2018

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Funder: Safefood

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Journal of Public Health | Vol. 40, No. 4, pp. 827–834 | doi:10.1093/pubmed/fdx137 | Advance Access Publication October 20, 2017

Optimization of folic acid supplementation in the prevention of neural tube defects

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ABSTRACT

Background We examined the relationship between timing and duration of folic acid (FA) supplementation in achieving red blood cell (RBC) folate levels in early pregnancy which are optimal (>906 nmol/l) for the prevention of neural tube defects (NTDs).

Methods Clinical, FA supplementation and dietary folate details were computerized at the first antenatal visit. Maternal blood samples were analysed for RBC and serum folate.

Results Of the 502 women, 98.2% (n = 493) reported taking FA. There was a positive correlation between duration of supplementation and both RBC folate (r = 0.43, P < 0.001) and serum folate (rho = 0.29, P < 0.001). The optimal RBC folate level was achieved in 80.4% (n = 46) of women who started FA 400 μg 4–8 weeks before their LMP compared with only 53.6% (n = 153) in women who started 4–8 weeks after their LMP (P < 0.001).

Conclusions This study provides, for the first time, information on both the timing and duration of FA that will achieve the optimum RBC folate levels associated with the prevention of NTDs. Women who are taking FA (400 μg) need to start before they conceive.

Keywords dietary folate, food fortification, neural tube defects, folic acid supplements, red blood cell folate, serum folate

Introduction

Neural tube defects (NTDs) are a group of serious congenital neurodevelopmental malformations which carry a heavy burden of illness.[1,2] Evidence that they may be prevented by folic acid (FA) comes from two landmark randomized controlled trials (RCT).[3,4] Subsequently, national guidelines published after 1992 recommended periconceptional FA supplementation to prevent both the occurrence and the recurrence of NTDs.[5,6] This supplementation strategy has, however, made little impact on NTD rates worldwide.[1,7] Thus, since 1998 a growing number of countries have implemented a policy of mandatory food fortification with FA.[2,7] One meta-analysis estimated that mandatory FA fortification halves the incidence of NTDs nationally.[8]

In 2006, the Food Safety Authority of Ireland (FSAI) recommended mandatory fortification of bread with FA.[9,10] However, 2 years later the FSAI recommended deferral because an unpublished survey suggested NTD rates were falling nationally, voluntary folate fortification levels had increased and there were theoretical concerns about the impact of fortification on rates of colonic cancer.[9,10] Recent European studies found that the levels of voluntary food fortification with FA have declined and that the prevalence of NTDs is not decreasing.[1,7,11] This led to an updated report from the FSAI in 2016 recommending mandatory

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fortification as an option for preventing NTDs.[12] There have also been renewed calls in the UK for mandatory food fortification with folate.[13]

In this study we examined the relationship between the timing and duration of FA supplementation on both maternal serum and RBC folate levels amongst women presenting for antenatal care in early pregnancy, in a country where food FA fortification remains voluntary.

Methods

The Hospital is one of the largest maternity hospitals in Europe and cares for women from all socioeconomic groups and from across the urban–rural divide. Women were recruited at their convenience at their first antenatal visit between June 2014 and March 2016 after sonographic confirmation of an ongoing singleton pregnancy. Exclusion criteria were multiple pregnancies, a history of a previous NTD or an inability to understand English. Informed written consent was obtained. Women's clinical and sociodemographic details were computerized routinely and updated immediately after delivery.

Once consent was obtained, at the time of taking the routine first visit blood tests, two additional samples were taken. These samples were transported in a cool box within 4 h of extraction for separation, storage at −70°C and analysis.[14] On receipt of the 2.7 ml serum tube in the laboratory, this tube was left to sit for ~30 min, to allow the clot to settle. The clot was removed from the tube and the tube recapped. This was centrifuged at 2200 revolutions per minute (rpm) for 10 min, and the serum was drawn off and stored in duplicate.

The 7.5 ml EDTA tube was placed on the blood mixer for 10 min. Haematocrit (Hct) was measured on the Hawksley Micro-Haematocrit Reader.

