

Papers

Does dietary folate intake modify effect of alcohol consumption on breast cancer risk? Prospective cohort study

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Abstract

Objective To evaluate the effect of dietary folate intake on the relation between alcohol consumption and breast cancer risk.

Design Prospective cohort study.

Setting Melbourne, Australia.

Participants 17 447 Anglo-Australian women resident in Melbourne, aged 40-69 years at recruitment in 1990-4, and followed up until 31 December 2003.

Main outcome measure Invasive breast cancers diagnosed during follow-up and ascertained through the Victorian cancer registry.

Results 537 invasive breast cancers were diagnosed. Compared with lifetime abstainers, the hazard ratio for breast cancer in women who consumed an average of 40 g or more of alcohol daily at baseline was 1.41 (95% confidence interval 0.90 to 2.23). No direct association was found between dietary folate intake and risk of breast cancer, but a high folate intake mitigated the excess risk associated with alcohol. The estimated hazard ratio of an alcohol consumption of 40 g/day or more was 2.00 (1.14 to 3.49) for women with intakes of 200 µg/day of folate and 0.77 (0.33 to 1.80) for 400 µg/day of folate ($P=0.04$ for interaction between alcohol and folate).

Conclusions An adequate dietary intake of folate might protect against the increased risk of breast cancer associated with alcohol consumption.

Introduction

Alcohol consumption is a known risk factor for breast cancer. Although the strength of the association is modest, its adverse effect on breast cancer is one of the most consistent findings among the many hypothesised dietary risk factors.¹ Pooled analyses of both case-control studies and cohort studies confirm a linear dose-response relation between alcohol consumption and risk of breast cancer.²⁻³ However, the mechanisms involved in alcohol associated carcinogenesis are still unknown. Ethanol itself is not considered a carcinogen, but increasing evidence suggests that its metabolite acetaldehyde is responsible for the co-carcinogenic effects of alcohol consumption.⁴

Folate is a B vitamin necessary for the production of red blood cells and the synthesis and normal methylation of DNA.⁵ The role of folate in colorectal carcinogenesis has been widely studied, and an inverse dose dependent relation has been found⁶⁻⁸; studies also fairly consistently show that people with high alcohol intake and low folate intake are at higher risk of colorectal neoplasia compared with those with low alcohol and high folate intake.⁹ Some studies have also reported an inverse association between folate from diet or dietary supplements and

risk of breast cancer¹⁰⁻¹³; the protective effect of folate on breast cancer is more pronounced for heavy drinkers.¹⁴⁻¹⁸ These findings suggest that folate and alcohol act in opposite directions in breast carcinogenesis and may interact with each other. To test this hypothesis, we used the Melbourne collaborative cohort study to investigate if the association between alcohol consumption and risk of breast cancer is modified by intake of dietary folate.

Methods

Participants

The Melbourne collaborative cohort study is a prospective cohort study of 41 528 people (24 479 women) aged between 27 and 75 years at baseline (99.3% aged 40-69). Recruitment took place between 1990 and 1994 in the Melbourne metropolitan area. Participants were recruited through the electoral rolls (registration to vote is compulsory for adults in Australia), advertisements, and community announcements in local media (such as television, radio, newspapers). Participants gave written consent and permission for the investigators to obtain access to their medical records.

We excluded women with a confirmed diagnosis of invasive breast cancer before baseline ($n=381$) and women who reported a diagnosis of angina, heart attack, or diabetes at baseline ($n=1461$), because their diets were not representative of the whole cohort and we could not rule out the possibility that they had changed their diet in response to a recent diagnosis. Other exclusions included women with missing data on alcohol intake or food items and extreme values for self reported total energy intake (<1 st centile and >99 th centile) ($n=439$). Of the 22 198 women left, 4751 (21%) were born in Greece or Italy. These women had a lower incidence of breast cancer than other women; few of them consumed alcohol (61% were lifetime abstainers compared with 31% of other women), and those that did had low levels of consumption (4% consumed at least 20 g/day compared with 13% of other women). Thus, they would have added little information about the association between alcohol consumption and risk of breast cancer. To avoid confounding by country of birth, we restricted the analyses to the 17 447 women born in Australia, New Zealand, or the United Kingdom.

