Folic Acid Fortification and Supplementation—Good for Some but Not So Good for Others

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Evidence has established the protective effect of folic acid (FA) fortification and periconceptional supplementation on neural tube defects (NTDs). Folic acid fortification and periconceptional supplementation of women may reduce the risk of certain childhood cancers in their offspring. However, recent human studies have suggested that FA supplementation and fortification may promote the progression of already existing, undiagnosed, preneoplastic and neoplastic lesions, thereby corroborating earlier observations from animal and in vitro studies. Following the success of mandatory FA fortification on the reduction of NTD rates in the United States and Canada, several countries are currently considering whether or not, and at what dose, to institute FA fortification. Future debates and decisions regarding FA fortification should take into consideration all potential adverse effects and dose-responses of such a measure because it may be associated with very serious consequences for many generations. In addition to careful monitoring of adverse effects, preclinical and population-based studies are warranted in order to determine the efficacy, safety, and potential deleterious effects of FA fortification and supplementation on cancer risk and other health outcomes.

Key words: cancer, colorectal cancer, folate, folic acid, neural tube defects

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INTRODUCTION

The controversy concerning the health benefits of folic acid (FA) fortification and supplementation has recently been heightened by the publication of several seminal articles investigating the effects of FA fortification and supplementation on disease prevention. The crux of the matter is while FA fortification and supplementation may prevent the development and progression of certain diseases, high-dose FA may be associated with potentially serious harmful effects.1,2 Folate (a water-soluble B vitamin that is present naturally in foods) and FA (the pharmaceutically packaged form of this vitamin that is used commercially in supplements and in fortified foods) have been considered nutritional stars for a decade. Folate has been generally regarded as safe and has long been presumed to be purely beneficial and an ideal functional food component for disease prevention.3 Indeed, the portfolio of observational epidemiologic studies has suggested a fairly convincing association between folate deficiency and development of anemia, atherosclerosis, neural tube defects (NTDs), adverse pregnancy outcomes, neuropsychiatric disorders, and cancer.4,5 However, results from several large FA intervention trials in humans have been inconsistent and generally have not been supportive of the cardioprotective effect of FA supplementation.6–8

One exception to this is the overwhelming evidence for the protective effect of periconceptional FA supplementation on the development of NTDs,9–11 which led to the eventual mandatory FA fortification in the United States and Canada in 1998.11–13 Indeed, mandatory FA fortification in these two countries has dramatically increased dietary intake and blood measurements of folate and decreased plasma homocysteine (an accurate inverse indicator of folate status).14,15 Coinciding with the significantly improved folate status, mandatory FA fortification has been shown to be associated with a significant reduction (~15–50%) in the prevalence and incidence of NTDs in the United States and Canada in preliminary studies.16–20 A recent population-based study from Canada further supports the protective effect of mandatory FA fortification on NTDs.21 This study clearly demonstrated that the prevalence of NTDs decreased significantly, from 1.58 per 1000 births before fortification (1993–1997) to 0.86 per 1000 births during the full-
fortification (1998–2003) period, a 46% reduction (95% confidence interval, 40–51)\textsuperscript{21}. The decrease was greatest in areas in which the baseline rate was high.\textsuperscript{21} Therefore, mandatory FA fortification, probably the most important science-driven intervention in nutrition and public health in decades,\textsuperscript{22} appears to be highly successful in achieving its primary objective, that is, reducing NTD rates, and should justifiably be considered as a public health triumph.

**CONTINUING DEBATE ON FA FORTIFICATION**

Despite the unequivocal success in reducing NTD rates, the debate over mandatory FA fortification has not ceased and, as a matter of fact, the controversy over this public health policy has intensified, partly because of an uncertain role of folate in cancer development and progression.\textsuperscript{23,24} Prior to 1990, when a flurry of publications began to appear reporting epidemiologic observations that suggested an inverse association between folate status and the risk of several human malignancies, the role of folate in cancer was entirely related to antifolate-based cancer chemotherapy. In the 1940s, shortly after FA was discovered, clinical investigators led by Sidney Farber\textsuperscript{25} at the Children’s Hospital in Boston administered FA polyglutamate conjugates in children with leukemia based on the observation by Lewisohn et al.\textsuperscript{26} at the Mt. Sinai Hospital in New York City that “folic acid concentrate” caused regression of breast cancer in mice. To their surprise, the administration of FA accelerated the progression of leukemia,\textsuperscript{25} which suggested that the proliferation of leukemia cells might be limited by the supply of FA, or, as learned later, its active tetrahydrofolate cofactor metabolites. Thus, Sidney Farber obtained FA antagonists from Lederle Laboratories, and in a historic article in the New England Journal of Medicine in 1948, Farber et al.\textsuperscript{27} reported that one of these agents, aminopterin, produced complete remissions in children with acute leukemia. This discovery heralded the beginning of the modern era of antifolate-based chemotherapy.

