Published in final edited form as:

Breast Cancer Res Treat. 2009 August ; 116(3): 551–562. doi:10.1007/s10549-008-0167-2.

Alcohol and folate intake and breast cancer risk in the WHI Observational Study

Christine M. Duffy,

Division of General Internal Medicine, Rhode Island Hospital, Brown University Medical School,
111 Plain Street, Providence, RI 02903, USA

Annlouise Assaf,

Global Epidemiology and Risk Management Pfizer, Inc., New London, CT, USA

Michele Cyr,

Division of General Internal Medicine, Brown University Medical School, Providence, RI, USA

Gary Burkholder,

College of Health & Sciences, Walden University, Minneapolis, MN, USA

Elizabeth Coccio,

Memorial Hospital of Pawtucket, Pawtucket, RI, USA

Tom Rohan,

Department of Epidemiology and Population Health, Albert Einstein College of Medicine, New
York, NY, USA

Anne McTiernan,

Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA, USA

Electra Paskett,

Division of Epidemiology, Ohio State University, Columbus, OH, USA

Dorothy Lane, and

Department of Preventive Medicine, Stony Brook University Medical School, Stony Brook, NY,
USA

V. K. Chetty

Department of Family Medicine, Boston University Medical School, Boston, MA, USA

Christine M. Duffy: cduffy@lifepsan.org

Abstract

Background—Alcohol increases breast cancer risk. Epidemiological studies suggest folate may modify this relationship.

Objective—To examine the relationship among breast cancer, alcohol and folate in the Women's Health Initiative-Observational Study (WHI-OS).

Methods—88,530 postmenopausal women 50–79 years completed baseline questionnaires between October 1993 and December 1998, which addressed alcohol and folate intake and breast cancer risk factors. Cox proportional hazards analysis examined the relationship between self-reported baseline alcohol and folate intake and incident breast cancer.

Results—1,783 breast cancer cases occurred over 5 years. Alcohol was associated with increased risk of breast cancer (RR = 1.005, 95% CI 1.001–1.009). Risk increased with consumption of alcohol (up to 5 g/d, adjusted HR = 1.10, 95% CI 0.96–1.32; >5–15 g/d HR = 1.14, 95% CI 0.99–1.31; and >15 g/d HR = 1.13 95% CI 0.96–1.32). We found no significant interaction between alcohol and folate in our adjusted model.

Conclusions—We found no evidence for folate attenuating alcohol's effect on breast cancer risk in postmenopausal women. Our results may be due to misclassification of folate intake or the relatively short follow-up period.

Keywords

Folate; Folic acid; Alcohol; Breast cancer; WHI

Introduction

In 1977, Williams and Horm [1] noted an increased rate of breast cancer in women who consumed alcohol. Subsequent observational cohort studies [2–5], as well as several meta-analyses [6–8], have demonstrated a relatively consistent positive association between alcohol consumption and breast cancer risk. More recently, several studies [9–13], but not all [14], have reported that folate may attenuate alcohol's effects on breast cancer risk.

Folate is a water-soluble B-vitamin found naturally in green leafy vegetables, beans and legumes, citrus fruits, and in enriched flour and grain products [15]. Folate plays a key role in DNA replication and cell division because of its involvement in the biosynthesis of purines and thymidylates, which are essential building blocks for DNA synthesis and repair. Folate deficiency can result in ineffective DNA synthesis leading to chromosome breaks and disruption of DNA repair. In addition, low folate levels can reduce the availability of S-adenosylmethionine for DNA methylation which may influence gene expression [16]. Alcohol is a folate antagonist and may decrease circulating levels of folate [17], particularly in those who drink heavily. Its primary metabolite, acetaldehyde, in combination with low folate intake may also lead to the derangement of DNA metabolism and repair, and subsequent inappropriate gene expression [18–21].

We sought to examine the relationship between breast cancer and intake of both alcohol and folate, using data from the Women's Health Initiative Observational Study (WHI-OS). We hypothesized that alcohol would increase breast cancer risk, and that folate would attenuate the increased risk of breast cancer observed in alcohol consumers.

Materials and methods

Study population

The Women's Health Initiative-Observational Study (WHI-OS) is a large prospective cohort study of post-menopausal women in the United States, designed to examine the epidemiology of diseases common in older women. A total of 93,676 women were enrolled in the study. Details regarding the recruitment and structure of the WHI-OS have been published elsewhere [22]. In brief, the WHI-OS enrolled postmenopausal women aged 50–79 years at 40 clinical centers throughout the US. Although recruitment occurred between October 1993 and December 1998, the majority of WHI-OS participants were recruited in the last years of recruitment (1997–1998). Eligibility criteria included premenopausal status, free from any medical condition might result in death in the next 3 years, and not enrolled in any other clinical trial. All patients completed the process for informed consent and institutional review boards at all participating institutions approved study protocols and procedures.

Follow-up

Annual questionnaires were mailed to the study participants. The follow-up rates for medical history updates in years 1, 2, 4, 5, and 6 (years in which medical histories were collected by mail) were 96, 94, 94, 95 and 94% respectively. As of August 2002, 3.7% of women had stopped participation or had been lost to follow-up and 3.2% had died.

Exposure assessment

Information on exposure variables was obtained at enrollment. Demographic information including age, ethnicity, marital status, education level and income were self-reported at ascertainment of eligibility. A medical history questionnaire captured information regarding breast cancer risk factors (age of menarche and menopause, number of breast biopsies, number of pregnancies, breastfeeding practices (ever versus never), body mass index (BMI [weight (kg)/ height (m²)]), family history (first degree affected versus none), and use of exogenous hormones (ever use of estrogen and progestin versus never). Weekly energy expenditure (expressed in metabolic equivalents—METs) was calculated from self-reported usual physical activity. Tobacco use was classified as previous, current or non-smoker.

Women completed a self-administered 122-item food frequency questionnaire (FFQ) at enrollment. Dietary folate was computed from responses to food items on the FFQ, using the University of Minnesota Nutrition Coding Center nutrient database, version 4.03, 1992 (Minneapolis, MN) [23]. The Pearson correlation coefficient between dietary folate intake measured by FFQ and 8-day dietary intake (four 24-h food recalls and 4 day food records) was 0.57 in a validation study using a sub-sample of WHI participants [24].

