A Prospective Study of Plasma Total Cysteine and Risk of Breast Cancer

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Abstract

Cysteine is the precursor of glutathione, a powerful intracellular antioxidant and an important detoxifying agent of carcinogens. However, data relating plasma total cysteine to breast cancer risk are sparse. We conducted a prospective nested case-control study among 32,826 women in the Nurses' Health Study who provided blood specimens during 1989–1990. Through 1996, a total of 712 incident breast cancer cases were identified and individually matched to 712 controls by year of birth, time of day that blood was drawn, fasting status, month of blood sampling, recent use of postmenopausal hormones at the time of blood collection, and menopausal status. Conditional logistic regression with adjustment for other risk factors for breast cancer was used to estimate the relative risks and 95% confidence intervals of breast cancer by levels of plasma total cysteine. Higher plasma total cysteine concentrations were significantly associated with a lower risk of breast cancer. For women in the highest quintile of plasma total cysteine, compared with those in the lowest quintile, the multivariate relative risk was 0.44 (95% confidence interval, 0.26–0.74). This association was dose dependent (P for trend = 0.002) and independent of plasma measures of folate, vitamin B_6, vitamin B_{12}, and total homocysteine. The inverse association between plasma total cysteine concentrations and risk of breast cancer was not significantly modified by other risk factors for breast cancer, except that a stronger association was observed among women who were leaner. The findings from this prospective study suggest that higher plasma concentrations of total cysteine predict a reduced risk of breast cancer. Cysteine or its precursors might have the potential to be chemopreventive against breast cancer.

Introduction

Cysteine is the rate-limiting amino acid in the intracellular synthesis of glutathione, a tripeptide consisting of cysteine, glutamate, and glycine (1). Cysteine is derived from methionine via the transsulfuration pathway through homocysteine (2, 3). Vitamin B_6, vitamin B_{12}, and folate are involved in a number of reactions that regulate cysteine derivation (2, 3). As the most powerful intracellular antioxidant, glutathione protects cells from oxidative DNA damage through scavenging of reactive oxygen species (4) and is considered to be the principal defense within the body against free radicals (1). Glutathione is also an important detoxifying agent that facilitates the elimination of heavy metals, drug metabolites, DNA-damaging chemicals, and carcinogens (4–6). Moreover, cysteine, glutathione, and *N*-acetylcysteine, a synthetic precursor of cysteine, can modulate immune responses (7–9). Cysteine and cysteine derivatives inhibit both the transcription factor nuclear factor-κB that is induced under physiological conditions by reactive oxygen species and the expression of several nuclear factor-κB-dependent genes including interleukin-2 receptor α chain, tumor necrosis factor α, the MHC, and c-*fos* (7–9). Cysteine and cysteine derivatives also stimulate DNA synthesis of several cycling T-cell clones and activate cytotoxic T cells and thus play a regulatory role in T-cell-mediated immune responses (7–9). In many experimental models, *N*-acetylcysteine has antigenotoxic and anticarcinogenic properties mediated at different stages of the carcinogenesis process (6). Although a protective effect of cysteine against carcinogenesis is biologically possible, data relating circulating cysteine concentrations to breast cancer risk are limited. We therefore conducted a prospective study to evaluate plasma total cysteine concentrations in relation to subsequent breast cancer risk in the Nurses' Health Study.

Materials and Methods

Study Population. In 1976, the Nurses' Health Study was established when 121,700 female registered nurses aged 30–55 years living in 11 United States states completed a mailed questionnaire about their medical history and lifestyle. Biennial follow-up questionnaires have been sent to cohort members to update their health-related information and to ascertain newly diagnosed diseases. During 1989–1990, 32,826 participants who were then 43–69 years provided blood specimens. Details regarding the blood collection in the Nurses' Health Study have been published previously (10). Briefly, blood was collected via overnight courier; 97% of the samples arrived within 26 h of

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being drawn. Approximately 75% of the samples were drawn at least 8 h after the last meal. On arrival at our laboratory, the samples were processed and aliquoted into plasma, WBC, and RBC components. Since collection, blood samples have been stored at −130°C or a colder temperature in continuously alarmed and monitored liquid nitrogen freezers. As of 1996, the follow-up rate among women who provided blood specimens was 99%. The research protocol was approved by the Use of Human Subjects in Research Committee at the Brigham and Women's Hospital and the Human Subjects Committee at the Harvard School of Public Health.

