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Folate fortification and supplementation—Are we there yet?

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ABSTRACT

Background: Folic acid fortification of flour has significantly decreased the incidence of neural tube defects (NTDs). We aimed at examining whether Ontario women of child-bearing age exhibit protective levels of RBC folate.
Methods: We reviewed laboratory databases on RBC folic acid from pre and post fortification years. The data included age, gender, RBC folate, hemoglobin, mean cell volume and pregnancy test. We examined a sub-set of females at ages 14–45 years who were non-anemic and normocytic. Complete protection against NTD was defined as RBC folate concentration above 900 nmol/L.
Results: In 2006, 40% of the women of child-bearing age and 36% of pregnant women, exhibited RBC folate levels below 900 nmol/L, rendering them sub-optimally protected against NTD.
Conclusion: A considerable proportion of pregnant women is still at risk of having a baby with NTD. This should be remedied by increasing the mandatory concentrations of folic acid required in flour, complemented by public education and increasing the folic acid in prenatal supplements.
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1. Introduction

Low folate levels and complication in pregnancy started to appear in literature in the 1960's [1–6]. Maternal folate deficiency was linked with low birth weight, high incidence of abortion, abruptio placentae and fetal malformation [7]. The 1977 editorial in Lancet concluded that "If the association is confirmed, then the next step will be to judge its casualty directed directly, by preconception vitamin supplementation in mothers at high risk of neural tube defect" [8]. Folate is required during cell and tissue growth and deficiency in the early stages of pregnancy might reasonably be expected to cause defective nucleic-acid formation, impaired cell-growth and replication, damage to the fetus and placenta with defective implantation and organogenesis [9].

The role of folate in preventing neural tube defects (NTDs) was established by two randomized placebo control studies [10,11]. Later, Daly et al. [12], in a case controlled study, characterized the dose response relationship between RBC folic acid levels and the incidence of NTD in Ireland. In this study they reviewed all women attending their first antenatal clinic in one of three hospitals in Dublin between March 1986 and March 1990 and collected 50,049 samples representing 70% of births at these hospitals and 25% of all Ireland. They documented graded risk reduction. NTD risk increases to more than three folds at levels below 700 nmol/L as compared with those above 900 nmol/L. At 900 nmol/L the odds ratio was 1.0 and this level has been accepted as the optimal folate concentration needed to minimize the incidence of NTD.

During pregnancy the folate requirement is increased by almost four folds. It is now well established that periconceptional supplementation with folic acid can reduce the risk of both the first occurrence as well as the recurrence of NTD [13,14]. Folate fortification at 140 μg/100 g and 150 μg/100 g of flour respectively, became mandatory in 1998 in the USA and Canada [15,16]. Since it is now almost ten years into folate fortification in North America, one would expect that population folate levels would have stabilized. The objective of the present study was to assess the percentage of women of reproductive age in Ontario who have protective levels of folate.

2. Method

We reviewed RBC folic acid test requests submitted to four general practice laboratories and two hospital laboratories in Ontario, Canada. All tests were requested by attending physicians as part of clinical care. Data were available for the following years: 1995 and 1997 (pre fortification), 1998 (start of fortification), and for most years from 2000 to 2006 (post fortification). All (six) laboratories were located in the Greater Toronto area. All samples were anonymous. Available data included age,

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gender, RBC and serum folic acid, hemoglobin, mean cell volume and pregnancy test (βHCG). We carefully selected our study population where the respective tests of interest were done at the same time. Ethnicity or differential diagnosis was not available to us. We focused on women between 14 and 45 years as child-bearing age. Since the patient's presenting diagnosis or symptoms were unknown to us, we selected our population who were both non-anemic (normal hemoglobin female: 120–160 g/L) and normocytic (MCV: 75–94 fL) for our analysis. The same calculations were repeated on a smaller sub-set of women who had positive pregnancy test (βHCG greater than 50 IU/L). We followed RBC folate levels since it more closely reflects tissue folate stores whereas plasma folate levels fluctuate significantly with diet. Following dietary deprivation of folate, plasma levels will decrease within 3 weeks whereas the RBC folate levels, which reflect storage form, remain normal for 3–4 months. Furthermore, many laboratories in the Greater Toronto region have stopped offering serum folic acid and as a result serum data from only a small cohort was available to us. Serum/plasma data is not presented here. Chi square analysis was used to compare the proportion of women below 900 nmol/L among the years in the target population. Mann–Whitney Rank Sum Test was used to compare medians between the years 2002 and 2006.