Assessment of alcohol consumption and diet

At baseline we used a structured interview schedule to obtain information on potential risk factors, including age, sex, country of birth, education, reproductive history, and alcohol consumption. We asked participants a series of questions about their intake of alcoholic drinks. People who had never consumed at

Table 1 Baseline characteristics of women in the Melbourne collaborative cohort study. Values are numbers (percentages) unless stated otherwise

Alcohol consumption	Folic acid ($\mu\text{g/day}$)				Total
	Mean (SD)	<270*	270-360*	>360*	
Abstainers†	333 (131)	1827 (32)	1733 (30)	1911 (32)	5471 (31)
Ex-drinkers‡	333 (133)	189 (3)	165 (3)	198 (3)	552 (3)
1-19 g/day	330 (123)	2918 (51)	3067 (53)	3121 (53)	9106 (52)
20-39 g/day	324 (110)	602 (10)	610 (11)	568 (10)	1780 (10)
≥ 40 g/day	302 (105)	222 (4)	182 (3)	134 (2)	538 (3)

*Thirds of distribution of folate intake.

†Women who never drank at least 12 alcoholic drinks in a year.

‡Women who drank at least 12 alcoholic drinks in a year, but did not drink at baseline.

least 12 alcoholic drinks in a year were considered lifetime abstainers. We asked non-lifetime abstainers at baseline about their current average quantity and frequency of intake of beer, wine, and spirits. We then asked them about the intake of alcoholic beverages on each day during the week before the interview (diary). Participants who were not lifetime abstainers but did not consume alcohol at baseline were classified as ex-drinkers. We used Australian food composition tables to calculate alcohol consumption from alcoholic beverages.¹⁹ We categorised total alcohol consumption into lifetime abstainers, ex-drinkers, and current drinkers with low intake (1-19 g/day), medium intake (20-39 g/day), or high intake (40 g/day or more), according to guidelines of the Australian National Health and Medical Research Council.²⁰

Participants completed a dietary questionnaire that included a 121 item food frequency questionnaire without portion sizes, specifically developed for the Melbourne collaborative cohort study.²¹ The questionnaire had 26 items on intake of vegetables, including fresh and cooked vegetables. We calculated nutrient intakes by using mean sex specific portion sizes from weighed food records. We used British data to calculate intake of folate.²² We calculated intake of energy from Australian food composition tables.¹⁹ Total energy intake included energy from the food frequency questionnaire and energy from alcohol. We calculated body mass index from measured height and weight.

Cohort follow-up and ascertainment of invasive breast cancer cases

Cases were women notified to the Victorian cancer registry with a first diagnosis of invasive breast cancer during follow-up to 31 December 2003. We did not count women with in situ breast cancer as cases.

We determined addresses and vital status of all participants by record linkage to electoral rolls, Victorian death records, and the national death index; from electronic phone books; and from responses to mailed questionnaires and newsletters. By the end of follow-up on 31 December 2003, 351 (2%) of the women included in this analysis were known to have left Victoria and 627 (4%) had died.

Statistical analysis

Follow-up began at baseline and continued until diagnosis of breast cancer, death, date of leaving Victoria, or 31 December 2003, whichever came first. We estimated hazard ratios by using Cox regression with age as the time metric. For both alcohol consumption and folate intake, we compared linear and more complex polynomial relations with the log hazard rate using fractional polynomials.²³ Fractional polynomials are a method of analysing dose-response curves that make no a priori assumption about the shape of the curves. We also fitted a model in which alcohol intake was categorised according to the National Health and Medical Research Council categories.²⁰ We studied the interaction between alcohol consumption and

dietary folate intake. We compared non-nested models with the Akaike information criterion.²⁴

We adjusted all analyses for total energy intake. Other potential confounders examined included education, body mass index, age at menarche, hormone replacement therapy, parity, and use of multivitamins. We did not include any of these variables in the final analyses, as their inclusion changed the estimated hazard ratios for the association with alcohol consumption by less than 5%. We used S-PLUS 6.2 (Insightful Corporation, Seattle, WA) and Stata 8.2 (Stata Corporation, College Station, TX) for all statistical analyses.

Results

We identified 537 incident cases of invasive breast cancer (536 were histologically verified) over an average of 10.1 person years of follow-up between 1990 and 2003 in the 17 447 women eligible for analysis. The mean age at baseline was 54.7 (SD 8.8) years; 36% of the women were under 50, 16% between 50 and 55, and 50% over 55 years.

Table 1 summarises the distribution of alcohol consumption and folate intake at baseline. Most women (86%) drank on average less than 20 g of alcohol a day, and only 538 (3%) reported an average daily intake of alcohol of at least 40 g. The Spearman's correlation coefficient between the reported daily average alcohol consumption during the current decade of age at baseline and that from the diary of the week before baseline was 0.87. The mean folate intake was 330 (SD 124) $\mu\text{g/day}$.

