Chapter 20
Folic Acid to Prevent Neural Tube Defects: Success and Controversies

Philippe De Wals

Key Points

- The impact of folic acid food fortification in Canada is described and current controversies are discussed.
- Mandatory fortification of many cereal products was implemented in 1998.
- Level of fortification is set at 0.15 mg/100 g for flour and cornmeal.
- The estimated average additional intake is approximately 150 μg/day.
- Folate deficiency in the population was virtually eliminated.
- The overall frequency of neural tube defects was reduced by a factor of 2 and geographical variation almost disappeared.
- A decrease in the birth frequency of congenital cardiovascular malformations, of Wilms' tumor and neuroblastoma in children, and of stroke mortality in adults was also observed.
- Currently, there is no consensus regarding the folic acid supplement dose to recommend for women of childbearing age, the safety and potential effectiveness of increasing folic acid fortification level, and the potential impact and feasibility of fortifying food with vitamin B12.

Keywords Fortification • Folic acid • Congenital anomalies • Cardiovascular diseases • Cancers • Canada

Abbreviations

FA Folic acid
NTD Neural tube defect

Introduction

This chapter contains the story of folic acid (FA) food fortification in Canada. The first section describes the context in which the decision was made to pass a regulation requiring flour producers to fortify a wide range of products with the aim of preventing neural tube defects. In Part II, the results

P. De Wals, MD, PhD (✉)
Department of Social and Preventive Medicine, Laval University,
CRIUCPQ, 2725 Chemin Ste-Foy, Quebec City, QC, Canada G1V4G5
e-mail: philippe.dewals@criucpq.ulaval.ca

V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_20,

of impact studies are described: increase in dietary intake, improvement in folate status, and reduction in the frequency of neural tube defects and possibly other congenital anomalies, cardiovascular disease, and some pediatric cancers. The difficulties involved in studying the adverse effects of fortification are discussed. Finally, three current controversies are summarized: the folic acid recommendation for women of childbearing age, increasing FA fortification levels, and fortifying food with vitamin B12.

Implementation of Folic Acid Fortification in Canada

How the Decision Was Made in Canada

Irrefutable evidence of the protective effect of folic acid was provided when the results of a randomized trial piloted by the Medical Research Council at 33 centers in 7 countries were published in 1991 [1]. This sparked an immediate debate in the United States regarding the merits of different preventive strategies such as improved nutrition, taking vitamin supplements, and fortifying the food chain [2, 3]. The debate received a lot of publicity and involved a large number of public organizations (Centers for Disease Control and Prevention, Food and Drug Administration, Institute of Medicine), professional bodies (American Medical Association, American College of Preventive Medicine, American College of Medical Genetics, American Academy of Paediatrics, American College of Obstetrics and Gynaecology, American Academy of Family Physicians), the industry (flour producers, vitamin manufacturers), and academia. With some difficulty, a consensus was reached to recommend a policy of fortifying a wide range of food products [4, 5]. Impact analyses were done to determine which food products should be fortified in order to increase the dietary intake of the largest possible number of women of childbearing age while ensuring that average daily intakes remained within safe limits. In the end, a regulation was passed making the fortification of certain foods mandatory starting January 1, 1998 [6].

In Canada, regulations involving food are the responsibility of Health Canada. At that time there was no public health agency operating with a certain degree of independence of the government as was the case with the Centers for Disease Control in the United States. The debate received little attention and took place behind the scenes at Health Canada. In March 1996, a workshop was organized to examine the issue of the prevention of neural tube defects [7]. There was not enough support for a regulation making fortification mandatory, and the only recommendation was to conduct a fortification pilot project (which was never done). In the next few years, pressure was exerted by the agrifood industry in general and flour producers in particular, who were unhappy about having to meet different standards for products sold in the USA and Canada. This pressure, combined with pressure from the public health network in the United States, finally persuaded Health Canada to support a common policy. The Food and Drug Regulations were amended in December 1996 to allow the fortification of certain products with folic acid [8] and then again in November 1998 making fortification mandatory in order to bring the fortification levels of flour in Canada in line with those in the United States [9].

How the Program Was Implemented in Canada

The level of fortification set in Canada is 0.15 mg/100 g for flour and cornmeal and 0.20–0.27 mg/100 g for pasta, as in the United States. In the USA, but not Canada, rice may be enriched at 0.154 mg/100 g up to a maximum of 0.308 mg/100 g and corn grits and farina at 0.15 mg/100 g. Breakfast cereals may be enriched up to 400 μg per serving in the USA and up to 60 μg per serving in Canada. In order to meet US requirements for imported flour on January 1, 1998, the Canadian industry started fortification early in 1997 [10]. Biochemical analysis of food products in Canada suggested that actual FA levels

in food could be somewhat higher than recommended as manufacturers were extra careful about meeting required levels [11].

There are no specific data concerning the extra cost of this fortification in Canada. Synthetic folic acid is not expensive to make, and flour producers should already have been doing some fortification to offset the losses in the cooking process. In the United States, the total cost of the operation was estimated to be three million dollars per year, or less than 1 cent per person [12]. For the industry, this cost is minimal and did not result in any price increase for the fortified products.

Although the avowed aim of the policy was to prevent neural tube defects, virtually no consideration had been given to measuring the impact of the policy. Canadian researchers had to step in to study this aspect.

Impact of Folic Acid Food Fortification in Canada

Intake of Folic Acid

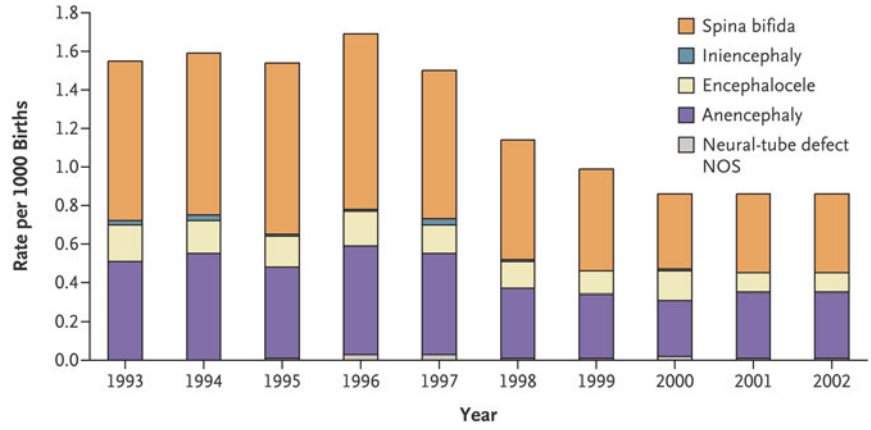
The goal of fortification was to provide an additional 100 μg/day of FA to women of childbearing age [9]. A study of pre-fortification vs. post-fortification folate levels in Ontario women of childbearing age found geometric mean red cell folate levels of 527 nmol/L vs. 741 nmol/L, with serum B12 levels remaining unchanged [13]. On the basis of the increase in serum folate concentration reported in Ontario, it was estimated that the fortification program increased folic acid consumption by 150 μg/day [14]. Based on four provincial dietary surveys conducted in Canada since fortification and assuming addition at the levels required by regulation, the average estimated additional intake was 131 μg/day among women 19–30 years of age (Lee NS, Food Directorate, Health Canada, written communication, August 25, 2006).

Folate Status

A cross-sectional study was conducted between August 1996 and July 1997 among pregnant women in Newfoundland during the first prenatal visit to obtain pre-fortification baseline data [15]. On the basis of the interpretive criteria used for red blood cell folate status, 11 % of the women were deficient (<340 nmol/L) and a further 13 % were classified as indeterminate (340–420 nmol/L). In 2007–2009, the folate status of a nationally representative sample of Canadians, including a subset of women of childbearing age, was assessed [16]. Less than 1 % of Canadians showed folate deficiency (red blood cell folate <305 nmol/L) and 40 % showed high folate concentrations (>1,360 nmol/L). Among women of childbearing age, 22 % showed concentrations below those considered optimal for maximum neural tube defect risk reduction (<906 nmol/L). No differences by age or income were found among women of childbearing age. Today, folate deficiency is virtually nonexistent in the Canadian population. As a result, ordering folate assays is no longer recommended for the investigation of anemia [17].

Neural Tube Defects

Changes in the frequency of neural tube defects before and after food fortification with folic acid were assessed in a multicentric study in seven Canadian provinces from 1993 to 2002 [10]. The study population included 1.9 million live births, stillbirths, and terminations of pregnancies because of fetal



All neural-tube defects	1.55	1.59	1.55	1.69	1.50	1.14	0.99	0.86	0.86	0.86
Spina bifida	0.83	0.84	0.89	0.91	0.77	0.62	0.53	0.39	0.41	0.41
Iniencephaly	0.02	0.03	0.01	0.01	0.03	0.01	0.00	0.01	0.00	0.00
Encephalocele	0.19	0.17	0.16	0.18	0.15	0.14	0.12	0.15	0.10	0.10
Anencephaly	0.51	0.55	0.47	0.56	0.52	0.36	0.33	0.29	0.34	0.34
Neural-tube defect NOS	0.00	0.00	0.01	0.03	0.03	0.01	0.01	0.02	0.01	0.01

