

Published in final edited form as:

Int J Cancer. 2012 August 15; 131(4): E518–E529. doi:10.1002/ijc.26455.

Folate, vitamin B₆, vitamin B₁₂, methionine and alcohol intake in relation to ovarian cancer risk

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Abstract

Folate, methionine, vitamin B₆, and vitamin B₁₂ may influence carcinogenesis due to their roles in the one-carbon metabolism pathway which is critical for DNA synthesis, methylation, and repair. Low intake of these nutrients has been associated with an increased risk of breast, colon, and endometrial cancers. Previous studies that have examined the relation between these nutrients and ovarian cancer risk have been inconsistent and have had limited power to examine the relation by histologic subtype. We investigated the association between folate, methionine, vitamin B₆, vitamin B₁₂, and alcohol among 1910 women with ovarian cancer and 1989 controls from a case-control study conducted in eastern Massachusetts and New Hampshire from 1992 to 2008. Diet was assessed via food frequency questionnaire. Participants were asked to recall diet one-year before diagnosis or interview. Logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (95% CIs). We also examined whether the associations varied by ovarian cancer histologies using polytomous logistic regression. We observed an inverse association between dietary vitamin B₆ (covariate-adjusted OR=0.76, 95% CI 0.64–0.92; $p_{\text{trend}}=0.002$) and methionine intake (covariate-adjusted OR=0.72, 95% CI=0.60–0.87; $p_{\text{trend}}<0.001$) and ovarian cancer risk comparing the highest to lowest quartile. The association with dietary vitamin B₆ was strongest for serous borderline (covariate-adjusted OR=0.49, 95% CI=0.32–0.77; $p_{\text{trend}}=0.001$) and serous invasive (covariate-adjusted OR=0.74, 95% CI=0.58–0.94; $p_{\text{trend}}=0.012$) subtypes. Overall, we observed no significant association between folate and ovarian cancer risk. One-carbon metabolism related nutrients, especially vitamin B₆ and methionine, may lower ovarian cancer risk.

Keywords

ovarian cancer; folate; alcohol; methionine; B-vitamins

INTRODUCTION

Folate, methionine, vitamin B₆, and vitamin B₁₂ may influence carcinogenesis due to their roles in the one-carbon metabolism pathway which is critical for DNA synthesis, methylation, and repair. In addition, alcohol and methionine, may influence folate's physiologic effects. Alcohol inhibits folate at many levels including intestinal absorption, transport to tissues, storage, and release by the liver¹ while methionine contributes to DNA

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methylation. Furthermore, some studies have found low intake of folate, methionine, vitamin B₆, vitamin B₁₂, and higher intake of alcohol have been associated with an increased risk of breast^{2,3}, colon cancer⁴⁻⁶, and endometrial cancer⁷⁻⁹. Folate receptor alpha, which is overexpressed in 90% of serous ovarian cancers¹⁰, has been shown to impart a growth advantage *in vitro* and has been identified as a potential target for immunologic therapies^{11,12}. Furthermore, folate receptor alpha expression is correlated with stage and grade of ovarian cancer, suggesting this pathway may be relevant to ovarian carcinogenesis and progression¹⁰.

Case-control studies that have examined the relation between dietary folate and ovarian cancer in women observed no association¹³⁻¹⁶, while most prospective studies have suggested a modest inverse association¹⁷⁻¹⁹. The few studies that have examined the associations of methionine and vitamin B₆ with ovarian cancer risk have observed no association^{13,19}, and the one study that examined the association with vitamin B₁₂ reported an inverse association¹⁶. Results for alcohol have been inconsistent²⁰⁻³⁴; but, a pooling project with 10 cohort studies reported no association³⁵. Most of the previous studies examining the relation between these nutrients and ovarian cancer had limited power to examine the relation by histologic subtype. In addition, these studies occurred in populations not exposed to folic acid grain supplementation which was implemented by the United States in 1998.

In this study, we investigated whether folate, methionine, vitamin B₆, vitamin B₁₂ and alcohol intake were associated with ovarian cancer risk in the New England Case-Control Study. We also examined whether the associations were modified by the time period before and after folic acid grain supplementation, alcohol consumption, two polymorphisms of 5,10-methylene-tetrahydrofolate reductase (*MTHFR*), and histologic subtype.

MATERIALS AND METHODS

Study population

Data from the New England Case-Control Study (NECC) of ovarian cancer come from three enrollment phases (phase 1 1992–1997, phase 2 1998–2002, phase 3 2003–2008) corresponding to three funding periods. Details regarding case and control enrollment are described elsewhere³⁶. Briefly, 3957 women residing in eastern Massachusetts or New Hampshire with a diagnosis of incident ovarian cancer were identified through hospital tumor boards and statewide cancer registries. Of these 3083 (78%) were eligible and 2203 (71%) agreed to participate. Controls were identified through a combination of random digit dialing, drivers' license lists, and town resident lists. In the first phase, 420 (72%) of the eligible women identified through random digit dialing agreed to participate and 102 (51%) of the eligible women identified through townbooks agreed to participate. In the second and third phases, 4366 potential controls were identified, 2940 (67%) were eligible, 1362 (46%) declined to participate by phone or by mail via an "opt-out" postcard, and 1578 (54%) were enrolled. Controls were frequency matched to cases on age and state of residence.

All study participants were interviewed at the time of enrollment about known and suspected ovarian cancer risk factors. To avoid the possible impact of pre-clinical disease on exposure status, cases were asked about exposures that occurred at least one-year before diagnosis, and controls were asked about exposures that occurred more than one year before the interview date. This study was approved by the Institutional Review Boards of Brigham and Women's Hospital and Dartmouth Medical School; each participant provided a signed informed consent.

Dietary assessment

Dietary data was collected using a semiquantitative food frequency questionnaire (FFQ), which asks how frequently food and beverage items were consumed (using common serving sizes) during the past year. Possible responses range from “almost never” to “six or more times per day”. Intake of folate, methionine, vitamin B₆ and vitamin B₁₂ were calculated by multiplying the frequency of consumption by the nutrient content of a serving size determined from the food composition values available from the US Department of Agriculture³⁷. Total folate, total vitamin B₆, total vitamin B₁₂ include intake from multivitamins and supplements, while the dietary folate, dietary vitamin B₆, and dietary vitamin B₁₂ do not. The FFQ has been previously validated^{38–40} and has been shown to provide valid estimates of vitamin B₆, vitamin B₁₂ and alcohol intake with correlation coefficients between the FFQ and 1-week diet records of 0.58 for vitamin B₆⁴⁰, 0.56 for vitamin B₁₂³⁸, and 0.90 for alcohol intake³⁹. In addition, total folate as measured by the FFQ and erythrocyte folate concentration were correlated in Nurses’ Health Study ($r=0.55$)⁴¹. Methionine intake has not been validated with diet records. All dietary factors were adjusted for total energy using the residual method⁴².