Participants brought in their supplements and/or vitamins to their WHI clinic and study personnel recorded folic acid as single supplements (folic acid alone), supplement mixtures (e.g. B-complex containing folic acid), or one-a-day multi-vitamins (with folic acid). These were summed to give folate intake from supplement sources. Dietary and supplement intake were summed to provide a total daily folate intake in micrograms per day. The Pearson

correlation coefficient between total folate intake and 8-day dietary intake which included supplement intake (four 24-h food recalls and 4 day food records) was 0.69 [24].

Alcohol intake (including portion size—small, medium or large) over the previous 3 months was categorized into beer, wine, and liquor on the FFQ. A medium portion was defined as one 12 oz can or bottle of beer, one 6 oz glass of wine, or one 1.5 oz shot of liquor with ethanol estimates of 12.6, 13.8 and 17.1 g, respectively [3, 24]. Daily intake of alcohol was expressed in grams of alcohol per day. The Pearson correlation coefficient between alcohol intake by FFQ versus 8 day dietary intake (four 24-h recalls and a 4 day food record) was 0.89 in a validation subsample [24].

Outcome measurement

Women completed annual follow-up questionnaires regarding health status, including information about new cancer diagnosis. Breast cancer cases were adjudicated by blinded study physicians and trained coders. This process included evaluation of pathology reports, discharge summaries, operative reports and radiology reports for all biopsies and surgeries. Only cases of invasive breast cancer were used in analysis.

Statistical analysis

SAS statistical software version 8.2 and S-Plus version 6.2 were used for all analyses. Women were excluded from the analysis if they reported a history of breast cancer at enrollment ($n = 5,021$) or had implausible (<600 kcal $> 3,500$ kcal) [24] dietary intake ($n = 113$), including folate outliers ($n = 2$), identified by influence analysis (calculating leverages). A total of 88,540 women remained available for analysis.

We created a four-level alcohol consumption variable: no alcohol consumption (<1 drink/mos), any consumption up to 5 g/d of alcohol (< 1 drink/mos to ~ 3 drinks/week), between 5–15 g/d (between ~ 3 –7 drinks/week) and 15+g/d (> 1 drink/day). Our cut-points reflect roughly the mean alcohol intake (5 g/d), the equivalent of one drink per day (15 g/d), and are consistent with other studies [10, 12, 14]. We categorized folate intake into quartiles, a similar approach taken by previous researchers [10, 11, 14].

Cox proportional hazards regression was used to model risk in association with the exposures of interest, with age as the time-scale. This approach has been recommended for epidemiologic cohort studies by several authors [25–27]. In addition, this approach is increasingly used in cohort studies examining factors potentially associated with cancer risk, including breast cancer [28–33].

Follow-up time for cases was accrued from date of birth until the date of diagnosis of breast cancer, and for non-cases from the date of birth until the date of death, loss to follow up, or administrative censor date (August 31, 2002), whichever was earliest. Women with missing data for any of the exposure or outcome variables ($n = 9,794$) were excluded, leaving a total of 78,746 women available for the Cox regression analysis. Data on income ($n = 6,568$) and diet ($n = 3,583$) were most often missing. Women with missing income information had slightly higher rates of breast cancer than those with income information. However, this difference in breast cancer incidence was quite small (2.11 vs. 2.05%). In addition, models

with a dummy variable for missing income were not significantly different from the main model. We also checked our model with a dummy variable for folate as well as alcohol, and these were not significantly different from the main model.

Other covariates considered in our model included the demographic variables ethnicity, income, education, breast cancer risk factor variables (ever use of combined hormone therapy, family history of breast cancer, age of menarche, age of menopause, number of breast biopsies, number of term pregnancies, ever breastfed, BMI, METS, and smoking status). The model was adjusted for energy intake and folate was energy adjusted using the residual method [34].

We performed extensive model diagnostics including assessing the validity of the Cox proportional hazards assumptions by calculating martingale and Schoenfeld residuals. Race/ethnicity, education and income all failed to meet the proportional hazards assumptions, and hence the analysis was stratified by these variables. Our Cox model includes different intercepts for each stratified group, but common slope parameters. This approach is recommended by Therneau and Grambsch [35] and we follow their technique. Because the number of breast cancer cases was quite small in non-Caucasians (e.g. African Americans $n = 74$, Hispanics $n = 43$), we lacked statistical power to examine outcomes among minority groups.

The effect of source of folate (diet versus supplement) was evaluated using several additional models. We examined our model using only dietary folate intake, then supplemental folate intake alone. We also treated dietary and supplemental folate as separate variables, including both in the model. For our final model, we combined dietary and supplemental folate and treated it as a single variable. We found no significant differences among models and so we report combined dietary and supplemental folate intake in our final model.

Results

During a mean follow-up of 5.5 years (SD = 1.3 years), 1,783 cases of invasive breast cancer were identified. Table 1 summarizes demographic and risk factor information for women who developed breast cancer subsequent to baseline and those who did not. Women who developed breast cancer were older, more likely to be Caucasian, had higher mean incomes and education levels, were more likely to use hormone therapy, had earlier menarche and later menopause, lower mean number of pregnancies, and were more likely to have smoked.

Baseline alcohol and folate intakes are shown in Table 2. For the entire cohort, intake was 5.6 g/d (SD 11.2), and baseline total folate was 446.0 (SD 289). The percentage of women taking folate from any supplement source was higher among women who developed breast cancer versus those who did not (51 vs. 48.5% $P < 0.01$). The relative contribution of supplements or multivitamins (MVI) to total folate increased with increasing total folate consumption. In the top quartile of folate intake, 59% of intake was from supplements, versus 3% in the bottom quartile.

