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# Folate, vitamin B12 and homocysteine in relation to birth defects and pregnancy outcome

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Increased folate intake reduces the risk of neural tube defects, other malformations and also possibly, pregnancy complications. Increasing evidence suggests that the beneficial effect of folate may be related to improved function of methionine synthase, a vitamin B12-dependent enzyme that converts homocysteine to methionine. In India, the majority of the population adheres to a vegetarian diet known to be deficient in vitamin B12. In such a population, increased folate intake may offer minimal protection against birth defects, whereas vitamin B12 administration should be considered. In this review, is described the metabolism of and interrelations between folate, vitamin B12 and homocysteine. This is followed by a brief discussion of some of the proposed mechanisms for their biological effects in relation to birth defects and pregnancy outcome.

**Folate: Neural tube defects: Pregnancy complications**

Periconceptional intake of folic acid reduces the incidence of neural tube defects (NTD), one of the most common birth defects, by more than 50% (MRC-Vitamin-Study-Research-Group, 1991; Czeizel & Dudas, 1992; Berry *et al.* 1999; Botto *et al.* 1999). Folic acid may have a beneficial effect on other malformations (Czeizel 1993; Allen, 1996) and pregnancy complications as well (Scholl *et al.* 1996; Neggers *et al.* 1997; Leeda *et al.* 1998). Recent studies suggest that impaired vitamin B12 status (Adams *et al.* 1995; Rowland *et al.* 1995; Wilson *et al.* 1999), and elevated blood homocysteine (Hcy) (Burke *et al.* 1992; Steegers-Theunissen *et al.* 1992; de Vries *et al.* 1997; Ray & Laskin, 1999; Vollset *et al.* 2000) may also be associated with birth defects and common pregnancy complications such as spontaneous abortions, placental abruption, pre-eclampsia and low birth weight. It is now recommended that all women in their reproductive years should increase their folate intake to at least 400 μg per day, whereas the possible significance of vitamin B12 status has received less attention.

In this presentation, the focus will be on the metabolism of and interrelations between folate, vitamin B12 and Hcy, and then some of the proposed mechanisms for their biological effects will be discussed.

## Interrelation between folate, vitamin B12 and homocysteine

Homocysteine is formed from methionine as a product of numerous S-adenosylmethionine (AdoMet)-dependent transmethylation reactions (Fig. 1) (Mudd *et al.* 1995). Hcy so formed is either directed to the transsulfuration pathway which irreversibly converts Hcy to cysteine. Alternatively, it is remethylated to methionine, a reaction which in most tissues is catalysed by methionine synthase. This enzyme uses vitamin B12 as a cofactor and methyltetrahydrofolate (methyl-THF) as a substrate (Finkelstein, 1990), and this explains the close relation between folate, vitamin B12 and Hcy. Deficiency of either vitamin leads to elevated total Hcy (tHcy) level in plasma or serum (Allen *et al.* 1994), referred to as hyperhomocysteinemia.

The provision of methyl-THF for the methionine synthase reaction is from two sources. The major fraction derives from the common cellular folate pool and is provided by the methylenetetrahydrofolate reductase (MTHFR) reaction (Fig. 1) (Rozen, 1996). A second source, particularly in dividing cells, is through uptake of the circulating methyl-THF monoglutamate (=serum folate). The initial demethylation of this newly absorbed folate through the methionine synthase reaction is critical for providing the cells with the folates used in DNA synthesis reaction. Moreover, the THF formed becomes polyglutamated, a process which ensures that the cellular folates are retained (Shane & Stokstad, 1985). Interestingly, both Hcy elimination and methyl-THF formation are under strict regulation of AdoMet and therefore the methionine level (Finkelstein, 1990). When AdoMet is in excess, the transsulfuration pathway is activated, leading to elimination of methionine. On the other hand, when AdoMet is low, MTHFR is stimulated, and this directs the folates from DNA and RNA synthesis to methionine conservation (Scott & Weir, 1981).

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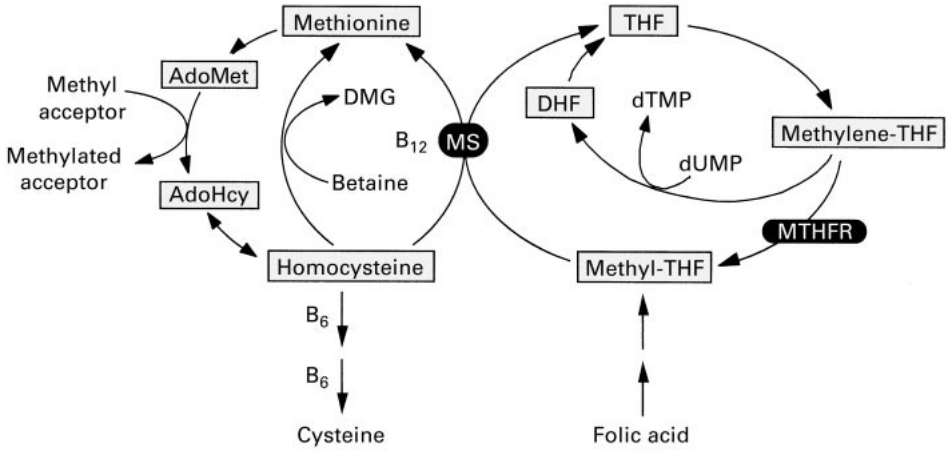


