European Journal of Clinical Nutrition (2013), 1–6

www.nature.com/ejcn

REVIEW

Vitamin B12: one carbon metabolism, fetal growth and programming for chronic disease

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This review brings together human and animal studies and reviews that examine the possible role of maternal vitamin B12 (B12) on fetal growth and its programming for susceptibility to chronic disease. A selective literature review was undertaken to identify studies and reviews that investigate these issues, particularly in the context of a vegetarian diet that may be low in B12 and protein and high in carbohydrate. Evidence is accumulating that maternal B12 status influences fetal growth and development. Low maternal vitamin B12 status and protein intake are associated with increased risk of neural tube defect, low lean mass and excess adiposity, increased insulin resistance, impaired neurodevelopment and altered risk of cancer in the offspring. Vitamin B12 is a key nutrient associated with one carbon metabolic pathways related to substrate metabolism, synthesis and stability of nucleic acids and methylation of DNA which regulates gene expression. Understanding of factors regulating maternal–fetal one carbon metabolism and its role in fetal programming of non communicable diseases could help design effective interventions, starting with maternal nutrition before conception.

European Journal of Clinical Nutrition advance online publication, 13 November 2013; doi:10.1038/ejcn.2013.232

Keywords: vitamin B12; methyl donor; one-carbon metabolism; fetal growth; programming; chronic disease

INTRODUCTION

Across the life course, the dietary supply of the methyl donors: folate, vitamin B12, betaine, methionine and choline, is essential for normal growth, development and physiological functions. One carbon metabolism refers to a network of interrelated biochemical pathways that donate and regenerate one–carbon units, including the methyl group (Figure 1). Maternal diet is the primary source of nutrient availability to the conceptus,[1,2] and the placenta has a vital regulatory role.[3] The developmental pathway of the child defines it's phenotype and the balance between future health and disease.[4] Critical periods of cell division and differentiation occur *in utero*.[5] Optimal organogenesis, growth and development of the foetus is dependent on the maternal diet and supply of nutrients, including the methyl donors.

Folate, the key methyl donor, has been extensively studied and world-wide there is recommendation for supplementation of women who plan to become pregnant. In more than 50 countries fortification of the food supply with folate is mandated but the extent of implementation and effectiveness vary.[6] On the other hand, vitamin B12 is often deficient in pregnant women who are ovo-lacto vegetarians or eat little or no meat,[7] and this continues to be a major nutritional problem in parts of the world where the population is predominantly vegetarian. Even in countries, such as Canada, where fortification has been effective there remains a residual problem with B12 deficiency.[8]

MATERIALS AND METHODS

We performed a selective literature review and identified studies and reviews that investigated the association of maternal B12 status with metabolic pathways and future health of offspring. Over 250 articles were identified, and we selected those that focussed on Vitamin B12, one carbon metabolism, fetal growth and programming for chronic disease and, where possible, were published in the last 10 years.

ONE CARBON METABOLIC PATHWAYS

The importance of dietary methyl donors, and in particular vitamin B12, the subject of this review, requires a broad understanding of the exquisite interrelationship and balances of one carbon metabolic pathways. A figure (Figure 1) integrating metabolic pathways involved in one carbon metabolism was constructed.

One carbon metabolism describes reactions including the addition, transfer or removal of 1-C units in cellular metabolic pathways. The central methylation pathway, the methylation cycle (Figure 1) occurs in the cytoplasm of every cell where the formation of S-adenosyl methionine from adenosine triphosphate and methionine is catalysed by methionine adenosyl transferase. In turn, S-adenosyl methionine is converted to S-adenosyl homocysteine and then to homocysteine. Methionine is regenerated when a methyl group from 5-CH3-tetrahydofolate is transferred to homocysteine. This last step of the cycle requires the presence of vitamin B12 as a cofactor for methionine synthase. When concentrations of available vitamin B12 are insufficient, folate becomes trapped as 5-methyltretrahydrofolate, and the regeneration of methionine is inhibited, and the concentrations of homocysteine and its metabolites increased.[9] Methionine deficiency is a commonly used animal model to demonstrate intrauterine growth retardation and fatty liver disease.[10]