Dietary information was collected using semiquantitative food frequency questionnaires in 1980, 1984, 1986, and 1990. The average intakes of nutrients over this 10-year period were calculated to best represent long-term intakes of these nutrients. The food frequency questionnaires assessed the average consumption during the past year of a specific amount of each food and allowed nine responses, ranging from "never" to "six or more times per day." Nutrient intake was computed by multiplying the frequency response by the nutrient content of the specified portion sizes. Information on alcohol consumption and multivitamin use was obtained from the 1990 dietary questionnaire because blood specimens were collected during 1989–1990. The validity and reliability of the food frequency questionnaires used in the Nurses' Health Study have been described in detail elsewhere (11–15). The correlation coefficients between the average intakes calculated from the 1980, 1984, 1986, and 1990 food frequency questionnaires and plasma concentrations were 0.49 for folate from foods and supplements, 0.33 for folate from foods only, 0.52 for vitamin B_6 from foods and supplements, 0.25 for vitamin B_6 from foods only, 0.25 for vitamin B_{12} from foods and supplements, and 0.08 for vitamin B_{12} from foods only among control subjects (16). The comparable correlation coefficients between nutrient intakes calculated from the 1990 food frequency questionnaire and plasma concentrations were 0.55 for folate from foods and supplements, 0.35 for folate from foods only, 0.58 for vitamin B_6 from foods and supplements, 0.18 for vitamin B_6 from foods only, 0.23 for vitamin B_{12} from foods and supplements, and 0.02 for vitamin B_{12} from foods only.

Identification of Cases and Controls. Incident cases of breast cancer were initially identified by self-report on biennial questionnaires and then confirmed by reviewing medical records. Through May 31, 1996, we documented 735 incident cases (carcinoma *in situ* and invasive) among women who provided blood specimens and were free of cancer before blood collection. Time from blood collection to diagnosis of breast cancer ranged from less than 1 month to 82 months (mean, 40 months). Each breast cancer case was individually matched to one control participant with no history of cancer by year of birth (±1 year), time of day that blood was drawn (2-h intervals), fasting status (≥10 h *versus* <10 h), month of blood sampling, use of postmenopausal hormones within 3 months before blood collection, and menopausal status. Most control matches were exact; however, when needed, the criteria of matching factors were slightly relaxed.

Laboratory Analyses. Plasma total cysteine concentrations were measured using high-performance liquid chromatography with fluorescence detection at the Jean Mayer United States Department of Agriculture Human Nutrition Research Center on Aging at Tufts University (17). As described elsewhere, plasma concentrations of total homocysteine, folate, vitamin B_6, and vitamin B_{12} were also measured among all cases and control subjects at the same laboratory (16). All matched case-control pairs were handled identically and together, shipped in the same batch, and assayed in the same analytical run. The blood specimens were labeled by number only. The order of specimens within each case-control pair was randomly determined. All assays were conducted without knowledge of the case and control status by the investigators and laboratory personnel. Seventy-five pairs of pooled plasma quality control samples, which were indistinguishable from the real samples, were interspersed to assess laboratory precision; the mean coefficient of variation was 6.8% for plasma total cysteine.

Statistical Analysis. We excluded 23 case-control pairs with missing information on plasma measures in one member of a case-control pair, thus 712 case-control pairs were included in this analysis. Plasma concentrations of total cysteine, folate, vitamin B_6, vitamin B_{12}, and total homocysteine were categorized into quintiles based on the distribution of control participants. Mixed effect regression models were used to test the differences in means of plasma total cysteine between cases and controls while adjusting for the correlation between cases and controls within the matched set (18). Plasma total cysteine concentrations were $\log_e$-transformed to improve normality.

Conditional logistic regression was used to calculate the RRs[6] and 95% CIs for the analysis of plasma total cysteine concentrations in relation to breast cancer risk to account for matching factors. In multivariate analysis, we further controlled for age at menarche (<12, 12, 13, or ≥14 years), parity (nulliparous, 1 or 2, 3 or 4, or ≥5), age at first birth (nulliparous, <25, ≥25 to <30, or ≥30 years), history of breast cancer in mother or a sister (yes or no), history of benign breast disease (yes or no), alcohol intake (0, >0 to <5, ≥5 to <15, ≥15 to <30, or ≥30 g/day), body mass index at age 18 (<21, ≥21 to <23, ≥23 to <25, or ≥25 kg/m^2), body mass index at blood collection (<23, ≥23 to <25, ≥25 to <27, ≥27 to <30, or ≥30 kg/m^2), and duration of postmenopausal hormone use (continuous). We also conducted an analysis to further adjust for plasma folate, vitamin B_6, vitamin B_{12}, and total homocysteine concentrations. Tests for trend were conducted by using the median values for quintiles of plasma total cysteine as a continuous variable. Log likelihood ratio tests were used to compare models with or without interaction terms between plasma total cysteine for a 125 nmol/ml increment (approximately 2 SDs) and other risk factors in relation to breast cancer risk. All *P*s were two-tailed.

A generalized linear model was used to calculate the Pearson correlation coefficients between plasma concentrations of total cysteine and plasma measures and intakes of nutrients that have been related to risk of breast cancer after controlling for matching factors and body mass index at blood collection. Only control participants were included in this analysis. Variables in continuous form were $\log_e$-transformed in this analysis to improve normality.

