Published in final edited form as:
Int J Cancer. 2010 May 1; 126(9): 2191–2198. doi:10.1002/ijc.24723.

Relationship between dietary and supplemental intake of folate, methionine, vitamin B_6 and folate receptor α expression in ovarian tumors

Joanne Kotsopoulos[1], **Jonathan L. Hecht**[2], **Jonathan D. Marotti**[2], **Linda E. Kelemen**[3], and **Shelley S. Tworoger**[1,4]

[1]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

[2]Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts

[3]Department of Population Health Research, Alberta Cancer Board, Calgary, Alberta

[4]Department of Epidemiology, Harvard School of Public Health, Boston

Abstract

Because folate receptor α (FRα) is frequently over-expressed in epithelial ovarian tumors, we hypothesized that its association with folate may differ by FRα expression or by the timing of intake. We examined the association between folate and other cofactors in 152 ovarian cancers evaluated for FRα expression from the Nurses' Health Study. Multivariate odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using unconditional logistic regression. FRα expression was higher in serous invasive and advanced stage ovarian tumors. Recent dietary folate intake ≥ 300 μg/day compared to < 300 μg/day was associated with decreased risk of developing ovarian cancer (OR=0.62; 95%CI 0.40-0.96). There was suggestion of an increased risk with total folate (dietary and supplemental) (OR=1.42; 95%CI 0.94-2.14 for past and OR=1.53; 95%CI 0.99-2.37 for recent intake). These results did not vary by FRα status of the tumor. Methyl group score, a marker of high dietary folate and methionine intake but low alcohol consumption, was inversely associated with risk of ovarian cancer (OR=0.44; 95%CI 0.23-0.84 for past and OR=0.46; 95%CI 0.24-0.88 for recent intake). There were no clear individual associations between methionine, vitamin B_6, or multivitamin use and ovarian cancer risk overall or by FRα tumor status. Our data do not support the hypothesis that the relationship between factors involved in one-carbon metabolism and ovarian cancer risk differs by FRα status of the tumor.

Keywords

folate receptor α; folate; methionine; ovarian cancer

Introduction

Epidemiological evidence suggests an inverse relationship between folate status and the risk of developing breast and colon cancer [1, 2]. Despite this, the evidence to date has been limited and inconsistent for ovarian cancer. Four case-control studies have generally reported no association between folate intake and the risk of ovarian cancer [3-6]; whereas,

Corresponding author: Joanne Kotsopoulos, PhD, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115, Phone: 617-525-2691, Fax: 617-525-2008, nhjok@channing.harvard.edu.

the results from three of four prospective cohort studies, including one from the Nurses' Health Study (NHS), have suggested an inverse relationship with dietary folate intake, although none of the associations reached statistical significance 7-10. The inconsistencies in the results for ovarian cancer have been attributed to the modification by other factors including alcohol consumption, intake of other folate cofactors involved in one-carbon metabolism (i.e. methionine, vitamins B_6 and B_{12}) and genetic variation in cellular folate metabolism 11.

Many epithelial ovarian tumors overexpress folate receptor α (FRα) 12. FRα expression has been associated with higher stage, grade, and worse survival 12-14. However, it is not clear whether FRα expression is an early or late event in tumorigenesis 12 and whether the timing of intake of folate or other methyl donors influences the risk of developing FRα positive ovarian tumors. Since few modifiable risk factors for ovarian cancer exist, it is important to clarify the role of folate in the etiology of this disease. We examined whether intake of folate and other nutrients involved in methyl group metabolism (e.g., methionine and vitamin B_6) and multivitamin use were associated with ovarian cancer risk stratified by FRα status of the tumor. We also examined whether the association differed by the timing of intake.

Materials and Methods

Study population

The NHS was initiated in 1976, when 121,700 U.S. female registered nurses aged 30-55 years completed a self-administered questionnaire about various risk factors for disease 15-17. Study participants have been followed biennially by questionnaire to update exposure status and disease diagnoses. In 1980, we included a 61-item food frequency questionnaire (FFQ) that asked about the use of vitamin and mineral supplements; the questionnaire was expanded to 131 items in 1984. Participants completed the expanded FFQ in 1986, 1990, 1994, 1998 and 2002. Follow-up for this cohort of women was >95% of person-years through 2002.

Assessment of exposure and covariate information

Details on the semi-quantitative FFQ and its reproducibility have been published previously 18-21. Briefly, for each food and beverage item, the questionnaire specifies a common serving size and asks participants to indicate their average intake of that food item during the past year (responses range from “almost never” to “six or more times per day”). We calculated intakes of dietary folate, methionine, and vitamin B_6 by multiplying the frequency of food item consumption by the nutrient content of the specified serving size, using food composition values from the US Department of Agriculture 22-24. Nutrient intakes are computed with and without vitamin and mineral supplements. Because of changes in food composition and fortification, our nutrient database is year-specific. For example, we take into account folate fortification for questionnaires completed in 1998 and beyond. All nutrient intakes were adjusted for total energy intake using the nutrient residual method 25. If a woman did not provide intake information for a particular food item in a certain year, that food was not included in the calculation of her intake of that nutrient in that year. However, this was rare since we only included FFQs that had missing data for 10 or fewer food items. Participants also provided information on the dose and duration of any vitamin supplements used, including brand of multivitamin. We used this information to calculate total folate and vitamin B_6 intakes from both diet and supplements. Other dietary factors, including lactose intake, total calories, and alcohol consumption were assessed similarly.

