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Cancer Research

Plasma Homocysteine and Cysteine and Risk of Breast Cancer in Women

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Abstract

Homocysteine and cysteine are associated with oxidative damage and metabolic disorders, which may lead to carcinogenesis. Observational studies assessing the association between circulating homocysteine or cysteine and breast cancer are very limited, and findings have been inconsistent. We prospectively evaluated plasma levels of homocysteine and cysteine in relation to breast cancer risk among 812 incident cases of invasive breast cancer and 812 individually matched control subjects from 28,345 women in the Women's Health Study; these women were ≥45 years old, provided blood samples, and had no history of cancer and cardiovascular disease at baseline. Logistic regression controlling for matching factors and risk factors for breast cancer was used to estimate relative risks (RR) and 95% confidence intervals (95% CI). All statistical tests were two sided. Homocysteine levels were not associated with overall risk for breast cancer. However, we observed a positive association between cysteine levels and breast cancer risk; the multivariate RR for the highest quintile group relative to the lowest quintile was 1.65 (95% CI, 1.04–2.61; *P* for trend = 0.04). In addition, women with higher levels of homocysteine and cysteine were at a greater risk for developing breast cancer when their folate levels were low (*P* for interaction = 0.04 and 0.002, respectively). Although our study offers little support for an association between circulating homocysteine and overall breast cancer risk, higher homocysteine levels may be associated with an increased risk for breast cancer among women with low folate status. The increased risk of breast cancer associated with high cysteine levels warrants further investigation. *Cancer Res; 70(6); 2397–405.* ©*2010 AACR.*

Introduction

Homocysteine, a thiol-containing amino acid, is produced through the catabolism of the essential amino acid methionine. When methionine is in excess, homocysteine is degraded to cysteine through the transsulfuration pathway in vitamin B_6–dependent reactions (1). However, under conditions of negative methionine balance, homocysteine is remethylated to methionine in a process that requires methionine synthase with vitamin B_{12} as a cofactor and methyltetrahydrofolate as a cosubstrate (1). Cysteine, converted through homocysteine from methionine, is also a precursor amino acid for synthesis of proteins, glutathione, CoA, and γ-glutamylcysteinylglycine (2). Tissue concentrations of both homocysteine and cysteine are maintained at low levels by tight regulation (2). Disrupted metabolism of homocysteine, resulting from a defect in the transsulfuration or remethylation pathways of homocysteine or by deficiencies of B vitamins required for the pathways, may cause elevation of homocysteine levels (3). Cysteine levels may be elevated with the accumulation of homocysteine or when its catabolism is impaired due to low cysteine dioxygenase (2, 4, 5).

In vitro studies have shown that homocysteine levels are positively associated with proliferation rates of cells in a variety of tumors, including breast tumors (6, 7), as well as with oxidative damage to cells (2, 8, 9). Cysteine has been considered to possess antioxidant properties through its rate-limiting role in biosynthesis of glutathione, the intracellular antioxidant and detoxifying agent (10, 11). However, recent evidence from *in vivo* and *in vitro* studies have suggested that cysteine may act as a pro-oxidant agent that causes DNA oxidative damage as a result of the overproduction of free radicals and hydrogen peroxide, leading to gene mutation and subsequent cancer development (2, 4, 5, 8, 9, 12–14). Elevated levels of homocysteine and cysteine are also associated with several metabolic disorders, including high body mass index (BMI), high plasma triglyceride levels, hypertension, and abnormal oxidation of low-density lipoproteins (9, 15–19), which may lead to the development of several cancers, including breast cancer (20, 21).

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Observational studies assessing the association between circulating homocysteine and cysteine and overall breast cancer risk are very limited, and findings have been inconsistent. One case-control study reported a positive association between homocysteine levels and breast cancer risk (22), whereas the other cohort study did not observe such an association (23). To date, circulating cysteine and overall breast cancer risk has only been assessed in one cohort study that reported an inverse association (24). In this case-control study nested in a large female cohort, we prospectively evaluated the association of plasma levels of total homocysteine and cysteine with risk for developing overall breast cancer as well as breast cancer subtypes according to hormone receptor status. Because the efficient metabolism of homocysteine and possibly cysteine relies on adequate levels of B vitamins, we also examined whether B-vitamin status, including plasma levels of folate, pyridoxal 5′-phosphate (PLP; the principal active form of vitamin B_6), and vitamin B_{12} affected the relation of plasma homocysteine and cysteine to overall breast cancer risk.