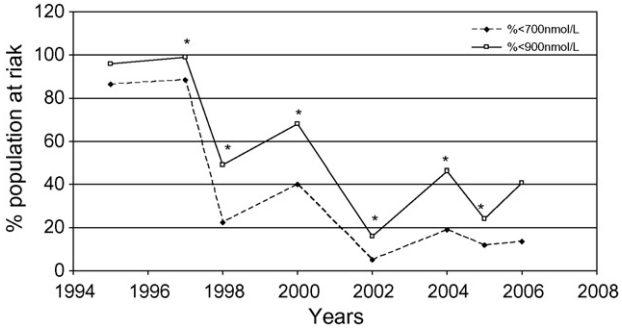

Fig. 1. Proportion of study women not achieving protective levels of RBC folate of 900 nM (female ages 14–45, hemoglobin 120–160 g/L; mean cell volume 75–94 fL) *There was a significant increase in protection of women with sub-optimal RBC folic acid levels for NTD prevention between 1997 and 1998. This proportion increased between 2002–2004 and 2005–2006 (all $p < 0.001$).

3. Results

Although our total sample size was large, in many cases there was one or the other test result missing. Once we corrected for child-bearing age, hemoglobin and MCV, the final dataset for analysis was smaller. The data from 2002 and 2006 are from the same general practice laboratory and data from 2004 and 2005 are from the same primary care hospital laboratory. Both these laboratories used the same method (Beckman-Coulter, Instrument-DXI), for RBC folate analysis and therefore allowed us to study trends in folate levels. Even though the methods for these years were the same, we have restricted our comments to data for the year 2006 since this is the most recent dataset and reflects the most recent state of folate fortification.

RBC folic acid levels have risen significantly over the years since the introduction of fortification (Table 1). Although, the medians between 2002 and 2004 or 2004 and 2005 were not significantly different from each other, there was a significant difference in the medians between 2005 and 2006 ($p < 0.01$). Overall there was a significant decrease in the population at risk (RBC folate below either 700 or 900 nmol/L ($p < 0.001$) (Fig. 1) from 1998 to 2002. This trend reversed after 2002 with an increase in proportion of women with levels below 900 nmol/L from 24% in 2005 to 40% in 2006 (Fig. 1). In a sub-set of 82 pregnant women who had a positive βHCG test, 36% had RBC folic acid levels below the optimal level of 900 nmol/L and 16% below 700 nmol/L (Table 2).

4. Discussion

Folic acid plays a major role as a coenzyme in one-carbon metabolism and is a key participant in the biosynthesis of DNA and RNA. The body's requirement for folate is thus related to the amount of cellular reproduction occurring at any particular time. Pregnancy imposes a unique requirement for additional folate due to pregnancy-associated increase in blood volume and active cell proliferation rates critical for fetoplacental growth and development. Although NTD is multifactorial, possibly due to the combined effects of several genes and environmental factors [17], Hibbard [2,7], Smithells et al. [18] were among the first to show that folate deficiency may be correlated with it.

Neural tube defects are serious congenital abnormalities of the central nervous system. Although some cases of NTDs are induced by hyperhomocysteinaemia, resulting from genetic polymorphism of a thermo labile enzyme, in the majority of cases the cause is unknown [19]. The mechanism by which FA rescues a sub-set of embryos from being malformed is also poorly understood. Open NTD result from failure of the neural tube to close during early embryogenesis. Seventy-five percent of NTD-affected pregnancies end in miscarriage or stillbirth. The incidence of these lethal or severely debilitating birth defects varies considerably according to geography, socioeconomic status and ethnicity [20,21].

