Short Communication

Interaction of Dietary Folate Intake, Alcohol, and Risk of Hormone Receptor-defined Breast Cancer in a Prospective Study of Postmenopausal Women[1]

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Abstract

Alcohol intake is an established risk factor for breast cancer, but the underlying mechanism remains unknown. Four recent studies have described interactions of alcohol and low folate intake. We examined this interaction on the risk of postmenopausal breast cancer stratified by tumor receptor status for estrogen (ER) and progesterone (PR). The Iowa Women's Health Study is a prospective cohort study of 34,393 at-risk women. Alcohol use and folate intake from diet and supplements were estimated at baseline in 1986 through a semiquantitative food frequency questionnaire. Through 1999, 1,875 cases of breast cancer were identified through linkage to the Iowa Surveillance, Epidemiology, and End Results registry. Compared with nondrinkers with folate intakes above the 50th percentile, women with low folate and high alcohol were at 1.43-fold greater risk (1.02–2.02). When stratified by tumor receptor status for ER or PR, the risks for low folate/high alcohol were 2.1 (1.18–3.85), 1.0 (0.76–1.42), 1.2 (0.88–1.70), and 1.2 (0.69–2.02) for ER−, ER+, PR+, and PR− tumors, respectively. Because the results were limited primarily to ER− tumors, one plausible interpretation of these data is that alcohol influences breast cancer through its metabolite, acetaldehyde, rather than through effects on ER levels and receptor-mediated pathways.

Introduction

There is consistent evidence that alcohol intake increases risk of breast cancer (1). A recent pooled analysis of >300,000 women and 4,335 cases suggests that intake of 2–5 drinks/day increases risk by roughly 40% (2). The underlying mechanisms through which this occurs are not firmly established (3) but may include an influence on circulating levels of estrogens (4), immune function, enhanced permeability of chemical carcinogens, decreased absorption of essential nutrients (5), or through metabolism of alcohol to acetaldehyde, a known carcinogen (6).

There are now four large epidemiological studies that suggest that risks associated with intake of alcohol may be greater in women with low intakes of dietary folate. The original report, from the Nurses Health Study (7), was quickly replicated with analyses of the Canadian National Breast Cancer Screening Trial (8), a case-control study from Italy (9), and the Iowa Women's Health Study (10). The consistency of this interaction lends credence to the possibility that the results are real and not merely a chance observation.

The current report describes an updated analysis of the Iowa Women's Health Study. We reported previously that alcohol is a risk factor for breast cancer (11), primarily for tumors lacking receptors for ER[3] and PR (12), and corroborated the interaction between low folate intake and alcohol consumption (10). The current analyses bring together these previous observations to examine the interaction within strata defined by tumor receptors for ER and PR.

Materials and Methods

Definition of Cohort. Detailed methods of the Iowa Women's Health Study have been published elsewhere (13). Briefly, the cohort represents 41,836 licensed drivers ages 55–69 years who responded to a mailed survey.

Diet and Risk Factor Assessment. The questionnaire solicited information on factors known or suspected to be relevant to breast cancer risk, including alcohol use, and a 127-item semiquantitative food frequency questionnaire that included use of multivitamins and supplements. Average daily alcohol intake over the previous year was assessed by summing the products of frequency of use of specific beverages by their ethanol content. The reliability and accuracy of the instrument is good (14).

Exclusion Criteria. We excluded women at baseline if they were not postmenopausal ($n = 569$), had a total or partial mastectomy ($n = 1,870$), or had any cancer other than skin cancer ($n = 2,293$). We also excluded women if ≥ 30 items on the food frequency questionnaire were left blank or if their responses resulted in extreme energy intake values (<600 or $\geq 5,000$ kcal/day; $n = 2,712$). These exclusions left a total of 34,393 women eligible for follow-up.

[3] The abbreviations used are: ER, estrogen; PR, progesterone; RR, risk ratio; CI, confidence interval.

