Improvements in the Status of Folate and Cobalamin in Pregnant Newfoundland Women Are Consistent with Observed Reductions in the Incidence of Neural Tube Defects

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ABSTRACT

Background: Historically, the province of Newfoundland and Labrador had one of the highest rates of neural tube defects (NTDs) in North America (1976-1991: 3.2/1000 births), which could be partially explained by the sub-optimal status of folate and cobalamin in this population. In order to gain evidence of the impact of folate fortification programs, as well as prenatal education programs, a cross-sectional study was conducted to obtain data on the folate and cobalamin status of pregnant Newfoundland women in the post-folate fortification era. Additionally, the rates of NTDs were determined.

Methods: Blood samples were collected during the first prenatal clinic (at approximately 16 weeks gestation) from 365 pregnant women in Newfoundland in 2002. Samples were analyzed for serum folate, cobalamin and homocysteine. In addition, rates of neural tube defects were calculated from data collected from the Provincial Medical Genetics Program, Newfoundland. Data were compared to historical data from a similar population of pregnant women in Newfoundland.

Results: The status of both folate and cobalamin has significantly improved ($p < 0.0001$) in the post-fortification era, concurrent with a significant reduction in the number of neural tube defects (NTDs), from 4.67 (years 1992-1996) to 1.01 (years 1998-2002) per 1000 total births.

Interpretation: These data provide evidence that both folate and cobalamin status of pregnant Newfoundland women have improved since 1997. The data for cobalamin provide evidence that strategies in addition to folate fortification programs are contributing to the improvements of poor water-soluble vitamin status in this population, thus providing a partial explanation for the dramatic reductions in NTD rates observed since 1995.

MeSH terms: Folic acid; cobalamin; homocysteine; neural tube defects

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

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In 1998, the fortification of cereal-grain products, such as flour, with folic acid became mandatory in Canada and the US.^{1,2} This fortification program was instituted primarily to help increase folate intake by women of childbearing age, due to the strong evidence to support a reduction in the risk for having an infant with a neural tube defect (NTD) when folate status is improved through folic acid supplementation.³⁻⁵ The province of Newfoundland and Labrador, in Canada, has traditionally had one of the highest rates of NTDs in the world, with rates exceeding 4/1000 total births.⁶ In order to assess folate status, we conducted a cross-sectional survey, from August 1996 to July 1997, in 1,424 pregnant women at their first prenatal visit, during the pre-folic acid fortification period.⁷ In our previous study,⁷ we documented that approximately 25% of the women had poor to marginal folate status, providing a partial explanation for the observed high rates of NTDs. With these historical data available, we conducted the current study as a follow-up to test the following hypothesis: The folate status of pregnant Newfoundland women has improved in the post-folic acid fortification era, and this improvement has translated to reductions in the rates of NTDs.

METHODS

An anonymous, cross-sectional survey was conducted to collect blood samples from 365 pregnant Newfoundland women at their first prenatal visit, from four different regions across the island during the period of January to March 2002. This sample represented approximately one of every four prenatal screens conducted during this period. Ethics approval for the study was obtained from the Human Investigation Committee, Memorial University of Newfoundland and the concerned hospital ethics committees. For each subject, blood was collected for the preparation of sera, and the samples, linked by identification numbers, were shipped frozen to the Public Health Laboratory in St. John's, Newfoundland and Labrador. Folate and cobalamin concentrations in sera were determined using the AXSYM7 folate and cobalamin systems (Abbott Laboratories), as per the manufacturer's instructions. Serum homo-

Figure 1. Cumulative frequency histograms highlighting the changes between the pre- and post-folic acid fortification periods for A) serum folate, B) serum cobalamin, and C) serum homocysteine

Median values as well as P values from Wilcoxon Ranked Sums test (pre vs. post) are presented within the figure pane.

