

## Hypothesis

# A Temporal Association between Folic Acid Fortification and an Increase in Colorectal Cancer Rates May Be Illuminating Important Biological Principles: A Hypothesis

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## Abstract

Nationwide fortification of enriched uncooked cereal grains with folic acid began in the United States and Canada in 1996 and 1997, respectively, and became mandatory in 1998. The rationale was to reduce the number of births complicated by neural tube defects. Concurrently, the United States and Canada experienced abrupt reversals of the downward trend in colorectal cancer (CRC) incidence that the two countries had enjoyed in the preceding decade: absolute rates of CRC began to increase in 1996 (United States) and 1998 (Canada), peaked in 1998 (United States) and 2000 (Canada), and have continued to exceed the pre-1996/1997 trends by 4 to 6 additional cases per 100,000 individuals. In each country, the increase in CRC incidence from the prefortification trend falls significantly outside of the downward linear fit based on nonparametric 95% confidence intervals. The statistically

significant increase in rates is also evident when the data for each country are analyzed separately for men and women. Changes in the rate of colorectal endoscopic procedures do not seem to account for this increase in CRC incidence. These observations alone do not prove causality but are consistent with the known effects of folate on existing neoplasms, as shown in both preclinical and clinical studies. We therefore hypothesize that the institution of folic acid fortification may have been wholly or partly responsible for the observed increase in CRC rates in the mid-1990s. Further work is needed to definitively establish the nature of this relationship. In the meantime, deliberations about the institution or enhancement of fortification programs should be undertaken with these considerations in mind. (Cancer Epidemiol Biomarkers Prev 2007;16(7):1325–9)

This communication highlights a temporal association between folic acid fortification of enriched cereal grains in the United States and Canada and an increase in the incidence of CRC in these two countries. The possibility that folic acid fortification was causally responsible for this increase in CRC incidence is consistent with the known biological functions of folate and with several preclinical and clinical observations that are briefly reviewed in this article. However, the observations and integration of knowledge presented here merely create a hypothetical foundation on which further research will be required to determine whether true causality exists.

## Background

In March of 1996, the U.S. Food and Drug Administration mandated that all enriched flour and uncooked cereal grains sold in the United States should be fortified with 140 µg folic acid/100 g of flour no later than January of 1998 (1). Although mandatory fortification did not begin until 1998, large food companies were well aware of the new mandate that was about to be codified and therefore began to roll in voluntary fortification of their products almost immediately after the 1996 report. Canada also chose to fortify in the similar manner (150 µg/100 g of flour), although the legislation did not allow for voluntary fortification to begin until the end of December 1996, with a deadline for mandatory fortification of November 1998 (2). The rationale for these programs arose from the observations of large, randomized clinical trials conducted in Europe in the late 1980s and early 1990s that established that maternal folate supplementation during the preconceptional period markedly reduced births complicated by the neural tube defects, spina bifida, and anencephaly (3, 4).

Following the institution of fortification in these two countries, population-based studies showed the effectiveness of this measure: plasma levels of folate in the adult population increased ~2-fold as a result (5, 6) and the incidence of births complicated by neural tube defects was variously reported to decline by 20% to 50% (7–9). The rather large increase in folate status was perhaps not surprising because analyses of several cereal grains that were purchased after the institution of fortification showed that in many instances the actual amount of folate was 150% to 300% greater than the mandate (10), suggesting that in this early era of fortification, manufacturers often included “overage” to ensure that they were meeting

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The data used for the analyses presented in this article were extracted from publicly available websites, as cited in the text. J.B. Mason takes responsibility for the accuracy of the data analyses and assumes final responsibility for the decision to submit for publication.

