Following Up Folate and Its Function in Colorectal Carcinogenesis

Regina G. Ziegler

Correspondence to: Regina G. Ziegler, PhD, MPH, Epidemiology and Biostatistics Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Executive Plaza South 8098, Bethesda, MD 20892–7246 (e-mail: regina.ziegler@nih.gov).

In this issue of the Journal, Song et al. (1) report that in the Women’s Antioxidant Folic Acid Cardiovascular Study (WAFACS), a randomized, placebo-controlled trial, a combination supplement of folic acid (2.5 mg/day), vitamin B$_6$ (50 mg/day), and vitamin B$_12$ (1 mg/day) did not statistically significantly increase or decrease the risk of colorectal adenoma or advanced adenoma in older women (median age = 62 years at study entry) who were followed up for as long as 9 years. These were high doses, with folic acid, B$_6$, and B$_12$ at 10.4, 33.3, and 417 times, respectively, the recommended dietary allowances, which are the levels of intake considered sufficient to meet the nutritional requirements of 97.5% of healthy individuals (2); yet results were impressively null. Furthermore, no statistically significant effect modification was noted in women who were above or below the median circulating level of folic acid (2.5 mg/day), vitamin B$_6$, and vitamin B$_12$ at study entry. A previous publication on this trial reported that risk of colorectal cancer was not statistically significantly increased or decreased by the combination supplement (3), but analyses were limited by small numbers. Although 355 colorectal adenomas, of which 133 were advanced, were detected by endoscopy during trial follow-up, only 40 cases of incident colorectal cancer were diagnosed.

Initially, suboptimal levels of folate and the other B vitamins required for one-carbon metabolism, specifically vitamins B$_2$ (riboflavin), B$_6$, and B$_12$, were thought to increase the possibility of carcinogenesis because efficient one-carbon metabolism is necessary for DNA synthesis, repair, and methylation (4). More recently, folate has been proposed to play a dual role, with high concentrations promoting tumor development once premalignant lesions are established (5,6). Because the WAFACS trial administered high doses of folic acid and compliance was high, with 83% of the women taking at least two-thirds of the pills throughout the trial (1), the null results during the lengthy follow-up strongly suggest that neither high levels of folate intake nor the metabolic route whereby synthetic folic acid is converted into 5-methyltetrahydrofolate, the naturally occurring form of folate, nor unmetabolized folic acid in circulation increases the risk of colorectal adenoma, at least not within the first 5–10 years. This finding is reassuring with respect to the US decision to fortify cereal-grain products with folic acid, beginning in 1998, to reduce neural tube defects. This mandate was estimated to have increased mean folic acid intake by about 200 µg/day (7), an increase that is approximately 8% of the high folic acid dosages used in the trial. However, the null results of the trial are less pertinent to the protective potential of folate, B$_6$, and B$_12$, when given to women with suboptimal micronutrient intake. Because the trial was carried out during the folic acid fortification era and the women volunteering were likely to be health-conscious, with a history of vitamin supplement use, the median intake of folate, B$_6$, and B$_12$ at study entry was relatively high, at 108%, 167%, and 292% of the respective recommended dietary allowances (1).

The lack of effect of high-dose folate/B$_6$/B$_12$ supplementation on adenoma risk in a well-nourished population agrees with a recent combined analysis of the three large randomized, placebo-controlled trials of folic acid supplementation, which were carried out in the United States, Canada, the United Kingdom, and Denmark among patients with an adenoma history (2632 men and women) (8). After up to 3.5 years of folic acid use, at 0.5 or 1.0 mg/day, risk of adenoma, or advanced adenoma, was not statistically significantly increased or decreased. Interestingly, folic acid was associated with non-statistically significant reductions in risk among subjects in the two lowest quintiles of circulating folic acid at study entry. This combined analysis is encouraging, even though more follow-up is needed. The largest of the three trials included in this meta-analysis had previously reported potentially adverse effects: a statistically nonsignificant increased risk of adenoma, especially advanced adenoma, after 3 and 6–8 years of follow-up (9).