Table 1   Association of alcohol and folate with risk of hormone receptor-specific breast cancer[a]

Daily intake[b]	ER+ RR (95% CI)	ER− RR (95% CI)	Missing ER RR (95% CI)	PR+ RR (95% CI)	PR− RR (95% CI)	Missing PR RR (95% CI)
Folate with supplements (μg)						
$\leq$ 186	1.04 (0.82–1.31)	1.14 (0.66–1.95)	1.60 (1.11–2.29)	1.10 (0.84–1.42)	1.04 (0.68–1.59)	1.41 (1.00–1.99)
187–270	1.07 (0.91–1.26)	1.37 (0.96–1.96)	1.22 (0.94–1.59)	1.14 (0.96–1.37)	1.11 (0.83–1.50)	1.16 (0.90–1.48)
271–351	0.89 (0.76–1.06)	0.80 (0.53–1.20)	1.02 (0.79–1.32)	0.88 (0.73–1.06)	0.87 (0.64–1.18)	1.00 (0.79–1.27)
> 351	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Folate without supplements (μg)						
$\leq$172	1.06 (0.82–1.36)	1.21 (0.69–2.13)	1.70 (1.16–2.50)	1.14 (0.86–1.51)	0.98 (0.62–1.54)	1.55 (1.08–2.21)
173–239	1.02 (0.85–1.23)	1.05 (0.69–1.60)	1.02 (0.76–1.38)	1.08 (0.88–1.32)	0.99 (0.72–1.38)	0.95 (0.72–1.26)
240–294	0.92 (0.78–1.10)	0.99 (0.67–1.46)	1.10 (0.85–1.44)	1.02 (0.84–1.22)	0.75 (0.54–1.04)	1.06 (0.83–1.35)
> 294	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Alcohol (g)						
0	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
$\leq$4	1.06 (0.91–1.22)	1.40 (1.00–1.96)	0.96 (0.76–1.22)	1.04 (0.89–1.23)	1.24 (0.95–1.62)	1.01 (0.81–1.26)
> 4	1.07 (0.90–1.26)	1.64 (1.14–2.35)	1.02 (0.79–1.32)	1.12 (0.93–1.34)	1.28 (0.96–1.71)	1.00 (0.79–1.28)

[a] All models account for age, caloric intake, education, family history of breast cancer, age at menarche, age at menopause, oral contraceptive use, hormone replacement therapy, parity, age at first birth, body mass index, waist-to-hip ratio, height, weight, body mass index at age 18, smoking status, physical activity, and methionine.
[b] Folate categories reflect lowest 10th, 11th–30th, 31st–50th, and upper 50th (ref) percentiles. Alcohol categories are based on a median split among drinkers.

Follow-Up. We mailed questionnaires in 1987, 1989, 1992, and 1997 to establish vital status and change of address. We ascertained cancer incidence and ER/PR status through annual record linkage to the Iowa Cancer Registry. We ascertained deaths in Iowa through the Cancer Registry supplemented through linkage with the National Death Index.

Through 14 years of follow-up, we identified 1875 cases of breast cancer (1,633 invasive and 242 in situ) among the cohort at risk. Of these, 1191 (63.5%) were ER+, 225 (12.0%) were ER−, 989 (52.8%) were PR+, and 353 (18.8%) were PR−. A total of 459 tumors (24.5%) were missing ER receptor status, 533 (28.4%) were missing PR receptor status, and 457 (24.4%) were missing both.

Analytic Approach. We calculated the length of follow-up for each individual from baseline until the date of breast cancer diagnosis, estimated date of move from Iowa, date of death, or December 31, 1999.

We calculated RRs and 95% CIs using Cox proportional hazards regression, with survival modeled as a function of age (15). To isolate those at the extreme of low folate intake, we defined four percentile categories: >50th [referent], 31st-50th, 11th-30th, and $\leq$10th. We categorized use of alcohol into three levels: never drinkers, $\leq$4 grams/day, >4 grams/day. The cut-point among drinkers was approximately the median of use. Models considered dietary folate alone and folate including supplements. Two sets of regression models were fit to the data: one adjusting only for age and total caloric intake, and one adjusting for additional potential confounding variables.

Results

A cross-tabulation of folate intakes (with and without supplements) with reported alcohol consumption confirmed that the two exposures were independent. Table 1 presents multivariate-adjusted RRs for folate and alcohol by tumor hormone receptor status. Low folate intake from diet alone is associated primarily with breast cancers with missing data on ER or PR. Note that most of these 459 cases were either in situ (41.2%) or localized (42.5%). When folate from supplements is considered, the same general pattern remains. The possible exception is the pattern with ER− tumors in which the graded dose-response trend disappears. Conversely, use of alcohol is associated primarily with ER− and PR− tumors.