Table 2 shows the relationship between alcohol and total folate intake and risk of breast cancer in our adjusted Cox model. We present models using alcohol and folate as continuous variables, as well as categorical variables. In both models, alcohol was associated with an increased risk of breast cancer. For each gram of alcohol per day, there was a 0.5% increased risk of breast cancer ($P = 0.009$). There was evidence for an increase in risk of breast cancer in individuals in the upper two categories of alcohol intake, though these relationships were not statistically significant ($P = 0.08$ and $P = 0.15$ respectively). Folate intake was not related to breast cancer incidence in either the continuous or the categorical model. There was no evidence for an interaction between alcohol and folate ($P = 0.34$).

We tested for interactions between alcohol and the following variables: race/ethnicity, family history of breast cancer, use of hormone therapy, and BMI. We repeated these analyses for folate. None of the interactions were statistically significant. In addition, we tested for three-way interactions between alcohol, folate, and the above variables. None of these interactions were significant, although our power to test for these interactions was limited.

To determine whether folate was protective among those with higher alcohol intakes, we examined risk of breast cancer among levels of folate by increasing alcohol consumption (Table 3). We found no evidence for a protective effect of high folate among those in the uppermost intake level of alcohol consumption. To determine the effect of very low folate levels on breast cancer risk, we examined risk of breast cancer for the lowest 10% of folate intake as compared to the upper 90%, but found no significant differences in risk in that population (OR = 1.05 $P = 0.63$).

Finally, our models using different sources of folate (dietary folate only, supplemental folate only, dietary and supplement folate entered separately into the model) yielded similar results to the main model using combined dietary and supplement folate intake (data not shown).

Discussion

Our results are consistent with the conclusions from several pooled analyses and reviews [7, 8, 18, 36] that have suggested increased risk of breast cancer with low levels (i.e. 3 drinks per week) of alcohol consumption. Contrary to most [9–13, 37], though not all [14, 38, 39] previous large cohort studies, we did not find that folate attenuated the increased risk of breast cancer observed among alcohol consumers. Strengths of our study are the diversity of the WHI-OS participants, the prospective design, the high rates of follow-up, and strict criteria for breast cancer end points.

Methodological differences could explain the discrepancies between our results and those from previous cohort studies. Researchers have noted the difficulty in capturing both folate [15, 40, 41] and alcohol intake [42, 43]. Of eight large cohorts of women [9–14, 37–39, 44] in which alcohol, folate and breast cancer risk were explored in detail [Table 4] all used a similar FFQ for folate measurement [12, 14, 37, 45, 46], as was used in the WHI-OS [24], except two [13] [9]. Correlation coefficients for dietary folate (0.57), total folate (0.69), and alcohol (0.89) in the WHI [24] were equal or superior to those in the other studies. Hence,

our results are unlikely to be explained by differences in validity of the measurement tools for alcohol and folate.

It is possible that we underestimated dietary folate intake because of changes in the fortification of cereals and grains which began in the early 1990s and was completed by the end of 1997 as part of a federal mandate [47]. Recruitment for the WHI-OS (and hence completion of the FFQs) occurred between Oct 1993 and Dec 1998. However, recruitment for the WHI-RCTs (which was conducted concurrently with the WHI-OS) was a priority so most of the women in the WHI-OS were recruited during the last year of recruitment, when folate supplementation was nearly complete. Therefore, we likely underestimated folate dietary intake. Folate fortification in cereals and grains has increased dietary folate by ~100 µcg/d µcg/day in women, although the increase in women 45 and older is higher (~200 µcg/d) [48]. It is possible that we failed to see a protective effect with increasing folate intake among alcohol consumers because nearly all these postmeno-pausal women were obtaining adequate folate. However we examined the lowest 10% of folate intake and failed to find any attenuation of breast cancer risk among alcohol consumers. Still, this remains a possible explanation for our findings.

One limitation of our study is the use of only one baseline measurement of alcohol and folate. However, most previous cohort studies exploring alcohol, folate and breast cancer share similar limitations [9–11, 14, 37] yet found a protective effect for folate on breast cancer risk in alcohol consumers. Thygesen et al. [49] found the strong relationship between baseline alcohol intake and breast cancer risk was markedly attenuated when recent alcohol use was included. If, as the authors' theorize, alcohol's effects on breast cancer risk are not immediate but take several years to realize, then using baseline measures is most appropriate.

This cohort's median intake of dietary folate was similar to that reported in several other studies (238 vs. 209–294 µcg/d) [10–13], but lower than that in a more recent US cohort study (660 µcg/d) [44]. Not all studies had information on non-food sources of folate (supplements and multivitamins). In the WHI-OS, 44% reported taking a supplement that contained folate, which is higher than in the Iowa Women's Health Study cohort (28%) [11, 37], but much lower than the more recent study by Stolzenberg-Solomon (85%) [44]. Essentially all MVIs contain folate, and most studies did report on MVI intake. In the WHI-OS, MVI intake (just over 40%) is higher than some cohorts [9, 11], lower than others [44] and similar to several others [12, 13, 14]. If failure to detect a protective effect of folate were related to higher folate intake from MVI and supplement use, we would have expected the model in which supplement use was an independent variable (not combined with food intake), to indicate this. However, we found no significant differences.

The median alcohol consumption in this study (4.33 g/d) was similar to previous cohort studies of postmenopausal women (4.0 g/d – 4.9 g/d) [11–14, 37], and the magnitude of the increased risk of breast cancer is consistent with the estimates obtained in two large meta-analyses examining alcohol and breast cancer risk [7, 8].

Our length of follow-up (5.5 years) was substantially shorter than in the previous cohort studies (10–16 years) [9–13], with the exception of two studies [14, 44]. If folate's protective effect is exerted early in carcinogenesis, cases occurring within the first 6 years of follow-up may not demonstrate the postulated protective effect of high folate intake. Giovannucci et al. [50] observed that it took at least 10 years before any protective effect against colorectal cancer was observed among high folate consumers. It is notable that Feigelson et al. [14], with a comparable length of follow-up, also failed to find that folate modified the relationship between alcohol and folate.