Results

The mean concentration of plasma total cysteine was 301 ± 58 nmol/ml among breast cancer cases, which was nonsignficantly lower than the mean of 305 ± 59 nmol/ml among control subjects ($P = 0.10$). The associations between plasma total cysteine concentrations and baseline characteristics of study participants are listed in Table 1, and the correlations between plasma total cysteine concentrations and plasma measures and intakes of nutrients are presented in Table 2. Women who had

[6] The abbreviations used are: RR, relative risk; CI, confidence interval.

Table 1 Age-standardized characteristics by plasma total cysteine concentrations in 712 controls in the Nurses' Health Study[a]

Characteristics	Quintile				
	1 (lowest)	2	3	4	5 (highest)
Age (yrs)	54	56	58	59	60
Plasma folate (ng/ml)	8	9	9	9	9
Plasma vitamin B_6 (pmol/ml)	76	73	76	71	78
Plasma vitamin B_{12} (pg/ml)	448	438	485	473	446
Plasma total homocysteine (nmol/ml)	9	10	11	11	13
Alcohol intake ≥15 g/day (%)	9	8	12	11	9
Postmenopausal (%)	65	68	69	71	69
Age at menarche (yrs)	13	12	12	13	12
Age at first birth (yrs)	24	25	25	24	25
Parity (births)	3	3	3	3	3
Body mass index at age 18 years (kg/m^2)	21	21	21	21	21
Body mass index at blood collection (kg/m^2)	24	25	25	26	26
Mother or sister with breast cancer (%)	13	13	13	18	14
History of benign breast disease (%)	48	55	45	50	53
Multivitamin supplement use (%)	39	38	40	38	40
Folate from foods and supplements (μg/day)[b]	412	407	399	410	397
Vitamin B_6 from foods and supplements (mg/day)[b]	8	9	8	8	7
Vitamin B_{12} from foods and supplements (μg/day)[b]	10	10	11	10	10

[a] All factors except age are directly standardized. Values presented here are means unless indicated otherwise.
[b] Average intakes calculated from the 1980, 1984, 1986, and 1990 food frequency questionnaires. Nutrients are adjusted for total energy intake.

higher concentrations of plasma total cysteine tended to be older, postmenopausal, and weighed more. However, the correlations between plasma total cysteine and age ($r = 0.15$, $P < 0.001$) and body mass index at blood collection ($r = 0.10$, $P = 0.007$) were weak. Women who had higher concentrations of plasma total cysteine were also more likely to have higher concentrations of plasma total homocysteine ($r = 0.50$, $P < 0.001$). Plasma total cysteine concentrations were not significantly correlated with plasma measures and intakes of folate, vitamin B_6, vitamin B_{12}, and intakes of major types of fat and dietary fiber, except for a weak positive correlation with intake of vitamin B_{12} from foods only ($r = 0.12$, $P = 0.04$).

Higher plasma total cysteine concentrations were significantly associated with a lower risk of breast cancer in analyses controlling for matching factors; the RR comparing the highest with the lowest quintile was 0.54 (95% CI, 0.34–0.87; Table 3). When we controlled for additional established risk factors for breast cancer, the association was slightly strengthened (RR, 0.44; 95% CI, 0.26–0.74, top *versus* bottom quintile). The inverse association between plasma total cysteine and risk of breast cancer was dose dependent (P for trend = 0.002). This association did not change appreciably after further adjustments for plasma concentrations of folate, vitamin B_6, vitamin B_{12} and total homocysteine (RR, 0.51; 95% CI, 0.27–0.94, top *versus* bottom quintile). To address the potential bias that breast cancer itself, before it was diagnosed, might have affected blood nutrient concentrations, we excluded cases that were diagnosed within the first 2 years after blood collection and their matched controls (198 case-control pairs); the inverse association remained (RR, 0.50; 95% CI, 0.27–0.93, top *versus* bottom quintile). When we examined only invasive cases and their matched controls (600 case-control pairs), the associations became slightly stronger (RR, 0.38; 95% CI, 0.21–0.68, top *versus* bottom quintile; Table 3). When we excluded 203 case-control pairs whose blood specimens had visible hemolysis in any one member of a case-control pair, the associations were also strengthened (RR, 0.37; 95% CI, 0.20–0.72, top *versus* bottom quintile).

The inverse association between plasma total cysteine concentrations and risk of breast cancer was slightly stronger among premenopausal women; the multivariate RRs comparing women in the highest quintile with those in the lowest quintile were 0.23 (95% CI, 0.07–0.84) for premenopausal women and 0.52 (95% CI, 0.28–0.96) for postmenopausal women. However, test for the interaction between plasma total cysteine and menopausal status and breast cancer risk was not statistically significant (P for interaction = 0.22). The inverse association between plasma total cysteine concentrations and risk of breast cancer did not differ according to other risk factors for breast cancer, except that there was a significant interaction between plasma total cysteine, body mass index at blood collection, and risk of breast cancer (P for interaction = 0.04; Table 4). The inverse association between plasma total cysteine and risk of breast cancer appeared to be stronger among women who were leaner.