cysteine concentrations were measured using reverse-phase HPLC with fluorescence detection.⁸ For the period 1992 to 2002, NTDs that were identified in the Newfoundland and Labrador Medical Genetics Program Neural Tube Defects Database were analyzed. Total NTDs, including anencephaly, spina bifida, and encephalocele, were expressed as a rate per 1000 total births, which included stillbirths and therapeutic abortions for NTDs. Biochemical data from the current study were compared to historical data derived from the pre-fortification phase⁷ (collected in 1996-1997 using a similar sample collection protocol from four different regions) using nonparametric analysis (Wilcoxon Ranked Sums test, SAS V. 8.2, SAS Institute, Cary, NC, USA). The proportion of NTDs in the pre-fortification period (1992-1996) was compared to that in the post-fortification period (1998-2002) by means of chi-squared analysis (SAS). The NTD data for 1997 were not included in the statistical analysis due to the fact that this period reflected a period of transition in which some cereal-grain products entering Canada were fortified with folic acid.⁹

RESULTS

The data presented in Figure 1 reflect the key findings relative to vitamin status. The median age of pregnant women in the present study was 28 (range 15-43), as compared to a median age of 28 (range 15-47) in the pre-fortification study. Serum folate concentrations have significantly increased (Panel A; $p < 0.0001$) since 1997, as indicated by a significant shift to the right in

Figure 2. Annual incidence of neural tube defects (NTDs) in Newfoundland (# per 1000 total births*) from 1992 to 2002

Difference between pre- and post-folic acid fortification era is significant ($p < 0.0001$) as determined by chi-squared analysis.

* Includes live births, stillbirths and therapeutic abortions for NTD.

the cumulative frequency curve. Over the same time period, serum cobalamin status has also improved (Panel B; $p < 0.0001$). We also observed a significant reduction in serum homocysteine concentrations (Panel C; $p < 0.0001$), an amino acid whose metabolism is highly dependent on an adequate supply of both folate and cobalamin.¹⁰ The annual rates of NTDs are given in Figure 2. A dramatic and significant reduction ($p < 0.0001$) in the observed rates was found between the pre- (1992-1996) and post- (1998-2002) folic acid fortification period.

INTERPRETATION

Our data provide strong evidence that an improvement in the folate status of Newfoundland women has occurred since the initiation of folic acid fortification strategies. These data are consistent with improvements in the folate status of women of reproductive age in another region of Canada.¹¹ The significance of the current study lies in the observation that the rates of NTDs have declined dramatically in this population, concurrent with improvements in folate status. The average

rates of NTDs in Newfoundland have decreased by almost 80%, from 4.7 to 1.0/1000 births in the pre- and post-folic acid fortification periods respectively. In other Canadian provinces, the rates of NTDs have also declined significantly, albeit not to the same extent as observed in Newfoundland, during the post-fortification period: Ontario – 51% reduction¹² (1.13 to 0.55/1000 births); Quebec – 32% reduction⁹ (1.89 to 1.28 /1000 births); Nova Scotia¹³ – 55% reduction (2.58 to 1.17 /1000 births). Therefore, our data, coupled with data from other provinces, lend support to the original thrust behind the folic acid fortification program – namely the protection against NTDs. The dramatic decline in NTD rates (80%) observed in Newfoundland reflects a very high pre-folic acid fortification rate (4.7/1000 births), as folic acid supplementation elicits greater percentage reductions when initial rates are higher.¹⁴

Poor folate status^{7,15} and genetic predisposition¹⁶ likely contributed to the historically high NTD rates in Newfoundland. Additionally, cobalamin (vitamin B₁₂) status may play a contributing role. A recent systematic review of the literature examining the relationship between cobalamin status and the risk for NTDs reported a moderate association between low maternal cobalamin status and NTDs.¹⁷ In our previous study, we documented that 44% of pregnant women in Newfoundland were of poor to marginal cobalamin status. As reflected in Figure 1b, cobalamin status has also improved since 1997. As cobalamin is not added to cereal grain products, our data provide evidence that strategies in addition to the fortification of cereal grain products with folic acid may be playing a contributing role in reducing NTDs in this population. The observed improvements in cobalamin status in pregnant Newfoundland women may reflect an increase in the usage of vitamin supplements containing both folic acid and cobalamin. This finding may reflect recent educational strategies in Canada designed to make women of child-bearing age more aware of the relationship between folic acid and NTDs. However, as samples were collected anonymously, it is not possible to determine which route (supplementation vs. fortification) had a greater impact on folate status. Additionally, we cannot say

