European Journal of Clinical Nutrition (2004) 58, 548–554
 0954-3007/04 $25.00
www.nature.com/ejcn

ORIGINAL COMMUNICATION

Effects of once-a-week or daily folic acid supplementation on red blood cell folate concentrations in women

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Objective: Our aim was to determine if a once-a-week folic acid supplement increases women's red blood cell folate to concentrations (>905 nmol/l) that are associated with a low risk of bearing a child with a neural tube defect.
Design: Randomized control trial.
Setting: General community.
Subjects: In total, 114 nonpregnant women (18–40 y) volunteers, with red blood cell folate concentrations between 295 and 905 nmol/l at screening.
Intervention: Women were randomized to receive a once-a-week 2800 μg folic acid supplement, a daily 400 μg folic acid supplement or a daily placebo for 12 weeks.
Results: The mean (95% CI) red blood cell folate concentrations increased during the 12-week intervention from 608 (553–668) to 900 (828–978) in the weekly folic acid group ($P<0.05$) and from 615 (560–677) to 1053 (957–1158) nmol/l in the daily group ($P<0.05$) during the trial. At week 12, 49% of women ingesting the weekly folic acid supplement had red blood cell folate concentrations greater than 905 nmol/l compared to 74% of women ingesting the daily supplement.
Conclusion: A once-a-week 2800 μg folic acid supplement can increase women's red blood cell folate to concentrations associated with a reduced risk of bearing a child with a neural tube defect, but is less effective than a 400 μg daily supplement. Use of a weekly folic acid supplement over at least 12 weeks before conception by women of child-bearing age may prevent neural tube defects.
Funding: The Otago Medical Research Foundation (Laurenson Award). Healtheries (New Zealand) provided the supplements.
European Journal of Clinical Nutrition (2004) **58,** 548–554. doi:10.1038/sj.ejcn.1601843

Keywords: folic acid; neural tube defects; red blood cell folate; women of reproductive age; folate

Introduction

A folic acid supplement taken daily by women during the periconceptional period will reduce the risk of having a child with a neural tube defect (MRC Vitamin Study Research Group, 1991; Czeizel & Dudas, 1992). In the UK-MRC Vitamin Study, a 4 mg per day dose of folic acid reduced the incidence of neural tube defects by 72% (MRC Vitamin Study Research Group, 1991). Evidence that a lower dose of folic acid is equally effective at reducing risk comes from a recent population-based prevention campaign involving 130 142 women in two regions of China (Berry *et al*, 1999). In the Northern region, where the incidence of neural tube defects was high (5–6 per 1000 births), risk of an affected pregnancy was reduced by 79% in women taking 400 μg of folic acid per day.

It is possible that the maximum reduction in risk of neural tube defects may not require a daily 400 μg folic acid supplement, but might be attained by women achieving and maintaining a high folate status. In a case–control study of 56 049 women in Ireland, Daly *et al* (1995) reported that the risk of neural tube defects declined continuously as red blood cell folate concentrations increased; the lowest

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***Contributors*: CMS and TG had the original idea and obtained funds for the project. All authors were responsible for planning the study. BN and CA recruited subjects and were responsible for sample collection and laboratory analysis. All authors contributed to statistical analysis and writing of the report.**
Received 19 March 2003; revised 4 June 2003; accepted 19 June 2003

category of risk occurring in women with folate concentrations greater than 905 nmol/l. Women can increase their red blood cell folate concentrations above 905 nmol/l by ingesting an additional 200 μg of folic acid per day, over a 6-month period (Daly *et al*, 1997). Mandatory folic acid fortification of flour in the United States was implemented in 1997 to provide women with an additional 100 μg per day. The fortification programme has coincided with a 19% decrease in the neural tube defect birth prevalence rate (Honein *et al*, 2001). In Canada, where a similar fortification programme was introduced in late 1998, the age-adjusted prevalence of open neural tube defects—among live births, stillborns, and therapeutic terminations—in the province of Ontario has declined by 38% (Ray *et al*, 2002).