Fig. 20.1 Prevalence of neural-tube defects, according to diagnostic category, in seven Canadian provinces from 1993 through 2002 (Reprinted with permission from the New England Journal of Medicine [10])

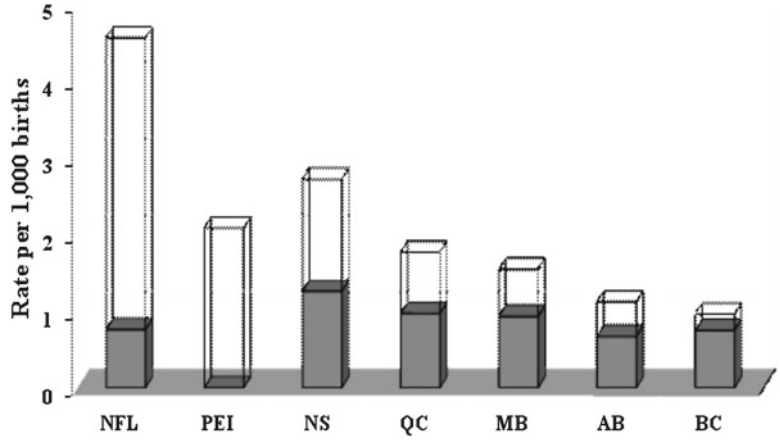


Fig. 20.2 Prevalence of neural tube defects in seven Canadian provinces before (*upperline*) and after (*lower line*) folic acid food fortification. *NFL* Newfoundland and Labrador; *PEI* Prince Edward Island; *NS* Nova Scotia; *QC* Quebec; *AB* Alberta; *BC* British Columbia (Adapted from the New England Journal of Medicine [10])

anomalies. The prevalence of neural tube defects decreased from 1.58 per 1,000 births before fortification to 0.86 per 1,000 births during the full-fortification period, a statistically significant reduction of 46 % (Fig. 20.1). The observed reduction in rate was greater for spina bifida (a decrease of 53 %) than for anencephaly and encephalocele (decreases of 38 % and 31 %, respectively).

In Canada the prevalence of neural tube defects was historically higher in the eastern than the western provinces. Interestingly, a greater reduction in rates was observed in regions with a higher baseline prevalence of neural tube defects than in regions with a lower prevalence (Fig. 20.2). The greatest reduction in birth prevalence was seen in Newfoundland and Labrador, which had a difference in rate of 3.80 per 1,000 births, as compared with British Columbia, which had a difference in

rate of 0.21 per 1,000 births. During the post-fortification period, geographical differences almost disappeared although a small east–west gradient persisted with rates of 1.26/1,000 in Nova Scotia, 0.97/1,000 in Quebec, 0.93/1,000 in Manitoba, 0.67/1,000 in Alberta, and 0.75/1,000 in British Columbia.

For spina bifida, frequency reduction following FA fortification was higher for the more severe upper cranial, cervical, and thoracic lesions than for the less severe lumbar and sacral defects [18]. No significant decrease was seen for the pathogenically distinct lipomyelomeningocele [19]. The effect of supplementation was also evaluated among women who underwent maternal serum screening in Ontario, and the prevalence of open neural tube defects declined from 1.13 per 1,000 pregnancies before fortification to 0.58 per 1,000 pregnancies thereafter [20].

Other Congenital Anomalies

The use of multivitamin supplements during the periconceptional period has been associated with a decreased risk of several categories of congenital anomalies. Results of a meta-analysis of controlled trials and observational studies suggested consistent protection against cardiovascular and limb defects but a lower degree of consistency for cleft palate and/or oral cleft, urinary tract anomalies, and hydrocephalus [21]. No statistically significant protection could be demonstrated in another meta-analysis including randomized and quasi-randomized trials only, but the small number of subjects limited the power to detect a beneficial effect [22]. Results of studies on multivitamin supplementation cannot be readily extrapolated to fortification using a single vitamin with a much lower dose.

In the province of Quebec, live and stillborn infants with severe congenital heart defects were identified in three administrative databases and time-series analyses were performed [23]. There was no change in the prevalence of heart defects in the 9 years before fortification (1990–1998), while in the 7 years after fortification (1999–2005), there was a statistically significant steady decrease (Fig. 20.3). In the study, medical records were not reviewed and congenital anomaly cases in pregnancy terminations were not included. Results should thus be interpreted with caution. The frequency rate of heart defects started to decrease in 2001, as compared with 1998 for neural tube defects, and the shapes of the two curves were also very different [10]. In Ontario, a retrospective study of all women who underwent maternal serum screening at 15–20 weeks gestation was performed and there was no evidence of a decline in the prevalence of orofacial clefts and trisomy 21 in the offspring following fortification [24, 25]. More studies are needed to assess the effect of folic acid food fortification on the risk of congenital anomalies other than neural tube defects.

