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B-vitamins and one-carbon metabolism during pregnancy: health impacts and challenges

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Folate, vitamin B_{12}, vitamin B_6 and riboflavin interact by functioning as cofactors within one-carbon metabolism (OCM), a network of interrelated cellular pathways essential for numerous biological processes, including the biosynthesis of DNA, amino acid interconversions and methylation reactions. The pathways of OCM are influenced by endocrine signals and genetic polymorphisms and are particularly responsive to relevant B-vitamin intakes. Physiological changes in healthy pregnancy, leading to a steady decline in B-vitamin status, add another layer of complexity to the regulation of OCM. Although significant advances have been made to improve our understanding of these pregnancy-related changes, no specific reference ranges yet exist for B-vitamin biomarkers in pregnancy to support normal fetal growth without depleting maternal stores. The lack of pregnancy-related criteria for adequacy of B-vitamin status is in turn a major limitation in identifying pregnant women most at risk of B-vitamin deficiency. Another challenge is that the evidence is very limited to provide a basis for establishing pregnancy-specific dietary recommendations for B-vitamins to support successful pregnancy outcomes. In terms of preventing adverse outcomes, periconceptional folic acid supplementation has a proven role, established more than 30 years ago, in protecting against neural tube defect-affected pregnancies and this has been the major focus of public health policy worldwide. This review evaluates the emerging evidence for the less well recognised role of B-vitamins in preventing hypertensive disorders in pregnancy and the intergenerational effects of B-vitamins on offspring neurodevelopment and cognitive performance during childhood. We also consider the underlying biological mechanisms.

Keywords: Folate: Folic acid: Vitamin B_{12}: Vitamin B_6: Riboflavin: Pregnancy: Offspring

Maternal nutrition is paramount not only for the successful outcome of pregnancy, but it also has well proven effects in influencing the health and wellbeing of both mother and child in later years. In this respect, the optimal provision of B-vitamins during pregnancy is vital in building the foundation for normal development of the child and maintaining the health of the mother[1,2]. This review will focus on the metabolically interrelated B-vitamins, folate, vitamin B_{12}, vitamin B_6 and riboflavin, and consider the physiological changes in pregnancy affecting the status of these vitamins and the implications for dietary B-vitamin recommendations in pregnancy.

Abbreviations: DFE, Dietary Folate Equivalents; DHFR, dihydrofolate reductase; DRV, dietary reference value HDP, hypertensive disorders of pregnancy; MTHFR, methylenetetrahydrofolate reductase; NTD, neural tube defects; OCM, one-carbon metabolism; RDA, recommended dietary allowance; RNI, recommended nutrient intake; THF, tetrahydrofolate.

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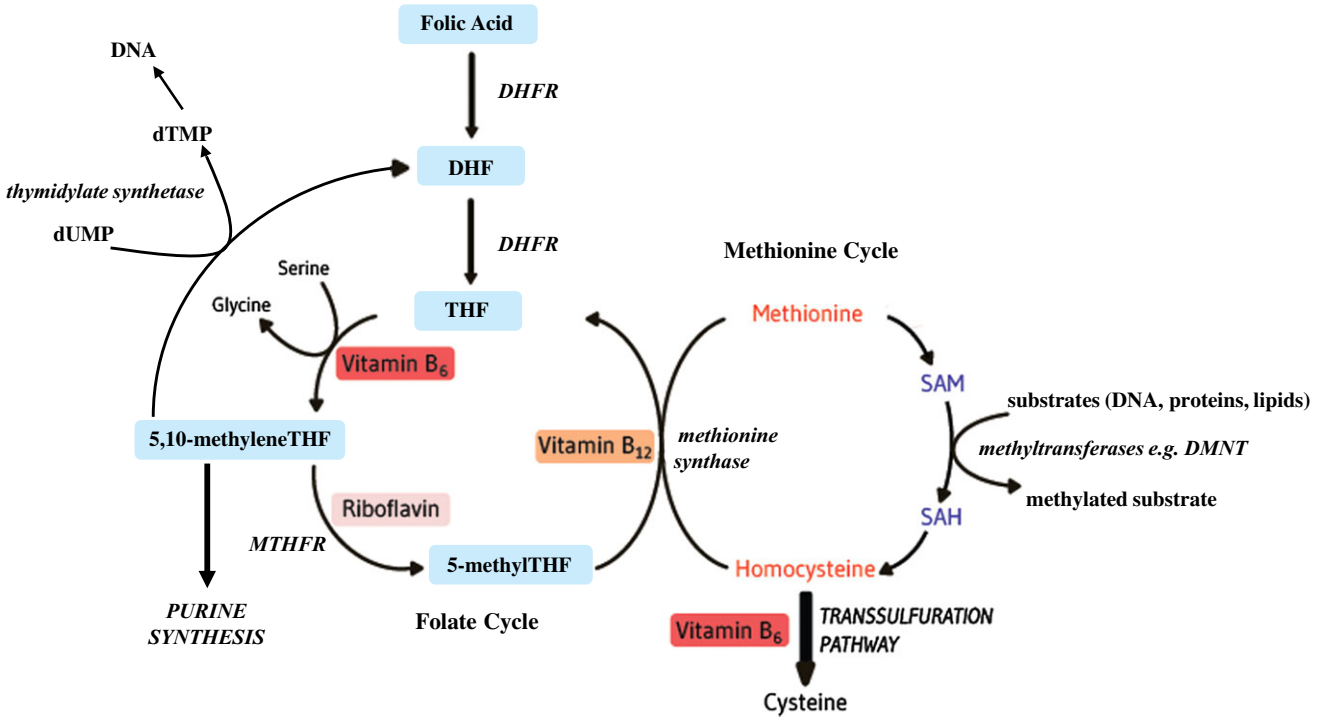

Fig. 1. Overview of the role of B-vitamins in one-carbon metabolism. DHF, dihydrofolate; DHFR, dihydrofolate reductase; DMNT, DNA methyltransferase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate.

Additionally, we explore the emerging evidence for the less well recognised role of B-vitamins in preventing hypertensive disorders in pregnancy and the intergenerational effects of B-vitamins on offspring neurodevelopment and cognitive performance during childhood. The underpinning biological mechanisms are also considered.