Enrollment phase was used as a proxy for exposure to folic acid grain supplementation which was mandated by the United States in 1998. Participants in phase 2 (1998–2002) and phase 3 (2003–2008) were classified as exposed to folate supplementation (post-supplementation) while participants in phase 1 (1992–1997) were considered not exposed to supplementation (pre-supplementation).

Genotyping methods

A detailed description of genotyping methods has been described previously.⁴³ Briefly, over 95% of participants provided a blood sample. We genotyped *MTHFR* SNPs C677T (rs1801133) and A1298C (rs1801131) on whole genome amplified DNA from phase 1 and 2 participants at the Dana Farber Harvard Cancer Center High Throughput Polymorphism Detection Core. Genotyping assays were performed by the 5′ nuclease assay (Taqman®) on the Applied Biosystems Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, California). Concordance between the replicate samples was 100%.

Statistical analysis

Women were excluded if they did not complete a food frequency questionnaire ($n=134$) had an implausibly high (> 3500 calories) or low (< 500 calories) total energy intake ($n=123$). Intake of folate, methionine, vitamin B₆, vitamin B₁₂, and alcohol were categorized into quartiles based on the distribution among the controls. The quartile cutpoints for each nutrient were: dietary folate 268.6, 268.7–332.2, 332.3–400.6, 400.7 (mcg/day), total folate 325.5, 325.6–486.2, 486.3–724.3, 724.4 (mcg/day), methionine 1.5, 1.6–1.7, 1.8–2.0, 2.1 (g/day), dietary vitamin B₆ 1.5, 1.6–1.7, 1.8–2.0, 2.1 (mg/day), total vitamin B₆ 1.8, 1.9–2.7, 2.8–4.2, 4.3 (mg/day), dietary vitamin B₁₂ 3.6, 3.7–4.8, 4.9–6.0 (mg/day), 6.1, total vitamin B₁₂ 4.7, 4.8–8.3, 8.4–12.5, 12.6 (mg/day) and alcohol 0, 0.1–2.2, 2.3–8.2, 8.3 (g/day).

We calculated odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression. In the simple model we adjusted for age (continuous), study center (Massachusetts or New Hampshire) and total energy intake (continuous). In covariate-adjusted models, we adjusted for the following *a priori* potential confounders: oral contraceptive use (< 3, 3–24, 25–60, 61 months), parity (0, 1, 2, 3 liveborn), tubal ligation (yes, no), and family history of ovarian cancer (yes, no). In addition, race/ethnicity, education, and lactose intake were also assessed but were not included in the final models as they did not materially alter the risk estimates. Tests for trend were calculated using a trend

variable based on the median nutrient value for each quartile category. To examine the joint effects of folate, methionine, and alcohol we calculated a methyl score⁴. A high methyl score was defined as alcohol intake <5 g/day and intake of folate and methionine in the highest quartile and a low methyl score was defined as alcohol intake ≥15 g/day and intake of folate and methionine in the lowest quartile. All other combinations were considered an intermediate methyl score.

Effect modification by alcohol intake (<5 g/day, ≥5 g/day), *MTHFR* polymorphism, and implementation of folate supplementation were assessed with a likelihood ratio test comparing a model with interaction terms and main effects to a model with only main effects. Analyses involving the *MTHFR* SNPs were restricted to Caucasian study participants because allele frequencies varied by race. Polytomous logistic regression was used to test for heterogeneity in the effect estimates by ovarian cancer histologies (serous borderline, serous invasive, mucinous, endometrioid, clear cell). Statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc, Cary, NC).

RESULTS

A total of 1910 women with ovarian cancer and 1989 controls were included in the final analytic sample. Cases were more likely than controls to be nulliparous but were less likely to have taken oral contraceptives or have had a tubal ligation (Table 1). Total energy (kcal/day) was higher among cases while energy-adjusted intake of dietary and total folate and vitamin B₆ were slightly lower in cases than controls, but standard deviations were large.

We observed a non-significant inverse association between dietary folate and ovarian cancer risk (covariate-adjusted OR=0.88, 95% CI 0.74–1.06) comparing the highest to lowest quartile (Table 2). This association was similar for total folate, which includes folate from non-dietary sources (covariate-adjusted OR=0.90, 95% CI=0.75–1.08). We observed no association between multivitamins (which make up approximately 30% of the total folate variable) and ovarian cancer (covariate-adjusted OR = 0.95, 95% CI=0.83–1.08). When we considered the association between dietary folate and ovarian cancer before the Food and Drug Administration (FDA) required folic acid supplementation of grains in 1998, the reduction in ovarian cancer risk for women in the highest quartile of folate intake was more apparent for women enrolled prior to supplementation (covariate-adjusted OR=0.57, 95% CI=0.37–0.87) compared to those enrolled after supplementation (covariate-adjusted OR=1.02, 95% CI=0.81–1.28) ($p_{\text{interaction}}=0.001$) (Table 3).

We observed an inverse association between methionine intake and ovarian cancer risk (Table 2). Compared to women in the lowest quartile of methionine intake, women in the highest quartile had a significant reduction in ovarian cancer risk (covariate-adjusted OR=0.72, 95% CI=0.60–0.87; $p_{\text{trend}}<0.001$). An inverse association was also observed between dietary vitamin B₆ and ovarian cancer risk (covariate-adjusted OR=0.76, 95% CI 0.63–0.91; $p_{\text{trend}}=0.002$). This association was similar for total vitamin B₆ intake (covariate-adjusted OR=0.73, 95% CI=0.61–0.88), however the trend was no longer significant ($p_{\text{trend}}=0.076$). No association was observed for dietary vitamin B₁₂ or total vitamin B₁₂. When we considered mutual adjustment for folate, vitamin B₆, vitamin B₁₂, and methionine the association with methionine remained significant (covariate-adjusted OR=0.75, 95% CI=0.60–0.93; $p_{\text{trend}}=0.003$) while the association with vitamin B₆ was borderline significant (covariate-adjusted OR=0.80, 95% CI 0.64–1.01; $p_{\text{trend}}=0.059$).