Red blood cell folate

A 1% solution of ascorbic acid was freshly prepared and 900 µl of this solution was aliquoted into each of two labelled 2 ml plastic tubes. The whole-blood was mixed carefully. A 100 µl of this whole-blood was added to each of the tubes containing the 900 µl of 1% ascorbic acid solution. The contents of these tubes were mixed well by pipetting up and down. The whole-blood/ascorbic acid mixture was left at room temperature for 30 min so that endogenous serum folate conjugase could convert folate polyglutamates released from the lysed red cells to the assayable monoglutamate form. These ascorbic acid lysates were then stored at −40°C pending further analysis.

Separation of serum

The blood in the clotting blood tubes was centrifuged to yield a pellet and a clear supernatant. The centrifuge may or may not be refrigerated. The separated serum was aspirated carefully from the packed cells and aliquoted into duplicate labelled plastic tubes.

Red blood cell and serum folate microbiological assays

Appropriately diluted serum and whole-blood haemolysate was then added to an assay medium containing *Lactobacillus casei* in addition to all nutrients needed for the growth of *L. casei* except folate. This method has been previously described in detail and is the recognized gold-standard for serum and RBC folate estimation.[14] This inoculated medium was incubated for 42 h at 37°C, and total folate was then assessed by measuring the turbidity of the inoculated medium at 590 nmol/l in a microplate reader.

For the assessment of RBC folate, the measured whole-blood value is related to the measured Hct according to the following equation:

$$\text{RBC folate(nmol/l)} = (\text{Whole blood hemolysate folate} * 10) - \text{Serum folate}(1 - \text{Hct}/100)/\text{Hct}/100$$

A detailed questionnaire about all supplement intakes was completed under the supervision of a research dietitian (S.C.). Daily compliance with FA supplement use was assessed by asking the woman how many days per week she took a FA supplement and also how many tablets per day she consumed. To collect estimated habitual food intakes for analysis of total dietary folate (natural folate and synthetic FA from fortified foods) women were asked to complete a retrospective 4-day diet history (DH) under supervision. This was combined with a customized food frequency questionnaire (FFQ) to capture the brand names of foods fortified with FA because in Ireland, FA is added to food on a voluntary basis and the amount added varies according to the brand. This method of dietary data collection has previously been validated for the collection and calculation of dietary folate intake.[15] Assessment of energy under- and over-reporting was calculated using standard equations for females.[16,17]

Quantitative food intake data from the 4-day DH was divided by four to get an average daily intake for each food listed. These data were entered into Nutritics Version 3.7 to convert the reported food intakes into nutrient intakes derived from these foods.[18] Food recipes for foods not included in the existing databases of Nutritics (e.g. branded foods which have been fortified with FA such as breads, spreads, cereals,

cereal bars and fortified milks) were manually generated to accurately capture nutrient intakes. Information on socio-economic status was assessed using questions from the EU Survey on Income and Living Conditions.[19]

Data from all questionnaires were anonymised and coded on a Microsoft Excel® spreadsheet and statistical analysis was conducted using SPSS for Windows, Version 20 (IBM Corporation, Armonk, New York). The distribution of continuous data was assessed for normality. Descriptive statistics were first used to characterize the study cohort. One-way ANOVA and independent-samples *t*-tests were used to compare mean RBC folate levels according to the time of FA supplement initiation, as these data were normally distributed. Inferential Chi-square tests for independence were then used to analyse differences in categorical variables (e.g. achieving optimal RBC folate level for NTD prevention) between different population groups.

Kruskal–Wallis and Mann–Whitney *U* tests were used to compare median dietary folate intakes and median serum folate levels according to time of FA supplement initiation as data from these variables were non-normally distributed. Binary logistic regression analysis was finally performed to assess the independent associations between a number of putative predictor variables and the likelihood of supplementing with FA prior to pregnancy. Factors were included in the multivariate model based on a statistically significant finding from previous mutually adjusted univariate analyses examining preconceptional FA supplementation ($P < 0.05$).