**Fig. 1.** Interrelation between folate, vitamin B12 and homocysteine metabolism. AdoHcy, adenosylhomocysteine; AdoMet, adenosylmethionine; DHF, dihydrofolate; DMG, dimethylglycine; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate.

Both folate and vitamin B12 deficiency will lead to reduced methionine formation, and if provision of methionine is limited, the MTHFR activity will be stimulated, thereby providing more methyl-THF for methionine synthase. During vitamin B12 deficiency, however, the enzyme function remains impaired, and a situation referred to as the methylfolate trap develops (Herbert & Zalusky, 1962; Scott & Weir, 1981): The methyl-THF accumulates at the expense of the other cellular folates, and the uptake of folate from serum is prevented. Thus, the biochemical effects of both folate and vitamin B12 deficiency are quite similar and include a functional folate deficiency, hyperhomocysteinemia, and a methionine level that is too low relative to the Hcy level. The possible consequences of these three biochemical conditions will be now discussed.

## Cellular folate deficiency

In addition to being a substrate in the methionine synthase reaction, folate is required for synthesis of both DNA and RNA. In severe folate deficiency, cell division is impaired, and a characteristic morphologic picture arises, i.e. the megaloblastic changes (Wickramasinghe, 1999). Presumably, severe deficiency is incompatible with normal fetal growth and development. Originally, it was believed that the cause of megaloblastosis was explained by reduced folate dependent formation of dTMP from dUMP (Fig. 1) and possibly also inhibited purine synthesis (Wickramasinghe, 1999). However, more recent data suggest that under conditions of low folate, uracil will frequently be incorporated into DNA instead of thymine, and the normal repair processes to remove the misincorporated uracil often fails. This will ultimately lead to double strand break and chromosome instability which again promotes apoptosis (Blount *et al.* 1997; Koury *et al.* 1997). It appears that chromosomal damage also occurs in subjects without clinical symptoms but with low folate or vitamin B12 status or increased tHcy (Blount *et al.* 1997; Fenech *et al.* 1998).

Perhaps the strongest evidence against the theory that congenital malformations are related to inhibited folate dependent dTMP formation are the observations related to the common C677T polymorphism in the MTHFR gene. Homozygosity for this polymorphism, the TT genotype, is associated with NTD (Van der Put *et al.* 1995; Christensen *et al.* 1999; Shields *et al.* 1999). This enzyme variant has low activity, and the afflicted subjects frequently have hyperhomocysteinemia. Notably, TT subjects usually have a normal or even high folate content, but their methyl-THF level is low (Bagley & Selhub, 1998). These data point in the direction of impaired methionine synthase function or a disturbed ratio between methionine and Hcy as the cause of major malformations (Lucock *et al.* 1997). In further support of this, is a recent finding that the common A66G polymorphism in methionine synthase reductase (MTRR), the enzyme that activates cobalamin-dependent methionine synthase, increases NTD risk when cobalamin status is low (Wilson *et al.* 1999).

## Imbalance between methionine and Hcy levels

Methionine is required for protein synthesis and it is the precursor of AdoMet which is required in polyamine synthesis and in the numerous transmethylation reactions (Finkelstein, 1990). In relation to birth defects, it is particularly AdoMet and its role in the transmethylation reactions that has received attention (Fig. 1). AdoMet is used in the methylation of phospholipids, proteins, DNA, RNA, amino acids, neurotransmitters and a number of other small molecules. Methylation and demethylation of critical CpG loci in DNA may cause gene silencing and gene activation, respectively, thereby regulating mammalian gene expression and cellular differentiation. In addition, methylation of ribosomal RNAs plays an important role in mRNA function and integrity (Chiang *et al.* 1996). Thus, a disturbed methylation activity may interfere with normal fetal growth and development in a number of different ways.