Discussion

The results from this large prospective nested case-control study indicated that higher plasma total cysteine concentrations were associated with lower risk of breast cancer. Women in the highest quintile of plasma total cysteine had an approximately 50% lower risk of breast cancer. Except for a stronger association among lean women, this inverse association did not differ

Table 2 Multivariate Pearson correlation coefficients[a] between plasma total cysteine concentrations and other characteristics among control subjects

Variable	Plasma total cysteine (nmol/ml)	
	Correlation coefficient	*P*
Age (yrs)[b]	0.15	<0.001
Body mass index at age 18 years (kg/m^2)[c]	0.06	0.15
Body mass index at blood collection (kg/m^2)[c]	0.10	0.007
Plasma		
Folate (ng/ml)	0.06	0.11
Vitamin B_6 (pmol/ml)	0.04	0.35
Vitamin B_{12} (pg/ml)	0.02	0.66
Total homocysteine (nmol/ml)	0.50	<0.001
Intake[d]		
Folate from foods and supplements (μg/day)	<0.001	0.99
Folate from foods only (μg/day)[e]	0.09	0.14
Vitamin B_6 from foods and supplements (mg/day)	−0.005	0.90
Vitamin B_6 from foods only (mg/day)[e]	0.06	0.24
Vitamin B_{12} from foods and supplements (μg/day)	0.03	0.51
Vitamin B_{12} from foods only (μg/day)[e]	0.12	0.04
Methionine (g/day)	0.05	0.18
Saturated fat (g/day)	0.03	0.44
Monounsaturated fat (g/day)	0.03	0.50
Polyunsaturated fat (g/day)	−0.04	0.25
Trans unsaturated fat (g/day)	−0.04	0.32
Dietary fiber (g/day)	−0.03	0.37

[a] Adjustments for matching factors and body mass index at blood collection (<23, ≥23 to <25, ≥25 to <27, ≥27 to <30, or ≥30 kg/m^2).
[b] Age was excluded.
[c] Body mass index at blood collection was excluded.
[d] Average intakes calculated from the 1980, 1984, 1986, and 1990 food frequency questionnaires.
[e] Supplement users were excluded.

Table 3 RR and 95% CI of breast cancer according to quintiles of plasma total cysteine concentrations in the Nurses' Health Study

	Quintile					*P* for trend
	1 (lowest)	2	3	4	5 (highest)	
Total cysteine (nmol/ml)	<259	259–283	284–311	312–351	>351	
Invasive cancer and *in situ*						
Cases/controls	152/142	160/143	147/142	136/143	117/142	
Simple RR[a] (95% CI)	1.0	1.01 (0.72–1.42)	0.88 (0.63–1.25)	0.74 (0.50–1.09)	0.54 (0.34–0.87)	0.008
Multivariate RR[b] (95% CI)	1.0	0.96 (0.66–1.39)	0.88 (0.60–1.28)	0.68 (0.44–1.05)	0.44 (0.26–0.74)	0.002
Multivariate RR[c] (95% CI)	1.0	1.03 (0.70–1.51)	0.99 (0.66–1.49)	0.80 (0.49–1.31)	0.51 (0.27–0.94)	0.04
Invasive cancer only						
Cases/controls	126/116	131/119	126/117	119/125	98/123	
Simple RR[a] (95% CI)	1.0	0.98 (0.67–1.42)	0.90 (0.62–1.30)	0.73 (0.48–1.10)	0.50 (0.30–0.84)	0.006
Multivariate RR[b] (95% CI)	1.0	0.97 (0.64–1.47)	0.90 (0.59–1.37)	0.71 (0.45–1.13)	0.38 (0.21–0.68)	0.001
Multivariate RR[c] (95% CI)	1.0	1.02 (0.66–1.57)	1.00 (0.64–1.56)	0.80 (0.47–1.36)	0.38 (0.19–0.76)	0.01

[a] Adjustments for matching factors only.
[b] Adjustments for matching factors, age at menarche, parity, age at first birth, history of breast cancer in mother or a sister, history of benign breast disease, alcohol intake, body mass index at age 18, body mass index at blood collection, and duration of postmenopausal hormone use.
[c] Additional adjustments for plasma concentrations of folate, vitamin B_6, vitamin B_{12} and total homocysteine.

significantly according to other risk factors for breast cancer. The prospective design and high follow-up rates in this study minimize the possibility that our findings are due to methodological biases. Because controlling for established risk factors for breast cancer had minimal effect on the RRs, our results are unlikely to be explained by residual confounding by these factors. The inverse association between plasma total cysteine concentrations and risk of breast cancer among women is also unlikely to be explained by plasma concentrations of folate, vitamin B_6, vitamin B_{12}, and total homocysteine because the RRs did not change appreciably after additionally adjusting for these plasma estimates in the model. Our results are also unlikely to be explained by the potential bias that breast cancer itself, before it was diagnosed, might have affected plasma total cysteine concentrations because the RRs after excluding cases and their matched controls within the first 2 years after blood collection were similar to those using all cases and controls.