On the basis of the results from prevention trials, the best advice to prevent neural tube defects remains for women to take a daily 400 μg folic acid supplement; however, the results of surveys indicate that only a minority of women follow this practice (Anonymous, 1999; Schader & Corwin, 1999). The reasons for the low use of folic acid supplements at the critical time of pregnancy involve a multitude of knowledge and behaviour factors, however, it is possible that women who are not planning a pregnancy or who find taking a daily supplement difficult might be more willing to take a high-dose folic acid supplement periodically. In New Zealand, 52% of people who use dietary supplements use them less than daily (Smith, 2001). The purpose of the present study was to determine if a once-a-week folic acid supplement taken by women can increase red blood cell folate to concentrations that will reduce the risk of neural tube defects.

Participants and methods

Protocol

Women from Dunedin, New Zealand, aged 18–40 y volunteered in response to advertisements in local newspapers and recruitment posters during May 2000. Women were excluded if they consumed a supplement containing more than 100 μg/day of folic acid in the previous 3 months, if they were taking drugs known to interfere with folate metabolism, or if they had established chronic disease. We also excluded women who had been pregnant in the previous year, were planning a pregnancy, or had a prior history of a neural tube defect-affected pregnancy. Women meeting these criteria received a screening blood test to determine their folate status. Screening took place from May to June, 2000. Women with red blood cell folate concentrations between 295 and 905 nmol/l were eligible and invited to participate. Those women with red blood cell folate greater than 905 nmol/l were excluded because they already had blood concentrations previously shown to be consistent with a very low risk (Daly *et al*, 1995). Women with red blood cell folate concentrations less than 295 nmol/l were classified as having folate deficiency and were advised to contact their physician for treatment. All women were enrolled within 3 months of screening. Participants were advised to withdraw from the study if they suspected they were pregnant. The Human Ethics Committee of the University of Otago approved the study and women gave written and informed consent to participate.

Design

We conducted the 12-week, randomized controlled trial between July and October 2000. Women were assigned to receive a daily placebo, a once-a-week 2800 μg folic acid supplement, or a daily 400 μg folic acid supplement. We choose 2800 μg as the weekly dose because it was the same amount of folic acid given to participants in the daily supplement group. Participants were given a 12-week supply of supplements. Participants attended a clinic at baseline, and at weeks 6 and 12 and were given fortnightly telephone calls between clinics to encourage compliance. At the baseline clinic, participants completed a demographic and lifestyle questionnaire and were issued supplements with verbal and written instructions. Participants were instructed to avoid taking tablets within 24-h of blood collection to avoid a transient increase in plasma folate that occurs soon after taking a supplement. Women were asked to maintain their usual dietary practices during the study; if consuming folic acid-fortified breakfast cereals they were asked not to change the frequency or amount consumed.

The participants were asked to bring any unused tablets with them to clinic at weeks 6 and 12. Compliance was calculated as the proportion of prescribed pills taken during the study and was assessed in the daily and placebo groups by weighing the remaining pills and in the once-a-week group by counting the unused pills. Participants completed a 3-day, weighed diet record between weeks 6 and 12. Energy and nutrient content of the diets was calculated based on the New Zealand Food Composition Database.

Assignment and masking

Treatment assignment took place after all women had been screened. In rank order of red blood cell folate concentrations women were randomized to the intervention groups. Supplements were in sealed bottles and were identical in size and similar in colour. The treatment codes remained sealed until all data analyses had been completed. A weekly placebo was not used in the study, consequently, participants assigned to take the once-a-week supplement would have known they were receiving folic acid.

Laboratory methods

All blood samples were collected by venipuncture after a 10–12 h overnight fast. At each clinic visit two tubes of blood were drawn from each participant, one no-additive tube for preparation of serum and one EDTA tube for whole-blood and plasma measures. Plasma was obtained by centrifuging

whole blood at 4°C for 15 min at 1200 *g*. Haematocrits were measured by centrifugation. Plasma folate and whole-blood folate concentrations were determined using the microtitre technique exactly as described by O'Broin and Kelleher (1992) with chloramphenicol-resistant *Lactobacillus casei* as the test microorganism. Red blood cell folate was calculated from whole-blood folate by subtracting plasma folate and correcting for haematocrit. The interassay coefficient of variation was 9.6% for plasma ($n=106$) and 8.7% for whole blood ($n=106$), based upon repeated measurements of pooled samples. An external whole-blood standard (National Institute for Biological Standards and Control, South Mimms, UK) with a certified folate concentration of 29.4 nmol/l was included on all microtitre plates on which a standard curve was measured. We measured a mean $\pm$ s.d. folate concentration in the external standard of 29.9 ± 1.6 nmol/l ($n=14$). Serum cobalamin (vitamin B_{12}) was determined with an Abbott IMX analyzer, reagents, and calibrators (Abbott Laboratories, Abbott Park, IL, USA). The between-run coefficient of variation was less than 10% based on the controls provided by the manufacturer. Serum homocysteine concentrations were measured at weeks 0 and 12, but are reported elsewhere (Adank *et al*, 2003).

