OVERVIEW

Sickle cell disease (SCD) is an autosomal recessive disorder that affects over 3,000 Canadian children (1). A variant in the gene encoding hemoglobin produces dysfunctional, sickled red blood cells (RBC). Children with SCD are considered to have high requirements for folate (a B-vitamin required for DNA synthesis and erythropoiesis), owing to chronic hemolytic anemia and higher erythropoiesis and RBC turnover (2,3). As such, high-dose supplementation with 1-5 mg/d folic acid, the synthetic form of folate, has been a long-standing recommendation for children with SCD (1). Even 1 mg/d folic acid provides 6 times higher folate than that recommended for healthy children aged 1-3 years (4).

However, there is mounting concern about whether Canadian children with SCD need such high doses of folic acid, especially following mandatory folic acid fortification of flour in Canada in 1998. In addition, new advancements in medical therapy (e.g. drug development of hydroxyurea) and clinical care warrant the reassessment of this guideline. A 2016 study reported that folate deficiency was non-existent in Canadian children with SCD (n=87) who were supplemented with 1 mg/d folic acid (5). A 2017 study showed that discontinuation of 1 mg/d folic acid for ~80 days did not change RBC folate concentrations in children with SCD (n=72); and even after discontinuation, RBC folate concentrations remained extremely high (2.2 times higher than 'normal' values) (6).

Problem: There is scant evidence that high-dose folic acid supplementation improves hematological or clinical outcomes in individuals with SCD, and some evidence of potential harm. With doses of synthetic folic acid as low as 0.2 mg/d, unmetabolized folic acid (UMFA) begins to accumulate in the blood (7). This occurs because of limited enzyme activity to convert synthetic folic acid to a form that can be utilized by the body, or when the liver is fully saturated with folate (8). There is growing concern that excess synthetic folic acid and circulating UMFA may lead to adverse health outcomes, such as an increased risk of some types of cancers (9–12). Further, excess folic acid can cause imbalances in folate metabolism; thus, could affect DNA methylation and gene expression (13,14).

Plan: To inform the efficacy and potential harm of high-dose folic acid supplementation in children with SCD, we propose a double-blind randomized controlled cross-over trial. Children with SCD (n=40, aged 2-19 y) will be recruited from BC Children's Hospital and randomized to 1 mg/d folic acid or a placebo for 12-weeks (wk). After a 12-wk washout period, treatments will be reversed. Blood samples will be collected at baseline and 12-wk of each treatment period. We will measure plasma and RBC folate concentrations, circulating forms of folate (including UMFA), folate metabolites (homocysteine, S-adenosyl-homocysteine [SAH], and S-adenosyl-methionine [SAM]), and clinical outcomes (anemia prevalence and acute pain crises).

Hypotheses: There will be no difference in mean RBC folate concentrations across folic acid and placebo groups after 12-wk, and none of the children will have folate deficiency. Compared to children taking placebo, children taking high-dose folic acid for 12-wk will show no difference in clinical outcomes, but higher plasma UMFA concentrations.

Significance: There is a need to determine if the current clinical practice of high-dose folic acid supplementation is efficacious, safe, and warranted.

BACKGROUND

Overview of folate metabolism

Folate is a water-soluble B-vitamin found naturally in dark green vegetables, beans, and lentils (4). Folate plays a critical role in cell growth and division, and nucleotide synthesis (15). It functions as an

acceptor or donor of one-carbon groups, and is required for erythropoiesis, and DNA synthesis, repair, and methylation (16). Folate also plays a critical role in the methionine cycle, where it is needed to convert homocysteine to methionine (17). Folate acts as a methyl donor for methionine to homocysteine conversion, thus, normal homocysteine metabolism requires an adequate folate supply (18) (**see Figure 1**). Vitamins B12, B6 and riboflavin are also closely involved in folate and homocysteine metabolism (19). Poor status and/or impaired metabolism of folate has been linked to increased risk of chronic diseases, such as cardiovascular disease and cancer (15). Folate also plays an important role in the prevention of neural tube birth defects, as it is required for the closure of the neural tube in the first trimester (20). **Utilization:** Folate from natural sources in food is in reduced form and enters circulation directly as 5-methyltetrahydrofolate (5-methyl-THF) (**see Figure 2**). **In circulation:** there are several forms of folate in the blood in reduced form. 5-methyltetrahydrofolate (5-methyl-THF) is the most common form (21), constituting >80% of total folate (22). Other reduced folate forms include dihydrofolate (DHF), tetrahydrofolate (THF), 5,10-methenyl-THF, 5,10-methylene-THF and 5-formyl-THF (22). Most often, 'total' folate is measured, inclusive of all reduced folate forms in blood.