Materials and Methods

Study population. Participants in this study were drawn from the Women's Health Study, a completed randomized trial evaluating low-dose aspirin and vitamin E for the primary prevention of cancer and cardiovascular disease (25–27). Beginning in 1992, 39,876 U.S. female health professionals ages ≥45 years and free of cancer and cardiovascular disease participated in the study and completed a questionnaire about their medical history and lifestyle factors. Blood samples were collected from 28,345 women at baseline. Baseline characteristics of women who gave blood samples were largely similar to those who did not (28).

Identification of case and control subjects. Every 6 months during the first year of follow-up and then annually thereafter, participants were asked whether they had been newly diagnosed with breast cancer. We then confirmed the diagnosis for women who reported a diagnosis of breast cancer and those who were deceased through a review of medical records and extracted detailed information on the diagnosis of breast cancer. In the present study, we included 812 women who had a confirmed diagnosis of invasive breast cancer during an average follow-up of 10 years between 1992 and 2004. Each breast cancer case was individually matched to one control with no diagnosis of cancer on age (up to 5 years of difference), ethnicity, menopausal status (premenopausal, postmenopausal, or uncertain), postmenopausal hormone use (never, past, current), and trial randomization date (12-month difference), yielding a total of 812 matched controls.

Dietary assessment. Upon enrollment into the study, participants also completed a 131-item food frequency questionnaire, which asked the average intake of food and beverages during the past year. Participants chose from nine possible answers ranging from "never or less than once per month" to "six or more times per day." The response for each food item was then converted into an average daily intake of the food item in servings per day. Nutrient values in foods were computed by multiplying the frequency of responses by the nutrient content of specified portion sizes based on the U.S. Department of Agriculture food composition data (29) and supplemented by food manufacturers.

The validity and reproducibility of the food frequency questionnaire have been assessed previously (30, 31). The Pearson correlation coefficients between nutrient intakes from the food frequency questionnaire and that from four 1-week dietary records spaced over a year were 0.68 for vitamin B_2, 0.71 for vitamin B_6, 0.65 for vitamin B_{12}, 0.8 for vitamin E (31), 0.57 for vitamin A, and 0.59 for vitamin C (30). The relationships between total intakes of B vitamins and their respective blood levels have been assessed previously in our study; the correlation coefficients between total intakes and plasma levels were 0.52 for folate, 0.46 for vitamin B_6, and 0.24 for vitamin B_{12} (32).

Laboratory analysis. Both assays of homocysteine and cysteine were conducted at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. Plasma homocysteine and cysteine were determined using high-performance liquid chromatography with fluorescence detection (33). Blood samples for 812 cases and 812 controls were handled identically in random order and shipped in the same batch. Each pair was assayed together and all pairs were analyzed in one run. The laboratory personnel were blinded to case or control status. The mean coefficients of variation for quality control samples (78 repeated samples) were 13.4% and 8.8% for homocysteine and cysteine, respectively.

Statistical analysis. We first $\log_e$-transformed plasma levels of homocysteine and cysteine, which were both skewed from normality. We then categorized the two plasma markers into quintiles based on the distribution in the controls. Differences between case-control pairs in mean concentrations of plasma markers and other continuous covariates were tested using a paired *t* test. McNemar's test was used to compare the difference between case-control pairs in proportions of covariates as categorical variables.

Conditional logistic regression was used to estimate relative risks (RR) and 95% confidence intervals (95% CI) for invasive breast cancer, with adjustment for matching factors (described previously), age (in years), randomized treatment assignment (aspirin versus placebo, vitamin E versus placebo), and additionally for risk factors for breast cancer assessed at baseline, including BMI (<25, 25 to <30, ≥30 kg/m^2), physical activity (energy expenditure in kcal/wk, in quartiles), family history of breast cancer in a first-degree relative (yes, no), history of benign breast disease (yes, no), age at menarche (<11, 12–13, ≥14 years), parity (0, 1–2, 3–4, ≥5 children), age at first birth (<19, 20–24, 25–29, ≥30 years), smoking status (never, past, current), alcohol consumption (none,>0 to <5, 5 to<15, ≥15 g/d), and age at menopause (<45, 45 to <50, 50 to <52, ≥52 years).

