Will mandatory folic acid fortification prevent or promote cancer?\textsuperscript{1–3}

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ABSTRACT

An overwhelming body of evidence for a protective effect of periconceptional folic acid supplementation against neural tube defects (NTDs) led to mandatory folic acid fortification in the United States. The effectiveness of folic acid fortification in improving folic acid status has already been shown to be quite striking, with a dramatic increase in blood measurements of folic acid in the United States. Preliminary reports also suggest a significant reduction (\textasciitilde15–50\%) in NTDs in the United States. The success of folic acid fortification in improving folic acid status and in reducing NTD rates is truly a public health triumph and provides a paradigm of collaboration between science and public health policy. Although folic acid is generally regarded as safe, there continues to be concern that folic acid fortification may have adverse effects in subpopulation groups not originally targeted for fortification. In this regard, an emerging body of evidence suggests that folic acid supplementation may enhance the development and progression of already existing, undiagnosed premalignant and malignant lesions. Over the past few years, the US population has been exposed to a significant increase in folic acid intake, for which essentially no data on safety exist. The potential cancer-promoting effect of folic acid supplementation needs to be considered in carefully monitoring the long-term effect of folic acid fortification on the vast majority of the US population, who are not at risk of NTDs. Am J Clin Nutr 2004;80:1123–8.

KEY WORDS Folic acid fortification, folate, cancer, carcinogenesis, DNA methylation, neural tube defects

Folate is a water-soluble B vitamin that appears to play an important role in the pathogenesis of several disorders in humans, including anemia, cardiovascular disease, thromboembolic processes, neural tube defects (NTDs) and other congenital defects, adverse pregnancy outcomes, neuropsychiatric disorders, and cancer (1). Folic acid is the fully oxidized monoglutamyl form of this vitamin and is used commercially in supplements and in fortified foods. The expanding role of folate nutrition in health and disease has major public health implications. For example, evidence from intervention trials (2–4) and observational studies (5) for a protective effect of periconceptional folic acid supplementation against NTDs was considered to be sufficiently conclusive and led public health policy makers, including the US Public Health Service in 1992 and the Institute of Medicine in 1998, to recommend that all women who were of reproductive age or were capable of becoming pregnant consume 400 \mu g of folic acid from supplements or fortified foods in conjunction with consumption of folate-rich foods (6, 7). This recommendation was followed by a US Food and Drug Administration regulation in 1996 requiring that all flour and uncooked cereal-grain products in the United States be fortified with folic acid (140 \mu g/100 g) by January 1998 (8). Mandatory folic acid fortification was also implemented in Canada in 1998 (9) and in Chile (10), and limited voluntary folic acid fortification of specified foods was implemented in Western Australia in 1995 (11).

The effectiveness of folic acid fortification in improving folic acid status has already been shown to be quite striking, with a dramatic increase in blood measurements of folate (serum, plasma, and red blood cell) and a substantial decrease in plasma homocysteine (an accurate inverse indicator of folate status) concentrations in the United States and Canada (10–17). Preliminary reports suggest a significant reduction (\textasciitilde15–50\%) in the prevalence and incidence of NTDs in the United States, Canada, and Western Australia (18–23). However, it is impossible to definitely attribute the decrease in the incidence of NTDs in the United States solely to fortification (24) because NTD rates in the United States (and worldwide) were decreasing even before fortification began, possibly as a result of factors such as improved nutrition or prenatal diagnosis and termination.

