Is natural (6S)-5-methyltetrahydrofolic acid as effective as synthetic folic acid in increasing serum and red blood cell folate concentrations during pregnancy? A proof-of-concept pilot study

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Study protocol

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Abstract

Background: Health Canada recommends 0.4 mg/day folic acid before conception and throughout pregnancy to reduce the risk of neural tube defects. Folic acid is a synthetic form of folate that must be reduced by dihydrofolate reductase and then further metabolized in the small intestine. Recent evidence suggests that the maximal capacity for this process is limited and unmetabolized folic acid has been detected in circulation. The biological effects of unmetabolized folic acid are not fully understood. A natural form of folate, (6S)-5-methyltetrahydrofolic acid (Metafolin®), may be a superior alternative because it does not need to be reduced in the small intestine. Metafolin® is currently used in some prenatal multivitamins, however it has yet to be evaluated during pregnancy.

Methods: This double-blind randomized trial will recruit 60 pregnant women aged 19-42 years. Women will receive either 0.6 mg/day folic acid or an equimolar dose (0.625 mg/day) of (6S)-5-methyltetrahydrofolic acid for 16-weeks. The trial will be initiated at 8-21 weeks gestation (after neural tube closure) to reduce the risk of harm should (6S)-5-methyltetrahydrofolic acid prove less effective. All women will also receive a prenatal multivitamin (not containing folate) to ensure adequacy of other nutrients. Baseline and endline blood samples will be collected to assess primary outcome measures, including serum folate, red blood cell folate and unmetabolized folic acid. Change in primary outcomes from baseline to endline in each group will be calculated using a paired-t-test.

Discussion: This proof-of-concept trial is needed to obtain estimates of the effect of (6S)-5-methyltetrahydrofolic acid compared to folic acid on circulating biomarkers of folate status during pregnancy. These estimates will inform the design of a definitive trial which will be powered to assess whether (6S)-5-methyltetrahydrofolic acid is as effective as folic acid in raising blood folate concentrations during pregnancy. Ultimately, these findings will inform folate supplementation policies for pregnant women.

Background

Overview of folate

Folate refers to a group of water-soluble B-vitamins which play a critical role in DNA synthesis and methylation reactions.¹ All folate vitamers consist of a common parent structure, but differ based on whether the pteridine ring is in an oxidized or reduced state, addition of one-carbon substituents at positions N5 and/or N10 and number of glutamic acid residues.^{1,2} Food folates are reduced and have a polyglutamate tail consisting of up to nine glutamate residues.^{1,3} Folic acid is a synthetic form of folate which is oxidized and contains one glutamic acid residue; this is the most stable form, therefore folic acid is used in food fortification and most supplements.¹⁻³

Bioavailability and metabolism of folates differ due to their respective chemical structures (*Fig 1*).^{1,3} Food folates are estimated to be about 50% bioavailable, as they must be hydrolyzed during digestion in

the gut by a brush border hydrolase.^{1,4} Conversely, folic acid is a monoglutamate and can be absorbed as such.² Under fasting conditions, folic acid is almost 100% bioavailable and when consumed with food, it is approximately 85% bioavailable.^{1,4} Ultimately, all forms of folate are metabolized to 5-methyltetrahydrofolate (5-MTHF).² However unlike food folates, folic acid must first be reduced to tetrahydrofolate in a two-step reaction via dihydrofolate reductase (*DHFR*). Tetrahydrofolate is then methylated to 5-MTHF via methylenetetrahydrofolate reductase (*MTHFR*).² A natural form of folate (Metafolin®) is currently used in some nutrition supplements in place of folic acid. Metafolin® is a naturally occurring calcium salt of (6S)-5-methyltetrahydrofolic acid.^{2,5} It does not require reduction by *DHFR* and can enter circulation directly for use in the body.²

Figure 1 Intestinal absorption of natural folate and folic acid

Legend: 1) *Food folates* are hydrolyzed during digestion to the monoglutamate form; 2) *Folic acid* must be reduced to tetrahydrofolate in a two-step reaction by *DHFR*; 3) Supplements in the natural form of folate, (6S)-5-methyltetrahydrofolic acid, may enter circulation directly for use in the body as 5-MTHF; 4) Both *food folates* and *folic acid* are ultimately metabolized to 5-MTHF by *MTHFR*

