


BMJ Open Effect of once weekly folic acid supplementation on erythrocyte folate concentrations in women to determine potential to prevent neural tube defects: a randomised controlled dose-finding trial in Malaysia

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ABSTRACT

Introduction Folic acid (0.4 mg) taken prior to and during early pregnancy reduces the risk of neural tube defects (NTDs). Because these birth defects occur early in pregnancy, before women may know they are pregnant, many countries have mandated the addition of folic acid to food staples. In countries where fortification is not possible, and weekly iron folic acid programmes exist to reduce anaemia, the WHO recommends that 2.8 mg (7×0.4 mg) folic acid be given instead of the current weekly practice of 0.4 mg. Currently, there is a lack of evidence to support if the 2.8 mg folic acid per week dose is sufficient to raise erythrocyte folate concentrations to a level associated with a reduced risk of a NTD-affected pregnancy. We aim to conduct a three-arm randomised controlled trial to determine the effect of weekly folic acid with iron on erythrocyte folate, a biomarker of NTD risk.

Methods and analysis We will recruit non-pregnant women (n=300; 18–45 years) from Selangor, Malaysia. Women will be randomised to receive either 2.8, 0.4 or 0.0 (placebo) mg folic acid with 60 mg iron weekly for 16 weeks, followed by a 4-week washout period. The primary outcome will be erythrocyte folate concentration at 16 weeks and the mean concentration will be compared between randomised treatment groups (intention-to-treat) using a linear regression model adjusting for the baseline measure.

Ethics and dissemination Ethical approval was obtained from the University of British Columbia (H18-00768) and Universiti Putra Malaysia (JKEUPM-2018-255). The results of this trial will be presented at scientific conferences and published in peer-reviewed journals.

Trial registration numbers ACTRN12619000818134 and NMR-19-119-45736.

INTRODUCTION

In 2015, an estimated 260 000 infants were born globally with a neural tube defect

Strengths and limitations of this study

- This is the first study to determine the optimal dose of folic acid to be included in weekly iron folic acid supplements to reduce neural tube defect (NTD) risk.
- Women of reproductive age will be randomised to a weekly iron (60 mg) supplement with either 0.0 mg, 0.4 mg (current practice) or 2.8 mg (WHO recommendation) folic acid.
- The primary outcome is erythrocyte folate, a biomarker of NTD risk, concentration after 16 weeks by treatment group.
- Erythrocyte folate is inversely associated with NTD risk.
- NTD as an outcome would not be ethical or feasible.

(NTD).¹ NTDs, such as anencephaly and spina bifida, are caused by the failure of the neural tube to close properly around 28 days postconception and are a significant cause of mortality and morbidity. Evidence from controlled trials has shown that up to 80% of NTDs could be prevented if women were to take folic acid supplements prior to and during early pregnancy.^{2–4} As such, the WHO recommends that women planning to become pregnant take 0.4 mg folic acid per day.⁵ Because many pregnancies are unplanned and the neural tube closes early in pregnancy,^{6,7} before women may know they are pregnant, many countries have mandated the addition of folic acid to wheat flour or other grain staples.⁸ This has led to a reduction of NTDs in these countries.^{9,10} In many countries where wheat flour is not the staple, or where milling of staple grains is at the



village or household level, fortification is difficult.¹¹ Alternative strategies are needed to deliver folic acid to women of reproductive age.

In countries where the prevalence of anaemia is $\geq 20\%$, the WHO recommends blanket intermittent supplementation of all women of reproductive age with weekly iron (60 mg) and folic acid (2.8 mg). The dose of folic acid was chosen as it is seven times the 0.4 mg daily dose found to be effective in reducing NTDs in controlled trials.⁴ However, there is a reluctance to change the dose of folic acid to 2.8 mg weekly for non-pregnant women due to the lack of evidence that this higher dose will reduce NTDs. Further, the global prevalence of anaemia due to folate deficiency is low and there have been calls to remove folic acid from these supplements altogether.¹²

Further randomised trials with NTDs as an outcome would be unethical. However, erythrocyte folate in women of reproductive age is accepted as a proxy indicator of NTD risk. In a case control study from Dublin, Ireland, erythrocyte folate measured early in pregnancy was inversely associated with NTD risk, with an erythrocyte folate >906 nmol/L considered desirable for maximal prevention.¹³ There are no studies which compare the current effect of weekly 0.4 mg folic acid, the dose currently contained in most weekly supplements on the market, with the recommended weekly 2.8 mg folic acid on erythrocyte folate concentration in non-pregnant women of reproductive age.