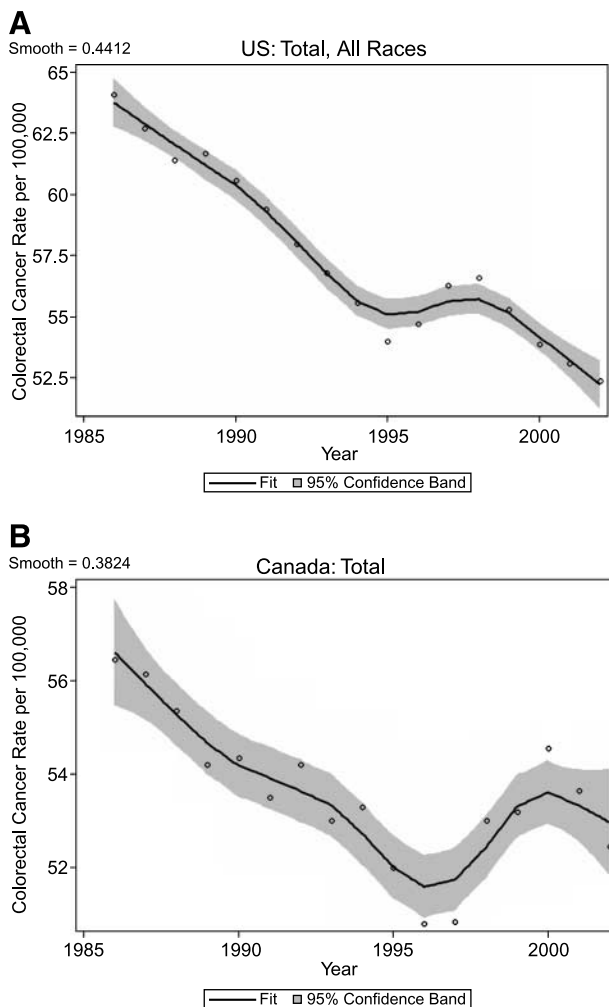
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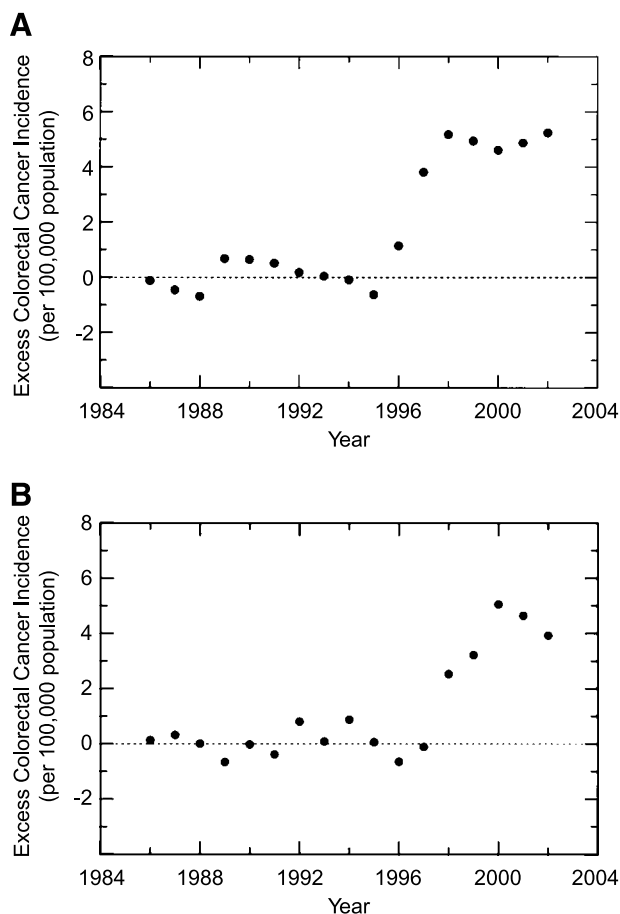
**Figure 1.** Colorectal cancer: age-adjusted incidence in the United States and Canada. Age-adjusted CRC incidence from 1986 to 2002 in the United States (**A**) and Canada (**B**) based on nationally representative databases (for Canada, the incidence is based on the average of the rates for men and women).  $\circ$ , data points. A nonparametric loess smoother was fitted to the data and 95% confidence bands (gray areas) were drawn by using PROC LOESS of SAS for Windows, version 9.1.2, with its default settings.

the minimal level of mandated fortification. Several other countries such as Costa Rica and Chile have also instituted fortification and the reduction in neural tube defect incidence in those countries has been as large as, or larger than, the North American experience (11, 12). A large-scale trial in China confirmed the robust nature of the benefits of supplementation in an Asian population (13). Folic acid fortification has therefore been hailed by most as a great step forward in public health policy and occasional calls continue to be voiced to further increase the level of fortification (14). To date, all nationwide fortification programs have used the fully oxidized and pharmaceutical form of the vitamin, folic acid, rather than the chemically less stable reduced forms of folate that occur naturally in foods.