For women in the highest two quartiles of alcohol intake there was the suggestion of an inverse association with ovarian cancer (covariate-adjusted OR for third quartile=0.77, 95% CI=0.64–0.92 and fourth quartile=0.86, 95% CI=0.72–1.03) compared to women in the

lowest quartile although the trend was not significant ($p_{\text{trend}}=0.206$) (Table 2). When women with any intake of alcohol were compared to those with no alcohol intake, alcohol drinkers had a 17% lower risk of ovarian cancer compared to non-drinkers (95% CI=0.73–0.96), but this was attenuated after adjustment for education and race (OR=0.89, 95% CI=0.77–1.03).

Alcohol consumption was most strongly inversely associated with risk among women whose total folate was below the median value in the controls (486.3 mcg/day). The covariate-adjusted OR in this group was 0.70 (95% CI=0.55–0.90; $p_{\text{trend}}=0.002$) comparing the highest to lowest quartile. The corresponding value for women above the median was 1.06 (95% CI=0.82–1.36; $p_{\text{trend}}=0.286$) (data not shown). In addition, the association between total folate and ovarian cancer differed by alcohol consumption (<5 g/d vs ≥ 5 g/d) ($p_{\text{interaction}}=0.049$). Among women consuming <5 g/day a statistically significant inverse association was observed (covariate-adjusted OR=0.78, 95% CI=0.62–0.98; $p_{\text{trend}}=0.047$) while total folate was not associated with ovarian cancer risk in women consuming ≥ 5 g/d. A similar pattern was observed for dietary folate, dietary B₆ and B₁₂, and total B₆ and B₁₂ but these results were not significant (all $p_{\text{interaction}}>0.05$) (Table 4). The association between methionine and ovarian cancer did not vary by alcohol consumption.

A high methyl score (alcohol intake <5 g/day and intake of folate and methionine in the highest quartile) was non-significant inverse associated with ovarian cancer risk with a covariate-adjusted OR of 0.57 (95% CI 0.30–1.11) compared to those with a low methyl score (alcohol intake ≥ 15 g/day and intake of folate and methionine in the lowest quartile) (Table 2); however, these results are based on small numbers in the low methyl group.

No interactions were observed by the C677T polymorphism (Supplemental Table 1) and only one significant interaction was observed by the A1298C polymorphism (Supplemental Table 2). Methionine was statistically significantly inversely associated with ovarian cancer risk among women who were wildtype for A1298C (covariate-adjusted OR=0.59, 95% CI 0.40–0.86), while this association was not significant among women who were heterozygous or homozygous for the minor allele ($p_{\text{interaction}}=0.011$).

We also evaluated the association between dietary factors and risk of ovarian cancer defined by histologic subtypes. The association with dietary vitamin B₆ differed significantly by histologic subtype ($p_{\text{heterogeneity}}=0.027$) with the strongest inverse associations observed for the serious borderline (covariate-adjusted OR=0.49, 95% CI=0.31–0.76; $p_{\text{trend}}=0.001$) and serous invasive (covariate-adjusted OR=0.74, 95% CI=0.58–0.94; $p_{\text{trend}}=0.012$) subtypes. No statistically significant differences by histologic subtype were observed for the other nutrients or alcohol (Table 5).

DISCUSSION

Overall, we observed a decreased risk of ovarian cancer with intake of methionine and dietary vitamin B₆ but no overall association with folate or B₁₂. However, we observed an association between dietary folate and ovarian cancer before the mandatory folate supplementation of grains in 1998. In addition, there was the suggestion of a nonlinear inverse association between alcohol intake and ovarian cancer.

Four previous case-control studies and four cohort studies have evaluated the association between folate and ovarian cancer. All four case-control studies reported no significant association between dietary folate and ovarian cancer risk^{13–16}, though one reported a non-significant increase in risk¹⁶ and another reported a non-significant decreased risk limited to the fifth quintile¹⁴. Among three of the cohort studies, dietary folate was inversely associated with ovarian cancer risk in some subgroups^{17–19} while in one study dietary folate was associated with a non-significant increase in risk²⁴. Total folate was associated with a

non-significant increase in ovarian cancer risk in both studies that examined this association^{19, 24}. We observed effect estimates that were inverse but not significant for both dietary and total folate in our main analyses. Different associations between folate intake and ovarian cancer observed across studies may be due to varying ranges of folate intake in different populations; the cutpoints for dietary folate categories varied across studies from 204 µg/day in the highest category for the Swedish Mammography Cohort¹⁷ to >425 µg/day in the Western New York Diet Study¹⁴. Our effect estimates for dietary folate assessed from 1992–1997, the time period that overlaps with previous studies, showed a decreased risk of ovarian cancer with increasing dietary folate intake, a result that is consistent with the most of the cohort studies as well as the other U.S. based case-control study.

Some have expressed concern that folic acid fortification may unintentionally increase cancer risk as folate has been shown to influence carcinogenesis differently depending on the dose and timing of exposure^{44, 45}. Folate deficiency in normal cells may play a part in initiating carcinogenesis while folate supplementation may promote the growth of existing tumors⁴⁶. In our study, we observed a decreased risk of ovarian cancer risk with dietary folate intake before supplementation and no association after supplementation. As our study asked participants to recall dietary folate one year before diagnosis, this finding may suggest that higher levels of dietary folate do not have a promoting effect on preclinical tumors and may help prevent ovarian cancer carcinogenesis in a low folate setting. To our knowledge no previous studies of folate and ovarian cancer have stratified by supplementation as most studies in the United States were before 1998 or in countries that do not supplement the food supply. However, in the Nurses' Health Study II (NHS II) no association was observed between folate and breast cancer⁴⁷ while in the Nurses' Health Study (NHS) an inverse association was observed between folate intake and ER-negative breast cancer⁴⁸. The authors suggest this may partially be due to the higher levels of post-fortification folate intake in the NHS II population as this population had folate levels in the first quintile that were in the range of the second quintile in NHS⁴⁷.

To date, only one study¹⁹, has examined the direct association between methionine and ovarian cancer risk, while two studies^{13, 19} have examined the association with vitamin B₆, and no associations were observed between these nutrients and ovarian cancer. In contrast, we observed significant inverse associations between methionine and dietary vitamin B₆ and ovarian cancer risk. In addition, we observed that the associations with dietary vitamin B₆ varied by histologic subtype with the strongest associations for serous borderline and serous invasive. Differences between our study and previous results may be due to differences in the distribution of ovarian cancer histologies in these studies as well as the range of dietary exposures in each population. In addition to playing a role the one-carbon metabolism pathway vitamin B₆ may influence ovarian cancer risk through its antioxidant properties. Laboratory studies have demonstrated that vitamin B₆ is effective at scavenging free radicals which if not properly controlled can promote carcinogenesis⁴⁹.

