HHS Public Access

Author manuscript

Nutr Cancer. Author manuscript; available in PMC 2015 April 04.

Published in final edited form as:

Nutr Cancer. 2015 April ; 67(3): 442–448. doi:10.1080/01635581.2015.1002623.

The association between circulating total folate and folate vitamers with overall survival after post-menopausal breast cancer diagnosis

Archana Jaiswal McEligot[a,d], **Argyrios Ziogas**[b], **Christine M Pfeiffer**[c], **Zia Fazili**[c], and **Hoda Anton-Culver**[b]

[a]Department of Health Science. California State University, Fullerton 800 N. State College Blvd., Room KHS-121 Fullerton, CA 92834

[b]Epidemiology Division, Department of Medicine, University of California, Irvine, 224 Irvine Hall, Irvine, CA 92697-7550.

[c]Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, GA 30341.

Abstract

We studied the relationship between plasma total folate and folate vitamer concentrations (5-methyltetrahydrofolic acid [5-methylTHF], pteroylglutamic acid [folic acid] and tetrahydrofolic acid [THF] with overall survival after breast cancer diagnosis. A secondary aim was to assess the relationship between folic acid supplement use with circulating total folate and folate vitamer concentrations. Participants were post-menopausal women diagnosed with breast cancer (n = 498) with an average follow-up of 6.7 years. Plasma total folate and folate vitamers were measured by isotope-dilution LC-MS/MS in samples collected at or post-diagnosis. Cox proportional multivariate hazards models (controlled for stage, age at diagnosis, body mass index, parity, HRT use, treatment, alcohol use, folic acid use, and energy intake), were used to assess overall survival after breast cancer diagnosis. We found that the relative risk of dying for women with plasma total folate concentrations in the highest quartile was 59% lower (HR: 0.41, 95% CI: 0.19 –0.90) compared with the lowest quartile. Data on supplement use showed that women taking folic acid supplements had significantly higher circulating total folate and folate vitamer concentrations ($p < 0.0001$), suggesting that increased folate consumption through diet and/or supplementation may improve prognosis after breast cancer diagnosis.

Keywords

folate; folate vitamers; breast cancer survival; epidemiological study

[d]Corresponding author: Archana Jaiswal McEligot, PhD Associate Professor Department of Health Science. California State University, Fullerton 800 N. State College Blvd., Room KHS-121 Fullerton, CA 92834 amceligot@fullerton.edu; Phone: (657) 278-3822; Fax: (657) 278-5317.

The present manuscript has not been published elsewhere and it has not been submitted simultaneously for publication elsewhere.

INTRODUCTION

Folate-mediated metabolism, also referred to as one-carbon metabolism, has a critical role in DNA methylation, synthesis and repair, and therefore has been linked to carcinogenesis (1-4). Epidemiologic studies have found an inverse association between folate intake and colon cancer risk (4-5), but data on the relationship between folate and risk for other cancers, including breast cancer have been mixed and reviewed extensively (6-19).

Some epidemiologic studies have examined the association between dietary folate, but even fewer have assessed circulating folate concentrations with survival/prognosis after breast cancer diagnosis (15, 20-26). Three studies on dietary folate intakes suggest protection after breast cancer diagnosis (20, 23-24). Also, earlier findings from the Iowa Women's Health study showed that postmenopausal women in the highest folate tertile had a reduced risk of dying compared with the lowest tertile (HR: 0.88; 95% CI: 0.44–1.76) (24). However, other reports show either poor or no association between dietary folate intakes and survival after breast cancer diagnosis (24, 26), and even fewer have studied the association between circulating folate concentrations and survival after breast cancer diagnosis (6, 25). Therefore, additional studies investigating the relationship between dietary folate (both supplemental and from diet), and circulating folate concentrations, need to be conducted to more clearly delineate the role of folate with overall survival after breast cancer diagnosis.

Studies have yet to report on the relationship between circulating folate vitamer concentrations and breast cancer outcomes. The role of dietary and supplemental folate on circulating folate vitamers has been studied in pregnant women and older adults (27-28). The primary circulating folate vitamer in plasma is 5-methyltetrahydrofolic acid (5-methylTHF), while the actual bioactive form of folate is tetrahydrofolic acid (THF). Pteroylglutamic acid (folic acid) is primarily derived from supplements. Investigating circulating folate vitamer concentrations may further elucidate the role of folate vitamers, dietary folate and supplemental folate (prior to folate fortification) on survival after breast cancer diagnosis.