The time interval 4–8 weeks after the first day of the last menstrual period (LMP) has been used as the reference for comparison with other time intervals for FA supplement commencement. This interval includes the time around 5–6 weeks amenorrhoea when pregnancy is usually confirmed by a positive urinary human chorionic gonadotropin (HCG) test. Also, in this interval, women who are not taking FA supplements before pregnancy usually start FA and the neural tube closes (Supplementary Figure 1).

Results

All 502 women had their serum and RBC folate levels measured. Of these, 458 women completed the detailed FA supplement questionnaire. For the remaining 44 (8.8%) women who did not have time to complete this questionnaire, additional information on FA supplementation was obtained from their medical records. These data described the dose of FA used and whether FA supplementation had started before pregnancy or not. Of the 458 women who completed the detailed FA supplement questionnaire, only 437 reported the time of initiation of FA.

Of the 502 women, 493 (98.2%) reported taking FA during the pregnancy. Of the 493 women, 447 women reported on their compliance with 93.9% reporting daily intake of FA. Of the women who knew the dose or brand of FA that they were taking at presentation ($n = 440$), 1.1% ($n = 5$) were taking <400 μg FA/day, 91.8% ($n = 404$) were taking 400 μg FA/day and 7.0% ($n = 31$) were taking >400 μg FA/day.

The characteristics of the study population are shown in Table 1 and were similar to the hospital population.[20] None of the pregnancies were complicated by a NTD. The women who initiated FA supplementation before pregnancy were compared with those who did not using univariate analysis (Table 1). Binary logistic regression analysis was applied to adjust for the other putative predictors of prepregnancy FA supplementation which had emerged on univariate analyses (age, pregnancy intention, marital status, smoking status and third level education (defined as third level non-degree qualification (e.g. diploma) or higher)). Those who planned their pregnancy and who had third level education were more likely to take FA before their LMP (odds ratio (OR) = 9.1 (95% CI: 5.2–15.6); $P < 0.001$ and OR = 2.3 (95% CI: 1.4–3.8); $P < 0.001$, respectively) in these multivariate analyses. Those who were self-reported smokers were less likely to take FA before their LMP (OR = 0.18 (95% CI: 0.1–0.5); $P < 0.001$) on multivariate analysis. The results of the multivariate analysis is now shown in greater detail within Supplementary Table 1.

Table 2 shows the timing of FA supplementation initiation in relation to the LMP in bands of 4 weeks. Of the 458 women who completed the detailed FA questionnaire, 437 provided details about when they initiated FA supplement use. The majority (56%) of women did not initiate FA until after their LMP (Supplementary Figures 2 and 3). Initiation after the LMP was clustered in the 4–8 weeks interval. Supplementary Figure 4 shows timing of women's initiation of FA ($n = 437$) according to whether they had planned their pregnancy; 89% ($n = 170$) of women who started FA before their LMP ($n = 192$) had planned their pregnancy compared with 43.2% ($n = 106$) who started after their LMP ($n = 245$) ($P < 0.001$). It is clear from Supplementary Figure 5 that the percentage of unplanned pregnancy increased moving from left to right on the bar chart.

Supplementary Figure 1 shows when women started FA during the pregnancy.

In Tables 3 and 4, the time interval 4–8 weeks after the first day of the LMP has been used as the reference for comparison with other time intervals for FA supplement commencement (In this interval the majority of women who are not taking FA before the pregnancy start to take FA (Supplementary Table 1).