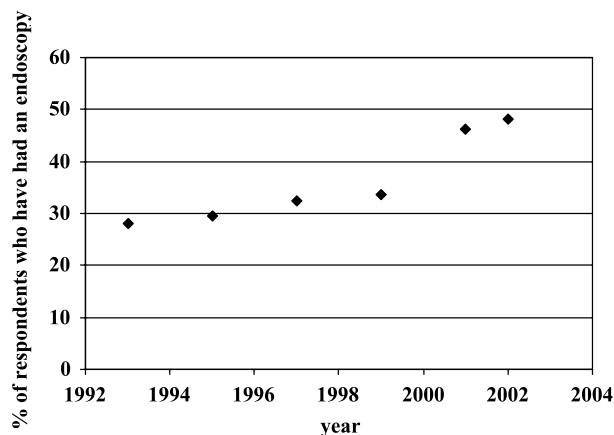
Compelling observations from many large epidemiologic studies indicate an inverse relationship between folate status, measured by habitual dietary intake or blood levels, and the risk of developing CRC (reviewed in ref. 15). Emerging data also suggest that folate may be protective against other cancers, such as those of the breast, uterine cervix, and others (16). Additional evidence for a true mechanistic role for folate comes from epidemiologic observations of a common poly-

morphism in the folate-dependent enzyme methylenetetrahydrofolate reductase, which is associated with a 40% to 50% reduction in the risk of developing colorectal neoplasms (reviewed in ref. 17). Further, preclinical studies in both cell culture and animal models provide strong evidence that this relationship is a true causative one: such studies indicate that the central role of folate in biological methylation and nucleotide synthesis goes awry in states where there is folate inadequacy (18). Because aberrations in DNA methylation and nucleotide synthesis and repair are among the most common pathways identified in carcinogenesis, these mechanistic studies provide biologically plausible explanations for the apparent protective effect of folate.

However, a very frequent feature of neoplastic cells is that they have much higher rates of proliferation than their normal counterparts. This seems to be the case in colorectal adenomas and cancers (19). Therefore, the pivotal role of folate in nucleotide synthesis, including its role as a cofactor in a rate-limiting step for DNA synthesis (20), also makes it a potential growth factor for neoplastic cells. The potential for folate to serve as a growth factor for neoplastic cells is further amplified by the tendency of most cancer cells to up-regulate the membrane receptors that mediate their uptake of folate, as well as some of the critical folate-dependent enzymes necessary for DNA synthesis (21, 22). Moreover, this is not just a theoretical concern: two groups of clinical investigators inadvertently provided us with explicit proof of this in the



**Figure 2.** The excess incidence of CRC in the United States and Canada compared with prefortification trends. The excess incidence of CRC was calculated as deviations from linear regressions based on the years preceding the institution of voluntary fortification: the 1986-1995 trend in the United States (**A**) and the 1986-1996 trend in Canada (**B**).



**Figure 3.** Endoscopy rates of the colon and/or rectum in the United States from 1993 to 2003. Endoscopy rates of the colon and/or rectum from 1993 to 2003 based on the Centers for Disease Control and Prevention Behavioral Risk Factor Surveillance System Surveys (32). A positive response was one in which a subject reported that they had undergone the procedure.