Statistical analysis

Differences in baseline participant characteristics of the treatment groups were tested using a one-way ANOVA for continuous variables and χ^2 analyses for categorical variables. Plasma and red blood cell folate concentrations were log-transformed to better fit a normal distribution; group results are presented as geometric means (95% confidence intervals). The differences in folate concentration between the treatment groups at each time point were determined by regression analysis, controlling for baseline values. Indicator (ie binary) variables were used in the regression analysis to model the three treatment groups. The regression coefficient is the difference in the log-transformed folate concentrations between two treatment groups at a given time point; the antilog of the coefficient is the ratio of the folate concentrations in the two groups. We multiplied this ratio by the geometric mean of the folate concentrations in the placebo group to calculate the baseline-adjusted folate concentration in the folic acid supplement groups. The percentage of participants in each intervention group achieving a red blood cell folate concentration greater than 905 nmol/l was compared using a χ^2 test. Statistical analyses were performed using Version 10 of SPSS for Macintosh software (SPSS Inc, Chicago, USA). The trend for linearity of red blood cell folate concentration across the time points in the weekly and daily folic acid supplement groups was tested using the Curvefit command in SPSS. Pearson's correlation coefficients were calculated to describe the relation between red blood cell folate concentrations at weeks 0 and 12. When designing the study we calculated that 40 women were needed in each group to detect a 10% difference in red blood cell folate concentrations between treatments with a power of 80% and an alpha of 0.05.

Results

Participant flow and follow-up

In all, 279 women attended a screening clinic for assessment of red blood cell folate status (Figure 1). Laboratory measures were obtained for 255 of these women, 205 (80%) of whom had a red blood cell folate concentration between 295 and 905 nmol/l. Of these, 138 women with the lowest red blood cell folate concentrations in this range, who were available and willing to begin the intervention, were randomized to the treatment groups. In all, 125 women completed the trial, and 13 withdrew from the study due to time constraints. No adverse side effects were reported from the women who withdrew or from those who completed the study. In all, 11 women with missing plasma and red blood cell folate data at week 6 or 12 were excluded from the final statistical analysis. Exclusion of these women made little difference to the magnitude of the treatment effects. For simplicity of the statistical analysis and presentation of the results in tables, we only included women for whom a complete set of blood results was available. Compliance was not a criteria for exclusion. The final statistical analysis included 114 partici-

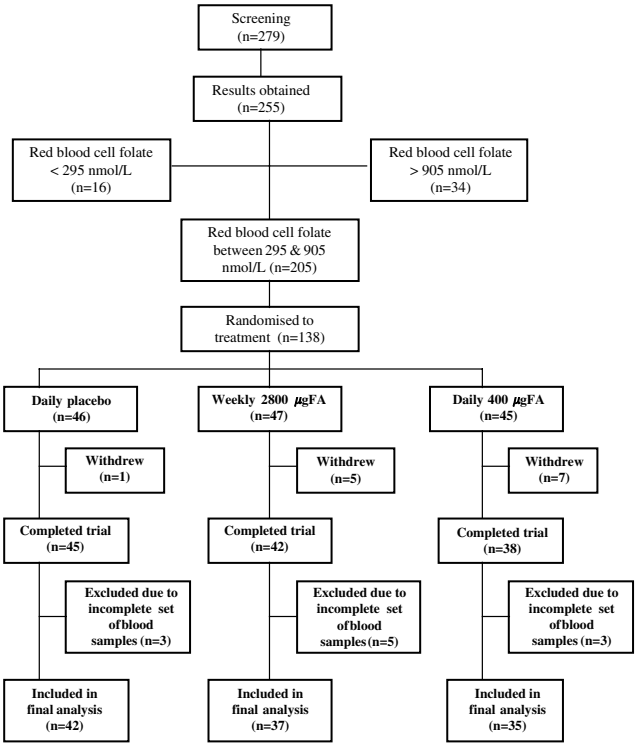

Figure 1 Participant flow and follow-up. Screening of all participants was completed before randomization.

pants: 42 in the placebo group, 37 in the weekly group, and 35 in the daily group.

