Review Article

The Relationship Between Folate, Vitamin B12 and Gestational Diabetes Mellitus With Proposed Mechanisms and Foetal Implications

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Received January 2021; Revised and accepted June 2021

Abstract

The incidence of gestational diabetes mellitus (GDM) is rising, which warrants attention due to the associated complications during pregnancy and in the long term for both mother and offspring. Studies have suggested a relationship between maternal folate (vitamin B9) and vitamin B12 status and GDM risk. Seemingly the most problematic scenario occurs when there is B-vitamin imbalance, with high folate and low vitamin B12. This nutritional state can occur in vitamin B12 deficient women who exceed the recommended folic acid supplementation. However, the pathological mechanisms behind this relationship are currently unclear and are explored in this review article. A high folate/low B12 can lead to a functional folate deficiency through the methyl-trap phenomenon, impairing re-methylation of homocysteine and regeneration of folates for DNA synthesis and repair. Consequently elevated homocysteine concentration leads to endothelial dysfunction and oxidative stress. Vitamin B12 deficiency also leads to an impairment of the conversion of methylmalonyl-CoA to succinyl-CoA, which has been associated with insulin resistance. Insulin resistance is thought to contribute to the etiology of GDM. More studies are needed to confirm the impact of these and other mechanisms on disease development. However, it highlights a potential avenue for GDM risk modification through a vitamin B12 supplement and improvement of maternal metabolic health.

Keywords: Pregnancy; Gestational Diabetes; Vitamin B 12; Folic Acid; Insulin Resistance

Introduction

Many studies to date have identified a link between maternal B vitamin imbalance, with high folate (vitamin B9) and low vitamin B12 status, to an increased risk of developing gestational diabetes mellitus (GDM) (1-3). The incidence of GDM is rising (4, 5), which is concerning due to the associated complications for both mother and offspring (6). Currently, the pathological mechanisms behind this association are not completely understood. This review article explores the potential contributory effects of the methyl-trap, a high plasma homocysteine concentration and an impaired methylmalonyl-CoA to succinyl-CoA conversion to the development of GDM.

Body

Folate and vitamin B12 (B12): Folate and cobalamin (B12) play important roles in cellular metabolism, with their sites of action being closely related (Figure 1).

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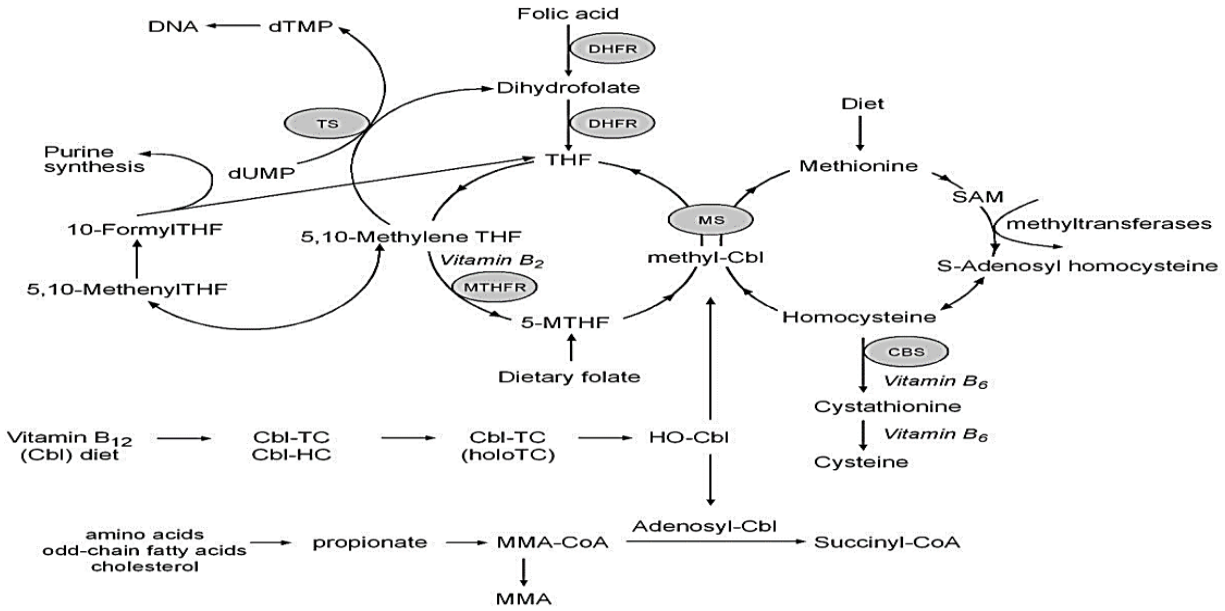

Figure 1: Absorption and metabolic pathways involving folate and vitamin B12.
Cbl (cobalamin), HC (haptocorrin), TC (transcobalamin), holoTC (holotranscobalamin), HO-Cbl (hydroxocobalamin), MS (methionine synthase), THF (tetrahydrofolate), 5-MTHF (5-methyltetrahydrofolate), DHFR (dihydrofolate reductase), dUMP (deoxyuridine monophosphate), dTMP (deoxythymidine monophosphate), TS (thymidylate synthase), MTHFR (methylene tetrahydrofolate reductase), CBS (cystathionine beta-synthase), SAM (S-Adenosyl methionine), methylmalonic acid (MMA), MMA-CoA (methylmalonyl-coenzyme A). In the absence of methyl-Cbl, 5-MTHF becomes metabolically trapped in this form producing a pseudo folate-deficient state.

Folate and B12 are both involved in one-carbon metabolism, in which methyl groups are donated or utilized (7). These reactions are essential for nucleic acid and protein synthesis, amino acid metabolism and methyl group biogenesis (8). Part of this cycle includes the conversion of homocysteine to methionine via methionine synthase (MS), in which methyl groups from 5-methyltetrahydrofolate are donated to form methylcobalamin (metabolically active B12), a cofactor for MS. In the absence of B12, 5-methyltetrahydrofolate becomes metabolically trapped in this form producing a pseudo folate-deficient state (methyl-trap) (9) whereby 5-methyltetrahydrofolate cannot be utilized for methionine and tetrahydrofolate formation. Outside of one-carbon metabolism, B12 (adenosylcobalamin form) is essential for the conversion of methylmalonyl-CoA to succinyl-CoA in mitochondrial energy metabolism (10, 11).

Folate and/or B12 deficiency result in macrocytic anemia (12), neurological manifestations including cognitive decline and psychological disorders (13) as well as severe developmental delay in infants (14). Folate deficiency has long been implicated in the foetal development of neural tube defects (NTD) and congenital heart defects (15, 16). As a result, to decrease the risk of NTD many countries introduced a mandatory fortification of flour, wheat and rice (17). UK guidelines recommend that women should supplement with 400μg folic acid pre-conceptually and in the first 12 weeks post-conception, with the dose increasing to 5mg in those at high risk of NTD and in women with diabetes mellitus (18).

Currently, in the UK, there are no recommendations for a B12 supplement during pregnancy, yet in the UK 6% of adults under 60 years of age and closer to 20% of adults over 60 have low B12 (19). Depending on the criterion used, the prevalence of low B12 status amongst vegetarians ranges from 11% to 90% (20). Low B12 status is also highly prevalent during pregnancy (21), however accurate deficiency rates are difficult to attain given that the assessment of B12 status is challenging and pregnancy-specific reference ranges for B12 markers are currently not available (22). Hemodilution and hormonal changes during pregnancy affect the concentration of B12 markers in serum (e.g. significant decline of serum B12 is observed throughout pregnancy) rendering this test less reliable for B12 assessment (13, 22).

Furthermore, a methyl-trap could also occur in B12 deficient pregnant women who exceed folic acid supplements above recommendations or in those supplementing 5 mg daily due to previous NTD pregnancies or diabetes mellitus (23, 24). A recent study in UK pregnant women found 26% of women with B12 <150 pmol/L (a commonly used cut-off for B12 deficiency in all populations) compared to only 1.5% for folate below deficiency cut-off (<7 nmol/L), supporting a potential imbalance between these B-vitamins (21).

