

# Methionine and Homocysteine Metabolism and the Nutritional Prevention of Certain Birth Defects and Complications of Pregnancy

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## Abstract

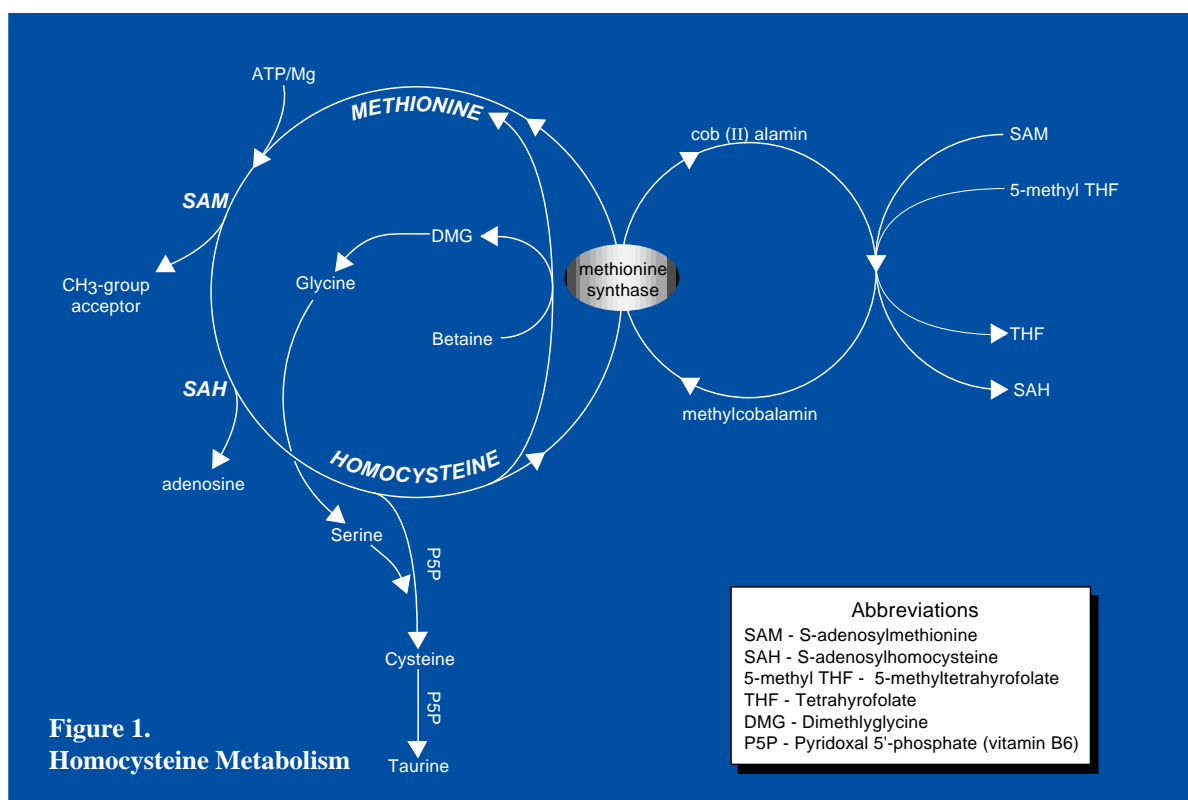
Defective metabolism of the essential amino acid methionine, resulting in overt hyperhomocysteinemia or situational hyperhomocysteinemia (after a methionine load), has been established as an independent risk factor for atherosclerotic heart disease. Nutrients involved in the pathways of homocysteine degradation, including folic acid, vitamins B6 and B12 all have a connection to negative pregnancy outcomes, which may be related to their impact on homocysteine. Dietary intake and metabolism of folic acid, the nutrient most closely identified with neural tube defects, has been studied in depth for the past fifteen years. The information from these studies has illuminated the mechanisms of these congenital defects, and has led to the discovery of connections with other nutrients related to homocysteine metabolism which may also be involved in negative pregnancy outcomes, including spontaneous abortion, placental abruption (infarct), pre-term delivery, and low infant birth weight.

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## Introduction

Approximately 4,000 pregnancies in the U.S. each year are affected by neural tube defects, the most commonly occurring manifestations being spina bifida and anencephaly. At a monetary cost of approximately \$295,000 per case throughout the affected individual's lifetime, spina bifida ranks as the third most expensive birth defect. From 1983 through 1990, the incidence rate for spina bifida in the U.S. was approximately 4.6 cases per 10,000 births, with rates varying from state-to-state. The incidence rate is highest in Hispanics and lowest in Asians and Pacific Islanders.<sup>1</sup> This variance between ethnic groups points toward a genetic component in the development of this congenital defect. Genetically-induced biochemical defects and/or nutritional deficiencies seem to be involved in a large number of these cases, as well as other negative pregnancy outcomes, including spontaneous abortion, placental abruption (infarct), pre-term delivery, and low infant birth weight.

Hyperhomocysteinemia has received increasing attention during the past decade and has joined smoking, dyslipidemia, hypertension, and obesity as an independent risk factor for cardiovascular disease. In addition to its possible role in cardiovascular disease, increased homocysteine levels have been implicated in a variety of other clinical conditions, including neural tube defects, spontaneous abortion, placental abruption, osteoporosis, renal failure, diabetic



microangiopathy, neuropsychiatric disorders, and pre-menstrual syndrome.

Studies of healthy men and women indicate that certain acquired and genetic determinants may impact total plasma homocysteine (tHcy). Women tend to have lower basal levels than men, and both contraceptives and hormone replacement therapy do not seem to significantly alter the levels.<sup>2</sup> Homocysteine concentrations are significantly higher in postmenopausal women than in premenopausal women; however, the above-mentioned sex differences in tHcy concentrations persist in elderly populations.<sup>3-5</sup> Nutrition impacts tHcy concentrations in both men and women. Those individuals in the lowest quartiles for serum folate and vitamin B12 (nutrients which significantly impact homocysteine metabolism) have significantly higher concentrations of tHcy, and men in the lowest quartile of serum pyridoxal-5'-phosphate (vitamin B6, another essential homocysteine-degrading nutrient) also have increased tHcy concentrations.<sup>2</sup> The fetus, the neonate, and the pregnant woman have an increased requirement for folic

acid and vitamin B12, and are more likely to suffer from a deficiency of these vitamins.<sup>6</sup>

## Homocysteine Metabolism

Metabolism of the amino acid methionine, a limiting amino acid in the synthesis of many proteins, affects several biochemical pathways involving the production of nutrients which are essential to the optimal functioning of the cardiovascular, skeletal, and nervous system.

