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Article

Protein Intake by Source and Breast Cancer Incidence and Mortality: The Women's Health Initiative

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Abstract

Background: Prior studies of dietary protein intake and breast cancer have been mixed and were 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 in 100 024 Women's Health Initiative 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 queries. All statistical tests were 2-sided. **Results:** After 14 years of follow-up, there were 6340 incident breast cancers, 764 deaths from breast cancer, and 2059 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 (hazard ratio [HR] = 0.98, 95% confidence interval [CI] = 0.96 to 0.99, $P_{trend} = .006$) and statistically significantly lower risk of death after breast cancer (HR = 0.93, 95% CI = 0.91 to 0.97, $P_{trend} < .001$) 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 to 1.06, $P_{trend} = .02$) 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) of 1.07 (95% confidence interval [CI] = 1.01 to 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 6348 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 ($P_{trend} = .02$ and .003, respectively), and increasing quintiles of animal protein intake were associated with lower risk of deaths from breast cancer ($P_{trend} = .044$) (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

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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 (8) examined biomarker-calibrated total protein consumption and breast cancer risk in the WHI Dietary Modification (DM) trial comparison group (n = 21 711) and WHI Observational Study (OS) (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 to 1.38) and higher breast cancer incidence (HR = 1.24, 95% CI = 1.11 to 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 (8), 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 4 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 3 years. Eligibility for the DM trial required baseline dietary fat intake equal to or more than 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 OS and women in the WHI Clinical Trial (CT) (n = 93 676), limited to those participants not randomly assigned to the intervention group of the DM trial (n = 19 541) (total N = 122 970). After exclusion of participants with no follow-up (n = 30), with caloric intake less than 500 or greater than 5000 kcal/d (n = 5533), underweight (<18.5 kg/m^2) (n = 1327), with prior breast cancer (n = 5420), or missing calibration (n = 7370) 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 CT group, women were queried twice per year through 2005 and annually thereafter for medical outcomes, including breast cancer. OS 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 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 or food group items, 19 adjusted items, and 4 summary questions (10). In the DM trial, FFQs were obtained at baseline and after 1 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), because 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 (7), in the WHI Nutritional Biomarkers Study (NBS), 544 women from 12 clinical centers of the DM trial participated in a doubly labeled water protocol to estimate total energy expenditure over a 2-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 (11) 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 BMI, 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 WHI website (www.whi.org). In past analysis, studies of calibrated protein intake have shown favorable associations with frailty (12) and physical function (13) that were attenuated in analyses based on FFQ measures without biomarker calibration.

The calibration equation that was derived through the NBS 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 NBS, the previously developed calibration equations were used.

To determine the intake of animal protein vs vegetable protein, the FFQ was used to determine each participant's percent ratio of animal vs 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 (grams per day). Bootstrap variance estimators were used for all of the log hazard ratio (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 reconsent and follow-up beyond 2010 (and ongoing) for Extension 2 again required reconsent. Reconsent was obtained from 73% of surviving participants in the OS in 2005 and 83% in 2010. Reconsent was obtained from 82.4% of surviving participants in the CT in 2005 and 85.2% in 2010. Survival information was enhanced by serial National Death

Table 1. Participant characteristics at time of baseline FFQ by quintile of calibrated protein intake