Stratifying analysis for the association between the two plasma markers and breast cancer risk was first conducted according to B-vitamin status, including plasma folate

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Table 1. Baseline characteristics (mean ± SD or %) among invasive breast cancer cases and their matched controls in the Women's Health Study

Characteristics	Cases (n = 812)	Controls (n = 812)	P
Age (y)	56.1 (7.2)	56.0 (7.0)	Matched
BMI (kg/m^2)	25.4 (4.4)	25.6 (4.8)	0.47
History of benign breast disease (%)	42.8	37.8	0.03
Family history of breast cancer (%)	7.8	7.5	>0.9
Mammogram screening test (%)*	63.3	66.3	0.21
Current smoking (%)	13.6	9.5	0.01
Current use of multivitamins (%)	30.3	27.3	0.17
No. of participants with vitamin E treatment (%)	51.6	49.4	0.37
Physical activity (kcal/wk)	936 (1,135)	981 (1,314)	0.37
Age at menarche (y)	12.3 (1.0)	12.4 (1.1)	0.07
Age at first birth (y)	25.1 (3.6)	24.4 (3.4)	0.0005
Parity	2.8 (1.7)	3.1 (1.7)	0.0003
Nulliparous (%)	14.9	11.7	0.06
Postmenopause (%)	62.9	62.9	Matched
Age at menopause (y)	48.1 (5.4)	47.1 (5.6)	0.0002
Current postmenopausal hormone use (%)	62.5	62.5	Matched
Alcohol intake (g/d)	5.2 (9.0)	4.5 (8.1)	0.08
Methionine intake (g/d)†	1.92	1.92	0.98
Cystine intake (g/d)†	1.03	1.04	0.27
Plasma homocysteine levels (nmol/mL)‡	8.8 (5.4–15.9)	8.4 (5.4–15.5)	0.09
Plasma cysteine levels (nmol/mL)‡	204.6 (166.8–236.1)	203.1 (164.6–236.6)	0.27

*Information was obtained from the 12-month follow-up questionnaire.
†Nutrient intakes were energy adjusted.
‡Median value (5th–95th range).

[<8.4 (median levels), ≥8.4 ng/mL], PLP [<57.59 (median), ≥57.59 pmol/mL], and vitamin B_{12} [<460.5 (median), ≥460.5 pg/mL]. We also examined whether the overall association with the two plasma markers was affected by risk factors for breast cancer, including menopausal status (premenopause, postmenopause), hormone therapy use (never, past, current), BMI (<25, ≥25 kg/m^2), alcohol consumption (none or <9, ≥9 g/d), multivitamin use (yes, no), current smoking (yes, no), and benign breast disease (yes, no), as well as dietary intakes of antioxidants, including vitamin A, vitamin C, and β-carotene (in quintiles) and vitamin E treatment (treatment versus placebo). We also examined the association according to tumor hormone receptor status [estrogen receptor positive (ER+), ER negative (ER−), progesterone receptor positive (PR+), PR negative (PR−)]. All stratified analyses were performed by unconditional logistic regression controlling for matching factors as well as risk factors for breast cancer as mentioned above. Tests for multiplicative interactions between plasma markers and modifiers were performed by entering each product term into the multivariate model with a Wald statistic. Tests for trend were performed by fitting the median of the plasma marker for each quintile as a continuous variable in the model. We used SAS statistical software (version 8.2; SAS Institute) for all analyses. All P values were two sided.

Results

Among the baseline characteristics presented in Table 1, breast cancer patients were different from control subjects with higher prevalence of history of benign breast disease and current smoking. In addition, cancer patients had slightly higher levels of plasma homocysteine and cysteine than control subjects, although differences between the cancer and control groups were not statistically significant. Baseline intakes for dietary methionine and cystine (oxidized dimer of cysteine) were also not different in the cancer and control groups.

Plasma levels of homocysteine and cysteine were moderately correlated with each other, but neither was related to dietary intakes of methionine and cystine (Table 2). Homocysteine levels were moderately inversely correlated with B-vitamin levels, dietary folate intake, and intakes of antioxidants, whereas cysteine levels were only weakly correlated with B-vitamin levels. In addition, the two plasma markers were not correlated with alcohol consumption and current cigarette smoking. Cysteine was somewhat related to BMI, whereas no relation could be observed between homocysteine and BMI (Table 2).

Plasma levels of homocysteine were not associated with overall breast cancer risk in models with simple and multivariate

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Table 2. Partial correlation coefficients between plasma levels of homocysteine and cysteine and related markers among control subjects (n = 812) adjusted for matching factors

	Plasma levels	
	Homocysteine (nmol/mL)	Cysteine (nmol/mL)
Plasma levels*		
Homocysteine (nmol/mL)	1.00	0.53†
Cysteine (nmol/mL)	0.53†	1.00
Cysteinylglycine (nmol/mL)	0.54†	0.44†
Folate (ng/mL)	−0.31†	0.18†
PLP (pmol/mL)	−0.18†	0.10
Vitamin B_{12} (pg/mL)	−0.20†	0.15†
Dietary intake*		
Methionine (g/d)	−0.02	0.03
Cystine (g/d)	−0.03	0.02
Folate (g/d)	−0.23†	0.03
Total vitamin A intake (IU/d)	−0.20†	−0.01
Total vitamin C intake (mg/d)	−0.17†	0.02
Total β-carotene intake (μg/d)	−0.15†	−0.02
Alcohol consumption (g/d)	0.01	0.002
Vitamin E (treatment vs placebo)	−0.04	0.003
Tobacco consumption (cigarette/d)	0.08	0.004
BMI (kg/m^2)	0.02	0.13

NOTE: Matching factors include age, month, and year of blood return; date of blood draw; fasting status; postmenopausal status; postmenopausal hormone use; and trial randomization date.