Although these randomized trials do suggest that high-dose folic acid is not a promising chemopreventive agent for colorectal cancer, they do not completely resolve the role of folate and other B vitamins in colorectal carcinogenesis. In 2010, in a pooled analysis of 13 prospective studies of folate intake and colon cancer, including 5720 incident cases and up to 7–20 years of follow-up, both dietary folate intake and total folate intake (from diet and supplements) were inversely, although modestly, associated with risk (comparing extreme quintiles, RRs = 0.92, 95% CI = 0.84 to 1.00 and 0.85, 95% CI = 0.77 to 0.95, respectively) (10). The stronger association with total folate intake might be due to an expanded range of intake, enhanced bioavailability of the synthetic folic acid in supplements, or the presence of other B vitamins in the multivitamins that provided most of the supplemental folate. More recently, in analyses of incident colorectal cancer diagnosed in the folic acid fortification era, the American Association of Retired Persons cohort reported a statistically significant 30% reduction in colorectal cancer risk comparing extremes of total folate intake (6484 incident cases) (11); and the Cancer Prevention Study II reported a statistically significant 19% reduction comparing extreme quintiles of total intake (1023 incident cases) (12). Although the strength of these associations with total folate intake...
could have been underestimated due to imprecise assessment of exposure, the analysis by Song et al. would have had limited power to detect modest inverse associations of the magnitude reported in these colorectal cancer studies (1). In addition, the majority (63%) of the adenomas detected in the WAFACS trial were not advanced and unlikely to progress to colorectal cancer and would have diluted any stronger associations with high-risk lesions.

Prospective studies of diet and colorectal cancer may also suggest a role for vitamin B₁₂. In a meta-analysis of eight prospective studies of B₆ intake and colorectal cancer risk, including about 3700 cases, B₆ was inversely associated with risk (comparing extreme quintiles, RR = 0.80, 95% CI = 0.69 to 0.92) (13). However, the inverse relationship was weakened (RR = 0.90) and became non-statistically significant when a ninth cohort, responsible for heterogeneity among the study-specific results, was included. Because only three studies included intake from supplements and the association was stronger in studies with a wider range of exposure, the reduction in risk may have been underestimated.

Prospective studies have also measured circulating levels of folate and other B vitamins in relation to subsequent incidence of colorectal cancer. Results for folate status have been inconsistent. One challenge to integrating and interpreting study results has been the use of competitive binding assays for folate. These kits bind the multiple forms of bioactive folate with different affinities, can also bind some inactive folate degradation products, and are, therefore, less accurate than the microbiological assays and the emerging liquid chromatography/tandem mass spectrometry assays, which can measure essentially all of the bioactive forms and indeed give similar results (14,15). However, circulating folate levels are not robust and are noticeably affected by blood processing and interim storage procedures (16). Liquid chromatography/tandem mass spectrometry assays may be the only way to measure the once-active folate metabolites generated during folate degradation.

The largest of the published analyses of circulating folate, a nested case–control study in the European Investigation into Cancer and Nutrition (EPIC) cohort, included 1367 incident colorectal cancer cases, did use a microbiological assay, and excluded samples with concerns about processing (17). Comparing the highest to lowest quintiles of circulating folate, risk was non-statistically significantly reduced by only 6%. However, in the same study, circulating levels of B₁₂ and B₆, but not B₉, were each statistically significantly associated with reduced risk (comparing extreme quintiles, RR for B₁₂ = 0.71, 95% CI = 0.56 to 0.91; RR for B₆ = 0.68, 95% CI = 0.53 to 0.87) (18). Additional support exists for a role for B₁₂ in carcinogenesis, but B₉ is rarely studied. In a meta-analysis of four prospective studies of circulating B₆ levels and risk of colorectal cancer, including 883 cases, which did not include the as-yet-unpublished EPIC analysis, B₆ was inversely associated with risk in each study, statistically significantly so in three of the four (comparing extreme quintiles, summary RR = 0.52, 95% CI = 0.38 to 0.71) (13).

These observational epidemiology results, from prospective studies of diet and supplements or circulating biomarkers, are provocative and imply that the role of folate and other B vitamins in colorectal carcinogenesis merits continued exploration. The relatively consistent, though modest, reductions in risk with increased total intake and higher circulating levels suggest that B vitamins, or a strong dietary correlate or possibly a lifestyle correlate, need more scrutiny. One-carbon metabolism may not be the only pathway by which B vitamins modulate carcinogenesis because B vitamins provide the cofactors essential for hundreds of enzymatic reactions in multiple metabolic pathways (19). Agnostic exploration of associations with metabolic pathways using platforms that concurrently measure and identify multiple metabolites might be informative. Follow-up of the folate and colorectal cancer relationship is not yet complete.

References

12. Stevens VL, McCullough ML, Sun J, Jacobs EJ, Campbell PT, Gapstur SM. High levels of folate from supplements and fortification are not associated with increased risk of colorectal cancer. Gastroenterology. 2011;141(1):98–105, e1.

**Funding**
Intramural Research Program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute.

**Notes**
The author has no conflicts of interest to disclose. The funder did not have a role in the writing of the manuscript and the decision to submit the manuscript for publication.

**Affiliation of author:** Epidemiology and Biostatistics Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD.