



## 5-Methyltetrahydrofolate

### Introduction

5-methyltetrahydrofolate (5-MTHF) is the most biologically active form of the B-vitamin folic acid, also known generically as folate. 5-MTHF functions, in concert with vitamin B12, as a methyl-group donor involved in the conversion of the amino acid homocysteine to methionine. Methyl (CH<sub>3</sub>) group donation is vital to many bodily processes, including serotonin, melatonin, and DNA synthesis. Therapeutically, 5-MTHF is instrumental in reducing homocysteine levels, preventing neural tube defects, and improving vascular endothelial function. Research on folate supplementation suggests it plays a key role in preventing cervical dysplasia and protecting against neoplasia in ulcerative colitis. Folate also shows promise as part of a nutritional protocol to treat vitiligo, and may reduce inflammation of the gingiva. Furthermore, certain neurological, cognitive, and psychiatric presentations may be secondary to folate deficiency. Such presentations include depression, peripheral neuropathy, myelopathy, restless legs syndrome, insomnia, dementia, forgetfulness, irritability, endogenous depression, organic psychosis, and schizophrenia-like syndromes. 5-MTHF supplementation may be a more favorable method of folate repletion.

### Biochemistry

Folic acid is a water-soluble member of the B-complex family of vitamins. It is composed of three primary structures, a hetero-bicyclic pteridine ring, para-aminobenzoic acid (PABA), and glutamic acid. Because humans cannot synthesize this compound, it is a dietary requirement. Dietary folate is a complex and variable mixture of folate compounds, including polyglutamate (multiple glutamate molecules attached) conjugate compounds, reduced folates, and tetrahydrofolates. 5-MTHF is the most biologically active form of folate and is the molecule to which folic acid must be converted in the body to be utilized. Although folates are abundant in the diet, cooking or processing destroys these compounds. The best folate sources in foods are green, leafy vegetables; sprouts, fruits, brewer's yeast, liver, and kidney also contain high amounts of folates.

### Pharmacokinetics

Human pharmacokinetic studies indicate folic acid has high bioavailability, with large oral doses of folic acid substantially raising plasma levels in healthy subjects in a time- and dose-dependent manner. Red blood cells (RBCs) appear to be the storage depot for folate, as RBC levels remain elevated for periods in excess of 40 days following discontinuation of supplementation. Folic acid is poorly transported to the brain and rapidly cleared from the central nervous system. The primary methods of elimination of absorbed folic acid are fecal (through bile) and urinary.<sup>1-4</sup>

After ingestion, the process of conversion of folic acid to the metabolically active coenzyme forms is relatively complex. Synthesis of the active forms of folic acid requires several enzymes, adequate liver and intestinal function, and adequate supplies of riboflavin (B2), niacin (B3), pyridoxine (B6), zinc, vitamin C, and serine. After formation of the coenzyme forms of the vitamin in the liver, these metabolically active compounds are secreted into the small intestine with bile (the folate enterohepatic cycle), where they are reabsorbed and distributed to tissues throughout the body.

Despite the biochemical complexity of this process, evidence suggests oral supplementation with folic acid increases the body's pool of 5-MTHF in healthy individuals.<sup>5</sup> However, enzyme defects, malabsorption, digestive system pathology, and liver disease can result in impaired ability to activate folic acid. In fact, some individuals have a severe congenital deficiency of the enzyme methylenetetrahydrofolate reductase (5-MTHFR), which is needed to convert folic acid to 5-MTHF. Milder forms of this enzyme defect likely interact with dietary folate status to determine risk for some disease conditions.<sup>6-10</sup> In individuals with a genetic defect of this enzyme (whether mild or severe), supplementation with 5-MTHF might be preferable to folic acid supplementation. Lamers et al found significantly greater RBC folate concentrations after 24 weeks of supplementation with 5-MTHF compared to folic acid and placebo.<sup>11</sup> A single, high-dose pharmacokinetic study of 5-MTHF or folic acid administration (5 mg) in patients with coronary artery disease demonstrated significantly higher bioavailability of 5-MTHF, which resulted in a 700-percent higher plasma folate concentration after 5-MTHF dosing compared to folic acid. This difference was irrespective of the patient's enzymatic genotype.<sup>12</sup> Others have found equivalent increases in plasma folate with either folic acid or 5-MTHF supplementation.<sup>13-15</sup>

## Mechanisms of Action

The mechanism of action of 5-MTHF is through its role as a methyl donor in a range of metabolic and nervous system biochemical processes, as well as being indirectly necessary for DNA synthesis. Serine reacts with tetrahydrofolate, forming 5,10-methylenetetrahydrofolate, the folate derivative involved in DNA synthesis. The enzyme 5-MTHFR converts 5,10-methylenetetrahydrofolate to 5-MTHF, which donates its methyl group to cobalamin (vitamin B12), forming methylcobalamin. The enzyme methionine synthase catalyzes the donation of methylcobalamin's methyl group to the amino acid metabolite homocysteine, converting it to the amino acid methionine. Methionine subsequently is converted to S-adenosylmethionine (SAME), a methyl donor involved in numerous biochemical processes.

