Interaction between excess folate and low vitamin B12 status

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Abstract

Current epidemiological evidence suggests that an imbalance of high folate status and low vitamin B12 status is associated with negative health outcomes in older adults and children. Such an imbalance during pregnancy also predisposes women to diabetes and their offspring to insulin resistance and adiposity and low birthweight. In older adults, vitamin B12 status can remain low despite adequate intake due to age-related decline in vitamin B12 absorption. Pregnant women are exposed to folic acid at varying doses depending on the prenatal care prescribed in different countries. This review summarizes the current knowledge on the interaction between folate and vitamin B12 and the associated health outcomes.

Keywords: Folate, folic acid, vitamin B12, cobalamin

1. Introduction to folate and vitamin B12

The B vitamins folate and vitamin B12 play central roles in one carbon metabolic pathway. One carbon pathway generates precursors for nucleotide biosynthesis and methyl groups for methylation reactions. Conversion of homocysteine to methionine using methyl group from 5-methyl tetrahydrofolate by methionine synthase is a vitamin B12-dependent reaction. In the absence of sufficient vitamin B12, 5-methyl tetrahydrofolate remains trapped in this form, since the synthesis of 5-methyl tetrahydrofolate from 5, 10-methylene tetrahydrofolate is an irreversible reaction. This reduces availability of nucleotide precursors for DNA synthesis prior to cell division, resulting in megaloblastic anemia, which is one of the clinical outcomes associated with vitamin B12 deficiency. When sufficient folate is available, the 'methyltrap' is overcome and anemia is resolved even if vitamin B12 deficiency persists. This phenomenon was observed in the early 20th century when folic acid became available for treatment of pernicious anemia. In pernicious anemia, patients cannot absorb vitamin B12 from diet due to lack of intrinsic factor. Untreated, this condition can result in neurological degeneration. Patients with pernicious anemia were treated with high doses of folic acid, which resolved their anemia, but since vitamin B12 deficiency was left untreated, it resulted in subacute combined degeneration of spinal cord (Ross et al. 1948). The frequency of development or progression of spinal cord degeneration was higher in those received 10-15 mg/d of folic acid, when compared to those who received 5 mg/d or less suggesting a deleterious effect of large doses of folic acid (Ross et al. 1948). When the dosage for folic acid fortification of cereal grains was being debated, one of the issues under consideration was the possibility that vitamin B12 deficiency will go undetected for a longer period of time. In the past decade, many studies have reported an adverse interaction between high folate and low vitamin B12 status in older adults, children and pregnant women. In older adults, vitamin B12 status can remain low despite adequate intake due to age-related decline in vitamin B12 absorption. Pregnant women are exposed to folic acid at varying doses depending on the prenatal care prescribed in different countries. This review summarizes the current

knowledge on the interaction between folate and vitamin B12 and the associated health outcomes.

2. Folate status and biochemical markers of vitamin B12 insufficiency

There are 2 vitamin B12 dependent reactions in mammalian cells; conversion of methyl malonyl CoA to succinyl CoA by methyl malonyl CoA mutase, and methylation of homocysteine to form methionine by methionine synthase. Vitamin B12 deficiency results in increase in the concentrations of functional biochemical markers of vitamin B12 status namely, homocysteine and methylmalonic acid, in circulation. Selhub et al (Selhub et al. 2007) determined if the improved folate status due to consumption of fortified foods and supplement use is associated with worsening of biochemical indicators of vitamin B12 deficiency using data from National Health and Nutrition Examination Survey (NHANES) of the general US population aged 20 y and older, collected from NHANES 1991-1994 (n=4940) and 1999-2002 (n=5473). The results of this study showed that in vitamin B12 deficiency (<148 pmol/L) higher plasma folate was associated with higher concentrations of homocysteine and methyl malonic acid. The trend for higher concentration of biomarkers was observed with increase in plasma folate above approximately 20 nmol/L. The results of this study were confirmed in a second cohort that had been exposed to folic acid fortification (Sacramento Area Latino Study on Aging, n=1535), among individuals ≥ 60 y (Miller et al. 2009). The study participants were divided into 4 groups based on plasma concentrations of vitamin B12 (<148 or ≥ 148 pmol/L) and plasma folate ($\leq$ or >45.3 nmol/L). When the 4 groups were compared, participants with low vitamin B12 and elevated plasma folate (>45.3 nmol/L) had the highest concentration of homocysteine and methyl malonic acid and lowest concentration of holotranscobalamin in plasma. This was true even when comparison was made between the 2 groups with low vitamin B12, but elevated vs non elevated plasma folate. Individuals with the elevated plasma folate also had significantly higher RBC folate in this cohort. Thus the combination of low vitamin B12 and high plasma folate in

this cohort is not the result of methyl-trap of folate resulting in accumulation of monoglutamate form of folate that is then detected in plasma.