The present study does not confirm the previously described protective effect of folate on breast cancer risk for postmenopausal women who are moderate alcohol consumers. Although the increased risk of breast cancer with alcohol consumption is relatively small (13% for approximately 1 drink/day), our result emphasizes the need to include breast cancer risk among the list of hazards for even the low levels of alcohol consumption considered "safe" according to the National Institute on Alcohol Abuse and Alcoholism (NIAAA) guidelines [51]. Folate may no longer attenuate the risk of breast cancer in alcohol users simply because nearly all women are receiving adequate amounts of folate in their diet. Future cohorts studies conducted entirely in the post-fortification era should examine this issue. Finally, further follow-up of this cohort is warranted to determine if a protective effect of folate emerges over time, and to examine the effects of folate fortification in the diet.

Acknowledgments

This work was funded by NHLBI grant #1RO3CA99491-01.

References

1. Williams RR, Horm JW. Association of cancer sites with tobacco and alcohol consumption and socioeconomic status of patients: interview study from the Third National Cancer Survey. *J Natl Cancer Inst.* 1977; 58:525–547. [PubMed: 557114]
2. van den Brandt PA, Goldbohm RA, Van't Veer P. Alcohol and breast cancer: results from The Netherlands Cohort Study. *Am J Epidemiol.* 1995; 141:907–915. [PubMed: 7741120]
3. Rohan TE, Jain M, Howe GR, Miller AB. Alcohol consumption and risk of breast cancer: a cohort study. *Cancer Causes Control.* 2000; 11:239–247. [PubMed: 10782658]
4. Schatzkin A, Jones DY, Hoover RN, Taylor PR, Brinton LA, Ziegler RG, Harvey EB, Carter CL, Licitra LM, Dufour MC, et al. Alcohol consumption and breast cancer in the epidemiologic follow-up study of the first National Health and Nutrition Examination Survey. *N Engl J Med.* 1987; 316:1169–1173. [PubMed: 3574367]
5. Friedenreich CM, Howe GR, Miller AB, Jain MG. A cohort study of alcohol consumption and risk of breast cancer. *Am J Epidemiol.* 1993; 137:512–520. [PubMed: 8465803]
6. Longnecker MP, Berlin JA, Orza MJ, Chalmers TC. A meta-analysis of alcohol consumption in relation to risk of breast cancer. *JAMA.* 1988; 260:652–656. [PubMed: 3392790]
7. Longnecker MP. Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. *Cancer Causes Control.* 1994; 5:73–82. [PubMed: 8123780]
8. Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, Graham S, Holmberg L, Howe GR, Marshall JR, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA.* 1998; 279:535–540. [PubMed: 9480365]

9. Baglietto L, English DR, Gertig DM, Hopper JL, Giles GG. Does dietary folate intake modify effect of alcohol consumption on breast cancer risk? Prospective cohort study. *BMJ*. 2005; 331:807. [PubMed: 16087654]
10. Rohan TE, Jain MG, Howe GR, Miller AB. Dietary folate consumption and breast cancer risk. *J Natl Cancer Inst*. 2000; 92:266–269. [PubMed: 10655445]
11. Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, Olson JE, Thorneau TM, Folsom AR. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. *Epidemiology*. 2001; 12:420–428. [PubMed: 11416780]
12. Zhang S, Hunter DJ, Hankinson SE, Giovannucci EL, Rosner BA, Colditz GA, Speizer FE, Willett WC. A prospective study of folate intake and the risk of breast cancer. *JAMA*. 1999; 281:1632–1637. [PubMed: 10235158]
13. Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, Colditz GA, Hankinson SE. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. *J Natl Cancer Inst*. 2003; 95:373–380. [PubMed: 12618502]
14. 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 Bio-markers Prev*. 2003; 12:161–164.
15. Rosenberg IH. Virtual folate: virtual success? *Am J Clin Nutr*. 1999; 70:177–178. [PubMed: 10426691]
16. Costello JF, Plass C. Methylation matters. *J Med Genet*. 2001; 38:285–303. [PubMed: 11333864]
17. Homann N, Tillonen J, Salaspuro M. Microbially produced acetaldehyde from ethanol may increase the risk of colon cancer via folate deficiency. *Int J Cancer*. 2000; 86:169–173. [PubMed: 10738242]
18. Singletary KW, Gapstur SM. Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. *JAMA*. 2001; 286:2143–2151. [PubMed: 11694156]
19. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. *Br Med Bull*. 1999; 55:578–592. [PubMed: 10746348]
20. Choi S, Mason J. Folate and carcinogenesis: an integrated scheme. *J Nutr*. 2000; 130:129–132. [PubMed: 10720158]
21. Kim YI. Folate and carcinogenesis: evidence, mechanisms, and implications. *J Nutr Biochem*. 1999; 10:66–88. [PubMed: 15539274]
22. Anderson G, Cummings S, Freedman LS, et al. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials*. 1998; 19:61–109. [PubMed: 9492970]
23. WHI. Women's Health Initiative Semi-Annual Progress Report: March 1 2002 to August 31 2002. 2003
24. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol*. 1999; 9:178–187. [PubMed: 10192650]
25. Commenges D, Letenneur L, Joly P. Re: "Serum trans-ferrin saturation, stroke incidence, and mortality in women and men. The NHANES I Epidemiologic Followup Study". *Am J Epidemiol*. 1997; 146:683–684. [PubMed: 9345125]
26. Korn EL, Graubard BI, Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. *Am J Epidemiol*. 1997; 145:72–80. [PubMed: 8982025]
27. Thiebaut AC, Benichou J. Choice of time-scale in Cox's model analysis of epidemiologic cohort data: a simulation study. *Stat Med*. 2004; 23:3803–3820. [PubMed: 15580597]
28. Chang SC, Ziegler RG, Dunn B, Stolzenberg-Solomon R, Lacey JV Jr, Huang WY, Schatzkin A, Reding D, Hoover RN, Hartge P, et al. Association of energy intake and energy balance with postmenopausal breast cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev*. 2006; 15:334–341. [PubMed: 16492925]
29. Doody MM, Freedman DM, Alexander BH, Hauptmann M, Miller JS, Rao RS, Mabuchi K, Ron E, Sigurdson AJ, Linet MS. Breast cancer incidence in U.S. radiologic technologists. *Cancer*. 2006; 106:2707–2715. [PubMed: 16639729]