Despite the observed beneficial effects of folic acid fortification on folic acid status and NTDs in countries that adopted either mandatory or limited voluntary fortification, there continues to be some concern that folic acid fortification may have adverse effects in subpopulation groups not originally targeted for fortification. Although folate is safe and almost free of toxicity (25), concerns that folic acid fortification may mask symptoms of vitamin B-12 deficiency, primarily in the elderly population, have been raised (7). Vitamin B-12 deficiency has been estimated to affect up to 10–15\% of the population over 60 y of age (7). Because of this concern, the amount of fortification chosen was estimated to provide on average 100 \mu g additional folic acid/d, with only a very small proportion of the population receiving \textasciitilde1 mg (7). The upper limit of 1 mg was a round number


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Received April 27, 2004.
Accepted for publication July 13, 2004.
chosen by the Institute of Medicine as unlikely to produce masking (7), although the folate intake that produces masking is controversial, with some arguing that intakes < 1 mg may cause this effect (24). Several European countries decided not to adopt mandatory folic acid fortification, and the United Kingdom’s Food Standards Agency Board and the Dutch Health Council even recommended against mandatory folic acid fortification, in part because of the potential for masking the diagnosis of a vitamin B-12 deficiency (5). The debate on the folic acid fortification controversy has become highly emotional; delaying folic acid fortification in some European countries was even labeled as public health malpractice (26).

A more logical alternative to generalized mandatory folic acid fortification might be targeted folic acid supplementation in a specific group at risk of NTDs (eg, women with a previous NTD-affected pregnancy). However, efforts to increase periconceptional folic acid supplements have been proven to be disappointing, and public health efforts to influence those persons most at risk are generally perceived to have been a failure. Surveys taken in the United States, Puerto Rico, Netherlands, and Western Australia have shown that the public health policy recommendations and massive folic acid education and promotion programs advocating daily consumption of supplements containing folic acid among women of childbearing age fail to substantially increase the proportion of women of reproductive age who take a daily supplement containing folic acid (5). What is most disturbing in these surveys is that although most of these women had knowledge of the importance of folic acid, only 30%–35% of women of reproductive age reported taking a daily supplement containing folic acid (5). Knowing that folic acid awareness does not necessarily translate into behavior change and that the neural tube closes during the fourth week of gestation, a time when many women are unaware of their pregnancy, public health policy makers opted for generalized folic acid fortification instead of targeted folic acid supplementation in subpopulations at risk of NTDs. However, there is evidence of success regarding reductions in NTDs and increased compliance with recommendations to take folic acid supplements in targeted geographic areas (27). For example, in response to the NTD Intervention Awareness Campaign in South Carolina, overall NTD rates decreased significantly, and no NTD recurrences were reported in women with a previous NTD-affected pregnancy who periconceptionally consumed supplements containing folic acid (27). The drop in overall NTD rates preceded fortification and coincided with higher reported supplemental folic acid intakes (27).

Implicit in the folic acid fortification recommendation and policy was that improving folate status in the general population may provide other health benefits in addition to the reduction in NTD rates. Over the next few years, there will likely be many epidemiologic studies that will attempt to elucidate the long-term effect of folic acid fortification on the incidence of anemia, cardiovascular disease, thromboembolic processes, congenital defects, adverse pregnancy outcomes, neuropsychiatric disorders, and cancers in countries that adopted mandatory and limited folic acid fortification. In this regard, a recent Canadian study (28) reported evidence supporting other potential health benefits of folic acid fortification. Using the database of the Pediatric Oncology Group of Ontario, which captures 95% of all pediatric cancers in Ontario, this study determined the effect of folic acid fortification on the incidence of neuroblastoma among children aged ≤17 y (28). The study showed that folic acid fortification was associated with a significant (60%) reduction in the incidence of neuroblastoma (from 1.57 cases per 10,000 births in 1996 to 0.62 cases per 10,000 births after 1997, when folic acid fortification of food became mandatory in Canada) (28). However, the incidence of infant acute lymphoblastic leukemia and hepatoblastoma remained almost the same (28). The results of this study corroborate those of previous epidemiologic studies, which reported a protective effect of the prenatal and perinatal maternal use of folic acid against the incidence of brain tumors in offspring (29–31). Therefore, the findings of this Canadian study suggest that, in addition to reducing NTD rates, mandatory folic acid fortification may prevent the development of certain cancers. Because of significant public health implications, the potential health benefits, including cancer prevention, of improved folate status in the general population make a strong case for mandatory folic acid fortification and for providing even higher amounts of folic acid as argued for by some proponents of mandatory generalized folic acid fortification.

