Women’s compliance with current folic acid recommendations and achievement of optimal vitamin status for preventing neural tube defects

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BACKGROUND: The timing of folic acid supplement usage is critical to preventing pregnancies affected by neural tube defects (NTDs) because the neural tube closes by Day 28 post-conception. We investigated compliance of pregnant women with current folic acid recommendations (400 µg/day from preconception to 12 weeks) in relation to achieving a folate status associated with lowest risk of NTDs.

METHODS: From a sample of 296 women with singleton uncomplicated pregnancies attending an antenatal clinic in Northern Ireland, those who reported taking folic acid in the first trimester (n = 226) were investigated. Samples were taken at 14 weeks gestation to measure serum concentrations of folate and vitamin B12 (related to folate and an independent predictor of NTD), and dietary B-vitamin intake and folic acid usage were investigated.

RESULTS: Although the majority of the overall sample (84%) reported taking folic acid supplements in the first trimester, only 19% had started before conception, as recommended. Multigravidae compared with primigravidae women were less likely to have followed the recommendations correctly (P = 0.001). At 14 weeks, red cell folate (considered a reliable biomarker of previous 3 months, covering time of neural tube closure) was correlated (r = 0.320, P < 0.001) with the reported duration of folic acid usage, and was lower (P < 0.0001) in women who started folic acid after conception.

CONCLUSIONS: Red cell folate concentrations in women not complying with recommendations were suboptimal in relation to NTD risk. The findings generally support the recent official recommendation to the Chief Medical Officer for mandatory fortification of food with folic acid in the UK.

Key words: maternal compliance / folic acid intake recommendations / folate / vitamin B12 / neural tube defect prevention

Introduction

Conclusive evidence establishing the protective effect of folic acid against both first occurrence (Czeizel and Dudas, 1992) and recurrence (MRC, 1991) of neural tube defects (NTDs) has existed for almost 18 years, leading to recommendations by governments worldwide for women to take 400 µg/day folic acid from preconception until the end of the first trimester of pregnancy (CDC, 1992; Department of Health, 1992). Over the years health promotion campaigns were introduced to encourage women to follow the recommendations correctly. Studies examining the effectiveness of such campaigns at reaching the target population report that, although awareness of folic acid has increased considerably amongst pregnant women, the proportion of women who take folic acid from before conception is still very low (Sillander and Pring, 2000). Globally, preconception use of folic acid is estimated to be <50% (Ray et al., 2004), with particular concern that young women from minority ethnic groups and lower socio-economic backgrounds are the least likely to follow the recommendations (Stockley and Lund, 2008). Furthermore, an estimated 50% of pregnancies are unplanned and therefore the period from preconception until the 28th day of the pregnancy (where folic acid is protective against NTD) may have passed before the woman is even aware of the pregnancy (Department of Health, 2002). Perhaps of greatest concern is evidence...
from a large multicentre study examining 13 million birth records from nine European countries, including the UK, showing that there has been no detectable impact on incidence of NTD in any country over the 10-year period from 1988 to 1998, covering the time before and after current folic acid recommendations were introduced and actively promoted (Botto et al., 2005). NTD rates have, in contrast, declined markedly by between 27 and 50% in the USA and Canada coinciding with the introduction of mandatory population-wide folic acid fortification of food (Honein et al., 2001; De Wals et al., 2007). Apart from preventing NTD, there is good evidence that periconceptional folic acid use also reduces congenital heart defects in infants (Van Beynum et al., 2010).

Mandatory folic acid fortification remains very controversial, however, with many opposed to the introduction of such a policy, primarily on safety grounds. Traditionally these relate to the potential risk (though unproven) that long-term exposure to high-dose folic acid might mask the anaemia of vitamin B12 deficiency in older people (Savage and Lindenbaum, 1995), but more recent evidence has raised the new concern that it might promote colorectal tumourigenesis in patients with pre-existing lesions (Cole et al., 2007). Because of these concerns, many governments worldwide have delayed decisions to implement population-based folic acid fortification similar to the policies in place for over 10 years in North America. However, in the UK, following consideration of new evidence on folic acid and cancer risk, the Scientific Advisory Committee on Nutrition (SACN) has recently reconfirmed the advice it published in 2006 recommending mandatory folic acid fortification in order to reduce the number of pregnancies affected by NTDs. This latest recommendation by SACN (2009), notified to the Chief Medical Officer in October 2009, has once again focused attention on the folic acid fortification controversy. At the heart of the debate is whether folic acid recommendations—in the absence of food fortification—can reach the target population and result in an optimal maternal folate status in sufficient time to cover the critical period when the neural tube is closing.