30. Harvie M, Howell A, Vierkant RA, Kumar N, Cerhan JR, Kel-emen LE, Folsom AR, Sellers TA. Association of gain and loss of weight before and after menopause with risk of post-menopausal breast cancer in the Iowa women's health study. *Cancer Epidemiol Biomarkers Prev.* 2005; 14:656–661. [PubMed: 15767346]
31. Radimer KL, Ballard-Barbash R, Miller JS, Fay MP, Schatzkin A, Troiano R, Kreger BE, Splansky GL. Weight change and the risk of late-onset breast cancer in the original Framing-ham cohort. *Nutr Cancer.* 2004; 49:7–13. [PubMed: 15456630]
32. Suzuki R, Rylander-Rudqvist T, Ye W, Saji S, Wolk A. Body weight and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status among Swedish women: a prospective cohort study. *Int J Cancer.* 2006; 119:1683–1689. [PubMed: 16646051]
33. Tehard B, Lahmann PH, Riboli E, Clavel-Chapelon F. Anthropometry, breast cancer and menopausal status: use of repeated measurements over 10 years of follow-up-results of the French E3 N women's cohort study. *Int J Cancer.* 2004; 111:264–269. [PubMed: 15197781]
34. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* 1986; 124:17–27. [PubMed: 3521261]
35. Therneau, TM. Grambsch PM: modeling survival data: extending the Cox Model. 1. Springer; New York: 2000.
36. Ellison RC, Zhang Y, McLennan CE, Rothman KJ. Exploring the relation of alcohol consumption to risk of breast cancer. *Am J Epidemiol.* 2001; 154:740–747. [PubMed: 11590087]
37. Sellers TA, Vierkant RA, Cerhan JR, Gapstur SM, Vachon CM, Olson JE, Pankratz VS, Kushi LH, Folsom AR. Interaction of dietary folate intake, alcohol, and risk of hormone receptor-defined breast cancer in a prospective study of postmenopausal women. *Cancer Epidemiol Biomarkers Prev.* 2002; 11:1104–1107. [PubMed: 12376515]
38. Zhang SM, Lee IM, Manson JE, Cook NR, Willett WC, Buring JE. Alcohol consumption and breast cancer risk in the Women's Health Study. *Am J Epidemiol.* 2007; 165:667–676. [PubMed: 17204515]
39. Tjønneland A, Christensen J, Olsen A, Stripp C, Nissen SB, Overvad K, Thomsen BL. Folate intake, alcohol and risk of breast cancer among postmenopausal women in Denmark. *Eur J Clin Nutr.* 2006; 60:280–286. [PubMed: 16234832]
40. Ubbink J. Should all elderly people receive folate supplements? *Drugs Aging.* 1998; 16:415–420. [PubMed: 9883397]
41. Lewis CJ, Crane NT, Wilson DB, Yetley EA. Estimated folate intakes: data updated to reflect food fortification, increased bioavailability, and dietary supplement use. *Am J Clin Nutr.* 1999; 70:198–207. [PubMed: 10426695]
42. Giovannucci E, Stampfer MJ, Colditz GA, Manson JE, Rosner BA, Longnecker MP, Speizer FE, Willett WC. Recall and selection bias in reporting past alcohol consumption among breast cancer cases. *Cancer Causes Control.* 1993; 4:441–448. [PubMed: 8218876]
43. Feunekes GI, Van't Veer P, van Staveren WA, Kok FJ. Alcohol intake assessment: the sober facts. *Am J Epidemiol.* 1999; 150:105–112. [PubMed: 10400547]
44. Stolzenberg-Solomon RZ, Chang SC, Leitzmann MF, Johnson KA, Johnson C, Buys SS, Hoover RN, Ziegler RG. Folate intake, alcohol use, and postmenopausal breast cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Am J Clin Nutr.* 2006; 83:895–904. [PubMed: 16600944]
45. Munger RG, Folsom AR, Kushi LH, Kaye SA, Sellers TA. Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. *Am J Epidemiol.* 1992; 136:192–200. [PubMed: 1415141]
46. Flagg EW, Coates RJ, Calle EE, Potischman N, Thun MJ. Validation of the American Cancer Society Cancer Prevention Study II Nutrition Survey Cohort Food Frequency Questionnaire. *Epidemiology.* 2000; 11:462–468. [PubMed: 10874556]
47. Administration FaD. Food standards: amendment of standards of identity for enriched grain products to require the addition of folic acid, final rule. *Fed Regist.* 1996; 61:8781–8797.
48. Bentley TG, Willett WC, Weinstein MC, Kuntz KM. Population-level changes in folate intake by age, gender, and race/ethnicity after folic acid fortification. *Am J Public Health.* 2006; 96:2040–2047. [PubMed: 17018833]

49. Thygesen LC, Mørch LS, Keiding N, Johansen C, Gronbaek M. Use of baseline and updated information on alcohol intake on risk for breast cancer: importance of latency. *Int J Epidemiol*. 2008; 37:669–677. [PubMed: 18390878]
50. Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE, Willett WC. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Ann Intern Med*. 1998; 129:517–524. [PubMed: 9758570]
51. Helping patients with alcohol problems. (<http://www.niaaa.nih.gov/publications/Practitioner/HelpingPatients.html>)