Often neglected and lost in public health policy making and debate concerning folic acid fortification is the effect of folate on cancer development and progression. Perhaps one of the most speculative and provocative new medical applications of folate nutrition is the potential role of folate as a cancer preventive agent (1, 32). The concept that folate deficiency enhances, whereas folate supplementation reduces, the risk of neoplastic transformation appears counterintuitive and contradictory to our conventional understanding of folate biochemistry. Folate is an essential cofactor for the de novo biosynthesis of purines and thymidylate, and in this capacity, folate plays an important role in DNA synthesis and replication. Consequently, folate deficiency in tissues with rapidly replicating cells results in ineffective DNA synthesis. In neoplastic cells, in which DNA replication and cell division occur at an accelerated rate, interruption of folate metabolism causes ineffective DNA synthesis, resulting in inhibition of tumor growth (32, 33). Indeed, this has been the basis for cancer chemotherapy with several antifolate agents (eg, methotrexate) and 5-fluorouracil (32, 33). Furthermore, folate deficiency has been shown to induce regression and suppress the progression of pre-existing neoplasms in experimental models (34–36). In contrast to the inhibitory and promoting effect of folate deficiency and supplementation, respectively, on established neoplasms, folate status appears to have the opposite effect in normal tissues. An accumulating body of epidemiologic, clinical, and experimental evidence over the past decade 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 (1, 32). The potential causal relation between folate status and cancer risk has been further strengthened by the existence of several biologically plausible mechanisms relating to the sole biochemical function known for folate (mediating the transfer of one-carbon moieties), by which folate status may modulate the development and progression of cancer in normal tissues (32, 33). As an essential cofactor for the de novo biosynthesis of purines and thymidylate, folate plays an important role in DNA synthesis, stability and integrity, and repair, aberrations of which have been implicated in colorectal carcinogenesis (32, 33). Folate may also modulate DNA methylation, which is an important epigenetic determinant in gene expression, maintenance of DNA integrity and stability, chromosomal modifications, and the development of mutations (32, 33). A growing body of evidence from in vitro,
animal, and human studies indicate that folate deficiency is associated with DNA strand breaks, impaired DNA repair, increased mutations, and aberrant DNA methylation and that folate supplementation can correct some of these defects induced by folate deficiency (32, 33).

Epidemiologic studies have suggested an inverse association of folate with the risk of cancer of the colorectum, lungs, pancreas, esophagus, stomach, cervix, ovary, and breast and the risk of neuroblastoma and leukemia (1, 32). The precise nature and magnitude of the inverse relation between folate status and the risk of these malignancies, however, have not yet been clearly established (1, 32). The role of folate in carcinogenesis has been best studied for colorectal cancer (CRC) in the general population and in persons with chronic ulcerative colitis (1, 5, 32, 37). Most of the published epidemiologic and clinical studies found either a significant inverse relation between folate status (assessed by using dietary folate intakes or measurement of blood folate concentrations) and the risk of CRC or its precursor, adenoma, or an equivocal inverse relation that was not significant. In the case of equivalent inverse relations, either those relations became nonsignificant after adjustment, or folate status could not be distinguished from other factors in its relation to the risk of CRC or adenoma (1, 5, 32, 37). A recent meta-analysis of 11 prospective epidemiologic studies from the United States, Canada, Netherlands, and Sweden that included >500,000 male and female subjects showed a significant inverse association between folate intake (dietary and supplemental) and the risk of CRC (DJ Hunter, personal communication, 2003). This meta-analysis also showed a 20% reduction in the risk of CRC in subjects with the highest folate intake compared with those with the lowest intake. In some epidemiologic studies, the observed inverse association between folate status and CRC risk was further modified by the intake of alcohol and other nutritional factors (eg, methionine and vitamins B-6 and B-12) that are involved in the folate metabolic pathway and by polymorphisms of critical genes (eg, methylene tetrahydrofolate reductase gene 677C→T) that are involved in folate metabolism (1, 5, 32, 37). At present, human intervention trials provide no conclusive evidence supporting the protective effect of folate supplementation against CRC, although several small pilot studies have shown that folate supplementation may improve or reverse surrogate endpoint biomarkers of CRC (1, 32), and some epidemiologic studies have shown a beneficial effect of multivitamin supplements containing ≥400 μg folic acid on CRC risk and mortality (38, 39).