## Deficiency States and Symptoms

Folate deficiency is considered to be one of the most common nutritional deficiencies. The following may contribute to a deficiency of folic acid: deficient food supply; defects in utilization, as in alcoholics or individuals with liver disease; malabsorption; increased needs in pregnant women, nursing mothers, and cancer patients; metabolic interference by drugs; folate loss in hemodialysis; and deficiencies in enzymes or cofactors needed for the generation of active folic acid.<sup>16</sup> Absorption of folic acid appears to be significantly impaired in HIV disease, irrespective of the stage of the disease.<sup>17</sup> Signs and symptoms of folate deficiency include macrocytic anemia, fatigue, irritability, peripheral neuropathy, tendon hyper-reflexivity, restless legs syndrome, diarrhea, weight loss, insomnia, depression, dementia, cognitive disturbances, and psychiatric disorders.<sup>18-23</sup> Elevated plasma homocysteine can also indicate a dietary or functional deficiency of folic acid.

## Clinical Indications

### Anemia

Folic acid has a long history of use in conjunction with vitamin B12 for the treatment of macrocytic anemia. Depending on the clinical status of the patient, the dose of folic acid or 5-MTHF required to reverse macrocytic anemia varies, but the therapeutic dose is usually 800-1,000 mcg (1 mg) daily. Duration of therapy to reverse macrocytic anemia can be as short as 15 days after initiation of supplementation, or it may require prolonged supplementation.

### Hyperhomocysteinemia

Elevated plasma homocysteine, the de-methylated derivative of the amino acid methionine, is an independent risk factor for cardiovascular disease. Hyperhomocysteinemia has been connected to increased risk of neural tube defects and other birth defects, as well as to schizophrenia, Alzheimer's disease, cognitive decline, osteoporosis, rheumatoid arthritis, kidney failure, and cancer.<sup>24-28</sup> 5-MTHF is needed for optimal homocysteine metabolism, since it acts as a methyl donor, providing a methyl group to vitamin B12. The methylated form of vitamin B12 (methylcobalamin) subsequently transfers this methyl

group to homocysteine. The result is a recycling of homocysteine to methionine, resulting in reduction in elevated plasma homocysteine.

In healthy subjects even low doses of folic acid can lower homocysteine levels. A dose of 400 mcg folic acid or 416 mcg 5-MTHF daily for 24 weeks reduced homocysteine significantly in 144 healthy females; there was no difference between supplemented groups.<sup>29</sup> In subjects with cardiovascular disease, 800 mcg folic acid daily resulted in an average decrease in homocysteine levels of 23 percent,<sup>30</sup> while 2.5 mg daily resulted in an average decrease of 27 percent.<sup>31</sup> Evidence suggests individuals with higher initial homocysteine levels are likely to experience a greater reduction following folic acid supplementation.<sup>31</sup> Studies comparing oral folic acid and 5-MTHF supplementation have noted similar homocysteine-lowering capacity of either supplement.<sup>11,32-34</sup>

### *Cardiovascular Disease*

In addition to reducing blood levels of homocysteine, 5-MTHF improves blood flow by increasing nitric oxide (NO) production in vascular endothelial cells. Impaired endothelial NO production occurs early in the development of cardiovascular disease, particularly atherosclerosis. In fact, most of the risk factors for atherosclerosis are associated with poor vasodilation due to insufficient NO production. Chronic exposure of the vascular endothelium to homocysteine compromises the production of adequate amounts of NO. This leads to injury of the endothelial lining and the initiation of atherosclerosis, including increased adhesiveness of monocytes and platelets, increased smooth muscle proliferation, and thrombus formation. 5-MTHF appears to improve NO synthesis by: reducing plasma homocysteine levels; enhancing the availability of key endothelial NO cofactors, such as tetrahydrobiopterin; reducing the production of superoxide anions; and by substituting for tetrahydrobiopterin as a cofactor in the enzyme nitric oxide synthase, the net effect of which is improvement of peripheral blood flow.<sup>35-40</sup>