Information on the potential confounding variables, including body mass index (BMI), reproductive history, and postmenopausal hormone (PMH) use was asked at baseline and updated on the biennial study questionnaires. Oral contraceptive (OC) use was asked at baseline through 1982, by which time use was rare because of the age distribution of the cohort. Women were not asked about a family history of ovarian cancer until 1992; they were then subsequently queried about this in 1996 and 2000.

Ascertainment of cases, ovarian tumor block collection, and control selection

Incident cases of epithelial ovarian cancer were identified by biennial questionnaire from 1976 to 2002. For women reporting a new ovarian cancer or cases identified via death certificate 26, we obtained pathology reports and related medical records. A gynecologic pathologist, unaware of exposure status, reviewed the records to confirm the diagnosis and classify cancers by invasion, histologic type, and stage. Paraffin-embedded tissue blocks with representative samples of the ovarian carcinoma and one or more blocks of uninvolved tissue (e.g. uterine) were requested for each of the confirmed ovarian cancer cases. Records were reviewed to determine the accession number of the primary surgery to remove the tumor. Appropriate blocks were then selected by the pathologist to isolate primary tumor tissue for a tissue microarray (TMA). The tissue blocks containing ovarian carcinoma were matched to their corresponding hematoxylin and eosin stained slides and the histologic diagnosis was confirmed. The initial fixation, processing and storage conditions of these tissues is largely unknown as blocks came from multiple hospitals across the United States but are assumed to follow standard practices of clinical labs. Four control women per case were randomly selected from the NHS study participants and matched to the case on year of birth. Eligible women had no prior bilateral oophorectomy (i.e. at least one ovary), and no history of cancer, other than non-melanoma skin cancer, at the time of diagnosis of the ovarian case.

Tissue microarray construction and immunohistochemistry

TMAs were assembled by taking three 0.6 mm core biopsies from each tissue block and re-embedding them with a spacing of 0.8 mm into an arrayed “master” paraffin block at the Pathology Core of the Brigham and Women's Hospital 27. Slides were cut from the TMA block for staining. Microarray slides were processed and stained within two weeks of cutting. Five-micron sections were soaked in Xylene overnight to remove any adhesive from the tape transfer system. Sections were stained using the primary antibody mAb343 (courtesy of Wilbur Franklin and Philip Low) according to published methods 28. Staining was graded by a gynecologic pathologist (JLH). Staining was scored as the number of reactive versus total cells and was categorized as 0, 1-10, 11-25, 26-50, and >50%. Three spots from the same case were independently assessed. Spots where tissue was missing from the slide or where only a few cell clusters (<20 cells) were present were designated as uninterpretable. Concordance (Cohen's Kappa [κ]) with a second pathologist (JDM) for the individual cores was 0.82 and for the maximum stain of the three cores was 0.85.

Statistical Analysis

The cores were dichotomized into FRα positive (+) if >10% of cells stained positive and FRα negative (-) if ≤10% stained positive, based on the maximum value of the three cores. The Fisher's exact test was used to examine if staining positivity for FRα differed by histologic type, invasiveness and stage. Because folate may have differential effects early (prior to tumor formation) versus late (after establishment of precursor lesion) in ovarian carcinogenesis 29, all the dietary exposures were assessed in two ways. First, we used baseline intake reported in 1980 as a measure of past intake since prior intake may reduce the risk of ovarian cancer. Second, we examined intake in the questionnaire cycle immediately preceding the report of ovarian cancer. This provided a measure of recent

intake that may increase risk by promoting the growth of existing malignant cells. For the analyses, we created dichotomous variables for intake based on the distribution in the control subjects or to match cut-points at which associations were observed in our previous studies 10(see Tables for cut-points). Median (10^{th} - 90^{th} percentile) dietary and total folate intake among the controls was 286.70 (191.00-383.00) and 403.26 (215.33-722.00) μg/day, respectively. We also created a methyl group score based on alcohol consumption and dietary folate or methionine intake 30. A high methyl group score was defined as alcohol intake of <3 g/d (< median) and dietary folate or methionine in the top tertile; a low methyl group score was defined as alcohol intake ≥3 g/d (≥ median) of alcohol and dietary folate or methionine in the lowest tertile; and all other scores were defined as intermediate. Multivitamin and folic acid supplement use (not asked until 1984) was coded as regular use (yes, no).

Unconditional logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of developing ovarian cancer comparing high versus low intakes of the dietary factors. We used polytomous unconditional logistic regression 31 to estimate risk for each case group (FRα positive [+] and FRα negative [-]) referenced to the controls, simultaneously. To determine whether the ORs across case groups differed, we compared a model holding the association of the exposure variable and ovarian cancer constant across case groups to one allowing the association to vary, using the likelihood ratio test 31. *A priori*, models were adjusted for the following potential confounders: age, number of pregnancies, menopausal status at diagnosis, and lactose intake. Tests for trend were conducted by modeling the quintile median concentrations and calculating the Wald statistic. *P* values were based on two-sided tests and considered significant at ≤0.05. Analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Results