Cysteine is derived from methionine via the transsulfuration pathway through homocysteine (2). Methionine and homocysteine are readily interconvertible. When methionine is in excess, homocysteine is catalyzed into cysteine via cystathionine, which is facilitated by two pyridoxal 5′-phosphate-dependent enzymes, cystathionine β-synthase and γ-cystathionase (2, 3). When methionine is needed, homocysteine is remethylated to methionine using a methyl group provided by methyltetrahydrofolate; this reaction is facilitated by methionine synthase, a vitamin B_{12}-dependent enzyme, or betaine-dependent methylation (2, 3). These interrelationships explain the positive association between plasma concentrations of total cysteine and total homocysteine we observed.

An inhibitory effect of cysteine on breast tumor initiation could be in part related to its role as the rate-limiting amino acid in the intracellular synthesis of glutathione (1). Glutathione is the most powerful intracellular antioxidant as well an important detoxifying agent (4–6). However, glutathione measures in plasma are strongly influenced by sampling method, processing, and storage, which limits their use in epidemiological studies. Even minor hemolysis (0.1–1%) can cause incorrectly high plasma values of glutathione because RBCs contain approximately 500 times higher glutathione than plasma (19). In addition, blood specimens must be collected with the use of preservation solution designed to inhibit autooxidation and enzymatic degradation (19, 20). Plasma glutathione is diminished by oxidation that occurs with a half-time of about 5 min at room temperature (21, 22). To the best of our knowledge, there are no prospective data relating prediagnostic blood glutathione levels to breast cancer risk. In three case-control studies involving approximately 20 cases each, breast cancer cases had levels of blood glutathione either similar to (23) or higher than that of controls (24, 25), which may reflect the influence of tumors on circulating glutathione levels.

Cysteine is commercially available as a dietary supplement. Based on data from the 1988 to 1994 Third National Health and Nutrition Examination Survey, cysteine intakes from food and supplements in the United States were 1.0 g/day at the 50th percentile and 2.0 g/day at the 99th percentile (26). Because of neural toxicity observed in rodents with high doses, there is some concern that cysteine itself may not be ideal for therapeutic use (27). Single oral administration of 5 or 10 g of cysteine has been shown to cause nausea and light-headedness or dissociation in normal humans (28). No reports were found on long-term cysteine supplementation in humans (26). The data on adverse effect of cysteine and/or cystine (the oxidized form of cysteine) intake from supplements were not considered sufficient for a dose-response assessment and derivation of an upper intake level by the Institute of Medicine (26). However, a whey-based cysteine donor, an oral supplement with a large amount of glutamylcystine, appeared to be well tolerated in 20 healthy young adults supplemented with 20 g/day (10 g/dose, twice daily) for 3 months, except that some subjects complained of bloating and occasional queasiness (29). Supplementation with this whey-based cysteine donor also resulted in a significant increase in lymphocyte glutathione levels (29). *N*-Acetylcysteine, a synthetic precursor of cysteine and glutathione, is commonly used as a mucolytic agent and as an antidote against acetaminophen-induced hepatotoxicity (30). *N*-Acetylcysteine appears safe, even at high doses (31). In a large clinical trial, the most frequently reported side effect of *N*-acetylcysteine (600 mg daily) was dyspepsia (32). *N*-Acetylcysteine has effectively blocked DNA binding by a variety of carcinogens including benzo(*a*)pyrene, acetylaminoflurorence, and cigarette smoke (33). *N*-Acetylcysteine also inhibits cell proliferation and tumor metastasis, increases DNA repair by protecting related enzymes, and modulates immune responses as well as gene expressions of a variety of tumor suppressor genes and oncogenes (6). Thus, *N*-acetylcysteine has been considered to be one of the most promising drugs for cancer chemoprevention (34, 35).