In a six-week, randomized, crossover study of 52 individuals with coronary artery disease, folic acid (5 mg/day) significantly improved flow-mediated dilation (FMD) at the brachial artery, a measurement of endothelial function. In the same study, 10

patients were administered 5-MTHF intra-arterially, which also improved FMD.<sup>39</sup> This effect was independent of any homocysteine-lowering effect, both in this study and a subsequent study by the same research group.<sup>41</sup> In a double-blind, placebo-controlled, crossover study of individuals with coronary artery disease, researchers found supplementation with high-dose folic acid (30 mg per day) improved blood flow to the heart muscle via the coronary arteries. Using positron emission tomography (PET scanning), researchers noted significant improvement in coronary blood flow with folic acid supplementation compared to placebo. The improvement was especially enhanced in areas of the heart that had shown reduced blood flow prior to supplementation. Folic acid supplementation also significantly lowered the blood pressure of study participants. The findings from this high-dose folate study demonstrate another significant cardiovascular mechanism for this nutrient.<sup>42</sup> Addition of vitamin C, L-arginine, tetrahydrobiopterin, and polyunsaturated fatty acids has been suggested as a means of enhancing the effect of folic acid on endothelial NO production.<sup>35</sup>

### *Inflammatory Bowel Disease*

Patients with inflammatory bowel disease (IBD) often have folate deficiencies, caused in part by the drug sulfasalazine, prescribed for IBD but also known to inhibit folate absorption.<sup>43</sup> Evidence suggests folate supplementation lowers the risk, in a dose-dependent fashion, of colonic neoplasia in patients with ulcerative colitis (UC). A review of 99 UC patient records found folic acid supplementation was associated with a 62-percent decreased risk of neoplasia compared to patients not taking a folate supplement.<sup>43</sup> In a similar study, the files of 98 UC patients disclosed dose-dependent protection from neoplasia by folic acid. The relative risk of developing neoplasia was 0.76 for 400 mcg folate and 0.54 for those taking 1 mg folate for at least six months compared to those not supplemented.<sup>44</sup>

### *Neuropsychiatric Applications*

Neuropsychiatric diseases encompass a number of neurological, cognitive, and psychiatric presentations that may be secondary to folate deficiency. Such presentations include dementia, schizophrenia-

like syndromes, insomnia, irritability, forgetfulness, endogenous depression, organic psychosis, peripheral neuropathy, myelopathy, and restless legs syndrome.<sup>18-22</sup> Lower serum and RBC folate concentrations have an association with depression, and deficiency might predict a poorer response to some antidepressant medications.<sup>23,45-52</sup> Several studies have documented improvement in depression in some patients subsequent to oral supplementation with 5-MTHF at doses of 15-50 mg daily.<sup>52-54</sup> Folic acid (500 mcg per day) significantly improved the antidepressant action of fluoxetine in subjects with major depression.<sup>55</sup> Limited evidence implies supplemental folic acid might positively affect morbidity of some bipolar patients placed on lithium therapy.<sup>56</sup> A syndrome characterized by mild depression, permanent muscular and intellectual fatigue, mild symptoms of restless legs, depressed ankle-jerk reflexes, diminution of vibration sensation in the legs, stocking-type hypoesthesia, and long-lasting constipation appears to respond to folic acid supplementation (5-10 mg per day for 6-12 months).<sup>57</sup>

### *Cervical Dysplasia*

Research points to an association between folate status in women and cervical dysplasia;<sup>58-60</sup> however, its role as an efficacious therapeutic intervention is unclear. One report suggests folic acid supplementation (10 mg folic acid daily for three months) reverses cervical dysplasia in women taking oral contraceptives.<sup>61</sup> In another study, 154 individuals with grade 1 or 2 cervical intraepithelial neoplasia were randomly assigned either 10 mg folic acid or placebo daily for six months. No significant differences were observed between supplemented and unsupplemented subjects regarding dysplasia status, biopsy results, or prevalence of human papilloma virus type-16 infection.<sup>62</sup> It is possible certain subsets of women (perhaps those with an oral contraceptive-induced deficiency) might be more amenable to treatment; however, additional research is required to clarify the therapeutic role of folic acid in cervical dysplasia.

### *Periodontal Disease*

Folic acid can increase the resistance of the gingiva to local irritants and lead to a reduction in inflammation. A mouthwash containing 5 mg folate per

5 mL of mouthwash used twice daily for four weeks, with a rinsing time of one minute, appears to be the most effective manner of application. The effect of folate on gingival health appears to be moderated largely, if not totally, through a local influence.<sup>63-65</sup>