Homocysteine is an intermediate product of methionine metabolism and is itself metabolized by two pathways: the re-methylation pathway which regenerates methionine, and the trans-sulfuration pathway which degrades homocysteine into cysteine and then taurine.

The re-methylation pathway (see Figure 1) is comprised of two intersecting biochemical pathways and results in the transfer of a methyl group (CH<sub>3</sub>) to homocysteine by either methylcobalamin (which receives its methyl group from S-adenosylmethionine or 5-methyltetrahydrofolate, an active form of folic acid) or

betaine (trimethylglycine). Methionine can then be utilized to produce S-adenosylmethionine (SAM), the body's "universal methyl donor", which participates in several other key metabolic pathways, including methylation of DNA and myelin (see section on methionine).

The trans-sulfuration pathway of methionine/homocysteine degradation (see Figure 1) produces the amino acids cysteine and taurine, and is dependent on adequate intake of vitamin B6 and the hepatic conversion of B6 into its active form, pyridoxal 5'-phosphate (P5P). Also necessary is the amino acid serine, a downline metabolite generated from betaine via the homocysteine-remethylation pathway.

Betaine supplementation has been shown to reduce homocysteine levels while resulting in modest increases of plasma serine and simultaneous increases of plasma cysteine levels.<sup>7</sup> Serine levels are depressed in some individuals with excess homocysteine who are treated with folic acid, cobalamin, and vitamin B6.<sup>8</sup> Because serine is required for: 1) the conversion of folic acid to its active form, 2) as a shuttle for methyl groups between the cytosol and the mitochondria, and 3) as a co-factor in the trans-sulfuration pathway of methionine/homocysteine metabolism, supplementation with betaine should be included with folic acid, cobalamin and pyridoxal-5'-phosphate in order to optimize the interrelated pathways of homocysteine metabolism.

### **Homocysteine and Pregnancy**

Research in progress in The Netherlands demonstrates that a derangement of methionine-homocysteine metabolism could be the underlying mechanism of the pathogenesis of neural tube defects and may be the mechanism of prevention observed with folic acid supplementation. Derangement of methionine-homocysteine metabolism was found in approximately 20% of cases with NTD,

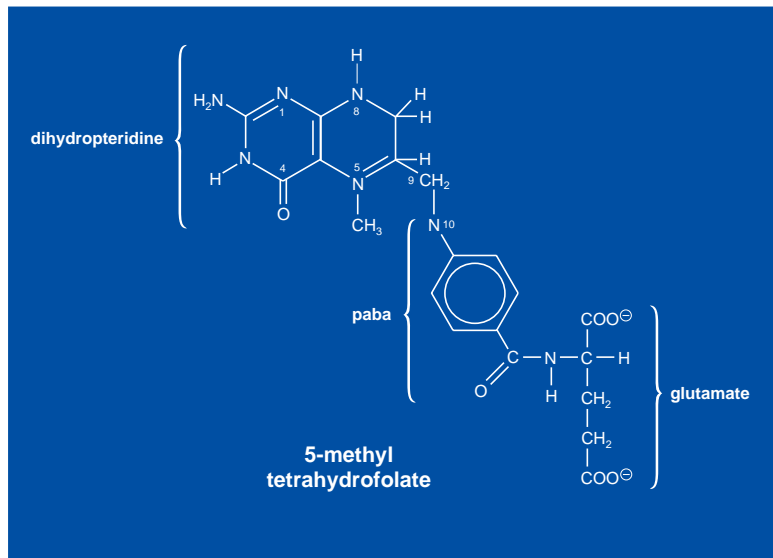
recurrent miscarriage and placental infarcts (abruption) and offers new possibilities for primary prevention in these three areas.<sup>9</sup>

Approximately 25-33% of women with a history of NTD-affected pregnancy show increased homocysteine levels after methionine loading,<sup>10</sup> possibly due to decreased remethylation of homocysteine to methionine secondary to decreased enzymatic activity of the folate-and-B12-dependent enzyme homocysteine methyltransferase (methionine synthase).<sup>11</sup> This abnormal metabolism and the resultant increased homocysteine levels may be an indirect indicator of aberrant folate metabolism, or may be a direct cause of teratogenicity. Homocysteine itself may be toxic to the embryo<sup>12</sup> or may be an indicator of reduced availability of SAM for methylation of DNA.

Animal studies also suggest that a decreased conversion of homocysteine to methionine could be a crucial step in causing neural tube defects. It has been shown that rat embryos in culture require methionine for neural tube closure.<sup>13</sup>

Homocysteine may also participate in placental abruption. Homocysteine levels were evaluated in 46 women with a normal pregnancy and 84 women with placental abruption or infarction. Elevated levels were seen in only 9% of the controls as opposed to 31% of those with placental abruption or infarction. Serum vitamin B12 and whole blood pyridoxal-5'-phosphate were also lower in the cases than the controls; however red cell folate levels were found to be normal. Median fasting plasma homocysteine concentrations were significantly higher in women who experienced placental abruption or infarction in their first pregnancy than women who had the same event after one or more uncomplicated pregnancies.<sup>14</sup>

**Figure 2.**  
**Structure of 5-methyltetrahydrofolate**



homocysteine metabolism) and methylidynetetrahydrofolate (involved in purine synthesis), as well as functioning on its own in the generation of thymine side chains for incorporation into DNA.