The literature regarding alcohol and ovarian cancer is mixed with studies reporting null results^{20, 22, 25, 29–32} as well as increased^{17, 28, 33} and decreased^{21, 23, 24} risk of ovarian cancer with some associations varying by folate intake. A large pooling project involving ten cohort studies reported no association between alcohol³⁵ and ovarian cancer. Our results suggested a decreased risk with alcohol intake that may be limited to women with lower intake of folate, though confounding by socioeconomic status cannot be ruled out.

We are the first to investigate whether *MTHFR* SNPs C677T and A1298C modify the association between one-carbon metabolism related nutrients and ovarian cancer. A previous analysis in a subset of this population found no association between either SNP and overall ovarian cancer risk but significant associations were observed for both SNPs and serous

ovarian cancer. However, these findings were not replicated in the Nurses' Health Study or the Mayo Clinic Ovarian Cancer Case-Control Study due to differences in the case populations⁴³. We observed a stronger inverse association for methionine among women who were wildtype for A1298C perhaps indicating methionine is more effective in reducing ovarian cancer risk among individuals without reduced *MTHFR* activity. However, given our overall findings between *MTHFR* SNPs and ovarian cancer differed from other studies these results should be interpreted with caution.

Our study has limitations stemming from its case-control design. Our dietary assessment is limited to a short time period that may not be the most relevant time period in relation to ovarian cancer development. In order to avoid capturing changes to diet immediately before or after diagnosis, we have asked women to recall their diet one year before diagnosis or interview for controls. Despite these efforts, women with preclinical disease one year before diagnosis may have changed their dietary habits due to symptoms, making it difficult to distinguish whether their diet influenced disease onset or their disease influenced dietary changes. Secondly, as with any case-control study, recall bias is a possibility. However, we observed associations with dietary and as well as known risk factors for ovarian cancer that were similar to those reported by cohort studies, making dietary changes due to preclinical symptoms or recall bias less likely. In addition, one-carbon metabolism related nutrients have not been established as risk factors for ovarian cancer and thus it is unlikely that cases reported their intake of foods containing these nutrients differently than controls. Finally, women with the most aggressive cases may have died before they could be enrolled in the study; therefore, dietary factors associated with survival could influence our results.

In addition, limited variability in the diet across the study population may have restricted our ability to detect an effect of a particular nutrient on cancer risk. For instance, the median alcohol intake in our population is two g/d which corresponds to less than half a drink per day. Considering 10 g/d of alcohol is needed to increase breast cancer risk 10%⁵⁰, an association of similar magnitude with ovarian cancer could be easily missed in our study. Similarly, FDA-mandated supplementation essentially eliminated folate deficiency in the United States and thereby diminished our ability to detect an association between folate and ovarian cancer risk. Since our study with the introduction of folate supplementation a spurious association could be observed between folate and ovarian cancer if cases and controls are not enrolled at the same time. Consequently, we adjusted for time of enrollment to minimize this potential bias. Additionally, our results may not be generalizable to populations folate supplementation is not mandatory. Finally, as we examined associations between nine exposure variables and three potential effect modifiers, as well as by histologic subtype, the potential for chance associations cannot be ruled out.

Strengths of our study include its large sample size. With over 1900 cases and 1900 controls, we are able to evaluate not only main effects but also histologic subtypes and potential effect modifiers. Additionally we have comprehensive dietary data and information on many important covariates.

In conclusion, we observed that vitamin B₆ and methionine may decrease ovarian cancer risk and that the association between dietary folate and ovarian cancer differed before and after folic acid grain supplementation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by National Cancer Institute grants CA054419 and P50 CA105009 and the Department of Defense Ovarian Cancer Academy.

Abbreviations

OR	odds ratio
95% CI	95% confidence interval
MTHFR	5,10-methylene-tetrahydrofolate reductase
NEC	New England Case-Control Study
SNP	single-nucleotide polymorphism
mcg	microgram
g	gram
mg	milligram
kcal	kilocalorie

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Novelty and impact

This study investigated whether one-carbon metabolism related nutrients were associated with ovarian cancer risk and whether these associations were modified by the time period before and after folic acid grain supplementation, alcohol consumption, two polymorphisms of 5,10-methylene-tetrahydrofolate reductase (*MTHFR*), and histologic subtype. Few studies have had the power to examine these potential interactions and to our knowledge no previous studies of folate and ovarian cancer have stratified by supplementation as most studies in the United States were before 1998 or in countries that do not supplement the food supply. We observed that vitamin B₆ and methionine may decrease ovarian cancer risk and that the association between dietary folate and ovarian cancer differed before and after folic acid grain supplementation. In addition, the association between total folate intake and ovarian cancer risk differed by alcohol consumption.

Table 1

Characteristics of study participants, New England Case-Control Study, 1992–2008

	Case	Control
n	1910	1989
Age (years), mean (SD)	54 (12)	53 (13)
Age at menarche, mean (SD)	13 (1)	13 (2)
BMI (kg/m ²) at reference, mean (SD)	26 (6)	26 (6)
Multivitamin Use, n (%)		
Yes	979 (52)	1038 (53)
No	919 (48)	928 (47)
Oral contraceptive use, n (%)	1003 (53)	1263 (64)
Number liveborn, n (%)		
0	616 (32)	353 (18)
1	272 (14)	253 (13)
2	500 (26)	634 (32)
3	522 (27)	749 (38)
Tubal ligation, n (%)	256 (13)	392 (20)
Family History of Ovarian cancer, n (%)	90 (5)	54 (3)
Total Calories (kcal), mean (SD)	1886 (574)	1851 (563)
Nutrients, mean (SD)		
Dietary folate (mcg/day)	346 (128)	350 (121)
Total Folate (mcg/day)	539 (283)	560 (299)
Methionine (g/day)	1.7 (0.4)	1.7 (0.4)
Dietary B ₆ (mg/day)	1.7 (0.5)	1.8 (0.5)
Total B ₆ (mg/day)	9.3 (25.7)	10.1 (27.0)
Dietary B ₁₂ (mg/day)	5.6 (3.5)	5.4 (3.0)
Total B ₁₂ (mg/day)	12.8 (16.3)	13.7 (19.5)
Alcohol (g/day) among drinkers, mean (SD)	8.8 (10.5)	8.7 (9.9)
Non-drinkers, n(%)	671 (35)	602 (30)
Tumor histology, n (%)		
Serous Borderline	226 (12)	
Serous Invasive	791 (41)	
Mucinous	217 (11)	
Endometrioid	306 (16)	
Clear Cell	243 (13)	
Other/undifferentiated	127 (7)	