Overview of B-vitamins within one-carbon metabolism

B-vitamins are chemically heterogeneous water-soluble organic compounds that cannot be endogenously synthesised by humans but are essential for normal physiological functions which requires their continuous supply in small amounts by the diet. In general, B-vitamins have small body pools at a cellular level and due to their fast turnover, deficiency can be developed rapidly under conditions of restricted dietary intake; in this regard, vitamin B_{12} is the only exception as the development of deficiency generally takes much longer time[3]. B-vitamins are absorbed in the small intestine through elaborated mechanisms involving high-affinity transporters and/or receptors[4]. In addition, microbiota in the colon can synthesise some B-vitamins, however their contribution to B-vitamin status in humans is still unclear. B-vitamins act as cofactors in a wide range of diverse metabolic reactions which encompass almost all cellular functions.

This review focusses specifically on four B-vitamins interlinked through one-carbon metabolism (OCM) as detailed below. Folate and vitamin B_{12} are important for cell division and tissue growth through their involvement in pyrimidine and purine nucleotide synthesis for DNA and RNA, maintenance of genomic stability and methylation reactions[5–7]. Riboflavin is a cofactor in oxidation-reduction reactions essential for energy generation, intermediary metabolism and antioxidant defence system[8]. Vitamin B-6 is involved in amino acid metabolism, neurotransmitter synthesis, modulation of steroid hormone receptors and Hb synthesis[9,10].

Metabolic interaction of folate and related B-vitamins. Folate, vitamin B_{12}, vitamin B_6 and riboflavin interact by functioning as cofactors within OCM, a network of interrelated cellular pathways essential for numerous biological processes, including the biosynthesis of DNA, amino acid interconversions and methylation reactions[5]. Folate is the core one-carbon donor and it acts synergistically with vitamin B_{12}, vitamin B_6 and riboflavin which are cofactors for some of the key enzymes in OCM (Fig. 1). Folate enters OCM as tetrahydrofolate (THF) which acquires a carbon unit from serine in a B_6-dependent reaction to form 5,10-methyleneTHF. The latter is then either irreversibly reduced to 5-methylTHF or serves as the one-carbon donor in the synthesis of nucleic acids, where it is required by thymidylate synthetase in the conversion of deoxyuridine to deoxythymidine for pyrimidine biosynthesis or is converted to other folate cofactor forms essential for purine biosynthesis. Methylenetetrahydrofolate reductase (MTHFR) is a riboflavin (FAD)-dependent enzyme that catalyses the irreversible reduction of 5,10-methyleneTHF to 5-methylTHF. Within the methionine cycle, 5-methylTHF acts as a co-substrate in the

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remethylation of homocysteine to methionine via the B_{12}-dependent enzyme methionine synthase. Methionine is activated by adenosine triphosphate leading to the generation of S-adenosylmethionine (SAM), which is a ubiquitous methyl group donor as it donates methyl groups in transmethylation reactions involved in the synthesis or activation of a wide range of bioactive compounds such as DNA, proteins, neurotransmitters, and membrane phospholipids, all of which are regulators of important physiological processes[5]. As a result, SAM is converted subsequently to S-adenosylhomocysteine and homocysteine which in turn, can be remethylated back to methionine or can be further metabolised in the transsulfuration pathway to form cysteine, in a B_6-dependent process[11].

A balanced and adequate supply of folate, vitamin B_{12}, vitamin B_6 and riboflavin as well as some other nutrients (i.e. choline) is required for the effective functioning of OCM. If the provision of some of these vitamins is insufficient, then a compensatory shift in pathways within OCM may occur[12] but also it might have a negative impact on SAM synthesis with impairment of transmethylation reactions[11]. In addition, genetic polymorphisms on B-vitamin-dependent enzymes in OCM, can result in disruption of OCM pathways which can lead to adverse phenotypes, even if dietary folate and related B-vitamin intakes are adequate[13]. For example, individuals homozygous for the common *MTHFR* C677T polymorphism have a reduced activity of MTHFR enzyme, impaired folate metabolism and high homocysteine concentrations and may have higher requirements for folate and riboflavin[14]. Meta-analyses showed that the MTHFR C677T polymorphism increases the risk of high blood pressure and hypertension in pregnancy by 24–87 %[15–18], which will be discussed in more depth later in this review.

Physiological changes in pregnancy affecting B-vitamin biomarkers

Numerous physiological changes occur in all the systems in the body during normal pregnancy with the aim of providing optimal conditions for the growth and development of the fetus and supporting the placental unit. These changes are predominantly driven by the dramatic increase in the production of progesterone and oestrogen[19]. The corpus luteum, that develops from an ovarian follicle during the luteal phase of the menstrual cycle, is initially responsible for the synthesis of these hormones but with the advancement of pregnancy their production is shifted from the corpus luteum to the placenta by the 7th–9th week of gestation[20]. Most of the oestrogen and progesterone produced by the placenta enter maternal circulation and are exported to the other maternal tissues. The brief overview below is focused on physiological changes in pregnancy which potentially affect B-vitamin status biomarkers in pregnant women.

Plasma volume and red blood cell mass

A measurable expansion of plasma volume already exists at the 6th–8th week of gestation and this increase continues up to the 34th–36th week of gestation[21]. In a singleton pregnancy the total increase of plasma volume accounts to 1200–1400 ml which is around 40 % above the volume of a non-pregnant woman, whereas in a multiple pregnancy this expansion is much bigger[22]. A simultaneous rise of red blood cell (RBC) count by 30 % is also observed in pregnancy, however due to the higher increase of plasma volume than RBC mass these changes lead to 'physiological anaemia' of pregnancy which usually becomes evident in the third trimester[23]. The reason for the increased blood volume is still unclear but one of the mechanisms under consideration relates to oestrogen activation of the renin-angiotensin-aldosterone system which promotes water and sodium retention and subsequently leads to hypervolemia[24]. The pregnancy induced hypervolemia generally is considered beneficial for the pregnant woman as it maintains the hemodynamic stability with the blood loss at the time of delivery[25]. It also might be advantageous for the fetus as the less viscous blood would have an improved uterine and intervillous perfusion with more efficient transport of nutrients and oxygen to the fetus. However, the hypervolemia in pregnancy could lead to decreased concentrations of various blood constituents including B-vitamins. Moreover, the increased production of RBC is associated with higher requirements for folate, vitamin B_{12}, vitamin B_6 and riboflavin due to their involvement either in the generation of new cells or in the synthesis of Hb, which in turn would further compromise the status of these B-vitamins.

