

Dietary Folate Intake and Incidence of Ovarian Cancer: The Swedish Mammography Cohort

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Background: Mounting evidence suggests that a low intake of the water-soluble B vitamin folate is associated with breast and colorectal carcinogenesis, especially among alcohol drinkers. However, epidemiologic data specifically linking folate intake to ovarian cancer risk are limited. **Methods:** We examined the association between dietary folate intake (i.e., folate from food sources) and the incidence of total epithelial ovarian cancer and its subtypes by analyzing data from the Swedish Mammography Cohort, a population-based prospective cohort of 61 084 women, aged 38–76 years, who, at baseline (i.e., from 1987 to 1990), were cancer-free and had completed a food-frequency questionnaire. Through June 30, 2003, 266 incident cases of invasive epithelial ovarian cancer were diagnosed. We used Cox proportional hazards models to estimate multivariable relative risks (RRs) of ovarian cancer with 95% confidence intervals (CIs). All statistical tests were two-sided. **Results:** Overall, dietary folate intake was weakly inversely associated with total epithelial ovarian cancer risk (RR for highest versus lowest quartile of intake = 0.67, 95% CI = 0.43 to 1.04; $P_{\text{trend}} = .08$). Among women who consumed more than 20 g of alcohol (approximately two drinks) per week, there was a strong inverse association between dietary folate intake and total epithelial ovarian cancer risk (RR for highest versus lowest quartile of intake = 0.26, 95% CI = 0.11 to 0.60; $P_{\text{trend}} = .001$), but among women who consumed 20 g or less of alcohol per week, there was no such association (RR for highest versus lowest quartile of intake = 1.00, 95% CI = 0.59 to 1.70; $P_{\text{trend}} = .80$). The absolute risk of epithelial ovarian cancer for the lowest three quartiles versus the highest quartile of folate intake was 8 per 100 000 person-years (95% CI = 0 to 16 per 100 000 person-years) overall and 26 per 100 000 person-years (95% CI = 10 to 42 per 100 000 person-years) among those who consumed more than 20 g of alcohol per week. The association between dietary folate intake and cancer risk did not vary substantially among subtypes of epithelial ovarian cancer. **Conclusion:** A high dietary folate intake may play a role in reducing the risk of ovarian cancer, especially among women who consume alcohol. [J Natl Cancer Inst 2004;96:396–402]

Folate is a water-soluble B vitamin with pivotal roles in DNA synthesis, repair, and methylation; much attention has recently focused on folate because aberrations in these processes have been implicated in carcinogenesis (1,2). Epidemiologic evidence accumulated over the past decade suggests that a low intake of folate may increase the risk for breast and colorectal cancers, especially among individuals who regularly consume alcohol (3–11). Alcohol consumption may increase folate requirements by reducing folate absorption and by interfering with folate metabolism, intestinal absorption, transport, and storage, and its release by the liver (12). Although many studies have examined the association between folate intake and the risks of breast and colorectal cancers, only three case-control studies (13–15) and one cohort study (16) have examined the association between folate intake and the risk of ovarian cancer. However, none of those four studies examined whether the association between folate intake and ovarian cancer risk is modified by alcohol consumption.

We used data from the Swedish Mammography Cohort, a large population-based prospective cohort study of 61 084 women, to examine the association between dietary folate intake (i.e., folate from food sources) and epithelial ovarian cancer risk. We also investigated the hypothesis that the inverse association between folate intake and risk of ovarian cancer is stronger among women who regularly consume alcohol.

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SUBJECTS AND METHODS

The Swedish Mammography Cohort

From 1987 to 1990, all women who lived in Uppsala County of central Sweden and were born in 1914 through 1948 ($n = 48\,517$) and all women who lived in the adjacent Västmanland County ($n = 41\,786$) and were born in 1917 through 1948 received an invitation by mail to participate in a population-based mammography screening program. The invitation included a six-page questionnaire that sought information on diet, parity, age at first birth, family history of breast cancer, weight, height, and educational level. A total of 66 651 (74%) women returned a completed questionnaire. Information on age at menarche, age at menopause, and history of oral contraceptive and postmenopausal hormone use was obtained only from women in Uppsala County at their mammography examination.

In 1997, a follow-up questionnaire was sent to all cohort members; the follow-up questionnaire requested information on age at menarche, history of oral contraceptive use, age at menopause, postmenopausal hormone use, and lifestyle factors, such as cigarette smoking history and the use of dietary supplements. The response rate for the follow-up questionnaire was 68%.