Descriptive characteristics of 152 ovarian cancer cases and 649 control subjects

The characteristics of the 152 ovarian cancer cases with FRα expression data and 649 healthy control subjects are shown in Table 1. We have previously shown that eligible cases in the NHS cohort through 2002 (n=672), those with any tissue sample (i.e., a tumor block or slide; 51%of eligible cases), and those cases included in our tissue microarrays (26% of eligible cases) were similar with respect to age at diagnosis, stage, and known risk factors for ovarian cancer 27. Of the cases, 60 (39%) expressed and 92 (61%) did not express FRα. Controls were more likely to have used oral contraceptives or had a tubal ligation compared to cases. Ovarian cancer risk factors did not appear to differ between the FRα+ and FRα- cases. There was a significant difference in the distribution of FRα staining tumors according to histologic subtype (P<0.001). Expression of FRα in the serous, endometrioid, mucinous, and clear cell subtypes was 55%, 30%, 7%, and 14%, respectively. A higher proportion of the stage 1 or 2 cancers were FRα- (80%) while the prevalence of the FRα+ and FRα- stage 3 or 4 tumors was similar (54% and 46%, respectively) (P<0.001). FRα expression also was significantly associated with tumor morphology (P=0.004). Ninety-four percent of the borderline tumors did not express FRα while 43% of invasive tumors were FRα+ and 57% were FRα-.

Past and recent folate, methionine, and vitamin B_6 intake

Dietary folate intake, in the past and more recently, were both inversely associated with the risk of developing ovarian cancer (Table 2); however, the association only achieved statistical significance with recent intake of ≥300 μg/day compared to < 300 μg/day (OR=0.62; 95%CI 0.40-0.96). The results did not vary by FRα status of the tumor (P-heterogeneity=0.40). In contrast, we observed a non-significant positive association between

total folate (dietary and supplemental) intake of ≥500 versus <500 μg/day and risk (OR=1.42; 95%CI 0.94-2.14; *P*-trend = 0.16 for past intake and OR=1.53; 95%CI 0.99-2.37 *P*-trend=0.19 for recent intake). The relationship between total folate intake and ovarian cancer was similar for FRα+ and FRα- tumors (*P*-heterogeneity ≥0.57).

We did not observe any clear associations between dietary vitamin B_6 intake of ≥2 versus <2mg/day and risk (e.g., OR=0.90; 95%CI 0.54-1.48; *P*-trend=0.83 for past intake)(Table 2) (*P*-heterogeneity=0.32). There was no significant association between past (≥1.26 versus <1.26 g/day; OR=1.23; 95%CI 0.56-2.71) or recent (OR=0.99; 95%CI 0.54-1.83) intake of methionine with risk; however, the association with past intake varied somewhat by FRα status (*P*-heterogeneity=0.07)(data not shown). Lower past intake was not clearly associated with developing FRα+ (OR=1.89; 95%CI 0.44-8.05; *P*-trend = 0.05) or FRα- cancer (OR=1.03; 95%CI 0.42-2.52; *P*-trend = 0.72).

Past and recent multivitamin or folic acid supplement use, and methyl group score

Overall, there was no association between past or recent multivitamin use and risk of ovarian cancer (OR=1.16; 95%CI 0.79-1.69 and OR=0.94; 95%CI 0.65-1.35, respectively) (Table 3); although, there was evidence for heterogeneity in the association between recent multivitamin use based on FRα status of the tumor (*P*-heterogeneity = 0.05). Women who recently used multivitamins for more than two years were at a suggestive increased risk of developing FRα+ (OR=1.63; 95%CI 0.35-7.50) and possibly decreased risk of developing FRα- (OR=0.75; 95%CI 0.45-1.27) ovarian tumors; however, the confidence intervals were wide and should be interpreted with caution. Overall, use of folic acid supplements was not clearly associated with risk of developing ovarian cancer compared to non-users (OR=1.51; 95%CI 0.68-3.36). There was no evidence for heterogeneity in the association based on FRα expression (*P*-heterogeneity=0.70).

Women with the highest methyl group score were at a significantly lower risk of developing ovarian cancer compared to the reference group (OR=0.44; 95%CI 0.23-0.84 and OR=0.46; 95%CI 0.24-0.88, past and recent intakes, respectively)(Table 3). An inverse association among women in the intermediate methyl group was also observed, although this only achieved statistical significance for past intake (OR=0.47; 95%CI 0.32-0.70). The relationship between the recent methyl group score and risk was similar for FRα+ and FRα- tumors (*P*-heterogeneity=0.36); however, the decreased risk with a high (versus low) past methyl group score was stronger among FRα- tumors (*P*-heterogeneity=0.09), with a significant 76% reduction in the risk of developing FRα- tumors (95%CI 0.09-0.62).

The results were similar when we used the cumulative average of intakes reported an all the previous FFQ's compared with intake from the questionnaire cycle immediately preceding the report of ovarian cancer (recent intake) (data not shown). In an analysis of daily alcohol consumption, we observed a significantly increased risk of ovarian cancer with consumption of ≥5 compared with <5 mg of alcohol per day irrespective of timing of intake and FRα status of the tumor (OR=2.11; 95%CI 1.45-3.06 and OR=1.63; 95%CI 1.09-2.44, past and recent, respectively) (data not shown).