Folic acid is a synthetic, man-made form of folate that is used in supplements or food fortification (e.g. fortified wheat flour). **Utilization:** Folic acid is different from naturally occurring folate, as it is in oxidized form, has a different biochemical structure (23), and is more bioavailable (it is rapidly absorbed across the intestine) (24). Folic acid must first be converted to DHF by an enzyme called dihydrofolate reductase (DHFR), before entering the folate cycle. DHF is then methylated to THF, and further to 5-methyl-THF, before entering circulation (19). However, the activity of DHFR is limited (8) and the gut cannot metabolize more than ~0.2 mg of synthetic folic acid at a time (25,26). This results in the accumulation of unmetabolized folic acid (UMFA) in the blood (25,26) (**see Figure 2**). UMFA is an un-used form of synthetic folic acid that accumulates and circulates in plasma when DHFR enzymatic capacity is limited, or when the liver is fully saturated with folate (27). Thus, plasma UMFA is thought to be a proxy indicator of excess folic acid intakes (28).

Sickle cell disease

SCD is an autosomal recessive disorder that affects over 3,000 Canadian children (1). The *HBB* E6A variant (*rs*334) in the gene encoding the beta-globin subunit of hemoglobin produces dysfunctional hemoglobin (type S) and damages red blood cells. This leads to SCD and an increased risk of hemolytic anemia, pneumococcal infection, acute pain crises (due to vasculature damage), stroke, and death (29–31). A study in the US reported that children with SCD have a 300-fold increased risk for stroke (30,32). Some children also require life-long blood transfusions to prevent further morbidity and mortality (31). Health statistics from the USA indicate that ~40% of children with SCD require at least one hospitalization each year (33). This disease poses a huge burden on affected children, their families, and the healthcare system. Given the importance of early detection, the burden of disease severity, and the increasing incidence of affected children born in Canada, SCD was added to the list of screened genetic disorders in the BC/Yukon Newborn Screening Program in 2009 (34). All infants born in BC and the Yukon are now screened for this genetic variant (using a heel-prick blood sample obtained in the first weeks of life). Infants screened positive in BC and the Yukon are referred to BC Children's Hospital for follow-up every 3-6 months.

Individuals with SCD are thought to have high folate requirements. Chronic hemolytic anemia, and greater erythropoiesis and RBC turnover in individuals with SCD increases folate requirements (2,3). Studies conducted between 1975 to 2001 showed that individuals with SCD had low blood folate concentrations (35–37). As such, high-dose folic acid supplementation in children with SCD has been a long-standing recommendation (38,39). Currently, the Canadian Haemoglobinopathy Association

recommends 1-5 mg/d folic acid in children with SCD (1). Just 1 mg/d of folic acid provides ~6 times more folate than the daily amount recommended for healthy children aged 1-3 years (4).

However, <u>there is scant evidence that folic acid supplementation improves hematological or clinical outcomes in individuals with SCD</u> (3). Effectiveness of folic acid supplementation was largely investigated in the 1960-70s; the evidence was inconclusive and is now outdated (37–41). A 1983 trial among 117 Jamaican children with SCD examined the effect of 5 mg/d folic acid versus a placebo (42). Although authors observed an increase in serum folate after one year, no effect on hemoglobin, growth, or other clinical events was observed (42). However, the study had many limitations and a high risk of bias; thus, it is difficult to draw conclusions from this trial (3). *No well-designed randomized controlled trial of folic acid supplementation on individuals with SCD has been conducted to date.*

Current problem in Canada

Canadian children with SCD may not need such high doses of folic acid, especially following mandatory folic acid fortification of flour in Canada in 1998 (43). Further, the care of children with SCD has improved drastically over the past decade (44), resulting in less complications associated with SCD (45). As such, Canadian children with SCD may be receiving too much folic acid.