GDM: There has been increasing interest in the relationship between folate and B12 status and GDM (1-3). GDM is a common complication of pregnancy, defined as insulin resistance (IR) and the resultant glucose intolerance with onset/first recognition during pregnancy. IR, a decreased ability of target tissues such as liver, adipose tissue and muscle to respond to normal circulating concentrations of insulin, is thought to contribute to the etiology of GDM. Gestation naturally predisposes to IR and this is consequent to the physiological adaptation necessary to provide glucose to the growing fetus (25). To compensate for IR, there is an increased production of insulin from beta cells. However, the insulin-producing capacity of pancreatic beta cells gradually falls, leading to a reduction in insulin production, and in some cases the condition progresses to GDM (25).

The prevalence of GDM is rising (4, 5), with the condition estimated to be affecting 17% of pregnancies globally, and with certain Asian countries having an approximate prevalence of over 20% (26). GDM is associated with an increased long-term risk of type 2 diabetes mellitus (T2DM), cardiovascular disease and metabolic syndrome in both the mother and the offspring (27). It is also linked to complications during pregnancy and birth such as pre-eclampsia, macrosomia in newborns and a four-fold increased risk of stillbirth (6). The maternal micronutrient intake, including optimum folate and B12, may modulate this risk.

Associations of folate and B12 status with GDM and other metabolic states: A number of studies in the last twelve years have linked high folate in combination with low B12 status in mothers with an increased risk of GDM (1, 2, 28), as well as IR in offspring (29). One study in a group of Chinese pregnant women demonstrated that the highest risk of GDM is observed in mothers with combined B12 deficiency and high folate concentrations with an odds ratio (OR) of 3.08, compared to high folate alone (OR=1.98), and that high B12 concentrations reduce the risk of GDM (OR=0.30) (1). Li *et al.* (1) also showed an even greater increase in GDM risk when a high folate/low B12 status was accompanied with higher maternal age and pre-pregnancy BMI, indicating that nutrient imbalances and maternal factors can act synergistically to increase GDM risk. Agreeable conclusions were drawn from a study on a group of pregnant women at 26 weeks gestation, demonstrating that the highest odds of GDM (OR=1.97) were observed in women with combined B12 insufficiency and high folate status, compared to high folate status alone, OR=1.29 (2). Furthermore, a study looking into third trimester maternal B12 showed that low B12 status alone is a risk factor for the development of GDM with an OR of 2.40 (21). It was also reported that folic acid supplementation in the first trimester increased the risk of GDM (OR=2.25), potentially through exacerbating B12 deficiency (30). Therefore, high folate and low B12 status could be a contributory factor in the pathogenesis of GDM.

The mechanisms linking high folate/low B12 status and increased GDM risk are currently unknown (29). The methyl-trap leading to elevated homocysteine levels and impaired methylation reactions, as well as alterations in mitochondrial metabolism may be contributory factors.

Poor B12 status has been associated with a higher prevalence of IR in other population groups. A study into non-diabetic obese male and female adults found that B12 concentration negatively correlated with fasting plasma glucose levels and prevalence of IR (31). Ho *et al.* (32) reported that nearly a third of non-diabetic obese adolescents aged 10-17 years with IR had low (<148 pmol/L) or borderline B12 (148 to 221 pmol/L). Furthermore, low B12 status has also been implicated in patients with features of metabolic syndrome, other than just IR. A study on an Indian adult population found that participants with metabolic syndrome (identified according to weight, waist circumference, BMI, fasting blood sugar and fasting blood lipid levels) had lower plasma B12 compared to healthy control subjects (33). This relationship was also shown by Guven *et al.* (34) who found that the participants with metabolic syndrome had statistically significant lower B12 concentrations (mean 157 pmol/L), compared to the healthy controls (mean 181 pmol/L), $p<0.01$. Additionally, low B12 concentrations have been linked to adverse lipid

profiles (35). Interestingly, a study on patients with T2DM found that B12 supplementation was able to significantly improve glycaemic control and IR (36). This highlights that low B12 status can be attributed to a range of states of metabolic dysfunction, and future research should focus on determining how B12 supplementation could impact these health outcomes.