Table 1

Baseline characteristics of cases and non-cases

	Non cases <i>n</i> = 86,747	Breast cancer cases <i>n</i> = 1,783	<i>P</i> -value
<i>Demographic characteristics</i>			
Age (SD)	63.5 (7.13)	64.3 (7.37)	<i>P</i> <0.0001
Ethnicity (%)			
American Indian or Alaskan	0.3	0.3	<i>P</i> <0.0001
Asian Pacific-Islander	3.0	2.0	
Black or African American	8.2	5.2	
Hispanic/Latino	4.0	2.4	
Non-Hispanic White	83.3	89.4	
Other	1.1	0.6	
Marital status (%)			
Never married	4.6	4.8	<i>P</i> = 0.62
Divorced/separated	15.9	14.5	
Widowed	17.3	17.8	
Presently married	60.5	61.2	
Marriage-like relationship	1.6	1.6	
Income ^{a,b} (mean)	4.2	4.3	<i>P</i> <0.01
Education ^{a,c} (mean)	7.3	7.6	<i>P</i> <0.0001
<i>Risk factors</i>			
Family history (%)	17.6	23.7	<i>P</i> <0.0001
Hormone use (ever) (%)	28.8	38.0	<i>P</i> <0.0001
Years of age at menarche (SD)	12.7 (1.33)	12.6 (1.36)	<i>P</i> <0.05
Years of age at menopause (SD)	48.4 (1.46)	49.6 (1.48)	<i>P</i> <0.0001
Number of breast biopsies (SD)	1.5 (0.71)	1.5 (0.84)	<i>P</i> = 0.11
Number of pregnancies (SD)	3.6 (1.98)	3.4 (1.89)	<i>P</i> <0.01
BMI (SD)	27.2 (5.86)	27.2 (5.93)	<i>P</i> = 0.83
Tobacco ever (%)	48.6	51.3	<i>P</i> <0.05
METS/week ^d (SD)	13.7 (14.4)	13.5 (13.8)	<i>P</i> = 0.60

^aResponses were categorical, not continuous^bIncome based on categorical values; 1 = 10,000, 2 = 10–19,000, 3 = 20–34,999, 4 = 35–49,999, 5 = 50–74,999, 6 = 75–99,999, 7 = 100–149,999, 8 = 150,000 or more^cEducation based on categorical values 1 = no school; 2 = grade school 1–4; 3 = grade school 5–8; 4 = some high school 9–11; 5 = high school or GED; 6 = vocation/training school; 7 = some college or Associates degree; 8 = college or baccalaureate degree; 9 = some post-graduate or professional; 10 = Master's degree; 11 = Doctoral degree^dMETS = “metabolic equivalents”; includes sum of walking and mild, moderate, and strenuous physical activities

Note: SD = standard deviation; BMI = Body Mass Index

Table 2

Adjusted Cox proportional hazards ratios for developing breast cancer (all participants with complete information)^{a,b}

<i>Baseline alcohol and folate intakes N = 86,747</i>		
Alcohol g/d (SD)		
Mean alcohol (g/d) ^b (SD) (Range 0–244.5)	5.6 (11.2)	
Folate µcg/d (SD)		
Dietary	254.2 (108.9)	
Supplemental	202.1 (259.2)	
Total folate	446.0 (289.0)	
<i>Alcohol & folate continuous variables N = 78,746</i>		
Alcohol (g/d)	1.005	1.001, 1.009, <i>P</i> = .009
Folate (µcg/d)	1.000	1.000, 1.001, <i>P</i> = 0.62
<i>Alcohol & folate categorical values</i>		
Alcohol g/d (# cases/total)		
None ^c (561/32,286)	1.00	
Up to 5 g/d (470/23,624)	1.10	0.97, 1.24
5–15 g/d (322/13,386)	1.14	0.99, 1.31
>15 g/d (246/9,450)	1.13	0.96, 1.32
Total Folate (µcg/d) (# cases/total)		
1st quartile (0–227.6) (340/19,686)	1.00	
2nd quartile (227.6–411) (411/19,697)	1.01	0.87, 1.16
3rd quartile (411–642) (428/19,686)	1.05	0.91, 1.22
4th quartile (642+) (420/19,687)	0.97	0.84, 1.12

^aStratified by race/ethnicity, income and education

^bAdjusted for tobacco consumption, BMI, history of breast biopsy, number of pregnancies, ever breast fed, family history, previous combined HRT use, age at menarche, age at menopause, weekly METs)

^cOperationalized as less than 1 drink per month

Test for an interaction between alcohol and folate was NS (*P* = 0.34)

Note: g/d = grams per day; µcg/d = micrograms per day

Table 3Hazard ratios of developing breast cancer by level of alcohol and folate consumption^{a,b,c}

Total folate intake (in mg/d)	Alcohol intake (g/d) (# cases within strata)			
	No alcohol <i>n</i> = 32,097	5 g/d <i>n</i> = 23,313	>5 <15 g/d <i>n</i> = 13,624	15 g/d <i>n</i> = 9,746
Quartile 1 (0–227.6)	1.00 (144)	1.10 (0.95–1.05) (140)	1.16 (0.98–1.02) (145)	1.12 (0.97–1.03) (132)
Quartile 2 (227.6–411.6)	1.00 (0.89–1.13) (81)	1.10 (0.91–1.10) (128)	1.16 (0.96–1.04) (125)	1.07 (0.88–1.14) (136)
Quartile 3 (411.6–642.7)	1.05 (0.91–1.09) (61)	1.14 (0.93–1.07) (93)	1.20 (0.98–1.02) (86)	1.10 (0.90–1.11) (82)
Quartile 4 (642.7–)	0.97 (0.82–1.21) (54)	1.13 (0.91–1.10) (50)	1.19 (0.96–1.05) (72)	1.09 (0.88–1.14) (70)

^a Stratified by race/ethnicity, education and income^b Adjusted for tobacco consumption, BMI, history of breast biopsy, number of pregnancies, ever breast fed, family history, previous combined HRT use, age at menarche, age at menopause, weekly METs^c Numbers add up to more than 78,746 (Table 3) due to inclusion of observations with missing values for co-variables other than alcohol and folate in the current analysis

