Protein Intake by Source and Breast Cancer Incidence and Mortality: The Woman’s Health Initiative

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Abstract

Background: Prior studies of dietary protein intake and breast cancer have been mixed with previous studies limited by dietary self-report measurement error.

Methods: Biomarker-calibrated total protein intake and estimated vegetable protein and animal protein intake were determined from baseline food frequency questionnaires (FFQ) in 100,024 Women’s Health Initiative (WHI) participants. Associations between total, animal and vegetable protein intake and breast cancer incidence, deaths from breast cancer, and deaths after breast cancer were estimated using Cox proportional hazards regression. Breast cancers were verified by medical record review and survival outcomes enhanced by National Death Index (NDI) queries. All statistical tests were 2-sided.

Results: After 14 years follow-up, there were 6,340 incident breast cancers, 764 deaths from breast cancer and 2,059 deaths after breast cancer. In multivariable analyses, higher calibrated total protein intake was not associated with breast cancer incidence or deaths from or after breast cancer. Vegetable protein intake was associated with statistically significantly lower breast cancer incidence (HR = 0.98, 95% CI = 0.96-0.99, \( P_{trend} = 0.006 \)) and statistically significantly lower risk of death after breast cancer (HR = 0.93, 95% CI = 0.91 to 0.97, \( P_{trend} <0.001 \)) but not with deaths from breast cancer. In contrast, higher animal protein intake was associated with statistically significantly higher breast cancer incidence (HR = 1.03, 95% CI 1.01-1.06, \( P_{trend} = 0.02 \)) but not with deaths from or after breast cancer.

Conclusions: Calibrated total protein intake was not associated with breast cancer incidence or mortality. Higher vegetable protein intake was associated with lower breast
cancer incidence and lower risk of death after breast cancer. Higher animal protein intake was associated with higher breast cancer incidence.
Findings on the relationship between dietary protein intake and breast cancer incidence and outcome have been inconsistent. In 2016, a meta-analysis of 46 prospective cohort studies, nested case-control studies, and case-cohort studies demonstrated that total red meat intake was associated with higher breast cancer risk, with relative risk [RR] 1.07 (95% confidence interval [CI] 1.01-1.14) for each increase in servings of red meat\(^1\). In contrast, greater protein intake has been associated with better breast cancer survival in several prospective studies\(^2-4\) including one from the Nurses’ Health Study (NHS)\(^5\). The NHS findings were recently updated after 16 years of follow-up. Among 6,348 women diagnosed with early stage breast cancer, increasing quintiles of post diagnosis total and animal protein intake were statistically significantly associated with lower breast cancer recurrence risk (\(P_{trend} = 0.02\) and \(P_{trend} = 0.003\), respectively), and increasing quintiles of animal protein intake were associated with lower risk of deaths from breast cancer (\(P_{trend} = 0.044\))\(^6\). Vegetable protein results were not statistically significant.

During the period of these reports, investigators in the Women’s Health Initiative (WHI) conducted a biomarker study (n=544) to evaluate the accuracy of self-reported energy and protein consumption from food frequency questionnaires (FFQ) using doubly labeled water for energy and urinary nitrogen for protein\(^7\). Using this approach, FFQ was found to considerably underestimate total energy intake by about 30%, modestly underestimate protein intake by about 15%, and overestimate the percentage of energy from protein. As a result, regression equations incorporating participant characteristics were developed to account for differential reporting errors in dietary data.
These equations are then used to adjust self-reported total protein intake for measurement error in WHI analyses.

Using this approach, Prentice and colleagues examined biomarker-calibrated total protein consumption and breast cancer risk in the WHI Dietary Modification trial comparison group (n=21,711) and WHI Observational Study (n=59,105) based on follow-up through 2005 with 1703 breast cancer cases. Calibrated total protein intake was positively associated with higher total cancer incidence (HR 1.18, 95% CI 1.11-1.38) and higher breast cancer incidence (HR 1.24, 95% CI 1.11-1.38), with this positive association essentially attributable to correlation between protein and energy consumption.