Raised concentrations of homocysteine within the cell are toxic and are actively regulated. Metabolism of homocysteine occurs through intersecting enzymatic pathways: (1) remethylation,

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Received 20 March 2013; revised 7 October 2013; accepted 10 October 2013

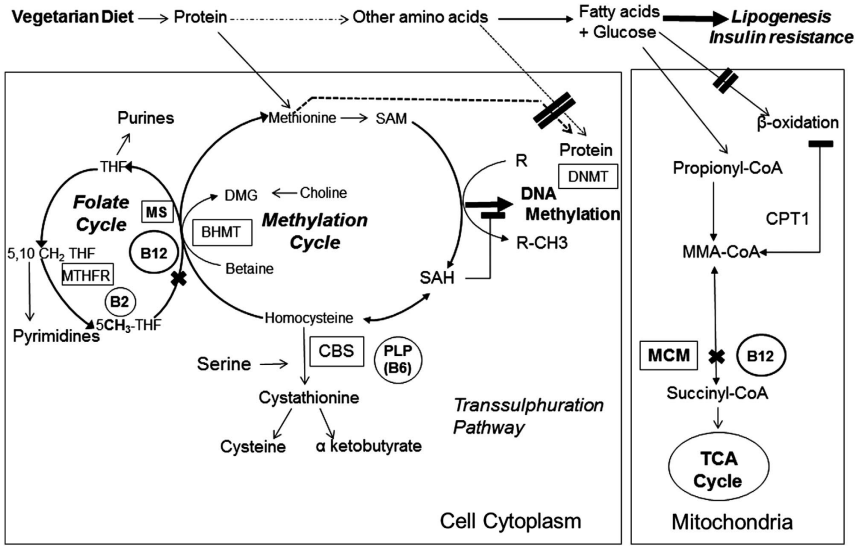

Figure 1. One carbon metabolic pathways, vegetarian diet and effects of B12 insufficiency: X blocked; ‖ 'secondarily' inhibited; → stimulated; ⊣ inhibited by metabolite. BHMT, Betaine-homocysteine S-methyltransferase; CPT1, Carnitine palmitoyltransferase; CBS, Cystathionine-β-synthase; DNMT, DNA methyltransferase; GNMT, Glycine N-methyltransferase; MCM, Methylmalonyl-CoA mutase; MMA-CoA, Methylmalonyl-CoA; MTR, Methionine synthase; MTHFR, ethylenetetrahydrofolate reductase; MS, Methionine Synthase; R Methyl acceptors, including adenosine and cytosine; R-CH3 Methylated acceptor; SAH, *S*-adenosyl homocysteine; SAM, *S*-adenosyl methionine; THF, Tetrahydrofolate.

which requires vitamins B12 and B2, (2) transsulphuration, which requires vitamin B6 and (3) the catalysis by betaine-homocysteine methyltransferase of the transfer of one of the amino groups of betaine to homocysteine to form methionine. The importance of labile methyl groups and homocysteine remethylation in liver function and diabetes has been demonstrated in humans and animals.[11]

The remethylation of homocysteine to methionine intersects with the folate cycle where methylenetetrahydrofolate reductase catalyses the reduction of 5,10 methyl tetrahydrofolate to 5 methyl tetrahydrofolate, which is a co-substrate for the methylation of homocysteine to methionine (Figure). Folate is the methyl acceptor and donor in this cycle where methionine is regenerated. The folate cycle is essential for purine and pyrimidine nucleotide synthesis, which are essential for the formation and stability of DNA, RNA and nucleoside triphosphates such as adenosine triphosphate.[12,13]

A critical step in the central methylation cycle is where S-adenosyl methionine serves as a methyl donor and is converted to S-adenosyl homocysteine. Methyl groups may be added to many methyl acceptor substrates including DNA. Methylation of DNA nucleotides is an important epigenetic mechanism for control of gene expression. This control of gene expression is particularly important during critical periods of growth and development and may help explain why nutritional imbalances are associated with fetal phenotypes, which increase the risk for subsequent diseases.[14]