In an intervention trial of vitamin B12 deficient elders conducted in Chile (Brito et al. 2016), those who had vitamin B12 below 148 pmol/L were given a single intramuscular injection of 10 mg cyanocobalamin (along with 100 mg pyridoxine and 100 mg thiamine). This treatment improved vitamin B12 status in the subjects as determined by a combination of serum vitamin B12, homocysteine, methylmalonic acid and holotranscobalamin concentrations. However, the improvement of vitamin B12 status was lower in those with serum folate ≥ 33.9 nmol/L when compared to those with serum folate below 33.9 nmol/L. In Chile flour is fortified with 220 μ g/100g as opposed to 150 μ g/100g in the US, and the mean folate intake among the subjects was 652 ± 265 μ g DFE/d. Majority of these subjects in the study were consuming a micronutrient food supplement that provides 1.7 μ g vitamin B12/d and 72.5% were consuming vitamin B12 at Estimated Average Requirement (2 μ g/d).

Mills et al (Mills et al. 2011) investigated the effect of high folate/low vitamin B12 imbalance on metabolic indicators of vitamin B12 status in a younger cohort of 2507 university students with a mean age of approximately 22 years. The participants were divided into 4 groups based plasma concentrations of vitamin B12 (<148 or ≥ 148 pmol/L) and plasma folate ($\leq$ or >30 nmol/L). In this cohort, a combination of high folate and low vitamin B12 was not associated with higher concentrations of homocysteine or methyl malonic acid, when compared to other combinations of these two vitamins. It is possible that the metabolic abnormalities due to the imbalance between high folate and low vitamin B12 status will be more pronounced in older adults when compared to a younger population.

3. Folate status and clinical outcomes associated with vitamin B12 deficiency

3.1 Anemia

One of the concerns with high folate intake is the possibility that it can resolve vitamin B12 deficiency anemia, thus delaying a diagnosis for vitamin B12 deficiency. Neurological damage from vitamin B12 deficiency can be irreversible if not treated early. In the elderly, the symptoms of neurological damage such as cognitive dysfunction, dementia, paresthesia etc may also have other etiology.

The question of folic acid consumption resolving vitamin B12 deficiency anemia was investigated by Wyckoff and Ganji (Wyckoff and Ganji 2007) using medical records from Rush University Medical Center, Chicago in the US. A comparison of individuals with low serum vitamin B-12 ($<258\text{pmol/L}$) during pre, peri and post-folic acid fortification periods ($n=86$, 138 and 409 respectively) showed that the mean corpuscular volume was significantly lower in post fortification period when compared to peri and pre fortification periods. The odds ratio for having low concentration of vitamin B12 in serum in the absence of macrocytosis was 3.0 (CI: $1.7, 5.2$) among those examined from post-fortification period when compared to pre-fortification period. This cohort included individuals ≥ 19 y old, however 58.5% of the individuals were ≥ 65 years old. Information regarding folate status or intake was not available in this cohort.

Other studies have not seen a difference in prevalence of anemia and macrocytosis in the presence of low plasma or serum vitamin B12 status after folic acid fortification. Among the participants of National Health and Nutrition Examination Survey (NHANES) of general US population >50 y old, there was no increase in prevalence of serum vitamin B12 deficiency ($<148\text{pmol/L}$) in the absence of anemia or macrocytosis after folic acid fortification when compared to that prior to fortification (Qi et al. 2014). In Chile, 108 elderly subjects aged ≥ 70 y were studied before and after folic acid fortification (Hirsch et al. 2002). Prior to fortification vitamin B12 deficiency was present in 27.6% of the population and this remained unchanged after fortification. In this cohort, MCV increased from 89.6 ± 4.7 to $90.6 \pm 4.4\text{ fL}$ after folic acid

fortification, suggesting that increase in folic acid consumption did not result in resolution of vitamin B12 deficiency anemia in this cohort.