Of the 66 651 women who responded to the baseline questionnaire, we excluded those who were younger than 38 years or older than 76 years ($n = 165$); those whose questionnaires had missing ($n = 707$) or incorrect ($n = 415$) national identification numbers, were not properly dated ($n = 608$), or lacked the date the woman moved out of the study area ($n = 79$); those with a missing date of death ($n = 16$); those with implausibly high or low energy intakes (i.e., three standard deviations below or above the mean value for log-transformed energy) ($n = 793$); and those diagnosed with cancer (other than nonmelanoma skin cancer) before receiving the baseline questionnaire ($n = 2429$). Furthermore, by linkage with the Swedish Inpatient Register, we identified and excluded all women who, prior to baseline, had a bilateral oophorectomy or a hysterectomy with an unknown number of ovaries removed ($n = 355$). After these exclusions, 61 084 women remained for this analysis.

Dietary Assessment

Diet was assessed with the use of a self-administered food-frequency questionnaire that included 67 food items (including alcoholic beverages) commonly consumed in Sweden. Women were asked how often, on average, they had consumed each type of food or beverage during the past 6 months. There were eight pre-specified response categories that ranged from never/seldom to four or more times per day. We used age-specific (<53, 53–65, >65 years) portion sizes that were based on mean values obtained from 213 randomly chosen women from the study area whose food intake for 5922 days was weighed and recorded (Wolk A: unpublished data). Intake of nutrients was calculated by multiplying the frequency of consumption of each food item by its nutrient content per serving, using composition values obtained from the Swedish National Food Administration Database (17).

We performed a methodologic study to determine the validity of the food-frequency questionnaire among 129 randomly selected women from the cohort; Pearson correlation coefficients between data from four 1-week diet records (obtained 3–4 months apart) and the food-frequency questionnaire were 0.5 for dietary folate intake and 0.9 for alcohol consumption. Among

this group of women, the mean estimated daily dietary folate intake was 211 $\mu\text{g}/\text{day}$ (standard deviation = 61 $\mu\text{g}/\text{day}$), and the mean total energy intake was 1702 kcal/day (standard deviation = 358 kcal/day).

Ascertainment of Ovarian Cancer Cases and Follow-Up of the Cohort

We identified incident ovarian cancer cases for the period from March 1, 1987, through December 31, 1997, through linkage of the cohort with the Swedish Cancer Registry. We identified incident cases of ovarian cancer for the period January 1, 1998, through June 30, 2003, by linkage with the Regional Cancer Registry in the study area. The Swedish cancer registry system, which includes the Swedish Cancer Registry and the Regional Cancer Registry, has been estimated to be 98% complete (18).

Deaths and migration from the study area were ascertained through the Swedish Death Registry and the Swedish Population Registry, respectively. This study was approved by the Ethics Committees at the Uppsala University Hospital (Uppsala, Sweden) and the Karolinska Institutet (Stockholm, Sweden). Completion of the self-administered questionnaire was considered to imply informed consent to participate in this study.

Statistical Analysis

We calculated person-years of follow-up for each woman from the date of her entry into the cohort (i.e., the date of her screening mammogram) to the date of an ovarian cancer diagnosis, the date of a bilateral oophorectomy or a hysterectomy with unknown number of ovaries removed, the date of death from any cause, the date of migration out of the study area, or June 30, 2003, whichever came first. We categorized women according to quartiles of dietary folate intake (from food sources) and computed incidence rates by dividing the number of incident cancer cases by the number of person-years of follow-up in each category. The relative risks (RRs) of ovarian cancer (with 95% confidence intervals [CIs]) were calculated by dividing the incidence rate in a specific quartile of folate intake by the incidence rate in the lowest quartile of folate intake. Pearson correlation coefficients were used to estimate correlations between dietary factors. The data conformed to proportional hazards assumptions, and we used Cox proportional hazards models for age-adjusted (5-year age categories) and multivariable analyses. The multivariable models simultaneously included age at baseline, body mass index, educational level achieved, family history of breast cancer, parity, age at first birth, oral contraceptive use, age at menarche, age at menopause, postmenopausal hormone use, alcohol consumption, and intake of lactose, fruits and vegetables, and total energy intake (as a proxy for physical activity). Dietary folate intake was adjusted for total energy intake by using the residual method (19). We conducted tests for trend by using the median value for each category of dietary folate intake as a continuous variable in the model. All P values are two-sided. Analyses were performed using SAS software (version 8.2; SAS Institute, Cary, NC).