Analysis

The majority of participants were nonsmoking (90%), young adult women (81% < 30 y) with no previous pregnancy (88%). Participant characteristics of the three treatment groups were not different (Table 1). Dietary intakes of folate and alcohol were not significantly different between the groups.

Plasma and red blood cell folate concentrations at baseline were not different in all the three groups (Table 2). Ingesting the weekly 2800 μg or the daily 400 μg folic acid supplement caused plasma folate concentrations to rise markedly so that by week 12 the mean (95%CI) concentrations were 62% (47–95) and 142% (109–179) higher, respectively, than in the placebo group. Red blood cell folate concentrations in participants ingesting the weekly and daily folic acid supplements were 39% (28–52) and 60% (47–75) higher, respectively, at week 12 than in those taking the placebo.

The rise in red blood cell folate concentrations in the folic acid supplement groups was linear ($P<0.001$) across weeks 0, 6, and 12 and did not plateau. Daily supplement users achieved a mean red blood cell folate concentration at week 12, adjusted for baseline values, that was 15% (5–26) or 137 nmol/l (47–237) higher than in weekly supplement users. The change in plasma folate concentration was not linear in the daily and weekly supplement groups; the increase was larger between weeks 0 and 6 than between

Table 1 Characteristics of study participants in each treatment group[a]

	Placebo (n=42)	*Weekly 2800 μg (n=37)*	*Daily 400 μg (n=35)*
Baseline characteristic			
Age (y)	24.7 (22.5–26.8)	22.9 (21.4–24.4)	24.7 (22.5–26.9)
BMI (kg/m^2)	24.0 (22.8–25.2)	24.1 (21.8–26.4)	23.7 (22.4–25.0)
OCA[b] users, *n* (%)	18 (43)	14 (40)	16 (47)
Smokers, *n* (%)	4 (10)	2 (5)	5 (14)
Previously pregnant, *n* (%)	8 (19)	4 (11)	2 (6)
Recent antibiotic users, *n* (%)	3 (7)	1 (3)	1 (3)
Serum vitamin B_{12} (pmol/l)	236 (205–271)	252 (215–296)	241 (183–317)
Daily dietary intakes during the study			
Energy (MJ)	7.7 (7.0–8.3)	7.9 (7.2–8.5)	7.4 (6.7–8.1)
Folate (μg)[c]	249 (219–283)	247 (212–288)	209 (184–236)
Vitamin B_{12} (μg)	3.0 (2.6–3.5)	2.7 (2.4–3.1)	2.9 (2.3–3.5)
Alcohol (g)[c]	3.8 (1.6–6.2)	3.0 (1.1–5.1)	2.6 (0.5–5.1)
% Compliance to treatment[e]	93 (90–96)	98 (95–99)[d]	98 (96–99)[d]

[a]Values are means (95% CI) unless otherwise stated.
[b]OCA, oral contraceptive agent.
[c]Exponent of log transformed number (ie geometric mean).
[d]$P<0.05$ compared to placebo group, χ^2 test.
[e]% of prescribed pills taken.

Table 2 Effect of daily or once-a-week folic acid supplementation on plasma and red blood cell folate concentrations in women of child-bearing age[a]

	Baseline	*Week 6*	*Week 12*	*Difference between treatment and placebo at Week 12*
Plasma folate		nmol/l		
Placebo	21 (19–24)	19 (17–22)*	19 (17 – 21)*	
Weekly 2800 μg	20 (17–24)[a]	28 (25–32)[b,†]	32 (28–36)[b,†]	13 (9–18)*
Daily 400 μg	18 (16–21)[a]	38 (32–43)[b,‡]	44 (39–49)[b,‡]	27 (21–34)[†]
Red blood cell folate		nmol/l		
Placebo	637 (583–695)	638 (593–686)*	663 (610–720)*	
Weekly 2800 μg	608 (553–668)[a]	747 (685–816)[b,†]	900 (828–978)[c,†]	265 (192–345)*
Daily 400 μg	615 (560–677)[a]	863 (797–935)[b,‡]	1053 (957–1158)[c,‡]	411 (325–504)[†]

[a]Values are geometric means (95% CI); placebo group, *n*=42; weekly folic acid group, *n*=37; daily folic acid group, *n*=35.
*,†,‡Within a column, means not sharing a common symbol superscript are significantly different at $P<0.001$. Differences between treatment groups were determined by regression analysis adjusting for baseline values.
[a,b,c]Within a row, means not sharing a common letter superscript are significantly different at $P<0.01$. Differences were determined by regression analysis.