Cardiovascular Disease

Epidemiological studies have shown a positive correlation between homocysteine plasma concentration and the risk of cerebral, coronary, and peripheral vascular disease [26]. Increasing folic acid intake results in the lowering of homocysteine plasma concentration, but the results of clinical trials to reduce the incidence of stroke and myocardial infarction in high-risk individuals have been disappointing [27–29]. The effect of folic acid at low doses in mostly low-risk individuals may be different. A time-series analysis of stroke mortality rate was conducted in the United States and Canada where folic acid fortification was implemented in 2008 and in England and Wales where no fortification is required [30]. An acceleration in the decline of stroke mortality following fortification was observed in the USA and Canada but not in the UK. It would be interesting to repeat this type of analysis in other countries.

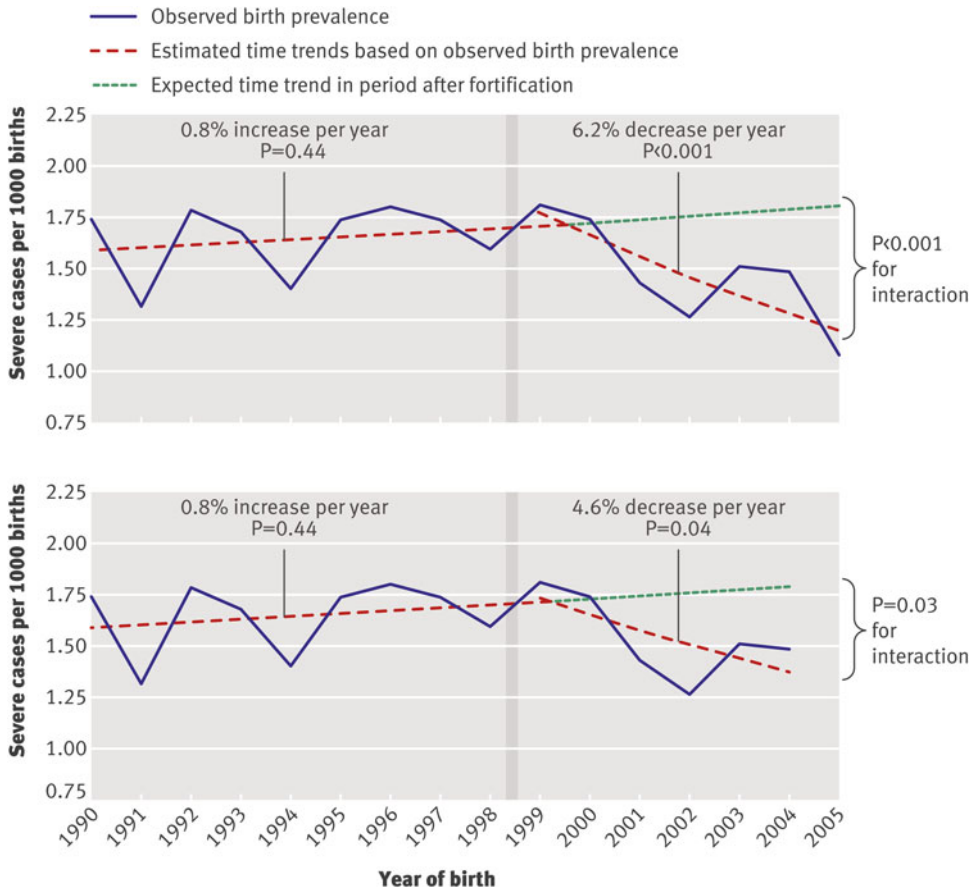


Fig. 20.3 Time trends in birth prevalence of severe congenital heart disease before and after January 1, 1999, cutoff representing introduction of mandatory fortification of flour and pasta products with folic acid in Canada (Reprinted with permission from the British Medical Journal [23])

Pediatric Cancer

The role of natural folates and folic acid in DNA biosynthesis, damage, and repair and in cell differentiation and multiplication is complex and not completely understood [31]. Observational studies were conducted in Ontario comparing the incidence of several types of pediatric cancers before and after the introduction of folic acid supplementation. A significant decrease in rate was observed for Wilms' tumor and neuroblastoma but not for acute lymphoblastic leukemia, brain cancers, and other embryonal cancers [32, 33]. Although there is a biological plausibility in these observations, more evidence is needed to establish causation.