Periconceptional folate requirements

Neural tube defects (NTDs) are severe, irreversible congenital anomalies which occur in the third to fourth week after conception.⁶ Although the etiology remains unclear, numerous clinical trials have demonstrated that folic acid supplementation is effective in reducing the risk of NTDs.^{7–10} Canadian guidelines recommend that all women at low risk for a NTD-affected pregnancy (which encompasses approximately 95% of pregnancies) supplement with 0.4 mg/day folic acid, starting 2–3 months before conception and throughout pregnancy.^{7,10} Due to many pregnancies being unplanned, several countries including Canada have introduced mandatory folic acid fortification of white flour, enriched pasta and cornmeal at a rate which aims to provide 0.1 mg/day.^{11,12} Following folic acid food fortification there has been an observed reduction in the rates of NTD subtypes, including a decrease of 53%, 38% and 31% for spina bifida, anencephaly and encephalocele respectively.¹²

Most pregnant women in Canada consume greater than 0.4 mg/day folic acid due to the combination of food fortification and prenatal multivitamins (which typically contain 0.6–1.0 mg per dose).¹³ In a large pregnancy cohort in Quebec (n = 1533), 87% of the women had total folic acid intakes (supplements and fortified foods) greater than the upper limit (1.0 mg/day).¹⁴ In two Toronto-based pregnancy cohorts, the mean red blood cell (RBC) folate was >2400nmol/L at 12–16 weeks gestation and at 36 weeks gestation, regardless of supplementation regimen.^{15,16} National surveys have identified that since the initiation of food fortification, folate deficiency (RBC folate <305 nmol/L) is essentially non-existent in Canada.^{17,18}

High intakes of folic acid are concerning because it appears that *DHFR* activity is limited,^{19–21} and the metabolic effects of unmetabolized folic acid (UMFA) are unknown. There is an acute appearance of

plasma UMFA following oral intakes of folic acid >0.2 mg, suggesting that intestinal absorptive capacity becomes saturated at this dose.^{22–24} Following single doses of folic acid, it appears that the body can clear plasma UMFA via mechanisms including tissue uptake and urinary excretion.^{25,26} However, in two studies which provided folic acid (0.4 mg/day) for 8 and 14 weeks, UMFA was detected in fasting plasma samples,^{27,28} suggesting that with regular daily intake, clearance mechanisms become saturated as well.^{25,27–29} A Toronto-based study evaluated the effects of 30-week folic acid supplementation (1.1 mg vs 5 mg) on UMFA in fasting plasma among women of reproductive age who are also exposed to folic acid fortification.²⁵ Interestingly, they found no significant difference in fasting plasma UMFA between those consuming 1.1 mg or 5 mg folic acid.²⁵ The proportion of women with detectable levels of UMFA in fasting plasma did increase after supplementation initiation, but these concentrations were variable between participants and increases were not sustained.²⁵ This supports findings that *DHFR* capacity is variable in humans, but suggests that there are mechanisms which limit systemic exposure to circulating UMFA following high-dose, long-term folic acid supplementation.²⁵ Nonetheless, it appears that folic acid intake in Canadian pregnant women likely exceeds the physiologic capacity to reduce and utilize it or clear it from plasma.³⁰ A Canadian study found plasma UMFA detectable in >90% of maternal and newborn (cord blood) samples (n = 368).¹⁶ Additionally, studies which have assessed breastmilk in Canadian women detected UMFA in 96% of milk samples.^{31,32}

Natural (6S)–5-methyltetrahydrofolic acid is approved by Health Canada as a source material of folate⁵ but it has never been evaluated in pregnancy. Previous studies have demonstrated that (6S)–5-methyltetrahydrofolic acid is more effective than folic acid in increasing serum and RBC folate in non-pregnant women of childbearing age^{33–36} and lactating women.¹⁵ (6S)–5-methyltetrahydrofolic acid may be a superior alternative to folic acid because it does not require reduction via *DHFR*, but it is critical to ensure that it can maintain serum and RBC folate concentrations throughout pregnancy in order to adequately supply the growing fetus. Thus, we aim to address these gaps in the current literature by conducting a pilot study which will inform the design of a definitive trial investigating whether (6S)–5-methyltetrahydrofolic acid is as effective as synthetic folic acid in increasing serum and RBC folate concentrations, while resulting in lower plasma UMFA, during pregnancy.