Therefore, we tested whether elevated plasma total folate and folate vitamer (5-methylTHF, folic acid and THF) concentrations, prior to all cereal-grain folate fortification in 1998, were associated with overall survival after breast cancer diagnosis in post-menopausal women. Sub-aims included assessing the degree and whether dietary and supplemental folate intakes were related to plasma total folate and folate vitamer concentrations. We hypothesized that elevated total folate and folate vitamer concentrations will be associated with improved overall survival after breast cancer diagnosis and that folate vitamers will be significantly associated with folic acid supplementation.

MATERIAL AND METHODS

Study Population and Recruitment

Eligible breast cancer cases were identified within 6 months of diagnosis via the Cancer Surveillance Program of Orange County (CSPOC), an existing population-based cancer registry (29-33), during a 1-yr period beginning in 1994. Participants consented and enrolled

into a population-based study assessing heredity and environmental factors associated with breast and ovarian cancer (32-33).

Methodology, recruitment and participation rates for the larger population-based study have been described previously (32-33). For the present study, women with complete dietary data, plasma samples, and other variable data, including body mass index (BMI), ethnicity, parity and hormone replacement therapy (HRT) use were included in this study. Briefly, of the 980 who completed the Food Frequency Questionnaire (FFQ), 691 were post-menopausal and/or were diagnosed at ≥ 50 yrs. Of the 691, 498 had plasma samples and complete reproductive, descriptive and dietary data, and are included in the folate vitamer correlation analyses. And of the 498, 471 had complete treatment data and are included in the survival analyses. The study protocol, including questionnaires, was approved by the Internal Review Board (IRB) of the California State University, Fullerton (CSUF, IRB#: 08-0321) and the University of California, Irvine (UCI, IRB #: HS91-137).

Dietary and Supplement Use Assessment

The 100-item NCI-Block FFQ (34) was used to assess the usual dietary intakes of participants enrolled in the present study. The FFQ was self-administered and completed via mail after enrollment into the study. Participants were provided specific instructions to answer all questions accurately and carefully and to complete the FFQ based on their "usual" dietary pattern. Women were also queried on supplement use, including folic acid use. Women diagnosed with breast cancer were instructed to complete the questionnaire based on dietary habits during the year prior to diagnosis. Details regarding development and dietary assessment capabilities of the FFQ have been previously published (34). Nutrient analysis was calculated by the DietSys 4.0 program.

Plasma Measurements

Blood samples were collected post-diagnosis by venipuncture into acidic citrate dextrose (ACD) vacutainers. Plasma samples collected for the parent study were stored at –80° C until analysis for the present study. Circulating total folate (sum of all folate vitamers) and folate vitamers (5-methylTHF, folic acid, THF, and 5-formylTHF/MeFox [pyrazino-s-triazine derivative of 4α-hydroxy-5-methylTHF]) were quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS) using stable-isotope labeled compounds for internal standardization ($^{13}C_5$-5-methylTHF, $^{13}C_5$-folic acid, $^{13}C_5$-THF, and $^{13}C_5$-5-formylTHF) (35-37). As we did not separate the two isobaric compounds 5-formylTHF and MeFox (an oxidation product of 5-methylTHF), we are not reporting those results. The between-run imprecision for three levels (one level for THF) of plasma QC (analyzed over 35-46 days with duplicate analysis each day) was 2.8-3.4% for 5-methylTHF (6.2-35 nmol/L, limit of detection [LOD] 0.5 nmol/L), 9.6-12.5% for folic acid (1.4-6.0 nmol/L, LOD 0.3 nmol/L) and 33% for THF (1.0 nmol/L, LOD 0.3 nmol/L).

Other measures

Age, stage at diagnosis and treatment were obtained through the cancer registry. Summary stage defined by the Surveillance, Epidemiology and End Results (SEER) program of the National Cancer Institute was used to define disease stage at diagnosis as follows: *in situ*

stage was defined as malignant, noninvasive carcinoma; localized disease was defined as invasive carcinoma confined to the breast; regional stage was defined as invasive carcinoma spread beyond the breast, by direct extension and/or to regional lymph nodes; and distant disease was defined as direct extension beyond adjacent organs specified as regional, metastasis to distant lymph nodes, or development of discontinuous secondary or metastatic tumors. TNM classification is summarized as the following: localized disease includes tumors T1-T3, N0, M0' regional disease includes tumors T4, N0, M0 or any T, N1 – N3, M0, and distant disease corresponds to any T, and N, M1. First course of treatment was categorized as completing chemotherapy, hormone therapy and radiation. All participants underwent surgery.