In liver, kidney, small intestine and pancreas, homocysteine may enter the trans-sulphuration pathway (lower panel, Figure 1) and be converted to cystathionine by the addition of serine. The active form of vitamin B6, pyridoxal-5-phosphate, is a cofactor for this step.[15]

In the mitochondria, β-oxidation of fatty acids requires that they are sequentially broken down into small even number carbon units, the units are linked with coenzyme A (CoA) and are then able to enter the tricarboxylic acid (TCA) cycle (Figure 1). The TCA cycle is responsible for the ultimate oxidation of acetyl (2-carbon) groups derived from lipids to carbon dioxide, water and energy. However, breakdown of uneven number carbon unit fatty acids results in the formation of propionyl-CoA, a three carbon unit. Propionyl-CoA is carboxylated to methylmalonyl CoA which then reversibly isomerizes to succinyl-CoA by the B12 dependent enzyme, methyl malonyl-CoA mutase. Deficiency of B12 blocks the production of succinyl-CoA, and leads to elevated methyl malonic acid (MMA) and MMACoA. Increased concentrations of malonyl CoA inhibit the activity of carnitine palmitoyltransferase (CPT1) the enzyme that controls the rate of long chain fatty acyl-CoA transfer into the mitochondria. The outcome is inhibition of β-oxidation. Thus, there is accumulation of fatty acids in the cytosol of the nucleus and increased inclusion of fatty acids into glycerolipids.

Thus, vitamin B12 influences folate-dependant reactions and mitochondrial energy and lipid metabolic pathways.

B12 absorption, transport, storage and biomarkers

In food, B12 is bound to protein. This bond is released by the action of gastric pepsin and acid and B12 binds to other proteins: R binders (haptocorrins) secreted in saliva. The gastric parietal cells secrete acid and a 50-kD glycoprotein called intrinsic factor. In the small intestine, the R-binders are hydrolyzed by pancreatic proteases and then the freed vitamin B12 binds to intrinsic factor. Most absorption of vitamin B12 occurs in the distal ileum via a specific receptor-mediated endocytotic process. The intrinsic factor is degraded in the cell lysosomes, and vitamin B12 is released into the cytosol of the gut epithelial cells. Vitamin B12 is released from these cells into intercellular fluid as a complex bound to a 38-kDa protein called (holo) transcobalamin II (TC-II). Most of the dietary B12 is absorbed in this way with a further 1% by passive diffusion.[16] High oral doses of B12 will have proportionately greater absorption by diffusion. Two additional B12-binding glycoproteins TC-I and TC-III are less specific and have a slower rate of turnover than TC-II and will bind inactive B12 analogues. Accordingly, TC-II is considered the best biomarker of active or available B12.

The liver may store up to 3 mg of B12, which is sufficient without repletion for 3–5 years. Efficient enterohepatic recycling of vitamin B12 ensures that loss of B12 is minimal. Yet while most dietary recommendations are for more than 2.4 μg/day of vitamin B12, the adequacy and practicality of this recommendation for optimal health needs of those whose diets do not include good sources of the vitamin need to be investigated further.[17]

As the liver stores of vitamin B12 become depleted TC-II concentration will start to fall before any decline of total serum B12 concentrations are observed. Therefore, TC-II is considered a more sensitive and early indicator of B12 insufficiency.[18] Conversely, with the repletion of vitamin B12, TC-II concentrations have been shown to rise rapidly,[19,20]and this fact has been used to design a test to measure absorption of vitamin B12.[21]

Also considered reliable markers of B12 insufficiency are elevated serum concentrations of homocysteine and MMA.[22] Elevated homocysteine is a non-specific marker influenced both by B12 and folate insufficiency. On the other hand, plasma MMA is a specific biomarker of B12 status.