Methods And Design

Study Design

In this two-arm, double-blind randomized trial, we will recruit 60 pregnant women aged 19–42 years living in Vancouver, BC, Canada. Participants will be randomized to supplement daily for 16-weeks with either 0.6 mg/day folic acid or an equimolar dose (0.625 mg/day) of (6S)–5-methyltetrahydrofolic acid (*Fig 2*). The 16-week intervention was based on the estimated half-life of RBC folate and to enable measurement of long-term folate status in RBCs (past 12–16 weeks).³⁷ All participants will also receive a prenatal multivitamin not containing any folate, to ensure adequacy of other nutrients (e.g. iron) during pregnancy.

Fasting venous blood samples will be collected at baseline and endline to measure primary outcome measures, including serum folate, RBC folate and UMFA; exploratory measures will include plasma S-adenosyl-methionine, S-adenosyl-homocysteine, total homocysteine, total cysteine, methionine, vitamin B-12, pyridoxal-5-phosphate, free choline and betaine. Genotyping of the *MTHFR* (677 C>T, rs1801133, and 1298 A>C, rs1801131) and *DHFR* (rs1643649 and rs70991108) variants and a complete blood count will also be determined. The study protocol has been developed in accordance with the 2013 SPIRIT guidelines (*Fig 3*)..³⁸ See Additional file 1 (SPIRIT checklist) for further details.

The primary objective of this pilot study is to gain estimates for the change in serum folate, RBC folate and UMFA following supplementation with (6S)-5-methyltetrahydrofolic acid and folic acid for 16-weeks of pregnancy to inform the design of an adequately powered definitive trial. The secondary objective is to obtain recruitment and participation rates to assist in the design and feasibility of a definitive trial.

Figure 2 Participant flow diagram

Figure 3 Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) schedule of enrollment, interventions and assessments

Participants, Recruitment & Informed Consent

Inclusion criteria: i) pregnant woman (singleton pregnancy); ii) living in the greater Vancouver area; iii) ≤ 21 weeks gestation at time of consent; iv) 19–42 years of age; v) able to provide informed consent.

Exclusion criteria: i) pre-existing medical conditions known to impact maternal folate status (malabsorptive and inflammatory bowel diseases, active celiac disease, gastric bypass surgery, atrophic gastritis, epilepsy, advanced liver disease, kidney dialysis, type 1 or 2 diabetes mellitus, sickle cell trait/anemia);⁷ ii) lifestyle factors known to impact maternal folate status (current smoking, alcohol consumption, recreational drug use);⁷ iii) medium to high risk for development of an NTD-affected pregnancy (applies to women or their male partner: personal or family history [parents or siblings] of other folate sensitive congenital anomalies, personal NTD history or a previous NTD-affected pregnancy);⁷ iv) medications known to interfere with B-vitamin metabolism (Chloramphenicol, Methotrexate, Metformin, Sulfasalazine, Phenobarbital, Phenytoin, Primidone, Triamterene, Barbiturates);⁷ v) pre-pregnancy body mass index (BMI) ≥ 30 kg/m²; or vi) allergy to any study supplement ingredients.

Sample Size: We will require 50 women (25 in each group) to reliably estimate the distributions of serum and RBC folate concentrations.³⁹ Thus, to account for dropouts or loss to follow up, we will recruit a total of 60 women (30 in each group).

Recruitment: Posters will be displayed at the BC Women's Hospital, in medical and prenatal clinics and in retail establishments for pregnant women (e.g. prenatal classes, fitness studios, maternity clothing stores) throughout Vancouver, BC, Canada. Study details will be shared with staff and health care professionals, including physicians, midwives, nurses and others as applicable. Advertising on social media (Facebook and Instagram) will be used and targeted towards pregnant women aged 19–42 years in the greater Vancouver, BC area.

Obtaining Informed Consent & Confirming Eligibility: Women interested in participating will contact the research coordinator (KMC) directly. At this time, study details, eligibility and informed consent will be described. Eligible participants who would like to be enrolled will be given a study ID and their baseline meeting will be scheduled. Participants will be instructed to continue any current prenatal multivitamin/folate supplementation until their baseline visit.

Study Visits

Study visits will be facilitated at the University of British Columbia, Food, Nutrition and Health Building (Vancouver, BC, Canada). *Baseline* (8–21 weeks gestation): Both the participant and the research coordinator (KMC) will sign the informed consent form; a copy will be scanned and e-mailed to the participant following the study visit. Participants will complete a baseline questionnaire to capture medical and nutrition history and demographic data. A validated food frequency questionnaire will be used to calculate dietary folate equivalents (DFEs). Weight and height will be taken using an electronic scale and stadiometer and recorded to 0.1 kg and 0.1 m respectively. A 12 mL 3 hr-fasting venous blood sample will be collected. Participants will receive their study supplements (either folic acid or (6S)–5-methyltetrahydrofolic acid) and be provided with a supplement diary to record daily intake. *Endline* (24–37 weeks gestation): A 12 mL 3-hr fasting venous blood sample will be collected, weight will be taken as described above and participants will complete an endline questionnaire to determine any changes in health status or medication use since baseline. Participants will return all supplement bottles (including any remaining capsules) and their supplement diary.