A recent Canadian study reported that folate deficiency was non-existent in Canadian children with SCD (n=87, aged 2-17 y) who were supplemented with 1 mg/d folic acid (5). Further, a 2017 study in the USA (a country which also has mandatory folic acid flour fortification) showed that discontinuation of 1 mg/d folic acid for ~80 days did not change RBC folate concentrations in children with SCD (n=72, aged 1-24 y); and even after discontinuation, RBC folate concentrations remained extremely high (~610 ng/mL, or 2.2 times higher than 'normal' values) (6). However, both studies lacked a control group and had several biases; thus, a rigorous trial is warranted in order to inform clinical practice.

There is emerging evidence of excess folic acid intakes in supplemented groups and/or countries with national fortification programs. The Canadian Health Measures Survey in 2011 showed that less than 1% of Canadians (n=5,248, 6-79 y) had folate deficiency (RBC folate <305 nmol/L) and 40% had very high folate concentrations (>1360 nmol/L)(46). Similarly, serum UMFA was detectable (>0.3 nmol/L) in >95% of Americans (n=2,707, 1-90 years) based on blood samples collected during the National Health and Nutrition Examination Survey (NHANES) in 2007-08 (47). Further, serum UMFA concentrations were significantly higher among supplement users (folic acid or multivitamin), as compared to non-supplement users (1.54 vs. 0.79 nmol/L, respectively, $P<0.05$) (47).

A recent study among 368 Canadian mothers who consumed perinatal folic acid supplements (between ~0.4 to 1 mg/d) showed that plasma UMFA was detectable in >90% of maternal and newborn cord blood samples (48). In another cohort of Canadian mothers (n=561), detectable UMFA concentrations were found in nearly all (~96%) breastmilk samples, regardless of supplement use (~70% self-identified as folic acid supplement users). The authors concluded that doses of folic acid >0.4 mg/d likely exceed the physiologic capacity to metabolize folic acid and should be reconsidered, especially among populations and/or countries consuming folic acid fortified staple foods (49). This raises serious concern for other populations consuming *high-dose* folic acid supplements (1 mg/d or higher), such as children with SCD.

Pilot Data in Canadian Children with SCD

Recent results from a pilot study conducted by our research group have illustrated that high serum folate levels are evident in a sample of BC children with SCD that are prescribed high-dose folic acid supplements (unpublished). Six individuals (50% male; median [IQR] age of participants: 14.95 years

[8.55-18.27]) with SCD were included in this pilot study. Half of the participants (n=3) were prescribed hydroxyurea (median dose: 500mg/d; 17.4, 25.5mg/kg/d), and all participants were prescribed 1mg/d folic acid (however, data on adherence was not available). Median non-fasted serum folate concentrations in this sample were 55.35 nmol/L (43.05, 71.88), and five out of six participants (83%) had serum folate levels above age-specific reference ranges, with levels 1.1-7.9 times the upper reference value (50). All participants (n=5) also had detectable unmetabolized folic acid levels (>0.2nmol/L) in blood plasma .

Too much synthetic folic acid may be harmful (25,51–55). With doses of synthetic folic acid as low as 0.2 mg/d in healthy individuals, UMFA becomes detectable in plasma (**see Figure 1**) (7). High synthetic folic acid intakes (>1 mg/d) and/or circulating UMFA have been associated with acceleration of some cancers, including colorectal cancer (9), prostate cancer (10), mammary tumors in rats (11), and breast cancer (12). Excess synthetic folic acid may interfere with folate metabolism and DNA methylation and gene expression (13,14,52). For example, high synthetic folic acid intakes (resulting in high levels of DHF) could potentially inhibit the formation of 5-methyl-THF, leading to a decreased synthesis of methionine and S-adenosyl-methionine (28) (**see Figure 1**). This is especially concerning for children consuming high-dose folic acid, who are undergoing rapid growth.

In summary, little is known about folic acid metabolism in Canadian children with SCD. As a result of the scant evidence of benefit and the potential risk of harm, there is serious controversy among clinicians regarding this clinical practice. This is a critical gap in patient care that urgently needs to be addressed.