Table 2 presents the joint effects of alcohol and folate (including supplements) by receptor status. A formal test for interaction between alcohol and total folate was not statistically significant ($P = 0.49$). To improve the stability of the risk estimates, the total folate intake categories were decreased from four to three (50:25:25 percentile split). Alcohol use was not associated with risk of any receptor-defined category of breast cancer if folate intake was above the median. Similarly, at all levels of folate and alcohol there was no appreciable association with risk of ER+ tumors. Rather, the largest RR was observed for ER− tumors among women with low total folate and alcohol above the median (RR = 2.14; 95% CI, 1.18–3.85). Notably, the risks for ER− tumors were also elevated when alcohol intake was above the median and folate intake was between the 25th and 50th percentiles.

Because methionine intake may play a role similar to folate in methylation pathways, we also examined the joint effects of alcohol and methionine. Overall, low methionine and high alcohol intakes were associated with a RR of 1.3 (0.90–1.92). Because this was weaker than the alcohol × folate joint effect, no additional analyses were performed.

Discussion

Epidemiological studies of folate and breast cancer have yielded inconsistent results (16, 17). A clearer picture began to emerge when studies examined the joint effects of folate and alcohol (7–10). The current report provides evidence that this interaction may apply primarily to tumors lacking the ER receptor. Because this is the first report to examine the inter-action by hormone receptor status, caution is warranted until this observation can be replicated, as it may reflect a chance finding.

There are at least two possible mechanisms that could account for the apparent joint effects of alcohol and folate on risk of ER− breast cancers. The first mechanism takes into consideration the direct carcinogenic effect of the primary metabolite of alcohol, acetaldehyde. Recent experimental evidence suggests that non-neoplastic human mammary epithelial cells treated with alcohol at physiologically relevant concentrations have increased rates of benzo(a)pyrene-DNA adduct formation (18). Rates of DNA adduct formation were even greater when cells were exposed to alcohol before administra-

Table 2 Joint association of alcohol and folate (including supplements) with risk of hormone receptor-specific postmenopausal breast cancer[a]

Daily intakes		ER+		ER−		PR+		PR−	
Folate (μg)[b]	Alcohol (g)[c]	n[d]	RR (95% CI)	n[d]	RR (95% CI)	n[d]	RR (95% CI)	n[d]	RR (95% CI)
>351	0	329	1.00 (ref)	50	1.00 (ref)	270	1.00 (ref)	88	1.00 (ref)
	≤4	150	0.97 (0.79–1.19)	33	1.32 (0.83–2.11)	123	0.99 (0.79–1.25)	48	1.04 (0.72–1.52)
	>4	121	1.01 (0.80–1.26)	23	1.11 (0.64–1.93)	98	1.02 (0.80–1.32)	39	1.05 (0.70–1.58)
252–351	0	152	0.86 (0.70–1.06)	18	0.65 (0.36–1.14)	128	0.88 (0.70–1.10)	30	0.62 (0.40–0.97)
	≤4	79	1.02 (0.78–1.32)	14	1.12 (0.60–2.09)	67	1.04 (0.78–1.38)	21	1.02 (0.63–1.66)
	>4	64	1.04 (0.78–1.39)	19	1.76 (0.98–3.15)	54	1.14 (0.81–1.53)	26	1.37 (0.85–2.20)
≤251	0	172	0.99 (0.80–1.22)	33	1.16 (0.71–1.90)	147	1.05 (0.83–1.32)	54	1.06 (0.72–1.55)
	≤4	68	1.13 (0.85–1.50)	16	1.54 (0.83–2.87)	49	1.07 (0.77–1.48)	28	1.48 (0.93–2.36)
	>4	56	1.04 (0.76–1.42)	19	2.14 (1.18–3.85)	53	1.22 (0.88–1.70)	19	1.18 (0.69–2.02)

[a] All models account for age, caloric intake, education, family history of breast cancer, age at menarche, age at menopause, oral contraceptive use, hormone replacement therapy, parity, age at first birth, body mass index, waist-to-hip ratio, height, weight, body mass index at age 18, smoking status, physical activity, and methionine.
[b] Categories are based on 50/25/25 split of folate intake in the entire cohort at risk.
[c] Alcohol categories are based on a median split among drinkers.
[d] n indicates number of breast cancer events.

tion of acetaldehyde. This type of genetic damage is independent of receptor-mediated events, and is, therefore, a plausible explanation for an association of alcohol with ER− breast cancers. Because folate is critical to certain DNA repair pathways (19), it should not be surprising that the association of alcohol with ER− tumors was stronger among women with low intakes of folate.