Now, with additional follow-up, we examined the association of biomarker-calibrated total protein intake with breast cancer incidence, deaths from breast cancer, and deaths after breast cancer, defined as breast cancer diagnosis followed by death from any cause. Additional analyses examined associations of estimated animal and vegetable protein with the same breast cancer outcomes. Given the prior findings associating higher biomarker-calibrated total protein consumption with higher breast cancer incidence, we did not anticipate that higher total protein intake would be favorably associated with breast cancer incidence and outcome.

### Methods

#### Study design

WHI investigators recruited 161,808 postmenopausal women to four clinical trials and an observational study at 40 US clinical centers between 1993 and 1998. Women were
eligible if they were between 50 and 79 years of age with plans to remain in the same area for the next three years. Eligibility for the Dietary Modification (DM) trial required baseline dietary fat intake $\geq 32\%$ of total energy intake by FFQ and additional eligibility requirements largely based on adherence issues. All women provided written informed consent and studies were approved by the institutional review boards at the clinical centers.

For the current analysis, the study population included women in the WHI Observational Study (OS) and women in the WHI Clinical Trial (CT) ($n = 93,676$), limited to those participants not randomized to the intervention group of the Dietary Modification (DM) trial ($n = 19,541$) (total $n = 122,970$). After exclusion of participants with no follow-up ($n = 30$), with caloric intake $<500$ or $>5000$ kcal/day ($n = 5,533$), underweight ($<18.5$ kg/m$^2$) ($n = 1,327$), with prior breast cancer ($n = 5,420$), or missing calibration ($n = 7,370$) or missing model covariate data ($n = 22,563$), $100,024$ were eligible (Table 1).

Details regarding the WHI study design, recruitment and implementation have been previously described$^9$. Medical, reproductive, and family histories were obtained by self-reported questionnaires. Height and weight were measured by study staff using standardized procedures with body mass index (BMI) calculated. In the Clinical Trial group, women were queried twice per year through 2005 and annually thereafter for medical outcomes including breast cancer. Observational Study women were queried annually for medical outcomes. Breast cancer reports were verified by medical record and pathology report review by centrally trained physician adjudicators at the clinical centers with final adjudication and staging per Surveillance Epidemiology and End
Results program (SEER) criteria at the WHI clinical coordinating center. Cause of death was determined by medical record or death certificate review at the clinical coordinating center, information from National Death Index queries and, in some cases by reports from participants’ relatives.

Dietary intake was assessed using FFQs including 122 individual food/food group items, 19 adjusted items, and 4 summary questions. In the DM trial, FFQs were obtained at baseline and after one year. In the OS, FFQs were obtained at baseline and at year 3. To avoid potential immortal status confounding, baseline FFQs were used in the current analyses for breast cancer incidence and breast cancer mortality for all except the subgroup of women in the DM trial comparison group. For the subgroup of women in the DM trial comparison group (N=19,541), since baseline FFQs were biased due to their use in trial eligibility screening (baseline dietary fat intake ≥ 32% of total energy intake was required), year 1 FFQs were used for analyses.

Biomarker-Calibrated Protein Estimation: As previously described, in the WHI Nutritional Biomarkers Study (NBS), 544 women from twelve clinical centers of the Dietary Modification trial participated in a doubly-labeled water protocol to estimate total energy expenditure over a two-week period and a urinary nitrogen protocol (determined by Kjeldahl method) to estimate protein consumption over a 24-hour period with PABAcheck used as a measure of complete urine collection with repeated measurements for quality control. The study design incorporated a 20% reliability subsample where the protocol was repeated after 6 months. Biomarker-calibrated total protein intakes were compared with concurrent self-reported FFQ dietary intake data. Calibration equations were then developed by using a linear regression of log-biomarker
estimates on corresponding log-FFQ estimates involving retained covariates of Body Mass Index, age, race-ethnicity, income, education, and an interaction term for FFQ · BMI. Analytic codes used in this report are available in a collaborative mode as described on the Women’s Health Initiative website (www.whi.org). In past analysis, studies of calibrated protein intake have shown favorable associations, with frailty\(^1\)\(^2\) and physical function\(^1\)\(^3\), that were attenuated in analyses based on FFQ measures without biomarker calibration.

The calibration equation that was derived through the Nutritional Biomarkers Study for calibrated dietary protein intake was applied to the dietary intake data for most participants in the current analysis\(^7\). For the few women included in the Nutritional Biomarkers Study, the previously developed calibration equations were used.