Adverse Effects

High doses of folic acid (≥5 mg/day) given to individuals with severe anemia caused by B12 deficiency have been associated with an improvement in the anemia, but they also could mask and even accelerate

the degeneration of the nervous system [34]. The additional intake of folic acid resulting from supplementation at the current levels is far beyond the therapeutic FA doses used at a time when it was difficult to differentiate folate from B12 deficiency [35]. As yet, there has been no good study on the incidence and severity of neurological disorders before and after FA supplementation. There are many reasons for this: the clinical symptoms of B12 deficiency are not very specific and are relatively mild at an early stage, patients usually have comorbidities and are not necessarily referred to neurologists or hospitalized, and there have been major changes in diagnostic tests in recent years, both for B12 status and neurological disorders.

An association between folic acid supplement use and twin pregnancies has been reported in some studies [36]. A systematic review of epidemiological studies comparing twinning rates before and after FA fortification in the USA and Chile provided conflicting results [37]. In recent years, there has been a steep rise in the frequency of multiple births in Canada, resulting mainly from increasing use of assisted reproduction technologies [38]. This factor is related at the individual level to nutrition and FA supplement use and at the ecological level to fortification. Thus, it is almost impossible to identify the potential role of FA fortification in the increase.

Epidemiological studies have shown an inverse relationship between dietary folate intake and the risk of colorectal cancer in the population [39–42]. Conversely, clinical trials using high FA doses in patients with precancerous colorectal lesions did not show a protective effect, and an increased risk of progression of advanced adenomas was observed in some studies [43–45]. A "dual-modulator" role of folate in colorectal carcinogenesis has been proposed, in which moderate dietary increases initiated before the establishment of neoplastic foci have a protective influence, whereas high intake once early lesions are established increases tumorigenesis [46]. In Canada, the incidence of colorectal cancer rose between 1980 and 1985 in both sexes and then declined until the mid-1990s, more markedly in females than males. Rates then rose through 2000, only to decline significantly thereafter, this time more markedly in males than females [47]. Whether FA fortification played a role in these trends is difficult to assess as there have been changes in nutrition patterns in the last decades as well as changes in screening and diagnostic practices.

Current Controversies

Folic Acid Recommendation for Women of Childbearing Age

In Canada, the first recommendations regarding folic acid supplementation for the prevention of neural tube defects were published in the early 1990s [48–50]. For low-risk women, a daily dose of 0.4–0.8 mg was recommended. In 2003, the Society of Obstetricians and Gynaecologists of Canada updated its recommendations, and 0.4–1.0 mg of folic acid was recommended for women who could become pregnant [51]. In 2007, when the impact of fortification was demonstrated, guidelines were changed: 0.4–1.0 mg was recommended for women with no personal health risks, planned pregnancy, good diet, and good compliance with supplement use on a daily basis, while 5 mg/day was recommended for all other women (the majority) [52]. Reasons for increasing the dose were as follows: FA intakes in the population were suboptimal at current fortification levels, 5 mg per day in young women is safe, and there may be a subset of women with certain genetic and/or physiological characteristics requiring high doses. As yet, this recommendation has not been endorsed by public health authorities.

Interestingly, two studies conducted in the fortification era in the USA did not show a clear benefit of multivitamin supplement use for the prevention of neural tube defects [53, 54].

Increasing Fortification Levels

After FA fortification was implemented in Canada, NTD rates decreased and then stabilized with a reduced but still discernible east to west gradient [10]. This is a powerful argument supporting an increase in FA food fortification levels to eliminate geographical variation that is most probably caused by nutritional and not genetic factors. In the USA, this debate is going on for years and no consensus has been achieved so far [55–57]. In the MRC trial, 72 % protection against NTD was recorded using a daily FA dose of 4 mg [1]. However, the confidence interval around this estimate was large. In a community intervention in China using pills containing 400 μg FA, the lowest NTD rates (0.6–0.7/1,000) were recorded among women who were highly compliant with supplementation during the periconceptional period [58]. These rates were also observed in the western part of Canada following fortification at low levels and may well represent the lowest achievable level with FA supplementation or fortification [10]. In Chile, fortification of wheat flour at 220 μg/100 g was associated with a 43 % reduction in NTD rate and an absolute rate of 1/1,000 births in exposed women [59]. These results are not very different than those reported in Canada using lower FA fortification doses [10]. Controlled trials testing the relative effects of different FA fortification levels would be almost impossible to organize, and indirect evidence could only be provided by high-quality studies on the effect of FA supplementation alone in a context of fortification.

