Declining Rate of Folate Insufficiency Among Adults Following Increased Folic Acid Food Fortification in Canada

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ABSTRACT

Objective: Canada introduced a mandatory folic acid food fortification program in November 1998. We investigated whether the rate of folate and vitamin B12 insufficiency among adults has changed since this mandatory fortification program was implemented.

Methods: We conducted a retrospective cross-sectional study using a large Ontario laboratory database. We included all individuals who underwent evaluation of their serum folate, red cell folate and serum vitamin B12 between April 1, 1997 to July 31, 1998 (Period A), August 1, 1998 to January 30, 1999 (Period B) and February 1, 1999 to March 31, 2000 (Period C).

Results: A total of 8,884 consecutive samples were analyzed during the period of study. Mean age was 57.4 years (SD 21.1), and 63.2% were female. The prevalence of serum folate insufficiency (below 3.4 nmol/L) fell from 0.52% in Period A to 0.22% in Period C [prevalence ratio (RR) 0.41, 95% confidence interval (CI) 0.18-0.93)]. The prevalence of red cell folate insufficiency (below 215 nmol/L) declined from 1.78% during Period A to 0.41% in Period C (RR 0.23, 95% CI 0.14-0.40). No significant difference was observed between periods in the prevalence of B12 insufficiency below 120 pmol/L (3.93% versus 3.11%, respectively; RR 0.79, 95% CI 0.62-1.01).

Conclusions: There has been a significant decline in the prevalence of folate, but not vitamin B12 insufficiency, following Canadian folic acid food fortification. These changes may have important implications for the prevention and detection of folate and vitamin B12 insufficiency, including identifying the benefits of folic acid food fortification and the need to further consider fortification or supplementation with vitamin B12.

La traduction du résumé se trouve à la fin de l'article.

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A Canadian national program was mandated in November 1998 to increase folic acid fortification of all flour and some corn and rice products, providing a daily average of 0.1 mg of folic acid. The goal of this initiative was to provide women of reproductive age with higher amounts of dietary folic acid in order to reduce the risk of neural tube defects.[1]

Since the introduction of this program, we expected that there would be a rise in both the average serum (Se) and red cell (RBC) folate concentrations, but we could not predict what would happen with serum vitamin B12 (Se B12).[2] Since vitamin B12 impairment might be more difficult to recognize in the presence of adequate folate stores, this might have widespread public health importance for Canadians.[2] In the following report, we examined whether there has been a change in both vitamins within the adult population since this program was implemented.

METHODS

Data collection

We retrospectively analyzed all consecutive, concomitant and non-redundant serum folate, RBC folate and Se B12 samples previously measured by MDS Laboratories (Toronto, Ontario), a large private laboratory that completes testing on approximately 30% of all B12 and folate measurements across the province of Ontario, paid for under the universal Ontario Health Insurance Plan. Samples were collected from April 1, 1997 to July 31, 1998 (Period A); August 1, 1998 to January 30, 1999 (Period B); and February 1, 1999 to March 31, 2000 (Period C) (Table I). Period B reflected a six-month interval spanning industry-compliance lead and lag times of three months each. The underlying reasons for folate and Se B12 testing were not available, but each patient's physician ordered the sample on clinical grounds. RBC folate, Se folate and Se B12 were measured by competitive protein binding (Bio-Rad Laboratories, Mississauga, Ontario). The maximum reporting limit was 45 nmol/L for Se folate, 1450 nmol/L for RBC folate and 1600 pmol/L for Se 12. The laboratory coefficient of variation (CV) was less than 7% for each of the three assays. Patient identifiers were removed to ensure patient confidentiality, and ethical approval to

TABLE I

Folate and Vitamin B12 Levels in Ontario Before (Period A), During (Period B) and After (Period C) the Introduction of Mandatory Folic Acid Fortification of all Canadian Flour in November 1998