*Nutrient intakes and concentrations were $\log_e$ transformed.

†$P < 0.0001$.

adjustments (Table 3). However, higher plasma cysteine was moderately associated with an increased risk for overall breast cancer risk (Table 3). Women in the highest quintile relative to those in the lowest group had a multivariate RR of 1.65 (95% CI, 1.04–2.61, P for trend = 0.04). Additional adjustment for screening mammography or for dietary intakes of methionine and cystine did not change the associations with the two plasma markers (data not shown). The associations were also unchanged when the two plasma markers along with cysteinylglycine levels were all included in the multivariate models (data not shown). Additional sensitivity analysis by excluding women diagnosed with breast cancer during the first 2 years of follow-up (n = 127 cases) revealed that the association with cysteine levels was somewhat attenuated (P for trend = 0.17). Dietary intakes of methionine and cystine did not seem to be related to breast cancer risk (Table 3).

B-vitamin levels seemed to affect the association between homocysteine levels and overall breast cancer risk (Table 3). Specifically, homocysteine levels were positively associated with risk for developing breast cancer among women with lower folate and PLP levels; the multivariate RRs in the highest quintile relative to the lowest group were 1.60 (95% CI, 0.93–2.74; P for trend = 0.01) for women with lower folate levels and 1.31 (95% CI, 0.80–2.15; P for trend = 0.06) for women with lower PLP status. Similarly, the positive association between cysteine levels and breast cancer risk was mainly present among women who had lower levels of folate and PLP (Table 4). The multivariate RRs in the highest group of plasma cysteine relative to the lowest group were 2.93 (95% CI, 1.73–4.96; P for trend = 0.0001) and 1.93 (95% CI, 1.16–3.21; P for trend = 0.005), respectively, among women with lower folate and PLP levels. By contrast, vitamin B_{12} status did not affect the overall association with either homocysteine or cysteine levels (Table 4).

Stratifying analyses according to breast cancer risk factors (including BMI, alcohol consumption, current smoking, multivitamin use, benign breast disease, menopausal status, and hormone therapy use) and dietary antioxidant intakes (including vitamins A and C, β-carotene, and vitamin E supplementation) did not significantly change the association of plasma homocysteine or cysteine with breast cancer risk (P values for interactions ≥0.12). Plasma homocysteine was also not significantly associated with risk for breast cancer subtypes according to tumor hormone receptor status (Table 5). However, higher plasma cysteine was marginally associated with an increased risk for developing ER+ and/or PR+ breast tumors. Women in the highest quintile group relative to those in the lowest group had a RR of 1.36 (95% CI, 0.92–2.02; P for trend = 0.05), 1.32 (95% CI, 0.87–2.01; P for trend = 0.06), 1.27 (95% CI, 0.83–1.95) for developing ER+, PR+, and ER+/PR+ breast cancer, respectively. In addition, higher homocysteine and cysteine levels were each associated with an increased risk for developing ER+ and/or PR+ breast cancer among women with lower folate status (P values for interaction ≤0.01).

Discussion

In this large prospective case-control study, we observed no association between plasma homocysteine and overall risk for developing breast cancer. However, higher homocysteine levels were associated with an increased risk among women with lower status of folate. Higher plasma cysteine was associated with an increased risk for breast cancer, and the positive association was primarily present among women with lower folate and, perhaps, PLP status. In addition, the increased risk for breast cancer with cysteine levels was more pronounced in ER+ and/or PR+ breast cancer. Dietary methionine and cystine intakes were not associated with risk for breast cancer.

Although total homocysteine levels were not associated with overall breast cancer risk in our study, women with higher homocysteine levels had an increased risk for breast cancer when their folate levels were low. Our finding of the

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joint relationship between plasma homocysteine and folate to breast cancer risk was also reported in another case-control study (22). Sufficient levels of B vitamins, including folate, are important for the metabolism of homocysteine, for which the plasma levels are elevated even when serum folate levels are in the low reference range (34, 35). It is, therefore, possible that homocysteine accumulation resulting from folate deficiency enhances its adverse effects on breast cancer development. More studies are warranted to confirm the relationship between homocysteine and folate in relation to breast cancer development.