Although animal studies performed in chemical carcinogen and genetically engineered murine models of CRC generally support a causal relation between folate depletion and CRC risk, these studies have shown that the dose and timing of folate intervention are critical in providing safe and effective chemoprevention; exceptionally high supplemental folate doses and folate intervention after microscopic neoplastic foci are established in the colorectal mucosa promote rather than suppress colorectal carcinogenesis (1, 32). For example, in a standard chemical carcinogen rodent model of CRC, supraphysiologic doses of folate (>20 times the basal daily dietary requirement) were shown to increase the development and progression of CRC (40–42). Furthermore, in 2 genetic models of CRC (Apc /M and Apc+/- x Msh2-/- mice), moderate folate deficiency enhanced, whereas modest doses of folate supplementation suppressed, the development and progression of CRC if folate intervention was started before the establishment of neoplastic foci in the intestine (43, 44). If, however, folate intervention is started after the establishment of neoplastic foci, dietary folate has the opposite effect on the development and progression of CRC (43, 44). Some animal studies have also shown that dietary folate deficiency inhibits rather than enhances the development of breast cancer in rats (45, 46), which is in contrast to the inverse association between folate status and breast cancer risk observed in epidemiologic studies (46). In conjunction with some clinical observations, these animal studies suggest that folate possesses dual modulatory effects on carcinogenesis depending on the timing and dose of folate intervention (1). Folate deficiency has an inhibitory effect, whereas folate supplementation has a promoting effect on the progression of established neoplasms (1, 32). In contrast, folate deficiency in normal epithelial tissues appears to predispose them to neoplastic transformation, and modest amounts of folate supplementation (4–10 times the basal dietary requirement) suppress, whereas supraphysiologic doses enhance, the development of tumors in normal tissues (1, 32).

Although some similarities do exist, tumor development in chemical and genetically engineered animal models of CRC differs in several important histologic, clinical, and molecular genetic aspects from that observed in humans. Therefore, any extrapolation of observations from these models to human situations should be made cautiously. Notwithstanding these limitations, however, the data from animal studies suggest that the optimal timing and dose of folate intervention should be established before folate supplementation can be used as a safe and effective chemopreventive agent against CRC. Although folate appears to be an ideal candidate for chemoprevention because of its proven safety and cost (25), the safe and effective dose range of folate supplementation and optimal timing of folate chemoprevention have not been clearly established in humans. Animal studies and some clinical studies have suggested that folate supplementation may increase cancer risk and accelerate tumor progression if too much is given or if it is provided after neoplastic foci are established in the target organ. Therefore, modest doses of folate supplementation should apparently be implemented before the development of precursor lesions in the target organ or in persons free of any evidence of neoplastic foci. However, determining the presence of neoplastic foci in the general population is obviously an almost impossible task. Furthermore, folate might prevent the progression of certain precursor or preneoplastic lesions to frank malignancy but promote the progression of other lesions. What constitutes safe precursor or preneoplastic lesions on which folate may exert a protective effect has not yet been established. For example, should folate chemoprevention be started before there is evidence of established premalignant lesions, such as aberrant crypt foci or microscopic adenomatous lesions in the colorectum, or should folate chemoprevention be started even after these lesions are present?