Characteristic	Quintile of calibrated protein intake					P_{trend}[a]
	1 No. (%)	2 No. (%)	3 No. (%)	4 No. (%)	5 No. (%)	
Mean age (SD), y	69.8 (6.1)	66.7 (6.4)	64.2 (6.2)	61.4 (5.9)	58.2 (5.2)	<.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 BMI (SD), kg/m^2	24.5 (3.5)	35.9 (3.9)	27.0 (4.4)	28.5 (5.0)	33.2 (7.3)	<.001
Mean physical activity (SD), MET-h/wk	11.9 (12.8)	13.3 (13.6)	13.6 (13.9)	13.5 (14.4)	12.0 (14.4)	.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)	<.001
Race or ethnicity						<.001[b]
White	14 444 (72.2)	16 599 (83.0)	17 358 (86.8)	17 710 (88.5)	18 014 (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)	
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						<.001
<$20k	4357 (21.8)	3223 (16.1)	2741 (13.7)	2358 (11.8)	2448 (12.2)	
$20 to <$35k	5132 (25.7)	4939 (24.7)	4553 (22.8)	4212 (21.1)	3972 (19.9)	
$35 to <$50k	3697 (18.5)	3818 (19.1)	3932 (19.7)	3868 (19.3)	3916 (19.6)	
$50 to <$75k	2895 (14.5)	3512 (17.6)	3834 (19.2)	4131 (20.6)	4222 (21.1)	
$75 to <$100k	1133 (5.7)	1484 (7.4)	1744 (8.7)	1979 (9.9)	2154 (10.8)	
≥$100k	1113 (5.6)	1600 (8.0)	1870 (9.3)	2250 (11.2)	2212 (11.1)	
Education						<.001
≤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)	
OC ever use	4889 (24.4)	6720 (33.6)	8050 (40.2)	9677 (48.4)	11331 (56.6)	<.001
HT use						<.001[c]
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)	10 417 (52.1)	10 393 (52.0)	
Hysterectomy	8681 (43.4)	8257 (41.3)	8129 (40.6)	7997 (40.0)	8193 (41.0)	<.001
Mean dietary intake (SD)						
Total energy[d], kcal/d	1868.9 (102.8)	1984.2 (91.6)	2069.7 (95.5)	2168.8 (103.7)	2383.6 (204.7)	<.001
Protein[d], g/d	60.7 (4.1)	68.6 (1.6)	74.0 (1.5)	79.8 (1.9)	90.2 (6.4)	<.001
Percent animal protein	64.0 (11.8)	67.1 (10.4)	68.8 (9.7)	70.0 (9.2)	72.0 (8.5)	<.001
Percent vegetable protein	35.9 (11.8)	32.8 (10.4)	31.1 (9.7)	29.8 (9.2)	27.9 (8.5)	<.001
Carbohydrate, g/d	149.4 (56.4)	180.7 (62.6)	198.8 (68.2)	216.7 (73.5)	248.5 (89.2)	<.001
Fat, g/d	38.2 (19.5)	47.7 (22.6)	54.5 (25.4)	61.5 (28.5)	77.6 (37.3)	<.001
Alcohol use						<.001[c]
Never	2949 (14.7)	2239 (11.2)	2025 (10.1)	1929 (9.6)	1915 (9.6)	
Former	4292 (21.5)	3751 (18.7)	3670 (18.3)	3765 (18.8)	4455 (22.3)	
Current	12 763 (63.8)	14 016 (70.1)	14 308 (71.5)	14 312 (71.5)	13 635 (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. BMI = body mass index; FFQ = food frequency questionnaire; HT = hormone therapy; MET = metabolic equivalent task; OC = oral contraceptive; SES = socioeconomic status.
[b]P value compares White vs non-White participants.
[c]P value compares current vs former or never users
[d]Calibrated nutrient value.

Index queries, complete through 2014, which identify 98% of deaths (14). Findings on longer term breast cancer incidence could possibly be influenced by reconsent status. Findings on deaths from breast cancer and deaths after breast cancer, which incorporated serial NDI queries, were not influenced by reconsent status of participants.

Outcomes

Biomarker-calibrated data apply 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

Table 2. Characteristics of invasive breast cancer by quintile of calibrated protein intake

Characteristic	Quintile of calibrated protein intake				
	1 No. (%[a])	2 No. (%[a])	3 No. (%[a])	4 No. (%[a])	5 No. (%[a])
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)
ER 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)
PR 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)
ER and PR 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+	16 (1.8)	14 (1.2)	14 (1.1)	11 (0.9)	17 (1.3)
ER−, PR−	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 to <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)

[a]Percentages based on nonmissing data only. Missing participants: histology, n = 72; estrogen receptor status, n = 434; progesterone receptor status, n = 500; estrogen or 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.

(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 SDs 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 2 models were conducted. Model 1 was adjusted for log-transformed calibrated daily energy intake, stratified by WHI component (OS or CT), 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 socioeconomic status, race or ethnicity, Breast Cancer Risk Assessment Tool 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 its corresponding 95% confidence interval and 2-sided *P* value calculated using a χ^2 test are presented, with *P* values less than .05 considered to be statistically significant. Additional analyses examined the association of protein intake with breast cancer subtypes: estrogen receptor, progesterone receptor, and 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 linear log–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.