Measure		Period A: 1/04/97-31/07/98 (n = 3257)*	Period B: 1/08/98-30/1/99 (n = 1456)*	Period C: 1/02/99-31/03/00 (n = 4171)*	Mean absolute change (p-value) between Period C vs. Period A
Age	Mean (SD)	57.5 (21.0)	57.9 (21.3)	57.0 (20.9)	-0.1 (p = 0.30)
Sex	No. (%) women	2057 (55.3)	896 (55.3)	2680 (54.8)	—
Serum folate (nmol/L)	Mean (95% CI)	18.5 (18.1-18.9)	27.2 (26.5-27.9)	27.1 (26.8-27.5)	8.6 (p < 0.001)
	5th percentile (95% CI)	6.3 (6.1-6.6)	10.1 (9.5-11.1)	10.9 (10.4-11.5)	—
Red cell folate (nmol/L)	Mean (95% CI)	680.3 (668.8-691.9)	804.1 (787.4-821.1)	851.6 (841.2-862.0)	171.3 (p < 0.001)
	5th percentile (95% CI)	297.0 (284.0-314.0)	405.0 (385.0-428.0)	450.0 (430.0-463.0)	—
Serum B12 (pmol/L)	Mean (95% CI)	293.4 (288.0-298.8)	298.3 (290.3-306.4)	292.9 (288.3-297.6)	-0.5 (p = 0.90)
	5th percentile (95% CI)	124.0 (122.0-129.0)	138.0 (129.0-145.0)	134.0 (129.0-140.0)	—

Data are presented as the geometric means and their 95% confidence intervals (CI), as well as the 5th percentile values

* Represents the number of serum folate samples obtained during each time period. See Table II for the exact number of samples for red cell folate and serum vitamin B12.

conduct this study was obtained by the Institutional Review Board of Sunnybrook and Women's College Health Sciences Centre.

Statistical analysis

The distributions of Se and RBC folate as well as Se B12 were found to be positively and significantly skewed. In accordance with other published data,[2,3] we applied logarithmic transformations to these measures, and used the transformed values for all subsequent analyses. Descriptive statistics for Se and RBC folate, and Se B12 included geometric mean concentrations and the 95% confidence intervals (CI), in addition to 5th percentile values and their 95% CI, estimated using quantile regression. Differences in mean concentrations between Period A and Period C were compared using an unpaired Student t-test. We used the widely accepted cut-off value of less than 3.4 nmol/L to define Se folate insufficiency, less than 215 nmol/L for RBC folate insufficiency, and below 120 pmol/L for Se B12 insufficiency. A Se B12 concentration between 120 to 150 pmol/L was used to define "indeterminate" Se B12 insufficiency.[2] Prevalence rate ratios (RR) were used to compare the rate of insufficiency in Period C versus Period A for each of three biochemical measures.

RESULTS

A total of 8,884 consecutive concomitant samples were analyzed during the period of study. There were minor discrepancies in the number of tests in each period because some individuals who had a measured Se folate did not have a concomitant RBC folate analysis. The mean age of all patients was 57.4 years (SD 21.1) and 63.2% were female; there were no significant differences in the distribution of age or sex between periods (Table I). The geometric mean serum folate level rose from 18.5 nmol/L in Period A to 27.1 nmol/L in Period C (mean change 8.6 nmol/L;