The primary aim of this randomised placebo-controlled trial is to compare the effect of the WHO recommended weekly folic acid dose of 2.8 mg versus the current weekly 0.4 mg folic acid practice or no folic acid (placebo) on erythrocyte folate concentrations after 16 weeks of treatment. Weekly iron folic acid programmes are often started when women are in secondary school.¹⁴ Women in school have several periods of school break during the year. Thus, we will include a 4-week washout period to determine the effect that a school break would have on erythrocyte folate concentrations. This research is needed by policy makers across the globe and will inform WHO guidelines on the optimal weekly dose of folic acid, if any, needed for NTD prevention.

METHODS AND ANALYSIS

Trial design

This is a three-arm, parallel-group, randomised controlled trial with a 16-week intervention period followed by a 4-week washout period. Participants will attend three clinic visits: baseline (0 weeks), endline (16 weeks) and washout (20 weeks). This trial started in September 2019 and is projected to run until February 2020.

Location

This study will take place at Universiti Putra Malaysia in Selangor, Malaysia. Malaysia was chosen for the study location as there is significant technical infrastructure in place and the overall prevalence of anaemia in Malaysia

in women of reproductive age is above 20%,^{15 16} falling within the criteria of the current WHO guideline for intermittent iron folic acid supplementation.¹⁴ Moreover, there is no folic acid fortification programme in Malaysia and folic acid supplement use is not widespread.¹⁷⁻¹⁹

Study population

A total of 300 women (18–45 years) from Selangor, Malaysia will be recruited.

Eligibility criteria

Potential participants will be approached by research staff at Universiti Putra Malaysia.

Inclusion criteria

Women must meet the following criteria to be enrolled in the study:

- ▶ Be between the ages of 18 and 45 years.
- ▶ Not be pregnant.
- ▶ Plan on living, working or studying near Universiti Putra Malaysia for 4 months following enrolment.
- ▶ Be able to give informed consent.

Exclusion criteria

Women must not have any of the following criteria to be enrolled in this study:

- ▶ Are pregnant (self-reported) or planning to become pregnant.
- ▶ Taking folic acid containing supplements.
- ▶ Participating in another nutritional intervention.
- ▶ Taking any medication known to inhibit folate status (methotrexate, certain anticonvulsants or sulphasalazine).

Study treatments

The supplements will be packaged in bottles containing 30 tablets. Each supplement will contain 60 mg of elemental iron as ferrous fumarate and either 0.0 mg, 0.4 mg or 2.8 mg of folic acid to be taken weekly. Supplements have been produced to US Pharmacopeia standards. Supplements have been manufactured by Unison Nutraceutical Sdn. Bhd. in Ayer Keroh, Malacca, Malaysia and approved by the National Pharmaceutical Regulatory Agency in Malaysia. Supplements will be dispensed in glass opaque bottles containing 30 tablets. The tablets, bottles and labels on the bottle will be identical except for a coloured sticker which identifies the different treatments.

Monitoring adherence to study treatment

Study staff will be responsible for storage and dispensing of the supplements. Supplements will be administered by study staff at the baseline clinic visit. Participants will be asked to take their first supplement at the clinic and then asked to take the supplement at the same time each week. Participants will be reminded weekly by short message service (SMS) to take their supplement to encourage adherence and asked to reply by SMS when they have taken the supplement. If they do not reply, research assistants will contact them by phone. If the participant fails

Table 1 Assessment time points

Visit (V)	Assessment time points			
	Screening	V1	V2	V3
Time per study site session				
Week		Week 0	Week 16	Week 20
Enrolment and randomisation				
Eligibility assessment	X			
Randomisation		X		
Implementation				
Questionnaire		X		
Blood collection		X	X	X
Adverse event reporting			X	X
Supplementation				
Tablet distribution		X		
Tablet count			X	

to take the supplement within the 5 days following their designated day, the supplement will not be taken, and the participant will be considered to have missed treatment that week. Adherence will be assessed by trained research staff by counting remaining tablets in the bottles at 16 weeks.

Outcome measures

The primary outcome will be erythrocyte folate concentration at 16 weeks.

Key secondary outcomes:

- ▶ Erythrocyte folate concentration at 20 weeks.
- ▶ Plasma folate concentration at 16 and 20 weeks.