In contrast to the inhibitory effect of antifolate (or folate deficiency) on tumors, an accumulating body of epidemiological, clinical, and experimental evidence suggests that folate deficiency in normal tissues appears to predispose them to neoplastic transformation, and folate supplementation suppresses the development of tumors in normal tissues.\textsuperscript{28} Epidemiologic studies collectively suggest an inverse association (in some cases, dose-dependent) between folate status (measured by dietary and supplemental intake or blood levels) and the risk of several malignancies including cancer of the colorectum, oropharynx, esophagus, stomach, pancreas, lungs, cervix, ovary, and breast, neuroblastoma, and leukemia, among which the best evidence exists for colorectal cancer.\textsuperscript{28}

Although not uniformly consistent, the portfolio of retrospectively and prospectively conducted epidemiologic studies, including two recent meta-analyses,\textsuperscript{29,30} suggests a 20–40% reduction in the risk of colorectal cancer or its precursor, adenomas, in subjects with the highest folate status compared with those with the lowest status.\textsuperscript{28} Furthermore, the Nurses’ Health Study reported a 75% reduction in colorectal cancer risk in women using multivitamin supplements containing ≥400 μg FA for ≥15 years compared with those not taking FA.\textsuperscript{31} Small human intervention studies have also reported that FA supplementation (400 μg–10 mg/day for 3 months to 2 years) can improve or reverse several functional biomarkers of folate and one-carbon metabolism and colorectal cancer.\textsuperscript{28} Animal studies have generally corroborated the inverse association between dietary folate intake and colorectal cancer risk observed in epidemiologic studies.\textsuperscript{28,32} However, animal studies have also provided critical information concerning dual modulatory effects of folate on colorectal cancer development and progression depending on the timing and dose of folate intervention. Folate deficiency has an inhibitory effect whereas FA supplementation has a promoting effect on the progression of established colorectal neoplasms.\textsuperscript{28,32}

In contrast, folate deficiency in normal colorectal mucosa appears to predispose it to neoplastic transformation, and modest levels of FA supplementation (4–10 times above the basal dietary requirement) suppress it, whereas supra-physiologic supplemental doses enhance the development of colorectal cancer in normal colorectal mucosa.\textsuperscript{28,32}

**DUAL MODULATOR ROLE OF FOLATE IN COLORECTAL CARCINOGENESIS**

The dual modulatory role of folate in colorectal cancer development and progression observed in animal studies is readily explained by several biologically plausible mechanisms\textsuperscript{28,33} (Figure 1). As an essential cofactor for the de novo biosynthesis of purines and thymidine, folate plays an important role in DNA synthesis, stability and integrity, and repair, aberrations of which have been implicated in colorectal carcinogenesis.\textsuperscript{28,33} Furthermore, folate plays an important role in mediating one-carbon transfer for the provision of S-adenosylmethionine, the primary methyl group donor for most biological methylation reactions, including that of DNA.\textsuperscript{28,33} Aberrant DNA methylation is implicated in colorectal carcinogenesis.\textsuperscript{28,33} The accumulating body of in vitro and in vivo evidence indicates that in normal colorectal epithelial cells, folate deficiency induces DNA strand breaks, chromosomal and genomic instability, uracil misincorporation, impaired DNA repair, increased
mutations, and possibly aberrant DNA methylation, while folate supplementation can correct some of these defects.\textsuperscript{28,33} In contrast, in preneoplastic or neoplastic colorectal epithelial cells, folate deficiency causes ineffective DNA synthesis, resulting in inhibition of tumor growth and progression.\textsuperscript{28} Mechanistically, the most likely means by which FA supplementation may promote the progression of established preneoplastic and neoplastic lesions in the colorectum is the provision of nucleotide precursors to rapidly replicating cells for accelerated proliferation and growth.\textsuperscript{28} Another mechanism may be de novo methylation of promoter CpG islands of tumor suppressor genes leading to gene inactivation leading to tumor progression.\textsuperscript{28}