Plasma protein binding

The concentrations of different plasma binding proteins are reported to decrease during pregnancy, mainly because of the dilutional effect of plasma volume expansion[26]. Plasma albumin concentrations are up to 80 % lower compared with those in non-pregnant women[22]. The fall of plasma albumin is of relevance to the concentrations of folate, vitamin B_6 and riboflavin which are normally bound to albumin in the circulation[5,27,28]. Some of the protein unbound (free) B-vitamins are reported to be prone to hydrolysis and destruction[29] which may also contribute to their low blood concentrations in pregnancy.

Renal clearance and glomerular filtration rate

Glomerular filtration rate and renal clearance start to increase in the middle of the first trimester and continue gradually to rise up to the third trimester with a total increase of 30–50 %[30]. However, it has been reported that the renal reabsorption process is unchanged in pregnancy[31] suggesting that there is no loss of important nutrients through the urine. In support of this are the results from a small but tightly controlled intervention study with stable isotopes which showed that the excreted amounts of total folate as well as folate catabolites in 24-h

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urinary samples were similar in second-trimester pregnant women and non-pregnant controls who were maintained for 12 weeks on a diet providing folate at either 450 μg/d or 850 μg/d[32]. According to our knowledge, there are not such precise investigations with stable isotopes conducted for the other B-vitamins, however a cross-sectional study in age matched (non-supplement users) pregnant, lactating and non-pregnant women with B-vitamin intake within the recommended levels, did not find significant differences among the three groups of women in the 24-hour urinary excretion of vitamin B_6, riboflavin and vitamin B_{12}[33]. These findings indicate that despite the considerable increase of glomerular filtration rate, it is unlikely that urinary B-vitamin excretion is affected by any way throughout pregnancy.

Gastric changes

Pregnancy is associated with an increase of gastric pH and hypochlorhydria[34] which theoretically may affect the bioavailability of some B-vitamins by reducing their release from foods where they are trapped in the food matrix bound to macromolecules. Furthermore, the change in the pH may affect the binding ability of some of the carriers/receptors involved in the active transport of B-vitamins in the upper small intestine which in turn might result in reduced B-vitamin bioavailability. Malabsorption of folate and vitamin B_{12} has been reported in non-pregnant individuals and older adults with hypochlorhydria and atrophic gastritis in different studies[35,36]. However, these chronic conditions are too extreme and the parallel with the situation in pregnancy is probably unreasonable. In order to examine whether the reproductive state has an impact on the response to folate intervention, West *et al.* (2012) conducted a controlled feeding trial in age matched pregnant, lactating and non-pregnant women maintained for 10–12 weeks on diets containing equivalent amounts of folate (400 μg/d natural folate and a supplement of 750 μg/d) and demonstrated comparable serum folate responses among the three groups, suggesting that there are insignificant changes in the intestinal absorption of folate during pregnancy[37]. It is unknown whether the same results would be obtained if the supplemental folic acid intake was in line with the recommendation of 400 μg/d. The same research group also investigated the effects of pregnancy and lactation on vitamin B-12 status response to a controlled vitamin B_{12} intake of 8·6 μg/d provided for 10–12 weeks[38]. The results of this trial showed that at the end of the intervention despite the equivalent B_{12} intakes pregnant women had 21 % lower serum B_{12} concentrations compared with the non-pregnant controls but the concentrations of holotranscobalamin (biologically active fraction of B_{12}) were not significantly different between the two groups. However, it is unknown whether these changes in vitamin B_{12} biomarkers of status are related to altered absorption. According to our knowledge, there are no published human studies investigating the impact of pregnancy on the absorption and bioavailability of ingested vitamin B_6 and riboflavin. Of note, a study using labelled pyridoxine in pregnant and non-pregnant control rats did not find significant differences in the intestinal absorption of radioisotope between the two groups[39]. Further research is required to clarify whether the absorption of B-vitamins is affected in pregnancy.

Transfer of B-vitamins to placenta and fetus

The placenta is actively involved in the transport of nutrients including B-vitamins from maternal circulation to the fetus. Folate transporters such as folate receptor-α, folate reduced carrier and proton coupled folate transporter are found to be expressed in the syncytiotrophoblast from the early stages of placenta development[40,41]. Transcobalamin receptor which is responsible for the uptake of biologically active fraction of B_{12}, holotranscobalamin, is also expressed in human placenta[42]. RFVT3 protein in placenta has been associated with the transfer of riboflavin derivatives FAD and FMN to the fetus[43]. As a result of the activity of these placental transporters, the concentrations of folate, vitamin B_{12} and riboflavin derivatives in the intervillous blood and in the fetus are 2–4 times higher than those in maternal blood[44–46]. Although carrier proteins and receptors in relation to vitamin B_6 have not been identified in placenta yet, studies have reported up to 4 times higher plasma PLP concentrations in the newborn compared with those in their mothers[47–49], suggesting the involvement of some unknown active mechanism that transfers vitamin B_6 against its concentration gradient. The substantial flow of B-vitamins from the mother to the fetus might represent a physiological adaptation to satisfy the high demands for these micronutrients during fetal development but with potential implications for maternal B-vitamin status.

Changes of activities of enzymes involved in OCM

Substantial and dynamic changes in the metabolic rates of the pathways in OCM have been reported in women with uncomplicated pregnancy. A study using tracer isotopes of methionine and phenylalanine showed that compared with non-pregnant women, the rate of transsulfuration was higher in the first trimester of pregnancy, however the differences disappeared towards the late gestation[50]. The higher activity in the maternal transsulfuration pathway in early pregnancy is probably a compensatory reaction in response to the high demands for cysteine during the fetal development coupled with the inability of the fetus to synthesise cysteine on its own due to the absence of transsulfuration activity in the fetal liver[51]. The study by Dasarathy *et al.* (2010) also showed a higher rate of transmethylation of methionine in the late gestation compared to non-pregnant women which may be related to high methylation demands by the growing fetus and the placenta[45]. The changes in the activities of the OCM pathways ultimately suggest that the requirements for folate and metabolically related B-vitamins acting as cofactors within OCM may be also increased.