SCIENTIFIC METHODOLOGY

Study design: To inform the efficacy and potential harm of high-dose folic acid supplementation in children with SCD, we propose a **double-blind randomized controlled cross-over trial**. Children with SCD (n=36, aged 2-19 y) will be recruited from British Columbia Children's Hospital in Vancouver and randomized to 1 mg/d folic acid or a placebo for 12-wk. After a 12-wk washout period, treatments will be reversed. Blood samples will be collected at baseline and 12-wk of each treatment period. The 12-wk duration of the intervention and washout periods is based on the estimated half-life of RBCs in children with SCD receiving hydroxyurea (~80 days) (56,57). Approximately 60% of the children at BCCH are prescribed hydroxyurea (58). The 12-wk washout period will minimize the risk of any carryover effect of the first treatment.

Design rationale: The distinguishing advantage of a cross-over trial from a conventional parallel-arm trial is that each child will serve as his/her own control; thus, reducing the bias of confounding variables (e.g. age, sex) (59). This design will also allow us higher power (lower sample size requirement) to determine a statistically significant treatment effect (59).

Inclusion criteria: Children with SCD aged 2-19 y attending British Columbia Children's Hospital.

Exclusion criteria: (i) Children with a blood transfusion in the prior 12-wk, (ii) children unable to swallow tablet forms of medication

Hypotheses: We hypothesize that there will be no difference in RBC folate concentration between folic acid and control groups after 12-wk, and none of the children will have folate deficiency. Compared to children taking placebo, children taking high-dose folic acid for 12-wk will show no difference in clinical outcomes, but higher plasma UMFA concentrations.

Aims and outcomes:

Aim 1. To assess the ***efficacy*** of high-dose folic acid supplementation by measuring the effect of 12-wk of 1 mg/d folic acid in children with SCD, compared to placebo.

- Primary outcome: RBC folate concentrations (nmol/L).
- Secondary outcomes: Serum folate concentrations (nmol/L), mean corpuscular volume (MCV, fL), and clinical outcomes (anemia prevalence and acute pain crises).

Aim 2. To assess the ***potential harm*** of high-dose folic acid supplementation by measuring the effect of 12-wk of 1 mg/d folic acid in children with SCD, compared to placebo.

- Primary outcome: plasma UMFA concentrations (nmol/L).
- Secondary outcomes: proportions of UMFA of total blood, proportions of children with UMFA >0.2 nmol/L, plasma S-adenosyl-methionine, S-adenosyl-homocysteine, and homocysteine concentrations (μmol/L).

Adherence: Adherence to the supplement regime will be assessed using a supplement diary and capsule counts.

Assessment of dietary folic acid intakes. The potential confounding bias of dietary folic acid intakes in children will be minimized due to the cross-over design of the trial (each child will serve as his/her own control). Thus, estimated dietary folic acid intakes will not be included in any statistical analyses in the cross-over trial. However, we will collect three 24-hr dietary recalls with a 5-step multiple pass method, completed at each 12-wk study period, to estimate dietary folic acid intakes in children (60) and to estimate the amount of folic acid that is coming from fortified food sources in the diet. We will use food models to increase the accuracy of food portion estimates. Total dietary folate equivalents (DFEs) will be calculated, which takes into account the differences in bioavailability of dietary folate and synthetic folic acid (1 DFE = 1 μg dietary folate or 0.6 μg folic acid added to food) (61).

Blood collection: A 3-hr fasting venous blood sample will be collected at British Columbia Children's Hospital. A 3-hr fast is needed to avoid the confounding influence of recent food or synthetic folic acid intakes on blood folate levels (8). We will advise caregivers in advance for children to not to take the supplement/placebo the day of the blood collection.

Biochemical folate status:

- **Plasma methylmalonic acid (MMA):** a marker of vitamin B12 status (62), will be measured using LC-MS (18,63) and *pyridoxal-5'-phosphate* (nmol/L), a marker of vitamin B6, will be measured using HPLC (64).
- **Complete blood count** (including *hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, reticulocyte count,* and *red cell distribution width*): will be performed using an automated hematology analyzer (Sysmex XNL550, Kobe, Japan).
- **Gene variants associated with folate metabolism:** Genomic DNA will be extracted from buffy coat using the QiaAmp Blood DNA kit. *MTHFR* (677 C>T, *rs*1801133, and 1298 A>C, *rs*1801131) and *DHFR* (*rs*1643649 and *rs*70991108) variants (54) will be detected using PCR (65,66).
- **Plasma and RBC total folate**: Plasma or serum folate (nmol/L) reflects recent status or dietary intake; RBC folate indicates longer term status (e.g. previous 3-4 months). We will assess serum and RBC total folate using two methods, as globally recommended (67,68): plasma folate using a microbiological assay (69) and RBC folate using LC-MS (70). *Plasma UMFA* and *RBC folate forms* (THF, 5-methyl-THF, 5-formyl-THF, and 5,10-methenyl-THF) will be measured using LC-MS (70). UMFA is not incorporated into RBCs; rather, it accumulates and circulates in the plasma (28). Thus, UMFA will be measured in plasma.
- **Folate metabolites**: S-adenosyl-methionine, S-adenosyl-homocysteine, and homocysteine (71,72) using LC-MS (73,74).

IMPACT OF THE RESEARCH

One in every 2,500 babies in Canada will be born with SCD (75), and incidence continues to rise with increased global migration to Canada (76,77). High gene frequencies of the sickle cell variant in tropical countries are a result of the conferred protection against malaria (78). Prevalence has been steadily increasingly in the West, as migration from tropical countries increases rapidly. The global number of migrants with sickle cell variants increased from ~1.6 million in 1960, to ~3.6 million in 2000 (76), and Canada could see further increases with proposed immigration policy changes in the USA. Ultimately, this translates to an increasing disease burden among families and the Canadian health care system (76,77). This trial will contribute to the evidence base for safe and effective nutritional therapy for Canadian children with SCD.

SAMPLE SIZE

Sample size was estimated for primary outcome in Aim 1 (efficacy) in consultation with a clinical epidemiologist (Dr. Joel Singer) and biostatistician (Dr. Arianne Albert, BC Women's Hospital Research Institute). We used data from the recent Nguyen et al. study in the USA to estimate means and SD for RBC folate from children with SCD who were discontinued 1 mg/d folic acid (6). Children in the Nguyen et al. study are similar to our clinical population in terms of folic acid fortification policy and clinical management (e.g. hydroxyurea adherence). A non-inferiority approach was used to determine the sample size needed to assess the efficacy of 1 mg/d folic acid as compared to the placebo. Non-inferiority is the ideal approach given that treatment (supplementation) may be unnecessary in children and folate concentrations are speculated to be high regardless of treatment group. Assuming a SD in RBC folate of 150 ng/mL, a difference in means between groups of 30 ng/mL (Nguyen et al.), power of 0.8, alpha of 0.05, and a non-inferiority margin of 100 ng/mL, we would need 28 children (14 in each treatment order) to confirm that the placebo is at most, 100 ng/mL lower than the folic-acid group.

We rounded up to 36 children (18 in each treatment order) to account for attrition and to be powered to detect clinically meaningful differences in our secondary outcome (UMFA concentrations).

STATISTICAL ANALYSES

Analyses will be performed using Stata 15 (Stata Corp, Texas) in consultation with our biostatistician (Dr. Albert). Two-sided p-values less than 0.05 will indicate statistical significance.

Aim 1 (efficacy): First, we will confirm that the washout period was long enough to rule out a carryover effect by conducting a preliminary test: the sum of the values measured in the two periods (for RBC folate concentration) will be calculated for each subject and compared across the two sequence groups by means of a test for independent samples (59). The 95% CI of the difference in means between treatments will be compared to the non-inferiority margin. If it does not exceed the margin, then we will support the hypothesis that placebo is non-inferior to supplementation (59).

Aim 2 (potential harm): We will repeat the aforementioned analyses for our secondary outcomes (UMFA concentrations). We will also consider plasma UMFA >0.2 nmol/L as 'detectable' levels of UMFA (as reported in previous studies (47–49)) and will calculate the proportion of children with concentrations >0.2 nmol/L in each group. We will also measure and compare plasma UMFA as a proportion of total blood (% of total blood) across groups. Lastly, we will compare blood folate forms across the groups in proportions of total folate, reduced folate forms, 5-methyl-THF, and UMFA. The 'total folate' group will represent the sum of THF, 5-methyl-THF, 5,10-methyl-THF, 5-formyl-THF, and UMFA. The 'reduced folate' group will represent the sum of THF, 5-methyl-THF, 5,10-methenyl-THF, and 5-formyl-THF.