It is apparent from the above discussion that folic acid supplementation should not be adopted as a chemopreventive agent against CRC and other cancers until definitive evidence indicates that such supplementation is indeed safe and effective. However, what are the effects of folic acid fortification on the risk of CRC and other malignancies? Is there any reason to believe that folic acid fortification may actually increase the risk of certain cancers? Although folic acid fortification may prevent NTDs as evidenced by preliminary reports suggesting a significant reduction (=15–50%) in the prevalence and incidence of NTDs in the...
United States, Canada, and Western Australia (18–23), the long-term effect of folic acid fortification on the risk of CRC and other malignancies may not be as clear as that observed for NTDs. Although folic acid fortification may prevent the development of new cancers in persons without preexisting premalignant lesions or neoplastic foci, it may promote the progression of these lesions in persons harboring them. The addition of folate to established tumors has previously been shown to cause an “acceleration phenomenon” in humans. For example, children with acute leukemia treated with folate supplementation experienced an accelerated progression of leukemia (47). Analogous to this situation, β-carotene supplementation has been shown to promote the development of lung cancer in smokers who likely harbored preneoplastic or neoplastic foci before supplementation (48, 49).

The success of folic acid fortification in improving folate status and in reducing NTD rates is truly a public health triumph and provides a paradigm of collaboration between science and public health policy. Improved folate status in the general population resulting from folic acid fortification may also lead to reduction in anemia, cardiovascular disease, thromboembolic processes, congenital defects, adverse pregnancy outcomes, and neuropsychiatric disorders. However, an emerging body of evidence suggests that folic acid supplementation may promote the progression of preexisting, undiagnosed premalignant and malignant lesions. Over the past few years, the US and Canadian populations have been exposed to a significant increase in folate intake, for which essentially no data on safety exist (24). No studies have been done to look directly or even indirectly for the adverse effects of greatly increased folate intakes (24). Several studies that assessed food composition and dietary intakes suggested that the increased postfortification folate intake in the US population may be about twice that originally anticipated (14, 50–52). However, these estimates of folate intake based on food-composition databases are likely underestimate because of limitations in the analytic methods previously used to analyze food folate (7).

Although the potential masking effect of folate on vitamin B-12 deficiency, especially in the elderly, has been the major concern of folic acid fortification, other adverse effects, such as the potential cancer-promoting effect of folic acid supplementation, need to be considered in carefully monitoring the long-term effect of folic acid fortification on health and disease in the vast majority of the US and Canadian populations, who are not at risk of NTDs. Furthermore, because intracellular and systemic folate concentrations are important determinants of the sensitivity of cancer cells to chemotherapy (53) and in the treatment of inflammatory and seizure disorders with the use of antifolates (25), the occurrence of resistance or tolerance to antifolate chemotherapy and antiinflammatory and antiseizure drugs should be added to the list of potential unwanted complications of folic acid fortification.