Table 1 Univariate analysis of sociodemographic characteristics of participants analysed according to whether they initiated folic acid (FA) supplementation before or after their last menstrual period (LMP) (n = 502)

	Total (n = *502)*	*Prepregnancy supplementation*[a] (n = *215)*	*Pregnancy supplementation*[b] (n = *287)*	P
Age [years; mean (SD)]	30.6 (5.5)	32.2 (4.3)	29.5 (6.0)	<0.001
Weight [kg; median (IQR)]	67.5 (17.40)	67.5 (17.2)	67.4 (18.3)	0.35
Body Mass Index [kg/m^2; median (IQR)]	24.7(5.9)	24.7 (5.6)	24.7 (6.2)	0.66
Obese [%; *n*]	19.3 (97)	20.0 (43)	18.8 (54)	0.83
Nulliparas [%; *n*]	42.6 (214)	44.7 (96)	41.1 (118)	0.48
Gestation at first visit [weeks; median (IQR)]	12.1 (1.6)	12.1 (1.4)	12.3 (1.6)	0.90
Planned Pregnancy [%; *n*]	62.7 (315)	87.9 (189)	43.9 (126)	<0.001
Married [%; *n*]	49.2 (247)	60.5 (130)	40.8 (117)	<0.001
Current smoker [%; *n*]	12.9 (65)	3.3 (7)	20.2 (58)	<0.001
Third level education[c]	60.1 (272)	77.1 (148)	47.7 (124)	<0.001
Consistent poverty [%; *n*][d]	4.2 (13)	2.1 (3)	6.0 (10)	0.17
History of infertility [%; *n*][e]	14.8 (74)	15.0 (32)	14.6 (42)	0.99
Living in the capital [%; *n*]	63.5 (319)	59.5 (128)	66.0 (191)	0.33
Irish nativity [%; *n*]	74.3 (373)	75.3 (162)	73.5 (211)	0.71

[a]Prepregnancy defined as before the LMP.

[b]Pregnancy supplementation defined as after the LMP.

[c]Data for n = 452 for whom third level education defined as third level non-degree qualification (e.g. diploma) or higher.

[d]Data for n = 308, consistent poverty defined as co-occurrence of relative income poverty and depriviation.[17]

[e]Data for n = 501 for those who reported a history of infertility at the antenatal booking visit.

Table 2 Timing of folic acid (FA) supplement initiation in relation to the first day of the last menstrual period (LMP)

	% (n)
≥12 Weeks before LMP	24.9 (109)
8–12 Weeks before LMP	6.2 (27)
4–8 Weeks before LMP	11.7 (51)
0–4 Weeks before LMP	1.1 (5)
0–4 Weeks after LMP	9.2 (40)
4–8 Weeks after LMP	38.2 (167)
≥8 Weeks after LMP	8.7 (38)
Total	100.0 (437)

Of the 502 women, overall the mean RBC folate level was 1137 (SD 443) nmol/l and median serum folate was 34.6 (IQR 59.5) nmol/l. Table 3 shows median serum folate and mean RBC folate for the 437 women who reported the time (in weeks) that they commenced FA relative to their LMP. Initiating FA supplementation at least four weeks before the LMP (6 weeks before conception approximately) was associated with an increased serum folate and an increased RBC folate compared with initiation 4–8 weeks after the LMP ($P < 0.05$ for all). When these findings were confined to women who took the recommended over-the-counter 400 µg FA (low risk women) (Supplementary Table 2), initiating FA supplementation at least 8 weeks before the LMP (or ~10 weeks before conception) was associated with an increased serum folate and an increased RBC folate compared with initiation 4–8 weeks after the LMP.

Table 4 shows the percentage of women with an optimal RBC folate for the prevention of NTDs (> 906 nmol/l).[21] Of the 437 women reporting the time of FA commencement, 65.7% (n = 330) overall had an optimal RBC folate level >906 nmol/l. Those who started FA ≥4 weeks before their LMP (86.4%) had a higher incidence of achieving optimal RBC folate compared to those who started FA 4–8 weeks after their LMP (53.3%) ($P < 0.001$). The findings were similar when this analysis was confined to the 394 women who took 400 µg FA (Supplementary Table 3).

Supplementary Figure 6 shows the positive correlation between mean RBC folate and the duration of FA supplementation in those women who reported taking 400 µg FA for ≤52 weeks (r = 0.43, $P < 0.001$). Supplementary Figure 7 shows the positive correlation between median plasma folate and the duration of FA supplementation in those women who reported taking 400 µg FA for ≤52 weeks (rho = 0.29, $P < 0.001$).