We estimated the absolute risk of ovarian cancer associated with a low dietary folate intake by using a program developed by Rothman (available at <http://members.aol.com/krothman/episheet.xls>). The absolute risk was defined as the difference between the age-standardized incidence rate of

ovarian cancer in the lowest three quartiles and the incidence rate in the highest quartile of dietary folate intake.

We also conducted analyses stratified by alcohol consumption (≤ 20 g/week, > 20 g/week) to assess possible effect modification by this factor. A cut point of 20 g of alcohol per week, which corresponds to approximately two alcoholic drinks per week, was used because it was a rounded value of the median alcohol consumption in the cohort. The statistical significance of an interaction between dietary folate intake and alcohol consumption was tested with a likelihood ratio test by comparing interactions between folate intake (in quartiles) and alcohol consumption (≤ 20 g/week, > 20 g/week) in models that contained or lacked the interaction terms.

RESULTS

During a mean follow-up of 13.5 years (823 572 person-years), 266 incident cases of invasive epithelial ovarian cancer were diagnosed, including 125 serous cancers, 48 endometrioid cancers, 21 mucinous cancers, five clear-cell cancers, and 67 undifferentiated cancers or cancers of other or unknown histologic subtypes. The mean age at epithelial ovarian cancer diagnosis was 64.0 years (standard deviation = 9.7 years). Table 1 shows the distribution of known and potential risk factors for ovarian cancer among the cohort by quartiles of dietary folate intake from food sources. Women with a higher dietary folate intake were more likely to have had 12 or more years of education and to have used postmenopausal hormones than women with a lower intake. Dietary folate intake was positively correlated with intakes of fruit ($r = .36$) and vegetables ($r = .63$). Other characteristics did not vary substantially across quartiles of dietary folate intake.

Overall, dietary folate intake was weakly inversely associated with total epithelial ovarian cancer risk in both age-adjusted analyses and multivariable analyses that were adjusted for all the covariates listed in Table 2. The multivariable relative risk of epithelial ovarian cancer was 0.67 (95% CI = 0.43 to 1.04) for women in the highest quartile of dietary folate intake compared with those in the lowest quartile. Additional adjustment for dietary intakes of methionine, vitamin B₆, and vitamin B₁₂ yielded virtually similar results (RR = 0.60, 95% CI = 0.37 to 0.98), as did further adjustment for dietary intakes of vitamin C, vitamin E, β -carotene, and fiber (RR = 0.64, 95% CI = 0.36 to 1.13). We also examined the association between dietary folate intake and the risks of different histologic subtypes of epithelial ovarian cancer and found that, although the inverse association with dietary folate intake was slightly stronger for endometrioid, clear-cell, and mucinous tumors than it was for serous tumors, the number of cases of endometrioid, clear-cell, and mucinous tumors was small (Table 2).

We also examined the association between dietary folate intake and ovarian cancer risk after stratifying women by alcohol consumption (Table 3). There was no association between dietary folate intake and epithelial ovarian cancer risk among women who consumed 20 g or less of alcohol per week. By contrast, we observed a strong inverse association between dietary folate intake and epithelial ovarian cancer risk among women who consumed more than 20 g of alcohol per week (RR for highest versus lowest quartile = 0.26, 95% CI = 0.11 to 0.60). The inverse association between folate intake and ovarian cancer risk was even stronger among women who consumed

more than 40 g of alcohol per week (the 50th and 75th percentiles of alcohol consumption in this subgroup were 57.3 g/week and 74.1 g/week, respectively); for those women, the multivariable relative risks of total epithelial ovarian cancer for increasing quartiles of dietary folate intake were 1.00 (referent), 0.62 (95% CI = 0.25 to 1.54), 0.36 (95% CI = 0.12 to 1.10), and 0.15 (95% CI = 0.03 to 0.82) ($P_{\text{trend}} = .01$). High dietary folate intake was inversely associated with the risks of all subtypes of epithelial ovarian cancer among women who consumed more than 20 g of alcohol per week (Table 3). A test for interaction between dietary folate intake and alcohol consumption and total epithelial ovarian cancer risk was statistically significant ($P = .03$). When dietary folate intake was analyzed as a continuous variable, an increment of 50 $\mu\text{g/day}$ of dietary folate intake (the approximate amount of folate in one orange) was associated with a 45% (95% CI = 20% to 62%) reduction in the risk of total epithelial ovarian cancer among women who consumed more than 20 g of alcohol per week (data not shown).