The aim of this study was to investigate the compliance of pregnant women with current folic acid recommendations (400 μg/day from preconception to 12 weeks) in relation to achieving a folate status associated with lowest risk of NTDs. In addition, given new evidence that low maternal vitamin B12 is a significant predictor (independent of folate) of NTD risk (Molloy et al., 2009), we determined serum concentrations of vitamin B12. We conducted the study in Northern Ireland where awareness of NTD might be expected to be high, given that NTD rates are among the highest in the world, and where pregnancy terminations are not legal (Botto et al., 2005).

**Materials and Methods**

**Subject recruitment and study design**

Between September 2005 and December 2006 women in the first trimester of pregnancy attending antenatal clinics at the Causeway Hospital, Coleraine, Northern Ireland, were invited to participate in the study. Participants were healthy women with singleton pregnancies, without current pregnancy complications and aged between 18 and 35 years (i.e. on the basis that pregnancy complications are generally more prevalent in women over 35 years of age). Women were excluded from participation if they had gastrointestinal, hepatic, renal or vascular disease, haematological disorders, epilepsy or a previous pregnancy with an NTD or were the first degree relative of a woman who had a pregnancy with an NTD or were themselves a sufferer of an NTD. Participants who were taking medication known to interfere with B-vitamin metabolism and those who had undergone IVF treatment were also excluded. Upon recruitment (at the first antenatal visit), women were asked to complete a screening questionnaire on general health and pregnancy details. As part of the screening process, supplementation usage was discussed in detail and particularly the consumption of folic acid and B-vitamin supplements. Only those women who had taken folic acid supplements at a dose of 400 μg/day in the first trimester of pregnancy were eligible for inclusion. Information was collected on the name of the supplement, the folic acid content of the supplement, when the supplement was started and the frequency of its usage. Ethical approval was granted by the Office for Research Ethics Committees (ref: 05/Q2008/21) and informed consent was obtained from each participant on recruitment.

**Blood sampling and laboratory analysis**

A non-fasting blood sample was collected from each participant at the time of recruitment (on average at the 14th gestational week). Samples were processed within 4 h of blood collection and aliquots were stored at –80°C for batch analysis at the end of the study. Blood samples were analysed for serum and red cell folate (Molloy and Scott, 1997), serum vitamin B12 (Kelleher and Broin, 1991) and plasma homocysteine (Leino, 1999). For all assays, samples were analysed blind, within 18 months of collection and quality control was provided by repeated analysis of stored batches of pooled samples covering a wide range of values. Intra- and inter-assay coefficients of variation were ≤8.2% for folate; ≤10.4% for vitamin B12 and ≤2.5% for homocysteine.

**Dietary analysis**

Dietary intakes of the relevant B-vitamins were evaluated using a 4-day food diary (which included 2 week and 2 weekend days) in combination with a food frequency questionnaire. The food frequency questionnaire was designed primarily to measure the intake of foods fortified with B-vitamins and included 29 food groups or specific branded products, such as ready-to-eat breakfast cereals, breakfast cereal bars, breads and fat spreads. Dietary analysis was carried out using the nutritional software package WISP version 3.0 (Tinuviel software, Warrington, UK), specifically modified to include the most up-to-date nutrient values for fortified foods and to enable the generation of separate values for the natural content of folate in food, and folic acid added to foods.

**Statistical analysis**

The sample size for the current study was estimated using the mean (SD) red cell folate concentrations from our previous investigation of pregnant women in Northern Ireland who had taken (or not taken) folic acid supplements (Holmes et al., 2005). We estimated that a sample size of 35 subjects per group would be able to discriminate a difference in red cell folate concentrations of 434 nmol/l between the different categories of pregnant women taking folic acid supplements with a power of 90% at α = 0.05 (DSS Research, 2010). A difference in red cell folate status of this magnitude has been reported by others to correspond to a reduction of NTD prevalence of 43% (Hertrampf and Cortes, 2008).