POTENTIAL RISK

The supplementation dose (1 mg/d folic acid) is standard clinical practice for children with SCD; thus, poses no additional risk of harm. During periods when no supplementation is provided (placebo and wash-out periods) there is the potential for folate deficiency to contribute to the development of megaloblastic anemia, even though rates of folate deficiency in the Canadian population has been reported to be less than 1% (46), due to fortification of our food system. In order to monitor for this risk, complete blood counts will be measured at each study period end-point to ensure participants do not develop megaloblastic anemia as a result of withholding supplements. Any participant that does develop megaloblastic anemia during a treatment period will have a serum folate measurement completed, via immunoassay, to determine if the cause of the megaloblastic anemia is related to folate deficiency. Participants who are found to have folate deficiency (<10 nmol/L) as a result of the intervention will have these results reported to the Data Safety Monitoring Board (DSMB) and will be withdrawn from the study to return to receiving folic acid supplementation, as prescribed by their physician according to the current standard of care.

Independent safety monitoring for the duration of the study will be continuous, with all adverse outcomes (pain crises and/or development of megaloblastic anemia based on complete blood counts) being reported at the end of each 12 week time period, according to protocol to our established, independent DSMB. Membership of the DSMB consists of: Dr. Nicholas Au (Hematopathology, BC Children's Hospital), Dr. Douglas Morrison (Medical Director of Transfusion Medicine Services, BC Women's and Children's Hospital), and Dr. Amanda Li (Hematology, Oncology, and Bone Marrow Transplant, BC Children's Hospital).

In addition to continuous reporting of adverse events to the DSMB, a full review of complete blood counts to determine megaloblastic anemia prevalence and occurrence of acute pain crises, in comparison with baseline measures, will be completed after a quarter of the participants (n=9) have completed the first 12-week treatment period to ensure the safety of the placebo intervention.

POTENTIAL BENEFITS

While there are no direct benefits for participation in the study, to account for the potential additional expenses that families may incur for extended clinic visits (due to study activities) and completion of non-routine study blood work, all families will be eligible for reimbursement of parking and gasoline expenses up to $25 per visit, upon submission of receipts.

INNOVATION AND ORIGINALITY

Effectiveness of folic acid supplementation in SCD was largely investigated in the 1960-70s; the evidence was inconclusive and is now outdated, given the advances in medical care of SCD over the past several decades (37–41). There is one randomized trial conducted to date: a 1983 trial among 117 Jamaican children with SCD examined the effect of 5 mg/d folic acid versus a placebo (42). Although authors observed an increase in serum folate after one year, no effect on hemoglobin, growth, or other clinical events was observed (42). However, the study had many limitations and a high risk of bias; thus, it is difficult to draw conclusions from this trial (3). *No well-designed randomized controlled trial of folic acid supplementation on individuals with SCD has been conducted to date*. Given the mandated fortification policy in Canada in 1998 (folic acid fortification) and recent advances in medical care (e.g. drug development of hydroxyurea), reassessment of this clinical practice guideline is warranted.

The assessment of UMFA and the different folate forms as potential biomarkers of harm has been recently investigated in pregnant and lactating women consuming prenatal folic acid supplements (47–

49,79), but to our knowledge, has not been investigated in children with SCD receiving high-dose folic acid supplements. This is a novel research question and we have assembled a team of experts who are well positioned to advance this area of research. Further, given our national folic acid fortification policy, this trial is specifically warranted in Canada.

Figures:

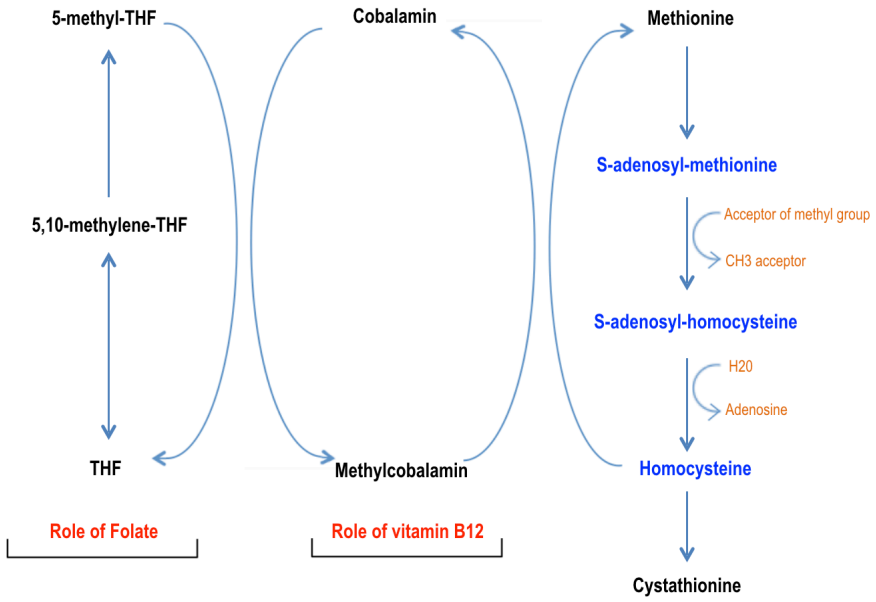

Figure 1: Homocysteine metabolism and the role of folate and vitamin B12

S-adenosyl-methionine, **S-adenosyl-homocysteine**, and **homocysteine** are important functional markers of folate metabolism. Deficiencies or excess of folate, vitamin B12, or the functional markers in the pathway, can cause abhorrent DNA methylation, elevated homocysteine concentrations (causing increased risk of endothelial damage), and other adverse outcomes.

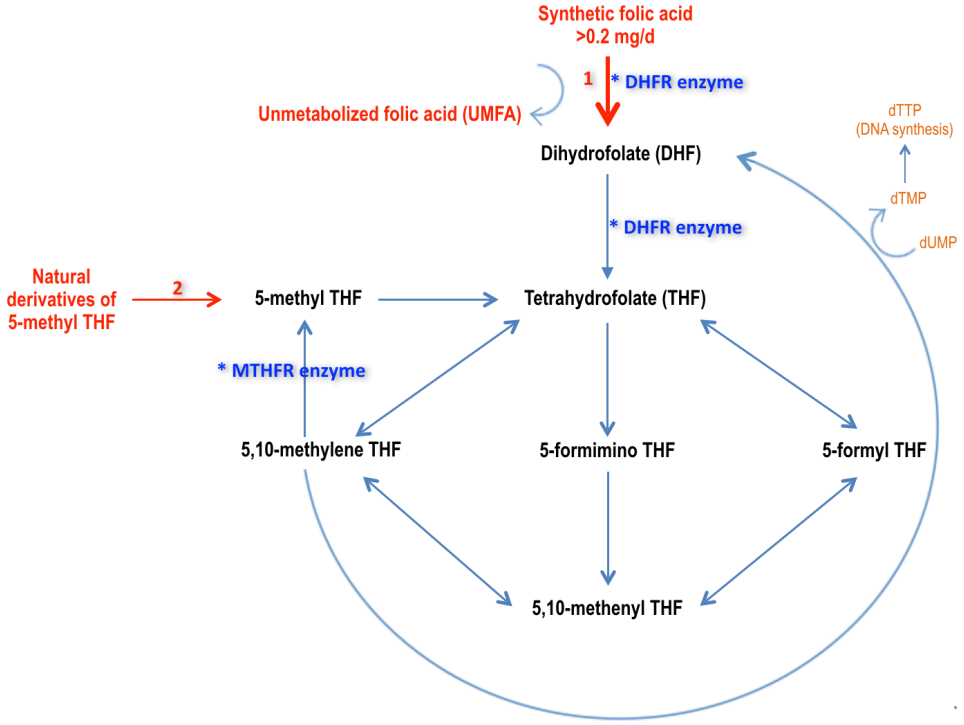

Figure 2: Folate metabolism

1 - With doses of **synthetic folic acid >0.2 mg/d**, unmetabolized folic acid (UMFA) begins to accumulate in the blood. Excess synthetic folic acid can interfere with normal folate metabolism and mask the symptoms of B12 deficiency.
2 - Supplementation with the **natural derivative of 5-methyl THF** does not require reduction or enzymatic activity (it is readily available for entry into the folate metabolism cycle) and does not interfere with folate metabolism.

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