We found no evidence for an overall association between alcohol (ethanol) consumption and epithelial ovarian cancer risk among the women in our cohort; the multivariable relative risks of epithelial ovarian cancer associated with quartile of alcohol consumption were 1.00 (referent) for women who consumed 0 to less than 7 g of alcohol per week, 0.95 (95% CI = 0.66 to 1.32) for women who consumed 7–15.6 g of alcohol per week, 1.35 (95% CI = 0.91 to 1.88) for women who consumed 15.7–27.2 g of alcohol per week, and 1.24 (95% CI = 0.84 to 1.81) for women who consumed 27.3 g or more of alcohol per week ($P_{\text{trend}} = .14$). Nevertheless, alcohol consumption was positively associated with epithelial ovarian cancer risk among women whose dietary folate intake was less than 178 $\mu\text{g/day}$, the median value for the cohort. The multivariable relative risks of total epithelial ovarian cancer in this subgroup of women from the lowest to the highest quartile of alcohol consumption were 1.00 (referent), 0.93 (95% CI = 0.55 to 1.58), 1.50 (95% CI = 0.89 to 2.49), and 1.62 (95% CI = 0.96 to 2.71) ($P_{\text{trend}} = .02$). The absolute risk (i.e., incidence rate difference) of epithelial ovarian cancer among women in the lowest three quartiles compared with women in the highest quartile of dietary folate intake was 8 per 100 000 person-years (95% CI = 0 to 16 per 100 000 person-years) overall and 26 per 100 000 person-years (95% CI = 10 to 42 per 100 000 person-years) among those who consumed more than 20 g of alcohol per week.

To assess the potential effect of misclassification of folate intake due to use of vitamin supplements, we repeated the analysis after excluding all women who, on the follow-up questionnaire, reported ever use of multivitamins, folic acid supplements, or B vitamins. Data from the follow-up questionnaire that was sent to cohort members in 1997 revealed that 22.8% of the women were currently taking multivitamins with minerals, 2.2% of the women were taking multivitamins without minerals, and 1.0% of the women were taking folic acid supplements (Swedish multivitamins contain about 200–400 μg folic acid). Among women who did not take vitamin supplements, the multivariable relative risks of epithelial ovarian cancer for women in the highest quartile of dietary folate intake compared with those in the lowest quartile were 0.61 (95% CI = 0.38 to 0.98) for all subjects and 0.24 (95% CI = 0.09 to 0.60) for those who consumed more than 20 g of alcohol per week. Thus, exclusion of these women did not substantially change the associations.

We also used data from the follow-up questionnaire to examine whether cigarette smoking history may have confounded the observed association between dietary folate intake and ovarian cancer risk in the subgroup of women who consumed more than 20 g of alcohol per week. There were 29 cases of invasive epithelial ovarian cancer (diagnosed between November 15, 1997, and June 30, 2003) among 15 013 women who consumed more than 20 g of alcohol per week and were free from ovarian cancer on November 15, 1997; the multivariable relative risks of ovarian cancer, adjusted for smoking status (never, past, or current) and for the covariates listed in Table 3, from the lowest to the highest quartiles of dietary folate intake were 1.00 (referent), 0.71 (95% CI = 0.26 to 1.95), 0.63 (95% CI = 0.22 to 1.80), and 0.13 (95% CI = 0.02 to 0.72) ($P_{\text{trend}} = .02$).

Finally, to eliminate potential effects of early undiagnosed epithelial ovarian cancers, we repeated our analyses after excluding ovarian cancer cases diagnosed during the first 3 years of follow-up. Results of these analyses did not differ substantially from those presented; for instance, in an analysis confined to women who consumed more than 20 g of alcohol per week and with follow-up from March 1, 1987, through June 30, 2003, the multivariable relative risk of ovarian cancer for women in the highest quartile of dietary folate intake compared with those in the lowest quartile was 0.20 (95% CI = 0.08 to 0.47).