Of the 502 women who had blood taken, 392 completed the retrospective 4-day DH. The median dietary folate was

Table 3 RBC and serum folate (nmol/l) according to timing for all folic acid (FA) supplement initiation ($n = 437$)

Timing of initiation of FA	*RBC folate (mean (SD))*	n	P	*Serum folate (median (IQR)*	n	P
≥12 Weeks before LMP[a]	1431.1 (460.2)	109	<0.001	39.0 (14.9)	109	<0.001
8–12 Weeks before LMP	1463.4 (385.1)	27	<0.001	39.1 (16.7)	27	<0.001
4–8 Weeks before LMP	1199.7 (903.0)	51	0.03	31.0 (16.0)	51	0.04
0–4 Weeks before LMP	1075.4 (251.0)	5	0.99	36.1 (22.5)	5	0.12
0–4 Weeks after LMP	966.9 (387.6)	40	0.99	28.1 (17.2)	40	0.23
4–8 Weeks after LMP	998.4 (361.4)	167	Reference category[b]	31.0 (16.6)	167	Reference category[b]
≥8 Weeks after LMP	884.7 (368.5)	38	0.67	20.2 (22.9)	38	0.30

[a]Last menstrual period (LMP).

[b]Denotes that reference category (4–8 weeks after LMP) used for all comparisons. One-way ANOVA used to assess differences in mean RBC folate between groups; Kruskall–Wallis and Mann–Whitney *U* tests used to assess differences in median serum folate between groups.

Table 4 Incidence of optimal RBC folate according to timing of folic acid (FA) supplement initiation for all doses ($n = 437$)

Timing of FA initiation	n	*RBC folate > 906 nmol/l (%,* n*)*	P
≥12 Weeks before LMP[a]	109	88.1 (96)	<0.001
8–12 Weeks before LMP	27	92.6 (25)	<0.001
4–8 Weeks before LMP	51	78.4 (40)	<0.001
0–4 Weeks before LMP	5	80.0 (4)	Not analysed[b]
0–4 Weeks after LMP	40	50.0 (20)	0.71
4–8 Weeks after LMP	167	53.3 (89)	Reference category[c]
≥8 Weeks after LMP	38	39.5 (15)	0.12

[a]Last menstrual period (LMP).

[b]Numbers in 0–4 weeks before LMP category too small for statistical analysis.

[c]Denotes that reference category (4–8 weeks after LMP) used for all comparisons.

Cross-tabulation used to assess differences in the percentage of people who achieve optimal RBC folate.

235.2 (IQR 148.2) μg/d. Of these 392 women the median natural folate was 190.7 (93.4) μg/d, median fortified FA was 25.6 (79.3) μg/d and median dietary folate equivalents (DFE) was 255.1 (185.2) μg/d. The mean (SD) fortified FA is 56.5 (82.6) μg/d which equates to a contribution of 21.3% of the means (SD) total folate (265.9 (235.2)) and the mean (SD) natural folate is 209.4 (122.8) μg/d which equates to a contribution of 78.7% of the mean (SD) total folate value. Of these women, the percentage of women with a daily intake of <600 μg DFE, which is the reference nutrient intake (RNI) for folate in early pregnancy as recommended to promote foetal development by the WHO, was 93.9% ($n = 368$).[22]

There was a weak correlation between DFE and maternal serum folate (rho = 0.21, $P = <0.001$), and between DFE and RBC folate ($r = 0.14$, $P=0.006$).

There was no difference in DFE according to the time of FA initiation ($P > 0.05$ for all) (see Supplementary Tables 4 and 5).