**THE ASPIRIN/FOLATE POLYP PREVENTION STUDY**

Based on the promising results from epidemiologic studies and small pilot intervention trials, a double-blind, placebo-controlled, 2-factor, phase 3, randomized, chemoprevention trial (The Aspirin/Folate Polyp Prevention Study) was conducted at nine clinical centers in the United States and Canada.\textsuperscript{34} Using a 3×2 factorial design, this trial compared 81 mg/d and 325 mg/d of aspirin with placebo and 1 mg/d of FA with placebo in persons with a history of colorectal adenomas. The findings regarding aspirin were reported in 2003 as follows: low-dose aspirin (81 mg/d) had a moderate, statistically significant chemopreventive effect, reducing the risk of colorectal adenomas by 19%, while high-dose aspirin (325 mg/d) provided no significant benefit.\textsuperscript{35} With regard to FA, participants were randomized to receive 1 mg/d of FA (N=516) or placebo (N=505) and followed with two colonoscopies (the first was at 3 years and the second was 3–5 years later).

The results regarding FA have recently been reported.\textsuperscript{34} Overall, there was no effect of FA supplementation on the recurrence of adenomas, with risk ratios (RRs) of 1.04 (95% confidence interval [CI], 0.90–1.20) at 3 years (N=987) and 1.13 (95%CI, 0.93–1.37) at the
second follow-up (N=607). Unexpectedly, however, at the second follow-up, there was a 67% increased risk of advanced lesions with a high malignant potential, defined as ≥25% villous features, high-grade dysplasia, size ≥1 cm, or invasive adenocarcinoma, (RR, 1.67; 95% CI, 1.00–2.80), along with a more than 2-fold increased risk of having at least three adenomas (RR, 2.32; 95% CI, 1.23–4.35). There was no significant effect modification by sex, age, smoking, alcohol use, body mass index, baseline plasma folate, or aspirin allocation. Another unexpected secondary finding from this trial was that the risk of cancers other than colorectal cancer was significantly increased in the FA-supplemented group (P=0.02). This was largely due to an excess of prostate cancer in the FA group (P=0.01). Furthermore, FA supplementation had no significant effect on the incidence of myocardial infarction, coronary revascularization, and stroke, which was expected to be reduced a priori because of the homocysteine-lowering effects of FA.

The potential tumor-promoting effect of FA supplementation observed in this trial is not entirely surprising and is consistent with some of the observations made in animal models. This trial was designed to address secondary rather than primary prevention of colorectal adenomas. The participants had previous colorectal adenomas removed before entry into the trial. These predisposed individuals were at high risk of developing colorectal adenomas and cancer and might have harbored microscopic precursors of colorectal cancer (e.g., aberrant crypt foci or microscopic adenomas). Therefore, FA supplementation might have promoted the progression of these already existing, undiagnosed preneoplastic lesions. Although there were no important differences in the baseline characteristics between the FA and placebo groups, the FA group had a higher proportion of subjects with large adenomas (≥1 cm in diameter) removed ≤16 months before recruitment (P=0.06). Large adenomas are considered to be advanced lesions with a higher malignant potential and as such, the subjects in the FA group might have been more predisposed to colorectal carcinogenesis than those in the placebo group. Another possibility, albeit unlikely, is that FA supplementation might have promoted the progression of adenomas missed on initial and first follow-up colonoscopies. The observed higher incidence of prostate cancer associated with FA supplementation is not surprising either and can be readily explained. The mean age of the study participants was 57 years (~64% were men); it is therefore highly likely that some of the male participants might have harbored precursor lesions in the prostate, which were allowed to progress more rapidly with FA supplementation. The take-home message from this important trial is that FA supplementation should not be given to individuals with previous colorectal adenomas, because their colons may already be predisposed to neoplastic transformation, and to those suspected of harboring precursor lesions of colorectal cancer in the colorectum. This of course applies to the large segment of the North American population as it has been estimated that approximately 25–50% of people by the age of 50 years in the United States and Canada harbor asymptomatic colorectal adenomas, and the prevalence increases with age. However, this study does not rule out the possibility that FA supplementation may prevent the development of new colorectal adenomas or cancer, and this issue can be addressed only by a primary prevention trial.

