

Published in final edited form as:

Am J Epidemiol. 2006 January 15; 163(2): 108–115.

Folate, vitamin B₆, multivitamin supplements, and colorectal cancer risk in women

Shumin M. Zhang^{1,2}, Steven C. Moore³, Jennifer Lin¹, Nancy R. Cook¹, JoAnn E. Manson^{1,2,4}, I-Min Lee^{1,2}, and Julie E. Buring^{1,2,5}

¹ Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

² Department of Epidemiology, Harvard School of Public Health, Boston, MA.

³ Department of Epidemiology, Yale School of Public Health, New Haven, CT.

⁴ Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

⁵ Department of Ambulatory Care and Prevention, Harvard Medical School, Boston, MA.

Abstract

The authors evaluated associations between intakes of folate and vitamin B₆ and colorectal cancer risk in women enrolled in a randomized trial of aspirin and vitamin E. During an average of 10.1 years of follow-up, 220 colorectal adenocarcinoma cases were documented among 37,916 women, aged 45 years or older, free of cancer and cardiovascular disease, who provided dietary information at baseline. Intakes of total folate and vitamin B₆ were not significantly associated with the risk of colorectal cancer. However, dietary intakes of folate and vitamin B₆ were significantly inversely associated with the risk of colorectal cancer among women not taking supplements containing folate and vitamin B₆. The multivariable relative risks (95% confidence intervals) comparing the highest to the lowest quintile were 1.16 (0.76, 1.79) for total folate, 1.14 (0.77, 1.69) for total vitamin B₆, 0.46 (0.26, 0.81) for dietary folate, and 0.69 (0.41, 1.15) for dietary vitamin B₆. The use of multivitamin supplements was not related to colorectal cancer risk. These findings suggest that dietary folate and vitamin B₆ may reduce the risk of colorectal cancer in women. An alternative explanation is that other factors related to dietary intakes of folate and vitamin B₆ account for the inverse associations.

Keywords

folate; vitamin B₆; colorectal cancer

Because of their roles in the maintenance of intracellular normal DNA synthesis and methylation (1-3), folate and vitamin B₆ have been hypothesized to be associated with reduced risk of colorectal cancer. Folate participates in regeneration of methionine, which is converted into S-adenosylmethionine, a methyl donor for DNA methylation (1, 4). Deficient folate thus can reduce the availability of S-adenosylmethionine for DNA methylation (1, 2) and may thereby influence gene expression (2). Aberrant DNA methylation patterns are frequently seen in colorectal tumors, with wide areas of hypomethylation along the genome accompanied by regional hypermethylation at specific sites, particularly cytosine-guanine rich areas, termed CpG islands (5). Folate and vitamin B₆ function as coenzymes in the synthesis of purines and thymidylate for DNA. Low levels of these vitamins may result in misincorporation of uracil into DNA, leading to chromosome breaks and disruption of DNA repair (2, 6, 7). Recent data from cell culture and animal studies also have suggested that vitamin B₆ may prevent the

development of colon cancer through several other mechanisms including the suppression of cell proliferation, oxidative stress, nitric oxide synthesis, and angiogenesis (8).

Although the findings from prospective cohorts and case-control studies in general suggest an inverse association between folate intake and the risk of colorectal cancer, data are not entirely consistent (9-28). In addition, data relating vitamin B₆ intake to the risk of colorectal cancer are limited. We thus conducted a prospective analysis of folate and vitamin B₆ intakes and the risk of colorectal cancer in a large cohort of women.

MATERIALS AND METHODS

Study Cohort

The Women's Health Study was established in 1992 when 39,876 female US health professionals, aged 45 years or older, who were free of cancer and cardiovascular disease at baseline were enrolled into a randomized, double-blind, placebo-controlled clinical trial evaluating the balance of benefits and risks of low-dose aspirin and vitamin E in the primary prevention of cancer (other than non-melanoma skin cancer) and cardiovascular disease (29). Upon enrollment in the study, all participants completed a baseline questionnaire about their medical history and lifestyle characteristics including potential risk factors for colorectal cancer. The current analysis was restricted to 37,916 women after excluding those who did not provide dietary information, had implausible total energy intake (<600 kcal/day or >3500 kcal/day), did not provide multivitamin supplement use information, or had newly diagnosed non-adenocarcinoma colorectal cancer.

Dietary Assessment

At baseline, 39,345 (98.7%) women in the Women's Health Study completed a 131-item food frequency questionnaire, a format that has been used in the Nurses' Health Study. The questionnaire assessed the average consumption over the past year of a specific amount of each food and allowed nine responses, ranging from "never" to "six or more times per day." Nutrient intake was calculated by multiplying the frequency response by the nutrient content of the specified portion sizes. The food frequency questionnaire included a section on current use of multivitamin supplements, which was completed by 96.6% women. Participants were asked about the exact brand and type of multivitamins used and how many times they had taken multivitamins per week (2 or less, 3-5, 6-9, 10 or more). Intakes of folate and vitamin B₆ from supplements were estimated by linking to a comprehensive database that was created by the Department of Nutrition, Harvard School of Public Health on the folate and vitamin B₆ content of the multivitamin preparations. Information about the status (never, past, and current) and duration of multivitamin supplement use (0-1, 2-4, 5-9, 10-14, 15-19, 20 or more years) were collected on the baseline enrollment questionnaire.