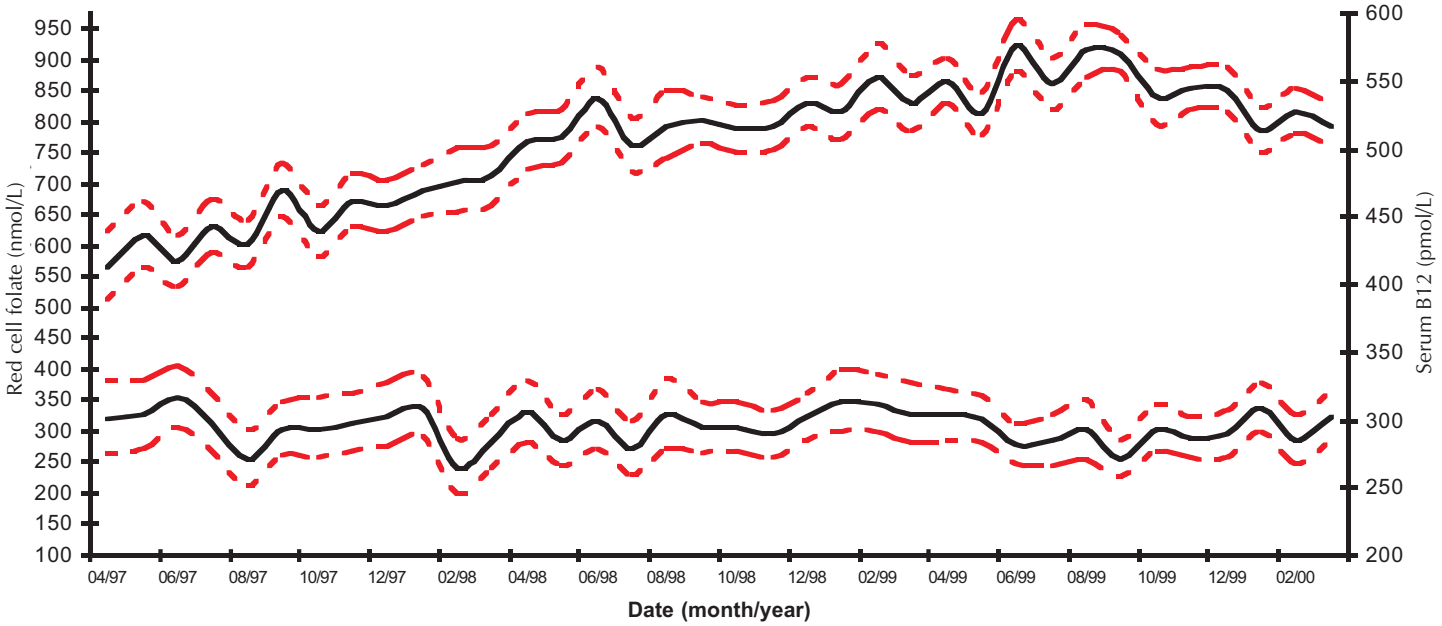

Figure 1. Trend in red cell folate (upper) and serum vitamin B12 (lower) after introduction of a Canadian folic acid fortification programme in 1998.
Geometric means are represented by solid lines and the 95 percent confidence limits by dashed lines

TABLE II

Prevalence of Normal and Abnormal Serum Vitamin B12, Serum Folate and Red Cell Folate Among Ontarians Who Underwent Testing for Clinical Reasons

	No. (%) Individual Samples			
Measure	**Period A: 1/04/97- 31/07/98**	**Period B: 1/08/98-30/1/99**	**Period C: 1/02/99-31/03/00**	**Rate Ratio (95% CI) Period C Versus Period A**
Serum folate (nmol/L)				
< 3.4 (deficient)	17 (0.52)	0 (0.0)	9 (0.22)	0.41 (0.18-0.93)
≥ 3.4	3240 (99.48)	1456 (100.0)	4162 (99.78)	–
All samples	3257 (100.0)	1456 (100.0)	4171 (100.0)	–
Red cell folate (nmol/L)				
< 215 (deficient)	57 (1.78)	8 (0.55)	17 (0.41)	0.23 (0.14-0.40)
≥ 215	3143 (98.22)	1442 (99.45)	4085 (99.59)	–
All samples	3200 (100.0)	1450 (100.0)	4102 (100.0)	–
Serum B12 (pmol/L)				
< 120 (deficient)	127 (3.93)	41 (2.78)	129 (3.11)	0.79 (0.62-1.01)
120-150 (indeterminate)	153 (4.73)	67 (4.55)	193 (4.65)	0.98 (0.80-1.21)
> 150	2953 (91.34)	1365 (92.67)	3825 (92.24)	–
All samples	3233 (100.0)	1473 (100.0)	4147 (100.0)	–

TABLE III

Number (%) and Rate Ratio of Individuals with Concomitantly Low Serum Vitamin B12 and Red Cell Folate Concentrations in Ontario Before (Period A) and After (Periods B and C) Mandatory Folic Acid Fortification

Period A (1/04/97 - 31/07/98)		**Serum B12 (pmol/L)**	
		< 150	≥ 150
Red cell folate (nmol/L)	< 215	12 (0.38)	45 (1.42)
	≥ 215	262 (8.27)	2848 (89.93)
Period B (1/08/98 - 30/1/99)		**Serum B12 (pmol/L)**	
		< 150	≥ 150
Red cell folate (nmol/L)	< 215	1 (0.07)	7 (0.49)
	≥ 215	104 (7.34)	1305 (92.10)
Period C (1/02/99 - 31/03/00)		**Serum B12 (pmol/L)**	
		< 150	≥ 150
Red cell folate (nmol/L)	< 215	1 (0.02)	16 (0.39)
	≥ 215	314 (7.71)	3742 (91.87)

unpaired t-test: $p < 0.001$) (Table I and Figure 1). A similar increase in red cell folate was observed (mean change 171.3 nmol/L, $p < 0.001$), but not in Se B12 (mean change -0.5 nmol/L, $p = 0.90$). The 5th percentiles of serum and red cell folate also increased substantially after fortification, while Se B12 levels did not (Table I).