Alcohol use data, and height and weight data to calculate BMI (kg/m^2) were obtained through the FFQ. Menopausal status, parity and HRT use were self-reported via a questionnaire. If a woman reported being post-menopausal or if this data item was missing (approximately 6% missing), but the woman was ≥ 50 years at time of diagnosis, then she was considered to be post-menopausal.

For the present analysis, follow-up data was obtained by the cancer registry and was available through January 1, 2003 (range = 5.0 – 101 months). Follow-up was ascertained from periodic reports from hospital-based registries and from annual linkage with the mortality records from the California Department of Vital Statistics, the Department of Motor Vehicles, the National Death Index, the National Change of Address and several other linkages with national and local databases. The mean follow-up/survival time was 81 months (SD: 16), and we had complete follow-up data on 98% of the sample.

Statistical Methods

Women who consumed < 600 Kcal or > 5000 Kcal (n = 62) were excluded from the analysis. All participants alive at last follow-up were treated as censored observations, and their survival time was computed from the date of diagnosis to date of last contact. The main outcome was death from any cause. In order to increase sample size and power, we included women diagnosed with *in situ* breast cancer (n = 75) and with metastatic disease (n = 5), and adjusted for stage in the analyses.

Total and dietary folate intakes, and circulating total folate and folate vitamer concentrations were log transformed in order to convert the data from a skewed distribution to an approximated Gaussian distribution. For samples in which folate vitamer concentrations were <LOD (21% for folic acid and 10% for THF), we used imputed values corresponding to the LOD divided by the square root of two (0.21 nmol/L for both folic acid and THF).

To test the primary hypotheses of whether circulating folate concentrations were associated with risk of dying after breast cancer diagnosis, we conducted cox proportional hazards multivariate regression models. Circulating total folate and folate vitamer concentrations were included as quartiles in the models. Total folate and folate vitamer concentrations were modeled separately. Hazard Ratios and 95% confidence intervals (CI) were calculated and are shown for the 2^{nd}, 3^{rd} and 4^{th} quartiles with the first quartile as the reference group. The multivariate models were adjusted for covariates shown previously to be related with breast

cancer risk and/or survival, including stage of disease (SEER summary stage), age at diagnosis, energy intake, BMI and parity were included as continuous variables. HRT use and first course of treatment were included as categorical variables in the model. HRT use was categorized as no use (reference category) vs. use of estrogen only, progesterone only and both estrogen and progesterone. Treatment was categorized as completed vs. not completed (reference category) for chemotherapy, radiation and hormone therapy. Folic acid supplement use and alcohol use were dichotomized as use or no use. We also conducted a linear test for significance across quartiles. In addition, we included a variable representing time since diagnosis to date of blood draw [mean (yrs) and SD: 1.19 (± 0.57)], however this was not significantly associated with overall survival and had little to no effect on the results, and therefore, it was not included in the model.

Means were calculated on dietary folate and circulating total folate and folate vitamer concentrations. Pearson correlation coefficients were used to assess linear correlations between total, supplemental and dietary folate intakes with plasma total folate and folate vitamer concentrations. We conducted *t*-test analyses to test for differences in dietary folate intakes, total folate intakes, circulating plasma total folate and folate vitamer concentrations between women taking and not taking folic acid supplements.

RESULTS

Demographic, descriptive, dietary and other prognostic characteristics have been published previously (23, 32-33). Briefly, the mean age at diagnosis was 64 (± 9) years, and overall 85 deaths were reported with a mean duration follow-up of 81 (± 16) months. A majority of the population was non-Hispanic white (n = 459; 92.2%), followed by Asians (n = 12; 2.4%), Hispanics (n = 12; 2.4%), and unknown/other (n = 15; 3.0%). Stage of diagnosis was as follows: 15.1% of the women were diagnosed with in situ, 60.2% with localized disease, 23.7% with regional stage, and 1.00% diagnosed with metastatic disease. A majority of the women underwent radiation (n = 293, 60.0%), while less than a quarter completed first round of chemotherapy (n = 99; 20.1%) and hormone therapy (n = 170; 34.8%).