Fortifying Food with Vitamin B12

Vitamin B12 deficiency is frequent in the Canadian population, especially in the elderly [60]. Results of a case–control study in Ontario showed a tripling risk of NTD in the presence of low maternal B12 status as compared to women in the highest quartile of serum holotranscobalamin levels, a marker of B12 status [61]. The authors of the study estimated that up to 34 % of NTD cases occurring in the context of folic acid fortification could be due to maternal B12 deficiency and could thus be prevented through additional B12 fortification [61]. Adding B12 in cereal products is technically feasible, of reasonable cost, and there is evidence that B12-fortified flour, consumed as bread, can improve B12 status among persons with no impairment of gastrointestinal absorption [62]. The implementation of this public health measure has been endorsed by the Society of Obstetricians and Gynaecologists of Canada [52]. In the USA, B12 fortification is a subject of a controversy as there are many scientific uncertainties regarding the potential effectiveness of the measure [62–64]. Up to now, there has been no controlled trial demonstrating the effectiveness of B12 supplementation or fortification to prevent the occurrence of neurological disorders in patients with mild subclinical deficiency (individuals with severe B12 deficiency caused by specific malabsorption will not respond to increased oral intake). The same can be said for neural tube defects and the only evidence is from epidemiological studies comparing NTD risk in women who had or not taken multivitamin containing B12 during the periconceptional period [21]. Randomized clinical trials will have to be conducted to justify a mandatory B12 food fortification policy as it was done for folic acid.

Conclusion

In Canada, a mandatory FA food fortification policy was implemented to reduce the occurrence of neural tube defects in the population and the effectiveness of the measure was higher than anticipated although the mean additional FA intake among women of childbearing age was quite low. There is certainly room for further NTD risk reduction if fortification levels are increased. However, because

of the uncertainty regarding the impact of this measure and the possibility of cancer promotion, public health authorities will request more evidence before considering such move. As history has told, decisions in Canada will most probably follow and be in line with those adopted in the USA. So, let us follow the debate south of the border.

References

1. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet. 1991;338:131–7.
2. Oakley GP. Folic acid-preventable spina bifida and anencephaly. JAMA. 1993;269:1292–3.
3. Crane NT, Wilson DB, Cook DA, Lewis CJ, Yetley EA, Rader JI. Evaluating food fortification options: general principles revisited with folic acid. Am J Public Health. 1995;85:660–6.
4. The Editors of Nutrition Reviews. Folate and neural tube defects: US policy evolves. Nutr Rev. 1993;51:358–61.
5. Gaull GE. Fortifying policy with science. The case of folate. Introduction. J Nutr. 1996;126(3):749S–50.
6. Department of Health and Human Services. Food and Drug Administration. 21 CFR. Part 101: Food labeling: health claims and label statements; folate and neural tube defects; proposed rule and final rule. 21 CFR Part 136, 137, and 139. Food standards: Amendment of standards of identity for enriched grain products to require addition of folic acid. 21 CFR. Part 172: Food additives permitted for direct addition to food for human consumption; folic acid (folacin); final rule. Washington, DC: Federal Register. 1996, vol. 61. p. 8750–807.
7. Bureau of Reproductive and Child Health. National workshop on the primary prevention of neural tube defects. Health Canada, Ottawa, March 1–3, 1995.
8. Canada Gazette. Part II: Regulatory impact analysis statement. SOR/96-527. 1996;130:3318–20.
9. Canada Gazette. Part II: Regulatory impact analysis statement. SOR/98-550. 1998;132:3029–33.
10. De Wals P, Tairou F, Van Allen MI, Uh SH, Lowry RB, Sibbald B, et al. Reduction in neural-tube defects after folic acid fortification in Canada. N Engl J Med. 2007;357:135–42.
11. Shakur YA, Rogenstein C, Hartman-Craven B, Tarasuk V, O'Connor DL. How much folate is in Canadian fortified products 10 years after mandated fortification? Can J Public Health. 2009;100:281–4.
12. Grosse SD, Waitzman NJ, Romano PS, Mulinare J. Reevaluating the benefits of folic acid fortification in the United States: economic analysis, regulation, and public health. Am J Public Health. 2005;95:1917–22.
13. Ray JG, Vermeulen MJ, Boss SC, Cole DEC. Declining rate of folate insufficiency among adults following increased folic acid food fortification in Canada. Can J Public Health. 2002;93:249–53.
14. Quinlivan EP, Gregory JF. The impact of food fortification on folic acid intake in Canada. Can J Public Health. 2002;94:154.
15. House JD, March SB, Ratnam S, Ives E, Brosnan JT, Friel JK. Folate and vitamin B_{12} status of women in Newfoundland at their first prenatal visit. CMAJ. 2000;162:1557–9.
16. Colapinto CK, O'Connor DL, Tremblay MS. Folate status of the population in the Canadian Health Measures Survey. CMAJ. 2011;183:E100–6.
17. Shojania AM, von Kuster K. Ordering folate assays is no longer justified for investigation of anemias, in folic acid fortified countries. BMC Res Notes. 2010;3:22.
18. De Wals P, Tairou F, Van Allen MI, Lowry RB, Evans JA, Van den Hof MC, et al. Spina bifida before and after folic acid fortification in Canada. Birth Defects Res A Clin Mol Teratol. 2008;82:622–6.
19. De Wals P, Van Allen MI, Lowry RB, Evans JA, Van den Hof MC, Crowley M, et al. Impact of folic acid food fortification on the birth prevalence of lipomyelomeningocele in Canada. Birth Defects Res A Clin Mol Teratol. 2008;82:106–9.
20. Ray JG, Meier C, Vermeulen MJ, Boss S, Wyatt PR, Cole DEC. Association of neural tube defects and folic acid food fortification in Canada. Lancet. 2002;360:2047–8.
21. Goh YI, Bollano E, Einarson TR, Koren G. Prenatal multivitamin supplementation and rates of congenital anomalies: a meta-analysis. J Obstet Gynaecol Can. 2006;28:680–9.
22. De-Regil LM, Fernandez-Gaxiola AC, Dowswell T, Peña-Rosas JP. Effects and safety of periconceptional folate supplementation for preventing birth defects. Cochrane Database Syst Rev. 2010;6:CD007950.
23. Ionescu-Ittu R, Marelli AJ, Mackie AS, Pilote L. Prevalence of severe congenital heart disease after folic acid fortification of grain products: time trend analysis in Quebec, Canada. BMJ. 2009;338:b1673.
24. Ray JG, Vermeulen MJ, Wyatt PR, Cole DE. Association between folic acid fortification and congenital orofacial clefts. J Pediatr. 2003;143:805–7.
25. Ray JG, Meier C, Vermeulen MJ, Cole DE, Wyatt PR. Prevalence of trisomy 21 following folic acid food fortification. Am J Med Genet A. 2003;120A:309–13.