The following may contribute to a deficiency of folic acid: a deficient food supply, a defect in utilization as in alcoholics, malabsorption, increased needs in pregnant women and in cancer patients, metabolic interference by drugs, folate losses in hemodialysis, and enzyme or cofactor deficiency needed for generation of active

### Folic Acid Metabolism

Folates function as carbon donors in the synthesis of serine from glycine, directly in the synthesis of purines and pyrimidine bases, indirectly in the synthesis of transfer RNA, and as a methyl donor to create methylcobalamin which is used for remethylation of homocysteine to methionine. Dietary folic acid is a mixture of folates in the form of polyglutamates, which are readily destroyed by cooking.

In plants, folic acid is formed from a hetero-bicyclic pteride ring, para-aminobenzoic acid (PABA), and glutamic acid (see Figure 2). Folate is initially deconjugated in the cells of the intestinal wall to the monoglutamate form. This is then reduced to dihydrofolate and then to tetrahydrofolate (THF) via folate and dihydrofolate reductase. Both of these enzymes require NADPH (niacin dependent) as a cofactor. Serine combines with pyridoxal-5'-phosphate to transfer a hydroxymethyl group to THF. This results in the formation of 5, 10-methylenetetrahydrofolate (methylene THF) and glycine. (see Figure 3) This molecule is of central importance, being the precursor of the metabolically-active 5-methyltetrahydrofolate (5-methylTHF, which is involved in

folic acid.

Folinic acid (5-formylTHF- available supplementally as calcium folinate—also known as leucovorin calcium) is an immediate precursor to 5, 10 methyleneTHF and 5-methylTHF. Folinic acid is more stable than folic acid and has a longer half-life in the body. Folinic acid also readily crosses the blood-brain barrier and is slowly cleared, compared to folic acid, which is poorly transported into the brain, and once in the CNS is rapidly cleared.<sup>15</sup>

### Folic Acid and Pregnancy

It has been firmly established that a low dietary intake of folic acid increases the risk for delivery of a child with a neural tube defect (NTD), and that periconceptional folic acid supplementation reduces the occurrence of neural tube defects, which include the major malformations spina bifida and anencephaly. Illustrating this, the U.S. Public Health Service recommended in September 1992 that all women of childbearing age consume 0.4 mg folic acid daily to reduce their risk of having a pregnancy affected with a neural tube defect.

Fifteen years ago (which indicates how long it takes to change prevailing attitudes and public policy) Laurence, et al. published their intervention study of folic acid supplementation and its effects on NTD recurrence in women with a previously-affected birth. Forty-four women took 4 mg folic acid daily before conception and during early pregnancy. There were no NTD recurrences in this fully-supplemented group, while four incidences were observed in the placebo group (n=51). Interestingly, 16 women initially in the supplemented group did not comply, and there were two NTD-affected births in this group. Therefore, there were six NTD births in the "unsupplemented" group (9.0%) versus none in the supplemented group (p=0.04).<sup>16</sup>

More recently, The Medical Research Council Vitamin Study Group found that periconceptual folic acid supplementation (4 mg/day) in 1195 women who were at high risk for a neural tube defect pregnancy due to a previous NTD-affected birth, resulted in 6 NTD-affected births in the folic acid group compared to 21 NTD births in the unsupplemented group; a relative risk of NTD of 0.28, or a 72% reduction in the recurrence of NTD.<sup>17</sup>

In a Cuban study, a 5 mg supplemental folic acid dose given periconceptionally to 81 women who had a previous NTD birth resulted in no recurrences, while in the 114-women unsupplemented control group, four recurrences were noted (3.5% recurrence).<sup>18</sup>

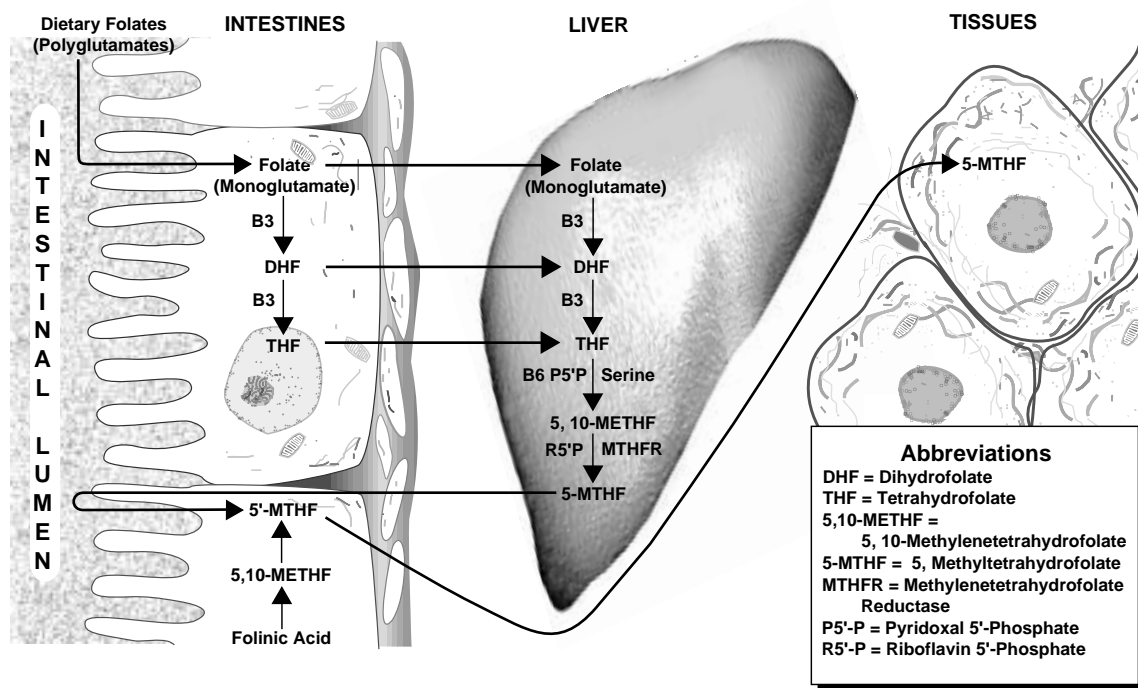
A 1989 study of first-occurrence of NTD in 22,776 pregnancies revealed a 0.35% occurrence rate of NTD in unsupplemented subjects vs. a 0.09% occurrence rate for women who took a multivitamin containing folic acid during the first six weeks or pregnancy.<sup>19</sup>