Table 1 presents data on the association of plasma total folate and folate vitamer concentrations with all-cause mortality. Women with plasma total folate concentrations in the highest quartile had a significantly reduced risk of dying compared with the lowest quartile (HR: 0.41, 95% CI: 0.19–0.90), with a significant ($p = 0.01$) linear test for trend. The highest quartile for all folate vitamers were inversely associated with risk of dying, but did not reach significance at $p \leq 0.05$.

Table 2 shows correlations between dietary, supplemental, and total folate intake with circulating total folate and folate vitamer concentrations. All folate vitamers as well as circulating total folate concentrations were significantly correlated among each other ($p < 0.05$). Folate intake from food alone was significantly ($p < 0.05$) associated with 5-methylTHF and circulating total folate concentrations. In contrast, supplemental folic acid and total folate intakes had a strong positive, significant ($p < 0.0001$) correlation with circulating total folate and all folate vitamer concentrations.

Pre-fortification means (± SD) for dietary folate intakes, circulating total folate and folate vitamer concentrations are shown in **table 3** for women taking and those not taking folic acid supplements. For the total sample, total folate intakes were 524.3 μg/d (± 113.48) with approximately half derived from food (252.6 ± 113.48) and the other half from folic acid supplements (271.61 ± 328.20). Mean circulating total folate concentration was 29.9 nmol/L (± 20.4) and was primarily comprised of 5-methylTHF (24.3 ± 15.5 nmol/L) followed by folic acid and THF. Significant ($p < 0.05$) differences between the no folic acid supplement group and those taking folic acid were observed for all variables.

DISCUSSION

We showed, in our sample of 471 post-menopausal women diagnosed with breast cancer, that elevated concentrations of plasma total folate were significantly associated with over a 50% reduced risk of dying after breast cancer diagnosis. The highest quartile of circulating 5-methylTHF, folic acid and THF were also inversely associated with risk of dying. Further, several associations between dietary folate intakes, including diet only and supplemental folate, and circulating folate vitamer concentrations were found.

Reports on cancer outcomes and total folate concentrations are mixed (9, 22) with some studies suggesting extremely high levels of folate and/or folate receptor over expression may contribute to reduced survival after cancer diagnosis (16, 22, 38). Yang and colleagues recently investigated cancer outcomes in the NHANES III cohort and reported that those in the lowest folate quintile were at significantly higher risk of dying after cancer diagnosis, and with no linear trend in the remaining quintiles (22). But, those in the highest quintile were at reduced risk of dying (0.85, 95%: 0.59 – 1.22). Our results, similar to Yang et al. and Rossi et al. (25), show that those in the highest quartile of circulating total folate concentrations were at significantly reduced risk of all-cause mortality after breast cancer diagnosis. Nonetheless, our total circulating folate concentrations were higher (29.9 nmol/L vs. 12.23 nmol/L) compared with NHANES III data (22). Discrepant findings of studies either reporting increased risk and/or nonlinear associations may be due to extremely high doses of folate (1,000 ug) administered, cancer types studied and/or population-wide data collection compared to the present study. Also, to further examine the role of folate in breast cancer outcomes, we examined plasma folate vitamers, as well as the association between dietary/supplemental folate, plasma total folate and folate vitamer concentrations. We found that plasma total folate concentrations were primarily comprised of 5-methylTHF followed by folic acid. We showed that although the bioactive form of folate, THF, and other vitamers including 5-methylTHF and folic acid were protective, the effects didn't reach significance, and a majority of the protection was derived from circulating total folate concentrations in our sample of post-menopausal women diagnosed with breast cancer.

Strong correlations ($r = 0.98$, $p < 0.001$) between circulating total folate concentrations with folic acid supplementation have been reported previously (39), however few studies have assessed the correlation between folate vitamers, dietary and supplemental folate intakes (27-28). We found significant correlations between total folate concentrations and folate vitamers, as well as between dietary folate and supplemental folate intakes with total folate concentrations. Overall, these results show that folic acid supplementation, compared with

folate from food only, significantly influences and increases both circulating total folate concentrations and folate vitamers (which were associated with reduced all-cause mortality after breast cancer diagnosis). Several studies have shown increased bioavailability of folate from folic acid supplements compared with folate from food (40).