The validity and reliability of the food frequency questionnaire has been assessed in the Nurses' Health Study (30-33), which has many characteristics similar to the Women's Health Study. In a sample of 188 participants from the Nurses' Health Study, the correlation coefficients between folate intake calculated from the 1980 dietary questionnaire and erythrocyte folate concentrations in 1987 were 0.55 for total folate (folate from foods and supplements) and 0.38 for dietary folate (folate from foods only) (34). In a sample of 712 participants who provided blood specimens during 1989-1990 and served as control subjects in a nested case-control study of breast cancer in the Nurses' Health Study, the correlation coefficients between the average intakes of folate and vitamin B₆ calculated from the 1980, 1984, 1986, and 1990 food frequency questionnaires and plasma levels were 0.49 for total folate and 0.52 for total vitamin B₆ (35). The corresponding correlation coefficients for dietary folate and dietary vitamin B₆ after excluding supplement users were 0.33 and 0.25, respectively (35).

Ascertainment of Colorectal Cancer Cases

Every six months during the first year of follow-up and then annually thereafter, participants were sent questionnaires asking about newly diagnosed endpoints including colon or rectal cancer. Deaths of participants were identified by reports from family members, postal authorities, and a search of the National Death Index. To date, follow-up rates for morbidity and mortality are 95% and 97%, respectively. For those who reported a diagnosis of colorectal cancer and for those who were deceased, we sought medical records and other relevant information, which were reviewed by an Endpoints Committee consisting of physicians to confirm medical diagnoses. Of the self-reports, medical record review confirmed 96%.

Statistical Analysis

For nutrient analysis, women were categorized by quintiles of nutrient intakes with the adjustment for total energy by the residual method to reduce measurement errors due to general over- or underreporting of food items (33). For the analysis according to tumor location (proximal colon, distal colon, and rectum), women were categorized by tertiles instead due to the modest numbers of cases.

We first compared the distribution of baseline risk factors for colorectal cancer by quintile of total folate and total vitamin B₆ to assess their potential for confounding. Person-years for each participant were calculated from the date of the randomization to the date of diagnosis of confirmed cancer, death, or 20 February 2004, whichever occurred first. Cox proportional hazards regression models were used to calculate the relative risks (RRs) and 95% confidence intervals (95% CIs). We first estimated the RRs according to category of nutrient intakes with adjustment for age (in years) and randomized treatment assignment (aspirin vs. placebo, vitamin E vs. placebo). We further performed a multivariate analysis that additionally adjusted for known or potential risk factors for colorectal cancer including body mass index (<23, ≥23 to <25, ≥25 to <27, ≥27 to <30, ≥30 kg/m²); family history of colorectal cancer in a first-degree relative (yes, no); history of self-reported colon polyps at baseline (yes, no); physical activity (total kcal/week in quartiles); smoking status (never, past, current); red meat intake (servings/day in tertiles); alcohol consumption (0, >0 to <15, ≥15 g/day); total energy intake (kcal/day in quintiles); menopausal status (premenopausal, postmenopausal, uncertain/unknown); and baseline postmenopausal hormone (PMH) use (never, past, current); and aspirin use before the trial (yes, no).

Additional analyses excluded incident colorectal cancer cases diagnosed within the first two years of follow-up, with further adjustment for colonoscopy or sigmoidoscopy for regular screening test (yes, no), which were asked on the 12-month follow-up questionnaire. Analyses for intake of total and dietary folate stratified by level of alcohol intake (<75th percentile of 5.0 g/day, or ≥75th percentile 5.0 g/day) were conducted to evaluate whether the association between folate intake and colorectal cancer risk may exist among those who consumed more alcohol. Because alcohol is a known folate antagonist (36, 37), women who consumed alcohol may have a higher requirement for folate. Tests for interaction between intakes of folate and alcohol in relation to colorectal cancer risk were conducted by using the median value for each quintile of folate intake as a continuous variable, an indicator variable for alcohol intake (<5 g/day vs. ≥5 g/day), and a product term of these two variables. The Wald test was used to assess the statistical significance of the multiplicative interaction term. Because multivitamins are major sources of supplements of folate and vitamin B₆, we also evaluated the relationship between the use of multivitamin supplements at baseline and the risk of colorectal cancer. The use of multivitamin supplements were categorized according to the status (never, past, or current use), the frequency of current use (none, ≤5/week, or >5/week, and the duration of past use or current use (<2 years, 2-4 years, 5-9 years, ≥10 years). Tests for trend were performed

using the median value for each nutrient category as a continuous variable. All *P*-values were two-sided.