In another study of first occurrence of NTD, Czeizel and Dudas found that, in a group of 2104 women supplemented periconceptionally with a multiple vitamin-mineral containing 5 mg folic acid per day, there

were no incidences of NTD. However, in 2052 women supplemented with only a "trace mineral supplement" (copper, manganese, zinc, and vitamin C), there were 6 cases (p = 0.029). These researchers also noted that there was no statistical difference between these groups regarding incidences of other congenital malformations.<sup>20</sup>

Higher levels of dietary folate intake have also been shown to decrease the occurrence of NTD.<sup>21-23</sup> However, women predisposed to NTD-related pregnancies may need to take in more dietary folate to reach the same plasma levels of women without NTD pregnancies.<sup>12</sup> Also, an increase in the dietary intake of folates may not lead to an increase in red cell folate (a reliable measure of tissue folate), especially in women with a history of NTD. Cuskelly et al, at the University of Ulster in Ireland, found that increasing the intake of folate-rich foods in the diet did not significantly raise red cell folate levels in the 41 women tested. However, folic acid given as a dietary supplement or added to food did increase red cell folate levels (p<0.01). This information underlies the importance of periconceptual folic acid supplementation, as the monoglutamate form (folic acid) is more stable and, from this study's results, is more bioavailable than the dietary (polyglutamate) form.<sup>24</sup>

Investigation of women with a history of two NTD-affected pregnancies has shown that they may have a defect in folate uptake and/or metabolism. Sixteen women with a history of two NTD-affected births had no correlation between dietary folate intake and serum or red blood cell folate levels, while controls, who had no history of NTD births, had significant increases in serum and RBC folate with increases in dietary folate.<sup>25</sup> In another study, investigators found that controls showed a direct relationship between serum folate and RBC folate, while women with a history of NTD births did not.<sup>26</sup> In 1987, Yates,



**Figure 3.**  
**Absorption and Activation of Folic Acid**

et al. noted that the mean RBC folate in a group of 20 women with two or more NTD-affected offspring was significantly lower than controls. A linear relationship was discovered between RBC folate (but not serum) levels and the number of NTD-affected pregnancies, while the lowest levels were found in those women with more than two affected births. One factor involved in the increased risk of NTD<sup>27</sup> with low red cell folate levels may be an inherited folate metabolism disorder which is not expressed until the cellular stress and increased need during pregnancy unmasks it.<sup>12</sup>

Researchers at Trinity College in Dublin, Ireland, have identified a gene responsible for an increased incidence of NTD. The gene, which is three times more prevalent in women with NTD infants than women without a history of NTD, regulates the activity of methylene tetrahydrofolate reductase (MTHFR), the enzyme responsible for converting 5,10-methylenethf into 5-methylthf, the form of folic acid involved in the remethylation of homocysteine to methionine. Therefore, these women could have adequate

dietary intake of folates, but improper conversion to the form needed for homocysteine disposition.<sup>28</sup> Others have suggested one or more metabolic defects in folate metabolism, the most likely being at or above MTHFR<sup>12</sup> (see Figure 3).

A genetic polymorphism, with the substitution of an alanine for valine on the enzyme MTHFR, a riboflavin-dependent enzyme, exists in some individuals. This substitution is likely to reduce the ability to produce the methyl form of folic acid which is needed along with cobalamin for one of the remethylation pathways of homocysteine to methionine. In a French-Canadian population, 51% of those sampled were heterozygous for this mutation and 12% were homozygous. The individuals, particularly with the latter genotype, had clearly elevated tHcy concentrations.<sup>2</sup> Dutch patients with premature cardiovascular disease also have been shown to have a relatively high frequency of heterozygosity (35%) and homozygosity (15%) for this amino acid substitution.<sup>29</sup>

Some anti-seizure medications are folate antagonists, and as such can increase the risk for NTD. Valproic acid may inhibit glutamate formyltransferase, the enzyme responsible for the conversion of THF to the oxidized forms 5-formyl and 10-formyl THF, which are involved in pyrimidine synthesis.<sup>30</sup> In a study of anticonvulsive drug use and congenital malformations, folic acid supplementation completely prevented the incidence of birth defects, which was 15% without supplementation.<sup>31</sup> Other drugs, including methotrexate, 5-fluorouracil, sulfasalazine, oral contraceptives, diphenylhydantoin, trimethoprim, and pyrimethamine can inhibit the absorption or conversion of dietary or supplemental folic acid. For example, sulfasalazine inhibits three separate enzymes involved in folate conversion; dihydrofolate, MTHFR, and serine transhydroxymethylase.<sup>32</sup>

A few studies have investigated if other birth outcomes besides NTD may be influenced by dietary or supplemental folic acid intake. In a group of 285 pregnant women, folate supplementation resulted in increased infant birth weight and Apgar scores, and a decreased incidence of fetal growth retardation and maternal infections.<sup>33</sup> In a recent study, the authors analyzed the outcomes of 832 births in Camden, NJ, specifically looking at preterm delivery (<37 wks) and low infant birth weight (<2500 g). Women with a low daily folate intake (<240 µg/d) had a significantly increased risk (>200%) of preterm delivery and low birth weight. These birth outcome parameters also corresponded to serum folate levels at week 28, with significant increases in adjusted risk as serum folate decreased.<sup>34</sup> This inverse correlation between serum folate levels in the third trimester and increased risk of low birth weight has been demonstrated in other investigations as well.<sup>35,36</sup>