Discussion

Ours is the first study that has examined whether the relationship between folate (and dietary factors involved in one-carbon metabolism) and risk of ovarian cancer differs by FRα expression of the tumor. We observed an inverse association between folate from food sources and risk; whereas, there was a positive association with total folate, which includes both food sources and supplemental intake. These relationships were similar irrespective of the timing of intake (past versus recent), as well as the FRα status of the tumor (FRα+ versus

FRα-). A high methyl group score (e.g., high methionine or folate and low alcohol intakes) also was associated with a lower risk, which was suggestively stronger for FRα- tumors. There were no clear associations between methionine, vitamin B_6, or multivitamin use and risk. Although based on a small sample of 152 cases, these data suggest that the inconsistencies in the literature thus far describing the association between folate co-factors and the risk of ovarian cancer cannot be attributed to a modifying role of timing of intake or FRα status.

In the only other study to investigate epidemiologic factors with FRα expression, multivitamin intake in the year prior to diagnosis was associated with decreased risk of ovarian cancer regardless of FRα tumor expression level 32, but multivitamin intake among cases was associated with a three-fold likelihood of having a strong-expressing FRα ovarian tumor 32. In contrast, we did not find an association between past multivitamin use and overall ovarian cancer risk; however, women who recently used multivitamins for at least two years were at a non-significantly 63% increased risk of developing FRα+ and 25% decreased risk of developing FRα- tumors compared to never users. The difference in the proportion of tumors with elevated FRα expression between the prior study and our study may explain the heterogeneity in the results. For example, 61% of tumors were classified as FRα strong-expressing tumors and 39% had absent/weak/moderate expression in the Kelemen *et al.* study 32, 33; we observed that 39% of tumors were FRα+ (i.e., >10% of cells stained positive). Although the classifications were different, one might have expected the lower cut-off for positivity in our study to have captured a larger proportion of FRα+ tumors if they were present. In a *post hoc* analysis of our data using the same classification as the Kelemen study, the results for multivitamin use did not change substantially (data not shown).

Technical explanations for the different results are unsatisfying. Differences due to the antibody used by Kelemen *et al.* (Pu17 polyclonal vs. mAb343 monoclonal antibody) are unlikely since recent analyses of the Mayo Clinic tumors using the mAb343 antibody similarly reported 72% of tumors staining positive for FRα 32, 33. We previously have shown no effect of tissue age on antigen preservation in this cohort of women 27 and we did not observe any differences in staining quality when stratified by year of diagnosis for the FRα stain (data not shown). Despite these differences, both studies 33 reported proportionately higher FRα+ staining among serous and endometrioid histologies and lower FRα + staining for mucinous tumors. Although speculative, another reason for the differences in the distribution of FRα+ staining tumors may be that the Kelemen cases were identified post-folic acid food fortification, while 96% of NHS cases were diagnosed in the pre-fortification period. Because these are the only two studies with representative population samples that report on the distribution of FRα in ovarian tumors, prospective follow-up for incident cases of ovarian cancer post-fortification in ours and other studies will be crucial to understanding the role of FRα expression in ovarian cancer.

Dietary intake of ≥300 μg of folate/day was associated with a 16-38% decrease in the risk of developing ovarian cancer in our study. This effect was strongest with recent rather than past intake. Collectively, three of four prospective evaluations have reported similar weak, non-significant inverse associations between dietary folate intake and the risk of ovarian cancer 8-10. Furthermore, the protective effect was strongest among women who consumed alcohol in all of the studies except for the prospective analysis of the NHS 7-10. The results from four case-control studies have been null and none of them looked at effect modification by alcohol intake 3-6. Our findings of a positive association with total folate intake (past and recent) are also in accordance with the results from the Iowa Women's Health Study and the larger NHS study, which both reported non-significant positive associations between total folate intake and risk (RR=1.73; 9; *P*-trend = 0.20 and RR=1.21; *P*-trend = 0.05,

respectively) 7, 10. The current study and our prior analysis in the entire NHS cohort had slightly different results (the current study observed stronger associations in general). This may be due to the use of cumulative average intake in our previous report 10 versus intake in the cycle prior to cancer diagnosis in this analysis. Additional differences between these two studies include the larger sample size of the earlier analysis and use of quintiles of exposure versus dichotomous intake. We found no evidence for heterogeneity of folate-related risk by FRα status suggesting that the potential effect of folate on ovarian carcinogenesis does not act through this receptor.

The relationship between past methionine intake and risk varied by FRα status of the tumor. There was suggestion of an increased risk with higher intakes for FRα+ but not FRα- tumors. No clear associations were observed for dietary or total B_6 intakes. These findings require further validation given the limited statistical power and lack of a dose-response relationship. In the prospective analysis of the NHS, Tworoger *et al.* reported no significant associations between methionine and total vitamin B_6 intakes and risk 10, but the authors did not explicitly examine associations by timing of intake. In their case-control study, Bidoli *et al.* similarly reported no association for vitamin B_6 intake 3. Finally, we observed that women with a high methyl score (past or recent) had a significant 54-56% reduction in ovarian cancer risk, irrespective of FRα expression and timing. This score was developed to take into account intake of important methyl group donors (i.e. folate and methionine) in addition to alcohol consumption, a known folate antagonist 34. The consistency in these results implies that overall methyl status, as opposed to intake of individual methyl group donors, may be more important in ovarian cancer prevention. The significant increase in risk with alcohol consumption requires further evaluation and is likely due to chance since Tworoger *et al.* found no evidence for an association of alcohol with ovarian cancer risk in the prospective analysis of the entire cohort 35.