RESULTS

The median length of follow-up was 10.1 years. During follow-up, 220 incident cases of invasive colorectal cancer were confirmed, including 89 cases of proximal colon cancer, 82 cases of distal colon cancer, 44 cases of rectal cancer, and 5 cases in which the site in the colon was not specified. Median baseline intakes were 348 $\mu\text{g}/\text{day}$ for total folate, 307 $\mu\text{g}/\text{day}$ for dietary folate, 2.3 mg/day for total vitamin B₆, and 2.0 mg/day for dietary vitamin B₆. There were 57% of women who took multivitamin supplements in the past and 29% who took multivitamin supplements currently.

Table 1 presents the distribution of baseline risk factors for colorectal cancer according to quintile intakes of total folate and total vitamin B₆. Women who consumed more total folate and total vitamin B₆ tended to be current users of multivitamins; 78% of women in the highest category of total folate and 75% of women in the highest category of total vitamin B₆ were current users of multivitamin supplements. Women who consumed more total folate and total vitamin B₆ tended to be older, leaner, more likely to be physically active, current users of postmenopausal hormones, and aspirin users before the trial, but they were less likely to be current smokers and report a family history of colorectal cancer in a first-degree relative. Women who consumed more total folate and vitamin B₆ also tended to undergo colonoscopy or sigmoidoscopy procedures for screening or for symptoms, consume less alcohol and red meat, but eat more dietary fiber. A personal history of colon polyps did not appear to differ significantly according to intakes of total folate and total vitamin B₆.

While intakes of total folate and total vitamin B₆ were not significantly associated with the risk of colorectal cancer, an inverse association was observed for intakes of dietary folate and dietary vitamin B₆ after exclusion of users of multivitamins or other supplements containing folate and vitamin B₆ (Table 2). The multivariable RRs (95% CIs) comparing the highest to the lowest quintile were 0.46 (0.26, 0.81) for dietary folate (*p* for trend = 0.02) and 0.69 (0.41, 1.15) for dietary vitamin B₆ (*p* for trend = 0.047). Additional adjustments for dietary fiber intake slightly attenuated the associations; the multivariable RRs and 95% CIs comparing the highest to the lowest quintile were 0.53 (0.28, 1.01) for dietary folate (*p* for trend = 0.12) and 0.74 (0.42, 1.32) for dietary vitamin B₆ (*p* for trend = 0.14). In addition, an apparent threshold effect of dietary folate and vitamin B₆ was observed; a reduced risk of colorectal cancer was observed in the second and fifth quintiles of dietary folate and in the higher three quintiles of dietary vitamin B₆ (Table 2). The multivariable RR (95% CI) comparing women in the higher three quintiles of dietary vitamin B₆ with those in the lower two quintiles was 0.66 (0.47, 0.93). When dietary intakes of folate and vitamin B₆ were evaluated jointly, the multivariable RRs (95% CIs) were 0.42 (0.21, 0.86) for women in the highest quintiles of both dietary folate and vitamin B₆ and 0.60 (0.38, 0.96) for women in other quintiles of both as compared with those in the lowest quintiles of both. There was no significant association between methionine intake and the risk of colorectal cancer (Table 2).

The associations between intakes of either total or dietary folate and the risk of colorectal cancer did not appear to differ according to level of alcohol intake; the multivariable *p* values for interaction were 0.40 for total folate and 0.58 for dietary folate. However, the number of cases was limited in this analysis.

The use of multivitamin supplements at baseline was not significantly associated with the risk of colorectal cancer; the multivariable RRs (95% CIs) were 0.94 (0.64, 1.37) for past users and 1.07 (0.72, 1.61) for current users (Table 3). The use of multivitamin supplements for 10 or

more years or for 6 or more times per week was also not significantly associated with the risk of colorectal cancer.

The associations with folate, vitamin B₆, and multivitamin supplements did not appreciably change in the analysis excluding cases diagnosed within the first two years of follow-up to address the potential bias that women might have changed their diet due to preclinical symptoms of colorectal cancer (data not shown). Also, there were no clear patterns for intakes of dietary folate and dietary vitamin B₆, and the use of multivitamin supplements in the analysis according to tumor locations. However, a positive association between intakes of total folate and total vitamin B₆ and the risk of distal colon cancer was observed. The multivariable RRs (95% CIs) comparing the highest to the lowest tertile of total folate intake were 0.75 (0.45, 1.24) for proximal colon cancer, 2.06 (1.11, 3.82) for distal colon cancer, and 0.94 (0.43, 2.03) for rectal cancer. The comparable multivariable RRs (95% CIs) comparing the highest to the lowest tertile of total vitamin B₆ intake were 0.81 (0.49, 1.36) for proximal colon cancer, 1.67 (0.95, 2.93) for distal colon cancer, and 0.82 (0.41, 1.64) for rectal cancer.