In 1998 folate fortification of 140 μg/100 g and 150 μg/100 g of flour respectively, became mandatory in the USA and Canada [15,22] In 2007, 54 countries had also made wheat-flour fortification mandatory, the greatest increase being in the Eastern Mediterranean countries from 4% in 2004 to 44% in [23]. The objective of fortification was to increase from 30% to 70% the average intake of folic acid by women of child-bearing age [16]. Indeed the impact of folate fortification has been significant as population folate levels have risen steadily. There are now several studies showing similar trends to ours, in an increase in mean and median folate levels post fortification [24–31]. Jacques et al. showed that mean serum folate increased from 4.6 ng/mL to 10.0 ng/mL in the US [32]. Prevalence of low serum folate of <3 ng/mL (<7 nmol/L) decreased from 22% to 1.7%. Lawrence et al. showed an increase in the mean serum folate from 12.6 ng/mL (27.6 nmol/L) to 18 ng/mL (40.7 nmol/L) while the

Table 1
Changes in RBC folate over the years in our study (female 14–45 years, hemoglobin 120–160 g/L and mean cell volume: 75–94 fL)

RBC folic acid, nmol/L (ng/mL)							
Lab.	Year	Number	Mean	Median	5%	95%	% below 900 nmol/L
#1	1995	221	514(226)	483(213)	251(110)	855(377)	95.7
#2	1997	95	424(187)	376(165)	207(91)	815(359)	98.9
#3	1998	5054	933(411)	881(388)	523(230)	1536(677)	48.9
#4	2000	141	814(359)	760(335)	381(168)	1487(656)	68
#5	2002	635	1235(545)	1207(532)	683(301)	1887(832)	16
#6	2004	155	1015(447)	928(409)	556(245)	1688(744)	46.2**
#7	2005	159	972(428)	910(401)	522(230)	1676(739)	24.1**
#8	2006	1537	1048(462)	972(428)*	577(254)	1827(806)	40.7**

* Mann–Whitney Rank Test for medians between 2005 and 2006, $p < 0.01$, NS between 2002 and 2004 or 2004 and 2005.
** Chi square, $p < 0.001$ for % change between the years 2002 and 2004; 2004 and 2005; 2005 and 2006.

Table 2
Proportion of pregnant women (positive βHCG) not achieving protective levels of RBC folate of 900 nM

RBC folic acid, nmol/L (ng/mL)							
Lab.	Year	Number	Mean	Median	5%	95%	% below 900 nmol/L
#8	2006	82	1113(491)	1051(463)	609(268)	2028(894)	36

percentage population of low values have decreased between 1994 and 1999 [28]. The National Health and Nutrition Examination Survey (NHANES) has been assessing trends in serum and RBC folic acid levels by race/ethnicity between the 1999–2000 and 2003–2004 surveys [33]. Their comparison shows that median serum and RBC folate concentrations among women of child-bearing age (14–45) decreased 16% and 8% respectively, from 1999–2000 to 2003–2004 and is consistent with our data (Fig. 1).

Pfeiffer and colleagues reported trends in blood folate and vitamin B12 concentrations in the United States 1988–2004. They found in the post fortification period 2003–2004 about 90% of the population still had RBC folate below 410 ng/ml (900 nmol/L) [34]. This is also consistent with our data which shows an initial increase in folate levels to 2002 and then a decline (Table 1). However, our study differs from many of the studies as our study population included only women of child-bearing age who were both non-anemic and normocytic and provides data up to the year 2006.