In an analysis of 1573 patients with vitamin B12 below 258 pmol/L in the Veteran's affairs Medical Center in Washington DC, the proportion of patients with low vitamin B12, but without anemia did not change between the periods before during and after fortification (Mills et al. 2003). When the cut off was lowered to 150 pmol/L there was still no difference in patients without anemia pre, peri and post-fortification periods, but the sample size for each period dropped to 50 patients or less. However the total folate intake or plasma folate of this cohort was not considered in the analysis. In this cohort, individuals younger than 60 years of age were more likely than older individuals to have low vitamin B12 concentration in plasma in the absence of anemia.

The above studies did not consider plasma folate status or folate consumption in their analysis. Rather than a generalized effect of folic acid fortification, analyses conducted after stratification based on the folate nutritional status measured via plasma concentration or intake might give a clearer picture of the effect of high folate on vitamin B12 deficiency anemia.

Morris et al (Morris et al. 2007) used data from senior participants ≥ 60 y from NHANES 1999-2002 (n=1459) to determine the prevalence of anemia and macrocytosis in relation to folate and vitamin B12 status. Low vitamin B12 status was indicated by plasma concentration of vitamin B12 < 148 pmol/L or elevated methyl malonic acid > 210 nmol/L in this study and high folate as > 59 nmol/L (80th percentile of the population). A combination of low vitamin B12 status and plasma folate of > 59 nmol/L (n=49) vs ≤ 59 nmol/L (n=297) was associated with increased anemia (OR: 3.1; 95% CI: 1.5, 6.6). However, since the low vitamin B12/high folate group had more multivitamin users than the other groups, it is possible that individuals with low vitamin B12 and high plasma folate have some undiagnosed vitamin B12 malabsorption such as pernicious anemia as has been previously suggested by Berry et al. (Berry et al. 2007). But, the

fact remains that those with the imbalance between folate and vitamin B12 had higher prevalence of anemia compared to those who had low vitamin B12 in the absence of elevated folate concentration.

Analysis of data from 2 cohorts in UK did not show an increase in prevalence of anemia and cognitive impairment due to the interaction between high folate status under low vitamin B12 status (defined as holotranscobalamin < 45 pmol/L) (Clarke et al. 2008). However, their study might have been underpowered due to the lower number of subjects with high plasma folate (>60 nmol/L). While there is no mandatory folic acid fortification in the UK, fortified breakfast cereals and spreads are available. In the US in addition to mandatory folic acid fortification of flour, many of the older adults take multivitamin supplements, increasing their folic acid intake and hence their plasma folate concentrations.

Effect of the imbalance between high folate status and low vitamin B12 status is generally thought to occur only in older adults. However, this interaction has been reported in school-age children (5-12 years old) in Colombia (Arsenault et al. 2009). In this cohort (n=2812) hemoglobin concentration was inversely associated with red blood cell folate, especially in children with marginal or deficient vitamin B12 status. Marginal or deficient vitamin B12 status was present in 16.6% of the children. Folic acid fortification was adopted in Colombia in 1996 at a dosage of 154 µg/100g flour. It is possible that the children who consumed more folic acid containing food also has less access to the highly bioavailable form of iron in the form of heme-iron containing food, resulting in low hemoglobin. However, the prevalence of iron deficiency in this cohort (assessed using ferritin concentration) was only 3.6%.

3.2 Cognition

Among the senior participants ≥ 60 y from NHANES 1999-2002 (n=1459) with low vitamin B12 status as indicated by plasma concentration of vitamin B12 <148 pmol/L or elevated methyl malonic acid >210 nmol/L, high plasma folate >59 nmol/L (n=42) was associated with increased

cognitive impairment (OR:2.6; 95% CI:1.1, 6.1) when compared to those with plasma folate ≤ 59 nmol/L (n=253) (Morris et al. 2007). In the original participants of Framingham Heart Study cohort who had not been exposed to folic acid fortification at the time of data and sample collection (n=549), a combination of low vitamin B12 status (<258 pmol/L) and plasma folate >20.2 nmol/L was associated with approximately 1 point decline in scores of Mini Mental State Examination that measures cognition (Morris et al. 2012). Similar results were also observed for low plasma vitamin B12 combined with use of supplemental folate (Morris et al. 2012). The authors concluded that the presence of low vitamin B12 in supplement users who hence had higher plasma folate were indicative of malabsorption of vitamin B12 leading to accelerated cognitive decline.