Of these 392 women who competed the 4-day DH, 34.7% ($n = 136$) were classified as dietary energy under-reporters. These under-reporters were younger, more likely to be obese and to be unmarried (all $P < 0.05$) (Data not shown). Total dietary folate intake, including that derived from fortified foods, was higher amongst the plausible reporters compared with the under-reporters (259.0 (IQR 129) μg/d versus 178.0 (IQR 106) μg/d; P <0.001). However, after excluding under-reporters, 91.2% of women ($n = 235$) were still taking less than the 600 μg DFE recommended by the WHO for early pregnancy.[22]

Discussion

Main findings of this study

In a recent updated evidence report and systematic review for the United States Preventive Services Task Force on FA supplementation for the prevention of NTDs, limited

information was found on the timing of supplementation and no information on duration was identified for the prevention of NTDs.[23] While our study did not examine NTD as an outcome, it examined the optimal RBC folate for NTD prevention. Our study in early pregnancy found that maternal RBC folate correlated positively with the duration of FA supplementation and, as result, women who started FA at least 6 weeks before conception (4 weeks before LMP) were more likely to achieve the RBC folate concentration associated with optimal NTD prevention compared to those who started 4–8 weeks after the LMP.

In an analysis based on week of initiation of FA in relation to conception we found that women who started FA during pregnancy usually started supplementation just before or after neural tube closure and thus this supplementation, particularly if started after conception, was unlikely to be effective in NTD prevention. 'However, in our study, initiation of FA supplementation was clustered around 4–8 weeks after the LMP because it is usually during this time interval that women realize they are pregnant and that it is confirmed by a positive HCG test.[24],

It is a concern that this is the point at which the majority of women commence supplementation with FA given that just over half of women who commence FA at this time point achieve an optimal level of RBC folate for NTD prevention.

Using a detailed dietary questionnaire with up to date brand information on FA fortification, we also found that only 6.1% of women in early pregnancy were meeting the WHO recommended intake of >600 μg/d DFE with energy under-reporters included in the analysis; this figure rose to 9.1% when under-reporters were excluded from the analysis. Maternal dietary folate levels were inadequate in early pregnancy despite voluntary food fortification with FA. These findings help explain why the current supplementation and voluntary food fortification strategies have failed to improve rates of NTDs in Europe over the last 25 years.[1,7]

What is already known on this topic

In one study, 144 healthy non-pregnant women aged 19–33 years were given 400 μg FA daily for 24 weeks duration.[25] This study took place in Germany where FA food fortification is voluntary. After 12 weeks of supplementation, serum plasma plateaued but RBC folate had not plateaued by the end of 24 weeks. Serum and RBC folate were measured using the microbiological assay. The authors suggested that the minimum recommended duration of FA supplementation before conception should be increased from >4 to >11 weeks. The same group have published on the pharmacokinetics of FA supplementation in healthy women who were not pregnant.[26] In this study, they estimated that the biological half-life is eight weeks, and reported that the increase in RBC folate concentration depends on the dose of the supplement and on baseline RBC folate levels. In a further study of 46 healthy women who were not pregnant, a RBC folate threshold of >906 nmol/l was reached after an average of 4.2 weeks (SD 3.5) but this required a higher dose of 800 μg FA daily.[27] This duration is shorter than that observed in our study even when all doses (including doses >400 μg) are included in the analysis, but this may be explained by different baseline levels of RBC folate.

In a further RCT from Germany, 198 non-pregnant healthy women who were off supplementation for 2 months and who were recruited as volunteers, were allocated to receive either 400 or 800 μg/d of FA.[28] Obese women were excluded. An immunoassay was used to measure RBC folate. Amongst the full cohort, RBC folate at baseline was deficient in 6.1% and suboptimal in 88%. In this non-obese group, the percentage of women with suboptimal RBC folate was higher than that observed in our study. After 4 and 8 weeks supplementation, serum and RBC folate levels were higher in the 800 μg group. Notably, the number of women with an optimal RBC folate after 4 weeks was 46% in the 800 μg group compared with 31% in the 400 μg group ($P < 0.04$), and after 8 weeks was 84% compared with 55% ($P < 0.001$). The authors concluded that in the absence of mandatory folate food fortification, a supplement of 400 μg was insufficient to achieve optimal RBC folate levels within 4–8 weeks which is similar to results from our study which suggest FA supplementation of 400 μg is needed for 6 weeks before conception to achieve an optimal RBC folate level.