Hypomethylation and altered gene expression

Alterations in the supply of one carbon units could influence DNA methylation and therefore gene expression by determining which genes are switched on and off and when.[12] Evidence from sheep models[12] and growing evidence in humans[2] suggests that environmental insults, particularly of availability of folate and B12 *in utero*, lead to differences in DNA methylation in the offspring. The patterns of DNA methylation that are established *in utero* could induce stable changes in gene expression lasting through the life of the individual. These epigenetic alterations can have profound and life-long effects on structure and function (phenotype). The cell cycle may be switched from proliferation to differentiation with adverse consequences for total cell number and function.[23] There is evidence that genetic polymorphism of the methylenetetrahydrofolate reductase modulates genomic DNA methylation.[24] Therefore, it is tempting to speculate that the balance of nutrient intake might ultimately affect patterns of epigenetic modifications such as DNA methylation in a population- and individual-specific manner.[25] This may help explain the associations of impaired intrauterine growth with the programming of the fetal endocrine and cardiovascular systems with consequences later in life.[26,27] Epigenetic changes related to one carbon metabolism and B12 status, for example, alteration in promotor methylation in cancer-relevant genes, have been associated with altered risk for cancer in offspring.[28]

Reduction in protein synthesis and deposition

Vitamin B12 is only derived from animal and microbial foods, and a vegetarian diet therefore contains little vitamin B12. For many Indian populations, religious and cultural beliefs and socioeconomic factors[29] contribute to low intakes of animal products, legumes and protein.[30] Such a diet is also a poor source of the essential amino acid, methionine.

Methionine is central to protein synthesis because its genetic codon is the most common start message for translation from mRNA. The methylation cycle is influenced by nutrients (methionine, B12, folate, B2 and B6) and hormones (insulin and glucagon) and by changes in redox state.[31] A plant-based diet may be insufficient in methionine and other essential amino acids and has been associated with hyperhomocysteinemia, reduced lean mass and increased fat.[32]

Lipogenesis and insulin resistance

There is some evidence that low B12 status in children is associated with increased lipogenesis, obesity and insulin resistance.[10,31,33,34] The possible biochemical-metabolic basis has been described above. Increased lipogenesis within the myocyte may be associated with increased intracellular accumulation of triglycerides, which has been associated with insulin resistance,[35] obesity and type 2 diabetes in some studies. Furthermore, from birth, Indian individuals have relatively more fat and less muscle compared to other ethnic groups,[36–38] and this could be associated with intergenerational dietary practices that are traditionally low in animal products, B12[29,39] and protein.[40] The reasons for this common dietary pattern include religious and personal beliefs, cultural practices and poverty.[29,39,41–44]

FETAL PROGRAMMING: CONCEPTS AND EVIDENCE

Studies have shown that children born during the Dutch Hunger Winter were at increased risk of non communicable diseases.[45] This is thought to be related to epigenetic changes induced by maternal undernutrition. Children conceived during the winter hunger had less DNA methylation of the imprinted IGF2 gene compared with their unexposed, same-sex siblings when studied six decades later. Other studies have reported that maternal folic acid supplementation in the periconceptional period was associated with increased methylation of IGF 2 in the offspring.[46]

Waterland *et al.*[47] studied establishment of metastable epialleles in early life development by comparing differences in DNA methylation by season of conception (dry or rainy). At the five loci investigated, conception during the rainy season resulted in significantly more methylation of DNA. Studying the influence of early environmental exposures on metastable epialleles and imprinted genes could offer insight into the mechanisms affecting the fetal epigenome and subsequent disease susceptibility.

Genetic stability is related to the supply of dietary one carbon nutrients in critical periods of growth. One of the most critical periods of growth is the intrauterine period[48] and the evidence for the importance of maternal-fetal B12 status on growth and programming of offspring is increasing. Selected references and reviews that relate early life nutrition to foetal growth and later function and disease are presented in Table 1, grouped by effects on body composition, neurodevelopment and metabolic pathways including insulin resistance and cancer.

Maternal nutrition and early growth

Associations of low maternal B12 concentrations and raised homocysteine with impaired foetal growth have been demonstrated in diverse populations[49–55] and have been recently reviewed.[56] Maternal whole body rate of protein metabolism is lower in the first trimester than second and third trimester[57] and the rate of transulphuration is higher during early gestation in contrast to the rate of transmethylation, which is higher in the later gestation period. This suggests a greater need for methyl donors, betaine and folate and protein in the later stages of pregnancy. Total body water and circulating blood volume increase in pregnancy, which results in hemodilution. This makes it more difficult to interpret serial changes in B12, folate and homocysteine concentrations as pregnancy proceeds.[58] Little is known about methyl donors in breastmilk, particularly for B12 deficient mothers. We do know that offspring who breastfed until at least two years of age have lower B12 and higher homocysteine concentrations than those who were weaned earlier.[59] Most of the evidence is from South Asian populations where the prevalence of vitamin B12 deficiency is high.