Participant timeline

Screening period

Women who are interested in participating in the study will be given a participant's information sheet and research assistants will answer any questions they may have. Written informed consent will be obtained from each woman who meets the screening criteria and is willing to participate in the study. The assessment time points are summarised in [table 1](#).

Visit 1 (baseline)

Participants will be asked to attend a clinic at Universiti Putra Malaysia following an overnight fast (since midnight). After reaffirming consent, blood samples will be collected by venipuncture into two evacuated tubes containing EDTA as an anticoagulant. Sociodemographic, health and anthropometric data will be recorded by the researchers. Women with severe anaemia (defined as a haemoglobin concentration <80 g/L) will be contacted within 3 days and referred to a local health centre for follow-up, but will not be excluded from the study unless their medical practitioner recommends withdrawal.²⁰

Visit 2 (~16 weeks)

Participants will attend a second clinic visit following an overnight fast. The second visit will be scheduled with a +2-week allowance to accommodate participant schedules. Participants will be advised to take their last pill no less than 48 hours before the blood draw and up to 1 week beforehand. The blood sample will be collected as described in the baseline visit. Adverse events will be recorded, and adherence determined via counting the remaining tablets.

Visit 3 (~20 weeks)

Following 4 weeks (+2 weeks) of not taking any supplements, women will return for a fasting blood sample. Adverse events will be recorded.

Sample size

To detect a clinically meaningful difference of 100 nmol/L in mean erythrocyte folate concentrations across treatment groups at the end of the intervention period (16 weeks), a sample size of 63 participants per group is required, assuming a SD of 202 nmol/L with 80% power while adjusting for baseline erythrocyte folate concentration.²¹ A two-sided α of 0.0167 will be used for pairwise comparisons between the three treatment groups (0.0 mg, 0.4 mg and 2.8 mg) for an overall α of 0.05. The sample size assumes a correlation between erythrocyte folate concentrations at baseline and 16 weeks of 0.6 and allows for a dropout rate of 10%. We will recruit 100 participants per arm to allow for some uncertainty in the assumed values.

Recruitment

Potential participants will be approached by trained research staff and informed about the purpose of the study, the protocol and potential risks and benefits of participation. Trained research staff will gather informed

consent, voluntary and free from coercion, to ensure that information about the trial is understood.

Randomisation

Following confirmation of eligibility and enrolment, participants will be randomised after the first blood draw using a web-based randomisation service. Participants will be randomly assigned to receive 60 mg of iron as ferrous fumarate and either 0.0, 0.4 or 2.8 mg of folic acid weekly. A computer-generated randomisation schedule will be prepared using `ralloc.ado` in Stata by an independent statistician who is not involved with trial participants or data analysis. The randomisation procedure will use randomly permuted blocks of size six to assign participants to one of six colour codes in the ratio 1:1:1:1:1:1. Two colour codes will be assigned to each treatment by an independent individual who will be responsible for labelling the study products but have no further involvement in the trial.

Blinding

Blinding will only be broken during the trial in the event of an emergency, when the investigator deems that a participant cannot be adequately treated without knowledge of the participant's treatment arm. The principal investigator will be contacted. In order to break the blinding for the affected participant, the investigator must contact the randomisation personnel. All attempts to avoid study withdrawal will be made, although participants can cease treatment as appropriate. Trial arms will only be unblinded once all data have been collected and entered in the study database and analysis of the primary and secondary outcomes has been completed.

Data collection, access and storage

Up to seven research staff, as needed, will be on site to obtain informed consent, collect data, randomise and distribute tablets. All efforts to ensure data quality will be taken. Study data will be collected and managed using REDCap electronic data capture tools hosted at the South Australian Health and Medical Research Institute (SAHMRI).^{22,23} Eleven investigators and research staff will have access to the data during the data collection period. All co-investigators will have access to the data at all stages of analyses and interpretation of data. Responsibilities concerning privacy and confidentiality will be reminded and discussed with all co-investigators and data entry staff.

Electronic data files will be stored on encrypted and password protected computers using secure servers. Any hard copies of data, consent forms, questionnaires or other papers containing data will be stored in locked filing cabinets in locked research rooms at Universiti Putra Malaysia in Selangor, Malaysia.

Blood collection and processing

A fasting venous blood sample will be collected at weeks 0, 16 and 20 of the trial into evacuated tubes containing EDTA.