Reduced methylation activity may occur when the AdoMet level is very low. Indeed, a recent study observed that women with low methionine intake had an increased

risk of having a NTD child (Shaw *et al.* 1997). However, a more common cause may be an unfavourable (low) ratio between AdoMet and adenosylhomocysteine (AdoHcy). This latter compound is the product formed after demethylation of AdoMet, and it is the immediate precursor of Hcy (Fig. 1). A high AdoHcy exerts a negative feed back on the transmethylation reactions. A high Hcy by increasing AdoHcy will have a similar effect. Notably, a deficiency of either vitamin B12 or folate will be particularly deleterious since both Hcy (AdoHcy) and methionine (AdoMet) change in an unfavourable direction. A high intake of methionine will circumvent the effect of a vitamin deficiency because the AdoMet: AdoHcy ratio is restored. Moreover, AdoMet has a folate sparing effect through its effect on MTHFR (Shane & Stokstad, 1985).

In this respect, a dietary vitamin B12 deficiency should be briefly mentioned. Vitamin B12 is only provided in animal derived proteins, and a vegetarian diet therefore contains little vitamin B12. Unfortunately, such a diet is also a poor source of methionine, thus, vegetarians may be at particularly high risk of developing conditions associated with reduced methylation activity. Indeed, low dietary intake or malabsorption of vitamin B12 may be the reason for the high risk of NTD in countries such as India (Sharma *et al.* 1994) and Mexico (Allen *et al.* 1995; Botto *et al.* 1999) where the reported incidence in some regions is nearly ten times higher than that observed in the United States (Lary & Edmonds, 1996; Botto *et al.* 1999). Notably, the harmful effect of vitamin B12 deficiency is not confined to birth defects and pregnancy complications. The babies of vitamin B12 deficient mothers have low stores of vitamin B12, and breast feeding may further aggravate the condition since maternal milk is low in B12 (Specker *et al.* 1990; Allen *et al.* 1995). Even a mild vitamin B12 deficiency in the mother may later in infancy cause growth retardation, delayed psychomotor development and in some instances, permanent effects on the developing brain (Schneede *et al.* 1994).

## Homocysteine accumulation

Hcy accumulation may have multiple biological effects. It may impair the methylation activity as described above. In addition, a high plasma tHcy level is believed to be thrombogenic and atherogenic (Refsum *et al.* 1998). The mechanism behind these vascular effects is not identified but platelet abnormalities, stimulated coagulation or inhibited fibrinolysis, smooth muscle cell proliferation, LDL oxidation and endothelial dyfunction have all been demonstrated in experimental systems (Refsum *et al.* 1998). The relevance of these findings *in vivo*, however, is uncertain.

Increasing evidence suggests that oxidative stress is a mediator of endothelial cell dysfunction and that it may contribute to the vascular complications of pregnancy (Davidge, 1998). In this regard, it is particularly interesting that hyperhomocysteinemia causes an acute endothelial dysfunction through mechanisms involving oxidative stress (Lentz, 1998). A recent clinical study elegantly demonstrated that Hcy interferes with nitric oxide function through its pro-oxidant effects (Chambers *et al.* 1999). If so, the relation between hyperhomocysteinemia and the pregnancy complications involving placental ischemia can be explained.

In relation to congenital abnormalities, an interesting observation is that Hcy interacts with the N-methyl-D-asparate (NMDA) receptor system which is involved in neuronal development and migration. A recent study showed that agents interfering with the NMDA receptor, are potent teratogens in animal embryo models (Andaloro *et al.* 1998), and it has been suggested that hyperhomocysteinemia may cause NTD through such mechanisms (Rosenquist *et al.* 1997).

## Conclusions

Worldwide, a unified strategy has been adopted to reduce the incidence of NTD, i.e. that all women in their reproductive years should substantially increase their folate intake (Locksmith & Duff, 1998), preferably by taking folic acid supplements (Cuskelly *et al.* 1996). However, in addition to folate status, low vitamin B12 level and elevated levels of tHcy have also been associated with birth defects and pregnancy complications. The biochemical basis for their effect on the fetus and the pregnant woman remains uncertain, but increasing evidence points in the direction of altered AdoMet dependent transmethylase activity, impaired methionine synthase function and hyperhomocysteinemia. From a practical point of view, the identification of the exact mechanisms may seem only of academic interest. However, an appropriate strategy for the prevention of these common conditions may critically depend on the underlying biochemical defect. For instance, increased folic acid intake may be appropriate for the Western society where impaired folate status and hyperhomocysteinemia usually are related to the C677T polymorphism, poor diet or unhealthy life style (Guttormsen *et al.* 1996; Nygård *et al.* 1998). However, intervention with folic acid alone may not only be inefficient, but may even cause harm to women living in regions where vitamin B12 deficiency is endemic. The scientists, clinicians and policy-makers in different countries should carefully investigate and evaluate the relevance of the present guidelines in their own populations.

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