Several randomized studies have investigated the effect of FA supplementation on cancer risk as a secondary endpoint. Two recently published large, randomized, placebo-controlled intervention trials designed to test the effect of FA supplementation in conjunction with other B vitamins on primary and secondary prevention of cardiovascular events have reported a nonsignificant trend toward an increased risk of total cancer (relative risk [RR], 1.22; 95% CI, 0.88–1.70) in the Norwegian vitamin (NORVIT) trial (N=3749; 800 µg FA/d for 40 months) and of colon cancer (RR, 1.36; 95% CI, 0.89–2.08) in the Heart Outcomes Prevention Evaluation (HOPE) II trial (N=5522; 2.5 mg FA/d for 5 years).

**HIGH FOLATE INTAKE: A CAUSE OF INCREASED COLORECTAL CANCER INCIDENCE?**

The major concern with mandatory FA fortification has been that while it protects against the development of NTDs, certain segments of the exposed population may benefit less and may even experience some adverse effects from an increased FA intake. In the United States and Canada, the average total folate intake in the post-mandatory fortification period is estimated to be approximately 400 µg/d in supplement non-users with approximately 200 µg/d consumed as FA provided in enriched products. For those taking multivitamins containing FA, the estimated total intake is approximately 800 µg/d. However, these estimates of folate intake are likely to be underestimates; indeed, several studies have suggested that the increased folate intake in the post-fortification era in the US population may be about twice that originally anticipated. Furthermore, in the National Health and Nutrition Examination Survey 1999–2000, after FA fortification began, 23% of the US population, 43% of children aged ≤5 years, and 38% of elderly persons had high serum folate concentrations (≥45.3 nmol/L). In addition to the drastic increase in dietary folate intake from mandatory FA fortification, 30–40% of the North American population consume supplemental FA for several possible but as yet unproven health
benefits. This begs an obvious question, namely, what is the effect of the dramatically increased folate status resulting from mandatory FA fortification and supplementation on cancer incidence in the United States and Canada?

To address this important public health concern, Mason et al. examined a temporal post-fortification trend of colorectal cancer incidence in the USA and Canada using the following two data sets from the respective countries: the Surveillance, Epidemiology and End Result registry and Canadian Cancer Statistics (by the Canadian Cancer Society, National Cancer Institute of Canada, and Statistics Canada). Their analysis demonstrates that concurrent with FA fortification, the United States and Canada experienced abrupt reversals of the downward trend in colorectal cancer incidence that the two countries had enjoyed in the preceding decades (Figures 2 and 3). Absolute rates of colorectal cancer began to increase in 1996 (US) and 1998 (Canada), peaked in 1998 (US) and 2000 (Canada), and have continued to exceed the pre-1996/1997 trends by 4–6 additional cases per 100,000 individuals (Figures 2 and 3). These investigators hypothesized that the institution of FA fortification may have been wholly or partly responsible for the observed increase in colorectal cancer rates in the mid-1990s. Changes in the rate of colorectal cancer screening by endoscopic procedures do not seem to account for this increase in colorectal cancer incidence. However, because of the lack of complete control of potential confounders inherent in the two data sets, these observations do not prove a causal link between FA fortification and increased rates of colorectal cancer in North America in the mid-1990s. Nevertheless, the observations provide a highly provocative impetus for further discussion, debate and research aimed at elucidating potential deleterious effects of FA fortification and supplementation.

**FOLIC ACID FORTIFICATION AND SUPPLEMENTATION AND PEDIATRIC CANCERS AND PERINATAL OUTCOMES**

However, the news is not all bad for FA fortification and supplementation concerning cancer risk. A Canadian study reported that FA fortification was associated with a significant 60% reduction in the incidence of neuroblastoma among children ≤17 years of age (from 1.57 cases per 10,000 births in 1996 to 0.62 cases per 10,000 births after 1997, when FA fortification became mandatory in Canada) using the Pediatric Oncology Group of Ontario, which captures 95% of all pediatric cancers in Ontario. However, the incidence of infant acute lymphoblastic leukemia and hepatoblastoma remained almost the same in this study. The results from this study corroborate those of previous epidemiologic studies, which reported a protective effect of preconceptional and periconceptional maternal use of FA on the incidence of brain tumors in the offspring. This study suggests that FA fortification and preconceptional and periconceptional maternal use of FA supplementation may prevent the development of “new” cancers in a site-specific manner.