The second mechanism is based on the observation that folate plays a key role in DNA methylation (20). Because DNA methylation is a key regulator of gene transcription (21), low folate intake could contribute to overall methyl deficiency and reduced global DNA methylation (22). In addition, the primary metabolite of alcohol, acetaldehyde, has been shown to inhibit methionine synthase activity *in vitro* (23). In animal models, methyl deficiency results in increased methyltransferase activity and regional hypermethylation (*e.g.,* promotor regions; Ref. 24). In breast cancer cells, hypermethylation of the promotor region of the ER gene is associated with reduced expression (25) and, *in vitro*, demethylation reverses this inhibition (26).

Limitations of the current study include imprecision in diet assessment and the reliance on only a single assessment at baseline. If study participants adopted significant changes in dietary habits during the course of follow-up, then substantial misclassification may have occurred. However, the likely effect of such misclassification would be to make it more difficult to detect associations. Our ability to generalize to other populations may be somewhat compromised given that our cohort is a predominantly white and consumption of alcohol is low. Data on hormone receptor status were not obtained using a common protocol; some differences in the accuracy of receptor status are possible. However, it is difficult to imagine how accuracy would systematically vary with dietary intakes of folate or alcohol, which would be unknown to the technicians measuring hormone receptors.

A more serious potential limitation is the absence of tumor receptor status on roughly a fifth of the cohort. Most of the tumors missing data on receptor status were either *in situ* (41.2%) or localized (42.5%). Determination of ER status is not performed on *in situ* tumors, and it is reasonable to speculate that the women with localized disease did not have receptor status determined on their tumors, either. These tumors are more likely to be ER+ than tumors without missing receptor status. Because the greatest association of alcohol and low folate on risk appears to be evident for ER− tumors, this would not be expected to be a major source of bias. In fact, we

performed a sensitivity analysis in which all of the cases with missing ER status were combined with ER+ tumors. The results were not materially changed.

Strengths of the current study include the large numbers of cases, the completeness of follow-up through the statewide registry, assessment of diet before diagnosis of breast cancer, extensive data on vitamin supplements, and the ability to adjust for multiple potential confounding factors.

In summary, the current study suggests that the apparent interaction between alcohol and folate is related primarily to tumors that lack receptors for ER and PR, an observation that should be examined by other investigators. These observations may help inform the underlying mechanism through which consumption of alcohol influences risk of postmenopausal breast cancer.

References

1. Longnecker M. P. Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. Cancer Causes Control, *5:* 73–82, 1994.

2. Smith-Warner S. A., Spiegelman, D., Yaun, S. S., van den Brandt, P. A., Folsom, A. R., Goldbohm, A., Graham, S., Holmberg, L., Howe, G. R., Marshall, J. R., Miller, A. B., Potter, J. D., Speizer, F. E., Willett, W. C., Wolk, A., and Hunter, D. J. Alcohol and breast cancer in women: a pooled analysis of cohort studies. JAMA, *279:* 535–540, 1998.

3. Singletary K. W., and Gapstur, S. M. Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. JAMA, *286:* 2143–2151, 2001.

4. Purohit V. Moderate alcohol consumption and estrogen levels in postmenopausal women: a review. Alcohol Clin. Exp. Res., *22:* 994–997, 1998.

5. Thomas D. B. Alcohol as a cause of cancer. Environ. Health Perspect., *103* (Suppl. 8)*:* 153–160, 1995.

6. Feron V. J., Til, H. P., de Vrijer, F., Woutersen, R. A., Cassee, F. R., and van Bladeren, P. J. Aldehydes: occurrence, carcinogenic potential, mechanism of action and risk assessment. Mutat. Res., *259:* 363–385, 1991.

7. Zhang S., Hunter, D. J., Hankinson, S. E., Giovannucci, E. L., Rosner, B. A., Colditz, G. A., Speizer, F. E., and Willett, W. C. A prospective study of folate intake and the risk of breast cancer. JAMA, *281:* 1632–1637, 1999.