We also found that elevated plasma cysteine was associated with an increased risk for developing overall breast cancer. Lower folate status, likely resulting in homocysteine accumulation and further conversion of cysteine, also seemed to amplify the positive association between cysteine and breast cancer risk. Our findings are in contrast with two other studies showing either an inverse association between cysteine levels and breast cancer risk (24) or an effect modification of the COMT genotype on the association (36). Discrepant findings among the three studies may be attributable to heterogeneity of the study populations, as we noted that cysteine levels in our study were much lower (mean levels were 203 nmol/mL in controls) relative to those in the other two studies (mean levels were >270 nmol/mL in controls). Nevertheless, the correlation coefficients of cysteine with homocysteine and B-vitamin levels in our study were of similar magnitude to those in another prospective study (24). Given the null relationship of cysteine levels to several antioxidant intakes, alcohol consumption, and smoking in this study population, it is possible that factors other than oxidative effects may explain the link between cysteine and breast cancer risk.

A recent study has suggested a possible causal role for cysteine in body weight regulation; elevated cysteine levels were associated with high BMI, independent of serum lipids, plasma cysteinylglycine, and serum γ-glutamyltransferase, an enzyme that converts glutathione into cysteine (37). In rats, increasing cysteine intake markedly increases body weight gain (38), whereas dietary restriction of methionine, the precursor for cysteine, decreased visceral fat mass (39). Obesity is a risk factor for breast cancer risk in postmenopausal women and a prognostic factor for breast cancer progression and mortality (40–44). It is, therefore, possible that the adverse effects of cysteine on energy balance may also contribute to breast cancer development. Nevertheless, cysteine levels were only weakly correlated with BMI in this

Table 3. RRs and 95% CIs of invasive breast cancer according to quintiles of plasma homocysteine and cysteine as well as of dietary methionine and cystine intakes in the Women's Health Study

	Quintile					P_{trend}
	1 (Lowest)	2	3	4	5 (Highest)	
Plasma levels						
Homocysteine (nmol/mL)	<6.79	6.79–<7.95	7.95–<9.13	9.13–<11	≥11	
n cases/controls	182/164	134/161	130/163	184/162	182/162	
Simple RR*	1.00	0.75 (0.54–1.05)	0.72 (0.51–1.01)	1.01 (0.73–1.40)	1.02 (0.73–1.43)	0.33
Multivariate RR†	1.00	0.61 (0.47–0.97)	0.63 (0.44–0.90)	1.00 (0.70–1.42)	0.92 (0.64–1.32)	0.61
Cysteine (nmol/mL)	<185.2	185.2–<198	198–<209	209–<219.7	≥219.7	
n cases/controls	138/163	150/162	196/163	172/162	156/162	
Simple RR*	1.00	1.23 (0.87–1.74)	1.73 (1.18–2.54)	1.55 (1.05–2.30)	1.41 (0.93–2.13)	0.11
Multivariate RR†	1.00	1.29 (0.89–1.88)	1.93 (1.27–2.94)	1.67 (1.08–2.57)	1.65 (1.04–2.61)	0.04
Dietary intake						
Methionine (g/d)	<1.61	1.61–<1.82	1.82–<2.0	2.0–<2.23	≥2.23	
n cases/controls	163/158	177/160	145/160	159/159	150/153	
Simple RR*	1.00	1.05 (0.76–1.44)	0.83 (0.60–1.14)	0.95 (0.69–1.32)	0.96 (0.69–1.33)	0.62
Multivariate RR†	1.00	1.06 (0.75–1.49)	0.82 (0.58_1.17)	0.98 (0.69–1.40)	0.93 (0.65–1.32)	0.55
Cystine (g/d)	<0.91	0.91–1	1–<1.08	1.08–<1.16	≥1.16	
n cases/controls	185/161	156/172	173/157	126/143	154/157	
Simple RR*	1.00	0.76 (0.56–1.04)	0.94 (.69–1.28)	0.76 (0.54–1.06)	0.86 (0.63–1.18)	0.43
Multivariate RR†	1.00	0.82 (0.59–1.15)	0.95 (0.68–1.31)	0.81 (0.56–1.16)	0.82 (0.58–1.15)	0.29

*Simple models were adjusted for matching variables and age (in year) and randomized treatment assignment to aspirin and vitamin E (aspirin versus placebo, vitamin E versus placebo).