DISCUSSION

In this large population-based prospective cohort study, we found a weak inverse association overall between dietary folate intake and risk of epithelial ovarian cancer. There was a strong inverse association between dietary folate intake and epithelial ovarian cancer risk among women who consumed more than 20 g of alcohol (approximately two alcoholic drinks) per week: those in the highest quartile of dietary folate intake had a 74% decreased risk of epithelial ovarian cancer relative to those in the lowest quartile. This association was independent of established major risk factors for ovarian cancer, including nulliparity and never-use of oral contraceptives, and was not explained by

differences in smoking habits or intakes of lactose, methionine, β -carotene, or vitamins B₆, B₁₂, C, or E. Although we cannot entirely rule out confounding by factors not accounted for or residual confounding due to imprecise measurement of important covariates, several factors argue against these possibilities as explanations for our findings. First, we obtained similar results using models that adjusted for consumption of fruits and vegetables (other nutrients or phytochemicals found in fruits and vegetables are the most likely confounders). Second, although misclassification of important covariates could lead either to an attenuation or accentuation of the relative risk, we do not believe that errors in measuring the covariates would be so extreme as to produce the observed association with folate because the age-adjusted and multivariable relative risks were essentially the same.

There are few published epidemiologic studies of the association between folate intake and ovarian cancer risk. In the prospective Iowa Women's Health Study, which had 139 cases of epithelial ovarian cancer, women in the highest quartile of total folate intake (>488.5 $\mu\text{g/day}$) had a statistically nonsignificant increased risk of ovarian cancer (RR = 1.63, 95% CI = 0.97 to 2.76) compared with women in the lowest quartile (<240.9 $\mu\text{g/day}$) (16). However, the positive association between folate intake and ovarian cancer risk observed in that study appeared to be attributable to folate intake from dietary supplements. A small hospital-based case-control study conducted in Mexico (84 epithelial ovarian cancer case patients and 629 control subjects) found that women in the highest tertile of dietary folate intake ($\geq 322 \mu\text{g/day}$) had a statistically nonsignificant increased risk of ovarian cancer (RR = 1.70, 95% CI = 0.95 to 3.05) compared with women in the lowest tertile (<197 $\mu\text{g/day}$) (14). A larger case-control study conducted in Italy (15) (1031 epithelial ovarian cancer case patients and 2411 control subjects) and a small case-control study conducted in the United States (13) (124 ovarian cancer case patients and 696 control subjects) found no associations between dietary folate intake and ovarian cancer risk.

Table 1. Age-standardized baseline characteristics according to quartiles of dietary folate intake among 61 084 women in the Swedish Mammography Cohort*

Characteristic	Quartiles of energy-adjusted dietary folate intake, $\mu\text{g/day}$			
	1 <155 (n = 15 241)	2 155 to <178 (n = 15 184)	3 178 to <204 (n = 15 245)	4 ≥ 204 (n = 15 414)
Median dietary folate intake, $\mu\text{g/day}$ (range) [†]	140 (108–154)	167 (156–177)	190 (179–202)	226 (205–307)
Mean age, y (SD)	53.1 (9.8)	53.4 (9.7)	53.7 (9.6)	54.6 (9.7)
Mean body mass index, kg/m^2 (SD)	24.6 (3.9)	24.6 (3.8)	24.6 (3.8)	24.9 (4.0)
Mean No. of children (SD)	2.1 (1.3)	2.1 (1.2)	2.1 (1.2)	2.1 (1.3)
Mean age at first birth, y (SD)	23.7 (4.6)	24.1 (4.5)	24.3 (4.6)	24.2 (4.6)
Mean age at menarche, y (SD)	13.3 (1.3)	13.3 (1.3)	13.3 (1.3)	13.2 (1.3)
Mean age at menopause, y (SD)	50.3 (4.7)	50.5 (4.7)	50.6 (4.7)	50.7 (4.9)
Family history of breast cancer, % [‡]	6.8	7.4	7.1	7.4
≥ 12 years of education, %	8.6	10.6	12.3	13.9
Ever used oral contraceptives, %	53.7	54.4	55.5	55.4
Ever used postmenopausal hormones, %	42.3	44.3	46.3	46.8
Dietary intake, mean (SD)				
Energy, kcal/day	1331 (392)	1356 (366)	1346 (365)	1286 (375)
Alcohol, g/wk	22.3 (3.8)	22.1 (3.2)	21.4 (2.9)	19.9 (2.6)
Lactose, g/day	11.1 (7.6)	12.0 (7.4)	12.5 (7.4)	13.1 (8.4)
Fruit, servings per week	6.8 (5.4)	9.3 (6.3)	11.2 (7.3)	14.6 (9.7)
Vegetables, servings per week	6.9 (4.3)	10.0 (5.1)	12.8 (6.2)	19.7 (11.7)

*SD = standard deviation.