To determine the intake of animal protein versus vegetable protein, the FFQ was used to determine each participant’s percent ratio of animal versus vegetable protein intake. Animal protein was defined as coming from animal products, including meats, eggs, and dairy foods. Vegetable protein was defined as coming from plant products. The individual percentages were then multiplied by the calibrated total protein to estimate the animal and vegetable protein intake (g/day). Bootstrap variance estimators were used for all of the log HR estimates and all models were adjusted for the log-calibrated energy intake.

Follow-up beyond the original protocol end date (2005) for Extension 1 required re-consent and follow-up beyond 2010 (and ongoing) for Extension 2 again required re-consent. Re-consent was obtained from 73% of surviving participants in the Observational Study in 2005 and 83% in 2010. Re-consent was obtained from 82.4% of
surviving participants in the Clinical Trial in 2005 and 85.2% in 2010. Survival information was enhanced by serial National Death Index queries, complete through 2014, which identify 98% of deaths\textsuperscript{14}. Findings on longer term breast cancer incidence could possibly be influenced by re-consent status. Findings on deaths from breast cancer and deaths after breast cancer, which incorporated serial NDI queries, were not influenced by re-consent status of participants.

**Outcomes**

Biomarker calibrated data applies to the analyses for total protein. The associations among intakes of calibrated total protein and estimated vegetable protein and animal protein were examined for breast cancer incidence, deaths from breast cancer (breast cancer followed by death directly attributed to the breast cancer) and deaths after breast cancer (breast cancer followed by death from all causes).

**Statistical analysis**

Demographics at the time of FFQ collection by quintiles of dietary protein intake are presented with frequencies and percentages for categorical variables and means with standard deviations for continuous variables. P-values were derived from linear (continuous, ordinal variables) or logistic (dichotomous variables) models, modeling the demographic variable as a function of linear trend over protein quintiles (Table 1). Characteristics of invasive breast cancer by quintiles of dietary protein intake are presented with frequencies and percentages (Table 2).
Associations between dietary protein intake and breast cancer incidence and breast cancer mortality were examined using Cox proportional hazards regression (Table 3). Findings from two models were conducted. Model 1 was adjusted for log-transformed calibrated daily energy intake, stratified by WHI component (Observational Study / Clinical Trial), 5-year age group, and time-dependent WHI trial period (WHI, Extension 1, Extension 2). Model 2 adjusted for Model 1 variables plus recreational physical activity, geographical socio-economic status, race/ethnicity, Breast Cancer Risk Assessment Tool (BCRAT) 5-year risk of breast cancer, parity, alcohol use and oral contraceptive use. Model 2 is additionally stratified by menopausal hormone therapy use and hysterectomy status. For each model, the hazard ratio for the protein intake parameter estimate as well as it’s corresponding 95% confidence interval and two-sided p-value calculated using a Chi-square test are presented, with p-values less than 0.05 considered to be statistically significant. Additional analyses examined association of protein intake with breast cancer subtypes: estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status. In exploratory analyses, the variability of total protein intake was examined over time in the subset of participants in the DM trial with serial FFQ analyses (n=1858).

The proportional hazards assumption was checked graphically looking at quintiles and also tested with the log linear calibrated protein intake by fitting a proportional hazards model with each of the outcomes as a function on the log calibrated protein and the interaction between log calibrated protein and the log follow-up time. In each of the models the proportional hazards assumption was not violated.
Follow-up time was calculated from the date of enrollment to the date of last contact or death through September 2016, whichever came first. All analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC).

Results

Comparing participants in the highest versus lowest quintile of calibrated total protein intake, women with higher protein intake had greater body mass index (BMI), were more likely to be White, be hormone therapy users, and have higher total energy intake and fat intake (Table 1). After an average of 14.8 years follow-up, there were 6,340 incident invasive breast cancers, 764 deaths from breast cancer and 2,059 deaths after breast cancer.