Study Supplements

The folic acid and (6S)–5-methyltetrahydrofolic acid will be provided separately from the prenatal multivitamin. This decision was made because some women report intolerance when consuming prenatal multivitamins; this is typically due to the high iron content and resolves after the first trimester.⁴⁰ Should temporary intolerance arise, participants may skip or take a half dose of their multivitamin, while continuing to supplement with the full dose of folate.

Bulk ingredients for the folic acid and prenatal multivitamins have been provided by Natural Factors (Coquitlam, Canada). Bulk (6S)–5-methyltetrahydrofolic acid (Metafolin®) has been provided by Merck &

Cie (Schaffhausen, Switzerland). All bulk ingredients have been compounded into vegetable gel-capsules at Natural Factors (Coquitlam, Canada). Folic acid and (6S)–5-methyltetrahydrofolic acid capsules are identical in appearance to accommodate double-blinding. The prenatal multivitamin contains the same micronutrient formulation as WN Pharmaceuticals Ltd *Prenatal* (NPN 80025456), except the folic acid has been removed. A notice of authorization for the clinical trials application (Submission No. 244456) was provided by the natural and non-prescription health products directorate of Health Canada on July 26th, 2019.

Double blinding & randomization

An independent research assistant from Natural Factors (Coquitlam, Canada) will assign blinding codes (A or B) to the folic acid and (6S)–5-methyltetrahydrofolic acid supplements. Folate supplement bottles will be labelled with “A” or “B” and indicate that “capsules are either folic acid or folate”. Both the participants and the primary research team will be blinded to the supplement allocations, which will be de-coded only once the final statistical analyses are completed. The randomization sequence will be computer-generated by an independent statistician, using blocks of four which each contain two participants per supplement group. The primary research team will be blinded to the randomization sequence. At each participant’s baseline visit, the research coordinator (KMC) will unblind the allocation (A or B) for that study ID only, in order to provide the allocated study supplements.

Concomitant Medications

Once enrolled, there will be no restrictions on medication intake throughout the intervention period, however all medications must be reported. The only exception will be folate/folic acid containing nutritional supplements, which will not be permitted throughout the intervention period.

Strategies to Enhance Adherence

Pregnant women tend to be highly motivated and are generally interested in participating in clinical research trials.⁴¹ As per a survey from the Public Health Agency of Canada, 94% of women in British Columbia supplement with prenatal multivitamins during pregnancy.⁴² Therefore, participants will likely be accustomed to daily supplementation upon enrollment. The research coordinator (KMC) will contact each participant at midline (8-weeks after their baseline visit) to address any questions or concerns. Additionally, the supplement diary will serve as a daily reminder to enhance adherence throughout the 16-weeks. For these reasons, we anticipate high adherence (>90%) to the study protocol.

Blood Sample Collection & Processing

Three-hour fasting venous blood samples (12 mL total) will be collected in a 6 mL EDTA tube, 2 mL EDTA tube and 4 mL serum tube (BD Diagnostics). After collection, tubes will be shielded from light, inverted gently as per manufacturers recommendations, placed in a cooler and transported to the laboratory for immediate processing.

For preparation of whole blood hemolysate, whole blood (0.3 mL) will be removed from the 6 mL EDTA tube and diluted 1/11 by adding 3.0 mL of a 1% ascorbic acid solution and subsequently incubated at 37°C for 30 minutes. The 6 mL EDTA tube will then be centrifuged at 3000 rpm for 15 minutes at 4°C. Plasma will be collected and remaining contents of the 6 mL EDTA tube will be processed for isolation of peripheral blood mononuclear layer cells (PBMCs) with the SepMate PBMC isolation protocol (Stemcell technologies, Vancouver, Canada). The 2mL EDTA tube will be used for a complete blood count. The 4 mL serum tube will be left at room temperature for ~30 minutes (until clotted) and then centrifuged at 3000 rpm for 10 minutes at 4°C; serum will be collected. All aliquots will be stored at –80°C until further analyzed.