Table 2 A summary of study blood analytes and methods

Analyte	Methods
Plasma folate	Microtiter technique with chloramphenicol-resistant <i>Lactobacillus casei</i> as the test micro-organism ²⁵
Erythrocyte folate	Calculated from whole blood folate by subtracting plasma folate and correcting for haematocrit
Plasma vitamin B ₁₂	Elecsys 2010 (Roche Diagnostics, Switzerland) automated electrochemiluminescence immunoassay
Plasma ferritin, sTfR, AGP, CRP and RBP	Single sandwich-enzyme linked immunosorbent assay (s-ELISA) ²⁶

AGP, α -1 acid glycoprotein; CRP, C-reactive protein; RBP, retinol binding protein; sTfR, soluble transferrin receptor.

Whole blood for haematocrit determination

One EDTA tube will be sent to Clinipath Malaysia Sdn. Bhd. (Selangor, Malaysia) for a full blood count determination using an automated haematology analyser.

Whole blood for plasma and erythrocyte folate, buffy coat and erythrocytes

The other tube will be inverted gently 8–10 times to ensure the blood is properly mixed. For erythrocyte folate, 100 μ L of whole blood and 1000 μ L of 1% ascorbic acid (~1 in 11), will be added to three separate tubes and then vortexed for 5 seconds. Samples will then be incubated at 38 for 30 min and subsequently placed on ice for storage at -80°C . Samples will be centrifuged at 3000 rpm for 10 min at 4°C . Plasma will be aliquoted into three labelled microtubes and stored at -80°C . Buffy coat will be aliquoted into a single labelled microtube and stored at -80°C .

Blood analyses

Deidentified blood samples will be shipped on dry ice to SAHMRI, Adelaide, Australia where plasma folate (nmol/L) and erythrocyte folate (nmol/L) concentrations will be determined using the folate microbiological assay harmonised by the Centers for Disease Control and Prevention.²⁴ Plasma ferritin ($\mu\text{g/L}$), soluble transferrin receptor (mg/L), α -1 acid glycoprotein (g/L), C-reactive protein (mg/L) and retinol binding protein ($\mu\text{mol/L}$) will be analysed at the VitMin lab in Germany (table 2).

Statistical analysis

The primary analysis will be performed on an 'intention-to-treat' basis, according to treatment allocation at randomisation. A secondary 'per-protocol' analysis will also be performed including only women who complete the study and are >80% adherent to the treatment regime. Continuous outcomes, including the primary outcome erythrocyte folate concentration at 16 weeks, will be analysed using linear regression models and binary outcomes will be analysed using log binomial regression models.

Independent variables will include randomised treatment group, time point (16 or 20 weeks) and a treatment group by time point interaction. Treatment effects (0.4mg vs 0.0mg, 2.8mg vs 0.0mg and 2.8mg vs 0.4mg) will be estimated for each time point separately (16 and 20 weeks) along with 95% CIs and two-sided p values. Clustering due to repeated measurements on the same individuals at different time points will be considered using generalised estimating equations. Adjustment will be made for the baseline measure of the analysed outcome as this is expected to be strongly related to the outcome; no adjustment for other baseline variables or subgroup analyses are planned. Missing data will be addressed using multiple imputation to create 100 complete datasets for analysis, with a sensitivity analysis performed on the available data. All analyses will follow a prespecified statistical analysis plan.

Dissemination

This trial is registered with the Australian New Zealand Clinical Trials Registry and the Malaysian National Medical Register.

Confidentiality

Participant confidentiality will be maintained throughout the trial. Confidentiality will extend to the biological testing of samples and additional medical information. The protocol and all study documents will be held in strict confidence. No information about the study or its data will be released to unauthorised third parties. Any medical information of the participants will not be released without the permission of the participant.

Use of data and publication policy

Publication of information regarding this protocol or its data in formats including, but not limited to, conference abstracts, posters or presentation, seminars, journal articles, public reports and internet postings. Approval of these activities must have the permission of all co-investigators before the event. The results of this trial will be published in peer-reviewed scientific journals and presented at conferences. Additionally, all results will be relayed to the relevant stakeholders, including the Ministry of Health and the WHO.