A dual modulatory role of folate in the etiology of colorectal and breast cancers depending on the timing and dose of the intervention has been described 36, 37. In normal epithelial tissues, folate deficiency predisposes and modest levels suppress neoplastic transformation; whereas, supraphysiological doses enhance the development of tumors 29, 38. In contrast, folate deficiency has an inhibitory effect whereas folate supplementation has a promoting effect on the progression of established neoplasms 29, 36. The data from the current study supports this hypothesis. Although not significant, there was suggestion of an increased risk of developing ovarian cancer with use of folic acid supplements and high levels of total folate (i.e. supplemental and dietary) while folate from food sources was protective, irrespective of the timing of intake. It is possible that folate from diet and supplements may act differently at the receptor level. Folic acid is more stable and has a higher bioavailability than dietary folate since the former is absorbed with almost twice the efficacy of dietary folate 39, 40. Normally following ingestion, folic acid is normally reduced and methylated by dihyrofolate reductase to 5-methyltetrahydrofolate, the main circulating form of folate. However, at levels > 300 μg this process is saturated and folic acid is transported in the unmetabolized form which may preferentially bind to the folate receptor 41, 42. Given the relatively few number of women who used folic acid supplement in this cohort, the suggestive positive association between folic acid supplement use and risk requires corroboration. Thus, similar to the literature for other cancers, our findings confirm that both the dose and formulation of folate may play important roles for ovarian carcinogenesis.

Similar to what has previously been described, we found that ovarian tumors that expressed FRα were mostly serous invasive and at advanced stage (i.e. either stage 3 or 4), suggestive of poorer prognosis compared with FRα- tumors 12¯14, 32. Despite this, only the associations between methionine intake and multivitamin use varied by FRα status of the tumor, and because of multiple statistical comparisons in the current paper, these findings

may be attributed to chance and should be replicated in future studies. Upregulation of FRα expression is a common feature in epithelial ovarian cancer; however, it is not clear whether FRα expression is an early or late event in tumorigenesis 12 or what is its exact function in tumorigenesis. While it was initially thought that FRα facilitated increased folate uptake to promote tumor cell differentiation, current evidence suggests that FRα works through another mechanism as it accounts for <25% of folate uptake in tumor cells 14, 43, 44. This may explain why FRα status did not differentially affect our results for folate intake as we initially hypothesized.

This study had several strengths and weaknesses. Due to the small sample size, we lacked the power to detect modest effects. Furthermore, we were not able to stratify our analyses by histologic subtype or evaluate a modifying role of other factors (e.g., alcohol). Despite these limitations, this is the first study to date that has evaluated whether the association between folate, methionine and vitamin B_6 with the risk of epithelial ovarian cancer varied by FRα status of the tumor. The prospective nature of the NHS allows for the detailed collection of unbiased risk factor information. More importantly, given the potential dual modulatory role of folate in carcinogenesis, we were able to evaluate dietary and supplemental exposures in the past as well as in more recent years. In addition, we used TMAs which minimized variability in the results due to differences in experimental conditions.

These data suggest that modest levels of dietary folate may reduce the risk of ovarian cancer, while higher levels of folate (supplemental and dietary) may increase risk. Given the small sample size, these findings require replication in future studies. It is imperative to clarify these relationships because folate intake in the United States has increased dramatically with mandated food fortification. More importantly, we observed increased risk at levels similar to the current recommended daily intake of 400 μg/day and is similar to the mean total folate intake in this study population (433 μg/day)45. Because few modifiable risk factors for ovarian cancer exist, along with the potentially important role of dose and formulation of this nutrient in carcinogenesis, the possible role of folate and FRα in the etiology of ovarian cancer risk warrants further consideration.

Acknowledgments

Financial support: This research was supported by Research Grants P01 CA87969, CA49449, and P50 CA105009 from the National Cancer Institute. J.K. is a Research Fellow of the Canadian Cancer Society supported through an award from the National Cancer Institute of Canada. LEK is supported by a Canadian Institutes of Health Research Investigator award.

References

1. Zhang SM. Role of vitamins in the risk, prevention, and treatment of breast cancer. Curr Opin Obstet Gynecol. 2004; 16:19–25. [PubMed: 15128003]
2. Kim YI. Folate, colorectal carcinogenesis, and DNA methylation: lessons from animal studies. Environ Mol Mutagen. 2004; 44:10–25. [PubMed: 15199543]
3. Bidoli E, La Vecchia C, Talamini R, Negri E, Parpinel M, Conti E, Montella M, Carbone MA, Franceschi S. Micronutrients and ovarian cancer: a case-control study in Italy. Ann Oncol. 2001; 12:1589–93. [PubMed: 11822759]
4. Salazar-Martinez E, Lazcano-Ponce EC, Gonzalez Lira-Lira G, Escudero-De los Rios P, Hernandez-Avila M. Nutritional determinants of epithelial ovarian cancer risk: a case-control study in Mexico. Oncology. 2002; 63:151–7. [PubMed: 12239450]
5. McCann SE, Freudenheim JL, Marshall JR, Graham S. Risk of human ovarian cancer is related to dietary intake of selected nutrients, phytochemicals and food groups. J Nutr. 2003; 133:1937–42. [PubMed: 12771342]