Comparing characteristics and stage of breast cancers across quintiles of calibrated total protein intake, no major differences in breast cancer characteristics by hormone receptor status, HER2 status or stage were apparent (Table 2). When calculated in models evaluating invasive breast cancer as a function of linear trend across 20% increments of total protein, higher calibrated total protein intake was not associated with breast cancer incidence (HR = 1.02, 95% CI 0.92-1.14, P for linear trend = 0.72) (Table 3). However, higher vegetable protein intake was associated with statistically significantly lower breast cancer incidence (HR = 0.98, 95% CI = 0.96-0.99, P for linear trend = 0.006). In contrast, higher animal protein intake was associated with statistically significantly higher breast cancer incidence (HR = 1.03, 95% CI 1.01-1.06, P for linear trend = 0.02).
Total protein intake, when analyzed based on a 20% increase in the protein variable, was not associated with deaths from breast cancer or deaths after breast cancer (HR = 0.79, 95% CI = 0.65 to 1.13, P for linear trend = 0.06) (Table 3). Higher vegetable protein intake was associated with statistically significantly lower risk of death after breast cancer (HR = 0.93, 95% CI = 0.91 to 0.97, P < 0.001) but not with lower risk of death from breast cancer (HR = 0.97, 95% CI = 0.92 to 1.02, P = 0.17) (Table 3). Animal protein intake was not associated with deaths from breast cancer or deaths after breast cancer.

All findings for breast cancer incidence and deaths from and after breast cancer are based on analyses of protein intake at entry. However, mean total protein intake levels remained relatively consistent through seven years follow-up (after year 1, mean = 68.3 g/day; after year 4, mean = 68.0 g/day; after year 7, mean = 67.6 g/day) (Supplementary Table 1).

Discussion
In a large prospective cohort of postmenopausal women with long-term follow-up, higher calibrated total protein intake was not associated with invasive breast cancer incidence, deaths from breast cancer or deaths after breast cancer. Vegetable protein intake was associated with statistically significantly lower breast cancer incidence and statistically significantly lower deaths after breast cancer while higher animal protein intake was associated with statistically significantly higher breast cancer incidence. The current findings do not support benefit of higher animal protein intake on breast cancer incidence or outcome.
Total protein intake in these analyses were estimated from the FFQ corrected for measurement error using regression calibration equations developed from objective measures of total energy expenditure (doubly labeled water) and dietary protein (24-hour urinary nitrogen) in the previously described WHI Nutritional Biomarkers Study. The utility of this correction was seen in the subsequent study of protein intake and incident frailty in Women’s Health Initiative participants. There, while higher biomarker-calibrated total protein intake was statistically significantly associated with a dose-response lower risk of incident frailty, using uncalibrated total protein measures underestimated the strength of the association.

Two meta-analyses of cohort studies have examined associations of protein source intake and breast cancer incidence. One report of eight cohort studies found statistically significant associations of breast cancer incidence with total red meat (dose-response RR 1.07, 95% CI 1.01-1.14) but not poultry, fish, egg, nuts, total milk, and whole milk intake. A second report of 13 cohort, three nested case-control studies and two clinical trials found that processed meat, comparing the highest to lowest category, was associated with 9% higher breast cancer risk (RR 1.09, 95% CI 1.03-1.16). These findings are concordant with current study results where higher animal protein intake was associated with statistically significantly higher breast cancer incidence.

In terms of breast cancer mortality, in an analysis of 6,348 women with breast cancer with findings measured from breast cancer diagnosis in the Nurse’s Health Study with 919 deaths attributed to breast cancer and 1,847 total deaths, there was an inverse association between post-diagnosis animal protein intake and deaths attributed to the cancer ($P_{trend} = 0.044$). The authors concluded “there is likely no advantage in
restricting protein intake” for women with a breast cancer history\textsuperscript{6}. The current WHI study findings did not directly address that question as protein intake in these analyses was determined on study entry, prior to breast cancer diagnosis. However, we did find that protein intake was stable at least through seven years of follow-up. These observational study findings of association of higher animal protein intake with higher breast cancer incidence are not consistent with the Nurse’s Health Study findings.