26. Rimm EB, Stampfer MJ. Folate and cardiovascular disease: one size does not fit all. Lancet. 2011;378:544–6.
27. Miller ER, Juraschek S, Pastor-Barriuso R, Bazzano LA, Appel LJ, Guallar E. Meta-analysis of folic acid supplementation trials on risk of cardiovascular disease and risk interaction with baseline homocysteine levels. Am J Cardiol. 2010;106:517–27.
28. Lee M, Hong KS, Chang SC, Saver JL. Efficacy of homocysteine-lowering therapy with folic acid in stroke prevention. A meta-analysis. Stroke. 2010;41:1205–12.
29. Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SI, Cooper J, et al. Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. Lancet. 2011;378:584–94.
30. Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H, et al. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation. 2006;113:1335–43.
31. Duthie SJ. Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. J Inherit Metab Dis. 2011;34:101–9.
32. French AE, Grant R, Weitzman S, Ray JG, Vermeulen MJ, Sung L, et al. Folic acid food fortification is associated with a decline in neuroblastoma. Clin Pharmacol Ther. 2003;74:288–94.
33. Grupp SG, Greenberg ML, Ray JG, Busto U, Lanctôt KL, Nulman I, et al. Pediatric cancer rates after universal folic acid flour fortification in Ontario. J Clin Pharmacol. 2011;51:60–5.
34. Institute of Medicine. Dietary reference intakes: thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: National Academy Press; 1998. p. 196–305.
35. Shakur Y, Garriguet D, Corey P, O'Connor D. Folic acid fortification above mandated levels results in a low prevalence of folate inadequacy among Canadians. Am J Clin Nutr. 2010;92:818–25.
36. Levy T, Blickstein I. Does the use of folic acid increase the risk of twinning? Int J Fertil Womens Med. 2006;51:130–5.
37. Muggli EE, Halliday JL. Folic acid and risk of twinning: a systematic review of the recent literature, July 1994 to July 2006. Med J Aust. 2007;186:243–8.
38. SOGC (Society of Obstetricians and Gynaecologists of Canada). Women's health information. Pregnancy. Multiple Birth. Ottawa, ON. Updated September 2010. Available from: http://www.sogc.org/health/pregnancy-multiple_e.asp
39. Sanjoaquin MA, Allen A, Couto E, Roddam AW, Key TJ. Folate intake and colorectal cancer risk: a meta-analytical approach. Int J Cancer. 2005;113:825–8.
40. Kim DH, Smith-Warner SA, Spiegelman D, Yaun SS, Colditz GA, Freudenheim JL, et al. Pooled analyses of 13 prospective cohort studies on folate intake and colon cancer. Cancer Causes Control. 2010;21:1919–30.
41. Kennedy DA, Stern SJ, Moretti M, Matok I, Sarkar M, Nickel C, et al. Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis. Cancer Epidemiol. 2011;35:2–10.
42. Lee JE, Willett WC, Fuchs CS, Smith-Warner SA, Wu K, Ma J, et al. Folate intake and risk of colorectal cancer and adenoma: modification by time. Am J Clin Nutr. 2011;93:817–25.
43. Ibrahim EM, Zekri JM. Folic acid supplementation for the prevention of recurrence of colorectal adenomas: metaanalysis of interventional trials. Med Oncol. 2010;27:915–8.
44. Fife J, Raniga S, Hider PN, Frizelle FA. Folic acid supplementation and colorectal cancer risk: a meta-analysis. Colorectal Dis. 2010;13:132–7.
45. Figueiredo JC, Mott LA, Giovannucci E, Wu K, Cole B, Grainge MJ, et al. Folic acid and prevention of colorectal adenomas: a combined analysis of randomized clinical trials. Int J Cancer. 2010;129:192–203.
46. Hubner RA, Houlston RS. Folate and colorectal cancer prevention. Br J Cancer. 2009;100:233–9.
47. Colorectal Cancer Association of Canada. 2011 Colorectal cancer statistics. Ottawa, ON Available from: http://www.colorectal-cancer.ca/en/just-the-facts/colorectal/
48. Van Allen MI, Fraser FC, Dallaire L, Allanson J, McLeod DR, Andermann E, et al. Recommendations on the use of folic acid supplementation to prevent the recurrence of neural tube defects. Can Med Assoc J. 1993;149:1239–43.
49. Canadian Task Force on the Periodic Health Examination. Periodic health examination, 1994 update: 3. Primary and secondary prevention of neural tube defects. Can Med Assoc J. 1994;151:159–66.
50. SOGC Genetics Committee. Recommendations on the use of folic acid for the prevention of neural tube defects. J Soc Obstet Gynaecol Can. 1993;15:41–6.
51. Wilson RD, Committee G. The use of folic acid for the prevention of neural tube defects and other congenital anomalies. J Obstet Gynaecol Can. 2003;25:959–73.
52. Wilson RD, Committee G. Pre-conceptional vitamin/folic acid supplementation 2007: the use of folic acid in combination with a multivitamin supplement for the prevention of neural tube defects and other congenital anomalies. J Obstet Gynaecol Can. 2007;29:1003–26.
53. Mosley BS, Cleves MA, Siega-Riz AM, Shaw GM, Canfield A, Waller DK, et al. Neural tube defects and maternal folate intake among pregnancies conceived after folic acid fortification in the United States. Am J Epidemiol. 2009;169:9–17.