DISCUSSION

In this large cohort of women, we observed no association between intakes of total folate and total vitamin B₆ and the risk of colorectal cancer. However, we found an inverse association between intakes of dietary folate and dietary vitamin B₆ and the risk of colorectal cancer among women who did not take these supplements.

Because of the prospective design, recall or selection biases are unlikely to explain our findings in this study, and the high follow-up rates minimize the concern that differential follow-up rates have affected our results. Symptoms of colorectal cancer might have caused some women to increase their intake of vitamins before clinical diagnosis. However, the similar results after exclusion of colorectal cancer cases diagnosed within the first two years of follow-up do not support this explanation. Although confounding by unknown variables cannot be excluded, it seems unlikely because adjustment for a number of potential risk factors for colorectal cancer had minimal effect on the relative risks. However, estimates of nutrient intake calculated from the food frequency questionnaire are subject to measurement error. Such error is most likely to be non-differential due to our prospective design and may result in attenuation of risk estimates. Since we assessed intakes of nutrients only at baseline, measurement error due to random withinperson variation may be inevitable. Finally, because the number of events was relatively modest, we had limited statistical power for stratified analyses and analyses according to tumor locations.

Although data are not totally consistent, the findings from prospective cohorts that have evaluated folate intake and the risk of colorectal cancer suggest an inverse association (9-18), particularly among individuals with a high-alcohol diet. In the Nurses' Health Study, women with folate intake of >400 µg/day were found to have a significantly lower (31%) risk of colon cancer than women with intake of ≤200 µg/day (11). Furthermore, women taking folic acid-containing multivitamins for ≥15 years were 75% less likely to develop colon cancer than were women who did not take multivitamins (11). Two (12, 38) of three (10, 12, 38) prospective studies have also suggested that high folate blood levels are related to reduced risk of colorectal cancer. Several case-control studies have reported on folate intake and colorectal cancer risk (19-28); most have found a lower risk of colorectal cancer associated with higher folate intake (19-22, 25-27). Compared with other cohorts in the North America (39), the level of alcohol intake in the Women's Health Study was much lower, which may limit our power to detect a difference in the association between folate intake and colorectal cancer risk by level of alcohol intake.

Few studies have assessed the relationship between vitamin B₆ and colorectal cancer risk. In the Nurses' Health Study, plasma vitamin B₆ was inversely associated with the risk of colorectal cancer and adenoma (40). In the Iowa Women's Health Study, higher vitamin B₆ intake was not independently associated with the risk of colon cancer but was significantly inversely associated with proximal colon cancer when combined with high folate intake (15). Several case-control studies reported a lower risk of colorectal cancer associated with higher vitamin B₆ intake (19, 24, 26). However, two other studies observed no overall associations (28, 41).

Our findings that suggest a possible inverse relationship of colorectal cancer risk with intakes of dietary folate and dietary vitamin B₆ rather than with total folate and total vitamin B₆ are intriguing. Such findings are consistent with the results from a recent meta-analysis of seven cohorts and nine case-control studies on folate intake and colorectal cancer risk, which reported a stronger inverse association for dietary folate than for total folate (42). However, these findings for folate seem to conflict with the fact that folic acid used in supplements is in the form of monoglutamate, which bypasses deconjugation for intestinal absorption, and is thus more bioavailable than dietary folate (43). One possible explanation is that dietary nutrient intakes (from foods only) calculated from the food frequency questionnaire may be more likely to reflect participants' long-term intakes than total nutrient intakes (from foods and supplements) as dietary intakes may have been consistent over long periods whereas widespread use of vitamin supplements is relatively recent. Because of cancer's long latent period, it is highly plausible that colorectal cancer may be related to a long-term or remote dietary exposure. In the Nurses' Health Study, a protective effect of multivitamin supplement use on colon cancer risk was mostly seen among users for 15 or more years (11). Due to a small number of cases, we were unable to evaluate the risk of colorectal cancer in the category of 15 or more years of multivitamin supplement use.

Another possible explanation is that folate and vitamin B₆ may reduce the risk of colorectal cancer in women with a diet low in these vitamins. Our finding that an inverse association for dietary folate and dietary vitamin B₆ was observed among women not taking these supplements appears to support such an explanation. It is also consistent with an apparent threshold effect observed for dietary folate and dietary vitamin B₆ (i.e., low intake increases risk but incremental intake above the threshold level may add minimum benefits). Because vitamin supplements are typically taken in excess of dietary intake, their removal from the analysis would strengthen the association between dietary folate or dietary vitamin B₆ intake and the risk of colorectal cancer. An alternative explanation for the present observations, however, is that dietary intake of folate or vitamin B₆ may merely serve as a marker for other constituents in foods that are rich in folate or vitamin B₆ (such as fiber or other potential protective constituents) or of lifestyle factors related to risk of colorectal cancer. In our analysis, additional controlling for dietary fiber slightly attenuated the associations for dietary folate. A null association between intake of total folate and total vitamin B₆ and colorectal cancer risk might also be a result of surveillance bias because of increased use of colonoscopy or sigmoidoscopy procedures among supplement users. However, this explanation is not supported by the unchanged results from the analysis excluding colorectal cancer cases diagnosed within the first two years follow-up in which the use of colonoscopy or sigmoidoscopy procedures was included in multivariable models. In a subgroup analysis of tumor locations, we also observed an increased risk associated with intakes of total folate and total vitamin B₆ for distal colon cancer, but not for proximal colon and rectal cancer. However, because we had limited number of cases in this analysis, these findings need to be interpreted with caution.