The current study findings of an association between higher vegetable protein intake and lower breast cancer risk suggest a potential contributing factor to the favorable effect seen in the WHI Dietary Modification randomized trial\textsuperscript{16} where the low-fat dietary intervention was associated with a statistically significant reduction in deaths from breast cancer. The WHI DM is an ongoing randomized clinical trial (intervention phase concluded in 2005) with breast cancer incidence as a primary endpoint evaluating a low-fat dietary intervention targeting reduced total fat intake and increased intake of fruits, vegetables, and grains. Caloric intake reduction and weight loss were not intervention targets\textsuperscript{17}. Participants in the intervention group reported compensating for the reduced fat intake by increasing carbohydrate and protein intake, specifically increasing plant protein intake which was statistically significant versus comparison group findings (P <0.001)\textsuperscript{18}. It is possible that the increase in plant protein contributed to the statistically significant reduction in deaths after breast cancer\textsuperscript{16,19} and statistically significant reduction in deaths from breast cancer seen in intervention group participants\textsuperscript{16}.

Study strengths include the large, diverse population of well-characterized postmenopausal women with long-term follow-up, the prospective study design, and
breast cancer cases verified by medical record review, biomarker-calibrated adjustment of total protein intake, analyses adjusted for biomarker-calibrated energy intake and long-term follow-up with mortality information enhanced by serial National Death Index queries.

This study has limitations. First, the observational design precludes causal inference. Second, the findings are based on baseline protein intake determinations with breast cancer outcomes identified years later. However, none of the participants were in a trial designed to change dietary intake and the substantial difference in characteristics among women in low versus high protein intake quintiles suggest dietary differences may be long-standing. Third, results regarding animal and vegetable protein should be considered hypothesis-generating given limitations of FFQ for certain foods including red meat and processed meat. Fourth, while statistically significantly lower breast cancer incidence and lower risk of death after breast cancer were seen in women with higher vegetable protein intake, the absolute differences were modest. Finally, detailed information regarding breast cancer therapy, which may influence mortality data, was not available.

Based on findings from biomarker-calibrated determination of total protein intake, higher total protein intake was not associated with breast cancer incidence or risk of deaths from or after breast cancer. Vegetable protein intake was associated with statistically significantly lower breast cancer incidence and statistically significantly lower risk of death after breast cancer while higher animal protein intake was associated with statistically significantly higher breast cancer incidence.
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Author contributions: KP and RTC wrote the initial analysis proposal and KP wrote the initial draft of the report. KP, RTC and JCL had full access to the data and take full responsibility for the integrity of the data and accuracy of the data analyses. JCL undertook the statistical analyses. All authors provided critical review of the manuscript for important intellectual content. RTC, RLP, JA Mortimer, JE Manson, LVH, TER, and DL collected the data and obtained study funding.
Prior presentation: A portion of the current findings was included in a presentation at the American Society of Clinical Oncology (ASCO) annual meeting on May 29, 2020. We thank the WHI investigators, staff, and trial participants for their dedication and commitment.
Data availability

The data underlying this article are available through the WHI online resource, https://www.whi.org/researchers/data/Pages/Home.aspx, while the WHI remains funded (currently through 2020) and indefinitely through BioLINCC, https://biolincc.nhlbi.nih.gov/studies/whi_ctos/.

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Table 1. Subject characteristics at time of baseline food frequency questionnaire by quintile of calibrated protein intake