1940s when they gave large doses of folic acid to individuals with acute leukemia and observed what Sidney Farber politely termed "the acceleration phenomenon" whereby the rate of expansion of the leukemic clone increased tremendously (refs. 23, 24; this, incidentally, prompted Farber and others to try antifolates for the treatment of childhood leukemia, and it was this latter step that is generally considered to be the beginning of the modern era of cancer chemotherapy). Controlled studies in a variety of animal models of colon cancer have provided similar findings: in settings where there is a particularly strong underlying predisposition to colon cancer or in a setting where neoplastic tumors are already established, supplemental folic acid is protective only before neoplastic foci appear in the intestine. Once such foci are established, the more folic acid that is given, the faster microscopic foci and macroscopic tumors arise (25, 26). Also consistent with this concern are contemporary human data. In a large multicenter trial 1,021 individuals with recently resected colorectal adenomas were randomized to receive either 1 mg of folic acid or a placebo over a period of 3 to 5 years (27). Analysis of the final follow-up colonoscopy did not reveal a protective effect of folic acid supplementation; rather, there was a small but significant increased risk in the multiplicity of recurrent adenomas with supplementation as well as a marginally significant increase in 'high-risk' adenomas. Two recent reviews have outlined the background science underlying this concern (28, 29). Further, one of us has previously stated the need for monitoring systems that would detect whether such concerns are borne out in the population (30).

### Observations and Analyses

We now present population-based observations from two data sets from the United States and Canada that suggest that such changes in CRC rates may have occurred as a result of folate fortification in North America. These are the first observations from nationally representative data sets to support the hypothesis that is presented in this communication.

Figure 1A and B presents graphs that depict the age-adjusted incidence of CRC in the United States and Canada, respectively, from 1986 to 2002. The U.S. data are derived from the nationwide Surveillance, Epidemiology and End Result

registry,<sup>4</sup> which currently collects cancer incidence and survival data from population-based cancer registries covering ~26% of the U.S. population. Canadian data were obtained from Canadian Cancer Statistics 2006,<sup>5</sup> which is published annually by the Canadian Cancer Society and the National Cancer Institute of Canada and is based on a nationwide registry of cancer incidence and mortality maintained by the Health Statistics Division at Statistics Canada. The open circles in Fig. 1A and B represent the data points. A nonparametric loess smoother was fitted to the data and 95% confidence bands (gray areas) were drawn by using PROC LOESS of SAS for Windows, version 9.1.2, with its default settings.

The graphs reflect the fact that each of these countries has generally enjoyed a modest decline in the age-adjusted incidence of colorectal cancer (CRC) over the past 15 years. However, a remarkable departure from this decline is noted for each country in the mid-1990s: in the United States beginning in 1996 and continuing through 1998, and in Canada beginning in 1997 and extending through 2000. Neither parametric nor nonparametric curve fitting procedures could adequately capture the apparent sharp bends in the data associated with the implementation of folic acid fortification. These abrupt changes in the prefortification trends are underscored by the fact that it is not possible to draw a straight line through the shaded area from end to end, indicating that the bends in the curves starting at 1996 (United States) and 1997 (Canada) are not the result of random variability about a straight line. In each instance, the sudden increase in CRC incidence represents a highly statistically significant deviation from the pre-1996/1997 trend, resulting in an excess of ~4 to 6 additional cases per 100,000 individuals. These deviations are quantified in Fig. 2A and B. For these analyses, a simple linear regression was generated based on the data points encompassing the prefortification years and then the deviation from this regression was calculated to produce the relationships seen in Fig. 2. As emphasized in these figures, the peak incidence of excess cases of CRC is achieved between 1998 and 2000, and in both countries this excess incidence had not yet returned to its earlier baseline by 2002. The same type of analyses done in Fig. 1A and B were also conducted independently on the data derived from men and women in each country, and the observed increase in CRC incidence in each instance deviated significantly from the 95% confidence band of the downward linear trend (data not shown), indicating that the cause of this increase in CRC affected both sexes.