[†]5th through 95th percentile.

[‡]Percentage of women with a family history of breast cancer among mother, sisters, or daughters.

Table 2. Relative risks (RRs) and 95% confidence intervals (CIs) of invasive epithelial ovarian cancer according to quartiles of dietary folate intake among 61 084 women in the Swedish Mammography Cohort*

	Quartiles of energy-adjusted dietary folate intake, $\mu\text{g}/\text{day}$				$P_{\text{trend}}^{\ddagger}$
	1 <155	2 155 to <178	3 178 to <204	4 ≥ 204	
All invasive epithelial tumors[‡]					
No. of cases	70	69	71	56	
No. of person-years	206 005	205 654	206 026	205 887	
Age-adjusted RR (95% CI)	1.00 (referent)	0.98 (0.70 to 1.36)	0.97 (0.70 to 1.36)	0.76 (0.54 to 1.08)	.13
Multivariable RR (95% CI)	1.00 (referent)	0.92 (0.65 to 1.30)	0.89 (0.62 to 1.29)	0.67 (0.43 to 1.04)	.08
Serous tumors					
No. of cases	31	36	30	28	
Age-adjusted RR (95% CI)	1.00 (referent)	1.15 (0.71 to 1.86)	0.90 (0.54 to 1.50)	0.85 (0.51 to 1.41)	.37
Multivariable RR (95% CI)	1.00 (referent)	1.08 (0.65 to 1.78)	0.84 (0.48 to 1.46)	0.78 (0.41 to 1.48)	.34
Endometrioid and clear-cell tumors					
No. of cases	15	14	14	10	
Age-adjusted RR (95% CI)	1.00 (referent)	0.93 (0.45 to 1.93)	0.93 (0.45 to 1.92)	0.66 (0.29 to 1.46)	.32
Multivariable RR (95% CI)	1.00 (referent)	0.70 (0.33 to 1.51)	0.62 (0.27 to 1.41)	0.38 (0.14 to 1.04)	.06
Mucinous tumors					
No. of cases	6	4	8	3	
Age-adjusted RR (95% CI)	1.00 (referent)	0.66 (0.19 to 2.34)	1.30 (0.45 to 3.75)	0.47 (0.12 to 1.87)	.47
Multivariable RR (95% CI)	1.00 (referent)	0.70 (0.19 to 2.66)	1.17 (0.33 to 4.13)	0.28 (0.05 to 1.56)	.20

*Multivariable RRs were adjusted for age (5-year categories), body mass index (quartiles), educational level (less than high school, high school, university), family history of breast cancer (yes or no), parity (nulliparous, 1 or 2, ≥ 3 children), age at first birth (nulliparous, <25, 25–29, ≥ 30 years), oral contraceptive use (ever or never), age at menarche (≤ 12 , 13, ≥ 14 years), age at menopause (<50, 50–54, ≥ 55 years), postmenopausal hormone use (ever or never), and quartiles of alcohol consumption, and fruit and vegetable, lactose, and total energy intake.

[†]Two-sided P values for trend were calculated with the Wald statistic using the median values for quartiles of dietary folate as a continuous variable.

[‡]Includes 67 cases that were of other or unknown histologic subtypes.

Unlike previous studies, our study may have detected an inverse association between folate intake and ovarian cancer because the overall folate intake in our cohort was quite low and because the association was essentially limited to alcohol drinkers. Previous studies (16) were conducted in populations in which the folate intakes were substantially higher than those in our cohort, and those studies did not specifically examine the interaction between dietary folate intake and alcohol consumption. Alcohol may interfere with the metabolism of folate thus increasing the minimal amount of folate required for adequate intake. An interaction between folate intake and alcohol consumption has previously been observed for both breast and colorectal cancers (3–11).