Another potential harmful consequence of folic acid fortification in relation to cancer risk modification concerns DNA methylation. DNA methylation of cytosine located within the cytosine-guanine (CpG) dinucleotide sequences is an important epigenetic determinant in gene expression (inverse relation), maintenance of DNA integrity and stability, chromatin modifications and remodeling, and the development of mutations (54). Neoplastic cells simultaneously harbor widespread genomic hypermethylation and more specific regional areas of hypomethylation (54). Genomic hypomethylation is an early and consistent event in carcinogenesis and is associated with genomic instability and increased mutations. In addition, site-specific hypermethylation at promoter CpG islands of tumor suppressor and mismatch repair genes is an important mechanism in gene silencing in carcinogenesis (54). Folate, in the form of 5-methyltetrahydrofolate, is involved in remethylation of homocysteine to methionine, which is a precursor of S-adenosylmethionine, the primary methyl group donor for most biological methylations, including that of DNA (55). Although the effect of folate deficiency on DNA methylation is highly variable and complex, folate supplementation appears to significantly increase the extent of genomic and site-specific DNA methylation in certain situations (55). This then begs a question: can folic acid fortification methylate normally unmethylated promoter CpG islands of tumor suppressor or mismatch repair genes and inactivate these genes, thereby promoting the development of cancer? This is a theoretical possibility and warrants investigation. In this regard, a recent animal study using viable yellow agouti (A<sup>vy</sup>) mice showed that maternal dietary methyl group supplementation with a modest amount of folic acid, vitamin B-12, choline, and betaine may permanently alter the phenotype of the offspring via increased CpG methylation at the promoter CpG site of the agouti gene (56). The investigators found that the methylation status of the promoter CpG region of the agouti gene was highly correlated with the methylation status of the adjacent transposon gene (56). Transposons are common and potentially mobile sequences of DNA that are scattered throughout the genome (57). More than 35% of human DNA is estimated to be derived from transposons (57). Depending on where they are inserted in DNA, transposons can end up silencing neighboring genes (57). Therefore, this study by Waterland and Jirtle (56) showed that there is a localized epigenetic instability in methylation that arises from an interaction between the transposon and its nearby genetic region and that genes that manifest a transposon region adjacent to a promoter region of DNA could be influenced by early nutrition containing methyl group donors, including folic acid. These investigators speculated that “population-based supplementation with folic acid, intended to reduce the incidence of NTD and long presumed to be purely beneficial, may have unintended deleterious influences on the establishment of epigenetic gene-regulatory mechanisms during human embryonic development (57).”

Some proponents of mandatory folic acid fortification have labeled the delay in folic acid fortification in some European countries as public health malpractice (26). However, a reasonable conclusion from the above discussion is that inertia on folic acid fortification in these European countries should not be construed as public health malpractice but should be regarded as public health prudence. The effect of folic acid fortification on cancer risk has a greater effect on public health than on NTDs because of the incidence and prevalence of cancer and premalignant precursors in the general US population. For example, in the United States, CRC is the fourth most frequently diagnosed and the second most common cause of cancer-specific death for both men and women (58). In 2004 alone, 146 940 new cases of CRC are expected to be diagnosed, and approximately 40% of these are expected to die within 5 y (58). In 2004 an estimated 56 370 deaths will have been caused by CRC (58). The lifetime risk of developing CRC is approximately 6% (59), and treatment costs nearly $6 billion annually (60). Colorectal adenomas, the well-established precursor of CRC (59), are found in approximately 25% of people by 50 y of age in the United States, and the prevalence increases with age.
(59). On the basis of autopsy series, which are probably less susceptible to selection and detection bias than are clinical series, the prevalence of adenomas is estimated to reach ≈50% by 50 y of age (59). It has been estimated that ≈25% of adenomas progress to CRC over 5–10 y (59). In contrast, NTDs occur in ≈1 of every 1000 births in the United States, and spina bifida and anencephaly, the most common NTDs, together affect ≈4000 pregnancies resulting in 2500–3000 US births annually (61). It is evident from this statistic that the potential effect of folic acid fortification on adenoma progression to CRC and on CRC progression to metastasis far outweighs the effect on NTD risk reduction. Given the prevalence and incidence of colorectal adenomas and CRC in the general population in the United States, therefore, whether or not folic acid fortification promotes the progression of adenomas to CRC in the colorectum is a legitimate public health concern and needs careful monitoring.

A recent study showing that folic acid fortification significantly reduced the incidence of neuroblastoma in Ontario (28) is an encouraging piece of information in this regard. However, long-term follow-up studies are urgently warranted to determine the effect of folic acid fortification on the incidence of cancer and on DNA methylation and other epigenetic regulatory machinery in countries that have adopted mandatory generalized folic acid fortification. Furthermore, safe and effective amounts of folic acid fortification need to be scientifically determined by using relevant animal, experimental, and clinical models. The potential cancer-promoting effect of folic acid fortification in the vast majority of the US population, who are not at risk of NTDs but have been unintentionally exposed to high amounts of folic acid, is a legitimate public health concern and needs careful monitoring.

The author had no conflicts of interest in connection with this article.

REFERENCES