Neurodevelopment

Throughout the life cycle, cognitive and neurological deficits are traditionally recognized as hallmark signs of vitamin B12 deficiency.[8,41,60–65] Neural tube defects are arguably the most severe effect. As reviewed by Godbole *et al.*[66] the reported incidence of neural tube defects in India is high, that is, between 0.5–11/1000 births. This is related to the poor B12 status of Indian women.[67] The closure of the neural tube takes place by 28 days of gestation[68] so optimisation of pre and periconceptional nutrition for the mother is a target. As reviewed by Black[64] neural tube defects and other neurological problems may be partially attributed to deficits in myelination and also to increased inflammation. Myelin is 80% lipid and the mechanism for

Table 1. Selected references that provide evidence for associations of B12 with growth and development, metabolism and future health

Reference	*Foetal outcome*	*Follow up*
Maternal nutrition and early growth		
Frery *et al.*[49]	Birthweight	
Muthayya *et al.*[51]	Birthweight	
Muthayya *et al.*[52]	Birthweight	
Yajnik *et al.*[55]	Neonatal size	
Obeid and Hermann[54]	Early abortion, pregnancy complications	
Lindblad *et al.*[50]	Intrauterine growth retardation	
Molloy *et al.*[62]	Birth defects and preterm delivery	
Neurodevelopment		
Black[64]	Cognition	Depression - adult
Bhate *et al.*[65]		Cognition- 9 year old
Dror and Allen.[41]	Neurological symptoms	
Godbole *et al.*[66]	Neural tube defect	
Godbole et al.[67]	Neural tube defect and genotype	
Ray *et al.*[8]	Neural tube defect	
Molloy *et al.*[62]	Neural tube defect	
Wang *et al.*[60]	Neural tube defect	
Lovblad *et al.*[75]	Retardation of myelination	
Metabolism		
Guerra-Shinohara *et al.*[76]	Accumulation of homocysteine	
Selhub[77]	Homocysteine and pre-eclampsia	Homocysteine and cardiovascular disease
Kalhan[10]	Intrauterine growth retardation	Fatty liver disease
Obeid and Hermann[54] [a]	Prenatal health	Postnatal health
Insulin resistance		
Sinclair *et al.*[12] [a]	Adiposity and insulin resistance	
Yajnik[78]		Adiposity and insulin resistance
Yajnik and Deshmukh[71]		Adiposity and insulin resistance
Cancer		
Ciappio *et al.*[28] [a]	Maternal one carbon nutrient intake	Epigenetic, cancer risk

[a]Review

defects in myelination may be related to accumulation of MMA and myelin destabilisation.

Insulin resistance

A series of longitudinal studies in Pune, India is providing unique insights into the developmental origins of phenotypic features and associations with susceptibility for chronic disease. It has been demonstrated that newborn Indian babies, who on average are 700 g lighter than European babies, have higher subcutaneous adiposity,[38] higher levels of intra-abdominal fat[69] and higher concentrations of insulin and leptin in cord blood[70] than in European babies. Moreover, maternal vitamin B12 deficiency and high folate status are associated with offspring insulin resistance at 6 years[71] and maternal B12 is also predictive of offspring cognition at 9 years.[65] In a study[72] in Mysore, there was an intriguing association between maternal vitamin B12 deficiency, obesity and gestational diabetes. In an earlier publication from the same group[73,74] hyperglycemia during pregnancy was shown to be associated with adiposity and insulin concentration of the children at 5 years. A combination of disturbed maternal one carbon metabolism associated with a diet high in carbohydrates and low in protein and vitamin B12 and high in folate appear to be important drivers of the intergenerational amplification of the diabetes epidemic in Indians.[13]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The assistance of Joseph Ding with the biochemistry is appreciated. The authors received no support in the form of grants and/or equipment and drugs for writing this review.

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