Methyl-trap and GDM: As previously stated, deficiency of B12 in the presence of normal/high folate inhibits the intracellular conversion of 5-methyltetrahydrofolate to tetrahydrofolate and homocysteine to methionine, resulting in a functional folate deficiency, reducing *de novo* purine and thymidine generation (9). The resultant impaired DNA synthesis could be a contributing factor to the development of IR. Crovetto *et al.* (37) found that women with GDM had reduced mitochondrial activity compared to women with physiological pregnancies, shown through a lower content of mitochondrial DNA in peripheral blood. An epigenetic study related a reduced mitochondrial DNA copy number in the adipose tissue of obese individuals with increased methylation of the D-loop region of the mitochondrial genome (38), a critical region controlling mitochondrial DNA replication, transcription and organization. This epigenetic modification was correlated with the development of IR (38).

High folate in low B12 conditions can also oxidize the cobalt of B12 (found at the center of the molecule), forming cob(II)alamin from the active methylcobalamin and cob(I)alamin forms of the enzyme (39). This prevents B12 from accepting the methyl group from 5-methyltetrahydrofolate as a highly reduced enzymatic state is needed for this reaction (39). This could potentiate the methyl-trap mechanism and the development of a functional folate deficiency and the worsening IR.

Excessive folic acid use and GDM: Supplementation with high doses of folic acid in pregnant women can also lead to the appearance of un-metabolized folic acid in plasma (40). In a cohort of Canadian pregnant women, over 90% of women had detectable un-metabolized folic acid in plasma and cord blood (41). The presence of un-metabolized folic acid is associated with altered natural killer (NK) cell cytoactivity (42) and this immune dysregulation has been suggested to be implicated in GDM pathology, through altering cell infiltration and signaling pathways (43). A study analysing NK cell cytokine production found a higher percentage of pro-inflammatory cytokine producing NK cells (TNF-α, IFN-γ) and a lower percentage of anti-inflammatory cytokine producing NK cells (TGF-β) in the blood of GDM women compared to non-GDM women (44). This indicates a potential inflammatory mechanism in the development of GDM, similarly as has been found before in T2DM in association with pancreatic islet cell damage; higher TNF-α levels were linked with worsening pancreatic beta cell function and IR (45). This potential pro-inflammatory mechanism in GDM pathology and its similarities to the inflammatory pathogenesis shown in T2DM could explain why patients with GDM are more prone to developing T2DM in the future (27).

Elevated homocysteine and low methionine and GDM: A deficiency of B12, as well as inducing a functional folate deficiency, also reduces the conversion of homocysteine to methionine. This can pose two potential problems: reduced methionine and elevated homocysteine concentrations.

Since methionine is an essential amino acid, its deficiency could reduce protein synthesis and lean tissue deposition (3, 29). As insulin sensitivity is impaired with increased adipose tissue volume, the reduction in lean tissue mass can promote IR (46). Methionine is also required for S-adenosylmethionine synthesis (Figure 1), needed for numerous methylation reactions, including DNA. Lambs born to sheep fed a 'methyl-deficient' diet had higher adiposity and IR rates (47), showing that maternal periconceptional B12 deficiency and methionine status can interfere with normal DNA methylation in offspring, impacting their metabolic health later in life. Methionine deficiency leads to DNA hypomethylation (48) and altered methylation has been implicated in the pathogenesis of T2DM (49). A similar mechanism may be implicated in GDM.

