>
</tr>
<tr>
<td>Multivariate†</td>
<td>1.00</td>
<td>1.14 (0.90-1.45)</td>
<td>1.42 (1.06-1.90)</td>
<td>1.20 (0.89-1.63)</td>
<td>1.97 (1.35-2.88)</td>
<td>.001</td>
</tr>
<tr>
<td>ER−/PR− cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>40</td>
<td>67</td>
<td>28</td>
<td>20</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>1.37 (0.92-2.03)</td>
<td>1.26 (0.78-2.05)</td>
<td>0.74 (0.43-1.26)</td>
<td>1.04 (0.55-1.99)</td>
<td>.44</td>
</tr>
<tr>
<td>Multivariate†</td>
<td>1.00</td>
<td>1.34 (0.89-2.00)</td>
<td>1.21 (0.73-2.00)</td>
<td>0.69 (0.39-1.23)</td>
<td>0.89 (0.43-1.84)</td>
<td>.28</td>
</tr>
</tbody>
</table>

Abbreviations: ER+, estrogen receptor positive; ER−, estrogen receptor negative; PR+, progesterone receptor positive; PR−, progesterone receptor negative.

*Test for trend calculated with median intake of each category of red meat intake as a continuous variable.

†Multivariate was stratified by age in months at start of follow-up and calendar year of the current questionnaire cycle and was simultaneously adjusted for height (<62, 62 to <65, 65 to <68, and ≥68 in), parity and age at first birth (nulliparous, parity ≥2 and age at first birth <25 years, parity ≥2 and age at first birth 25 to <30 years, parity ≥2 and age at first birth ≥30 years, parity ≥3 and age at first birth <25 years, and parity ≥3 and age at first birth ≥25 years), body mass index (calculated as weight in kilograms divided by the square of height in meters; <16.5, 16.5-19.9, 20.0-22.4, 22.5-24.9, 25.0-29.9, and ≥30.0), age at menarche (<12, 12 to 13, and ≥14 years), family history of breast cancer (yes, no), history of benign breast disease (yes, no), oral contraceptive use (never, <4 years in the past, ≥4 years in the past, currently ≤5 g/day, and currently >5 g/day), alcohol intake (nondrinkers, <1 to 3 g/wk, 1 to 6 g/wk, and ≥6 g/wk), age at first birth (nulliparous, parity ≥2 and age at first birth <25 years, parity ≥2 and age at first birth 25 to <30 years, parity ≥2 and age at first birth ≥30 years, parity ≥3 and age at first birth <25 years, and parity ≥3 and age at first birth ≥25 years), and energy intake (continuous). The numbers of cases were 1021 for total breast cancer, 512 for ER+/PR+ cancer, and 167 for ER−/PR− cancer.
lated to increased risk of ER+ breast cancer among postmenopausal women. We previously found that animal fat intake was positively related to breast cancer risk among premenopausal women and that the association was stronger with ER+ breast cancer risk.23 No other dietary factors have been specifically associated with hormone receptor–positive cancers, although, to our knowledge, red meat intake has not been examined in relation to hormone receptor–positive cancers.

Several biological mechanisms may explain the positive association between red meat intake and hormone receptor–positive breast cancer risk. Because hormonal risk factors are more strongly related to hormone receptor–positive cancers, meat intake may operate through hormonal pathways. First, cooked or processed red meat is a source of carcinogens, such as heterocyclic amines, nitroso–compounds, and polycyclic aromatic hydrocarbons, that increase mammary tumors in animals and have been hypothesized to increase breast cancer risk.3,13,39,40 A nested case-control study among postmenopausal women reported a positive association between doneness of red meat...
meat and breast cancer risk. A few studies have measured intake of heterocyclic amines, which are created during the cooking of red meat, and found positive association with overall breast cancer risk. None of these studies examined breast cancer risk by hormone receptor status. Heterocyclic amines are estrogenic and can stimulate ER-dependent gene expression and the expression of PR in vitro. Animal studies found that heterocyclic amines increase serum prolactin levels. We had only limited information on methods of preparation of red meat so we were not able to examine this hypothesis in detail. Second, exogenous hormone treatment of beef cattle for growth promotion, which is banned in European countries but not in the United States, has been of concern. Although long-term health effects of hormone residues in beef have not been investigated, theoretically they may preferentially affect hormone receptor-positive tumors. Third, red meat is a source of heme iron, a highly bioavailable form of iron and a major source of stored body iron, which has been shown to raise estrogen-induced tumor induction. Finally, fat intake in general has been hypothesized to raise steroid hormone levels. However, randomized trials provide little support for this hypothesis. Also, in our previous report, we found that intake of animal fat, but not vegetable fat, was related to elevated risk of breast cancer. It is not clear why only fat from animal-based food would affect hormone levels and breast cancer risk, unless it were because of correlated constituents.

This study had several strengths. The prospective design of the study avoided the biases of case-control studies, and few participants were lost to follow-up. With repeated measures of dietary intake, we examined diet in different ways. Our dietary assessment method was shown to be informative by a variety of methods, but some error is inevitable and would tend to underestimate the magnitude of associations. Error is reduced by the use of repeated measures, and this would not account for the positive association we observed with red meat intake only among hormone receptor–positive breast cancer cases. Also, we had information on a wide range of potential confounders and adjusted for them.

In conclusion, in this population of relatively young, premenopausal women, red meat intake was associated with a higher risk of hormone receptor–positive breast cancer but not with risk of hormone receptor–negative cancer. Given that most of the risk factors for breast cancer are not easily modifiable, these findings have potential public health implications in preventing breast cancer and should be evaluated further.

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