Table 4 Multivariate RR and 95% CI[a] of breast cancer for 125 nmol/ml increment in plasma total cysteine by levels of other breast cancer risk factors

Risk factor	Cases/controls	RR (95% CI)	*P* for interaction
Menopausal status			
Premenopausal	149/155	0.33 (0.13–0.83)	
Postmenopausal	487/484	0.72 (0.47–1.12)	0.22
Plasma folate			
<Median (7.5 ng/ml)	394/356	0.64 (0.41–1.02)	
≥Median	318/356	0.69 (0.44–1.08)	0.78
Plasma vitamin B_6			
<Median (48.5 pmol/ml)	405/356	0.78 (0.50–1.24)	
≥Median	307/356	0.57 (0.36–0.90)	0.21
Plasma vitamin B_{12}			
<Median (423 pg/ml)	355/356	0.67 (0.42–1.04)	
≥Median	357/356	0.64 (0.41–1.00)	0.88
Plasma total homocysteine			
<Median (10.2 nmol/ml)	361/356	0.79 (0.46–1.35)	
≥Median	351/356	0.55 (0.35–0.88)	0.23
Alcohol intake			
<15 g/day	641/641	0.67 (0.46–1.00)	
≥15 g/day	71/71	0.55 (0.22–1.35)	0.65
Body mass index at age 18 years			
<23 kg/m^2	589/560	0.65 (0.44–0.97)	
≥23 kg/m^2	123/152	0.66 (0.36–1.21)	0.97
Body mass index at blood collection			
<25 kg/m^2	387/401	0.52 (0.33–0.81)	
≥25 kg/m^2	325/311	0.88 (0.56–1.40)	0.04
Age at menarche			
<12 yrs	172/162	0.51 (0.28–0.95)	
≥12 yrs	540/550	0.70 (0.47–1.05)	0.29
Parity			
<3 births	285/282	0.68 (0.43–1.09)	
≥3 births	427/430	0.64 (0.41–0.98)	0.78
Mother or sister with breast cancer			
No	593/637	0.67 (0.45–1.00)	
Yes	119/75	0.53 (0.24–1.14)	0.53
History of benign breast disease			
No	299/406	0.61 (0.39–0.97)	
Yes	413/306	0.69 (0.44–1.09)	0.64

[a] Adjustments for matching factors, age at menarche, parity, age at first birth, history of breast cancer in mother or a sister, history of benign breast disease, alcohol intake, body mass index at age 18 years, body mass index at blood collection, and duration of postmenopausal hormone use.

In the dimethylbenzanthracene-induced rat mammary carcinogenesis model, the chemopreventive efficacy of *N*-acetylcysteine on breast cancer has yielded paradoxical results; a low dose of *N*-acetylcysteine modestly decreased the occurrence of mammary cancers, whereas a high dose of *N*-acetylcysteine significantly increased the occurrence (36). In a randomized trial among 2592 patients with head and neck or lung cancer (most of them were former or current smokers), 2 years of supplementation with *N*-acetylcysteine (600 mg daily) had no effect on survival, event-free survival, or secondary primary tumors (32). There are many possible explanations for these findings, including short duration and the heavily smoking population (32). The negative findings from this secondary prevention trial of neck and head cancer do not preclude the potential of *N*-acetylcysteine as a chemopreventive agent for other cancers.

Only one previous investigation that used a prospective nested case-control design has evaluated the association between plasma total cysteine and breast cancer risk (37). In that study including 112 cases and 113 controls, plasma total cysteine concentrations were significantly inversely associated with breast cancer risk among women with two high-activity catechol-*O*-methyltransferase alleles but not among women with low-activity catechol-*O*-methyltransferase alleles (37). In the present study including 712 case-control pairs, we found a strong inverse association between plasma total cysteine concentrations and risk of breast cancer. This inverse association was strong among women who were leaner, which has no obvious explanation but could be related to less masking effect by endogenous estrogens in this group of women. These results suggest a potential method for reducing risk of breast cancer independent of standard risk factors. Limited case-control data suggest that cysteine also might be related to cervical neoplasia. Plasma total cysteine levels were found to be inversely associated with risk of cervical dysplasia in a large case-control study (38). Lower blood levels of glutathione were also observed among patients with cervical dysplasia (39) or invasive cancer (39–42) as compared with control subjects.

In summary, the findings from this study suggest that higher circulating concentrations of total cysteine may contribute to reduction in breast cancer risk. Cysteine or its precursors might have the potential to be chemopreventive against breast cancer. The association between plasma total cysteine and risk of breast cancer needs to be evaluated in other well-designed large prospective studies.

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References

1. Faintuch, J., Aguilar, P. B., and Nadalin, W. Relevance of *N*-acetylcysteine in clinical practice: fact, myth or consequence? Nutrition, *15:* 177–179, 1999.

2. Cooper, A. J. L. Biochemistry of sulfur-containing amino acids. Annu. Rev. Biochem., *52:* 187–222, 1983.

3. Selhub, J., and Rosenberg, I. H. Folic acid. *In:* E. E. Ziegler and J. Filer, L. J. (eds.), Present Knowledge in Nutrition, 7th ed., pp. 206–219. Washington, DC: International Life Sciences Institute Press, 1996.