54. Ahrens K, Yazdy MM, Mitchell AA, Werler MM. Folic acid intake and spina bifida in the era of dietary folic acid fortification. Epidemiology. 2011;22:731–7.
55. Johnston RB. Will increasing folic acid in fortified grain products further reduce neural tube defects without causing harm?: consideration of the evidence. Pediatr Res. 2008;63:2–8.
56. Oakley GP. The scientific basis for eliminating folic acid-preventable spina bifida: a modern miracle from epidemiology. Ann Epidemiol. 2009;19:226–30.
57. Lucock M, Yates Z. Folic acid fortification: a double-edged sword. Curr Opin Clin Nutr Metab Care. 2009;12:555–64.
58. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, et al. Prevention of neural-tube defects with folic acid in China. N Engl J Med. 1999;341:1485–90.
59. Hertrampf E, Cortés F. National food-fortification program with folic acid in Chile. Food Nutr Bull. 2008;29(Suppl):S231–7.
60. McLean E, de Benoist B, Allen LH. Review of the magnitude of folate and vitamin B12 deficiencies worldwide. Food Nutr Bull. 2008;29(Suppl):S38–51.
61. Ray JG, Wyatt PR, Thompson MD, Vermeulen MJ, Meier C, Wong PY, et al. Vitamin B_{12} and the risk of neural tube defects in a folic-acid-fortified population. Epidemiology. 2007;18:362–6.
62. Allen LH, Rosenberg IH, Oakley GP, Omenn GS. Considering the case for vitamin B_{12} fortification of flour. Food Nutr Bull. 2010;31(Suppl):S36–46.
63. Green R. Is it time for vitamin B-12 fortification? What are the questions? Am J Clin Nutr. 2009;89(Suppl):712S–6.
64. Carmel R. Mandatory fortification of the food supply with cobalamin: an idea whose time has not yet come. J Inherit Metab Dis. 2011;34:67–73.