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[a]		Model 2[b]		Model 1[a]		Model 2[b]		Model[a]		Model 2[b]	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Total protein, 20% increase	1.15 (1.04 to 1.28)	.005	1.02 (0.92 to 1.14)	.72	0.87 (0.74 to 1.08)	.16	0.89 (0.72 to 1.20)	.36	0.88 (0.76 to 1.14)	.22	0.79 (0.65 to 1.13)	.06
Animal protein, 20% increase	1.05 (1.02 to 1.08)	<.001	1.03 (1.01 to 1.06)	.02	0.98 (0.91 to 1.06)	.68	0.98 (0.91 to 1.07)	.67	1.05 (1.00 to 1.10)	.06	1.04 (0.99 to 1.09)	.14
Vegetable protein, 20% increase	0.98 (0.96 to 1.00)	.01	0.98 (0.96 to 0.99)	.006	0.96 (0.91 to 1.01)	.09	0.97 (0.92 to 1.02)	.17	0.94 (0.91 to 0.97)	<.001	0.93 (0.91 to 0.97)	<.001

[a]Model 1: Adjusted for log-transformed calibrated daily energy intake and is stratified by Women's Health Initiative (WHI) component (observational study or clinical trial), 5-year age group, and time-dependent WHI trial period (WHI, Extension 1, Extension 2). CI = confidence interval; HR = hazard ratio.
[b]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, and oral contraceptive use. Model 2 is additionally stratified by hormone use and hysterectomy ever.

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 vs lowest quintile of calibrated total protein intake, women with higher protein intake had greater BMI and 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 6340 incident invasive breast cancers, 764 deaths from breast cancer, and 2059 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 to 1.14, $P_{\text{linear trend}}$ = .72) (Table 3). However, higher vegetable protein intake was associated with statistically significantly lower breast cancer incidence (HR = 0.98, 95% CI = 0.96 to 0.99, $P_{\text{linear trend}}$ = .006). In contrast, higher animal protein intake was associated with statistically significantly higher breast cancer incidence (HR = 1.03, 95% CI = 1.01 to 1.06, $P_{\text{linear trend}}$ = .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, $P_{\text{linear trend}}$ = .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, $P < .001$) but not with lower risk of death from breast cancer (HR = 0.97, 95% CI = 0.92 to 1.02, $P = .17$) (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 7 years follow-up (after year 1, mean = 68.3 g/d; after year 4, mean = 68.0 g/d; after year 7, mean = 67.6 g/d) (Supplementary Table 1, available online).

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, whereas 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 NBS

(7). The utility of this correction was seen in the subsequent study of protein intake and incident frailty in WHI participants. There, although 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 (12).

Two meta-analyses of cohort studies have examined associations of protein source intake and breast cancer incidence. One report of 8 cohort studies found statistically significant associations of breast cancer incidence with total red meat (dose-response RR = 1.07, 95% CI = 1.01 to 1.14) but not poultry, fish, egg, nuts, total milk, and whole-milk intake (1). A second report of 13 cohort, 3 nested case-control studies and 2 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 to 1.16) (15). 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 6348 women with breast cancer with findings measured from breast cancer diagnosis in the NHS with 919 deaths attributed to breast cancer and 1847 total deaths, there was an inverse association between postdiagnosis animal protein intake and deaths attributed to the cancer (P_{trend} = .044). The authors concluded "there is likely no advantage in restricting protein intake" for women with a breast cancer history (6). The current WHI study findings did not directly address that question because protein intake in these analyses was determined on study entry, before breast cancer diagnosis. However, we did find that protein intake was stable at least through 7 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 NHS findings.

This study's 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 DM randomized trial (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 (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 vs comparison group findings ($P < .001$) (18). It is possible that the increase in plant protein contributed to the statistically significant reduction in deaths after breast cancer (16,19) and statistically significant reduction in deaths from breast cancer seen in intervention group participants (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- vs 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, although 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, whereas 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.

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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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