Our results on differences in circulating total folate and folate vitamer concentrations between those taking folate supplementation compared with folate from food are similar to other studies (27, 28, 41), as well as confirm our correlation analyses. Compared with pre-fortification levels of the Framingham Cohort (19.5 nmol/L and 30.8 nmol/L, respectively without and with B vitamin supplements), our circulating total folate concentrations are similar (20.17 nmol/L and 36.29 nmol/L, respectively), but are higher compared with NHANES III data (41, 42). It is possible that women recently diagnosed with breast cancer may have more healthful diets and/or behaviors which could result in higher circulating folate concentrations. Further, Obeid and colleagues compared folate vitamer concentrations in pregnant women supplemented with 400 μg folic acid to levels in un-supplemented women in Germany (27). Pregnant women who were administered folic acid had significantly higher ($p < 0.05$) circulating total folate, 5-methylTHF and THF concentrations compared with women not supplemented with folic acid (27). Similarly, our data suggest that in women diagnosed with breast cancer, taking folic acid supplements significantly influences not only circulating total folate concentrations, but also contributes to an increase in vitamer concentrations; it is therefore conceivable that, increased overall survival after breast cancer diagnosis can be contributed to folic acid supplementation in addition to folate from food only. Nonetheless, a recent study has clearly demonstrated either a U-shaped curve for the association between cancer risk and/or outcomes with folate (43), therefore further studies investigating the appropriate (and protective) range for folate consumption and/or circulating folate concentrations should be conducted.

Limitations should be acknowledged. Our study has a relatively small sample size which may contribute to limited power to detect associations via stratified analyses, particularly between folate vitamers and treatment or stage. Another limitation includes data availability for the first round of treatment only and little to no data on breast cancer sub-types, which may influence breast cancer outcomes. Nonetheless, we controlled for key prognostic factors related to breast cancer, including age and stage at diagnosis, first round of treatment, HRT use and BMI, still permitting to detect an association between total plasma folate concentrations and overall survival in our study population.

In summary, the pattern of association with total circulating folate concentrations and reduced risk of mortality was observed in our sample of 471 postmenopausal women diagnosed with breast cancer. Folate vitamers may provide a protective effect, however, their association with overall survival after breast cancer diagnosis may be conferred via their cumulative influence on circulating total folate concentrations. Additionally, folic acid supplementation compared to dietary folate alone, was not only significantly associated, but also much more highly correlated with circulating total folate concentrations, suggesting that in the absence of folic acid fortification and/or consuming a low folate diet, folic acid supplementation may improve survival after breast cancer diagnosis. However, more studies are needed to elucidate the role of the relationship between dietary folate intakes and folate

supplementation with circulating folate vitamers, and their influence on survival after breast cancer diagnosis.

Acknowledgements

We'd like to thank Melinda Verissimo for providing technical assistance and support in manuscript preparation and submission.

Funding Source: The present study was supported by the National Cancer Institute, NIH grant, 5K07CA101964.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official views or positions of the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry, the National Institutes of Health, or the Department of Health and Human Services.