Although both the Women's Health Study and the Nurses' Health Study consisted of female health professionals, participants in the Women's Health Study were enrolled much later (1992 vs. 1976) and consumed a healthier diet. More than half of follow-up in the Women's Health Study took place after the mandatory fortification of grain products with folic acid in US

(44). Approximately half of participants in the Women's Health Study consumed at least 400 µg/day of total folate (Dietary Reference Intake of folate for adults (45)) at baseline. In contrast, only 29% participants in the Nurses' Health Study consumed this amount of total folate at baseline (11). In addition, the level of alcohol intake in the Nurses' health Study (39) was more than 2 times higher than that in the Women's Health Study. These factors may contribute to the discrepancy in the results from these two studies.

The data from present study support recommendations to improve folate and vitamin B₆ intakes from dietary sources for colorectal cancer prevention. However, the null data for multivitamin supplements alone cannot be definitely viewed as suggesting no role for multivitamin supplements in reducing risk, as some other studies have reported an inverse association between multivitamin supplement use and risk of colorectal cancer, especially among long-term users (39). On the other hand, the null data on use of multivitamin supplements argue against them playing a major role in colorectal cancer prevention.

In summary, the findings from this large prospective cohort of women suggest that higher dietary intake of folate and vitamin B₆ may reduce the risk of colorectal cancer in women. However, an alternative explanation is that other factors related to dietary intake of folate and vitamin B₆ may account for the inverse associations.

ACKNOWLEDGEMENTS

This study is supported by research grants CA-47988 and HL-43851 from the National Institutes of Health (Bethesda, MD). Shumin M. Zhang is supported in part by the National Cancer Institute Career Development Award (CA096619). We acknowledge the contributions of the entire staff of the Women's Health Study under the leadership of David Gordon, as well as Mary Breen, Susan Burt, Marilyn Chown, Georgina Friedenber, Inge Judge, Jean Mac-Fadyean, Geneva McNair, David Potter, Claire Ridge, and Harriet Samuelson. We also acknowledge the Endpoints Committee of the Women's Health Study (Dr. Wendy Y. Chen) and Seetha Medabalimi for her assistance with the manuscript and the 39,876 dedicated participants of the Women's Health Study.

Abbreviations:

RR, relative risk; CI, confidence interval; PMH, postmenopausal hormone.

References

1. Cooper AJL. Biochemistry of sulfur-containing amino acids. *Ann Rev Biochem* 1983;52:187–222.
2. Mason JB, Levesque T. Folate: effects on carcinogenesis and the potential for cancer chemoprevention. *Oncology* 1996;101:727–736. [PubMed: 8953590]
3. Selhub J, Rosenberg IH. Folic Acid. In: Ziegler, EE.; Filer, J.; L., J., editors. Present knowledge in nutrition. 7th. International Life Sciences Institute Press; Washington, DC: 1996. p. 206–19.
4. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993;270:2693–8. [PubMed: 8133587]
5. Frigola J, Sole X, Paz MF, et al. Differential DNA hypermethylation and hypomethylation signatures in colorectal cancer. *Hum Mol Genet*. 2004
6. Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. *Mutat Res* 2001;475:7–20. [PubMed: 11295149]
7. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci USA* 1997;94:3290–5. [PubMed: 9096386]
8. Matsubara K, Komatsu S, Oka T, Kato N. Vitamin B6-mediated suppression of colon tumorigenesis, cell proliferation, and angiogenesis (review). *J Nutr Biochem* 2003;14:246–50. [PubMed: 12832027]
9. 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]