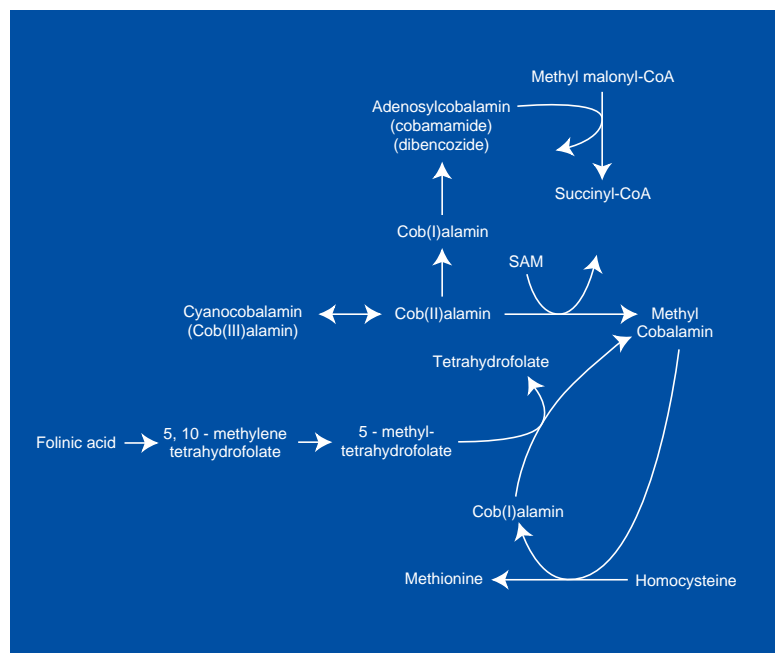
## Vitamin B12

The coenzyme form of vitamin B12 is a very complex molecule containing cobalt bound to 5 nitrogens and one carbon. The metal-carbon bond found on this coenzyme is the only known biological example of this type of linkage. Surrounding the cobalt is a corrin ring, which structurally resembles the porphyrin ring found in hemoglobin, the cytochromes and chlorophyll. The use of cobalt in the two biologically-active forms of cobalamin, adenosyl- and methylcobalamin, is the only known function of this metal in biological systems.

In humans, the cobalt in cobalamin exists in a univalent oxidation state, designated as cob(I)alamin. The compound commonly referred to as vitamin B12 has a cyanide molecule at the metal-carbon position and the oxidation state of the cobalt is +3 instead of the biologically active +1. In order to be utilized in the body, the cyanide molecule must be removed. It is thought that glutathione may be the compound that performs this function. Other available forms of vitamin B12 include hydroxocobalamin, and the two active forms: adenosylcobalamin (cobamamide) and methylcobalamin.

The absorption of dietary cobalamin requires the formation of a complex between dietary B12 and R-proteins and the secretion, by the stomach mucosa, of intrinsic factor. The B12 complex is split by pancreatic proteases and the released B12 attaches to intrinsic factor and is absorbed in the distal ileum. The amount of cobalamin required in the diet is very low and even people with pernicious anemia can generally absorb sufficient amounts if the coenzyme is supplemented at a high enough dosage.

Although the basic cobalamin molecule is only synthesized by micro-organisms, all mammalian cells can convert this into the coenzymes adenosylcobalamin and methylcobalamin. Adenosylcobalamin is the

**Figure 4. Cobalamin Metabolism**

major form in cellular tissues, where it is retained in the mitochondria. Methylcobalamin predominates in blood plasma and certain other body fluids, and in cells is found in the cytosol.

Adenosylcobalamin functions in reactions in which hydrogen groups and organic groups exchange places. In humans, adenosylcobalamin is required in only two reactions: the catabolic isomerization of methylmalonyl-CoA to succinyl-CoA and interconversion of alpha- and beta-leucine. After its formation from methylmalonyl-CoA, succinyl-CoA is either involved in the synthesis of porphyrin molecules (along with glycine) or transfers its coenzyme A to form acetyl coenzyme A. The latter reaction is magnesium dependent and the remaining succinate is fed into the citric acid cycle. Deficiencies in this coenzyme form of vitamin B12 result in increased amounts of methylmalonyl CoA and generally an increase in glycine levels.

Methylcobalamin's only known biological function in humans is in the remethylation of homocysteine to methionine via the enzyme methionine synthetase, also known as 5-methyltetrahydrofolate-homocysteine methyltransferase. In order to originally form

methylcobalamin from cyanocobalamin or other Cob(III)alamin or Cob(II)alamin precursors, S-adenosylmethionine (SAM) must be available to supply a methyl group. Once methylcobalamin is formed it functions in the regeneration of methionine by transferring its methyl group to homocysteine. Methylcobalamin can then be regenerated by 5-methyl-THF (see Figure 4). The cell's ability to methylate important compounds such as proteins, lipids and myelin will be compromised by a deficiency of either folate or vitamin B12.<sup>37</sup> Shortages of active folic acid, SAM, or a dietary deficiency

of cobalamin will lead to a decrease in the generation of methylcobalamin and a subsequent impairment in homocysteine metabolism. Since lack of methylcobalamin leads to depressed DNA synthesis, rapidly-dividing cells in the brain and elsewhere are affected.

At least 12 different inherited inborn errors of metabolism related to cobalamin are known. Abnormalities are detectable by urine and plasma assays of methylmalonic acid and homocysteine, and plasma and erythrocyte analysis of cobalamin coenzymes, which can reveal deficiencies of methylcobalamin or adenosylcobalamin.<sup>38</sup>

Low plasma vitamin B12 levels have been shown to be an independent risk factor for neural tube defect in one study.<sup>39</sup> This was an original finding and needs to be confirmed still in further studies. If methionine synthetase is the critical enzyme, methylcobalamin might be able to stimulate the abnormal enzyme as folic acid does, since active folic acid acts to provide the methyl group to cobalamin. It is quite probable that a deficiency in Vitamin B12, folic acid, or any of the cofactors required for their activation may result in a similar dysfunction.<sup>13</sup>



## **Methionine**

Methionine is a component of many proteins and cannot be manufactured from other dietary amino acids. It serves as a source of available sulfur for the synthesis of both cysteine and taurine, and as SAM it is the most important methyl-group donor in cellular metabolism.

SAM is formed by the transfer of an adenosyl group from ATP to the sulfur atom of methionine. This reaction requires magnesium as a cofactor. When methyl groups are transferred from SAM, S-adenosylhomocysteine is formed. This is then hydrolyzed to release the adenosine and results in the formation of homocysteine.