6. Pelucchi C, Mereghetti M, Talamini R, Negri E, Montella M, Ramazzotti V, Franceschi S, La Vecchia C. Dietary folate, alcohol consumption, and risk of ovarian cancer in an Italian case-control study. Cancer Epidemiol Biomarkers Prev. 2005; 14:2056–8. [PubMed: 16103462]
7. Kelemen LE, Sellers TA, Vierkant RA, Harnack L, Cerhan JR. Association of folate and alcohol with risk of ovarian cancer in a prospective study of postmenopausal women. Cancer Causes Control. 2004; 15:1085–93. [PubMed: 15801492]
8. Larsson SC, Giovannucci E, Wolk A. Dietary folate intake and incidence of ovarian cancer: the Swedish Mammography Cohort. J Natl Cancer Inst. 2004; 96:396–402. [PubMed: 14996861]
9. Navarro Silvera SA, Jain M, Howe GR, Miller AB, Rohan TE. Dietary folate consumption and risk of ovarian cancer: a prospective cohort study. Eur J Cancer Prev. 2006; 15:511–5. [PubMed: 17106331]
10. Tworoger SS, Hecht JL, Giovannucci E, Hankinson SE. Intake of folate and related nutrients in relation to risk of epithelial ovarian cancer. Am J Epidemiol. 2006; 163:1101–11. [PubMed: 16554344]
11. Strohle A, Wolters M, Hahn A. Folic acid and colorectal cancer prevention: molecular mechanisms and epidemiological evidence (Review). Int J Oncol. 2005; 26:1449–64. [PubMed: 15870856]
12. Toffoli G, Cernigoi C, Russo A, Gallo A, Bagnoli M, Boiocchi M. Overexpression of folate binding protein in ovarian cancers. Int J Cancer. 1997; 74:193–8. [PubMed: 9133455]
13. Toffoli G, Russo A, Gallo A, Cernigoi C, Miotti S, Sorio R, Tumolo S, Boiocchi M. Expression of folate binding protein as a prognostic factor for response to platinum-containing chemotherapy and survival in human ovarian cancer. Int J Cancer. 1998; 79:121–6. [PubMed: 9583724]
14. Figini M, Ferri R, Mezzanzanica D, Bagnoli M, Luison E, Miotti S, Canevari S. Reversion of transformed phenotype in ovarian cancer cells by intracellular expression of anti folate receptor antibodies. Gene Ther. 2003; 10:1018–25. [PubMed: 12776159]
15. Colditz GA. The nurses' health study: a cohort of US women followed since 1976. J Am Med Womens Assoc. 1995; 50:40–4. [PubMed: 7722205]
16. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. J Womens Health. 1997; 6:49–62. [PubMed: 9065374]
17. Colditz GA, Hankinson SE. The Nurses' Health Study: lifestyle and health among women. Nat Rev Cancer. 2005; 5:388–96. [PubMed: 15864280]
18. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol. 1985; 122:51–65. [PubMed: 4014201]
19. Colditz GA, Martin P, Stampfer MJ, Willett WC, Sampson L, Rosner B, Hennekens CH, Speizer FE. Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. Am J Epidemiol. 1986; 123:894–900. [PubMed: 3962971]
20. Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE. The use of a self-administered questionnaire to assess diet four years in the past. Am J Epidemiol. 1988; 127:188–99. [PubMed: 3337073]
21. Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B, Willett WC. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int J Epidemiol. 1989; 18:858–67. [PubMed: 2621022]
22. U.S. Department of Agriculture A. Agricultural Handbook No 8 Series. Washington, DC: Department of Agriculture, Government Printing Office; 1993. Composition of foods-raw, processed, and prepared.
23. U.S. Department of Agriculture A. Release 11: Nutrient Data Laboratory Home Page. Washington, DC: Department of Agriculture, Government Printing Office; 1996. USDA Nutrient Database for Standard Reference.
24. U.S. Department of Agriculture A. Release 14: Nutrient Data Laboratory Home Page. Washington, DC: Department of Agriculture, Government Printing Office; 2001. USDA Nutrient Database for Standard Reference.
25. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol. 1986; 124:17–27. [PubMed: 3521261]