†Multivariate models were adjusted for matching variables denoted in the legend and additionally for BMI [weight (kg)/height (m)^2: <25, 25–<30, ≥30 kg/m^2], physical activity (energy expenditure in kcal/wk, in quartiles), family history of breast cancer in a first-degree relative (yes, no), history of benign breast disease (yes, no), age at menarche (≤11, 12, 13, ≥14 years), parity (0, 1–2, 3–4, ≥5 children), age at first birth (≤19, 20–24, 25–29, ≥30 years), smoking status (never, past, current), alcohol consumption (never, 0.1–<5, 5–<15, ≥15 g/d), and age at menopause (<45, 45–<50, 50–<52, ≥52 years).

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Table 4. RRs and 95% CIs of invasive breast cancer and quintiles of plasma homocysteine and cysteine according to plasma B-vitamin levels in the Women's Health Study

	Homocysteine				Cysteine			
	Cases/ controls	Simple RR*	Multivariate RR†	$P_{interaction}$	Cases/ controls	Simple RR*	Multivariate RR†	$P_{interaction}$
Folate status				0.04				0.002
Folate <8.4 ng/mL								
Q*1	37/48	1.00	1.00		60/94	1.00	1.00	
Q2	53/62	1.12 (0.63–2.00)	1.06 (0.58–1.94)		70/79	1.45 (0.90–2.32)	1.46 (0.89–2.41)	
Q3	53/78	0.90 (0.51–1.57)	0.90 (0.50–1.62)		98/83	1.89 (1.21–2.95)	2.18 (1.35–3.51)	
Q4	106/95	1.47 (0.87–2.49)	1.63 (0.94–2.82)		80/78	1.61 (1.02–2.55)	1.62 (0.99–2.63)	
Q5	138/112	1.67 (1.00–2.80)	1.60 (0.93–2.74)		79/61	2.16 (1.33–3.51)	2.93 (1.73–4.96)	
P_{trend}		0.007	0.01			0.002	0.0001	
Folate ≥8.4 ng/mL								
Q*1	143/111	1.00	1.00		75/65	1.00	1.00	
Q2	80/97	0.64 (0.43–0.94)	0.59 (0.39–0.89)		78/82	0.82 (0.52–1.31)	0.78 (0.48–1.27)	
Q3	73/83	0.66 (0.44–0.99)	0.62 (0.40–0.95)		95/76	1.06 (0.67–1.68)	1.08 (0.66–1.76)	
Q4	75/64	0.90 (0.58–1.38)	0.84 (0.54–1.31)		89/81	0.94 (0.59–1.49)	0.90 (0.56–1.46)	
Q5	43/47	0.69 (0.42–1.14)	0.61 (0.36–1.02)		77/98	0.67 (0.42–1.07)	0.65 (0.40–1.06)	
P_{trend}		0.22	0.09			0.18	0.18	
PLP status				0.11				0.07
PLP <57.6 pmol/mL								
Q*1	60/63	1.00	1.00		69/87	1.00	1.00	
Q2	67/66	1.06 (0.64–1.77)	1.04 (0.61–1.78)		68/83	1.06 (0.67–1.68)	1.14 (0.70–1.85)	
Q3	57/95	0.64 (0.39–1.05)	0.67 (0.40–1.12)		115/86	1.68 (1.09–2.58)	1.94 (1.22–3.08)	
Q4	107/82	1.36 (0.85–2.18)	1.59 (0.97–2.62)		80/75	1.35 (0.86–2.14)	1.50 (0.92–2.43)	
Q5	120/93	1.36 (0.85–2.16)	1.31 (0.80–2.15)		79/68	1.42 (0.88–2.29)	1.93 (1.16–3.21)	
P_{trend}		0.05	0.06			0.07	0.005	
PLP ≥57.6 pmol/mL								
Q*1	120/96	1.00	1.00		66/72	1.00	1.00	
Q2	66/93	0.58 (0.38–0.88)	0.56 (0.36–0.87)		80/78	1.18 (0.74–1.89)	1.12 (0.69–1.83)	
Q3	69/66	0.85 (0.55–1.32)	0.78 (0.50–1.24)		78/73	1.25 (0.78–2.02)	1.25 (0.76–2.07)	
Q4	74/77	0.76 (0.49–1.17)	0.76 (0.48–1.19)		89/84	1.20 (0.76–1.90)	1.15 (0.71–1.87)	
Q5	61/66	0.77 (0.49–1.21)	0.69 (0.43–1.12)		77/91	1.01 (0.63–1.62)	1.00 (0.61–1.64)	
P_{trend}		0.37	0.23			0.95	0.96	
Vitamin B_{12} status				0.59				0.23
Vitamin B_{12} <460.5 pg/mL								
Q*1	64/62	1.00	1.00		75/86	1.00	1.00	
Q2	61/73	0.78 (0.47–1.29)	0.71 (0.41–1.20)		77/90	1.04 (0.66–1.62)	1.10 (0.69–1.76)	
Q3	61/80	0.74 (0.45–1.22)	0.73 (0.44–1.23)		100/93	1.23 (0.80–1.90)	1.37 (0.87–2.17)	
Q4	103/85	1.22 (0.76–1.95)	1.33 (0.81–2.19)		80/74	1.29 (0.82–2.04)	1.33 (0.82–2.15)	
Q5	106/99	1.07 (0.67–1.69)	1.01 (0.62–1.65)		63/56	1.31 (0.80–2.16)	1.54 (0.90–2.63)	
P_{trend}		0.25	0.27			0.16	0.07	
Vitamin B_{12} ≥460.5 pg/mL								
Q*1	116/97	1.00	1.00		60/73	1.00	1.00	
Q2	72/86	0.71 (0.47–1.08)	0.65 (0.42–1.01)		71/71	1.23 (0.76–2.00)	1.17 (0.71–1.93)	
Q3	65/81	0.67 (0.43–1.02)	0.63 (0.40–0.98)		93/66	1.77 (1.10–2.85)	1.89 (1.15–3.12)	
Q4	78/74	0.89 (0.58–1.36)	0.82 (0.52–1.29)		89/85	1.30 (0.82–2.06)	1.22 (0.75–1.97)	
Q5	75/60	1.05 (0.68–1.64)	0.95 (0.60–1.52)		93/103	1.14 (0.72–1.80)	1.16 (0.72–1.88)	
P_{trend}		0.75	0.89			0.62	0.58	