whether or not supplements were consumed during the critical peri-conceptional (to 21 days post-conception) period where protection against NTDs is highest. Furthermore, we are not able to further speculate the potential contribution of folate from supplements on the basis of the improvements in cobalamin status, as this would require knowledge of the ratio of folic acid to cobalamin in those particular supplements. The reduction in homocysteine concentrations does, however, support a sustained improvement in the status of one or both of the measured water-soluble vitamins, but this still may reflect post-conceptional events. Homocysteine is a sulphur amino acid, produced during the metabolism of the amino acid methionine. Both folate and cobalamin are important in the metabolism of homocysteine, as they help in converting homocysteine back to methionine in a process known as remethylation.¹⁰ Reductions in serum homocysteine concentrations may confer additional advantages to the Newfoundland population, due to the fact that higher homocysteine levels are linked to increased rates of cardiovascular disease and stroke,¹⁸ Alzheimer's disease and dementia,¹⁹ and, most recently, osteoporosis.²⁰ However, these beneficial effects remain to be demonstrated.

As other countries, such as the United Kingdom, consider the implementation of folic acid fortification strategies,²¹ our data support the role of such an initiative in

reducing the incidence of NTDs. Newfoundland has experienced dramatic reductions in the rates of NTDs in the post-folic acid fortification era. The fortification of staple foods or food ingredients with folic acid, coupled with education/awareness campaigns as to the benefit of improved peri-conceptional folate status, represent effective strategies for the reduction of NTDs. The question remains as to whether further reductions in NTD rates in Newfoundland are possible and whether additional strategies are required to realize them.

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RÉSUMÉ

Contexte : Par le passé, la province de Terre-Neuve-et-Labrador avait l'un des taux d'anomalies du tube neural (ATN) les plus élevés en Amérique du Nord (1976-1991 : 3,2 p. 1 000 naissances), ce qui s'expliquait en partie par le statut sous-optimal en folates et en cobalamine dans sa population. Afin d'évaluer l'impact des programmes d'enrichissement en folates et des programmes prénatals de sensibilisation, nous avons mené une étude transversale pour recueillir des données sur le statut en folates et en cobalamine des Terre-Neuviennes enceintes après l'enrichissement des aliments en folates. Nous avons aussi calculé les taux d'ATN.

Méthode : Nous avons prélevé du sang auprès de 365 femmes enceintes à Terre-Neuve en 2002 pendant leur première visite de soins prénatals (à environ 16 semaines de gestation), puis analysé les taux de folate sérique, de cobalamine et d'homocystéine dans ces échantillons. Nous avons calculé les taux d'ATN à partir des données recueillies par le programme provincial de génétique médicale de Terre-Neuve. Ces données ont été comparées aux données historiques d'une population semblable de Terre-Neuviennes enceintes.

Résultats : Le statut en folates et en cobalamine s'était significativement amélioré ($p < 0,0001$) après la mise en œuvre du programme d'enrichissement; en parallèle, il y a eu une baisse significative du nombre d'ATN, lequel est passé de 4,67 (1992-1996) à 1,01 (1998-2002) pour 1 000 naissances.

Interprétation : Ces données montrent que le statut en folates et le statut en cobalamine des Terre-Neuviennes enceintes se sont tous les deux améliorés depuis 1997. Les données sur la cobalamine montrent que les stratégies complémentaires aux programmes d'enrichissement en folates contribuent à l'amélioration du piètre statut en vitamines hydrosolubles dans cette population, ce qui expliquerait en partie la forte baisse des taux d'ATN observée depuis 1995.

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