SAM is known to be utilized in the synthesis of the following compounds: carnitine, coenzyme Q10, creatine, methylcobalamin from Cob(III)alamin, 1-methylnicotinamide, N-methyltryptamine, phosphatidylcholine, and polyamines. It is also utilized in methylation reactions as part of hepatic phase II detoxification.

Supplementation with methionine (70 mg/kg body wt) of Axd mutant mice, which are predisposed to NTD, has been shown to reduce the incidence of NTD by 41% when administered on days eight and nine of pregnancy. Supplementation of dams with a dose of 180 mg/kg body weight produced a reduction in NTD of 47%. Maternal supplements of folic acid (33 mg/kg) or vitamin B-12 (330 mg/kg) did not alter the incidence of NTD among these animals.<sup>40</sup>

Adequate dietary methionine seems to exert a protective effect in Wistar female rats fed a folic acid-deficient diet, while a diet deficient in both folic acid and methionine results in fetal underdevelopment.<sup>41</sup>

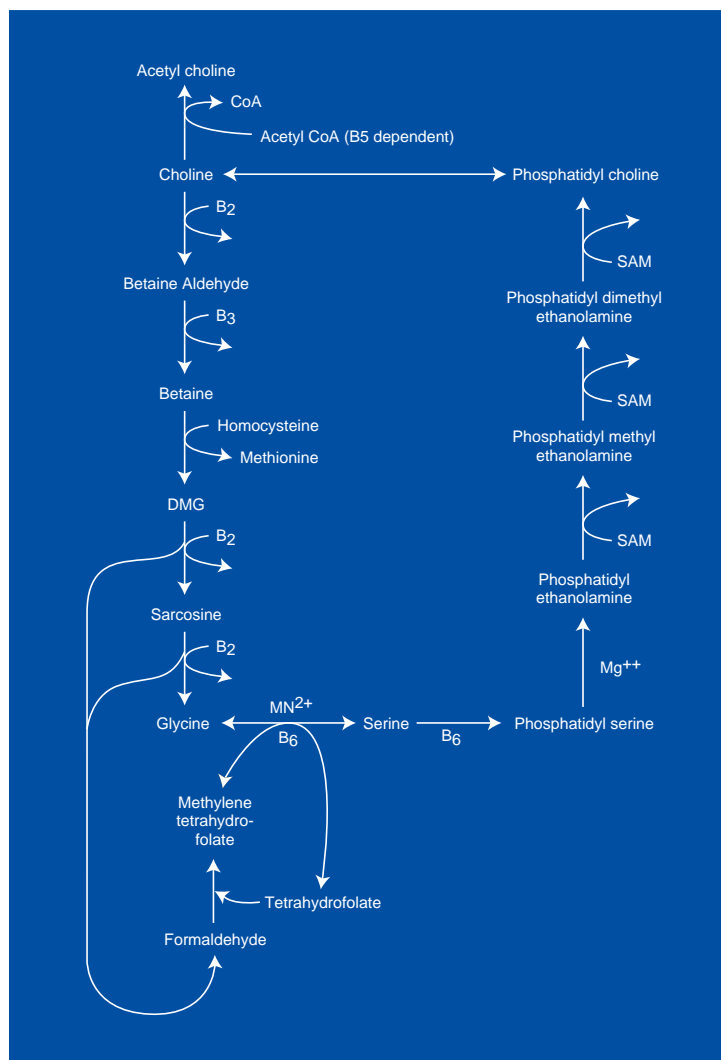
## **Phosphatidylcholine**

Phosphatidylcholine (PC) is a primary component of lecithin and is sometimes referred to as pure lecithin. It is the most fre-

quently encountered phospholipid in animals and is structurally related to phosphatidylserine and phosphatidylethanolamine. PC consists of a glycerol backbone that is esterified with fatty acids on carbon atoms 1 and 2, and with a phosphoric acid/choline complex in position 3. Although phosphatidylcholine is usually referred to as if it were a single compound, it is actually a group of related compounds which vary depending upon the fatty acid composition at position C-1 and C-2.

Dietary choline is derived primarily from PC, which after absorption by the intestinal mucosa, is metabolized to choline in the liver by the enzyme phospholipase D. Most choline is re-phosphorylated to PC; however, a small amount is carried to the brain via the blood stream, where it is converted to the neurotransmitter acetylcholine. If PC or choline are lacking in the diet they can be synthesized from phosphatidylserine and phosphatidylethanolamine. (Note: phosphatidylserine and phosphatidylethanolamine are interconvertible by means of decarboxylation reactions which require pyridoxal-5'-phosphate). Synthesis of PC is dependent on the availability of SAM as a methyl donor, since synthesis involves the transfer of methyl groups from 3 SAM molecules to phosphatidylethanolamine in order to generate one molecule of PC.

Choline, due to its role as a precursor to acetylcholine and PC, is a critical nutrient for brain and nerve development and function. In mammals, amniotic fluid has a ten-fold greater concentration of choline than that in maternal blood<sup>42</sup> and at birth, all mammals studied have plasma choline concentrations much higher than those found in adults.<sup>43</sup> Choline supplementation to pregnant rats between day 12 and 17 of pregnancy has been shown to permanently enhance the spatial memory of offspring.<sup>44</sup> Male albino rats exposed to choline chloride supplementation both prenatally (through the diet of pregnant rats) and postnatally (subcutaneous injections)

**Figure 5. Phosphatidylcholine Metabolism**

rapidly a diet deficient in choline will induce pathological changes.<sup>46</sup> The regeneration of methionine from homocysteine is accomplished in one of two ways. One involves the generation in the cytosol of methylTHF from methylene THF and the transfer of its methyl group to regenerate methylcobalamin, which then acts as a coenzyme in the regeneration of methionine. Since THF and its derivatives can only cross the mitochondrial membrane very slowly, inside the mitochondria regeneration of methionine relies on recovery of a methyl group from phosphatidylcholine. This is converted to choline which undergoes a two step process, requiring riboflavin and then niacin, to form betaine. Betaine donates one of its three methyl groups, via the enzyme betaine:homocysteine methyl transferase, to homocysteine resulting in the regeneration of methionine. After the donation of the methyl group, one molecule of dimethylglycine (DMG) remains. This molecule is oxidized to glycine and to two molecules of formaldehyde, by

riboflavin-dependent enzymes. The formaldehyde can combine with THF within the mitochondria to generate one of the active forms of folic acid, methyleneTHF, which can be converted to 5-methylTHF and subsequently used as a methyl donor (see Figure 5).