Table 2

Odds ratios (OR) and 95% confidence intervals (CI) of ovarian cancer by quartile of one-carbon metabolism related nutrients, New England Case-Control Study, 1992–2008

Nutrient	Controls n (%)	Cases n (%)	OR (95% CI)*	MV Adjusted [†] OR (95% CI)	P _{trend} [‡]
Dietary folate					
Q1	498 (25)	521 (27)	1.00	1.00	0.18
Q2	505 (25)	469 (25)	0.89 (0.75, 1.06)	0.89 (0.74, 1.06)	
Q3	458 (23)	409 (21)	0.85 (0.71, 1.02)	0.84 (0.69, 1.01)	
Q4	528 (27)	511 (27)	0.93 (0.78, 1.10)	0.88 (0.74, 1.06)	
Total folate					
Q1	502 (25)	527 (28)	1.00	1.00	0.43
Q2	481 (24)	431 (23)	0.83 (0.69, 1.00)	0.82 (0.68, 0.99)	
Q3	487 (24)	460 (24)	0.87 (0.73, 1.04)	0.85 (0.70, 1.02)	
Q4	519 (26)	492 (26)	0.90 (0.75, 1.07)	0.90 (0.75, 1.08)	
Methionine					
Q1	516 (26)	592 (31)	1.00	1.00	<0.001
Q2	421 (21)	412 (22)	0.85 (0.71, 1.02)	0.87 (0.72, 1.05)	
Q3	610 (31)	549 (29)	0.78 (0.66, 0.92)	0.79 (0.67, 0.94)	
Q4	442 (22)	357 (19)	0.71 (0.59, 0.85)	0.72 (0.60, 0.87)	
Dietary B ₆					
Q1	518 (26)	582 (30)	1.00	1.00	0.002
Q2	460 (23)	438 (23)	0.84 (0.70, 1.00)	0.85 (0.71, 1.02)	
Q3	538 (27)	465 (24)	0.76 (0.64, 0.90)	0.76 (0.64, 0.91)	
Q4	473 (24)	425 (22)	0.79 (0.66, 0.94)	0.76 (0.63, 0.91)	
Total B ₆					
Q1	507 (25)	594 (31)	1.00	1.00	0.08
Q2	472 (24)	379 (20)	0.65 (0.54, 0.78)	0.63 (0.52, 0.77)	
Q3	533 (27)	502 (26)	0.77 (0.65, 0.92)	0.78 (0.65, 0.93)	
Q4	477 (24)	435 (23)	0.75 (0.63, 0.90)	0.73 (0.61, 0.88)	
Dietary B ₁₂					
Q1	476 (24)	491 (26)	1.00	1.00	0.76

Nutrient	Controls n (%)	Cases n (%)	OR (95% CI)*	MV Adjusted [†] OR (95% CI)	P _{trend} [‡]
Q2	539 (27)	486 (25)	0.87 (0.73, 1.03)	0.91 (0.76, 1.09)	
Q3	465 (23)	422 (22)	0.87 (0.73, 1.05)	0.91 (0.76, 1.10)	
Q4	509 (26)	511 (27)	0.95 (0.80, 1.14)	0.95 (0.79, 1.14)	
Total B ₁₂					
Q1	466 (23)	463 (24)	1.00	1.00	0.56
Q2	522 (26)	502 (26)	0.94 (0.78, 1.12)	0.95 (0.79, 1.15)	
Q3	506 (25)	458 (24)	0.89 (0.74, 1.07)	0.89 (0.74, 1.08)	
Q4	495 (25)	487 (26)	0.97 (0.80, 1.16)	0.93 (0.77, 1.12)	
Alcohol					
Q1	602 (30)	671 (35)	1.00	1.00	0.21
Q2	390 (20)	376 (20)	0.86 (0.72, 1.03)	0.88 (0.73, 1.07)	
Q3	503 (25)	411 (22)	0.73 (0.62, 0.87)	0.77 (0.64, 0.92)	
Q4	494 (25)	452 (24)	0.83 (0.70, 0.98)	0.86 (0.72, 1.03)	
Methyl score [§]					
Low	23 (1)	28 (1)	1.00	1.00	0.14
Middle	1882 (95)	1813 (95)	0.79 (0.46, 1.38)	0.66 (0.37, 1.18)	
High	84 (4)	69 (4)	0.68 (0.36, 1.29)	0.57 (0.30, 1.11)	

* Adjusted for age, study center, total energy.

[†] Adjusted for age, study center, total energy, oral contraceptive use, parity, tubal ligation, and family history of ovarian cancer.

[‡] Tests for trend were performed using the median of the interval for each quartile and correspond to the MV adjusted analyses. Medians for dietary categories in controls are as follows: dietary folate (234.0 mg/day, 300.5, 362.5, 465.5), total folate (257.6 mg/day, 397.0, 613.4, 856.5), alcohol (0 g/day, 1.2, 4.6,

14.5) methionine (1.3 g/day, 1.6, 1.8, 2.2), dietary B₆ (1.3 mg/day, 1.6, 1.8, 2.2), total B₆ (1.5 mg/day, 2.1, 3.5, 7.3), dietary B₁₂ (2.9 mg/day, 4.2, 5.3, 8.2), total B₁₂ (3.7 mg/day, 6.0, 10.2, 25.0).

[§] A low methyl score was defined as alcohol intake < 15 g/d and folate and methionine intake in the lowest quartile; a high methyl score was defined as alcohol intake > 5 g/d and folate and methionine intake in the highest quartile; all other were considered the middle score.