References

1. Stern LL, Mason JB, Selhub J, Choi SW. Genomic DNA Hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. Cancer Epidemiol Biomarkers Prev. 2000; 9:849. [PubMed: 10952104]
2. Xu X, Chen J. One-carbon metabolism and breast cancer: an epidemiological perspective. J Genet Genomics. 2009; 36(4):203–214. [PubMed: 19376481]
3. Choi SW, Mason JB. Folate status: effects on pathways of colorectal carcinogenesis. J Nutr. 2002; 132(8):2413S–2418S. [PubMed: 12163703]
4. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr. 2000; 130(2):129–32. [PubMed: 10720158]
5. Sanjoaquin MA, Allen N, Couto E, Roddam AW, Key TJ. Folate intake and colorectal cancer risk: a meta-analytical approach. Int J Cancer. 2005; 113(5):825–828. [PubMed: 15499620]
6. Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality. Arch Intern Med. 2010; 170(18):1622–1631. [PubMed: 20937919]
7. Cho E, Hunter DJ, Spiegelman D, Albanes D, Beeson WL, et al. Intakes of vitamins A, C and E and folate and multivitamins and lung cancer: a pooled analysis of 8 prospective studies. Int J Cancer. 2006; 118(4):970–978. [PubMed: 16152626]
8. Maruti SS, Ulrich CM, White E. Folate and one-carbon metabolism nutrients from supplements and diet in relation to breast cancer risk. Am J Clin Nutr. 2009; 89(2):624–633. [PubMed: 19116331]
9. Ebbing M, Bonaa KH, Nygard O, Amesen E, Ueland PM, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B_{12}. JAMA. 2009; 302(19):2119–2126. [PubMed: 19920236]
10. Slatore CG, Littman AJ, Au DH, Satia JA, White E. Long-term use of supplemental multivitamins, vitamin C, vitamin E, and folate does not reduce the risk of lung cancer. Am J Respir Crit Care Med. 2008; 177(5):524–530. [PubMed: 17989343]
11. Stevens VL, Rodriguez C, Pavluck AL, McCullough ML, Thun MJ, Calle EE. Folate nutrition and prostate cancer incidence in a large cohort of US men. Am J Epidemiol. 2006; 163(11):989–996. [PubMed: 16554345]
12. Larsson SC, Giovannucci E, Wolk A. Folate and risk of breast cancer: a meta-analysis. J Natl Cancer Inst. 2007; 99(1):64–76. [PubMed: 17202114]
13. Stolzenberg-Solomon RZ, Chang SC, Leitzmann MF, Johnson KA, Buys SS, Hoover RN, et al. Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Am J Clin Nutr. 2006; 83(4):895–904. [PubMed: 16600944]
14. Zhang CX, Ho SC, Chen YM, Lin FY, Fu JH, Cheng SZ. Dietary folate, vitamin B6, vitamin B12 and methionine intake and the risk of breast cancer by oestrogen and progesterone receptor status. Br J Nutr. 2011; 16:1–8.

15. Zhang SM, Cook NR, Albert CM, Gaziano JM, Buring JE, Manson JE. Effect of combined folic acid, vitamin B6, and vitamin B12 on cancer risk in women. JAMA. 2008; 300(17):2012–2021. [PubMed: 18984888]
16. Ulrich CM, Potter JD. Folate supplementation: too much of a good thing? Cancer Epidemiol Biomarkers Prev. 2006; 15:189–93. [PubMed: 16492904]
17. Larsson SC, Giovannucci E, Wolk A. Folate intake and stomach cancer incidence in a prospective cohort of Swedish women. Cancer Epidemiol Biomarkers Prev. 2006; 15:1409–12. [PubMed: 16835345]
18. Vollset SE, Igland J, Jenab M, et al. The association of gastric cancer risk with plasma folate, cobalamin, and methylenetetrahydrofolate reductase polymorphisms in the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev. 2007; 16:2416–24. [PubMed: 18006931]
19. Feigelson HS, Jonas CR, Robertson AS, McCullough ML, Thun MJ, Calle EE. Alcohol, folate, methionine, and risk of incident breast cancer in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. Cancer Epidemiol Biomarkers Prev. 2003; 12:161–4. [PubMed: 12582027]
20. Harris HR, Bergkvist L, Wolk A. Folate intake and breast cancer mortality in a cohort of Swedish women. Breast Cancer Res Treat. 2012; 132(1):243–250. [PubMed: 22037788]
21. Holmes MD, Stampfer MJ, Colditz GA, Rosner B, Hunter DJ, Willett WC. Dietary factors and the survival of women with breast carcinoma. Cancer. 1999; 86(5):826–835. [PubMed: 10463982]
22. Yang Q, Bostick RM, Friedman JM, Flanders WD. Plasma folate and cancer mortality among U.S. adults: findings from the Third National Health and Nutritional Examination Survey linked mortality file. Cancer Epidemiol Biomarkers Prev. 2009; 18(5):1439–47. [PubMed: 19423522]
23. McEligot AJ, Largent J, Ziogas A, Peel D, Anton-Culver H. Dietary fat, fiber, vegetable, and micronutrients are associated with overall survival in postmenopausal women diagnosed with breast cancer. Nutr Cancer. 2006; 55(2):132–40. [PubMed: 17044767]
24. Sellers A, Alberts SR, Vierkant RA, Grabrick DM, Cerhan JR, Vachon CM, et al. High-folate diets and breast cancer survival in a prospective cohort study. Nutr Cancer. 2002; 44:139–144. [PubMed: 12734059]
25. Rossi E, Hung J, Beilby JP, Knuiman MW, Divitini ML, Bartholomew H. Folate levels and cancer morbidity and mortality: prospective cohort study from Busselton, Western Australia. Ann Epidemiol. 2006; 16:206–212. [PubMed: 16343942]
26. Xu X, Gammon MD, Wetmur JG, Bradshaw PT, Teitelbaum SL, Neugut AI, Santella RM, Chen J. Folate intake and breast cancer prognosis: a meta-analysis of prospective observational studies. Eur J Cancer Prev. 2014 (Epub ahead of print).
27. Obeid R, Kasoha M, Kirsch SH, Munz W, Herrmann W. Concentrations of unmetabolized folic acid and primary folate forms in pregnant women at delivery and in umbilical cord blood. Am J Clin Nutr. 2010; 92(6):1416–1422. [PubMed: 20844072]
28. Obeid R, Kirsch SH, Kasoha M, Eckert R, Herrmann W. Concentrations of unmetabolized folic acid and primary folate forms in plasma after folic acid treatment in older adults. Metabolism Clinical and Experimental. 2011; 60(5):673–680. [PubMed: 20727555]
29. Anton-Culver H, Culver BD, Kurosaki T, et al. Incidence of lung cancer by histological type from a population-based registry. Cancer Res. 1998; 48:6580–6583. [PubMed: 2846154]
30. Seiffert JE, Price WT, Gordon B. The California tumor registry: a state-of-the-art model for a regionalized, automated, population-based registry. Top Health Rec Manage. 1990; 11:59–73. [PubMed: 10108499]
31. North American Association of Central Cancer Registries. Standards for Cancer Registries: Vol I, Data Exchange Standards and Record Description; Vol. II, Data, Standards and Data Dictionary; Vol. III, Standards for Completeness, Quality, Analysis, and Management of Data. Cancer Surveillance and Control Program, California Department of Health Services; Sacramento, CA: 1994-1995.
32. Anton-Culver H, Cohen PF, Gildea ME, Ziogas A. Characteristics of BRCA1 mutations in a population-based case series of breast and ovarian cancer. Eur J Cancer. 2000; 36:1200–1208. [PubMed: 10882857]