10. Glynn SA, Albanes D, Pietinen P, et al. Colorectal cancer and folate status: a nested case-control study among male smokers. *Cancer Epidemiol Biomarkers Prev* 1996;5:487–94. [PubMed: 8827351]
11. Giovannucci E, Stampfer MJ, Colditz GA, et al. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Ann Intern Med* 1998;129:517–24. [PubMed: 9758570]
12. Kato I, Dnistrian AM, Schwartz M, et al. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. *Br J Cancer* 1999;79:1917–22. [PubMed: 10206314]
13. Su LJ, Arab L. Nutritional status of folate and colon cancer risk: evidence from NHANES I epidemiologic follow-up study. *Ann Epidemiol* 2001;11:65–72. [PubMed: 11164122]
14. Terry P, Jain M, Miller AB, Howe GR, Rohan TE. Dietary intake of folic acid and colorectal cancer risk in a cohort of women. *Int J Cancer* 2002;97:864–7. [PubMed: 11857369]
15. Harnack L, Jacobs DR Jr, Nicodemus K, Lazovich D, Anderson K, Folsom AR. Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. *Nutr Cancer* 2002;43:152–8. [PubMed: 12588695]
16. Konings EJ, Goldbohm RA, Brants HA, Saris WH, van den Brandt PA. Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study. *Cancer* 2002;95:1421–33. [PubMed: 12237910]
17. Flood A, Caprario L, Chatterjee N, Lacey JV Jr, Schairer C, Schatzkin A. Folate, methionine, alcohol, and colorectal cancer in a prospective study of women in the United States. *Cancer Causes Control* 2002;13:551–61. [PubMed: 12195645]
18. Larsson SC, Giovannucci E, Wolk A. A prospective study of dietary folate intake and risk of colorectal cancer: modification by caffeine intake and cigarette smoking. *Cancer Epidemiol Biomarkers Prev* 2005;14:740–3. [PubMed: 15767361]
19. Benito E, Stiggelbout A, Bosch FX, et al. Nutritional factors in colorectal cancer risk: a case-control study in Majorca. *Int J Cancer* 1991;49:161–7. [PubMed: 1652565]
20. Freudenheim JL, Graham S, Marshall JR, Haughey BP, Cholewinski S, Wilkinson G. Folate intake and carcinogenesis of the colon and rectum. *Int J Epidemiol* 1991;20:368–74. [PubMed: 1917236]
21. Ferraroni M, La Vecchia C, D'Avanzo B, Negri E, Franceschi S, Decarli A. Selected micronutrient intake and the risk of colorectal cancer. *Br J Cancer* 1994;70:1150–5. [PubMed: 7981067]
22. Meyer F, White E. Alcohol and nutrients in relation to colon cancer in middle-aged adults. *Am J Epidemiol* 1993;138:225–36. [PubMed: 8395140]
23. Boutron-Ruault MC, Senesse P, Faivre J, Couillaud C, Belghiti C. Folate and alcohol intakes: related or independent roles in the adenoma-carcinoma sequence? *Nutr Cancer* 1996;26:337–46. [PubMed: 8910915]
24. Slattery ML, Schaffer D, Edwards SL, Ma KN, Potter JD. Are dietary factors involved in DNA methylation associated with colon cancer? *Nutr Cancer* 1997;28:52–62. [PubMed: 9200151]
25. White E, Shannon JS, Patterson RE. Relationship between vitamin and calcium supplement use and colon cancer. *Cancer Epidemiol Biomarkers Prev* 1997;6:769–74. [PubMed: 9332757]
26. La Vecchia C, Braga C, Negri E, et al. Intake of selected micronutrients and risk of colorectal cancer. *Int J Cancer* 1997;73:525–30. [PubMed: 9389567]
27. La Vecchia C, Negri E, Pelucchi C, Franceschi S. Dietary folate and colorectal cancer. *Int J Cancer* 2002;102:545–7. [PubMed: 12432561]
28. Le Marchand L, Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). *Cancer Causes Control* 2002;13:239–48. [PubMed: 12020105]
29. Rexrode KM, Lee IM, Cook NR, Hennekens CH, Buring JE. Baseline characteristics of participants in the Women's Health Study. *J Womens Health Gend Based Med* 2000;9:19–27. [PubMed: 10718501]
30. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;122:51–65. [PubMed: 4014201]
31. Willett WC, Sampson L, Browne ML, et al. The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol* 1988;127:188–199. [PubMed: 3337073]

32. Salvini S, Hunter DJ, Sampson L, et al. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *Int J Epidemiol* 1989;18:858–867. [PubMed: 2621022]
33. Willett, WC. *Nutritional Epidemiology*. Oxford University Press; New York: 1998.
34. Giovannucci E, Stampfer MJ, Colditz GA, et al. Folate, methionine, and alcohol intake and risk of colorectal adenoma. *J Natl Cancer Inst* 1993;85:875–84. [PubMed: 8492316]
35. Zhang SM, Willett WC, Selhub J, et al. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. *J Natl Cancer Inst* 2003;95:373–80. [PubMed: 12618502]
36. Hillman RS, Steinberg SE. The effects of alcohol on folate metabolism. *Ann Rev Med* 1982;33:345–354. [PubMed: 6805415]
37. Weir DG, McGing PG, Scott JM. Folate metabolism, the enterohepatic circulation and alcohol. *Biochem Pharmacol* 1985;34:1–7. [PubMed: 3881098]
38. Ma J, Stampfer MJ, Giovannucci E, et al. Methylene tetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res* 1997;57:1098–102. [PubMed: 9067278]
39. Smith-Warner SA, Spiegelman D, Yaun S-S, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA* 1998;279:535–40. [PubMed: 9480365]
40. Wei EK, Giovannucci E, Selhub J, Fuchs CS, Hankinson SE, Ma J. Plasma vitamin B6 and the risk of colorectal cancer and adenoma in women. *J Natl Cancer Inst* 2005;97:684–92. [PubMed: 15870439]
41. Macquart-Moulin G, Riboli E, Cornee J, Charnay B, Berthezene P, Day N. Case-control study on colorectal cancer and diet in Marseilles. *Int J Cancer* 1986;38:183–91. [PubMed: 3015806]
42. Sanjoaquin MA, Allen N, Couto E, Roddam AW, Key TJ. Folate intake and colorectal cancer risk: a meta-analytical approach. *Int J Cancer* 2005;113:825–8. [PubMed: 15499620]
43. Gregory JF 3rd. Case study: folate bioavailability. *J Nutr* 2001;131:1376S–82S. [PubMed: 11285357]
44. Oakley GP Jr. Eat right and take a multivitamin. *N Engl J Med* 1998;338:1060–1061. [PubMed: 9535672]
45. Persson I. Estrogens in the causation of breast, endometrial and ovarian cancers - evidence and hypotheses from epidemiological findings. *J Steroid Biochem Mol Biol* 2000;74:357–64. [PubMed: 11162945]