Table 3

Odds ratios (OR) and 95% confidence intervals (CI) of ovarian cancer by quartile of one-carbon metabolism related nutrients, pre (1992–97) and post (1998–2008) folic acid grain supplementation enrollment status*, New England Case-Control Study

Nutrient	Participants enrolled between 1992–1997				Participants enrolled between 1998–2008			
	Cases n (%)	Controls n (%)	MV Adjusted [†] OR (95% CI)	P _{interaction} [‡]	Cases n (%)	Controls n (%)	MV Adjusted [†] OR (95% CI)	P _{interaction} [‡]
Dietary folate								
Q1	285 (54)	240 (48)	1.00	0.002	236 (17)	258 (17)	1.00	0.94
Q2	119 (23)	124 (25)	0.73 (0.53,1.00)		350 (25)	381 (26)	1.02 (0.80,1.29)	
Q3	67 (13)	76 (15)	0.65 (0.44,0.96)		342 (25)	382 (26)	0.96 (0.75,1.21)	
Q4	54 (10)	63 (13)	0.57 (0.37,0.87)		457 (33)	465 (31)	1.02 (0.81,1.28)	
Total folate								
Q1	275 (52)	248 (49)	1.00	0.70	252 (18)	254 (17)	1.00	0.38
Q2	95 (18)	130 (26)	0.67 (0.48,0.94)		346 (25)	366 (25)	0.86 (0.67,1.09)	
Q3	100 (19)	83 (16)	0.98 (0.69,1.39)		350 (25)	389 (26)	0.80 (0.63,1.02)	
Q4	55 (11)	42 (8)	1.12 (0.71,1.76)		437 (32)	477 (32)	0.87 (0.69,1.09)	
Methionine								
Q1	192 (37)	157 (31)	1.00	0.02	400 (29)	359 (24)	1.00	0.005
Q2	119 (23)	101 (20)	0.99 (0.70,1.40)		293 (21)	320 (22)	0.84 (0.67,1.04)	
Q3	135 (26)	144 (29)	0.75 (0.54,1.04)		414 (30)	466 (31)	0.80 (0.65,0.98)	
Q4	79 (15)	101 (20)	0.66 (0.46,0.97)		278 (20)	341 (23)	0.73 (0.59,0.91)	
Dietary B ₆								
Q1	201 (38)	141 (28)	1.00	<0.0001	381 (28)	377 (25)	1.00	0.16
Q2	102 (19)	107 (21)	0.65 (0.45,0.93)		336 (24)	353 (24)	0.94 (0.76,1.16)	
Q3	112 (21)	119 (24)	0.62 (0.44,0.88)		353 (25)	419 (28)	0.83 (0.67,1.02)	
Q4	110 (21)	136 (27)	0.49 (0.35,0.70)		315 (23)	337 (23)	0.88 (0.70,1.09)	
Total B ₆								
Q1	215 (41)	185 (37)	1.00	0.42	379 (27)	322 (22)	1.00	0.15
Q2	118 (22)	140 (28)	0.69 (0.50,0.97)		261 (19)	332 (22)	0.60 (0.48,0.76)	
Q3	104 (19)	85 (17)	1.01 (0.70,1.45)		398 (29)	448 (30)	0.72 (0.58,0.89)	

Nutrient	Participants enrolled between 1992–1997				Participants enrolled between 1998–2008				
	Cases n (%)	Controls n (%)	MV Adjusted [†] OR (95% CI)	P _{trend} [‡]	Cases n (%)	Controls n (%)	MV Adjusted [†] OR (95% CI)	P _{trend} [‡]	P _{interaction}
Q4	88 (17)	93 (18)	0.77 (0.53,1.11)		347 (25)	384 (26)	0.71 (0.57,0.89)		
Dietary B ₁₂									
Q1	158 (30)	124 (25)	1.00	0.57	333 (24)	352 (24)	1.00	0.93	0.59
Q2	128 (24)	139 (28)	0.75 (0.53,1.06)		358 (26)	400 (27)	1.00 (0.81,1.24)		
Q3	101 (19)	121 (24)	0.69 (0.47,0.99)		321 (23)	344 (23)	1.02 (0.82,1.28)		
Q4	138 (26)	119 (24)	0.85 (0.59,1.21)		373 (27)	390 (26)	1.01 (0.81,1.25)		
Total B ₁₂									
Q1	160 (30)	160 (32)	1.00	0.87	303 (22)	306 (21)	1.00	0.60	0.60
Q2	160 (30)	154 (31)	1.11 (0.80,1.54)		342 (25)	368 (25)	0.89 (0.71,1.12)		
Q3	110 (21)	109 (22)	1.01 (0.70,1.45)		348 (25)	397 (27)	0.86 (0.69,1.08)		
Q4	95 (18)	80 (16)	1.07 (0.73,1.58)		392 (28)	415 (28)	0.89 (0.71,1.12)		
Alcohol									
Q1	193 (37)	176 (35)	1.00	0.58	478 (35)	426 (29)	1.00	0.08	0.14
Q2	114 (22)	108 (21)	1.03 (0.73,1.46)		262 (19)	282 (19)	0.83 (0.67,1.04)		
Q3	100 (19)	116 (23)	0.86 (0.61,1.23)		311 (22)	387 (26)	0.74 (0.60,0.91)		
Q4	118 (22)	103 (20)	1.11 (0.78,1.57)		334 (24)	391 (26)	0.79 (0.64,0.97)		

* Enrollment phase was used as a proxy for exposure to folic acid grain supplementation. Participants in phase 2 (1998–2002) and phase 3 (2003–2008) were classified as post-supplementation and participants in phase 1 (1992–97) were classified as pre-supplementation.

[†] Adjusted for age, study center, total energy, oral contraceptive use, parity, tubal ligation, and family history of ovarian cancer.

[‡] Tests for trend were performed using the median of the interval for each quartile.

Table 4

Odds ratios (OR) and 95% confidence intervals (CI) of ovarian cancer by quartile of one-carbon metabolism related nutrients stratified by alcohol intake, New England Case-Control Study, 1992–2008

