Role of maternal vitamin B12 on the metabolic health of the offspring: a contributor to the diabetes epidemic?

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Abstract

There is increasing evidence, in humans and other mammals, that periconceptional nutritional status influences health in adulthood. This is called 'foetal programming' and is likely to be mediated through DNA methylation. Micronutrients, especially B vitamins such as folic acid and vitamin B12 play crucial roles in providing methyl groups for such reactions. This is called epigenetic regulation and may provide some clues to the epidemic of type 2 diabetes and cardiovascular disease. Evidence from mandatory folic acid fortification studies suggests that in the presence of adequate folic acid, neural tube defects due to B12 deficiency have tripled. Such 'imbalance of high folic acid and low vitamin B12' in the elderly causes cognitive impairment. A longitudinal study of young women in India showed that children born to those with 'high folic acid and low B12' had higher adiposity and insulin resistance. In addition to increased levels of folic acid, B12 deficiency is increasing in countries with mandatory folic acid fortification. Studies on the prevalence of vitamin B12 deficiency during pregnancy and in women of childbearing age, plus the effects of B12 supplementation are therefore urgently needed. This article reviews the role of vitamin B12 during pregnancy on the offspring's metabolic risk.

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Key words: cardiovascular risk, diabetes, epigenetics, foetal programming, folate, intrauterine environment, maternal vitamin B12, pregnancy

Abbreviations and acronyms

1-c	1-carbon
CVD	cardiovascular disease
MS	methionine synthase
MCM	methylmalonyl-CoA mutase
MMA	Methyl melanoic acid
SAM-e	S-adenosyl methionine
MM-CoA	methylmalonyl-CoA
NTD	neural tube defect
NHANES	National Health and Nutrition Examination Survey
CPT-1	carnitine palmitoyltransferase-1

Background

The incidence of type 2 diabetes is rapidly increasing worldwide and is reaching epidemic proportions.[1] The onset of type 2 diabetes is occurring earlier in life and the incidence of childhood type 2 diabetes is also increasing at an alarming rate.[2] This may partly be due to higher incidence of childhood obesity due to dietary factors and reduction in physical activity.[3] However, the intrauterine environment, due to maternal malnutrition (thrifty phenotype hypothesis),[4] and genetic factors affecting insulin secretion, glucose sensing and insulin resistance (foetal insulin hypothesis)[5] must also contribute to the increasing prevalence of childhood obesity as well as type 2 diabetes and CVD in adult life. Though evidence from monogenic diabetes supports the foetal insulin hypothesis this affects a relatively small number of people and is therefore an unlikely contributor to the epidemic of type 2 diabetes and CVD. On the contrary, several studies across different populations confirmed that the future metabolic risk (type 2 diabetes and CVD) is high in people born with low birth weight, especially if they become overweight adults,[6] suggesting this association is much more common and therefore unlikely to be due to genetic factors alone. It is more likely to be due to 'gene–diet' interaction during the periconceptional period. Such interaction is likely to 'programme the foetus' for the rest of its life. However, the factors and the mechanism by which such programming results in low birth weight is not clear.

Understanding the ethnic variations to the susceptibility to type 2 diabetes and CVD may offer some explanation. South Asians are at higher risk of these conditions and have distinctive adverse anthropometric (figure 1) and biochemical profile.[7,8] They also develop these conditions at an earlier age[8] and have worse adverse outcomes.[9,10] In addition, such adverse profiles seem to be present even at birth (figure 2),[11,12] suggesting that