Additionally, elevated homocysteine concentrations in plasma (hyperhomocysteinemia) have been associated with a number of diseases including cardiovascular disease, dementia, osteoporosis, pre-eclampsia and spontaneous pregnancy loss (50). Oxidative stress mediated apoptosis, endothelial and DNA damage are some of the pathogenetic consequences of high homocysteine (51). Whilst several studies have also linked elevated homocysteine to the development of T2DM through IR and endothelial dysfunction (52, 53), it is unclear whether this relationship is applicable to GDM risk. Some studies have shown that homocysteine concentrations are significantly increased in women with GDM compared to non-GDM pregnant women (54-56),

whilst other studies have not confirmed the same associations and have not found a link between plasma homocysteine and GDM risk (28, 57). Moreover, one study found that elevated homocysteine concentrations in pregnant women were found to be associated with lower fasting glucose levels and reduced GDM odds (2). The relationship between plasma homocysteine and GDM risk is unclear due to conflicting evidence. However, one study investigating non-alcoholic fatty liver disease found that elevated homocysteine altered systemic lipid homeostasis leading to raised plasma free fatty acids through an oxidative-stress mediated mechanism involving H_2O_2 accumulation (58). Raised plasma free fatty acids could contribute to GDM risk by promoting IR (59).

Interestingly, Seghieri *et al*. (55) demonstrated that raised homocysteine concentrations were related to post-load glucose concentrations more than to mean metabolic control, as monitored by glycated hemoglobin values. They also found that glucose tolerance, assessed via oral glucose tolerance tests, was impaired in pregnant women with high third-trimester homocysteine concentrations, but this relationship did not extend to impaired insulin sensitivity, assessed via the homeostasis model assessment of insulin resistance (HOMA-IR) which takes into account fasting glucose and fasting insulin levels (55). Potentially this indicates a different pathological mechanism between GDM and T2DM, as high homocysteine has been linked to IR in T2DM (52). However, the discrepancies in associations of plasma homocysteine and GDM as well as its relationship with insulin may be attributed to changes in homocysteine concentrations due to pregnancy. Firstly, homocysteine concentrations are normally much lower during pregnancy due to hemodilution, hormonal changes and folic acid supplementation. Secondly, subnormal plasma homocysteine was reported in non-diabetic hyperinsulinemic subjects in the prediabetic stage (60). Glomerular hyperfiltration observed in early diabetes, or a metabolic effect of high insulin levels were proposed as mechanism for low homocysteine (61, 62). Therefore, homocysteine concentrations may not be truly reflective of folate and B12 status during pregnancy.

Additionally, Idzior-Walus *et al.* (28) found that in GDM women, elevated homocysteine concentrations were significantly associated with low B12 and high folate, suggesting the importance of B-vitamins balance and the associated risks this poses for GDM development. A study in folate replete, B12 insufficient (plasma B12 <150 pmol/L) pregnant Indian women between 17 and 34 weeks gestation found that the addition of a B12 supplement at doses >1,000 μg per day reduced plasma homocysteine by 1.3 μmol/L in late pregnancy compared to the un-supplemented group ($p<0.006$) (63).

Furthermore, the impact of elevated homocysteine concentrations in women with GDM has been shown to extend to the post-partum period (64). Over a 4-year follow up period of women with GDM pregnancies, the women who went on to develop T2DM had significantly higher plasma homocysteine at 6-weeks postpartum, a relationship that was independent of age, BMI and family history of diabetes (64), suggesting that early post partum homocysteine concentrations could be used as a prognostic factor for later T2DM development in those with a history of GDM.

Elevated MMA and GDM: B12 is also a co-factor in the mitochondrial conversion of methylmalonyl-CoA from odd chain fatty acids, cholesterol and branched chain amino acids to succinyl-CoA, a major intermediate in the Krebs cycle (65). B12 deficiency leads to an elevated methylmalonic acid (MMA) concentration, a product of hydrolysis of the excessive concentration of methylmalonyl-CoA. Inhibition of fatty acid oxidation leads to adipocyte dysfunction and hence increased lipogenesis and IR as a result (3). The increase in circulating fatty acids and hepatic lipids may consequently lead to upregulation of methylmalonyl-CoA synthesis, additionally coupled with higher demands for B12.