A recent meta-analysis of seven articles selected out of 61 articles that investigated the effect of preconceptional multivitamin supplements containing FA on several pediatric cancers demonstrated a protective effect for childhood leukemia (odds ratio [OR], 0.64; 95%CI, 0.53–0.78), in particular acute lymphocytic leukemia (OR, 0.61; 95%CI, 0.50–0.74), and for pediatric brain tumors (OR, 0.73; 95%CI, 0.60–0.88), especially neu-
robastoma (OR, 0.53; 95%CI, 0.42–0.68). However, this meta-analysis is limited by the following factors: 1) the fact that all studies included in the analysis were retrospective and had the inherent limitations of this design; 2) there was wide variance among studies in the compositions of the multivitamins as well as the timing and duration of exposure; and 3) it was not possible to attribute the observed cancer-protective effect to specific components of the multivitamins. Nevertheless, this report supports a potential cancer-preventive effect associated with preconceptional and periconceptional maternal use of multivitamins containing FA on certain pediatric cancers in their offspring.

Additional good news for preconceptional and periconceptional multivitamin supplementation comes from a recent study that examined the effect of maternal multivitamin supplementation on perinatal outcomes. The double-blind trial was conducted in Tanzania and included 8468 pregnant women who were negative for human immunodeficiency virus infection; the results showed that daily multivitamins (including multiples of the recommended dietary allowance [RDA], e.g., for FA, it was twice the RDA at 0.8 mg) from the time of enrollment (gestational week 12–27) until 6 weeks after delivery significantly reduced the incidence of low-birthweight and small-for-gestational-age births but had no significant effects on prematurity or fetal death compared with placebo.

However, a recent population-based case-control study from Germany that included 1867 cases and 2057 controls showed that although maternal use of vitamin, folate, or iron supplementation is associated with a reduced risk of non-Hodgkin’s lymphoma and certain types of leukemia in offspring, these supplements are associated with an increased risk of neuroblastoma. Furthermore, among participants in a large trial (N=2928) of FA supplementation during pregnancy, women who received 5 mg FA/d had a 70% increased risk of total cancer mortality compared with those not on supplementation (hazard ratio, 1.70; 95%CI, 1.06–2.72). The risk of death from breast cancer in women taking 5 mg FA/d was twice that of women taking no supplementation in this study, albeit nonsignificant (HR, 2.02; 95%CI, 0.88–4.72). Although this study did not address the effect of FA supplementation on the offspring, it suggested that FA supplementation during pregnancy may have adverse effects on the mothers.

**CONCLUSIONS**

Evidence has established the protective effect of FA fortification and preconceptional/periconceptional supplementation on NTDs. Furthermore, FA fortification and preconceptional/periconceptional supplementation of women may reduce the risk of certain childhood cancers in their offspring. However, FA supplementation and fortification may promote the progression of already existing, undiagnosed preneoplastic and neoplastic lesions. Following the success of mandatory FA fortification on the reduction of NTD rates in the United States and Canada, Costa Rica and Chile have instituted FA fortification with similar success. Several European countries, Australia, and New Zealand are currently considering whether or not and at what dose level to institute FA fortification. In fact, the United Kingdom's Food Standards Agency has recently recommended mandatory FA fortification on some foods. Whether possible deleterious effects of FA fortification and supplementation at the dose recommended outweigh its known and potential health benefits will not be known in the near future.

An emerging body of evidence has recently warned
of new potentially harmful effects of FA supplementation including resistance or tolerance to antifolate-based chemotherapy, anti-inflammatory drugs, anti-seizure treatments, epigenetic instability, decreased natural killer cell cytotoxicity, and genetic selection of disease alleles.\textsuperscript{1,2} Future debates and decisions regarding FA fortification should take into consideration all potential adverse effects and dose-responses of such a measure because it may be associated with very serious consequences for many generations. In addition to careful monitoring of adverse events, preclinical and population-based studies are warranted to determine efficacy, safety, and potential deleterious effects of FA fortification and supplementation on cancer risk and other health outcomes.

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