All statistical analysis was performed using the Statistical Package for the Social Sciences software (version 15.0; SPSS UK Ltd, Cherssey, UK). Data on serum and red cell folate, serum vitamin B12 and plasma homocysteine concentrations were log transformed before analysis for normalization purposes. Participants were categorized into three groups on the basis of reported time that folic acid supplementation started: prior to conception; between conception and before the sixth gestational week; or after the sixth gestational week. Correlations between variables were...
performed using Pearson correlation coefficients. For continuous variables, differences between groups were examined using one-way analysis of variance with Bonferroni post hoc test. For categorical variables, differences were examined using the $\chi^2$ test. Results were considered significant when $P < 0.05$.

**Results**

Of the total available sample of 296 pregnant women, 248 (84%) reported taking folic acid supplements at any time in the first trimester (Fig. 1). Of these, 22 women were excluded as they were taking folic acid at a dose higher than the recommended dose of 400 $\mu$g/day. The remaining 226 women fulfilled the inclusion criteria and were deemed eligible for investigation. The general characteristics of this group are shown in Table I. The average duration of folic acid supplementation was 12 weeks, but this ranged from a minimum of 4 weeks to a maximum of 52 weeks. Dietary intakes for energy and B-vitamins compared favourably with current reference values as defined for pregnant females aged 19–50 years in the UK (Department of Health, 1991).

B-vitamin status and homocysteine concentrations at the 14th gestational week were examined in relation to duration of folic acid supplement usage (Table II). Only 57 women (19% of the total sample) had followed the recommendations correctly by starting to take folic acid supplements before conception and continuing until the end of the first trimester of pregnancy, while the majority had started folic acid later than recommended. Women with previous pregnancies were found to be significantly less likely to commence folic acid supplementation as recommended before conception when compared with primigravidae women (14 versus 29%; $\chi^2 = 11.38, P = 0.001$; not shown). A step-wise decrease was observed in the total number of weeks of folic acid usage, ranging from an average of 24 weeks in those starting folic acid before conception to just under 7 weeks in those who started after the sixth gestational week (Table II). Serum folate concentrations were lower, and corresponding plasma homocysteine higher, at the end of the first trimester of pregnancy in women who started taking folic acid six or more weeks after conception, compared with those starting it before conception or during gestational Weeks 0–6. There were no differences between the groups in vitamin B12 levels. Serum folate was negatively correlated with homocysteine in the two groups that started taking folic acid after conception, but not in those who started it preconception and were therefore taking folic acid for a more prolonged period (Fig. 2). Within the total sample there was a significant correlation between serum folate and dietary folate intake ($r = 0.301; P = 0.001$), but not between serum vitamin B12 concentrations and dietary B12 intake ($r = 0.02; P = 0.8$) (data not shown). There was no correlation between the number of weeks of folic acid supplement use and dietary intake of folate ($r = 0.09; P = 0.4$) or vitamin B12 ($r = 0.08; P = 0.4$).

Red cell folate concentrations at the 14th gestational week were correlated with the total number of weeks of folic acid usage reported by participants ($r = 0.320, P < 0.001$; not shown). Women who reported starting folic acid six or more weeks after conception had lower red cell folate status compared with those who started before conception or at gestational Week 0–6 (Fig. 3a). Using the cut-off

![Figure 1](https://academic.oup.com/humrep/article-abstract/26/6/1530/2914587/653000914587)
for optimal red cell folate concentration of 400 μg/l (907 nmol/l) or greater, (the maternal level associated with the lowest risk of having a pregnancy with an NTD; Daly et al., 1995), the proportion of women failing to achieve optimal folate status varied depending on when folic acid usage was commenced (Fig. 3b), from 27% in the preconception group to 38% and 53% in the 0–6 gestational week group and ≥6 gestational week group, respectively. Vitamin B12 concentrations were also considered because serum B12 concentrations >185 pmol/l (250 ng/l) at the end of the first trimester of pregnancy were recently found to be independently associated with a reduced risk of NTD (Molloy et al., 2009). Using the reported cut-off values associated with lowest risk of NTD as a basis for evaluating maternal B-vitamin status, the results showed suboptimal status of folate in 42% and of vitamin B12 in 31%, while 57% of participants were suboptimal in one or other nutrient (not shown).