In vitro studies have found that adipocytes in low B12 conditions displayed increased lipid accumulation (66). Adipocyte dysfunction was related to B12 deficiency through altering adipose-derived circulating micro-RNAs involved in gene expression regulation in IR pathways (67). The low B12 concentrations were associated with increased expression of genes involved in adipogenesis and lipogenesis, indicating that epigenetic mechanisms could also be implicated in GDM pathogenesis by potentiating IR (67). Furthermore, *in vivo* studies found that pups born to B12 deficient rats had higher levels of obesity and hypertriglyceridemia, which are both risk factors for diabetes mellitus, through down-regulation of proteins involved in the β-oxidation pathway (68). Therefore, maternal body fat distribution/metabolism during pregnancy may be implicated in determining circulating concentrations of micronutrients in pregnancy.

Fetal implications: Maternal folate/B12 status

also impacts on the health of the offspring years after birth. Based on their study in 6 year old children (N = 674), Yajnik *et al.* (29) found that *in utero* environment can have a lasting effect on metabolic function in later life. Using the HOMA-IR, higher maternal folate concentrations at 28 weeks gestation were associated with higher HOMA-IR in offspring ($p<0.001$) and low maternal B12 concentrations at 18 weeks of gestation were associated with higher HOMA-IR in offspring ($p=0.03$) (29). The offspring of women with a combination of high folate and low B12 concentrations were found to be the most insulin resistant ($p<0.001$) (29). The same study indicated that B12 concentration at 18 weeks of gestation was more strongly associated with IR in the offspring compared to 28 weeks of gestation, indicating that early-mid pregnancy could be a critical programming period for carbohydrate metabolism in the fetus (29). Krishnaveni *et al.* (69) also demonstrated that higher maternal folate concentrations at 30 weeks gestation were associated with higher HOMA-IR in the offspring at 9.5 years ($p=0.03$) and 13.5 years ($p=0.03$) of age. Conversely, they found no significant association between maternal gestational plasma B12 concentrations and IR in the N = 539 offspring participating in this study (69).

As well as contributing to IR, Krishnaveni *et al.* (70) in their study on N= 264 Indian adolescents, showed that low maternal gestational B12 concentration (<150 pmol/L) is associated with greater cortisol response to stress (induced through public speaking and mental arithmetic tasks in front of unfamiliar people) in offspring compared to those born to mothers with normal gestational B12 concentrations (>150 pmol/L) ($p<0.001$). The increased reactivity to stress is thought to be related to increased cardiovascular and mental disorders (70) and therefore could mean an increased future cardiovascular disease risk in offspring born to B12 deficient mothers. This builds on the idea of nutrient-mediated teratogenesis whereby micronutrient imbalances and deficiencies in the intrauterine environment lead to epigenetic programming in the fetus that can promote the development of non-communicable chronic disease later in life (71). Targeting micronutrient imbalances during pregnancy to optimise health outcomes for the child and possibly reduce the risk of diseases later in life is warranted.

Conclusion

Many studies to date have shown associations between maternal folate and B12 status and the presence of GDM. This is important in view of increasing GDM prevalence and the associated complications, both to mother and offspring. Longitudinal cohort studies affirming the relationship between GDM risk and folate and B12 status as well as vitamin B12 supplementation trials to establish the optimum dose of both folic acid and vitamin B12 to achieve the 'metabolic balance' of both vitamins throughout pregnancy are needed. Other markers of status, such as holotranscobalamin, homocysteine, MMA or red cell folate should be included in these studies to confirm the associations thus far and vitamin B12 status during each trimester of pregnancy. This would possibly support elucidation of the mechanisms linking maternal B-vitamins status with GDM development, facilitate B12 supplementation and formulate recommendations, which could offset GDM risk as well as improve post-pregnancy health and blood glucose control in women as well as their offspring.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

Renata Gorska of the Nutristasis Unit is thanked for her artistic skills in drawing the figure.

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Citation: Maher A, Sobczyńska-Malefora A. **The Relationship Between Folate, Vitamin B12 and Gestational Diabetes Mellitus With Proposed Mechanisms and Foetal Implications.** J Family Reprod Health 2021; 15(3): 141-9.