TABLE 1

Age-adjusted baseline characteristics according to quintile intakes of total folate and total vitamin B₆ among 37,916 women in the Women's Health Study.

	Total folate (µg/day) [*]				Total vitamin B ₆ (mg/day) [*]			
	Q1	Q3	Q5	P trend	Q1	Q3	Q5	P trend
Median intake	223	348	758		1.58	2.28	5.14	
Current multivitamin use (%)	8.5	12.4	77.8	<0.001	7.2	10.7	74.5	<0.001
Age (years)	52.9	54.2	54.5	<0.001	52.9	54.2	54.6	<0.001
BMI (kg/m ²)	26.6	25.9	25.5	<0.001	26.1	26.3	25.6	<0.001
Physical activity (kcal/week)	687	1024	1139	<0.001	723	1017	1124	<0.001
Current PMH use (%)	36.9	41.6	46.9	<0.001	37.8	40.8	45.6	<0.001
Aspirin use before the trial (%)	10.3	10.6	13.9	<0.001	10.3	10.2	14.1	<0.001
Current smokers (%)	21.4	9.8	9.9	<0.001	20.3	9.5	10.4	<0.001
Family history of colorectal cancer in a first-degree relative (%)	10.7	10.5	9.7	0.04	10.5	10.4	9.5	0.08
Colonoscopy or sigmoidoscopy during the past year for screening [†]	4.2	5.5	5.3	<0.001	4.5	5.7	5.2	0.003
Colonoscopy or sigmoidoscopy during the past year for symptoms [†]	2.1	2.2	2.5	0.004	2.0	2.0	2.5	0.009
Colorectal polyps (%)	2.8	2.5	2.3	0.06	2.8	2.4	2.4	0.13
Alcohol consumption (g/day)	4.7	4.4	4.2	0.004	4.8	4.4	4.0	<0.001
Red meat (servings/day)	0.9	0.7	0.6	<0.001	0.7	0.8	0.6	<0.001
Dietary fiber (g/day)	14.8	20.2	20.7	<0.001	15.5	20.2	20.5	<0.001
Total energy intake (kcal/day)	1608	1800	1618	<0.001	1548	1844	1608	<0.001

^{*}Energy-adjusted values.

[†]From the 12-month questionnaire.

TABLE 2
Relative risks and 95% confidence intervals (CIs) of colorectal cancer according to quintile intakes of folate and vitamin B₆ in the Women's Health Study