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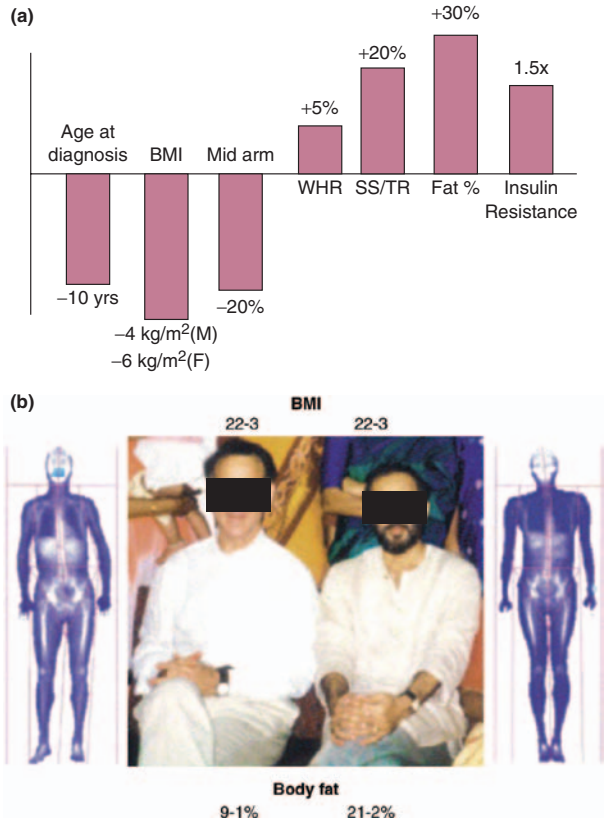
Figure 1. (a) The characteristic adverse anthropometry of South Asians with newly diagnosed type 2 diabetes. (b) Y-Y paradox: the central adiposity and higher body fat content of a South Asian compared with a Europid with the same BMI



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Key: BMI = body mass index; Fat % = total body fat content; Mid arm = mid arm skin fold thickness; SS/TR = ratio of subscapular to triceps skin fold thickness; WHR = waist–hip ratio.

Figure 2. The adverse anthropometry and cardiovascular risk factors of the South Asian children compared with Europid children despite lower birth weight and body mass index at birth

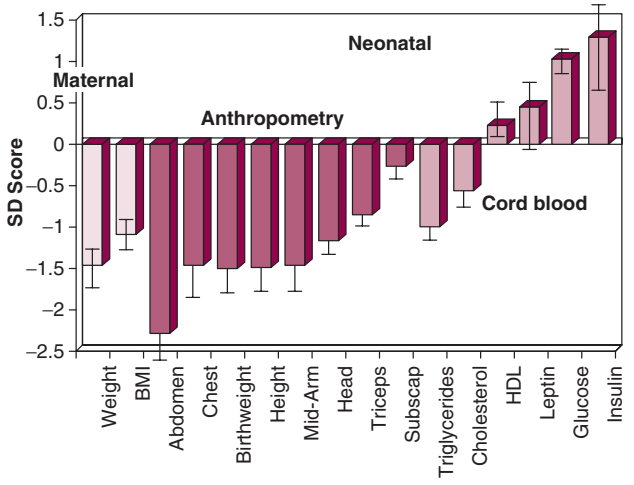


Figure reproduced with permission from Yajnik CS, et al *Int J Obes Relat Metab Disord* 2003;**27**:173–80.[11]

the often described 'genetic' and 'non-traditional risk factors' that contribute to the higher metabolic risk of South Asians may well be due to adverse prenatal or intra-uterine environment (periconceptional period). Recent evidence from a well designed longitudinal observational study suggests that micronutrients, especially folic acid and vitamin B12 may play a crucial role.[13] In addition to highlighting the importance of maternal B12 in predicting the offspring's metabolic risk, this review will also look at the potential mechanisms.