All these changes in pregnancy may lead to a decline in the concentrations of B-vitamin biomarkers in maternal circulation. The understanding of these changes and their profound impact on B-vitamin status and requirements in

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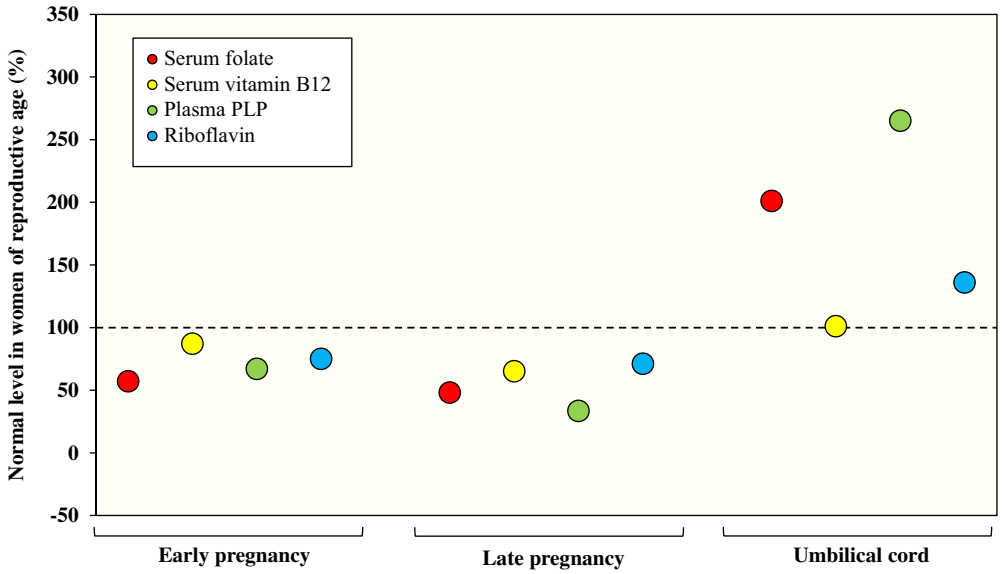

Fig. 2. B-vitamin status biomarkers in maternal and umbilical cord blood in unsupplemented pregnant women. Levels of B-vitamin biomarkers in pregnancy are presented as percentage of the concentrations of these biomarkers in non-pregnant, non-lactating unsupplemented women (punctured line). Data are based on: Kerr *et al.* (2005)[133]; Milman *et al.* (2006a)[54] and Obeid *et al.* (2005)[55] for serum folate; Kerr *et al.* (2005)[133]; McNulty *et al.* (2013)[124]; Molloy *et al.* (2013)[134] for serum vitamin B_{12}; Bjorke-Monsen *et al.* (2023)[49] and Zempleni *et al.* (1992)[48] for plasma pyridoxal 5´-phosphate (PLP); Bates *et al.* (1981)[65] and Shaw *et al.* (1993)[67] for erythrocyte glutathione reductase activation coefficient (EGRac).

pregnancy is essential for optimising maternal and fetal health.

B-vitamin biomarkers during pregnancy

Longitudinal studies of non-supplemented pregnant women at different stages in pregnancy generally show a decline of B-vitamin status with the advancing gestation (Fig. 2). Serum and RBC folate concentrations were reported to decrease by up to 50 % of those in non-pregnant state[52–54]. In contrast serum folate in the umbilical cord vein was reported to be around two times higher than in the maternal circulation[55].

Several research groups have found a progressive fall of approximately 30 % in serum vitamin B_{12} during pregnancy[56–58] which returns to pre-pregnancy concentrations shortly after delivery[58]. Importantly, holotranscobalamin, the fraction of vitamin B_{12} available for tissue uptake, was reported to remain relatively stable throughout pregnancy after a decline in early gestation[57,59]. The maintenance of stable holotranscobalamin in pregnancy is considered to be a result of mobilisation of maternal B_{12} pools to safeguard the adequate B_{12} supply to the fetus. In support of this is the fact that the concentrations of biomarkers of B_{12} function, methylmalonic acid and homocysteine increase in the later stages of pregnancy[57,58] suggesting some level of metabolic B_{12} depletion in pregnant women.

Studies investigating vitamin B_6 status throughout pregnancy have reported a dramatic decline of plasma pyridoxal phosphate (PLP) concentration with up to 70 % lower values in late pregnancy in comparison with the concentrations postpartum[49] or in age matched non-pregnant controls[60]. Some investigators found a compensatory increase of other vitamin B_6 derivatives in maternal circulation corresponding to the fall of PLP[61] and unchanged urinary excretion of the catabolic product 4-pyridoxic acid[60] leading to the conclusion that there are no substantial changes in vitamin B_6 status in pregnancy, however, this remains uncertain as others have failed to confirm these findings[62]. A recent study reported an increase of HKr index, a functional biomarker of vitamin B_6 status based on the activities of B_6-dependent enzymes in the kynurenine pathway, which indicates that there are some B_6 insufficiency at the metabolic level during pregnancy[49]. The intensive transfer of vitamin B_6 to the fetus demonstrated by up to 4 times higher concentrations of PLP in the umbilical cord vein than in maternal circulation in the late gestation[62] is still considered the main reason for the decline of B_6 status in pregnancy.

Riboflavin status, measured by the functional biomarker erythrocyte glutathione reductase coefficient (EGRac), has been found to decrease only marginally, up to 10 % during pregnancy, however this was reported to occur together with a progressive increase in the prevalence of riboflavin deficiency, especially towards the end of the third trimester[63–65]. The changes in riboflavin status have shown to be more pronounced with around 60 % prevalence of clinical deficiency in women with habitually low riboflavin intake in the range 0·5–1 mg/d[65,66]. Compared with the maternal EGRac values, the measurements of EGRac in the umbilical cord vein have indicated a better riboflavin status[67].