26. Stampfer MJ, Willett WC, Speizer FE, Dysert DC, Lipnick R, Rosner B, Hennekens CH. Test of the National Death Index. Am J Epidemiol. 1984; 119:837–9. [PubMed: 6720679]
27. Hecht JL, Kotsopoulos J, Gates MA, Hankinson SE, Tworoger SS. Validation of tissue microarray technology in ovarian cancer: results from the Nurses' Health Study. Cancer Epidemiol Biomarkers Prev. 2008; 17:3043–50. [PubMed: 18990746]
28. Shia J, Klimstra DS, Nitzkorski JR, Low PS, Gonen M, Landmann R, Weiser MR, Franklin WA, Prendergast FG, Murphy L, Tang LH, Temple L, et al. Immunohistochemical expression of folate receptor alpha in colorectal carcinoma: patterns and biological significance. Hum Pathol. 2008; 39:498–505. [PubMed: 18342661]
29. Kim YI. Will mandatory folic acid fortification prevent or promote cancer? Am J Clin Nutr. 2004; 80:1123–8. [PubMed: 15531657]
30. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low-methionine--low-folate diets, and risk of colon cancer in men. J Natl Cancer Inst. 1995; 87:265–73. [PubMed: 7707417]
31. Glynn RJ, Rosner B. Methods to evaluate risks for composite end points and their individual components. J Clin Epidemiol. 2004; 57:113–22. [PubMed: 15125620]
32. Kelemen LE, Sellers TA, Keeney GL, Lingle WL. Multivitamin and alcohol intake and folate receptor alpha expression in ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2005; 14:2168–72. [PubMed: 16172227]
33. Kalli KR, Oberg AL, Keeney GL, Christianson TJ, Low PS, Knutson KL, Hartmann LC. Folate receptor alpha as a tumor target in epithelial ovarian cancer. Gynecol Oncol. 2008; 108:619–26. [PubMed: 18222534]
34. Hillman RS, Steinberg SE. The effects of alcohol on folate metabolism. Annu Rev Med. 1982; 33:345–54. [PubMed: 6805415]
35. Tworoger SS, Gertig DM, Gates MA, Hecht JL, Hankinson SE. Caffeine, alcohol, smoking, and the risk of incident epithelial ovarian cancer. Cancer. 2008; 112:1169–77. [PubMed: 18213613]
36. Kim YI. Role of folate in colon cancer development and progression. J Nutr. 2003; 133:3731S–9S. [PubMed: 14608107]
37. Kim YI. Does a high folate intake increase the risk of breast cancer? Nutr Rev. 2006; 64:468–75. [PubMed: 17063929]
38. Kotsopoulos J, Sohn KJ, Martin R, Choi M, Renlund R, McKerlie C, Hwang SW, Medline A, Kim YI. Dietary folate deficiency suppresses N-methyl-N-nitrosourea-induced mammary tumorigenesis in rats. Carcinogenesis. 2003; 24:937–44. [PubMed: 12771039]
39. Sanderson P, McNulty H, Mastroiacovo P, McDowell IF, Melse-Boonstra A, Finglas PM, Gregory JF 3rd. Folate bioavailability: UK Food Standards Agency workshop report. Br J Nutr. 2003; 90:473–9. [PubMed: 12908910]
40. Hannon-Fletcher MP, Armstrong NC, Scott JM, Pentieva K, Bradbury I, Ward M, Strain JJ, Dunn AA, Molloy AM, Kerr MA, McNulty H. Determining bioavailability of food folates in a controlled intervention study. Am J Clin Nutr. 2004; 80:911–8. [PubMed: 15447898]
41. Kelly P, McPartlin J, Goggins M, Weir DG, Scott JM. Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. Am J Clin Nutr. 1997; 65:1790–5. [PubMed: 9174474]
42. Ghandour H, Chen Z, Selhub J, Rozen R. Mice deficient in methylenetetrahydrofolate reductase exhibit tissue-specific distribution of folates. J Nutr. 2004; 134:2975–8. [PubMed: 15514261]
43. Miotti S, Bagnoli M, Ottone F, Tomassetti A, Colnaghi MI, Canevari S. Simultaneous activity of two different mechanisms of folate transport in ovarian carcinoma cell lines. J Cell Biochem. 1997; 65:479–91. [PubMed: 9178098]
44. Corona G, Giannini F, Fabris M, Toffoli G, Boiocchi M. Role of folate receptor and reduced folate carrier in the transport of 5-methyltetrahydrofolic acid in human ovarian carcinoma cells. Int J Cancer. 1998; 75:125–33. [PubMed: 9426700]
45. Medicine Io. Folate Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press; 1998. p. 195-305.

Table 1

Characteristics of 152 Ovarian Cancer Cases and 649 Controls From the Nurses' Health Study

Characteristic	FRα +	FRα-	Controls
N (%)	60 (39%)	92 (61%)	649
Age at diagnosis, mean (SD)	63.8 (7.4)	60.02 (9.5)	n/a
Parity, mean (SD)[a]	4.3 (1.8)	3.6 (1.6)	4.0 (1.62)
Oral contraceptive use, ever, N (%)	24 (40%)	36 (39%)	293 (45%)
Tubal ligation, ever, N (%)	8 (13%)	13 (14%)	123 (19%)
Body mass index in 1990, mean (SD)	25.5 (4.7)	24.5 (4.7)	25.4 (4.6)
Histology, N (%)[b]			
Serous	42 (55%)	35 (45%)	n/a
Endometrioid	10 (30%)	23 (70%)	
Mucinous	1 (7%)	14 (93%)	
Clear cell	2 (14%)	12 (86%)	
Other[c]	5 (38%)	8 (62%)	
P[d]		<0.001	
Disease stage, N (%)			
I, II	13 (20%)	52 (80%)	n/a
III, IV	47 (54%)	40 (46%)	
P		<0.001	
Morphology, N (%)			
Borderline	1 (6%)	15 (94%)	n/a
Invasive	59 (43%)	77 (57%)	
P		0.004	

[a] Among parous women.

[c] The histological classification in Table 2 was based on abstraction of the pathology report.

[c] Other includes Brenner, carcinosarcoma, mixed, other, or unknown.

[d] *P* - value calculated using Fisher's exact test.