Source of folic acid and vitamin B12

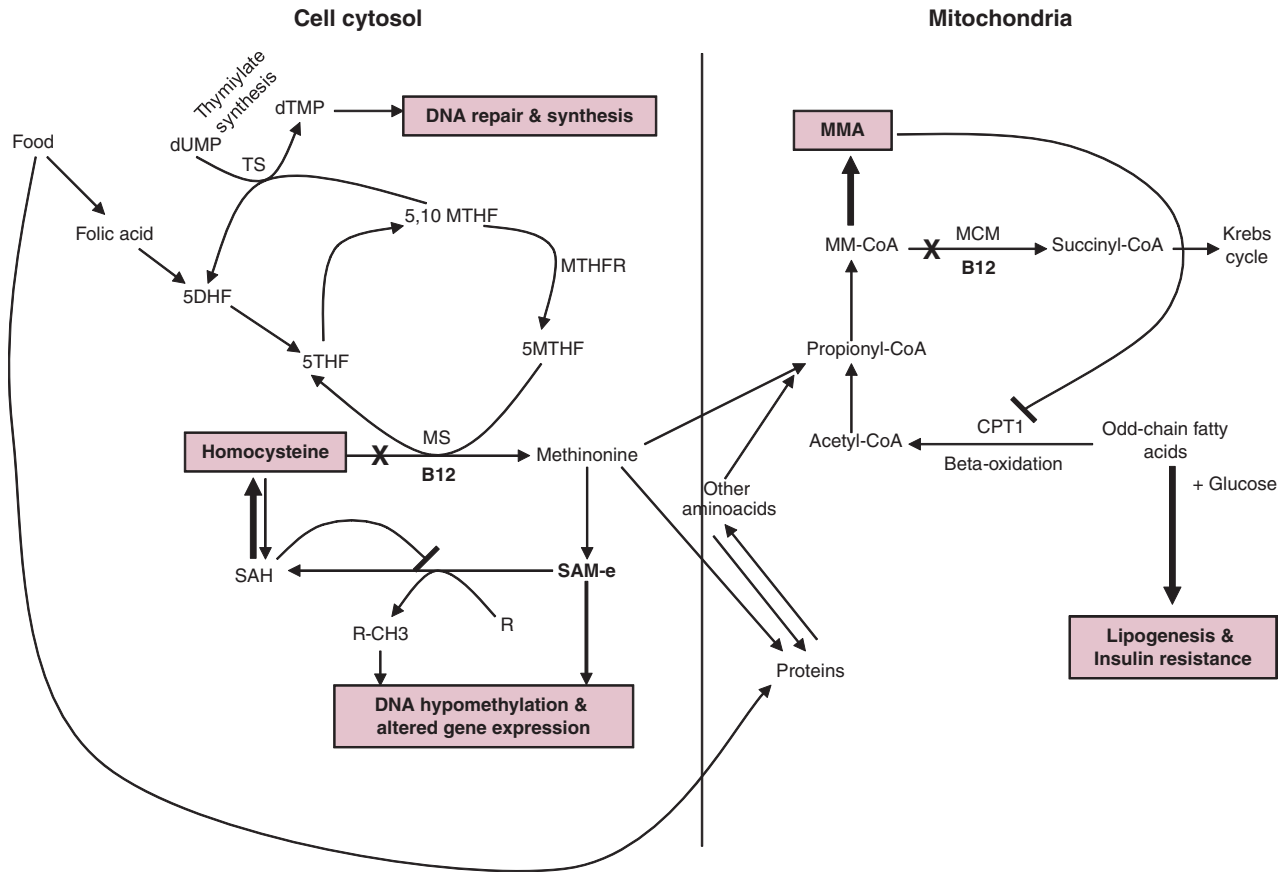
Folic acid is mainly present in green leafy vegetables and fresh fruits. It is also present in liver products and most cereals are fortified with folic acid. Folic acid deficiency is therefore rare and is mainly due to concomitant long-term use of certain drugs that interfere with its metabolism. These include anti-epileptics, antibiotics and anti-cancer drugs.

Vitamin B12 is naturally found in animal products including fish, meat, poultry, eggs, and milk products although it cannot be made by plants or animals as the only type of organisms that have the enzymes required for its synthesis are microorganisms. People of South Asian origin are particularly at risk of vitamin B12 deficiency because of a higher prevalence of vegetarianism. However, hygienic environments can destroy these microorganisms which can result in vitamin B12 deficiency in non-vegetarians as well. Maternal micronutrient deficiencies are particularly common in teenage pregnancies due to higher maternal need.[14] Poor socioeconomic status is an important factor in teenage pregnancy and therefore people from this group may also be particularly at risk of these micronutrient deficiencies.