There is a very close chronological relationship between these increases in the incidence of CRC and the remarkable increase in systemic folate status that occurred among adults as a result of folic acid fortification. As mentioned above, both countries experienced approximately a 100% increase in plasma folate levels between 1996 and 1999 (5, 6). The effect was also robust among habitual users of vitamin supplements: they experienced an absolute increase in plasma folate concentrations as great or greater than nonusers although, on a percentage basis, supplement users only experienced an increase of 62% because their plasma folate was substantially higher to begin with (5). Although mandatory fortification did not begin until 1998, it is important to recall that voluntary fortification in the United States began almost immediately after the March 1996 report. Voluntary fortification began somewhat later in Canada because the legislation did not allow for it until December 25, 1996. Thus, the increase in the incidence in CRC in each country was nearly contemporaneous with the increase in dietary folic acid intake and blood folate levels.

<sup>4</sup> <http://www.seer.cancer.gov>

<sup>5</sup> See "Canadian Cancer Statistics" under "Canada-wide" at <http://www.cancer.ca>.

To consider cancer screening as a potential confounding factor that could account for this apparent concordance between folate intake and CRC rates, we examined the U.S. national rates of colorectal endoscopy during the same era. This was done to ensure that the increasing acceptance of this procedure by the populace was not responsible for the aforementioned increase in the rate of CRC. These data (Fig. 3) are based on the Centers for Disease Control and Prevention Behavioral Risk Factor Surveillance System, a state-based, random-digit-dialed telephone survey of the civilian, U.S. noninstitutionalized population. Done yearly since 1984, the Behavioral Risk Factor Surveillance System tracks both health conditions and risk behaviors and collected national prevalence data for CRC screening biannually from 1993 to 2001 as well as in 2002 and 2004 (31). The database is an imperfect one for our purposes: during the 1990s the survey question variably inquired about "proctoscopies," "sigmoidoscopies," and "colonoscopies" and it did not distinguish those procedures done for screening versus those done for the diagnosis of CRC. It nevertheless is the only database providing biannual nationwide data on this matter during the 1990s. Figure 3 indicates that although there was a gradual increase in the screening rates during the early 1990s, no sudden increase occurred during the period before 1999. The upward inflection in the screening rate that occurred after 1999 has been described in a previous publication (32) and is ascribed to the "Katy Couric Effect" in which Couric's colonoscopy was televised nationwide in an attempt to encourage acceptance of the procedure. It is also relevant that Medicare coverage of screening colonoscopies for average risk individuals did not begin in the United States until July of 2001.

## Discussion

These observations do not prove a causal link between folic acid fortification and increased rates of CRC in North America in the mid-1990s. Whether the increased rate of CRC between 1996 and 2000 was due to the institution of folic acid fortification is a matter of speculation based on a temporal association between the two phenomena and a supportive literature that indicates that high intake of folic acid can accelerate the growth of established neoplasms. Because it is estimated that 35% to 50% of Americans over the age of 50 years harbor one or more colorectal adenomas (33) and that the vast majority of these growths lack any associated signs or symptoms, we suggest that the addition of substantial quantities of folic acid into the food supply in the mid-1990s may have facilitated the transformation of adenomas into cancers, or small cancers into larger ones: in each instance this would make the lesions more evident at the time of a routine screening colonoscopy. In addition, accelerating the growth of benign or malignant neoplasms to a size that is sufficient to produce symptoms may have prompted more individuals to seek out colonoscopies. Moreover, if neoplasms grew in size as a result of fortification, it would likely have made screening more efficient because the "miss rate" for larger lesions is less than for small lesions.

Although the stated limitations of the Behavioral Risk Factor Surveillance System preclude a definitive conclusion that no marked acceleration in the rate of colonoscopic surveillance occurred during the years in question, several features of the cancer incidence data argue against a simple screening effect, thereby corroborating the conclusions drawn from Fig. 3. First, the fact that the upswing in CRC incidence occurred in two countries with distinctly different health care systems suggests that a modification in screening recommendations or policy in one country would not translate into a similar increase in the apparent rate of CRC in both countries. Second, the fact that the increase in CRC incidence in Canada lagged 1 to 2 years

behind the increase in the United States is consistent with the fact that voluntary fortification in Canada was permitted nearly a year later than in the United States. The observation that the decline rates in CRC in Fig. 1A and B seem to be returning to their pre-1996/1997 downward trajectory may indicate that fortification "unveiled" a large number of covert neoplasms that would have otherwise never transformed into cancers or would have only gradually evolved into cancers over many years, and once this population of susceptible neoplasms was removed from the population, preexistent forces that were causing a downward trend in incidence were once again able to predominate. Lastly, the fact that the incidence of CRC in the United States and Canada has not returned to the pre-1996/1997 trends argues against a simple screening effect, in which case uncovering additional cases earlier would lead to a deficit in diagnosed cases later on.