NOTE: The ranges of homocysteine in the quintile groups were <6.79, 6.79 to <7.95, 7.95 to <9.13, 9.13 to <11, and ≥11. The ranges of cysteine in the quintile groups were <185.2, 185.2 to <198, 198 to <209, 209 to <219.7, and ≥219.7.

*Simple models were adjusted for the same variables as in Table 3.

†Multivariate models were adjusted for the same variables as in Table 3.

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Table 5. RRs and 95% CIs of invasive breast cancer and quintiles of plasma homocysteine and cysteine according to hormone receptor status in the Women's Health Study

	Homocysteine			Cysteine		
	Cases/controls	Simple RR*	Multivariate RR†	Cases/controls	Simple RR*	Multivariate RR†
ER status						
ER+						
Q*1	147/122	1.00	1.00	105/126	1.00	1.00
Q2	106/126	0.68 (0.48–0.97)	0.64 (0.44–0.93)	118/131	1.13 (0.79–1.64)	1.12 (0.76–1.63)
Q3	93/122	0.62 (0.43–0.90)	0.59 (0.41–0.87)	153/132	1.44 (1.01–2.05)	1.55 (1.07–2.25)
Q4	151/136	0.92 (0.65–1.30)	0.91 (0.64–1.29)	142/124	1.42 (0.99–2.04)	1.42 (0.97–2.07)
Q5	144/131	0.92 (0.65–1.29)	0.81 (0.57–1.17)	123/124	1.23 (0.84–1.79)	1.36 (0.92–2.02)
P_{trend}		0.78	0.78		0.12	0.05
ER–						
Q*1	29/32	1.00	1.00	21/26	1.00	1.00
Q2	20/26	0.91 (0.40–2.07)	0.95 (0.38–2.37)	25/23	1.49 (0.64–3.48)	1.87 (0.71–4.90)
Q3	22/29	0.83 (0.38–1.82)	0.90 (0.37–2.19)	33/22	2.20 (0.95–5.09)	3.18 (1.22–8.31)
Q4	21/16	1.55 (0.64–3.75)	1.95 (0.71–5.37)	20/22	1.22 (0.51–2.93)	1.37 (0.52–3.63)
Q5	30/19	1.90 (0.84–4.27)	1.78 (0.71–4.48)	23/29	1.03 (0.46–2.34)	1.07 (0.42–2.72)
P_{trend}		0.07	0.13		0.98	0.95
PR status						
PR+						
Q*1	127/109	1.00	1.00	94/114	1.00	1.00
Q2	96/118	0.66 (0.45–0.96)	0.61 (0.41–0.91)	104/117	1.13 (0.77–1.67)	1.12 (0.75–1.68)
Q3	89/108	0.67 (0.46–0.99)	0.63 (0.42–0.94)	129/115	1.41 (0.96–2.06)	1.49 (1.00–2.23)
Q4	127/120	0.90 (0.62–1.29)	0.89 (0.61–1.31)	131/107	1.52 (1.04–2.23)	1.52 (1.02–2.27)
Q5	130/112	0.99 (0.69–1.44)	0.88 (0.60–1.29)	111/114	1.21 (0.82–1.81)	1.32 (0.87–2.01)
P_{trend}		0.49	0.90		0.12	0.06
PR–						
Q*1	44/40	1.00	1.00	30/35	1.00	1.00
Q2	29/32	0.77 (0.38–1.56)	0.61 (0.28–1.34)	38/33	1.35 (0.67–2.69)	1.36 (0.64–2.90)
Q3	26/42	0.50 (0.25–0.98)	0.45 (0.22–0.94)	55/37	1.77 (0.91–3.46)	2.00 (0.97–4.12)
Q4	41/31	1.18 (0.61–2.28)	1.37 (0.66–2.87)	26/38	0.79 (0.38–1.61)	0.84 (0.39–1.80)
Q5	43/36	1.04 (0.54–1.98)	0.96 (0.48–1.95)	34/38	1.00 (0.51–2.00)	1.11 (0.52–2.39)
P_{trend}		0.57	0.55		0.64	0.92
ER+ and PR+						
Q*1	125/103	1.00	1.00	91/107	1.00	1.00
Q2	90/112	0.62 (0.42–0.92)	0.57 (0.38–0.84)	100/113	1.09 (0.73–1.62)	1.08 (0.71–1.63)
Q3	82/107	0.61 (0.41–0.90)	0.55 (0.37–0.84)	121/113	1.30 (0.88–1.92)	1.37 (0.91–2.06)
Q4	126/118	0.87 (0.60–1.26)	0.86 (0.59–1.27)	129/104	1.49 (1.01–2.21)	1.49 (1.00–2.24)
Q5	127/109	0.96 (0.66–1.40)	0.83 (0.56–1.23)	109/112	1.17 (0.78–1.76)	1.27 (0.83–1.95)
P_{trend}		0.58	0.97		0.16	0.09
ER– and PR–						
Q*1	27/26	1.00	1.00	18/19	1.00	1.00