Based upon the measured Se folate concentration, the rate of folate insufficiency fell from 0.52% in Period A to 0.22% during Period C [RR 0.41, 95% confidence interval (CI) 0.18-0.93] (Table II). Similarly, using RBC folate values, there was a significant decline in the rate of folate insufficiency between Period A and Period C (1.78% versus 0.41%, respectively; RR 0.23, 95% CI 0.14-0.40). No significant difference was observed between Period A and Period C for either B12 insufficiency (3.93% versus 3.11%; RR 0.79, 95% CI 0.62-1.01) or indeterminate B12 measures (4.73% versus 4.65%, respectively, RR 0.98, 95% CI 0.80-1.21) (Table II). Finally, among individuals whose vitamin B12 concentration was below 150 pmol/L, the rate of concomitant RBC folate insufficiency (i.e., below 215 nmol/L) declined significantly from 0.38% in period A to 0.02% in Period C (RR 0.06, 95% CI 0.01-0.50) (Table III).

DISCUSSION

We observed a significant rise in folate concentrations as well as a relative decline of between 59% and 77% in the rate of folate insufficiency since the introduction of increased folic acid fortification in Canada. From that time, the rate of folate insufficiency has dropped to below 0.5% in a large population of Canadians undergoing laboratory testing. Over the same time period, the rate of probable or indeterminate vitamin B12 insufficiency did not significantly change.

Our data might have been biased by several factors. First, we could not obtain information on the reasons for testing. It is likely that many individuals in the current study sample underwent testing for evaluation of either anemia or neuropsychiatric indications, so the actual rate of folic acid and vitamin B12 insufficiency in the general population may be lower. Furthermore, individuals who were previously folate deplete may have specifically demonstrated a more dramatic response to folic acid with food fortification.[4] Because we had no data on ethanol use, smoking status and dietary intake,[5] we cannot be certain how these factors may have influenced both test ordering and folate levels. Although increased use of folic acid-containing supplements may also explain the declining rate of folate insufficiency, the absence of similar changes in vitamin B12 status suggests that use of multivitamin tablets alone cannot explain this phenomenon. It is conceivable that Canada's national folic acid fortification program has had a major influence on these results, which are similar to those observed following folic acid fortification in the United States.[6,7] The substantial increase in folate concentrations in Period B could be explained by the fact that many flour producers began to fortify their products in anticipation of the mandatory deadline.[1] Experimental studies have shown that removal of dietary folic acid-fortified foods for 12 weeks can significantly reduce red blood cell folate concentrations in women,[8] such that 3 to 6 months of fortification would probably have been sufficient to alter folate levels in our study population.

This study raises several important issues regarding the evaluation, definition and prevention of both folate and vitamin B12

insufficiency. First, the pre-test probability (i.e., general population prevalence) of folate insufficiency may continue to remain less than 0.5%. Accordingly, in clinical practice, greater emphasis should be placed on other causes of both normocytic and macrocytic anemia, beyond an individual's folate status.[9,10] Second, due to the imprecision of direct measurement,[11] a greater understanding of the accuracy of various tests in the evaluation of both vitamin B12 and folate insufficiency is needed.[12] We previously demonstrated that a plasma total homocysteine concentration greater than 15 µmol/L could not accurately discriminate between either Se B12 concentrations below versus above 120 pmol/L (sensitivity 24.0%, specificity 90.0%) or indeterminate B12 levels between 120 to 150 pmol/L (sensitivity 11.0%, specificity 90.0%).[2] Thus, indirect measures of B12 may not provide a satisfactory solution, which is particularly an issue in any population susceptible to Se B12 insufficiency.[13] This problem may be shaped in part on the new understanding that there may be genetically or physiologically predisposed subpopulations, based in part on the recent identification of genetic polymorphisms associated with low Se B12 and hyperhomocysteinemic disorders, including neural tube defects[14] and cardiovascular disease.[15,16]