4. Hayes, J. D., and McLellan, L. I. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radic. Res., *31:* 273–300, 1999.

5. Quig, D. Cysteine metabolism and metal toxicity. Altern. Med. Rev., *3:* 262–270, 1998.

6. De Flora, S., Izzotti, A., D'Agostini, F., and Balansky, R. M. Mechanisms of *N*-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related end-points. Carcinogenesis (Lond.), *22:* 999–1013, 2001.

7. Dröge, W., Eck, H. P., Gmünder, H., and Mihm, S. Modulation of lymphocyte functions and immune responses by cysteine and cysteine derivatives. Am. J. Med., *91:* 140S–144S, 1991.

8. Dröge, W. Cysteine and glutathione deficiency in AIDS patients: a rationale for the treatment with *N*-acetyl-cysteine. Pharmacology (Basel), *46:* 61–65, 1993.

9. Dröge, W., Schulze-Osthoff, K., Mihm, S., Galter, D., Schenk, H., Eck, H. P., Roth, S., and Gmünder, H. Functions of glutathione and glutathione disulfide in immunology and immunopathology. FASEB J., *8:* 1131–1138, 1994.

10. Hankinson, S. E., Willett, W. C., Manson, J. E., Hunter, D. J., Colditz, G. A., Stampfer, M. J., Longcope, C., and Speizer, F. E. Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. J. Natl. Cancer Inst. (Bethesda), *87:* 1297–1302, 1995.

11. Willett, W. C., Sampson, L., Stampfer, M. J., Rosner, B., Bain, C., Witschi, J., Hennekens, C. H., and Speizer, F. E. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am. J. Epidemiol., *122:* 51–65, 1985.

12. Willett, W. C., Sampson, L., Browne, M. L., Stampfer, M. J., Rosner, B., Hennekens, C. H., and Speizer, F. E. The use of a self-administered questionnaire to assess diet four years in the past. Am. J. Epidemiol., *127:* 188–199, 1988.

13. Salvini, S., Hunter, D. J., Sampson, L., Stampfer, M. J., Colditz, G. A., Rosner, B., and Willett, W. C. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int. J. Epidemiol., *18:* 858–867, 1989.

14. Willett, W. C. Nutritional Epidemiology. New York: Oxford University Press, 1998.

15. Giovannucci, E., Colditz, G., Stampfer, M. J., Rimm, E. B., Litin, L., Sampson, L., and Willett, W. C. The assessment of alcohol consumption by a simple self-administered questionnaire. Am. J. Epidemiol., *133:* 810–817, 1991.

16. Zhang, S. M., Willett, W. C., Selhub, J., Hunter, D. J., Giovannucci, E. L., Holmes, M. D., Colditz, G. A., and Hankinson, S. E. Plasma folate, vitamin B_6, vitamin B_{12}, homocysteine, and risk of breast cancer. J. Natl. Cancer Inst. (Bethesda), *95:* 373–380, 2003.

17. Araki, A., and Sako, Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J. Chromatogr., *422:* 43–52, 1987.

18. Liang, K. Y., and Zeger, S. L. Longitudinal data analysis using generalized linear models. Biometrika, *73:* 13–22, 1986.

19. Jones, D. P., Carlson, J. L., Samiec, P. S., Sternberg, P., Jr., Mody, V. C., Jr., Reed, R. L., and Brown, L. A. Glutathione measurement in human plasma. Evaluation of sample collection, storage and derivatization conditions for analysis of dansyl derivatives by HPLC. Clin. Chim. Acta, *275:* 175–184, 1998.

20. Smith, C. V., Hansen, T. N., Martin, N. E., McMicken, H. W., and Elliott, S. J. Oxidant stress responses in premature infants during exposure to hyperoxia. Pediatr. Res., *34:* 360–365, 1993.

21. Anderson, M. E., and Meister, A. Dynamic state of glutathione in blood plasma. J. Biol. Chem., *255:* 9530–9533, 1980.

22. Lash, L. H., and Jones, D. P. Distribution of oxidized and reduced forms of glutathione and cysteine in rat plasma. Arch. Biochem. Biophys., *240:* 583–592, 1985.

23. Hietanen, E., Bartsch, H., Bériziat, J. C., Camus, A. M., McClinton, S., Eremin, O., Davidson, L., and Boyle, P. Diet and oxidative stress in breast, colon and prostate cancer patients: a case-control study. Eur. J. Clin. Nutr., *48:* 575–586, 1994.

24. Seven, A., Erbil, Y., Seven, R., Inci, F., Gülyasar, T., Barutcu, B., and Candan, G. Breast cancer and benign breast disease patients evaluated in relation to oxidative stress. Cancer Biochem. Biophys., *16:* 333–345, 1998.

25. Abou Ghalia, A. H., and Fouad, I. M. Glutathione and its metabolizing enzymes in patients with different benign and malignant diseases. Clin. Biochem., *33:* 657–662, 2000.

26. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Part I. Summary and Chapters 1–9. Panel on Macronutrients, Panel on the Definition of Dietary Fiber, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Institute of Medicine of the National Academies. Washington, DC: The National Academies Press, 2002.

27. Janáky, R., Varga, V., Hermann, A., Saransaari, P., and Oja, S. S. Mechanisms of L-cysteine neurotoxicity. Neurochem. Res., *25:* 1397–1405, 2000.

28. Carlson, H. E., Miglietta, J. T., Roginsky, M. S., and Stegink, L. D. Stimulation of pituitary hormone secretion by neurotransmitter amino acids in humans. Metabolism, *38:* 1179–1182, 1989.

29. Lands, L. C., Grey, V. L., and Smountas, A. A. Effect of supplementation with a cysteine donor on muscular performance. J. Appl. Physiol., *87:* 1381–1385, 1999.

30. Kelly, G. S. Clinical applications of *N*-acetylcysteine. Altern. Med. Rev., *3:* 114–127, 1998.

31. De Vries, N., and De Flora, S. *N*-Acetyl-L-cysteine. J. Cell. Biochem. Suppl., *17F:* 270–277, 1993.

32. van Zandwijk, N., Dalesio, O., Pastorino, U., de Vries, N., and van Tinteren, H. EUROSCAN, a randomized trial of vitamin A and *N*-acetylcysteine in patients with head and neck cancer or lung cancer. For the European Organization for Research and Treatment of Cancer Head and Neck and Lung Cancer Cooperative Groups. J. Natl. Cancer Inst. (Bethesda), *92:* 977–986, 2000.

33. Izzotti, A., D'Agostini, F., Bagnasco, M., Scatolini, L., Rovida, A., Balansky, R. M., Cesarone, C. F., and De Flora, S. Chemoprevention of carcinogen-DNA adducts and chronic degenerative diseases. Cancer Res., *54:* 1994s–1998s, 1994.

34. Kelloff, G. J., Crowell, J. A., Boone, C. W., Steele, V. E., Lubet, R. A., Greenwald, P., Alberts, D. S., Covey, J. M., Doody, L. A., Knapp, G. G., Nayfield, S., Parkinson, D. R., Prasad, V. K., Prorok, P. C., Sausville, E. A., and Sigman, C. C. NCI, DCPC Chemoprevention Branch and Agent Development Committee. Clinical development plan: *N*-acetyl-L-cysteine (NAC). J. Cell. Biochem. Suppl., *20:* 63–73, 1994.

35. Kelloff, G. J., Crowell, J. A., Boone, C. W., Steele, V. E., Lubet, R. A., Greenwald, P., Alberts, D. S., Covey, J. M., Doody, L. A., Knapp, G. G., Nayfield, S., Parkinson, D. R., Prasad, V. K., Prorok, P. C., Sausville, E. A., and Sigman, C. C. Strategy and planning for chemopreventive drug development: clinical development plans. J. Cell. Biochem. Suppl., *20:* 55–62, 1994.

36. Lubet, R. A., Steele, V. E., Eto, I., Juliana, M. M., Kelloff, G. J., and Grubbs, C. J. Chemopreventive efficacy of anethole trithione, *N*-acetyl-L-cysteine, miconazole and phenethylisothiocyanate in the DMBA-induced rat mammary cancer model. Int. J. Cancer, *72:* 95–101, 1997.

37. Goodman, J. E., Lavigne, J. A., Wu, K., Helzlsouer, K. J., Strickland, P. T., Selhub, J., and Yager, J. D. COMT genotype, micronutrients in the folate metabolic pathway and breast cancer risk. Carcinogenesis (Lond.), *22:* 1661–1665, 2001.

38. Goodman, M. T., McDuffie, K., Hernandez, B., Wilkens, L. R., and Selhub, J. Case-control study of plasma folate, homocysteine, vitamin B_{12}, and cysteine as markers of cervical dysplasia. Cancer (Phila.), *89:* 376–382, 2000.

39. Kumar, A., Sharma, S., Pundir, C. S., and Sharma, A. Decreased plasma glutathione in cancer of the uterine cervix. Cancer Lett., *94:* 107–111, 1995.

40. Balasubramaniyan, N., Subramanian, S., and Govindasamy, S. Status of antioxidant systems in human carcinoma of uterine cervix. Cancer Lett., *87:* 187–192, 1994.

41. Manju, V., Kalaivani Sailaja, J., and Nalini, N. Circulating lipid peroxidation and antioxidant status in cervical cancer patients: a case-control study. Clin. Biochem., *35:* 621–625, 2002.

42. Kolanjiappan, K., Manoharan, S., and Kayalvizhi, M. Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clin. Chim. Acta, *326:* 143–149, 2002.