Q2	14/20	0.66 (0.25–1.73)	0.75 (0.25–2.29)	21/19	1.28 (0.50–3.31)	1.55 (0.52–4.64)
Q3	15/28	0.46 (0.19–1.11)	0.52 (0.19–1.43)	25/20	1.50 (0.59–3.82)	2.22 (0.74–6.66)
Q4	20/14	1.29 (0.50–3.35)	1.58 (0.50–4.97)	18/19	1.07 (0.41–2.78)	1.06 (0.36–3.14)
Q5	27/16	1.68 (0.69–4.08)	1.45 (0.51–4.08)	21/27	0.84 (0.34–2.06)	0.88 (0.33–2.56)
P_{trend}		0.13	0.31		0.60	0.70

NOTE: The ranges of homocysteine in the quintile groups were <6.79, 6.79 to <7.95, 7.95 to <9.13, 9.13 to <11, and ≥11. The ranges of cysteine in the quintile groups were <185.2, 185.2 to <198, 198 to <209, 209 to <219.7, and ≥219.7.

*Simple models were adjusted for the same variables as in Table 3.

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population, suggesting that other unknown factors are also likely to play a role in the relationship between cysteine and breast cancer development.

We also found that the increased risk for breast cancer with elevated cysteine levels was more relevant to ER+ and/or PR+ breast cancer. *In vitro* studies have suggested that accumulation of homocysteine, a precursor of cysteine, results in elevated concentrations of *S*-adenosylhomocysteine, which bind to COMT and inhibits the COMT-mediated metabolism of several carcinogenic catechol estrogens, including 2-hydroxy-E2 and 4-hydroxy-E2 (45). The consequence may lead to an increased risk for developing estrogen-dependent cancers (45). It is possible that the accumulation of homocysteine causes elevated cysteine levels and results in pathogenic effects on estrogen-dependent breast tissue.

The limitations of this study include the representativeness of a single blood specimen to assess circulating homocysteine and cysteine as well as blood specimen stability over time in the freezers. However, prior work from other prospective studies has shown that correlations over time for several biochemical tests tend to be very stable with little or no sign of deterioration during storage (46–49). In addition, data showing that dietary methionine and cystine intakes were not correlated with plasma homocysteine and cysteine levels are unexpected. Although we cannot rule out the possibility of measurement errors in these two nutrient intakes, the tight regulation in circulating cysteine may be less affected by normal dietary intakes (5). The correlation between cysteine levels and dietary methionine intake obtained in our study was also comparable with that in the other large prospective study (24). Finally, our findings may be subject to chance as we have performed many comparisons.

In conclusion, our study suggests that women with higher homocysteine and cysteine levels may be at an increased risk for breast cancer when their folate levels are low. The mechanistic factors underlying the relationship between cysteine and breast cancer risk warrants more investigation in future studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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