Our finding of an increased risk of cancer associated with deficient folate status is biologically plausible. Folate deficiency can reduce the regeneration of *S*-adenosylmethionine, which has a central role in DNA methylation (1). Loss of DNA methylation might promote carcinogenesis by inducing genomic instability, possibly through elevated mutation rates (20). DNA hypomethylation may also result in the activation of oncogenes (21). Paradoxically, DNA hypomethylation might be followed by DNA hypermethylation, as was observed in folate- and methyl-deficient rats (22). Gras et al. (23) observed microsatellite instability, hypermethylation of the promoter for the DNA mismatch repair gene MLH-1, and mutations in DNA repeat sequences in tumors of the endometrioid and clear-cell subtypes of ovarian cancer. Folate is needed for *de novo* synthesis of thymidine from its precursor, uridine, and folate deficiency may result in the replacement of uracil for thymidine in DNA (24). Blount et al. (2) reported that folate-deficient humans have substantially elevated levels of uracil in DNA and increased numbers of chromosome breaks, and that folate supplementation reverses these lesions. Results of other studies (25,26) have indicated that

folate deficiency may, perhaps through impaired DNA repair, enhance the genetic damage caused by alkylating agents in Chinese hamster ovary cells, thereby providing a biologic basis for a role of folate in ovarian carcinogenesis in humans. It has been suggested that the effect of folate supplementation on carcinogenesis depends on the dose of folate and on the stage of tumor development (27). In this regard, it is noteworthy that pharmacologic doses of folate have been shown to promote, rather than suppress, tumor growth in animals with well-established cancers (28–30). Indeed, antifolate agents, including methotrexate and 5-fluorouracil, are used as antitumor therapy (31).

The major strengths of our study include its population-based design, the relatively large number of cases of epithelial ovarian cancer, and the completeness of identification of ovarian cancer cases through the Swedish cancer registries. Furthermore, the prospective nature of our study makes it highly unlikely that the associations we observed were due to recall or selection biases, which can lead to spurious associations in case-control studies. Another strength of this study was our ability to evaluate dietary folate intake in relation to ovarian cancer risk among women with low folate intakes.

Our study has several limitations. First, because dietary intake was assessed through a self-administered food-frequency questionnaire, measurement errors were inevitable. However, results of comparisons with dietary records suggest that we obtained a reasonable assessment of dietary folate intake. Second, we had no information about the use of vitamin supplements at baseline, and approximately 25% of the cohort reported ever-use of any vitamin supplement. Results of three nationally representative, cross-sectional surveys conducted in Sweden from 1980 through 1981, 1988 through 1989, and 1996 through 1997 showed that the use of any dietary supplement, including

Table 3. Relative risks (RRs) and 95% confidence intervals (CIs) of epithelial ovarian cancer according to dietary folate intake and alcohol consumption among 61 084 women in the Swedish Mammography Cohort*

Quartiles of dietary folate intake, $\mu\text{g}/\text{day}$	Alcohol consumption, ≤ 20 g/wk			Alcohol consumption, > 20 g/wk [†]		
	No. of cases	Age-adjusted RR (95% CI)	Multivariable RR (95% CI)	No. of cases	Age-adjusted RR (95% CI)	Multivariable RR (95% CI)
All invasive epithelial tumors[‡]						
<155	42	1.00 (referent)	1.00 (referent)	28	1.00 (referent)	1.00 (referent)
155 to <178	36	0.88 (0.56 to 1.37)	0.84 (0.53 to 1.33)	33	1.08 (0.65 to 1.79)	0.96 (0.57 to 1.64)
178 to <204	47	1.12 (0.74 to 1.70)	1.09 (0.69 to 1.72)	24	0.74 (0.43 to 1.28)	0.60 (0.32 to 1.11)
≥ 204	45	0.99 (0.65 to 1.51)	1.00 (0.59 to 1.70)	11	0.39 (0.19 to 0.78)	0.26 (0.11 to 0.60)
P_{trend}^{\S}		.82	.80		.004	.001
Serous tumors						
<155	20	1.00 (referent)	1.00 (referent)	11	1.00 (referent)	1.00 (referent)
155 to <178	18	0.92 (0.49 to 1.75)	0.87 (0.45 to 1.67)	18	1.47 (0.70 to 3.12)	1.31 (0.59 to 2.90)
178 to <204	21	1.05 (0.57 to 1.93)	1.00 (0.51 to 1.96)	9	0.64 (0.26 to 1.59)	0.51 (0.19 to 1.42)
≥ 204	24	1.10 (0.61 to 1.99)	1.15 (0.55 to 2.43)	4	0.35 (0.11 to 1.10)	0.22 (0.06 to 0.85)
P_{trend}^{\S}		.67	.62		.02	.01
Endometrioid, clear-cell, and mucinous tumors						
<155	13	1.00 (referent)	1.00 (referent)	8	1.00 (referent)	1.00 (referent)
155 to <178	9	0.71 (0.31 to 1.67)	0.60 (0.25 to 1.47)	9	1.05 (0.40 to 2.72)	0.83 (0.30 to 2.27)
178 to <204	16	1.25 (0.60 to 2.59)	0.92 (0.39 to 2.16)	6	0.71 (0.25 to 2.06)	0.49 (0.15 to 1.63)
≥ 204	8	0.57 (0.24 to 1.39)	0.33 (0.11 to 0.99)	5	0.64 (0.21 to 1.98)	0.38 (0.10 to 1.54)
P_{trend}^{\S}		.40	.08		.35	.14