In summary, the physiological changes in pregnancy with associated decline of B-vitamin status are well recognised, but specific reference ranges for B-vitamin biomarkers in pregnancy so far have not been developed. This greatly limits the timely diagnosis and management of B-vitamin deficiency in pregnant women.

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Table 1. Recommendations for dietary B-vitamin intakes during pregnancy

	UK_DH[69] (1991) RNI	US_IOM[72] (1998) RDA	FAO/WHO[73] (2004) RNI	EFSA[10,74,79,135] (2014–2017) DRV
Folate (DFE*/d or μg/d†)	300 (+100)†	600 (+200)*	600 (+200)*	600 (+270)*,‡
Vitamin B_{12} (μg/d)	1·5 (no increment)	2·6 (+0·2)	2·6 (+0·2)	4·5 (+0·5)‡
Vitamin B_6 (mg/d)	1·2 (no increment)	1·9 (+0·4)	1·9 (+0·4)	1·8 (+0·3)
Riboflavin (mg/d)	1·4 (+0·3)	1·4 (+0·3)	1·4 (+0·3)	1·9 (+0·3)

DH, Department of Health; RNI, recommended nutrient intake; IOM, Institute of Medicine; RDA, recommended dietary allowance; EFSA, European Food Safety Authority; DRV, dietary reference value.
RNI, RDA, DRV are defined as the average daily intake that is sufficient to meet the nutrient requirements of almost all (97·5 %) healthy pregnant women. Figures in brackets indicate the increment added to the value for non-pregnant non-lactating women.
*DFE, dietary folate equivalents.
†μg/d.
‡Adequate Intake (AI).

Recommendations for B-vitamin intake in pregnancy

The recommendations for B-vitamin intake in pregnant women vary substantially between countries as different standards and criteria have been adopted (Table 1). Depletion-repletion studies, which are traditionally used for deriving dietary recommendations are inappropriate in pregnancy for health safety and ethical issues, thus other types of studies and indirect approaches need to be utilised.

In view of the intensive tissue growth during pregnancy, health authorities worldwide have established higher folate intake recommendations for pregnant women than for non-pregnant/non-lactating women. Red blood cell folate concentration has been used as the main criterion to assess intake adequacy but not all authorities have expressed the recommended intake values as dietary folate equivalents (DFE) which considers the differences in the bioavailability of food folates and folic acid, the synthetic folate derivative used for supplementation and food fortification. Most of the authorities recommended an intake of 600 μg/d DFE based on a controlled metabolic study[68]. However, the UK Department of Health (1991) has derived its recommendations for folate intake in pregnancy at 300 μg/d[69] using evidence available at that time from several historic intervention studies with different folic acid doses[70,71] but there are some concerns that the requirements might be underestimated as folate intake through the diet in these studies has not been accurately measured. Of note is that these recommendations do not include the required amount of folic acid supplementation for neural tube defect (NTD) prevention.

The requirements for vitamin B_{12} intake in pregnancy set by most official authorities are based on the recommendations for adults with an increment (0·2–0·5 μg/d) to compensate for B_{12} accumulated by the fetus[72–74]. That extra amount was estimated by liver B_{12} content in infants born to mothers with an adequate B_{12} status and assuming that liver contains half of B_{12} in the body[75–77]. However, other authorities, have not considered any B_{12} increment in pregnancy with the view that the requirement for B_{12} in adults should be also sufficient for pregnant women who are with adequate body stores at the start of pregnancy[69].

Most, but not all, authorities consider that pregnant women have increased requirements for vitamin B_6 as a result of the intensive transfer of B_6 to the fetus. The required increment of 0·4–0·5 mg/d added to the recommended value for non-pregnant women has been estimated based on the gestational weight gain, B_6 tissue content and the vitamin bioavailability from food[10,72]. Some have taken the view that B_6 requirements in pregnancy are not different from those in non-pregnant women since the observed changes of vitamin B_6 biomarkers in pregnancy are physiological and are not related to any obvious signs of deficiency[69].

The increased requirements for riboflavin intake in pregnancy are generally based on the progressive decline of riboflavin status throughout gestation[65] and rely heavily on the evidence from one small intervention study in Filipino pregnant and non-pregnant women[78]. The amount of 0·3 mg/d in addition to the requirements of non-pregnant women has been estimated to be sufficient for the normal growth of maternal tissues and the fetus[69,72,73,79].

Considering the lack of standardised approach for deriving dietary recommendations for B-vitamin intakes in pregnancy, and the fact that most existing recommendations are based on extrapolation from values for non-pregnant women and estimated needs for tissue growth and fetal transfer, there is a need for well-designed controlled feeding trials to be conducted in pregnancy. These will facilitate the generation of evidenced-based B-vitamin requirements during pregnancy to help support successful pregnancy outcomes without compromising maternal health.

Clinical implications of B-vitamins in pregnancy

B-vitamins play an important role in maintaining maternal health, fetal development and successful delivery. Irrefutable evidence from over 30 years ago showed that maternal folic acid supplementation in early pregnancy could protect against NTD[80,81], leading to global recommendations for supplementation of women with folic acid at 400 μg/d from preconception to the end of the first pregnancy trimester. NTD are major birth defects that occur when the neural tube fails to close properly in the first four weeks of gestation, leading to death of the fetus or newborn, or to various disabilities

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involving the spinal cord, the most common form of which is spina bifida. The exact mechanism for the protective effect of folic acid against NTD is unknown but it is believed that it is through OCM. In support of this hypothesis there is evidence from several reports, although only observational, indicating that other methyl donors as well as B-vitamins involved in OCM are also implicated in the risk of NTD. For example, low maternal B_{12} status was reported to be associated with an increased risk of NTD-affected pregnancy independently of folate[82,83]. Furthermore, a recent population-based, case-control study of more than 40 000 US pregnancies showed that the concurrent high consumption of vitamin B_6, vitamin B_{12}, choline, betaine and methionine in addition to folic acid was associated with a higher NTD reduction compared with the increased intake of a single nutrient[84]. Thus, the effectiveness of NTD prevention by folic acid supplementation could be further improved with the intakes of B-vitamins and other methyl donors involved in OCM. B-vitamin deficiencies are also associated with various adverse pregnancy outcomes and complications including anaemia[85–89], early pregnancy loss[89–91], hypertension in pregnancy and preeclampsia[92–94], low birth weight and intrauterine growth restriction[95]. Furthermore, there is emerging evidence for the inter-generational effects of maternal B-vitamin status during pregnancy on the offspring development[89,96,97]. The impact of B-vitamins on hypertensive disorders of pregnancy (HDP) and offspring neurodevelopment during childhood are considered below.