| Characteristic                          | Quintile of calibrated protein intake |  |  |  |  |  |  |  |
|----------------------------------------|---------------------------------------|---|---|---|---|---|---|
|                                        | 1          | 2          | 3          | 4          | 5          |  |  |
|                                        | No. (%)    | No. (%)    | No. (%)    | No. (%)    | No. (%)    |  |  |
| Mean age, y (SD)                       | 69.8 (6.1) | 66.7 (6.4) | 64.2 (6.2) | 61.4 (5.9) | 58.2 (5.2) | <0.001 |  |
| <55                                    | 322 (1.6)  | 854 (4.3)  | 1390 (6.9) | 2676 (13.4)| 5486 (27.4)|  |  |
| 55 – 59                                | 1078 (5.4) | 2097 (10.5)| 3323 (16.6)| 5241 (26.2)| 6968 (34.8)|  |  |
| 60 – 64                                | 2405 (12.0)| 3867 (19.3)| 5299 (26.5)| 5909 (29.5)| 4927 (24.6)|  |  |
| 65 – 69                                | 4742 (23.7)| 5894 (29.5)| 5860 (29.3)| 4315 (21.6)| 2118 (10.6)|  |  |
| 70 – 74                                | 6442 (32.2)| 5257 (26.3)| 3316 (16.6)| 1612 (8.1) | 462 (2.3)  |  |  |
| ≥75                                    | 5015 (25.1)| 2037 (10.2)| 815 (4.1)  | 253 (1.3)  | 44 (0.2)   |  |  |
| Mean Body mass index, kg/m² (SD)       | 24.5 (3.5) | 35.9 (3.9) | 27.0 (4.4) | 28.5 (5.0) | 33.2 (7.3) | <0.001 |  |
| Mean Physical activity, MET-hr/wk (SD) | 11.9 (12.8)| 13.3 (13.6)| 13.6 (13.9)| 13.5 (14.4)| 12.0 (14.4)| 0.95 |  |
| Mean SES Index (SD)                    | 74.5 (9.5) | 76.0 (8.5) | 76.4 (8.1) | 76.4 (7.9) | 76.0 (8.0) | <0.001 |  |
| Race/ethnicity                         |            |            |            |            |            | <0.001b |  |
| White                                  | 14444 (72.2)| 16599 (83.0)| 17358 (86.8)| 17710 (88.5)| 18014 (90.0)|  |  |
| African American                       | 3048 (15.2)| 1590 (7.9) | 1200 (6.0) | 888 (4.4)  | 671 (3.4)  |  |  |
| Hispanic                               | 869 (4.3)  | 790 (3.9)  | 693 (3.5)  | 772 (3.9)  | 798 (4.0)  |  |  |
| Race            | $<20k | $20-$<50k | $50-$<75k | $75-$<100k | ≥$100k |
|-----------------|-------|-----------|-----------|------------|--------|
| Native American | 93 (0.5) | 81 (0.4) | 68 (0.3) | 74 (0.4) | 100 (0.5) |
| Asian           | 1159 (5.8) | 650 (3.2) | 444 (2.2) | 351 (1.8) | 235 (1.2) |
| Unknown         | 391 (2.0) | 296 (1.5) | 240 (1.2) | 211 (1.1) | 187 (0.9) |

Income

| ≤ High school | 5318 (26.6) | 4608 (23.0) | 4313 (21.6) | 4195 (21.0) | 4076 (20.4) |
| Some college  | 7660 (38.3) | 7475 (37.4) | 7207 (36.0) | 7249 (36.2) | 7562 (37.8) |
| ≥ College degree | 6884 (34.4) | 7794 (39.0) | 8330 (41.6) | 8438 (42.2) | 8188 (40.9) |

Education

OC ever use

| Never | 8640 (43.2) | 7749 (38.7) | 7291 (36.4) | 6945 (34.7) | 7101 (35.5) |
| Former | 3266 (16.3) | 2995 (15.0) | 2776 (13.9) | 2644 (13.2) | 2511 (12.6) |
| Current | 8098 (40.5) | 9262 (46.3) | 9936 (49.7) | 10417 (52.1) | 10393 (52.0) |
### Hysterectomy

| Mean Dietary intake (SD) | 8681 (43.4) | 8257 (41.3) | 8129 (40.6) | 7997 (40.0) | 8193 (41.0) | <0.001 |
|-------------------------|------------|------------|------------|------------|------------|--------|
| Total energy\(^a\), kcal/d | 1868.9 (102.8) | 1984.2 (91.6) | 2069.7 (95.5) | 2168.8 (103.7) | 2383.6 (204.7) | <0.001 |
| Protein\(^3\), g/d       | 60.7 (4.1)  | 68.6 (1.6)  | 74.0 (1.5)  | 79.8 (1.9)  | 90.2 (6.4)  | <0.001 |
| Percent Animal Protein   | 64.0 (11.8) | 67.1 (10.4) | 68.8 (9.7)  | 70.0 (9.2)  | 72.0 (8.5)  | <0.001 |
| Percent Vegetable Protein| 35.9 (11.8) | 32.8 (10.4) | 31.1 (9.7)  | 29.8 (9.2)  | 27.9 (8.5)  | <0.001 |
| Carbohydrate, g/d        | 149.4 (56.4) | 180.7 (62.6) | 198.8 (68.2) | 216.7 (73.5) | 248.5 (89.2) | <0.001 |
| Fat, g/d                 | 38.2 (19.5) | 47.7 (22.6) | 54.5 (25.4) | 61.5 (28.5) | 77.6 (37.3) | <0.001 |