### Table I General characteristics and dietary information for pregnant women at the 14th gestational week.a

<table>
<thead>
<tr>
<th></th>
<th>Whole group (n = 226)</th>
<th>Reference valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>27.6 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>Ethnicity (Caucasian, %)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Folic acid supplement usage (%)</td>
<td>100 100</td>
<td></td>
</tr>
<tr>
<td>Duration of folic acid supplement usage (week)</td>
<td>12.0 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>Parity (n)</td>
<td>0.9 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Gestation (week)</td>
<td>13.7 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Dietary intakes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
<td>1896 ± 399</td>
<td>2000</td>
</tr>
<tr>
<td>Total dietary folate (μg/day)</td>
<td>301 ± 119</td>
<td>400</td>
</tr>
<tr>
<td>Folic acid added to food (μg/day)</td>
<td>114 ± 103</td>
<td></td>
</tr>
<tr>
<td>Natural food folate (μg/day)</td>
<td>186 ± 51</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12 (μg/day)</td>
<td>4.0 ± 1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Vitamin B6 (mg/day)</td>
<td>2.4 ± 0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Riboflavin (mg/day)</td>
<td>1.7 ± 0.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

aValues are presented as mean ± SD or percentages.
bReference ranges for dietary values refer to reference nutrient intakes for pregnant women except for energy where the estimated average energy requirement value for pregnant women is given (Department of Health, 1991).

As per inclusion criteria (see text) only women taking folic acid at the time of conception were included in the analysis.

### Table II B-vitamin status and homocysteine concentrations at the 14th gestational week in pregnant women in relation to duration of folic acid supplement usage.1

<table>
<thead>
<tr>
<th>Time of commencement of folic acid supplements</th>
<th>Preconception (n = 57)</th>
<th>0–6 gestational weeks (n = 79)</th>
<th>≥6 gestational weeks (n = 90)</th>
<th>Significance3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of folic acid usage (week), range</td>
<td>23.8 ± 7.3a (13–55)</td>
<td>9.4 ± 2.4a (7–12)</td>
<td>6.7 ± 1.8a (1–6)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Serum folate (nmol/l)3</td>
<td>55.5 ± 24.0a</td>
<td>48.0 ± 19.8a</td>
<td>38.5 ± 23.5b</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Serum vitamin B12 (pmol/l)4</td>
<td>235 ± 84</td>
<td>245 ± 76</td>
<td>208 ± 80</td>
<td>P = 0.219</td>
</tr>
<tr>
<td>Plasma homocysteine (μmol/l)</td>
<td>6.2 ± 1.6a</td>
<td>6.3 ± 1.8a</td>
<td>7.2 ± 2.5b</td>
<td>P = 0.011</td>
</tr>
</tbody>
</table>

1Values are presented as mean ± SD.
2Values compared using analysis of variance with Bonferroni post hoc following log transformation of data for normalization purposes. Different superscript letters (a, b, c) denote significant differences between any two groups within a row, same superscript letters (a; a) indicate no significant difference between any two groups within a row.
3Values were converted from μg/l to nmol/l using a conversion factor of 2.267.
4Values were converted from ng/l to pmol/l using a conversion factor of 0.738.

### Discussion

In a convenience sample attending an antenatal clinic, our results showed that most women (84%) were aware of folic acid and had taken it during the first trimester of pregnancy. However, the majority (i.e. four in every five) of our overall sample were not meeting the specific recommendation to take 400 μg/day of folic acid from before conception until the 12th week of pregnancy. Women who did not start folic acid supplements as recommended before conception were twice as likely as those that did to have sub-optimal folate status, as indicated by a failure to achieve a red cell folate concentration associated with the lowest risk of a pregnancy affected by a NTD. Pregnancy concentrations of vitamin B12 were also of concern. Overall, a total of 57% of these women had a pregnancy B-vitamin status that would be associated with an increased risk of an NTD-affected pregnancy.