Impact on hypertensive disorders of pregnancy (HDP)

HDP are serious pregnancy complications that include chronic hypertension, gestational hypertension, preeclampsia/eclampsia, and preeclampsia superimposed on chronic hypertension[98]. These conditions affect up to 15 % of pregnancies and are recognised as one of the leading causes of maternal and fetal death globally[99]. In addition, HDP are also related to an increased risk for cardiovascular and metabolic diseases in later life of both mother and child[98,100]. The treatment of HDP is challenging as certain antihypertensive medications are associated with serious neonatal complications (intrauterine growth restriction, fetal bradycardia and distress and neonatal hypoglycaemia)[101]; therefore, the identification of effective nondrug approaches for managing hypertension in pregnancy could be particularly beneficial.

There is considerable observational evidence linking B-vitamins involved in OCM with the risk of HDP. The use of folic acid supplements in pregnancy has been related to a decreased risk of gestational hypertension and preeclampsia[92,102], whereas a systematic review of case-control studies showed that women with preeclampsia had significantly lower serum vitamin B_{12} concentrations than normotensive pregnant women[103]. A large observational study from our centre of over 2200 pregnancies found that riboflavin deficiency was associated with an almost 3-fold greater risk of developing hypertension during pregnancy[94]. However, the evidence so far from RCTs for a causal effect of B-vitamins is weak (Table 2)[104–108]. Inconsistencies in the literature might be explained to some extent by genetic differences among populations.

Emerging evidence from genome-wide association studies (GWAS) and epidemiological research has linked the C677T polymorphism in the gene encoding the folate-metabolising enzyme MTHFR with an increased risk of hypertension and HDP by up to 87 %, as mentioned earlier in this review[15–18]. Individuals with the homozygous variant TT genotype in *MTHFR* appear to have reduced MTHFR enzyme activity and thus impaired folate metabolism and OCM. Riboflavin, in the form of FAD, acts as a cofactor of MTHFR and molecular studies show that the variant enzyme becomes inactive as a result of an increased propensity to dissociate from FAD[109,110]. New evidence points to a novel gene-nutrient interaction of *MTHFR* with riboflavin in relation to blood pressure. Notably, our study of over 6000 Irish non-pregnant adults found that a deficient riboflavin status exacerbated the genetic risk of hypertension throughout adulthood, with a three-fold excess risk when the TT genotype occurred in combination with deficient riboflavin status compared to those without this polymorphism and normal riboflavin status[111]. Furthermore, in three previous RCTs of non-pregnant hypertensive patients, with and without CVD, we demonstrated that intervention with low-dose riboflavin (1·6 mg/d) for 16 weeks resulted in a lowering of systolic blood pressure by up to 13 mmHg, specifically in those with the TT genotype[112–114]. Together, these studies show that riboflavin plays an important role in modulating blood pressure phenotype in individuals with the variant TT genotype in *MTHFR*. This novel gene-nutrient interaction with implications for blood pressure management is important in public health terms, given that the frequency of the variant *MTHFR* 677TT genotype is 10–12 % in the UK and Ireland but it varies according to the geographical area with the highest frequency of 32–44 % is reported in Mexico[115–117]. Moreover, low to deficient riboflavin status is emerging as a much more widespread issue across the developed world than has previously been recognised[8,118]. Given the increased risk of HDP in pregnant women with *MTHFR* TT genotype, and the blood pressure-lowering effect of riboflavin in non-pregnant adults with this genotype, it is worth exploring further whether targeted supplementation with riboflavin in pregnancy has benefits as an effective preventive strategy to reduce the risk of HDP and decrease the burden of related adverse pregnancy outcomes in genetically at-risk women.

Impact on offspring neurodevelopment

There is emerging evidence linking maternal folate and vitamin B_{12} during pregnancy with offspring neurodevelopment and cognitive function in childhood. In terms of folate, two systematic reviews of observational studies concluded that maternal folate status or self-reported folate intake or usage of folic acid supplements were associated with improved cognitive function and reduced risk of behavioural and language problems of the child[119,120]. Of note is also that the supplementation with

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Table 2. Randomised trials investigating the effect of maternal B-vitamin supplementation on blood pressure in pregnancy

Author	Country	*n*	Maternal intervention, timing	Main findings
FA				
Charles *et al.*[104] 2005	Scotland, UK	2928	0·2 mg/d *v.* 5 mg/d *v.* placebo, < 30th GW	The odds of PE were lower in pregnant women receiving 0·2 mg/d *v.* 5 mg/d FA. Results were similar in those with the lowest serum folate status at baseline.
Hua *et al.*[105] 2016	UK, USA	3883	0·2 mg/d *v.* 5 mg/d *v.* placebo 0·8 mg/d *v.* placebo	The use of folic acid alone or as part of multivitamin supplementation could reduce the risk of hypertension in pregnancy by 40 %.
Wen *et al.*[106] 2018	Argentina, Australia, Canada, Jamaica & UK	2464	4 mg/d *v.* placebo, 8–16th GW until delivery	No effect of high-dose FA supplementation beyond the 1st trimester for the prevention of PE or related maternal & neonatal outcomes.
Zheng *et al.*[107] 2020	China	1576	0·4 mg/d *v.* 4 mg/d, from the first 3 months of pregnancy until delivery	Higher dose FA supplementation (4 mg/d) pre-pregnancy showed a significant reduction in recurrent PE *v.* Low-dose FA.
Vitamin B_6				
Salam *et al.*[108] 2015	Hungary, USA	1646	Pyridoxine (B_6) *v.* placebo	No significant differences in the risk of eclampsia or PE with administration of vitamin B_6.
Riboflavin				
Neugebauer *et al.* 2006[136]	Burkina Faso	815	15 m/d *v.* placebo, < 24th GW until delivery	No significant effects of riboflavin supplementation in PE prevention.
Combined B-vitamins and/or MMN				
Merchant *et al.* 2005[137]	Tanzania	1078	Combined MMN *v.* vitamin A *v.* MMN+vitamin A *v.* placebo, 12–27th GW until 3 months postpartum*	Combined MMN supplementation (compared to vitamin A alone) significantly reduced the risk of hypertension during pregnancy in HIV-infected women.
Rumiris *et al.*[138] 2006	Indonesia	60	Combined MMN *v.* control†	PE was significantly less frequent in the combined MMN group when compared to the control group.