### Alcohol Use

| Alcohol Use | Never | Former | Current |
|-------------|-------|--------|---------|
|             | 2949 (14.7) | 4292 (21.5) | 12763 (63.8) |
|             | 2239 (11.2) | 3751 (18.7) | 14016 (70.1) |
|             | 2025 (10.1) | 3670 (18.3) | 14308 (71.5) |
|             | 1929 (9.6)  | 3765 (18.8) | 14312 (71.5) |
|             | 1915 (9.6)  | 4455 (22.3) | 13635 (68.2) |

\(^a\) *P*\(_{trend}\) from either a linear (continuous, ordinal characteristics) or logistic (dichotomous characteristics) model with the characteristic of interest as a function of linear trend across protein quintile medians.

\(^b\) *p*-value compares White vs. non-White participants

\(^c\) *p*-value compares current vs. former/never users
| Characteristic               | Quintile of calibrated protein intake |  |  |  |  |  |
|-----------------------------|-------------------------------------|---|---|---|---|---|
|                             | 1                                  | 2                        | 3                        | 4                        | 5                        |
|                             | No. (%)<sup>a</sup>                | No. (%)<sup>a</sup>       | No. (%)<sup>a</sup>       | No. (%)<sup>a</sup>       | No. (%)<sup>a</sup>       |
| **Histology**               |                                     |                          |                          |                          |                          |
| Ductal                      | 629 (65.4)                          | 779 (63.5)                | 864 (65.1)                | 898 (65.2)                | 918 (66.8)                |
| Lobular                     | 110 (11.4)                          | 142 (11.6)                | 127 (9.6)                 | 138 (10.0)                | 114 (8.3)                 |
| Ductal and lobular          | 113 (11.7)                          | 187 (15.2)                | 187 (14.1)                | 178 (12.9)                | 186 (13.5)                |
| Other                       | 110 (11.4)                          | 119 (9.7)                 | 149 (11.2)                | 163 (11.8)                | 157 (11.4)                |
| **Estrogen receptor status**|                                     |                          |                          |                          |                          |
| Positive                    | 749 (83.1)                          | 985 (85.1)                | 1096 (87.5)               | 1122 (86.9)               | 1121 (86.0)               |
| Negative                    | 152 (16.9)                          | 172 (14.9)                | 157 (12.5)                | 169 (13.1)                | 183 (14.0)                |
| **Progesterone receptor status**|                                  |                          |                          |                          |                          |
| Positive                    | 601 (67.7)                          | 828 (71.9)                | 911 (74.0)                | 955 (74.7)                | 980 (75.9)                |
| Negative                    | 287 (32.3)                          | 323 (28.1)                | 320 (26.0)                | 324 (25.3)                | 311 (24.1)                |
| **Estrogen/progesterone receptor status**|                           |                          |                          |                          |                          |
| ER+, PR+                    | 585 (66.0)                          | 814 (70.8)                | 897 (72.9)                | 944 (74.0)                | 963 (74.6)                |
| ER+, PR-                    | 152 (17.1)                          | 164 (14.3)                | 180 (14.6)                | 163 (12.8)                | 147 (11.4)                |
|                          | ER-, PR+ | ER-, PR- | HER2 overexpression (+) | Triple negative tumor | Stage | Grading | Tumor size, cm | Positive lymph nodes |
|--------------------------|----------|----------|-------------------------|----------------------|-------|---------|---------------|---------------------|
|                          | 16 (1.8) | 14 (1.2) | 14 (1.1)                | 11 (0.9)             | 17 (1.3) |        |               |                     |
|                          | 134 (15.1) | 157 (13.7) | 140 (11.4) | 157 (12.3) | 164 (12.7) |        |               |                     |
| HER2 overexpression (+)  | 97 (13.9) | 127 (13.9) | 140 (13.3) | 144 (13.4) | 135 (11.9) |        |               |                     |
| Triple negative tumor    | 79 (11.4) | 93 (10.2) | 80 (7.6)                | 98 (9.2)             | 97 (8.6)  |        |               |                     |
| Stage                    |          |          |                        |                      |        |         |               |                     |
| Local                    | 729 (76.7) | 916 (75.5) | 1002 (76.1) | 1010 (74.1) | 998 (73.1) |        |               |                     |
| Regional or distant      | 221 (23.3) | 297 (24.5) | 315 (23.9) | 353 (25.9) | 367 (26.9) |        |               |                     |
| Grading                  |          |          |                        |                      |        |         |               |                     |
| Well differentiated      | 236 (27.6) | 339 (30.5) | 361 (30.0) | 342 (27.5) | 361 (28.5) |        |               |                     |
| Moderately differentiated| 405 (47.3) | 510 (45.8) | 548 (45.6) | 584 (46.9) | 591 (46.6) |        |               |                     |
| Poorly differentiated    | 215 (25.1) | 264 (23.7) | 293 (24.4) | 319 (25.6) | 316 (24.9) |        |               |                     |
| Tumor size, cm           |          |          |                        |                      |        |         |               |                     |
| <1                       | 284 (30.4) | 364 (30.7) | 382 (29.8) | 406 (30.5) | 436 (32.8) |        |               |                     |
| 1 - <2                   | 376 (40.2) | 485 (40.7) | 575 (44.9) | 547 (41.1) | 510 (38.3) |        |               |                     |
| ≥2                       | 275 (29.4) | 342 (28.7) | 323 (25.2) | 378 (28.4) | 385 (28.9) |        |               |                     |
| Positive lymph nodes     |          |          |                        |                      |        |         |               |                     |
| None                     | 588 (76.8) | 793 (45.2) | 896 (75.9) | 923 (75.3) | 918 (73.7) |        |               |                     |
| 1-3                      | 128 (16.7) | 181 (10.3) | 211 (17.9) | 223 (18.2) | 243 (19.5) |        |               |                     |
| ≥4                       | 50 (6.5) | 780 (44.5) | 73 (6.2) | 79 (6.4) | 85 (6.8) |        |               |                     |
Percentages based on non-missing data only. Missing participants: Histology n=72; Estrogen receptor status n=434; Progesterone receptor status n=500; Estrogen/progesterone receptor status n=507; HER2 overexpression n=1463; Triple negative tumor n=1498; Stage n=132; Grading n=656; Tumor size n=272; Positive lymph nodes n=169. ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2
Table 3. Association between sources of protein intake and breast cancer using Cox proportional hazards regression.