Nutrient	<5 g/day				5 g/day				Pinteraction
	Cases n (%)	Controls n (%)	MV Adjusted* OR (95% CI)	Ptrend [†]	Cases n (%)	Controls n (%)	MV Adjusted* OR (95% CI)	Ptrend [†]	
Dietary folate									
Q1	364 (29)	328 (26)	1.00	0.06	157 (25)	170 (24)	1.00	0.69	0.11
Q2	312 (24)	314 (24)	0.89 (0.71,1.12)		157 (25)	191 (27)	0.88 (0.64,1.21)		
Q3	261 (20)	288 (23)	0.77 (0.61,0.98)		148 (23)	170 (24)	0.99 (0.71,1.36)		
Q4	339 (27)	349 (27)	0.82 (0.65,1.02)		172 (27)	179 (25)	1.02 (0.74,1.40)		
Total folate									
Q1	367 (29)	319 (25)	1.00	0.05	160 (25)	183 (26)	1.00	0.17	0.05
Q2	298 (23)	308 (24)	0.80 (0.64,1.01)		134 (21)	178 (25)	0.87 (0.63,1.21)		
Q3	298 (23)	314 (25)	0.78 (0.62,0.99)		161 (25)	168 (24)	1.00 (0.73,1.39)		
Q4	313 (25)	338 (26)	0.78 (0.62,0.98)		179 (28)	181 (25)	1.18 (0.86,1.61)		
Methionine									
Q1	378 (30)	328 (26)	1.00	0.009	214 (34)	188 (26)	1.00	0.006	0.92
Q2	267 (21)	267 (21)	0.88 (0.70,1.11)		145 (23)	154 (22)	0.86 (0.63,1.17)		
Q3	377 (30)	392 (31)	0.80 (0.65,0.99)		172 (27)	218 (31)	0.76 (0.57,1.02)		
Q4	254 (20)	292 (23)	0.75 (0.59,0.94)		103 (16)	150 (21)	0.65 (0.46,0.90)		
Dietary B ₆									
Q1	387 (30)	320 (25)	1.00	0.0005	195 (31)	198 (28)	1.00	0.57	0.16
Q2	296 (23)	293 (23)	0.82 (0.65,1.03)		142 (22)	167 (23)	0.90 (0.66,1.23)		
Q3	309 (24)	344 (27)	0.71 (0.57,0.89)		156 (24)	194 (27)	0.84 (0.62,1.14)		
Q4	284 (22)	322 (25)	0.68 (0.54,0.85)		141 (22)	151 (22)	0.93 (0.68,1.28)		
Total B ₆									
Q1	414 (32)	304 (24)	1.00	0.002	180 (28)	203 (29)	1.00	0.24	0.91
Q2	258 (20)	312 (24)	0.55 (0.43,0.70)		121 (19)	160 (23)	0.82 (0.59,1.14)		
Q3	324 (25)	337 (26)	0.67 (0.54,0.84)		178 (28)	196 (28)	0.99 (0.73,1.34)		

Nutrient	<5 g/day				5 g/day				Pinteraction
	Cases n (%)	Controls n (%)	MV Adjusted* OR (95% CI)	Ptrend ‡	Cases n (%)	Controls n (%)	MV Adjusted* OR (95% CI)	Ptrend ‡	
Q4	280 (22)	326 (25)	0.58 (0.46,0.73)		155 (25)	151 (21)	1.11 (0.81,1.52)		
Dietary B ₁₂									
Q1	311 (24)	293 (23)	1.00	0.24	180 (28)	183 (26)	1.00	0.33	0.91
Q2	333 (26)	333 (26)	1.01 (0.80,1.27)		153 (24)	206 (29)	0.76 (0.56,1.03)		
Q3	283 (22)	298 (23)	0.93 (0.74,1.18)		139 (22)	167 (24)	0.87 (0.63,1.20)		
Q4	349 (27)	355 (28)	0.89 (0.71,1.12)		162 (26)	154 (22)	1.07 (0.78,1.47)		
Total B ₁₂									
Q1	311 (24)	284 (22)	1.00	0.05	152 (24)	182 (26)	1.00	0.10	0.18
Q2	348 (27)	334 (26)	0.94 (0.75,1.19)		154 (24)	188 (26)	0.95 (0.69,1.31)		
Q3	302 (24)	325 (25)	0.82 (0.65,1.04)		156 (25)	181 (25)	1.03 (0.75,1.42)		
Q4	315 (25)	336 (26)	0.79 (0.62,1.00)		172 (27)	159 (22)	1.24 (0.90,1.72)		

* Adjusted for age, study center, total energy, oral contraceptive use, parity, tubal ligation, and family history of ovarian cancer.

‡ Tests for trend were performed using the median of the interval for each quartile.

Table 5

Odds ratios* (OR) and 95% confidence intervals (CI) of ovarian cancer by quartile of one-carbon metabolism related nutrients by histologic subtype, New England Case-Control Study, 1992–2008

	Controls		Serous Borderline		Serous Invasive		Mucinous		Endometrioid		Clear Cell	
	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR
Dietary folate												
Q1	498 (25)	1.00	191 (24)	1.00	72 (33)	1.00	84 (28)	1.00	58 (24)	1.00	58 (24)	1.00
Q2	505 (25)	0.67 (0.46,0.98)	203 (26)	1.00 (0.79,1.28)	46 (21)	0.68 (0.45,1.01)	77 (25)	0.88 (0.63,1.24)	62 (26)	1.05 (0.71,1.54)	62 (26)	1.05 (0.71,1.54)
Q3	458 (23)	0.79 (0.54,1.15)	157 (20)	0.84 (0.65,1.08)	47 (22)	0.77 (0.52,1.14)	75 (25)	0.95 (0.67,1.34)	57 (24)	1.05 (0.70,1.55)	57 (24)	1.05 (0.70,1.55)
Q4	528 (26)	0.56 (0.38,0.84)	240 (31)	1.05 (0.83,1.33)	52 (24)	0.74 (0.50,1.09)	70 (23)	0.73 (0.51,1.03)	66 (27)	1.01 (0.69,1.48)	66 (27)	1.01 (0.69,1.48)
P _{heterogeneity} = 0.08												
P _{trend} = 0.01												
Total folate												
Q1	502 (25)	1.00	195 (25)	1.00	71 (33)	1.00	91 (30)	1.00	56 (23)	1.00	56 (23)	1.00
Q2	481 (24)	0.84 (0.58,1.22)	174 (22)	0.87 (0.68,1.12)	47 (22)	0.71 (0.48,1.06)	72 (23)	0.80 (0.57,1.12)	64 (26)	1.17 (0.79,1.72)	64 (26)	1.17 (0.79,1.72)
Q3	487 (25)	0.67 (0.48,1.05)	190 (24)	0.94 (0.74,1.20)	50 (23)	0.79 (0.54,1.17)	74 (24)	0.82 (0.58,1.15)	62 (26)	1.10 (0.74,1.62)	62 (26)	1.10 (0.74,1.62)
Q4	519 (26)	0.78 (0.52,1.16)	232 (29)	1.00 (0.79,1.26)	49 (23)	0.83 (0.56,1.23)	69 (23)	0.72 (0.51,1.02)	61 (25)	1.05 (0.70,1.55)	61 (25)	1.05 (0.70,1.55)
P _{heterogeneity} = 0.37												
P _{trend} = 0.17												
Alcohol												
Q1	602 (30)	1.00	288 (36)	1.00	63 (29)	1.00	113 (37)	1.00	81 (33)	1.00	81 (33)	1.00
Q2	390 (20)	0.92 (0.62,1.36)	149 (19)	0.89 (0.70,1.13)	46 (22)	1.06 (0.71,1.60)	48 (16)	0.66 (0.46,0.96)	55 (23)	1.09 (0.75,1.58)	55 (23)	1.09 (0.75,1.58)
Q3	503 (25)	0.76 (0.52,1.11)	163 (21)	0.81 (0.64,1.02)	41 (19)	0.69 (0.45,1.05)	72 (24)	0.77 (0.56,1.08)	58 (24)	0.87 (0.60,1.26)	58 (24)	0.87 (0.60,1.26)
Q4	494 (25)	0.80 (0.54,1.19)	191 (24)	0.90 (0.72,1.13)	67 (31)	1.26 (0.86,1.83)	73 (24)	0.81 (0.58,1.12)	49 (21)	0.73 (0.49,1.07)	49 (21)	0.73 (0.49,1.07)
P _{heterogeneity} = 0.15												
P _{trend} = 0.33												
Methionine												
Q1	517 (26)	1.00	245 (31)	1.00	66 (30)	1.00	79 (27)	1.00	64 (26)	1.00	64 (26)	1.00
Q2	421 (21)	0.78 (0.53,1.15)	163 (21)	0.85 (0.67,1.09)	46 (21)	0.88 (0.58,1.31)	77 (26)	0.93 (0.66,1.31)	58 (24)	1.16 (0.79,1.70)	58 (24)	1.16 (0.79,1.70)
Q3	610 (31)	0.69 (0.48,0.99)	226 (29)	0.79 (0.64,0.99)	62 (29)	0.83 (0.57,1.21)	74 (25)	0.89 (0.65,1.23)	71 (29)	0.98 (0.68,1.41)	71 (29)	0.98 (0.68,1.41)
Q4	442 (22)	0.50 (0.33,0.78)	157 (20)	0.79 (0.62,1.00)	43 (20)	0.80 (0.53,1.20)	62 (21)	0.67 (0.46,0.97)	50 (21)	0.95 (0.64,1.42)	50 (21)	0.95 (0.64,1.42)
P _{heterogeneity} = 0.23												
P _{trend} = 0.001												
Dietary B ₆												
Q1	518 (26)	1.00	230 (29)	1.00	67 (31)	1.00	96 (32)	1.00	66 (27)	1.00	66 (27)	1.00
P _{heterogeneity} = 0.04												
P _{trend} = 0.04												