A strength of this study is that we considered the maternal status of not only folate, well established as a determinant of NTD risk, but also vitamin B12, metabolically closely related to folate and recently identified as an independent predictor of NTD risk. We used published data from nested case-control studies within large population-based Irish cohorts that quantified the effect of each nutrient on NTD risk (Daly et al., 1995; Molloy et al., 2009) to assess the maternal biomarker status of both nutrients in this sample in relation to predicted NTD risk. The published data show a continuous dose-response inverse relationship between maternal red cell folate concentrations and NTD risk, with a red cell folate of 400 μg/l (906 nmol/l) or above linked to lowest risk (Daly et al., 1995). Although folate insufficiency is the major contributing factor, low maternal vitamin B12...
status has more recently been recognized as an independent risk factor for having a pregnancy affected by an NTD (Ray et al., 2007; Molloy et al., 2009). Almost one-third of our sample had a pregnancy vitamin B12 concentration below 250 ng/l (185 pmol/l), a level recently associated (independent of folate) with an almost three times higher risk of a pregnancy affected by an NTD compared with having a good B12 status (i.e., 400 ng/l; Molloy et al., 2009). This pregnancy level in turn equates to a pre-pregnancy B12 concentration of 300 ng/l (221 pmol/l), given an estimated 20–25% natural drop in serum B12 over 14 weeks from pre-pregnancy concentrations (Murphy et al., 2007). Thus, the value of 300 ng/l (221 pmol/l) vitamin B12 was recently proposed as a target for women entering pregnancy (Molloy et al., 2009) although no formal recommendation has ever been suggested for vitamin B12 in relation to preventing NTDs.

Our analysis of compliance relied on reported usage of folic acid supplements by pregnant women sampled at 14 weeks gestation who stated that they had taken folic acid supplements in the first trimester of pregnancy. Although self-reported compliance with the

![Figure 2](https://example.com/figure2.png)  
**Figure 2** Correlation between serum folate and plasma homocysteine levels at ~14 gestational weeks according to time of commencement of folic acid supplements. (a) Preconception, (b) 0–6 weeks gestation and (c) ≥6 weeks gestation.

![Figure 3](https://example.com/figure3.png)  
**Figure 3** (a) Mean (and SEM) red cell folate concentrations at ~14 gestational weeks according to time of commencement of folic acid supplement usage. Values compared using analysis of variance with Bonferroni post hoc following log transformation of data for normalization purposes. Different letters denote significant differences between groups (Preconception versus 0–6 weeks gestation, P = 0.095; Preconception versus ≥6 weeks gestation, P < 0.0001; 0–6 weeks gestation versus ≥6 weeks gestation, P = 0.026). (b) Proportion of pregnant women with a suboptimal red cell folate status at ~14 gestational weeks according to time of commencement of folic acid supplement usage. The cut-off for red cell folate concentration was taken to be ≥906 nmol/l (400 μg/l), the maternal concentration associated with the lowest risk of having a pregnancy with an NTD (Daly et al., 1995). *A significantly greater proportion of women with a suboptimal red cell folate status were found in both post-conception groups compared with the preconception group (χ² test: χ² = 11.03; P < 0.0001).
recommendations may be questionable, the reliability of these self-reported data is supported to some extent by the significant correlation between reported duration of folic acid usage and laboratory measures of folate status in these women. Red cell folate concentrations measured at 14 weeks were found to be significantly correlated with the total number of weeks of reported folic acid usage, and were lower in women who reported commencing folic acid after conception: among the latter group, the majority (53%) failed to achieve a red cell folate level associated with the lowest risk of an NTD-affected pregnancy, compared with only 27% of those who started folic acid supplements, as recommended, before conception. In other words, women not complying with the recommendations were twice as likely to have a folate status that would have placed them at an increased risk of a pregnancy affected by an NTD at the time of neural tube closure. Thus, although others have previously reported on compliance with folic acid recommendations (Ray et al., 2004; Nilsen et al., 2006), a strength of our study is that the compliance data were related to laboratory folate values, increasing the validity of our conclusions in relation to potential NTD risk. A limitation of this study is that we did not investigate the various confounding factors, such as socioeconomic status, which are likely to affect supplementation usage and B-vitamin concentrations.