Impact of folic acid on the reduction of NTD has been shown in various parts of the world. A significant decline in the prevalence of NTD was reported also from Canada [35–39] and Chile [40] where fortification became mandatory in 2000 and from China where folate supplementation has been given [41]. In the USA an estimated decline in prevalence of NTD from 10.6 cases per 10,000 live births in 1995–1996 (pre fortification) to 7.5 cases per 10,000 in 1999–2000 (post fortification) was reported [42]. Williams et al. reported 31% decrease in the prevalence of spina bifida from 5.15 cases per 10,000 births in 1995–1996 to 3.54 cases per 10,000 births in 1998–1999, and 16% decrease in anencephaly, from 2.43 cases per 10,000 births in 1995–1996 to 2.05 cases per 10,000 births in 1998–1999 [43]. Stevenson et al. reviewed the incidence of NTD also showing a decrease from 18.9 to 9.5 per 10,000 live births over a six-year period (1992–1998) coinciding with the increased periconceptional use of folic acid supplement among women of child-bearing age [44]. Honein et al. reviewed the birth records of 45 US States and Washington, DC over the period of 1990–1999. They found that the prevalence of NTD had decreased by 19% concluding that the decrease may be due to the fortification of flour but there may be other factors contributing to the decline [45]. Although this study did not include fetal deaths or still births, it nonetheless shows the impact of folate addition to flour.

De Wals et al., in a recent paper [39], studied a population that included live births, stillbirths, and terminations of pregnancies because of fetal anomalies in seven Canadian provinces. A total of 2446 subjects with NTDs were recorded among 1.9 million births. The prevalence of NTDs decreased from 1.58 per 1000 births before fortification to 0.86 per 1000 births during the full fortification period (46% reduction). The preexisting geographical differences almost disappeared after fortification began. The authors concluded that fortification with folic acid was associated with a significant reduction in the rate of NTDs in Canada.

To reduce the incidence of NTD, Chile in 2000 legislated fortification of wheat flour with 220 μg of folic acid to every 100 g of flour. An estimated mean 427 μg/d would be added to the diet of Chileans. Preliminary report suggests about 40% reduction in NTDs [30,40].

Berry et al. [41] reviewed the incidence of NTD in northern and southern regions of China. As part of public health initiative, pregnant women in areas of high risk for NTD, were asked to take 400 μg of folic acid. In this very large study from 1993 to 1995, there were 130,143 women who took folic acid at anytime before or during pregnancy and 117,689 women who did not take folic acid. For women who used periconceptional folic acid, the rates of NTD were 1.0 per 1000 in the northern region, as compared with 4.8 per 1000 pregnancies of at least 20 weeks' gestation in women who did not take any folic acid. With 400 μg folic acid supplementation, a protection of almost 80% was achieved. In the southern region for women who used periconceptional folic acid the rate was 0.6 per 1000 as compared with 1.0 per 1000 in women who did not take any folic acid, The greatest reduction in risk was observed among infants of a subgroup of women in the northern region with periconceptional use, who took folic acid pills more than 80% of the time.

While most of the studies reported on significant decline in NTD, the magnitude especially in United States and Canada was smaller than expected. Centers of Disease Control's recommendations had estimated a decline of 50% in the rate of NTD with supplementation of 0.4 mg of folic acid per day [46]. The reason could well be that the fortification of flour of 140 μg/100 g flour is not sufficient and the compliance of women in taking prenatal folate supplements is poor as our data suggest (Table 2). Indeed it has been shown that only 32% of women of reproductive age (18–45 years) take a daily supplement of folic acid in USA [47], 26–47% in the Netherlands [48] and 30% in Western Australia [49]. The compliance is poor also in other European countries [50,51]. Ray and colleagues in a systematic review summarized the available literature (52 reports) regarding the rate of folic acid supplementation periconceptionally. They concluded that in many countries less than 50% of women take folic acid supplements [52,53]. Our data (Table 2) shows that indeed 36% of pregnant women had folate levels that would put them at risk of having babies with NTD. There are several possible reasons for poor compliance to prenatal supplements. Size of the tablet, nausea and vomiting or just plain dislike in taking drugs during pregnancy [54] has been reported as some of the reasons for poor compliance to prenatal vitamins [55]. Studies showing poor dissolution characteristics of folic acid in prenatal vitamins may also result in inadequate folate levels due to poor bioavailability [56,57].