*Multivariable RRs were adjusted for age (5-year categories), body mass index (quartiles), educational level (less than high school, high school, university), family history of breast cancer (yes or no), parity (nulliparous, 1–2, ≥ 3 children), age at first birth (nulliparous, <25, 25–29, ≥ 30 years), oral contraceptive use (ever or never), age at menarche (≤ 12 , 13, ≥ 14 years), age at menopause (<50, 50–54, ≥ 55 years), postmenopausal hormone use (ever or never), and quartiles of fruit and vegetable, lactose, and total energy intake.

[†]The 50th and 75th percentiles of alcohol consumption in this subgroup were 32.5 g/wk and 45.5 g/wk, respectively.

[‡]Includes 67 cases that were of other or unknown histologic subtypes.

[§]Two-sided P values for trend were calculated with the Wald statistic using the median values for quartiles of dietary folate as a continuous variable.

vitamins and minerals, among adults has increased dramatically during the last two decades; for example, the prevalence of dietary supplement use among women aged 16–84 years was 22.9% in 1980–1981, 23.9% in 1988–1989, and 33.3% in 1996–1997 (32). Thus, although the proportion of women using multivitamins at baseline (i.e., from 1987 to 1990) was probably lower than that in 1997, some women might still have been misclassified with respect to their total folate intake. However, this kind of nondifferential misclassification would tend to attenuate the observed association between folate intake and ovarian cancer risk and thus could not explain our results. The reduction in ovarian cancer risk associated with higher dietary folate intake among all subjects as well as among alcohol drinkers remained practically unchanged when we excluded women who reported taking vitamin supplements in 1997 from the analysis. This finding suggests that the effect of the misclassification, due to lack of information about vitamin supplements at baseline, did not markedly influence our results.

Folate deficiency (i.e., an erythrocyte folate concentration <140 ng/mL or a plasma folate concentration <3 ng/mL) is among the most common nutritional deficiencies in both developing and Western countries (2). The Swedish Nutritional Recommendation for folate among adults is 300 $\mu\text{g}/\text{day}$ (33). Thus, the average intake of dietary folate among our study population of Swedish women, as determined from the median folate intake (211 $\mu\text{g}/\text{day}$) among a subsample of 129 cohort members who kept 28-day diet records, was less than this recommendation. It should be emphasized that the strong inverse association observed between dietary folate intake and ovarian cancer risk among women who consumed alcohol might not have been seen in populations that had a higher average folate intake (e.g., as in

countries where cereal and grain products are fortified with folic acid and multivitamin use is frequent). Future studies conducted in populations with high folate intakes (i.e., ≥ 400 $\mu\text{g}/\text{day}$) might need to examine subgroups of women with much higher alcohol consumption to detect a similarly strong interaction. The differences in folate intake and alcohol consumption between populations should also be considered when comparing and interpreting the results from different studies.

In conclusion, findings from this prospective population-based study suggest that a high folate intake from food sources is associated with a decreased risk of epithelial ovarian cancer, particularly among women who consume alcohol. Additional studies are needed to determine the generalizability of our results to other populations that have higher folate intakes and to evaluate the efficacy and safety of high doses of folate from supplements with respect to cancer. In the meantime, it may be prudent to increase folate intakes from folate-rich foods, such as fruits and vegetables, legumes, and whole grain. However, it may be premature to recommend increasing folate intake for the whole population through the use of dietary supplements because supraphysiologic levels of folate might enhance cancer progression among those with existing but yet undiagnosed cancer.

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NOTES

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