The comparison between the models of the log hazard rate using linear and fractional polynomials of current alcohol consumption provided no evidence for a departure from linearity ($P=0.64$; null hypothesis: the association is linear). From the model in which alcohol consumption was fitted as a linear term, the hazard rate increased by 1.03 (95% confidence interval 0.95 to 1.09) times for each additional 10 g a day intake of alcohol ($P=0.36$). Next, we modelled alcohol consumption as a categorical variable using the National Health and Medical Research Council categories²⁰ (table 2). The highest hazard ratio occurred in women who drank 40 g of alcohol a day or more; however, overall, the association of breast cancer incidence with alcohol was not significant ($P=0.29$).

The comparison between the regression models using linear and fractional polynomials of folate intake indicated that the more complex models did not fit better than the simple linear model ($P=0.27$; null hypothesis: the association is linear). However, folate was not significantly related to incidence of breast cancer (for 100 $\mu\text{g/day}$ increment of folate, relative risk = 1.01 (0.93 to 1.10); $P=0.79$).

We evaluated whether folate modified the association between alcohol and breast cancer by fitting a model with an interaction between alcohol and folate. Our model of choice included alcohol as a categorical variable and folate as a continu-

Table 2 Hazard ratios for association between average alcohol consumption at baseline and breast cancer in the Melbourne collaborative cohort study

Alcohol consumption	Cases	Person years*	Hazard ratio† (95% CI)
Abstainers‡	171	56 267	Reference
Ex-drinkers§	16	5 553	1.03 (0.62 to 1.73)
1-19 g/day	286	91 274	1.12 (0.93 to 1.36)
20-39 g/day	43	17 720	0.87 (0.62 to 1.22)
≥40 g/day	21	5 313	1.41 (0.90 to 2.23)

*Person years of follow-up of 17 447 women.

†Adjusted for total energy and folate intake and fitted as linear variable in Cox's proportional hazard model with age as time metric.

‡Women who never drank at least 12 alcoholic drinks in a year.

§Women who drank at least 12 alcoholic drinks in a year, but did not drink at baseline.

ous variable. We preferred this model to those including both variables as continuous because its results are easier to interpret. Table 3 shows estimates of the hazard ratios in relation to alcohol consumption for three values of folate intake. The values of folate were 200 µg/day, 330 µg/day (the mean value of folate intake), and 400 µg/day, which is the Australian recommended daily intake for women in childbearing age.²⁵ Compared with abstainers who consumed 200 µg/day of folate, the hazard ratio was twofold higher for those women who consumed the most alcohol (40 g/day or more), but the hazards ratios for lower levels of consumption were not elevated. None of the hazard ratios was elevated for women in any other categories of alcohol consumption or for women in the highest category of consumption who had higher levels of folate intake. The test for the interaction gave P values of 0.04. The model in which alcohol and folate were both linear did not give a better fit (AIC = 9138 *v* AIC = 9138 for the model with alcohol as categorical and folate as linear). The test for the interaction between alcohol and folate was not significant (P = 0.42), probably because, as table 3 shows, the interaction between alcohol and folate was not a simple linear term.

Discussion

Overall in this prospective cohort study, we did not observe a significant association of breast cancer with either alcohol consumption or folate intake, but we found a significant interaction between alcohol and folate intakes. Women who had high alcohol consumption and low intake of folate had an increased risk of breast cancer, but those women who had high alcohol consumption and moderate to high levels of folate intake had no increased risk.

Strengths and limitations

The strengths of our study include its prospective nature, extensive information on potential confounding variables, minimal

Table 3 Hazard ratios* (95% confidence intervals) for association between average alcohol consumption at baseline and breast cancer by intake of dietary folate in the Melbourne collaborative cohort study

Alcohol consumption	Folic acid		
	200 µg/day	330 µg/day†	400 µg/day
Abstainers‡	Reference	Reference	Reference
Ex-drinkers§	1.06 (0.52 to 2.16)	1.03 (0.62 to 1.73)	1.02 (0.56 to 1.87)
1-19 g/day	0.93 (0.71 to 1.23)	1.12 (0.92 to 1.35)	1.23 (0.99 to 1.53)
20-39 g/day	0.94 (0.58 to 1.54)	0.85 (0.61 to 1.20)	0.81 (0.52 to 1.25)
≥40 g/day	2.00 (1.14 to 3.49)	1.08 (0.60 to 1.93)	0.77 (0.33 to 1.80)

*Hazard ratio from Cox's proportional hazard model with age as time metric. The model includes total energy, folate fitted as a linear variable, alcohol as categorical variable, and interaction between folate and alcohol consumption.