In a study of 226 women aged 18–35 years presenting for antenatal care at a mean of 13.7 weeks gestation; of those who had taken FA 400 μg/d supplementation in the first trimester, 19% had started before pregnancy.[15] This study took place in Northern Ireland where FA food fortification is also voluntary. The incidence of optimal RBC folate was 73% in women who started before pregnancy compared with 47% who started after the sixth week of pregnancy ($P < 0.001$). RBC folate was measured using the microbiological assay. These data were not analysed by weeks in assessing the duration of supplementation or its timing in relation to conception. Nonetheless, it is consistent with the findings of our study as women who took FA preconceptionally were more likely to have an optimal RBC folate compared to women who did not take FA preconceptionally.

Total dietary folate intakes observed in the present study were slightly lower than those observed in other studies

amongst pregnant women in Ireland. When under-reporters were included in the analysis, as was the case with data reported in 2011 from Northern Ireland, median total dietary folate in the present study was 235.2 (IQR 148.2) μg/d compared with 301 μg/d in the data from 2011.[15] It was also lower than the median total dietary folate of 337 μg/d reported at our centre amongst plausible dietary energy reporters.[29] These results may be explained by differences in dietary assessment methods. Furthermore, given that a recent study has observed that voluntary FA fortification has declined over recent years it is possible that these results are reflective of declining levels of FA fortification in Ireland, given that total dietary folate intakes observed in the present study were slightly lower than those observed in previous studies.[11]

What this study adds

To our knowledge, for the first time this study provides detailed information on the dose and the duration of FA supplementation in pregnancy, with the latter expressed in weeks which can be timed relative to neural tube closure following a dating ultrasound. While other studies have examined how the timing of FA initiation affects RBC folate, these previous studies have not provided this detailed (by week of initiation of FA in relation to the LMP) breakdown of how time of initiation and dose of FA influences RBC folate levels.[15]. In addition, up to date and detailed information on natural folate and fortified FA, DFE and total dietary folate, and on maternal folate measurements were recorded simultaneously in the same cohort of women in early pregnancy. These data were augmented by clinical and sociodemographic information which were used to enhance the strength and integrity of our inferential multivariate analyses.[15]

Limitations of this study

The study was undertaken in a country with voluntary FA food fortification, thus the implications of the findings for countries with mandatory FA fortification require further study. Participants were recruited at their convenience because it was not feasible with limited resources to collect detailed clinical, dietary and supplement details in women presenting consecutively for antenatal care in a busy maternity hospital. Random selection was also not feasible given the limited resources.

Maternal serum folate levels are possibly more important for the closure of the neural tube than RBC folate which reflects storage of folate. There was also a positive relationship between the duration of FA supplementation and serum folate, however, a cut-off for optimal serum folate concentration for prevention of NTDs has not been established.

Conclusions

Based on our findings, we believe that national and international guidelines need to highlight the importance of women starting FA at least 6 weeks before conception (4 weeks before LMP). Women planning a pregnancy should be advised to start FA at least 6 weeks before trying to conceive if they are to optimize their RBC folate levels. As half of pregnancies are unplanned, our findings confirm that women who could potentially become pregnant should take a daily supplement of FA. Finally, the low total dietary folate intake observed in this study despite voluntary FA food fortification strengthens the need to implement mandatory FA fortification in European countries.

Acknowledgements

We thank the women who participated and our laboratory and midwifery colleagues, particularly Ruth Harley and Muireann Ní Mhurchu from the Hospital's Biobank, for their assistance.

Funding

This work was supported by *Safe*food, an all-island body funded under the Good Friday Agreement between the governments of the Republic of Ireland and the United Kingdom, Grant number [02–2015]. The researchers are independent from funders.

Conflicts of interest statement

The authors declare no conflict of interest.

Supplementary data

Supplementary data are available at the *Journal of Public Health* online.

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