Note: g/d = grams per day; µcg/d = micrograms per day

Table 4

Summary of cohort studies examining breast cancer, and alcohol & folate

Authors	Cohort & recruitment dates	N cases/ cohort	Years of f/u	Folate measurement	Mean/median	Alcohol measurement	Mean/median
Zhang et al. [12]	Nurse's Health Study Recruitment 1980–1996 Pre and post-menopausal	2,483/88,818	16	<ul style="list-style-type: none"> • Willett FFQ, correlation 0.56 in validation study • Cumulative intake (avg over time) • Self-reported supplement intake • Dietary median 209 µcg/d • Total median 385 µcg/d 	<ul style="list-style-type: none"> • FFQ, correlation 0.90 in validation study • Mean 5.5 g/d Median 4.9 g/d • Stratified by <15 g/d or 15 g/d 		
Rohan et al. [3]	Canadian National Breast Cancer Screening Study Recruitment 1980–1993 Pre and post-menopausal	1,469/56,837	8–12	<ul style="list-style-type: none"> • FFQ developed for the Canadian National Breast Screening Study (NBSS) FFQ • Baseline only • Dietary median 286 µcg/d (no supplement info) 	<ul style="list-style-type: none"> • FFQ • Mean/Median not reported • Stratified by <or 14 g/d (20% of women 14 g/d) 		
Sellers et al. [11, 37]	Iowa Women's Health Study Recruitment 1986 Postmenopausal	1,586/34,000	12	<ul style="list-style-type: none"> • FFQ, Nurse's Health Study, correlation 0.43 in validation study • Baseline only • Dietary median 294 µcg/d • Total median 351 µcg/d 	<ul style="list-style-type: none"> • FFQ, correlation 0.32 in validation study • Median 4.0 g/d • Stratified by 0, 4 g/d, >4 g/d 		
Zhang et al. [13]	Nurse's Health Study Recruitment 1989–1990 Pre and postmenopausal	712/32,826	16	<ul style="list-style-type: none"> • Serum folate baseline corroborated with dietary folate • Baseline only • Mean 6.9 & 7.6 µg/ml 	<ul style="list-style-type: none"> • FFQ • Mean 5.5 g/d, Median 5.2 g/d • Stratified by 15 g/d, 15 g/d 		
Feigelson et al. [14]	American Cancer Society Cancer Prevention Study II Nutrition Cohort Recruitment 1993–1998 Postmenopausal	1,303/66,561	5	<ul style="list-style-type: none"> • Modified Block FFQ, correlation 0.66 in validation study • Baseline only • Dietary mean 253 µcg/d, median = 231 • Total mean 320 µcg/d 	<ul style="list-style-type: none"> • FFQ, correlation 0.77 in validation study • Mean alcohol intake not given but <5 g/d • Stratified by 0, <5 g/d, 5–<10 g/d, 10–15 g/d, >15 g/d 		
WHI [23]	Women's Health Initiative Recruitment 1993–1999 Postmenopausal only	1,783/88,532	5	<ul style="list-style-type: none"> • FFQ for food, separate supplement form • Correlation 0.69 in validation study • Baseline only 	<ul style="list-style-type: none"> • FFQ, correlation 0.88 in validation study • Mean intake 5.6 g/d 		

Authors	Cohort & recruitment dates	N cases/ cohort	Years of f/u	Folate measurement Mean/median	Alcohol measurement Mean/median
				<ul style="list-style-type: none">Dietary mean 254 µg/d, median 238 µg/dSupplement mean 202 µg/d, median 0Total Mean 446 µg/d, total median 411 µg/d	<ul style="list-style-type: none">Stratified by abstainers; 0 4.33 g/d; 4.33–15.0 g/d; >15 g/d (roughly tertiles)
Stolzenberg-Solomon et al. [44]	Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial Cohort (USA) Recruitment 1993–2001 Postmenopausal	691/25,400	5	<ul style="list-style-type: none">FFQ, separate supplement info obtainedBaseline (last 12 mos) onlyTotal median folate 660 µg/d	<ul style="list-style-type: none">FFQMean/median not given, quintilesAlcohol lowest <0.01 g/d, 0.01–0.43 g/d, 0.43–1.39 g/d, >1.39– 7.62 g/d, >7.62 g/d
Baglietto et al. [9]	Melbourne Collaborative study Recruitment 1990–1994 Premenopausal & postmenopausal (40+)	537/17,447	10	<ul style="list-style-type: none">FFQ, based on WillettBaseline onlyNo information on supplement intakeDietary mean 330 µg/d	<ul style="list-style-type: none">Structured interviewNo mean or median intake givenAbstainers, ex-drinkers, 1–19 g/d (light), 20–39 g/d (mod), and 40 g/d (heavy)
Zhang et al. [38]	Women's Health Study (ASA/Vit E prevention of CAD) Recruitment 1992–1995 -Premenopausal & postmenopausal (45+)	1,484/38,454	10	<ul style="list-style-type: none">FFQ, based on Nurses Health StudyBaseline only (previous 12 months)Dietary median 228 µg/d, supplement 210 µg/d	<ul style="list-style-type: none">FFQ, (previous 12 months)Stratified by abstainers 37%, 0.1–4.9 g/d 38%, 5–10 g/d 11%, 10–14 g/d 6%, 15–30 g/d 5%, 30 g/d 2%
Tjønneland et al. [39]	EPIC—European prospective investigation into cancer and nutrition -Premenopausal & postmenopausal (35–75) Recruitment 1993–2004	4,285/274,688	6.4	<ul style="list-style-type: none">FFQ unique to each country, correlations 0.5–0.7 in validations studiesBaseline onlyDietary mean *300 µg/dNo supplement data	<ul style="list-style-type: none">FFQ, correlation 0.79 in validation studyMean alcohol 5.71 g/dAsked about intake in 20, 30, 40, 50 sStratified by 0 g/d (17.1%), >0–1.5 g/d (18.6), >1.5–4.7 g/d (18.6), >4.7–10 g/d (17.7%), >10–19 g/d (14.9%), >19 g/d (13.2%)
Author	Controlled for	Interaction between ETOH & folate	Results	Comments	
Zhang et al. [12]	Age, length of f/u, total energy intake, parity, age at first birth, menarche, menopause, FH, benign breast dx, ETOH intake, BMI at 18, weight change from age 18, height, HT	Yes $P = 0.02$	<ul style="list-style-type: none">RR = 1.32 (1.15–1.50) in women with low (<300 µg/d) total folate and high ETOH (15 g/d) versus high folate.(300 µg/d) low ETOH (<15 g/d)Folate from foods alone not correlated	<ul style="list-style-type: none">Highest quintile folate intake were supplement users;First 3 grps mostly dietary intakeFindings independent of beta-carotene, vit A, C, E-Stratified by methionine as well	