Measured folate or Se B12 may appear within normal limits, despite the presence of elevated methylmalonic acid or plasma total homocysteine concentrations.[2,17] Accordingly, further discussion must focus on whether current definitions of "abnormal" folate or Se B12 concentrations should be based on either a) statistical extremes (e.g., values below the 5th percentile) within a large population sample, or b) the point at which either folate- or B12-related disease is most likely to arise, using clinicopathological correlation. The next question concerns which disease one is attempting to either diagnose and treat (e.g., megaloblastic anemia, B12-related neurological disease, homocysteine-related cardiovascular disease) or prevent (e.g., neural tube defects), and whether they all share the same pathophysiological thresholds. Perhaps the term "impairment" should replace "insufficiency" to highlight the notion that there is probably no distinct concentration of folate or B12 that clearly represents a fixed threshold for any one particular disease.

Based on our data and those of others,[6,7] it appears that North America's folic acid fortification programs have been associated with a reduction in the prevalence of folate insufficiency, but this is not seen for vitamin B12, so that diseases related to vitamin B12 insufficiency may be more difficult to recognize.[13,18] Since the incidence of B12 insufficiency in North America may rise within a population that is aging[13] and increasingly turning toward ovo-lacto vegetarianism,[19] Se B12 insufficiency remains a potential health problem.[2] We support the notion to consider modifying current North American folic acid fortification programs to provide a minimum daily intake of 2.4 µg of synthetic vitamin B12 per day,[13,20] or at least monitoring the future trend of vitamin B12 concentrations and B12-related disease.

In conclusion, we have observed a significant decline in the rate of folate – but not vitamin B12 – insufficiency, since the increase in Canadian folic acid fortification. These changes may have important implications for the prevention and detection of folate and vitamin B12 insufficiency, including identifying the benefits of folic acid food fortification and the need to further consider fortification or supplementation with vitamin B12.

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RÉSUMÉ

Objectif : En novembre 1998, le Canada a lancé un programme d'enrichissement en acide folique obligatoire pour certains aliments. Nous avons voulu déterminer si le taux de carence en folate et en vitamine B12 chez les adultes a changé depuis la mise en œuvre du programme.

Méthode : Étude transversale rétrospective à partir d'une vaste base de données d'un laboratoire ontarien. Nous avons inclus toutes les personnes dont le folate sérique, le folate érythrocytaire et la vitamine B12 sérique ont été évalués entre le 1er avril 1997 et le 31 juillet 1998 (période A), entre le 1er août 1998 et le 30 janvier 1999 (période B) et entre le 1er février 1999 et le 31 mars 2000 (période C).

Résultats : En tout, 8 884 échantillons consécutifs ont été analysés durant la période de référence. L'âge moyen des sujets était de 57,4 ans (déviation sensible [DS] de 21,1), et 63,2 % étaient des femmes. Le taux de carence en folate sérique (< 3,4 nmol/L) a reculé, passant de 0,52 % pendant la période A à 0,22 % pendant la période C [ratio des taux de prévalence (RT) = 0,41, intervalle de confiance (IC) de 95 % = 0,18-0,93)]. Le taux de carence en folate érythrocytaire (< 215 nmol/L) a également baissé, passant de 1,78 % pendant la période A à 0,41 % pendant la période C (RT = 0,23, IC de 95 % = 0,14-0,40). Nous n'avons observé aucune différence significative d'une période à l'autre pour les taux de carence en vitamine B12 (< 120 pmol/L) (3,93 % contre 3,11 %, respectivement; RT = 0,79, IC de 95 % = 0,62-1,01).

Conclusions : Il y a eu une baisse significative du taux de carence en folate, mais non en vitamine B12, depuis l'augmentation de l'enrichissement en acide folique au Canada. Les changements observés pourraient avoir des conséquences importantes pour la prévention et la détection des carences en folate et en vitamine B12, en permettant notamment de déterminer les avantages de l'enrichissement des aliments en acide folique et la nécessité d'étudier plus avant l'enrichissement ou la supplémentation en vitamine B12.

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Received: August 17, 2001
Accepted: March 21, 2002