FA, folic acid; GW, gestational week; PE, preeclampsia; MMN, multiple micronutrient.
*Combined daily multivitamin comprised of 20 mg thiamine, 20 mg riboflavin, 25 mg vitamin B_6, 50 μg vitamin B_{12}, 500 mg vitamin C, 30 mg vitamin E, 0·8 mg FA *v.* Vitamin A (30 mg beta-carotene + 5000 μg preformed vitamin A/d).
†Combined daily MMN comprised of vitamin A 1000 μg, vitamin B_6 2·2 mg, vitamin B_{12} 2·2 μg, vitamin C 20 mg, vitamin E 400 μg, FA 0·4 mg, N-acetylcysteine 200 mg, Cu 2 mg, Zn 5 mg, Mn 0·5 mg, Iron 30 mg, calcium 800 mg, selenium 100 μg *v.* Control (Iron 30 mg, FA 0·4 mg).

folic acid at doses higher than 1000 μg/d was negatively associated with global verbal scores in children[121]. These findings are in contrast with the results of a noteworthy RCT of mother-child pairs which showed that periconceptional supplementation with a multivitamin product containing folic acid did not have impact on the child cognitive function at 11 months, 2 and 6 years[122,123] (see Table 3 for the summary of the RCTs in this area). However, a RCT from this centre, the Folic Acid Supplementation in the Second and Third Trimesters (FASSTT) trial, investigated the effects of extended maternal folic acid supplementation (at the recommended dose of 400 μg/d) in trimesters 2 and 3 of pregnancy, and thus beyond the periconceptional period recommended for preventing NTD[124]. FASSTT trial found direct evidence for positive effects of folic acid on various cognitive domains in the children at age 3, 7 and 11 years and particularly in the verbal domain at age 7 and 11 years[96,97]. In addition, an objective assessment of the brain function by magnetoencephalography of the FASSTT offspring at 11 years suggested more efficient processing of language in children from folic acid-supplemented mothers[97]. Thus, maintaining optimal maternal folate throughout pregnancy, well beyond the early period known to be protective against NTD, may be beneficial for brain development and functioning in the child. Furthermore, the dose of folic acid supplementation appears to be of importance as higher doses than recommended might have a negative impact on offspring neurodevelopment.

In relation to vitamin B_{12}, a systematic review of observational studies (*n* 7) showed inconsistent results with investigations from developing countries demonstrating reduced cognitive function in children of mothers with deficient B_{12} status or with low B_{12} intakes whereas there was no evidence of an association between self-reported maternal B_{12} intake and offspring cognitive performance in the cohorts from developed countries[119]. In support of these findings are the results from a systematic review that focused on studies conducted in India, where the prevalence of vitamin B_{12} deficiency among pregnant women is up to 70 % and is considered one of the highest in the world[125]. The observational studies (*n* 4) included in this review supported associations of lower maternal B_{12} and poorer cognitive functions in the child, whereas one RCT showed a beneficial effect of B_{12} supplementation on offspring language abilities at 30 months but no effect at 9 months[125] (Table 3). Similar results were obtained in a recent Cochrane systematic review on RCTs (three ancillary analyses of one trial) which concluded that vitamin B_{12} supplementation during pregnancy improved offspring B_{12} status that may have important health impacts in later life but its effect on child cognitive function is still inconclusive and requires further research in larger scale trials[89].

The biological mechanism linking folate and B_{12} during pregnancy with offspring neurodevelopment is likely to be

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Table 3. Randomised trials investigating the effect of maternal B-vitamin supplementation and cognitive performance of the offspring