Laboratory Analyses

Serum folate (nmol/L) and RBC folate (nmol/L) will be analyzed by microbiological assay, as globally recommended, using the microtitre plate method outlined by Molloy [et.al.](#)⁴³ RBC folate (nmol/L) will be calculated with the following formula:⁴⁴

$$\text{RBC Folate} = (\text{Whole blood hemolysate folate} * 11) - \text{Serum folate} (1 - \text{Hematocrit}/100)$$

Hematocrit/100

Plasma biomarkers including UMFA (nmol/), S-adenosyl-methionine (μmol/L), S-adenosyl homocysteine (μmol/L), total homocysteine (μmol/L), total cysteine (μmol/L), methionine (μmol/L), free choline (μmol/L) and betaine (μmol/L) will be analyzed using liquid chromatography mass spectrometry.^{45–47} Vitamin B–12 (pmol/L) will be analyzed using an immunoanalyzer. Pyridoxal–5-phosphate (nmol/L) will be analyzed using liquid chromatography-tandem mass spectrometry.⁴⁸ Gene variants will be genotyped by using taqman SNP genotyping assays.^{1,49} Complete blood count will be performed using an automated hematology analyzer (Sysmex XNL–550).

Additional Data to be Collected

Demographic, medical and nutrition data via structured questionnaires: Baseline data collection will include age, ethnicity, parity, education, occupation, household income, medical and medication history, reported pre-pregnancy weight, general diet (including vegan, vegetarian, ketogenic, gluten free, and any

foods/food groups avoided for any reason) and supplement use. Endline data collection will include any changes in overall health or medication use since the baseline visit.

Anthropometrics: Pre-pregnancy BMI will be calculated using self-reported pre-pregnancy weight and measured height. Participant measurements (weight and height) will be taken at baseline and endline for calculation of gestational weight gain throughout the intervention period and total weight gain throughout pregnancy (using self-reported pre-pregnancy weight and measured endline weight).

Dietary assessment: Total DFEs ($\mu\text{g/day}$) will be calculated using the Block Folic Acid/Dietary Folate Equivalents Screener (NutritionQuest, Berkeley, USA), a validated food frequency questionnaire.⁵⁰

Withdrawal Criteria

Withdrawal criteria includes spontaneous or planned termination of pregnancy, use of additional folate/folic acid containing nutritional supplements and following an adverse/serious adverse event associated with the supplements/trial intervention (as determined by the qualified investigator [CM]). Participants may also self-withdraw at any time and no further information will be collected. Regardless of the reason for withdrawal, all previously collected data will be retained for analysis.

Data Handling & Privacy

All data and blood samples will be identified using each participant's study ID and stored on an encrypted, password-protected computer in a secure server space with the BC Children's Hospital Research Institute. Data will be cleaned and double entry will be completed by the research coordinator (KMC) and an independent research assistant. KMC, JAH and CDK will have access to the final dataset. Paper documents will be stored in a locked filing cabinet and blood samples will be stored in a locked freezer housed inside the principal investigator's laboratory (CDK) at the University of British Columbia. Participants may be told their folate supplement allocation and blood folate results (including serum and RBC folate at baseline and endline) after final statistical analyses are completed.

Data and Statistical Analysis

Data will be assessed for normality and transformations will be considered if data do not follow a normal distribution. Statistical analyses will be performed using Stata (Stata Corp, Texas, USA). Two-sided p-values less than 0.05 will indicate statistical significance. Descriptive statistics will be used for participant baseline data. All quantitative variables will be reported with a mean $\pm$ standard deviation (or median and interquartile range if not normally distributed) and qualitative variables as absolute numbers per group. Missing data will be evaluated to identify whether it is random or non-random, and multiple imputation will be used to correct for missing data as appropriate.⁵¹

Primary objective: We will calculate the mean $\pm$ standard deviation for serum folate, RBC folate and plasma UMFA in each group at baseline and endline. The change from baseline to endline in each group will be evaluated using a paired t-test. These estimates will be used to inform the sample size calculation

of the definitive trial. As a secondary test, we will evaluate the difference in the mean change between groups using a two-sample-t-test. All tests will initially be completed on an intention-to-treat basis according to initial group allocation at baseline and without any imputation for missing data. Secondary per-protocol analyses will be conducted including those who fully completed the study and adhered to the study protocol (supplementation rate $\geq 90\%$). Exploratory analyses will be conducted using a multiple linear regression model, adjusting for explanatory variables (DFEs, exploratory biomarkers, demographic data).