In animal studies, a disturbance in the metabolism of either of these two methyl-donor pathways, due to limited availability of either choline, or folates and vitamin B12, has a direct impact on reducing the levels of nutrients in the coexisting pathway since now more of a drain will be placed on the other pathway as a source of methyl groups. Rats fed diets deficient in choline and methionine have hepatic folate concentrations half that of controls

show more accurate performance on both working and reference memory components of tasks compared to control litter mates. It was assumed that choline-induced performance differences were due to long-term enhancement of spatial memory capacity and precision.<sup>45</sup> These observations that perinatal choline supplementation results in improved spatial memory in rats suggests that the availability of choline and its metabolites is critical for optimal brain development.

The metabolic pathways of choline, methionine, and methyl-folate are interrelated, intersecting at the regeneration of methionine from homocysteine. The use of choline molecules as methyl donors in this process is probably the main factor that determines how

after five weeks.<sup>47</sup> During choline deficiency, hepatic SAM concentrations have also been shown to decrease by as much as 50%.<sup>46</sup> Similarly, THF deficiency results in decreased hepatic total choline levels.<sup>43</sup>

Patients with a congenital deficiency of the enzyme MTHFR, which is needed for the formation of 5-methylTHF, have reduced levels of both methionine and adenosylmethionine in the cerebrospinal fluid and show demyelination in the brain and degeneration of the spinal cord. Methionine is effective in the treatment of some of these patients; however betaine was shown to restore CSF S-adenosylmethionine levels to normal and to prevent the progress of neurological symptoms in all patients in whom it was tried.<sup>48</sup>

Due to its impact on the remethylation of homocysteine to methionine and the increased levels of betaine found in rat fetuses supplemented with choline, it is likely that supplementation of choline or its metabolites, such as PC or betaine, may play a critical role in optimizing human development as well. This redundant pathway protects the ability for the body to have a source of methyl groups, and although the primary attention has been placed upon folate as a methyl donor, it is likely that optimal function cannot occur without adequate supplies of the nutrients in both remethylation pathways.

## **Taurine**

Taurine is a unique amino acid because it carries a sulfonic acid group (-SO<sub>3</sub>H) instead of a carboxyl group (-CO<sub>2</sub>H). Taurine is widely distributed in foods of animal origin, with the exception of commercial milk and milk products, which contain very low levels. Taurine is biosynthesized from methionine or from cysteine via the trans-sulfuration pathway. As discussed previously, homocysteine can be remethylated to form methionine; however, it can also be degraded to form cysteine.

This degradation involves a two step process resulting in the formation of cystathionine and its subsequent cleavage to cysteine. Both of the enzymes involved require pyridoxal-5'-phosphate as a cofactor, and the committed first step in the degradation of homocysteine, cystathionine synthase, also requires serine. In humans, defects in both of these enzymatic reactions occur. Homocysteinuria resulting from an absence of cystathionine synthase can lead to mental retardation. Low levels of this enzyme can also lead to abnormally-high levels of homocysteine, especially when remethylation cofactors are also deficient. Cystathioninuria resulting from a deficient function of cystathioninase is also associated with mental defects.

Once cysteine is generated it can be directed into several different pathways including synthesis of glutathione, acetyl-CoA, and taurine.

During the neonatal period, total body levels and brain taurine concentrations reach a peak.<sup>49</sup> In 1984, the FDA approved fortification of human infant formulas with taurine, and while this will minimize the risk of postnatal taurine deficiency, it has no effect on the outcome of maternal consumption of a low taurine diet during pregnancy. At particular risk may be infants of strict vegetarian (vegan) mothers,<sup>50</sup> as well as others on a protein, methionine, or B6 deficient diet. A high dietary content of methyl donors may spare methionine and so may have a beneficial influence on taurine biosynthesis.<sup>51</sup>

Although dietary deficiency of taurine and its impact on fetal development in humans have not yet been demonstrated, it makes sense to optimize dietary levels of both protein and the nutrients required, especially vitamin B6, for its synthesis. This recommendation may be particularly important for vegetarian women who intend to have children, since virtually no taurine is present in plants and vegetables.

## CoQ10

Coenzyme Q10 is a fat soluble quinone occurring in the mitochondria of every cell. The primary biochemical action of CoQ10 is as a cofactor in the electron transport chain, the biochemical pathway that generates adenosine triphosphate (ATP). Since most cellular functions are dependent on an adequate supply of ATP, CoQ10 is essential for the health of virtually all human tissues and organs.

Biosynthesis of CoQ10 begins with the amino acid tyrosine. Pantothenic acid, pyridoxal-5'-phosphate and vitamin C are all required for the initial steps in its synthesis. An isoprenyl side chain from farnesyl diphosphate, an intermediate in cholesterol synthesis between 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) and squalene, is then added. An inadequate supply of this intermediate, which can be caused by HMG-CoA reductase inhibitors (cholesterol lowering drugs of the statin family) results in decreased levels of CoQ10.<sup>52</sup> In two of the final steps in the synthesis of CoQ10, methyl groups are provided by SAM. Adequate dietary methionine along with a sufficient supply of the nutrients required for the re-methylation of homocysteine to methionine (folic acid, cobalamin, and betaine) are required to generate sufficient SAM. Sub-optimal amounts of SAM may negatively impact on the ability of the body to synthesize sufficient CoQ10. This relationship between SAM and CoQ10 has been suggested in various animal studies.<sup>53,54</sup>