	Controls		Serous Borderline		Serous Invasive		Mucinous		Endometrioid		Clear Cell	
	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR	N (%)	MV Adjusted OR
Q2	461 (23)	0.97 (0.68,1.39)	177 (23)	0.82 (0.65, 1.05)	52 (24)	0.96 (0.65,1.42)	60 (20)	0.71 (0.50,1.01)	53 (22)	0.92 (0.63,1.36)		
Q3	538 (27)	0.65 (0.44,0.95)	204 (26)	0.79 (0.62, 0.99)	51 (24)	0.81 (0.55,1.20)	73 (24)	0.73 (0.53,1.03)	52 (21)	0.77 (0.52,1.13)		
Q4	473 (24)	0.49 (0.31 0.76)	180 (23)	0.74 (0.58, 0.94)	47 (22)	0.88 (0.59,1.31)	77 (25)	0.83 (0.60,1.16)	72 (30)	1.14 (0.79,1.65)		
P _{heterogeneity} [‡] =0.03												
Total B ₆												
Q1	507 (25)	1.00	224 (28)	1.00	73 (33)	1.00	97 (32)	1.00	61 (25)	1.00		
Q2	472 (24)	0.48 (0.32,0.72)	149 (19)	0.66 (0.52,0.85)	51 (24)	0.79 (0.53,1.16)	69 (22)	0.73 (0.52,1.03)	53 (22)	0.90 (0.61,1.34)		
Q3	533 (27)	0.67 (0.46,0.97)	213 (27)	0.79 (0.63,1.00)	50 (23)	0.78 (0.53,1.15)	75 (25)	0.73 (0.52,1.03)	74 (30)	1.16 (0.80,1.68)		
Q4	477 (24)	0.54 (0.36,0.82)	205 (26)	0.79 (0.63,1.01)	43 (20)	0.78 (0.52,1.18)	65 (21)	0.67 (0.47,0.96)	55 (23)	0.93 (0.63,1.39)		
P _{heterogeneity} [‡] =0.45												
Dietary B ₁₂												
Q1	476 (24)	1.00	179 (23)	1.00	60 (28)	1.00	81 (26)	1.00	65 (27)	1.00		
Q2	539 (27)	0.86 (0.59,1.25)	208 (26)	1.08 (0.85,1.38)	57 (26)	0.87 (0.59,1.28)	69 (23)	0.78 (0.55,1.11)	57 (23)	0.80 (0.55,1.17)		
Q3	465 (23)	0.73 (0.48,1.11)	169 (21)	0.95 (0.74,1.22)	50 (23)	0.96 (0.64,1.43)	76 (25)	1.00 (0.70,1.41)	60 (25)	0.98 (0.67,1.43)		
Q4	509 (26)	0.87 (0.59,1.29)	235 (30)	1.10 (0.87,1.40)	50 (23)	0.91 (0.61,1.36)	80 (26)	0.90 (0.64,1.27)	61 (25)	0.85 (0.58,1.24)		
P _{heterogeneity} [‡] =0.84												
Total B ₁₂												
Q1	466 (23)	1.00	176 (22)	1.00	61 (28)	1.00	74 (24)	1.00	51 (21)	1.00		
Q2	522 (26)	1.04 (0.72,1.50)	177 (22)	0.88 (0.69,1.14)	62 (29)	0.95 (0.65,1.39)	92 (30)	1.09 (0.78,1.53)	67 (28)	1.16 (0.79,1.72)		
Q3	506 (25)	0.82 (0.55,1.22)	185 (23)	0.87 (0.68,1.12)	54 (25)	0.96 (0.64,1.42)	71 (23)	0.87 (0.61,1.25)	67 (28)	1.18 (0.80,1.76)		
Q4	495 (25)	0.73 (0.47,1.13)	253 (32)	1.07 (0.84,1.36)	40 (18)	0.82 (0.53,1.26)	69 (23)	0.81 (0.56,1.17)	58 (24)	1.01 (0.67,1.53)		
P _{heterogeneity} [‡] =0.10												
P _{trend} [‡] =0.33												
P _{trend} [‡] =0.43												
P _{trend} [‡] =0.01												
P _{trend} [‡] =0.41												
P _{trend} [‡] =0.09												
P _{trend} [‡] =0.58												
P _{trend} [‡] =0.73												

* Adjusted for age, study center, total energy, oral contraceptive use, parity, tubal ligation, and family history of ovarian cancer.

[‡] Determined using polytomous logistic regression.

[‡] Tests for trend were performed using the median of the interval for each quartile.