33. Ziogas A, Gildea M, Cohen P, Bringman D, Taylor TH, Seminara D, et al. Cancer risk estimates for family members of a population-based family registry for breast and ovarian cancer. Cancer Epidemiol Biomark Prev. 2000; 9:103–111.
34. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol. 1986; 124:453–469. [PubMed: 3740045]
35. Pfeiffer CM, Fazili Z, McCoy L, Zhang M, Gunter EW. Determination of folate vitamers in human plasma by stable-isotope-dilution tandem mass spectrometry and comparison with radioassay and microbiologic assay. Clinical Chemistry. 2004; 50(2):423–432. [PubMed: 14670827]
36. Fazili Z, Pfeiffer CM. Measurement of folates in plasma and conventionally prepared whole blood lysates: application of an automated 96-well plate isotope-dilution tandem mass spectrometry method. Clin Chem. 2004; 50(12):2378–81. [PubMed: 15459090]
37. Hannisdal R, Ueland PM, Svardal A. Liquid chromatography–tandem mass spectrometry analysis of folate and folate catabolites in human plasma. Clin Chem. 2009; 55:1147–54. [PubMed: 19359539]
38. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA. 2007; 297(21):2351–9. [PubMed: 17551129]
39. Kalmbach RD, Choumenkovitch SF, Troen AM, D'Agostino R, Jacques PF, Selhub J. Circulating folic acid in plasma: relation to folic acid fortification. Am J Clin Nutr. 2008; 88:763–8. [PubMed: 18779294]
40. McNulty H, Penteiva K. Folate bioavailability. Proc Nutr Soc. 63(4):529–36. 20004. [PubMed: 15831124]
41. Pfeiffer CM, Johnson CL, Jain RB, Yetley EA, Picciano MF, Rader JI, et al. Trends in blood folate and vitamin B12 concentrations in the United States, 1988-2004. Am J Clin Nutr. 2007; 86:718–27. [PubMed: 17823438]
42. Quinlivan EP, Gregory JF. Reassessing folic acid consumption patterns in the United States (1999–2004): potential effect on neural tube defects and overexposure to folate. Am J Clin Nutr. 2007; 86:1773–9. [PubMed: 18065598]
43. Chuang SC, Stolzenberg-Solomon R, Ueland PM, Vollset SE, Midttun O, Olsen A, et al. A U-shaped relationship between plasma folate and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. Eur J Cancer. 2011; 47(12):1808–16. [PubMed: 21411310]