Although serum and red cell folate concentrations were both measured in the current study, red cell folate is considered to be the more reliable indicator of longer-term status because it represents tissue stores and reflects folate intake/status over the previous 3–4 months when circulating folate is incorporated into the maturing red cells. Thus, of relevance to the current study, red cell folate measured at 14 weeks gestation can be considered to reflect maternal folate status during the crucial period of between 21 and 28 days post-conception when the neural tube is closing. Plasma homocysteine (considered to be a functional marker of folate and, to a lesser extent, vitamin B12) is generally lower in pregnant compared with non-pregnant women (Andersson et al., 1992; Holmes et al., 2005), but evidence suggests that an elevated homocysteine concentration during pregnancy may be a cause of, or a contributor to, various pregnancy complications including NTD (Mills et al., 1995), pre-eclampsia, recurrent early pregnancy loss, low birthweight and intrauterine growth retardation (Vollset et al., 2000). In the current study pregnancy homocysteine concentrations were 1 μmol/l higher in women who started taking folic acid after the sixth gestational week than in those who started folic acid earlier. An elevation in homocysteine of this order of magnitude (i.e. 1–1.5 μmol/l) was associated with an increased risk of pre-eclampsia and premature of 36 and 41%, respectively, in a large study of 5883 women (Vollset et al., 2000). In the current study an inverse relationship between serum folate and homocysteine was evident only in participants who started folic acid supplementation after conception, and not in those who started taking folic acid, as recommended, before conception: the lack of a significant correlation between homocysteine and folate in the latter group is further evidence that, on average, these women had achieved an optimal folate status, given that the expected inverse relationship is found only where folate is limiting with respect to homocysteine metabolism.

The proportion of pregnant women (19%) who were correctly following current official recommendations is generally consistent with other studies (Ray et al., 2004; Nilsen et al., 2006). In a systematic review of 49 global studies carried out from 1992 to 2001, the reported rate of preconception folic acid usage varied greatly, from 0.9% in Southern Israel to 49% in Canada (Ray et al., 2004), with the average rate estimated to be around 30%. Of note, our results showed that women with a previous pregnancy were more likely to start folic acid supplementation later, with the majority starting after the sixth gestational week. Likewise, Norwegian women who were users of folic acid periconception were reported to be significantly more likely to have a lower parity (Nilsen et al., 2006). This may suggest that women on second and third pregnancies are becoming complacent with the folic acid recommendation, itself a cause of concern considering that higher parity is associated with an increased risk of NTD (Whiteman et al., 2000). Alternatively, poor pregnancy planning has been associated with both higher parity (Koren and Mawn, 2010; Rocca et al., 2010) and poor compliance with the recommendations for folic acid supplement usage during pregnancy (Inskip et al., 2009).

In any case, given the generally poor compliance with current folic acid recommendations shown in this and other studies (Ray et al., 2004; Nilsen et al., 2006), it is not surprising that no detectable improvement has been observed in the incidence of NTDs in the 13 European countries investigated (Botto et al., 2005).

There are important implications of our findings for emerging food fortification policy, particularly in the UK where a formal recommendation for the implementation of fortification in order to reduce the number of pregnancies affected by NTD has recently been made to Government (SACN, 2009). First, our results support the views of others (Botto et al., 2005) that, in the absence of population-wide food fortification, current recommendations are not being reached in the target population to optimize folate status in sufficient time to cover the critical period of pregnancy when the neural tube is closing. Thus, preventable incidences of NTDs are not necessarily being prevented whereas mandatory folic acid fortification has, in contrast, reduced the incidence of NTD by between 27 and 50% (Honein et al., 2001; De Wals et al., 2007). Second, the high prevalence of poor vitamin B12 status in this study suggests that attention may also need to be directed at improving vitamin B12 levels of women entering pregnancy in order to offer more complete protection against NTDs in pregnancy but this requires further investigation.

**Authors’ roles**

H.M.N. was the principal investigator and is the guarantor. B.M.N. executed the study, conducted the analysis and wrote the first draft of the paper. K.P., J.S., B.M. and H.M.N. planned, designed and supervised the study. M.W. and A.M. contributed to the dietary and laboratory analysis. All authors were involved in the data interpretation, manuscript revisions and the final drafting of the paper.

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