†Mean of distribution of folate.

‡Women who never drank at least 12 alcoholic drinks in a year.

§Women who drank at least 12 alcoholic drinks in a year, but did not drink at baseline.

loss to follow-up, and virtually complete ascertainment of cases through the population cancer registry. Limitations include the small numbers of women with high levels of alcohol consumption and folate intake, use of a single measure of self reported alcohol consumption at baseline, and limitations in the assessment of folate intake. Random error in measuring alcohol consumption and folate intake at baseline may have led to conservative biases in the association between breast cancer incidence and alcohol and folate.

We measured folate intake at baseline, but changes in folate intake due to use of multivitamins, folate supplementation of foods, or change in dietary habits may have occurred during the follow-up period. Use of multivitamin supplements at baseline was not common and did not affect the hazard ratios, but we had no information on subsequent use of supplements containing folic acid. Since 1995, more than 100 foods have been approved for fortification with folate in Australia for the prevention of neural tube defects.^{25, 26} Changes in serum folate concentration following the voluntary fortification of food in Australia have been shown to be very small, especially compared with those in the United States, where folate fortification has been mandatory.²⁷

Comparison with other studies

Epidemiological evidence for an effect of alcohol consumption on risk of breast cancer has been summarised in a collaborative reanalysis of individual data from 53 epidemiological studies.³ Compared with women who reported drinking no alcohol, the relative risk of breast cancer was 1.46 (95% confidence interval 1.33 to 1.61) for those consuming on average ≥ 45 g/day, a result in general agreement with our finding of a hazard ratio of 1.41 (0.90 to 2.23) for those consuming ≥ 40 g/day. According to the collaborative reanalysis, the relative risk of breast cancer increased by 7% for each additional 10 g/day intake of alcohol. Our weaker association of a 3% increase is consistent with another reanalyses of six prospective studies restricted to women who drank less than 60 g/day of alcohol, which found increases of 3-16% across individual studies.²

Few other studies have investigated the interaction between folate and alcohol.¹⁴⁻¹⁷ Two reports showed a higher risk of breast cancer in women with high intake of alcohol and low intake of folate,^{14, 17} whereas the other two studies focused on the protective effect of folate when associated with high consumption of alcohol.^{15, 16}

Possible mechanisms

Consistent evidence from animal experiments shows that ethanol modulates chemically induced carcinogenesis.⁴ The mechanisms by which alcohol induces carcinogenesis are hypothesised to include the induction of cytochrome P-4502E1 (CYP2E1), which metabolises ethanol to acetaldehyde and is also involved in the metabolism of various procarcinogens; nutritional deficiencies, including folate deficiency caused by either low intake or destruction by acetaldehyde; interactions with retinoids with effects on cellular growth and differentiation; changes in DNA methylation; alteration in the immune system; and alcohol mediated increases in oestradiol.⁴ Acetaldehyde is carcinogenic and mutagenic, binds to DNA and proteins, destroys folate, and causes hyperproliferation.²⁸ How these mechanisms are involved in breast carcinogenesis is not clear, but the impact of alcohol on hormonal status and cumulative exposure to oestrogens, particularly oestradiol, is likely to be a major contributor to the risk of breast cancer.¹ It is also not clear whether the modifying effect of folate is an early or late stage effect or whether total dose is important.⁷

What is already known on this topic

Alcohol consumption is a known risk factor for breast cancer

Some studies have reported that folate is inversely associated with breast cancer risk

Whether folate intake can mitigate the adverse effects of alcohol on risk of breast cancer is not well established

What this study adds

This study strengthens the evidence that an adequate dietary intake of folate might protect against the increased risk of breast cancer associated with alcohol consumption

Conclusion

Our results support the hypothesis that alcohol consumption may increase the risk of breast cancer through an interaction with folate and suggest that any adverse effect of alcohol consumption may be reduced by sufficient dietary intake of folate.

This study was made possible by the contribution of many people, including the original investigators and the diligent team who recruited the participants and who continue working on follow-up. Finally, we thank the many thousands of Melbourne residents who continue to participate in the study.

Contributors: GGG, DMG, and JLH were responsible for the initial study design. DRE and GGG supervised the conduct of the study. LB did the data analysis. All of the authors contributed to the final report. All the authors are guarantors.

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Ethical approval: The Cancer Council Victoria's human research ethics committee approved the study protocol.

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