Table 2

Relationship Between Folate, Methionine and Vitamin B_6 Intake and the Risk of Ovarian Cancer in All Cases and by FRα Expression

	All Cases		FRα +		FRα-		
	OR (95%CI)[1]	*P*-trend[2]	OR (95%CI)	*P*-trend	OR (95%CI)	*P*-trend	*P*-heterogeneity[3]
Past Dietary Folate[4]	1.00 (ref)		1.00 (ref)		1.00 (ref)		
<300 vs ≥300 μg/d	0.84 (0.56-1.28)	0.11	0.78 (0.41-1.50)	0.24	0.88 (0.53-1.45)	0.24	0.83
Recent Dietary Folate[5]	1.00 (ref)		1.00 (ref)		1.00 (ref)		
<300 vs ≥300 μg/d	0.62 (0.40-0.96)	0.13	0.49 (0.24-1.01)	0.12	0.70 (0.42-1.18)	0.43	0.40
Past Total Folate[4]	1.00 (ref)		1.00 (ref)		1.00 (ref)		
<500 vs ≥500μg/d	1.42 (0.94-2.14)	0.16	1.52 (0.82-2.81)	0.38	1.36 (0.83-2.24)	0.24	1.00
Recent Total Folate[5]	1.00 (ref)		1.00 (ref)		1.00 (ref)		
<500 vs ≥500μg/d	1.53 (0.99-2.37)	0.19	1.41 (0.71-2.78)	0.62	1.61 (0.96-2.69)	0.13	0.57
Past Dietary Vitamin B_6[4]	1.00 (ref)		1.00 (ref)		1.00 (ref)		
<2 vs ≥2 mg/d	0.90 (0.54-1.48)	0.83	0.88 (0.40-1.91)	0.37	0.91 (0.50-1.68)	0.42	0.83
Recent Dietary Vitamin B_6	[5] 1.00 (ref)		1.00 (ref)		1.00 (ref)		
<2 vs ≥2 mg/d	0.75 (0.50-1.13)	0.47	0.98 (0.49-1.94)	0.91	0.56 (0.32-0.99)	0.11	0.32
Past Total Vitamin B_6[4]	1.00 (ref)		1.00 (ref)		1.00 (ref)		
<3 vs ≥3 mg/d	1.30 (0.88-1.93)	0.69	1.36 (0.75-2.45)	0.78	1.26 (0.79-2.03)	0.80	0.95
Recent Total Vitamin B_6[5]	1.00 (ref)		1.00 (ref)		1.00 (ref)		
3 vs ≥3 mg/d	1.16 (0.79-1.71)	0.92	1.19 (0.61-2.34)	0.11	0.95 (0.56-1.62)	0.67	0.31

[1] Adjusted for age (continuous), menopausal status (premenopausal, postmenopausal), number of pregnancies (continuous), lactose intake (continuous).

[2] *P* for trend was determined using the quintile median concentrations and calculated using the Wald statistic.

[3] *P* for heterogeneity was calculated using polytomous logistic regression.

[4] N for past intake = 55, 93, and 613 for FRα+, FRα-, and controls respectively.

[5] N for recent intake = 39, 72, and 482 for FRα+, FRα-, and controls respectively.

Table 3

Relationship Between Multivitamin Use, Folic Acid Use and the Methyl Group Score and the Risk of Ovarian Cancer in All Cases and by FRα Expression

	OR (95%CI)[1]			
	All Cases	**FRα +**	**FRα-**	***P*-heterogeneity[2]**
Past Multivitamin Use[3]	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Regular Use (yes, no)	1.16 (0.79-1.69)	1.15 (0.65-2.04)	1.16 (0.74-1.83)	0.96
Recent Multivitamin Use[4]	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Regular Use (yes, no)	0.94 (0.65-1.35)	1.65 (0.86-3.18)	0.75 (0.45-1.27)	0.05
Past Folic Acid Supplement Use[5]	1.00 (ref)	1.00 (ref)	1.00 (ref)	n/a
Regular Use (yes, no)	n/a	n/a	n/a	
Recent Folic Acid Supplement Use[4]	1.00 (ref)	1.00 (ref)	1.00 (ref)	0.70
Regular Use (yes, no)	1.51 (0.68-3.36)	1.63 (0.35-7.50)	2.25 (0.78-6.52)	
Past Methyl Group Score[3,6]	1.00 (ref)	1.00 (ref)	1.00 (ref)	
2	0.47 (0.32-0.70)	0.51 (0.28-0.94)	0.46 (0.29-0.73)	
3	0.44 (0.23-0.84)	0.86 (0.38-1.94)	0.24 (0.09-0.62)	0.09
Recent Methyl Group Score[4,6]	1.00 (ref)	1.00 (ref)	1.00 (ref)	
2	0.81 (0.50-1.32)	0.96 (0.38-2.43)	0.69 (0.35-1.36)	
3	0.46 (0.24-0.88)	0.31 (0.08-1.16)	0.50 (0.22-1.15)	0.36

[1] Adjusted for age (continuous), menopausal status (premenopausal, postmenopausal), number of pregnancies (continuous), lactose intake (continuous).

[2] *P* for heterogeneity was calculated using polytomous logistic regression and modeling the quintile median concentrations.

[3] N for past intake = 55, 93, and 613 for FRα+, FRα-, and controls respectively.

[4] N for recent intake = 39, 72, and 482 for FRα+, FRα-, and controls respectively.

[5] n/a = not applicable because folic acid supplement use was not asked until the 1984 FFQ.

[6] Low methyl group score (reference group): ≥ 3 g/d of alcohol and dietary folate or methionine in lowest tertile; intermediate methyl group score (2): others; high methyl group score (3): < 3 g/d of alcohol and dietary folate or methionine in top tertile.