| Protein Source                  | Invasive Breast Cancer | Death from Breast Cancer | Death after Breast Cancer |
|--------------------------------|------------------------|--------------------------|--------------------------|
|                                | Model 1<sup>a</sup>    | Model 2<sup>b</sup>     | Model 1<sup>a</sup>    | Model 2<sup>b</sup>     |
|                                | HR (95% CI)            | P                        | HR (95% CI)            | P                        |
| Total Protein, 20% Increase    | 1.15 (1.04, 1.28)      | 0.005                    | 1.02 (0.92, 1.14)      | 0.72 (0.74, 1.08)        | 0.16 (0.72, 1.20)        | 0.46 (0.76, 1.14)        | 0.22 (0.65, 1.13)        | 0.06 (0.65, 1.13)        |
| Animal Protein, 20% Increase   | 1.05 (1.02, 1.08)      | <0.001                   | 1.03 (1.01, 1.06)      | 0.02 (0.91, 1.06)        | 0.68 (0.91, 1.07)        | 0.67 (1.00, 1.10)        | 0.06 (0.99, 1.09)        | 0.14 (0.99, 1.09)        |
| Vegetable Protein, 20% Increase| 0.98 (0.96, 1.00)      | 0.01                     | 0.98 (0.96, 0.99)      | 0.006 (0.91, 1.01)       | 0.09 (0.92, 1.02)        | 0.17 (0.91, 0.97)        | <0.001 (0.91, 0.97)      | <0.001 (0.91, 0.97)      |

<sup>a</sup> Model 1: Adjusted for log-transformed calibrated daily energy intake and is stratified by WHI component (observational study / clinical trial), 5-year age group, and time-dependent WHI trial period (WHI, Extension 1, Extension 2).

<sup>b</sup> Model 2: Model 1 plus additional adjustment for physical activity, geographical socio-economic status, race, gail 5 year risk of breast cancer, parity, alcohol use, oral contraceptive use. Model 2 is additionally stratified by hormone use and hysterectomy ever.