Table 3 Effect of daily or once-a-week folic acid supplementation on the number of women with red blood cell folate concentrations greater than 905 nmol/l

	n	*Baseline*	*Week 6*	*Week 12*
			Number (%)	
Placebo	42	3 (7)	1 (2)*	6 (14)*
Weekly 2800 μg	37	3 (8)	7 (19)†	18 (49)†
Daily 400 μg	35	2 (6)	8 (23)‡	26 (74)‡

*,†,‡Within a column, means not sharing a common symbol are significantly different at $P<0.05$, χ^2 test.

weeks 6 and 12. Mean plasma folate concentration at week 12, adjusted for baseline values, was 46% (26–70) or 14 nmol/l (8–22) higher in daily than weekly supplement users. Half of the women taking the weekly folic acid supplement achieved a red blood cell folate concentration greater than 905 nmol/l at week 12, but this was significantly less than the three quarters who reached 905 nmol/l in the daily supplement group (Table 3).

Participants in the daily and weekly supplement groups were divided into tertiles of baseline red blood cell folate concentrations. Red blood cell folate concentrations rose by 313, 263, and 312 nmol/l in the tertiles of lowest to highest baseline red blood cell folate in the weekly supplement group and by 462, 412, and 482 nmol/l in the corresponding tertiles in the daily group. The correlation between red blood cell folate concentrations at weeks 0 and 12 was 0.76 ($P<0.01$) in the weekly group, and 0.72 ($P<0.01$) in the daily group.

Discussion

Public health authorities in many countries recommend that women of child-bearing age take a daily 400 μg folic acid supplement to reduce the risk of having a child with a neural tube defect (Cornel *et al*, 1997; Skeaff & Mann, 1998). Surveys of women, however, indicate that only a small minority follow this advice (Anonymous, 1999; Schader & Corwin, 1999). For this reason the United States mandated that 140 μg of folic acid be added to every 100 g of all flour products and estimated that folic acid intakes would increase by approximately 100 μg per day in the target population (Anonymous, 1996). Recent evidence indicates that women are consuming almost twice as much as estimated (Choumenkovitch *et al*, 2002).

Authorities in the United States continue to recommend the use of folic acid supplements because mandatory fortification does not provide the majority of women with the additional 400 μg of folic acid per day recommended to prevent folate-responsive neural tube defects. Nevertheless, the incidence of neural tube defects has declined in the United States as folate status has improved (Honein *et al*, 2001). This supports the observations of Daly *et al* (1995), who reported that red blood cell folate concentrations in early pregnancy were inversely correlated with a woman's risk of having a child with a neural tube defect; the lowest category of risk associated with concentrations above 905 nmol/l.

The results of our study demonstrate that a once-a-week 2800 μg folic acid supplement is a strategy that women can adopt to increase their folate status, although it is less effective than a daily 400 μg supplement. Almost 50% of women who took the weekly supplement achieved a red blood cell folate concentration greater than 905 nmol/l. This percentage, though substantial, was significantly less than in the daily 400 μg supplement group where 74% of women exceeded 905 nmol/l. The increase in red blood cell folate concentrations in both folic acid groups during the intervention was linear and had not reached a plateau by 12 weeks, therefore, it is possible that a higher proportion of women in both groups would have exceeded 905 nmol/l with continued use of the folic acid supplements. The length of time to reach a steady state of red blood cell folate after initiating supplementation is not known (Schorah *et al*, 1998). Women in our study achieved a mean red blood cell folate concentration of 1053 nmol/l after 12 weeks of ingesting the daily 400 μg folic acid supplement, whereas, Daly *et al* (1997) reported that 6 months of taking the same daily dose increased concentrations to 1290 nmol/l in women with similar initial folate status. There was a nonlinear increase in plasma folate concentrations during the 12-week intervention indicating that plasma is a shorter-term index of folate status than red blood cells.