It might then be expected that decreased CoQ10 levels may correlate with increased homocysteine. While this has not been investigated to date, a recent study evaluated coenzyme Q10 levels in 483 pregnant females. It was found that plasma coenzyme Q10 levels rise throughout pregnancy beginning at week 18 and reach a high of about 50% above normal levels by week 36. Forty-nine patients who had spontaneous abortion and 19 patients

with threatened abortion had significantly decreased levels of CoQ10 when compared to women who had a normal pregnancy.<sup>55</sup> An earlier study also reported low CoQ10 levels in complicated pregnancy, particularly in cases of spontaneous abortion occurring before 12 weeks.<sup>56</sup>

## Vitamin A

Vitamin A is essential for human health, but concerns have arisen regarding its potential teratogenicity. In pregnant women, a high correlation has been found between elevated plasma retinol levels and low birth weights,<sup>57</sup> while epidemiological evidence suggests that a teratogenic effect might exist for exposures to high doses of vitamin A (above 40,000 IU). This effect seems to be related to the status of organ development at the time of exposure, with a several-fold higher incidence of birth defects correlating with high vitamin A intake during the first two months of pregnancy.<sup>58</sup>

In a study of 22,748 pregnant women, a significant increase in defects associated with cranial-neural-crest tissue was found among the babies born to women who consumed more than 15,000 IU of vitamin A per day from food and supplements or 10,000 IU of vitamin A as a supplement. The increased frequency of defects was concentrated among the babies born to women who had consumed high levels of vitamin A before the seventh week of gestation. The authors estimate that among the babies born to women who took more than 10,000 IU of vitamin A per day in the form of supplements, about 1 infant in 57 had a malformation attributable to the supplement.<sup>59</sup>

At this point in time, no adequate explanation exists for this teratogenic effect of vitamin A; however, evidence from an animal model suggests a possible link to folic acid metabolism. The activity of hepatic MTHFR, which plays a critical role in the regulation of liver folate metabolism, is suppressed in rats

fed a diet containing 1000 IU of retinol/gram body weight. This results in decreased 5-methylTHF synthesis (the cofactor required for methylation of cobalamin and the subsequent regeneration of methionine from homocysteine) and a decrease of S-adenosylmethionine in the liver.<sup>60</sup>

Practitioners should be familiar with the possible hazard of excessive dosages of vitamin A and its analogues in pregnancy. The available evidence suggests that consuming a multiple vitamin preparation with 6000 IU of vitamin A does not increase the risk of birth defects.<sup>20</sup> Supplementation of vitamin A at daily doses of higher than 6,000 IU in a multiple vitamin preparation or as a single nutrient is not necessary for good health during pregnancy and as such is not recommended. Beta-carotene, in contrast to vitamin A, has not been associated with toxicity or teratogenicity in humans or animals.<sup>61</sup>

## Discussion

Biochemical enzyme defects and nutritional deficiencies are receiving increasing attention for their role in preventing NTD as well as other negative pregnancy outcomes, including spontaneous abortion, placental abruption (infarct), pre-term delivery, and low infant birth weight. Recent evidence has suggested that derangement of methionine-homocysteine metabolism could be the underlying mechanism of pathogenesis of neural tube defects and may be the mechanism of prevention observed with supplementation of folic acid. It has been firmly established that a low dietary intake of folic acid increases the risk for delivery of a child with a neural tube defect, and that periconceptional folic acid supplementation reduces the occurrence of NTD. Research also indicates that supplemental folic acid intake results in increased infant birth weight and improved Apgar scores, along with a concomitant decreased incidence of

fetal growth retardation and maternal infections. A derangement in methionine-homocysteine metabolism has also been correlated with recurrent miscarriage and placental infarcts (abruption).

Because homocysteine metabolism through the re-methylation and transsulfuration pathways affects several biochemical pathways involving the production of nutrients which are essential to the optimal functioning of the cardiovascular, skeletal, and nervous system, it is not surprising that these other nutrients have been linked to complications of pregnancy in animal models and humans. Low plasma vitamin B12 levels have been shown to be an independent risk factor for NTD. Methionine has been shown to reduce the incidence of NTD by 41% in an animal model when administered on days eight and nine of pregnancy. This evidence indicates that a disturbance in the re-methylation pathway with a subsequent decrease in SAM may be a contributing factor to these complications of pregnancy.

Phosphatidylcholine, due to its role as a precursor to acetylcholine and choline, is acknowledged as a critical nutrient for brain and nerve development and function. Since the metabolic pathways of choline (via betaine), methionine, cobalamin and methyl-folate are interrelated, intersecting at the regeneration of methionine from homocysteine, a disturbance in the metabolism of either of these two methyl-donor pathways, due to limited availability of key nutrients or decreased enzyme activity, will have a direct impact on the body's ability to optimize levels of SAM.

Patients with a severe congenital deficiency of the enzyme MTHFR, which is needed for the formation of methylTHF, have reduced levels of both methionine and adenosylmethionine in the cerebrospinal fluid and show demyelination in the brain and degeneration of the spinal cord. Because of its direct impact in the activation of folic acid to

its methyl derivative, a milder version of this enzyme defect is also strongly suspected to increase the incidence of NTD.

It is established that high vitamin A intake during the first two months of pregnancy is associated with a several-fold higher incidence of birth defects. Although the mechanism of action remains to be elicited, in an animal model the activity of hepatic MTHFR is suppressed with high vitamin A levels, suggesting that its teratogenic effect during early pregnancy may be associated with a subsequent derangement in the re-methylation of homocysteine.

Since a significant correlation has been found between higher homocysteine levels in women experiencing placental abruption, infarction, and spontaneous abortion than in control women, and since homocysteine and CoQ10 synthesis are both dependent upon the methionine-SAM-homocysteine pathway, it is possible that low CoQ10 and elevated homocysteine independently found in complicated pregnancy may also in fact be found to be related conditions.

Nutritional intervention with the cofactors required for optimal metabolism of the methionine-homocysteine pathways offers a new integrated possibility for primary prevention of NTD and several other complications of pregnancy. Supplementation with betaine, and the active forms of cobalamin and folic acid, such as methylcobalamin and folinic acid, along with riboflavin-5'-phosphate (because of its role as a cofactor for the MTHFR enzyme), may play a significant role in reducing or preventing these emotionally-devastating outcomes.

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