Table 1

Multivariate hazard ratios (HR)[1] of death (all-cause mortality) of plasma folate and folate vitamer concentrations among post-menopausal women diagnosed with breast cancer (n = 471)

Variable	Q1	Q2	Q3	Q4	*p*-value for trend
Plasma total folate, nmol/L		14.04	25.21	40.56	0.01
HR (95% CI)	1.0, ref	1.11 (0.60 - 2.07)	0.72 (0.37 - 1.39)	0.41 (0.19 - 0.90)	
p-value		0.74	0.33	0.03	
5-MethylTHF, nmol/L		11.50	20.40	34.90	0.11
HR (95% CI)	1.0, ref	1.09 (0.59 - 2.01)	0.81 (0.41 - 1.56)	0.62 (0.30 - 1.28)	
p-value		0.78	0.52	0.20	
Folic acid, nmol/L		0.33	0.64	1.27	0.54
HR (95% CI)	1.0, ref	0.56 (0.29 - 1.08)	0.78 (0.43 - 1.48)	0.75 (0.39 - 1.43)	
p-value		0.08	0.45	0.38	
THF, nmol/L		0.84	1.69	2.85	0.12
HR (95% CI)	1.0, ref	1.06 (0.58 - 1.94)	1.13 (0.62 - 2.05)	0.51 (0.25 - 1.04)	
p-value		0.86	0.69	0.06	

[1] Dietary variables are controlled for stage of disease, age at diagnosis, BMI, parity, HRT use, treatment, alcohol use, folic acid supplement use and energy intake. All species are modeled separately.

Table 2

Pearson correlation coefficients between plasma total folate and folate vitamers and dietary, supplemental and total folate intakes (n = 498).

	5-MethylTHF	Folic acid	THF	Plasma total folate
5-MethylTHF (nmol/L), *r*	1.00	0.57	0.50	0.97
p		<0.0001	<0.0001	<0.0001
Folic acid (nmol/L), *r*	0.57	1.00	0.37	0.69
p	<0.0001		<0.0001	<0.0001
THF (nmol/L), *r*	0.50	0.37	1.00	0.56
p	<0.0001	<0.0001		<0.0001
Dietary folate (μg/d), *r*	0.17	0.009	0.06	0.15
p	0.0001	0.83	0.22	0.0009
Supplement folate (μg/d), *r*	0.46	0.35	0.31	0.47
p	<0.0001	<0.0001	<0.0001	<0.0001
Total folate intake (μg/d), *r*	0.48	0.32	0.30	0.49
p	<0.0001	<0.0001	<0.0001	<0.0001

Table 3

Mean (SD) for dietary folate, supplementary folate and circulating total folate and folate vitamer concentrations[1] (n = 498).

Variable	Total Sample (n = 498)[1]	No Folic Acid Supplement (n = 199)	With Folic Acid Supplement (n = 299)	*p*-value[4]
Dietary folate[2], μg/d	252.7 (113.5)[2]	240.79 (113.2)	260.5 (113.1)	< 0.02
Total folate intake, μ,g/d	524.3 (353.10)	240.8 (113.2)	712.9 (331.7)	< 0.0001
Plasma total folate, nmol/L	29.9 (20.4)	20.2 (15.6)	36.3 (20.7)	< 0.0001
Plasma 5-methylTHF, nmol/L	24.3 (15.5)	16.6 (12.5)	29.4 (15.1)	< 0.0001
Plasma folic acid, nmol/L	2.39 (8.39)	1.19 (4.69)	3.18 (10.1)	< 0.0001
Plasma THF, nmol/L	2.05 (1.57)	1.47 (1.34)	2.43 (1.60)	< 0.0001

3 P-values for differences between users and non-users of folic acid supplements.

[1]For samples in which folate vitamer concentrations were < LOD (21% for folic acid and 10% for THF), we used imputed values corresponding to the LOD divided by the square root of two (0.21 nmol/L for both folic acid and THF).

[2]Folate from diet alone.