Actions of folic acid and B12

Folic acid and vitamin B12 are essential nutrients for humans as they are necessary for new cell formation and maintenance by participating in several vital reactions.[15] Folic acid is involved in the activation, oxidation and reduction of single carbon atoms, referred to as 1-c metabolism. This folic acid dependent 1-c metabolism plays a vital role in amino acid metabolism as well as biosynthetic pathways of DNA, RNA, lipids and neurotransmitters. Vitamin B12 is an important co-enzyme for two crucial reactions: MS and MCM. MS converts homocysteine to methionine and then to SAM-e in the presence of B12 and folic acid. MCM is required for degradation

Figure 3. Pathways that involve vitamin B12 and the suggested mechanisms of increased adiposity and insulin resistance in maternal B12 deficiency



Vitamin B12 deficiency 'traps' folic acid as 5MTHF. This results in reduced synthesis of methionine and the powerful methyl donor SAM-e inside the cell cytosol. Inside the mitochondria, MM-CoA is converted into MMA, which is a potent inhibitor of beta-oxidation. Figure modified with permission from Yajnik CS, et al *Diabetologia* 2008;**51**:29–38.[13]

Key: dTMP = thymidine monophosphate; dUMP = deoxyuridine monophosphate; TS = thymidylate synthase; MTHF = methyl tetrahydrofolate; MTHFR = methylene tetrahydrofolate reductase; DHF = dihydrofolate; THF = tetrahydrofolate; MS = methionine synthase; SAH = S-adenosylhomocysteine; SAM-e = S-adenosyl methionine; R = methyl acceptor; R– = Methylated compound; MM-CoA = methylmalonyl-CoA; MCM = methylmalonyl-CoA mutase; CPT1 = carnitine palmitoyltransferase-1.

of odd-chain fatty acids and branched-chain amino acids, in particular conversion of MM-CoA to succinyl-CoA, which is an important substrate in the Krebs cycle. MM-CoA is derived from propionyl-CoA, which in turn comes from several amino acids including methionine and odd-chain fatty acids.

While the actions of MS (which happen inside the cell cytosol) are dependent on folic acid in addition to B12, the actions of MCM (which occur inside mitochondria) are dependent only on B12 (figure 3).[16] SAM-e is the common methyl donor required for DNA methylation, methylation of myelin sheath phospholipids and manufacture of neurotransmitters (e.g. serotonin) and catecholamines (e.g. dopamine).[17] In addition, the folic acid derivative, 5,10 methylene tetrahydrofolate is involved in thymidylate synthesis (DNA synthesis and DNA repair) (figure 3). Thus, both vitamin B12 and folic acid play crucial roles in the genomic stability of human cells by preventing chromosomal breakage, hypomethylation of DNA and myelin destabilisation as well as being involved in several other critical pathways during development and adult life.

Clinical manifestations of folic acid and vitamin B12 deficiency

Overt deficiency of either of these vitamins results in megaloblastic anaemia as rapid cell division in the bone marrow is impaired, especially erythropoiesis. Severe B12 deficiency also results in cognitive impairment especially in the elderly and

Figure 4. The characteristic phenotype of a South Asian baby compared to a Europid baby. Note the central adiposity and the lesser muscle bulk in the legs of the South Asian baby