Author	Controlled for	Interaction between ETOH & folate	Results	Comments
Rohan et al. [3]	Total energy intake, age, age at menarche, menopausal status, # births, FH, breast self-exam, ETOH intake, study group and randomization	NS	<ul style="list-style-type: none"> Findings same for pre- and postmenopausal women 	<ul style="list-style-type: none"> Checked correlation of folate with serum erythrocytes folate concentrations Predominantly white (97%)
Sellers et al. [11, 37]	Age, education, FH, age at menarche, menopause, first birth, OCP, HT, parity, BMI, waist-to-hip, height BMI at 18, tobacco, physical activity, other B vitamins	NS	<ul style="list-style-type: none"> IRR = 0.34 (0.18–0.61) in highest quintile of dietary folate intake (>354 µg/d) versus lowest (225 µg/d) among women consuming 14 g/d ETOH RR = 1.45 (0.95, 2.23) in women who consume >4.0 g/d ETOH and in lowest 10% intake folate, versus abstainers with above median folate Repeated analysis with 15 g/d cut point similar (though NS) 	<ul style="list-style-type: none"> 2001 All white Low folate intake alone was associated with increased risk of breast cancer (lowest 10th versus 50th or above) Low alcohol intake No info on supplements
Zhang et al. [13]	Age at menarche, menopause, and first birth, parity, FH, benign breast disease, ETOH intake, BMI at 18, BMI, duration of HT	NS	<ul style="list-style-type: none"> No relationship with ER+ tumors; only ER- tumors RR = 2.14 in women with ER- tumors with low folate (10%) and above median (4 g/d) for alcohol versus above median folate (351 µg/d) and alcohol Looked at diet only versus diet & supplements and results similar 	<ul style="list-style-type: none"> 2003 All white Specifically looking at folate, ETOH and ER status Low alcohol intake No info on supplements
Feigelson et al. [14]	ETOH, dietary folate, methionine, MVI, race, education, FH, h/o lump, mammographic history, HT use, parity, age at first birth, menopause, physical activity, BMI, adult weight gain, and energy	NS	<ul style="list-style-type: none"> RR = 1.26 (1.04, 1.13) in women with high ETOH intake (15 g/d), compared to non-users 	<ul style="list-style-type: none"> Inverse relationship between plasma folate and breast cancer risk was particularly strong in women consuming at least 15 g/d ETOH Findings similar among pre and postmenopausal women Predominantly white Short follow-up 97% white

Author	Controlled for	Interaction between ETOH & folate	Results	Comments
WHI [23]	Adjusted for age, HT, FH, age of menarche, menopause, # mammograms, # breast bx, # pregnancies, breastfed, weekly physical activity, BMI, tobacco	NS	<ul style="list-style-type: none"> Dietary folate, total folate, did not modulate alcohol and breast cancer risk relationship Found increasing breast cancer risk with increasing ETOH consumption HR = 1.005 (1.001, 1.009) for every g/d ETOH ($P < .05$) Folate did not modulate alcohol & breast cancer risk relationship 	<ul style="list-style-type: none"> Checked specifically for interaction between ETOH and MVI users More ethnically diverse (84% white) Short follow-up
Stolzenberg-Solomon et al. [44]	<ul style="list-style-type: none"> Adjusted for energy, education, HT, mammogram screening, OCP, benign breast disease, FH, age at menarche, age at menopause, age at first birth, # of live births 	Yes, total folate $P = 0.05$	<ul style="list-style-type: none"> HR = 2.10 (1.08, 4.07) in women consuming >7.6 g/d ETOH versus abstainers, among women in lowest quintile of total folate intake ($< 335.5 \mu\text{g/d}$) RR = 1.19 (1.01, 1.41) in high supplement users ($< 400 \mu\text{g/d}$) versus no supplements Folate from foods, and total folate, was not associated with breast cancer risk 	<ul style="list-style-type: none"> Examined effect of fortification by calendar yr & breast cancer risk, and by post-fortification foods, total folate & breast cancer risk, no effect Much higher total folate intake than previous studies More ethnically diverse, 86% white Short follow-up
Baglietto et al. [9]	<ul style="list-style-type: none"> Total energy intake, no other variables were significant & not adjusted for 	Yes, $P = 0.04$	<ul style="list-style-type: none"> HR = 2.00 (1.14–3.49) in high alcohol ($> 40 \text{ g/d}$) consumers versus abstainers among women with low dietary folate ($< 200 \mu\text{g/d}$) 	<ul style="list-style-type: none"> Nothing on supplemental folate, but use of MVI supplements did not change HR All white
Zhang et al. [38]	Adjusted for age, randomized tx assignment, age at menarche, age at first pregnancy, # of pregnancies, menopausal status, age at menopause, HT, BMI, FH, benign breast dx, physical activity, MVI use, total energy intake	NS $P = 0.96$	<ul style="list-style-type: none"> RR = 1.43 (1.02–2.02) for high alcohol ($> 30 \text{ g/d}$) versus abstainers Folate did not affect increased risk of breast cancer with alcohol consumption 	<ul style="list-style-type: none"> Breast cancer not a not a primary outcome Mostly white 95% Focus of analysis on ER/PR status
Tjønneland et al. [39]	Adjusted for age at menarche, parity, OCP, HT, menopausal status, tobacco, education, height and weight	NS $P = 0.59$	<ul style="list-style-type: none"> IRR = 1.03 (1.01–1.05) for every 10 g/d ETOH Folate did not affect the increased risk of breast cancer with alcohol consumption 	<ul style="list-style-type: none"> No relationship seen for previous alcohol intake (as measured in 20, 30 s, 40 s) European, mostly Caucasian

FH = family history, HT = hormonal therapy, OCP = oral contraceptive pills, BMI = body mass index, MVI = multi-vitamins, RR = Relative risk, IRR = incident relative risk, HR = hazard ratio IRR = incidence rate ratio