The fact that a pharmaceutical form of folate, folic acid, has been used for fortification may be of importance. Folic acid is converted to a natural biological form of the vitamin as it passes through the intestinal wall, with enzymatic reduction and methylation resulting in the circulating form of the vitamin, 5-methyltetrahydrofolate. Nevertheless, it has been known for some years that oral doses of folic acid in physiologic quantities can saturate this conversion mechanism, resulting in detectable levels of circulating folic acid, and there has been some concern that this oxidized, nonsubstituted form of folate might feasibly be detrimental because it is not a naturally occurring coenzymatic form of the vitamin. Several studies have shown that as little as 200  $\mu\text{g}$  of oral folic acid may produce the acute appearance of detectable levels of folic acid in the bloodstream (34-36), and more recently, it has been shown that the daily ingestion of 400  $\mu\text{g}$  produces a sustained appearance of folic acid in the blood (37). Up to 35% of the U.S. adult population is thought to regularly consume a supplement containing folic acid, most of which contain 400  $\mu\text{g}$  per pill (38). When this folic acid intake is added to that which appears in fortified foods such as breakfast cereals plus the additional quantity that is now consumed in the form of mandatory cereal grain fortification, it should come as no surprise that a substantial percentage of the population has detectable quantities of folic acid chronically circulating in the bloodstream. Consistent with these projections are the recent observations of Troen et al. (39), who reported detectable levels of folic acid in the blood among 78% of 105 healthy, free-living postmenopausal women, 54% of whom were taking a folate-containing supplement on a daily basis. Interestingly, the Troen et al. study also observed that increasing concentrations of plasma folic acid among the elder women who took folic acid supplements were inversely associated with decreases in the cytotoxicity of circulating natural killer cells. Natural killer cells are a population of lymphocytes thought to play a role in the destruction of arising clones of neoplastic cells (40). Thus, the coenzymatic form of folate used for fortification is perhaps relevant in determining whether a detrimental effect is produced from excessive intake.

It is extremely important that the public and the scientific community not misconstrue either these observations or the nature of our interpretations. We do not propose to ignore or negate the very compelling body of scientific evidence that has accrued over the past 15 years that indicates that supplemental folic acid protects against neural tube defects and that habitually high intakes of dietary folate are protective against CRC. Indeed, due to the strength of the existing epidemiologic and preclinical observations in conjunction with the known biological effects of folate, several of us remain strong advocates of this latter hypothesis. Instead, by presenting these data, we wish to highlight the potential complexity of the response to this nutrient and emphasize prior observations that have been made in both preclinical and clinical studies that indicate that administering high doses of folic acid to

susceptible individuals or in an inappropriate time frame may accelerate the growth of existing neoplasms. Clearly, further research needs to be conducted to determine whether our hypothesis that folic acid fortification was responsible for the increase in CRC rates in the 1990s is a valid one. Nevertheless, the potential ramifications of such a phenomenon, if real, are quite substantial. We therefore contend that until further research defines the nature of this relationship, this concern should be considered in the debate that is presently under way in many countries that are deciding whether or not to institute, or enhance, fortification. By bringing attention to these CRC trend data in relation to fortification, and pointing out their consistency with research on folic acid and cancer development, our aim is to encourage better monitoring and further research in this field. These considerations illustrate just how important a thorough understanding of these complex issues is if fortification programs to avoid neural tube defects or supplemental use of folate to prevent cancer are to be carried out in a safe manner.

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