Our study has a few drawbacks. Our study population is physician selected. Folate levels can be affected by diet as well as ethnicity, and, the reason for the tests request was unknown to us. We therefore selected only women of child-bearing age who were both non-anemic and normocytic and had RBC folate levels below 700 nmol/L and 900 nmol/L. Our analysis reveals that despite fortification about 40% of Canadian women in 2006 were not optimally protected by adequate RBC folic acid levels. More importantly 36% of the women who were pregnant (Table 2) most likely did not comply with prenatal supplements and/or their dietary habits were such that they did not get adequate amounts of folic acid to bring their RBC folate levels above 900 nmol/L. Our data is in concordance with Sherwood et al. [58] who interviewed 61 pregnant women and collected both dietary and supplement history. They conclude that one-third of their subjects did not meet their folate requirements from dietary sources alone. They suggest that during pregnancy or lactation, fortification at twice the mandated level, resulting

Table 3
Levels of fortifications in Canada and USA [39]

	Canada	USA
Flour/cornmeal	0.15 mg/100 g	0.14 mg/100 g
Pasta	0.20 to 0.27 mg/100 g	0.20 to 0.27 mg/100 g
Rice	NA[a]	0.154 mg to 0.308 mg/100 g
Corn grits and farina	NA	0.15 mg/100 g
Breakfast cereal	= or <60 μg/serving	= or <400 μg/serving

[a] NA: not available.

in mean dietary intakes 786 ± 132 μg/day and 716 ± 150 μg/day of dietary folate equivalent, respectively, would produce only a 3% prevalence of folate inadequacy and will improve the "population baseline" significantly.

The debate about the optimal level of folic acid fortification and supplementation has not subsided [50,53,59–68]. Wald et al. quantified the effect of different doses of folic acid as related to NTD, and calculated 36%, 57%, and 85% risk reduction of NTD on 0.4 mg/day, 1 mg/day, and 5 mg/day of folic acid respectively [64,69,70]. Oakley called for an increase in fortification to the level of 240 μg/100 g of flour from 140 μg/100 g flour and thereby prevent substantially more NTD cases [61]. While it is possible that the recommended doses of periconceptional vitamin supplementation would be sufficient *if* there was full compliance, many studies including ours suggest that compliance is less than 50% (range from 0.5 to 52%) [50,52,53]. Thus, both fortification and supplementation are needed.

Our study shows that initially folate from fortification did indeed improve substantially folate status and the percent of women at risk is consistent with NHANES data [33] and other studies [30,40]. Our data suggest that although there was a significant increase in the median RBC folic acid level in 2006 from 2005, there were still 40% of women of child-bearing age who had RBC folic acid below 900 nmol/L, and were exposed to the risk of having babies with NTD. The percentage of at-risk population is well explained by low compliance to periconceptional folic acid supplements and a sub-optimal level of fortification. The last few years have also been characterized by the "low carb" diet wave leading women to decrease the consumption of flour based products and decrease in their folate intake [16,71]. Recently a decrease of the folate content in breads containing enriched flour has also been reported [72]. This may also contribute to our documented decrease in folate levels after 2002.

Our findings document that the goal of optimal prevention of NTD has not yet been achieved, and there is a considerable proportion of pregnant women at risk of having a baby with NTD. Although these NTDs are multifactorial in origin, the potentially preventable cases should not be overlooked and both folate fortification and supplementation need to be reassessed. To achieve this, a multipronged approach needs to be taken. Fortification of breakfast cereals is considerably lower in Canada than in the United States (breakfast cereal = or <60 μg/serving vs. = or <400 μg/serving (Table 3) and most women in Canada are not receiving the recommended 0.4 mg/day [39]. Since almost 50% of the pregnancies are un-planned, and, prenatal supplementation needs to start pre conception, increase in folate fortification [58] will improve the "population baseline" significantly and should be addressed as a public health priority. Size of the tablet, reasons for nausea and vomiting and supplementation also needs to be addressed. Compliance monitoring by following RBC folate levels may be another practical approach in assuring adequate protection [73]. Our data is supportive of Wald's [69,70] suggestion that folate supplementation of 0.4 mg/day is insufficient, and that supplementation with 5 mg/day will result in almost full protection of pregnant women against preventable NTD. A 5 mg daily dose, starting when planning pregnancy will provide a booster dose whenever it is taken. We call for an urgent action in increasing fortification and supplementation as well as measures in increasing awareness and education needs to be undertaken.

Conflict of interest

None

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