	Quintile Intake					P trend
	1	2	3	4	5	
Total folate						
Intake (µg/day) *	<259	259-317	317-392	392-614	≥614	
No. cases	38	46	40	44	52	
Relative Risk [†]	1.00	1.13	0.93	1.01	1.17	0.46
95% CI		0.74, 1.74	0.60, 1.45	0.65, 1.56	0.77, 1.78	
Relative Risk [‡]	1.00	1.10	0.91	0.97	1.16	0.46
95% CI		0.71, 1.70	0.58, 1.44	0.62, 1.52	0.76, 1.79	
Dietary folate						
Intake (µg/day) *	≤244	244-288	288-329	329-385	≥385	
No. cases	48	32	50	48	42	
Relative Risk [†]	1.00	0.63	0.93	0.86	0.72	0.34
95% CI		0.40, 0.98	0.62, 1.38	0.58, 1.29	0.47, 1.09	
Relative Risk ^{‡§}	1.00	0.62	0.89	0.83	0.67	0.21
95% CI		0.40, 0.98	0.59, 1.34	0.55, 1.26	0.43, 1.03	
Dietary folate (excluding folate supplement users)						
Intake (µg/day) *	≤244	244-288	288-329	329-385	≥385	
No. cases	35	21	35	30	20	
Relative Risk [†]	1.00	0.59	0.97	0.80	0.52	0.07
95% CI		0.34, 1.01	0.61, 1.55	0.49, 1.31	0.30, 0.91	
Relative Risk ^{‡§}	1.00	0.58	0.92	0.75	0.46	0.02
95% CI		0.34, 1.00	0.57, 1.48	0.45, 1.25	0.26, 0.81	
Total vitamin B₆						
Intake (mg/day)	<1.78	1.78-2.09	2.10-2.52	2.53-3.99	≥4.00	
No. cases	46	39	30	44	61	
Relative Risk [†]	1.00	0.82	0.59	0.85	1.14	0.06
95% CI		0.53, 1.26	0.37, 0.93	0.56, 1.29	0.78, 1.67	
Relative Risk [‡]	1.00	0.84	0.58	0.85	1.14	0.07
95% CI		0.55, 1.30	0.36, 0.93	0.55, 1.30	0.77, 1.69	
Dietary vitamin B₆						
Intake (mg/day)	<1.69	1.69-1.91	1.92-2.12	2.13-2.39	≥2.40	
No. cases	47	47	42	32	52	
Relative Risk [†]	1.00	0.93	0.82	0.61	0.88	0.29
95% CI		0.62, 1.40	0.54, 1.25	0.39, 0.96	0.59, 1.31	
Relative Risk ^{‡§}	1.00	0.98	0.86	0.61	0.84	0.18
95% CI		0.65, 1.48	0.56, 1.31	0.39, 0.97	0.56, 1.27	
Dietary vitamin B₆ (excluding vitamin B₆ supplement users)						
Intake (mg/day)	<1.69	1.69-1.91	1.92-2.12	2.13-2.39	≥2.40	
No. cases	34	33	25	19	28	
Relative Risk [†]	1.00	0.93	0.70	0.54	0.72	0.07
95% CI		0.58, 1.50	0.42, 1.18	0.31, 0.94	0.44, 1.20	
Relative Risk [‡]	1.00	0.99	0.74	0.54	0.69	0.047
95% CI		0.61, 1.61	0.44, 1.26	0.31, 0.96	0.41, 1.15	
Methionine						
Intake (g/day)	<1.61	1.61-1.81	1.82-2.00	2.01-2.23	≥2.24	
No. cases	59	45	34	37	45	
Relative Risk [†]	1.00	0.83	0.63	0.72	0.88	0.36
95% CI		0.56, 1.23	0.41, 0.96	0.48, 1.09	0.60, 1.30	
Relative Risk ^{‡§}	1.00	0.90	0.68	0.77	0.89	0.43
95% CI		0.60, 1.33	0.44, 1.04	0.51, 1.18	0.60, 1.32	

* Some values were overlapped due to rounding.

[†] Models were adjusted for age and randomized treatment assignment.

[‡] Multivariate models were adjusted for age, randomized treatment assignment, body mass index, family history of colorectal cancer in a first-degree relative, history of colon polyps, physical activity, smoking status, red meat intake, alcohol consumption, total energy intake, menopausal status, baseline PMH use, and baseline aspirin use.

[§] Multivariate models were additionally adjusted for the use of multivitamin supplements.

TABLE 3
Relative risks and 95% confidence intervals (CIs) of colorectal cancer according to the use of multivitamin supplements in the Women's Health Study

Use of multivitamins	No. cases	Relative Risk [*]	95% CI	Relative Risk	95% CI
Status					
Never	37	1.00		1.00	
Past	112	0.93	0.64, 1.36	0.94	0.64, 1.37
Current	71	1.05	0.71, 1.57	1.07	0.72, 1.61
Duration					
Never	37	1.00		1.00	
Past					
<2 years	26	0.82	0.50, 1.36	0.82	0.50, 1.37
2-4 years	54	0.88	0.58, 1.35	0.88	0.57, 1.35
5-9 years	24	1.35	0.81, 2.27	1.36	0.81, 2.28
≥10 years	5	0.66	0.26, 1.69	0.69	0.27, 1.76
Current					
<2 years	11	1.10	0.56, 2.15	1.00	0.50, 2.03
2-4 years	19	1.11	0.64, 1.93	1.12	0.64, 1.95
5-9 years	14	0.97	0.52, 1.80	1.00	0.54, 1.86
≥10 years	27	1.07	0.65, 1.76	1.14	0.69, 1.88
Frequency					
None	149	1.00		1.00	
≤5/week	11	0.87	0.47, 1.61	0.91	0.49, 1.69
≥6/week	50	1.22	0.88, 1.68	1.24	0.90, 1.72
P for trend			0.26		0.23

^{*} Models were adjusted for age and randomized treatment assignment.

[†] Multivariate models were adjusted for age, randomized treatment assignment, body mass index, family history of colorectal cancer in a first-degree relative, history of colon polyps, physical activity, smoking status, red meat intake, alcohol consumption, total energy intake, menopausal status, baseline PMH use, baseline aspirin use, and dietary intakes of folate and vitamin B₆.