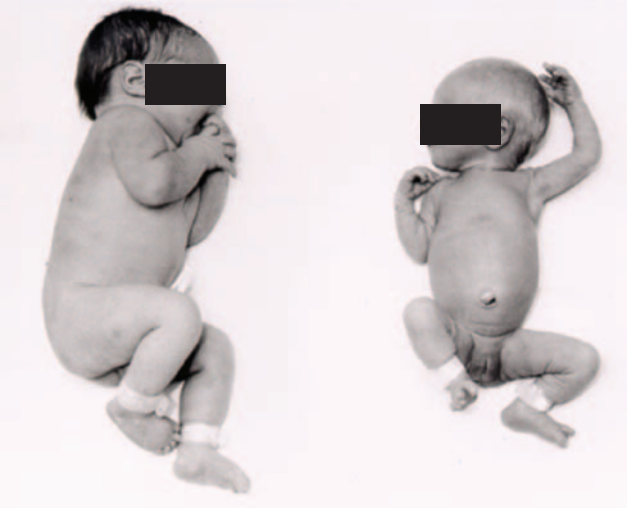
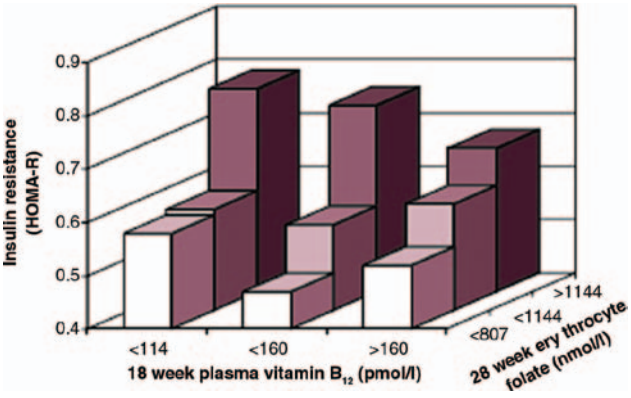


Figure 5. The impact of 'high folate/low vitamin B12'. Insulin resistance at 6 years of age is the highest in children born to mothers who had the lowest vitamin B12 and highest folic acid levels during pregnancy



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rarely subacute combined degeneration of the cord.[18] Thankfully overt deficiencies of these vitamins are rare and are diagnosed early. Folic acid deficiency in mothers is one of the major risk factors for NTDs and periconceptional supplementation reduces this risk by 50%.[19] This has resulted in mandatory folic acid fortification of wheat flour in many countries. In the UK although it is not mandatory, most breakfast cereals have fortification of folic acid. Maternal B12 deficiency is an independent risk factor for NTDs[20] and the risk is higher in folic acid sufficient individuals.[21] As folic acid deficiency is increasingly rare because of fortification of various foods, B12 deficiency has become the major modifiable risk factor for NTDs.[21]

Several recent studies report that vitamin B12 deficiency is common in infants too.[22,23] Low maternal B12 levels can result in low levels in the infants with resultant poor brain development[24] and cognitive impairment in children.[25] It has also been shown that the combination of low folate and B12 is associated with abnormal behaviour and development.[26]

In addition, deficiency of either of these vitamins cause high homocysteine levels due to lack of re-methylation of homocysteine to methionine.[27] B12 deficiency 'traps' folic acid as 5-methyltetrahyrofolate resulting in reduced synthesis of methionine and SAM-e (the most potent methyl donor) from homocysteine (figure 3).[28] Homocysteine is shown to be an independent risk factor for CVD[29] and it is believed that some of this mediated through atherosclerosis.[30] However, trials that used B-vitamins to reduce homocysteine levels have not shown a reduction in CVD.[31,32]

Lessons from homocysteine lowering studies

The randomised controlled trials using B-vitamins (folic acid, B12 and B6) did not show reductions in CVD outcomes despite significant reductions in homocysteine levels.[31,32] However, these results do not rule out the causal link between the B-vitamins and CVD. Several factors support this link: (a) these studies are done in non-deficient populations; (b) these vitamins, especially B12, may have other effects independent of homocysteine and (c) they may play a more crucial role during development rather than during adulthood. Indeed, homocysteine correlates strongly with low birth weight while folic acid has the opposite effect.[13,33] The folic acid independent effects of B12 (as a co-enzyme to MCM), in deficient states may also cause increased MMA resulting in increased lipogenesis due to inhibition of beta-oxidation by inhibiting CPT-1 (figure 3).[33] Such effects in intrauterine life will result in 'thin-fat' babies with higher body fat and lower lean mass (figure 4). These babies will in turn have higher insulin resistance and therefore be at higher risk of CVD in later life.[11,34] Thus homocysteine lowering using B12 and folic acid during periconception is likely to have a significant impact on the metabolic risk of the offspring.