Only 23% of women taking the daily folic acid supplement had a red blood cell folate concentration that exceeded 905 nmol/l after 6 weeks. This finding has important public health implications for advising women planning a pregnancy because it indicates that most women need to start taking folic acid longer than 4 weeks before pregnancy—the length recommended in many countries (Cornel *et al*, 1997)—to enter the conceptional period with blood folate at concentrations associated with the lowest category of risk of having a child with a neural tube defect. In trials that established the relationship between periconceptional folic acid supplementation and neural tube defects, women were asked to take the supplements from the time of enrolment. A woman was typically considered compliant if she consumed the supplement at least 4 weeks prior to conception and through to the end of the first trimester (MRC Vitamin Study Research Group, 1991; Czeizel & Dudas, 1992; Berry *et al*, 1999). Many women in these studies would have taken supplements for much longer than 1 month before becoming pregnant. In the Hungarian Trial of Czeizel *et al* (1996), for example, the average duration of supplement use before becoming pregnant was 3.8 months.

We need to emphasize that it is not clear that attaining a red blood cell folate concentration of 905 nmol/l represents a threshold for the maximum prevention of neural tube defects. A red blood cell folate concentration of 905 nmol/l

was the, somewhat arbitrary, lower bound of the upper quintile of red blood cell folate in the Irish case–control study. The authors of this report stress that the model for the relationship between red blood cell folate and NTD risk is continuous (Daly *et al*, 1995). Thus, a red blood cell folate concentration greater than 905 nmol/l may confer additional benefit. Nevertheless, most women in the population do not have red blood cell folate concentrations above 905 nmol/l (Wright *et al*, 1988), consequently, any strategy that increases folic acid intakes to above that concentration should reduce the NTD rate.

Fortifying foods with folic acid and encouraging the regular use of folic acid supplements by all women capable of becoming pregnant are advocated as the best strategies to reduce the primary occurrence of neural tube defects (Mills, 2000). Recent data from the United States and Canada indicate a 19% reduction in neural tube defect birth prevalence in the US and 38% reduction (prevalence among informed pregnancies) in Canada following implementation of mandatory folic acid fortification in 1998 (Honein *et al*, 2001; Ray *et al*, 2002). This reduction, attributed to fortification, is less than the 70% reduction attainable through universal use of folic acid supplements (Smithells *et al*, 1983; MRC Vitamin Study Research Group, 1991; Czeizel & Dudas, 1992; Berry *et al*, 1999), although some of this difference may be the result of the lower birth prevalence of NTDs in the United States than in the supplementation trial populations. Nevertheless, in women planning a pregnancy, taking a daily folic acid supplement remains an important preventive strategy. However, only 50% of pregnancies are planned (Forrest, 1994) and it is unlikely that most women will take a daily folic acid supplement throughout their reproductive years. In this regard, use of a once-a-week supplement may be an option for women not planning a pregnancy or for whom daily supplement use is difficult.

The immediate question that follows from the results of our trial is whether women will be more likely to take a weekly than a daily folic acid supplement. However, this question would be irrelevant without having demonstrated first that a once-a-week folic acid supplement can achieve a clinically meaningful increase in folate status of compliant women. Compliance with daily and weekly supplements was high in our study because women had volunteered for the study and were encouraged throughout to take the supplements; therefore, we cannot comment on whether women in a community setting would be more compliant to a once-a-week or daily regimen. In New Zealand, 52% of people who use dietary supplements use them less than daily. As there is an established pattern of using dietary supplements episodically, it seems reasonable to expect that a once-a-week high-dose folic acid supplement might be readily accepted by some. The practice of using a once-a-week 2800 μg supplement should be safe because the average dose is well below the Tolerable Upper Intake Level of 1000 μg/day set by the Food and Nutrition Board (1998) of the Institute of Medicine on the basis of its review of the evidence of possible adverse effects of folic acid.

In conclusion, a once-a-week high-dose folic acid supplement taken for 12 weeks can increase red blood cell folate to concentrations associated with a low risk of neural tube defects and offers women an additional strategy to prevent adverse pregnancy outcomes. Daily use of 400 μg of folic acid remains the best-proven practice to prevent neural tube defects.

Acknowledgements

We thank Sean O'Broin for advice on the microbiological assay; Margaret Waldron for blood collection; Steven Tzisavari, Ashley Duncan and Christian Thoma for their support with the laboratory analysis; Joanne Mackenzie for statistical advice.

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