8. Rohan T. E., Jain, M. G., Howe, G. R., and Miller, A. B. Dietary folate consumption and breast cancer risk. J. Natl. Cancer Inst., *92:* 266–269, 2000.

9. Negri E., La Vecchia, C., and Franceschi, S. Dietary folate consumption and breast cancer risk. J. Natl. Cancer Inst., *92:* 1270–1271, 2000.

10. Sellers T. A., Kushi, L. H., Cerhan, J. R., Vierkant, R. A., Gapstur, S. M., Vachon, C. M., Olson, J. E., Therneau, T. M., and Folsom, A. R. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. Epidemiology, *12:* 420–428, 2001.

11. Gapstur S. M., Potter, J. D., Sellers, T. A., and Folsom, A. R. Increased risk of breast cancer with alcohol consumption in postmenopausal women. Am. J. Epidemiol., *136:* 1221–1231, 1992.

12. Potter J. D., Cerhan, J. R., Sellers, T. A., McGovern, P. G., Drinkard, C., Kushi, L. R., and Folsom, A. R. Progesterone and estrogen receptors and mammary neoplasia in the Iowa Women's Health Study: how many kinds of breast cancer are there? Cancer Epidemiol. Biomark. Prev., *4:* 319–326, 1995.

13. Bisgard K. M., Folsom, A. R., Hong, C. P., and Sellers, T. A. Mortality and cancer rates in nonrespondents to a prospective study of older women: 5-year follow-up. Am. J. Epidemiol., *139:* 990–1000, 1994.

14. Munger R. G., Folsom, A. R., Kushi, L. H., Kaye, S. A., and Sellers, T. A. 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., *136:* 192–200, 1992.

15. Korn E. L., Graubard, B. I., and Midthune, D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. Am. J. Epidemiol., *145:* 72–80, 1997.

16. Graham S., Hellmann, R., Marshall, J., Freudenheim, J., Vena, J., Swanson, M., Zielezny, M., Nemoto, T., Stubbe, N., and Raimondo, T. Nutritional epidemiology of postmenopausal breast cancer in western New York. Am. J. Epidemiol., *134:* 552–566, 1991.

17. Wu K., Helzlsouer, K. J., Comstock, G. W., Hoffman, S. C., Nadeau, M. R., and Selhub, J. A prospective study on folate. B12, and pyridoxal 5'-phosphate (B6) and breast cancer. Cancer Epidemiol. Biomark. Prev., *8:* 209–217, 1999.

18. Barnes S. L., Singletary, K. W., and Frey, R. Ethanol and acetaldehyde enhance benzo[a]pyrene-DNA adduct formation in human mammary epithelial cells. Carcinogenesis (Lond.), *21:* 2123–2128, 2000.

19. Ames B. N. Micronutrients prevent cancer and delay aging. Toxicol. Lett. (Amst.), *102–103:* 5–18, 1998.

20. Herbert V. The role of vitamin B12 and folate in carcinogenesis. Adv. Exp. Med. Biol., *206:* 293–311, 1986.

21. Bergman Y., and Mostoslavsky, R. DNA demethylation: turning genes on. Biol. Chem., *379:* 401–407, 1998.

22. Jacob R. A., Gretz, D. M., Taylor, P. C., James, S. J., Pogribny, I. P., Miller, B. J., Henning, S. M., and Swendseid, M. E. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. J. Nutr., *128:* 1204–1212, 1998.

23. Kenyon S. H., Nicolaou, A., and Gibbons, W. A. The effect of ethanol and its metabolites upon methionine synthase activity *in vitro*. Alcohol, *15:* 305–309, 1998.

24. Pogribny I. P., Poirier, L. A., and James, S. J. Differential sensitivity to loss of cytosine methyl groups within the hepatic p53 gene of folate/methyl deficient rats. Carcinogenesis (Lond.), *16:* 2863–2867, 1995.

25. Ottaviano Y. L., Issa, J. P., Parl, F. F., Smith, H. S., Baylin, S. B., and Davidson, N. E. Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells. Cancer Res., *54:* 2552–2555, 1994.

26. Ferguson A. T., Lapidus, R. G., Baylin, S. B., and Davidson, N. E. Demethylation of the estrogen receptor gene in estrogen receptor-negative breast cancer cells can reactivate estrogen receptor gene expression. Cancer Res., *55:* 2279–2283, 1995.

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