### *Pregnancy*

Low dietary intake of folic acid increases the risk for delivery of a child with a neural tube defect (NTD). Periconceptional folic acid supplementation significantly reduces the occurrence of NTD.<sup>66-72</sup> Supplemental folic acid intake during pregnancy results in increased infant birth weight and improved Apgar scores, along with a concomitant decreased incidence of fetal growth retardation and maternal infections.<sup>73-75</sup> In a group of women of childbearing age, supplementation with 416 mcg 5-MTHF daily for 24 weeks resulted in higher RBC folate levels compared to folic acid supplementation. It took eight weeks of supplementation to reach RBC levels consistent with a significantly reduced risk of having a child born with a neural tube defect.<sup>11</sup>

### *Vitiligo*

In some individuals, administration of folate appears to be a rational aspect of a nutritional protocol to treat vitiligo. Degrees of re-pigmentation ranging from complete re-pigmentation in six subjects and 80-percent re-pigmentation in two subjects were reported in eight individuals who followed a three-year protocol with a dosage of 2 mg folic acid twice daily, 500 mg vitamin C twice daily, and intramuscular injections of vitamin B12 every two weeks.<sup>76</sup> A two-year study using a combination of folic acid, vitamin B12, and sun exposure for treatment of vitiligo reported positive results. One hundred patients with vitiligo were treated, with re-pigmentation occurring in 52 subjects. Total re-pigmentation was seen in six patients and the spread of vitiligo was halted in 64 percent of the patients. Re-pigmentation was most evident on sun-exposed areas.<sup>77</sup>

### *Drug-Nutrient Interactions*

A number of drugs can interfere with the pharmacokinetics of folic acid. Cimetidine and antacids appear to reduce folate absorption.<sup>78</sup> Sulfasalazine interferes with folic acid absorption and conversion

to 5-MTHF.<sup>79</sup> Continuous long-term use of acetaminophen and aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs appears to increase the body's need for folate.<sup>80</sup> Although the mechanism is unclear, anticonvulsants, antituberculosis drugs, alcohol, and oral contraceptives produce low serum and tissue concentrations of folate.<sup>79,81</sup> Folic acid reduces elevated liver enzymes induced by methotrexate therapy in rheumatoid arthritis; however, it had no effect on the incidence, severity, and duration of other adverse events.<sup>82</sup> Folate supplementation prevents nitric oxide synthase dysfunction induced by continuous nitroglycerin use.<sup>83</sup>

Anti-seizure medications, including carbamazepine and phenobarbital, appear to utilize folic acid during hepatic metabolism. Folic acid supplementation can increase metabolism of these drugs, thus lowering blood levels of the drugs and possibly resulting in breakthrough seizures. Initiating folic acid therapy after starting these drugs should be done with caution.<sup>84</sup> The anticonvulsant drugs phenytoin and valproic acid appear to interfere with folate absorption.<sup>85</sup> Supplementation may be helpful to prevent deficiency if taken at a time of day other than when taking an anticonvulsant.

There is conflicting information regarding the effects of folate supplementation in individuals treated with antifolate cancer chemotherapy medications such as methotrexate and 5-fluorouracil (5-FU). There is evidence folic acid might inhibit the activity of these drugs, although in some cases it may increase activity. The folic acid metabolite folinic acid (an upstream metabolite of 5-MTHF also known as 5-formyltetrahydrofolate and leucovorin) is often used to "rescue" normal tissue after methotrexate or 5-FU therapy.<sup>82</sup> Folic acid, folinic acid, or 5-MTHF supplementation does not appear to interfere with methotrexate's anti-arthritis or anti-inflammatory activity. However, since they might interfere with cancer chemotherapy, their indiscriminate use during chemotherapy is not recommended.

### Nutrient-Nutrient Interactions

Some concern exists that supplementation with high doses of folic acid could mask a vitamin B12 deficiency, resulting in neurological injury secondary to undiagnosed pernicious anemia.

Supplementation with 5-MTHF appears to sidestep this potential problem. Since 5-MTHF can only be converted to 5,10-methylenetetrahydrofolate (which is involved in DNA synthesis) after participating in homocysteine recycling with vitamin B12, it will not mask a B12 deficiency, as 5-MTHF is not active in DNA synthesis without the help of B12.<sup>13,15</sup>

### Side Effects and Toxicity

In doses typically administered for therapeutic purposes, 5-MTHF is considered non-toxic. At doses of up to 50 mg daily, gastrointestinal complaints, insomnia, irritability, and fatigue have been mentioned as occasional side effects. Folic acid and 5-MTHF are considered safe during pregnancy, with a recommended intake of 800 mcg daily.

### Dosage

The dose of 5-MTHF varies depending on the clinical condition. For lowering homocysteine, daily doses up to 5 mg are generally used. The most common therapeutic dose is in the range of 1-3 mg daily. Doses greater than 10 mg per day of folic acid have been used in conditions such as cervical dysplasia, and up to 50 mg daily of 5-MTHF has been used in neuropsychiatric conditions.

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