Author	Country	*n*	Maternal intervention, timing	Age of child	Cognitive assessment	Main findings
FA and/or MMN						
Dobo & Czeizel 1998[123]	Hungary	336 289	Multivitamin containing FA (0·8 mg/d) *v.* placebo, Preconception until 8th GW	2 years 6 years	Brunet-Lezine & Binet tests	No association between periconceptional multivitamin supplementation and cognitive development.
Christian *et al.* 2010[139]	Nepal	676	FA, Iron/FA, iron/FA/zinc or MMN, 11th GW to 3 months postpartum	7–9 years	UNIT & MABC	Iron/FA maternal supplementation associated with better intellectual, executive & motor function.
Campoy *et al.* 2011[140]	Europe	154	Fish oil and/or 5-MTHF *v.* placebo, 20th GW until birth	6·5 years	K-ABC^{KM}	No association between fish oil and/or 5-MTHF supplementation on cognitive performance.
Prado *et al.* 2012[141]	Indonesia	487	Iron/FA or MMN, Throughout pregnancy until 3 months postpartum	3·5 years	Motor, language, non-verbal cognitive & socio-emotional development	MMN supplementation associated with improved motor & cognitive abilities compared to iron/FA alone (specifically visual attention/spatial ability).
Catena *et al.* 2016[142]	Europe	136	Fish oil and/or 5-MTHF *v.* placebo, 20th GW until birth	8·5 years	Attention Network Test & EEG	Maternal 5-MTHF supplementation associated with improved conflict solving ability (executive function).
Prado *et al.* 2017[143]	Indonesia	2879	Iron/FA or MMN, Throughout pregnancy until 3 months postpartum	9–10 years	Motor, language, non-verbal cognitive & socio-emotional development	MMN supplementation long-term benefits for child cognitive development compared to iron/FA alone (specifically procedural memory and general intellectual ability).
McNulty *et al.* 2019[96]	Northern Ireland, UK	39 72	FA *v.* placebo, 2nd & 3rd trimesters	3 years 7 years	WPPSI	Maternal FA supplementation associated with increased cognition and word processing.
Caffrey *et al.* 2021[97]	Northern Ireland, UK	68	FA *v.* placebo, 2nd & 3rd trimesters	11 years	WISC & MEG	Maternal FA supplementation associated with increased processing speed, and verbal comprehension in girls only. Neuronal activity assessments using MEG indicated more efficient language processing.
Vitamin B_{12}						
Srinivasan *et al.* 2017[144]	India	178	50 µg/d *v.* placebo, ≤14th GW until 6 weeks postpartum	9 months	BSID-III	No effect of maternal B_{12} supplementation on early neurocognitive outcomes.
Thomas *et al.* 2019[145]	India	218	50 µg/d *v.* placebo, ≤14th GW until 6 weeks postpartum	30 months	BSID-III	Maternal B_{12} supplementation during pregnancy associated with higher expressive language scores.
Srinivasan *et al.* 2020[146]	India	132	50 µg/d *v.* placebo, ≤14th GW until 6 weeks postpartum	72 months	ERP	No effect of maternal B_{12} supplementation on childhood ERP measures. Elevated maternal MMA concentrations in the 3rd trimester were negatively associated with P300 amplitude in the child.
D'Souza *et al.* 2021[147]	India	74	50 µg/d (with or without MNN) *v.* placebo, preconception until delivery	2 years	BSID-III	Pre-conceptional B_{12} supplementation (*v.* placebo) improved maternal B_{12} status and offspring neurodevelopment in cognition & language domains. No effect of B_{12} + MMN *v.* placebo.
Chandyo *et al.* 2023[148]	Nepal	800	50 µg/d *v.* placebo, ≤ 15th GW until 6 months postpartum	6 months 12 months	BSID-III	B_{12} supplementation in pregnancy improved maternal B_{12} status but no corresponding effect on infant neurodevelopment.

FA dosage is 0·4 mg/d, unless otherwise stated. FA, folic acid; MMN, multiple micronutrient; GW, gestational week; UNIT, Universal Nonverbal Intelligence Test; MABC, Movement Assessment Battery for Children; 5-MTHF, 5-methyltetrahydrofolate; K-ABC^{KM}, Kaufman Assessment Battery for Children; EEG, electroencephalography; WPPSI, Weschler Preschool and Primary Scale of Intelligence; WISC, Weschler Intelligence Scale for Children; MEG, magnetoencephalography; BSID, Bayley Scales of Infant and Toddler Development; ERP, Event-related potentials; MMA, methylmalonic acid.

via the roles of these vitamins in OCM and the generation of SAM, which in turn is essential in donating methyl groups in numerous transmethylation reactions including the synthesis of neurotransmitters and DNA methylation[5,7]. DNA methylation is one of the key epigenetic mechanisms for regulation of gene expression

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which is currently a subject of intensive research and it was reported that maternal deficiency of folate and B_{12} could result in aberrant gene expression with consequential health outcomes[126,127]. By using a candidate gene approach, FASSTT trial, conducted in this centre, demonstrated that folic acid supplementation through trimesters 2 and 3 of pregnancy led to significant changes in DNA methylation in cord blood which also involved two genes related to brain development, IGF2 and BDNF[128]. The subsequently applied epigenome-wide screening on these samples identified differentially methylated regions[129], where a high proportion of the affected genes were associated with brain function as shown by examination through gene ontology analysis[130]. Furthermore, a study using a Mendelian randomisation approach provided evidence that DNA methylation plays a mediating and causal role in associations between maternal vitamin B_{12} status and offspring cognition[131]. These findings offer a biological basis to link maternal B-vitamin status with neuro-development of the offspring, but more thorough investigation of these diet-epigenome-brain relationships is currently ongoing within the international EpiBrain project, involving partners in the UK, Canada and Spain, with the aim of providing substantiation and translation of the evidence for health improvement strategies[132].

Conclusions

Pregnancy, a period of intense development with increased demands for folate and related B-vitamins (vitamins B_{12}, B_6 and riboflavin), is associated with substantial temporal changes in B-vitamin status. Despite this, there are no pregnancy-specific reference ranges for relevant B-vitamin biomarkers. This greatly limits the ability to identify and appropriately manage pregnant women most at risk of B-vitamin deficiency. The development of reference ranges for plasma B-vitamin concentrations in pregnancy to support placental and fetal growth and maintain maternal health is an urgent priority. Another challenge is the limited evidence and lack of standardised approach for deriving dietary recommendations for B-vitamin intakes in pregnancy, with current recommendations mostly based on extrapolation from reference values for non-pregnant women. Therefore, well-designed controlled feeding trials for defining evidenced-based B-vitamin requirements during pregnancy are needed, that will help support successful pregnancy outcomes without compromising maternal health.

Apart from the well-established protective effect of periconceptional maternal folic acid supplementation against NTD, the driver of international policy in this area, these B-vitamins are associated with a plethora of health impacts. Exciting new evidence indicates that the effectiveness of NTD prevention could be further enhanced with the simultaneous provision of folic acid along with other B-vitamins and methyl donors involved in OCM. Furthermore, continued supplementation with folic acid beyond the first trimester of pregnancy (the period covered by current folic acid recommendations and at the same dose of 400 μg/d), may have intergenerational effects by improving cognitive performance and neuronal functioning in the offspring into the second decade of life. If these results are confirmed in other populations, they may provide the basis for revising recommendations for folic acid usage during pregnancy with the aim of optimising brain health in the child. Emerging evidence also suggests that optimal riboflavin status may help to maintain healthier blood pressure in pregnancy and reduce the prevalence of HDP and related adverse consequences, particularly in pregnant mothers who are genetically at a higher risk of these conditions owing to impaired folate metabolism. However, this area of research is still at an early stage and requires further investigations using carefully designed randomised trials in pregnant women.

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N/A.

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There are no conflicts of interest.

Authorship

K.P. drafted the manuscript; A.C., B.D., M.W., M.C., M.K. and H. McN. critically revised the manuscript for important intellectual content. All the authors have read and approved the final manuscript.

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