Patient and public involvement

The development of the research question and outcome measures were not informed by the participants' priorities, experience and preferences. Participants were not involved in the design of this study. Participants will be provided with a summary of the trial findings and their personal results. Results will also be disseminated to policy makers and the Ministry of Health through briefing papers containing summaries of the main findings.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The Ethics Committee for Research Involving Human Subjects of Universiti Putra Malaysia (JKEUPM-2018-255) and The University of British Columbia Clinical Research Ethics Board (H18-00768) approved the research.

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REFERENCES

- 1 Imbard A, Benoist J-F, Blom HJ. Neural tube defects, folic acid and methylation. *Int J Environ Res Public Health* 2013;10:4352–89.
- 2 Mrc vitamin study research group. Prevention of neural tube defects: results of the medical Research Council vitamin study. MRC vitamin study Research Group. *Lancet* 1991;338:131–7.
- 3 Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832–5.
- 4 Berry RJ, Li Z, Erickson JD, et al. Prevention of neural-tube defects with folic acid in China. *N Engl J Med* 1999;341:1485–90.
- 5 World Health Organization. Who recommendation on antenatal care for positive pregnancy experience 2016.
- 6 Botto LD, Moore CA, Khoury MJ, et al. Neural-Tube defects. *N Engl J Med* 1999;341:1509–19.
- 7 Sadler TW. Embryology of neural tube development. *Am J Med Genet C Semin Med Genet* 2005;135C:2–8.
- 8 Crider KS, Bailey LB, Berry RJ. Folic acid food Fortification—Its history, effect, concerns, and future directions. *Nutrients* 2011;3:370–84.
- 9 Sayed A-R, Bourne D, Pattinson R, et al. Decline in the prevalence of neural tube defects following folic acid fortification and its cost-benefit in South Africa. *Birth Defect Res A* 2008;82:211–6.
- 10 De Wals P, Tairou F, Van Allen MI, et al. Spina bifida before and after folic acid fortification in Canada. *Birth Defect Res A* 2008;82:622–6.
- 11 Tsang BL, Moreno R, Dabestani N, et al. Public and private sector dynamics in scaling up rice fortification: the Colombian experience and its lessons. *Food Nutr Bull* 2016;37:17–28.
- 12 Bailey RL, West KP, Black RE. The epidemiology of global micronutrient deficiencies. *Ann Nutr Metab* 2015;66 Suppl 2:22–33.



- 13 Daly LE, Kirke PN, Molloy A, *et al.* Folate levels and neural tube defects. Implications for prevention. *JAMA* 1995;274:1698–702.
- 14 World Health Organization. Guideline: intermittent iron and folic acid supplementation in menstruating women 2011.
- 15 Institute for Public Health. National health and morbidity survey 2015 (NHMS 2015). Vol. II: *Non-Communicable Diseases, Risk Factors & Other Health Problems* 2015.
- 16 World Health Organization. Prevalence of anaemia in women of reproductive age. estimates by who region. *Glob. Heal. Obs. data Repos* 2017.
- 17 Country Profile - Malaysia. Food Fortif. Initiat, 2019. Available: http://ffinetwork.org/country_profiles/country.php?record=131
- 18 Khor GL, Duraisamy G, Loh SP, *et al.* Dietary and blood folate status of Malaysian women of childbearing age. *Asia Pac J Clin Nutr* 2006;15:341–9.
- 19 Green TJ, Skeaff CM, Venn BJ, *et al.* Red cell folate and predicted neural tube defect rate in three Asian cities. *Asia Pac J Clin Nutr* 2007;16:269–73.
- 20 World Health Organization. *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity*. Geneva, 2011. <http://www.who.int/vmnis/indicators/haemoglobin.pdf>
- 21 Norsworthy B, Skeaff CM, Adank C, *et al.* Effects of once-a-week or daily folic acid supplementation on red blood cell folate concentrations in women. *Eur J Clin Nutr* 2004;58:548–54.
- 22 Harris PA, Taylor R, Thielke R, *et al.* Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377–81.
- 23 Harris PA, Taylor R, Minor BL, *et al.* The REDCap Consortium: building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
- 24 Zhang M, Sternberg MR, Pfeiffer CM. Harmonizing the calibrator and microorganism used in the folate microbiological assay increases the comparability of serum and whole-blood folate results in a CDC Round-Robin study. *J Nutr* 2018;148:807–17.
- 25 O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. *J Clin Pathol* 1992;45:344–7.
- 26 Erhardt JG, Estes JE, Pfeiffer CM, *et al.* Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. *J Nutr* 2004;134:3127–32.