These results were confirmed in a study that combined data from 3 Australian cohorts (n=1354) to determine the association of vitamin B12 and serum folate status with Mini Mental State Examination scores (Moore et al. 2014). Participants with a combination of serum vitamin B12 <250 pmol/L and red cell folate >1594 nmol/L had 3.5 fold higher odds of cognitive impairment (OR 3.45; CI 1.6, 7.43) when compared to those with vitamin B12 ≥ 250 pmol/L and serum folate ≤ 1594 pmol/L. In this study, low vitamin B12 status was associated with cognitive impairment even in those with normal folate (OR:1.85; 95% CI: 1.37, 2.5) and high folate was associated with cognitive impairment even in those with normal vitamin B12 (OR:1.74; 95% CI: 1.03, 2.95) but the odds ratio for these groups were smaller when compared to the group with folate/vitamin B12 imbalance.

3.3 Peripheral neuropathy

In a recent study, we investigated the relationship between the 776 C>G polymorphism (rs1801198) in the gene for vitamin B12 transport protein transcobalamin (*TCN2*) and peripheral neuropathy in vitamin B12 sufficient elderly (Hathairat 2016). This polymorphism results in a Pro259Arg substitution in the protein and is associated with lower holotranscobalamin

concentration in plasma which may reduce the availability of vitamin B12 to tissues, even when vitamin B12 intake is adequate. The results showed that GG genotypes have higher odds for peripheral neuropathy (OR: 3.33; 95% CI: 1.15, 9.64) compared CC genotypes. When total folate intake was taken into consideration, the odds for peripheral neuropathy was 7-fold higher for the GG genotypes of *TCN2* (OR: 6.9; 95% CI: 1.31, 36.36) if they consumed more than twice the Recommended Dietary Allowance of folate (800 µg DFE/d), but not if they consumed ≤800 µg/d. The studies that have reported an interaction between high folate and low vitamin B12 status have so far depended on the plasma concentrations of the vitamin status. This raises the issue of artificially selecting a subgroup who consume multivitamins, but have vitamin B12 malabsorption and hence have higher plasma folate due to 5-methyltetrahydrofolate accumulating in plasma. All the subjects in the above mentioned study have sufficient vitamin B12 in plasma, and the adverse outcome is present in the GG genotypes only when folate intake is high, suggesting that the adverse interaction of high folate/low vitamin B12 status is not an artifact of sample selection during analysis.

4. B vitamin imbalance during pregnancy

It is standard practice to recommend supplements containing folic acid during pregnancy. In a study conducted in India, where vitamin B12 deficiency is quite common, pregnant women (n=774) were assessed for diabetes at 30 weeks of gestation (Krishnaveni et al. 2009). Vitamin B12 deficiency was present in 43% of the women and the median folate concentration was 35.4 nmol/L. In this cohort, incidence of gestational diabetes was higher among the vitamin B12 deficient women who were also in the upper tertiles of plasma folate.

Maternal nutrient status during pregnancy also has the potential to affect the health outcomes in the children. Interaction between maternal folate and vitamin B12 status and adiposity and HOMA-IR has also been reported in an Indian cohort (Yajnik et al. 2008). In this cohort (n=653), where vitamin B12 deficiency (<150 pmol/L) was prevalent among 60% of the women at

28 weeks of gestation, having higher redblood cell folate (>1144 nmol/L) or low vitamin B12 during pregnancy was associated with higher HOMA-IR in children at 6 years of age (Yajnik et al. 2008). HOMA-IR was highest among the children whose mothers had a combination of high redblood cell folate and low vitamin B12 status. Women in this study were given 100 tablets of folic acid (500 μ g/tablet) as well as iron (60mg/tablet) from 18 weeks of gestation.

In a second observational cohort of 654 mother child pairs in India, maternal plasma folate at 30 weeks of gestation was positively associated with fasting glucose in children at 5 years of age and fasting HOMA-IR in children at 9.5 and 13.5 years (Krishnaveni et al. 2014). However, in this study there was no interaction between maternal plasma folate and low vitamin B12 status (<150 pmol/L) in relation to fasting glucose, or HOMA-IR in children. The median plasma folate of this cohort of mothers was 35.4 nmol/L and the prevalence of vitamin B12 deficiency in this cohort was 41.4%. Supplement use by mothers was recorded at recruitment, but not at the time of blood collection. There was no association between supplement use at recruitment and plasma concentration of vitamin B12 and folate at the time of blood sampling in this cohort, suggesting that most of the participants may not have been taking the supplements by 30 weeks of gestation.