Does the combination of 'high folate and low B12' matter? Lessons from folic acid fortification studies

In support of the periconceptional supplementation with folic acid, population wide fortification of wheat flour achieved similar (50%) reductions in NTDs.[20] However, the potential ill effects of excess folic acid especially in the presence of B12 deficiency have only recently been studied. In Canada, the prevalence of vitamin B12 deficiency has increased since the introduction of mandatory folic acid fortification in 1997. In addition, NTDs attributable to B12 deficiency have tripled in the same period.[21] Data from NHANES III (survey conducted following folic acid fortification in the USA) showed that in the presence of B12 deficiency, high folic acid is associated

Key messages

- Vitamin B12 and folic acid are involved in crucial pathways including DNA methylation
- DNA methylation is one of the epigenetic mechanisms resulting in foetal programming of adult diseases
- Maternal imbalance of 'high folic acid and low vitamin B12' is associated with higher metabolic risk in the offspring

with anaemia and cognitive impairment of the elderly.[35] In the Pune maternal nutrition study, the children born to mothers with the combination of 'high folic acid and low B12' had higher truncal adiposity and insulin resistance (figure 5).[13] Thus, B12 deficiency during the periconceptional period, especially in the presence of high folic acid levels, may increase NTDs as well as the offspring's future risk of type 2 diabetes and CVD. Such 'imbalance' may also increase the risk of cognitive impairment in the elderly population.

Possible mechanisms of 'imbalance of B12/folate' on the metabolic risk of offspring

It is well known that altering vital developmental processes *in utero* can predispose to metabolic diseases in adulthood.[36,37] However, until recently the mechanism of specific nutrients on 'nutrient programming' was not known.[38] Programming of gametes and pre-implantation embryos by DNA methylation highlights the possibility that periconceptional availability of methyl groups might influence such programming with resultant altered adult status.[39] As SAM-e is the most powerful methyl donor for DNA methylation (figure 3), the diets used for providing methyl groups ('methylating cocktail') contain both vitamin B12 and folic acid. Studies in rodents showed that such a 'methylating cocktail' diet during pregnancy altered DNA methylation of candidate genes (agouti, PPAR-gamma and glucocorticoid receptor) which in turn affects regulatory mechanisms of these genes throughout the life of these animals.[40,41] More recently, an elegant study by Sinclair *et al.*[42] showed that the lambs born to sheep fed a 'methyl-deficient' diet had higher adiposity, higher insulin resistance and higher blood pressure. These animal studies clearly provide evidence for epigenetic alterations of DNA methylation by these nutrients during the periconceptional period. The only human evidence for this comes from the Pune maternal nutrition study.[13] Though causality from this study cannot be proven (this would need intervention studies), given the huge impact of such intervention, this study needs urgent replication in other populations. As subclinical vitamin B12 deficiency is not uncommon[43] and given its association with NTDs, several experts call for B12 fortification in the population.[44,45] However, given the strong influence on the methylation of DNA with potential lifelong epigenetic programming, such measures should only be taken after careful evaluation of such intervention.

Conclusions and future directions

Preventing the epidemic of type 2 diabtes and CVD is a huge public health challenge worldwide which needs careful long-term planning and implementation. Foetal programming, if proven, provides a fantastic opportunity to achieve this goal. As this is mediated through DNA hypomethylation, micronutrient status (especially vitamin B12 and folic acid) of young women during their periconceptional period is of paramount importance and provides a crucial window of opportunity to intervene. However, carefully designed *in vitro* studies, animal studies (using varying doses of vitamin B12/folic acid), observational studies in patients with higher risk (e.g. gestational diabetes and polycystic ovarian syndrome) and intervention studies using vitamin B12 need to be urgently undertaken before the introduction of population-wide food fortification with vitamin B12. These studies should be designed to look at the adverse effects of high-dose vitamin B12 supplementation (such as excess accumulation of cyanocobalamin).[46] In addition, analyses of the cost effectiveness of population-wide food fortification as well as targeted supplementation of B12 need to be carefully conducted prior to such implementation.

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