Secondary objective: Overall study participation rate will be estimated by dividing the total number of women enrolled by the total number of women who were interested in participating. Weekly recruitment rate will be estimated by dividing the total number of women enrolled by the total number of weeks it took to recruit them. Participant retention rate will be estimated by dividing the number of women who complete the full trial by the total number of women enrolled. Most effective recruitment strategies will be determined by recording number of successful participant acquisitions per recruitment site. Capsule counts will be used to calculate participant adherence and the supplement diary will be used to provide descriptive insight into adherence barriers.

Safety

Folic acid is provided at a dose that meets Canadian recommendations for pregnant women (0.4–1.0 mg/day); thus, is considered low risk. Calcium salt of (6S)–5-methyltetrahydrofolic acid is recognized as safe and is included in Canadian prenatal multivitamins with no established tolerable upper limit (no risk of harm is currently known). Randomization will occur between 8–21 weeks gestation (after neural tube closure). Considering the low risk of this pilot study, assembly of a data monitoring committee was not deemed necessary. All safety concerns and if necessary, the decision to terminate the trial, will be addressed by the principal investigators (CDK and JAH) and qualified investigator (CM) and reported to the research ethics board and Health Canada. Unblinding of the intervention codes may be undertaken at any time if deemed necessary for participant safety monitoring.

Discussion

These pilot data are critical to inform the design a definitive trial which will be powered to assess whether (6S)–5-methyltetrahydrofolic acid supplementation is as effective as synthetic folic acid in increasing serum and RBC folate concentrations during pregnancy, while resulting in lower UMFA. Ultimately, a definitive trial will help to inform the safest and most effective form of folate supplementation for pregnant women and their babies.

Trial Status

Date and version: November 18, 2019, version 1; As of September 2019, recruitment is ongoing and is expected to be completed April 2020.

Abbreviations

5-MTHF: 5-methyl-tetrahydrofolate; DFEs: dietary folate equivalents; *DHFR*: dihydrofolate reductase; *MTHFR*: methylene-tetrahydrofolate reductase; NTD: Neural tube defects; RBC: red blood cell; UMFA: unmetabolized folic acid; EDTA: ethylenediaminetetraacetic acid; BMI: body mass index

Declarations

Ethics approval and consent to participate: Ethics approval was obtained by the UBC Children's and Women's Research Ethics Board on September 16th, 2019 (H18–02635). The trial was registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT04022135), and the full protocol is posted there for public access. The World Health Organization Trial Registration Data Set provides further detail (see Additional file 2). Written informed consent will be obtained from all participants before the intervention begins. Any protocol amendments will be reported to the ethics board, ClinicalTrials registry and Health Canada. The results of the study will be published in a nutrition focused peer-reviewed journal and presented at national and international conferences.

Consent for publication: Not applicable.

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare no competing interests.

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Authors' contributions: Study concept: CM, RE, JAH and CDK. Protocol design and development: KMC, CM, AMD, RE, JAH and CDK. Drafting of the manuscript: KMC and CDK. Critical revision of the manuscript for important intellectual content: KMC, CM, AMD, RE, JAH and CDK.

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Figures

Figure 1

Intestinal absorption of natural folate and folic acid

Figure 2

Participant flow diagram

STUDY PERIOD								
	Enrollment	Allocation (baseline)	Post-allocation (intervention period)					Close-out (endline)
TIMEPOINT (weeks gestation)	<21	21*	21-37 (16 weeks of supplementation)					37
ENROLLMENT:								
Eligibility screen	X							
Informed consent	X							
INTERVENTIONS:								
<i>Folic Acid</i>		X						X
<i>(6S)-5-methyl- tetrahydrofolic acid</i>		X						X
ASSESSMENTS:								
<i>Blood sample</i>		X						X
<i>Baseline variables: Demographic information, medical and medication history, usual diet and supplement use</i>		X						
<i>Anthropometrics (weight and height)</i>		X						X
<i>Dietary folate intakes (food frequency questionnaire)</i>		X						
<i>Provision of study supplements</i>		X						
<i>Endline variables: updates in health or medications</i>								X
<i>Return of study supplements</i>								X

*Supplementation may begin at any point between 8-21 weeks gestation

Figure 3

Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) schedule of enrollment, interventions and assessments

Supplementary Files

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- [Additionalfile2WHOdataset.docx](#)