The interaction between folate and vitamin B12 status in mothers and the outcomes in children were also examined in an intervention trial in Nepal ($n=545$) (Stewart et al. 2011). In this study the women were given folic acid (400 μ g), folic acid with iron (60mg ferrous fumarate), folic acid+iron+zinc (30mg zinc sulfate) or multiple micronutrients containing folic acid, iron, zinc, vitamin B12 (2.6 μ g) and an additional 10 vitamins and minerals at 10 weeks of gestation. All supplements were given with 1000 RE of retinoyl palmitate and the control was vitamin A alone. Vitamin B12 deficiency was prevalent in 27% of mothers. Maternal vitamin B12 deficiency at baseline, but not at late pregnancy (32.6 ± 3.9 weeks) was associated with 26.7% increase in HOMA-IR in children. Supplementation of vitamin B12 deficient mothers with folic acid or other micronutrients did not have any effect on the children. The median folate concentration of

this cohort was 14.2 nmol/L, which was much lower than that of other studies that examined the relationship between high folate and low vitamin B12 status in mothers and the effect on offspring.

In a prospective study of pregnant women in India, low vitamin B12 intake concurrent with high supplemental folic acid in the second trimester was associated with having babies that were small for gestational age (Dwarkanath et al. 2013). Subjects were recruited at <13 week of gestation and were prescribed 5 mg/d folic acid until the end of first trimester which is a common practice in India. During the second trimester, the dose of folic acid prescribed was lower at 500µg/d, however 47% of the subjects continued taking 5mg of folic acid /d for ≥ 1 week in the second trimester. In addition to folic acid, the women were also prescribed 150 mg ferrous sulfate, 1000 mg Ca and 250 IU vitamin D3 per day. The percentage of participants non-compliant with supplement intake was lowest in the second trimester (7%), compared to first and third trimester (93% and 17% respectively).

The difference between the cohorts that demonstrate a high folate/low B12 interaction and those that do not could be the dose and duration of exposure of supplemental folic acid as well as the prevalence of vitamin B12 deficiency.

5. Possible mechanisms

It is currently not known how excess folate, mostly resulting from high folic acid intake, may interfere with vitamin B12 metabolism or worsen the health outcomes associated with vitamin B12 insufficiency. It has been suggested that folic acid may oxidize the cobalt of vitamin B12 which has to be in a highly reduced state (Cob I) to accept the methyl group from 5-methyl tetrahydrofolate (Selhub et al. 2007). However an *in vitro* study with purified methionine synthase bound to cobalamin in reduced stage (Cob I) and folic acid using stopped-flow analysis did not show oxidation of the cobalt in the enzyme by folic acid (Paul, Selhub and Matthews,

unpublished). We are currently investigating the mechanism behind the folate/vitamin B12 interaction using *in vivo* models.

6. Conclusions

Current epidemiological evidence suggests that an imbalance of high folate status or intake and low vitamin B12 status or intake is associated with negative health outcomes in older adults and children. Such an imbalance during pregnancy also predisposes women to diabetes and their offspring to insulin resistance and adiposity and low birthweight. The combination of high folate and low vitamin B12 in older adults could be the result of vitamin B12 malabsorption or non-resolution of deficiency even with supplementation, and hence the higher risk for negative health outcomes compared to those who have low vitamin B12, but not high folate. However, the same could not be said of the effect of exposure to this imbalance in utero due to folic acid supplementation during pregnancy and health effects on children, and the risk for peripheral neuropathy in those with *TCN2* polymorphism and high folate intake. It does appear that high folate status/intake may worsen conditions associated with vitamin B12 insufficiency. There is a large variation in the exposure to folic acid and the prevalence of vitamin B12 deficiency in the studies that have investigated this relationship and not all studies have shown this effect. Thus additional research is necessary to conclusively demonstrate this interaction. Since conducting such an experiment in humans would be unethical, this has to be conducted in animal models. In the meantime, the risk for the negative outcomes arising from folate/vitamin B12 imbalance maybe avoided by addressing vitamin B12 deficiency in pregnant women and the elderly.

Acknowledgments

Support from United States Department of Agriculture special cooperative agreement #58-1950-4-003. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the United States Department of Agriculture.

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