

# The Methylation, Neurotransmitter, and Antioxidant Connections Between Folate and Depression

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## Abstract

Depression is common – one-fourth of the U.S. population will have a depressive episode sometime in life. Folate deficiency is also relatively common in depressed people, with approximately one-third of depressed individuals having an outright deficiency. Folate is a water-soluble B-vitamin necessary for the proper biosynthesis of the monoamine neurotransmitters serotonin, epinephrine, and dopamine. The active metabolite of folate, 5-methyltetrahydrofolate (5-MTHF, L-methylfolate), participates in re-methylation of the amino acid metabolite homocysteine, creating methionine. S-adenosylmethionine (SAME), the downstream metabolite of methionine, is involved in numerous biochemical methyl donation reactions, including reactions forming monoamine neurotransmitters. Without the participation of 5-MTHF in this process, SAME and neurotransmitter levels decrease in the cerebrospinal fluid, contributing to the disease process of depression. SAME supplementation was shown to improve depressive symptoms. 5-MTHF also appears to stabilize, enhance production of, or possibly act as a substitute for, tetrahydrobiopterin (BH4), an essential cofactor in monoamine neurotransmitter biosynthesis. There are few intervention studies of folic acid or 5-MTHF as a stand-alone treatment for depression related to folate deficiency; however, the studies that have been conducted are promising. Depressed individuals with low serum folate also tend to not respond well to selective serotonin reuptake inhibitor (SSRI) antidepressant drugs. Correcting the insufficiency by dosing folate along with the SSRI results in a significantly better antidepressant response. (*Altern Med Rev* 2008;13:216-226)

## Introduction

Clinical depression is common; one in four people will experience depression in their lifetime. It can be debilitating, but is treatable; however, many people do not respond to antidepressant medications. As many as 60 percent of individuals treated with a selective serotonin reuptake inhibitor (SSRI) drug, the standard of depression care, do not achieve remission of depression. Others discontinue drug therapy due to side effects or cost. The causes of depression are manifold, and can include socioeconomic, situational, genetic, and biochemical mechanisms. Because successful drug treatment of depression is uncertain at best, the alternative practitioner has an opportunity to treat the person with efficacious, non-toxic methods. However, the cause of the depressive symptoms must be addressed. One biochemical mechanism that appears to be involved is one-carbon metabolism – a simple biochemical process that appears to be perturbed in a significant number of individuals. One-carbon donation, also called methyl donation, may be at the root of many biochemical disturbances seen in depression and may be improved by providing the cofactors, such as folate, necessary for its optimal metabolism.

Folate is a B-vitamin that, as per the definition of a vitamin, cannot be synthesized *de novo*; it must be derived from diet or supplementation. Dietary folate is found in leafy green vegetables, legumes, beans, liver, citrus fruits, and yeast. Multiple biochemical conversions

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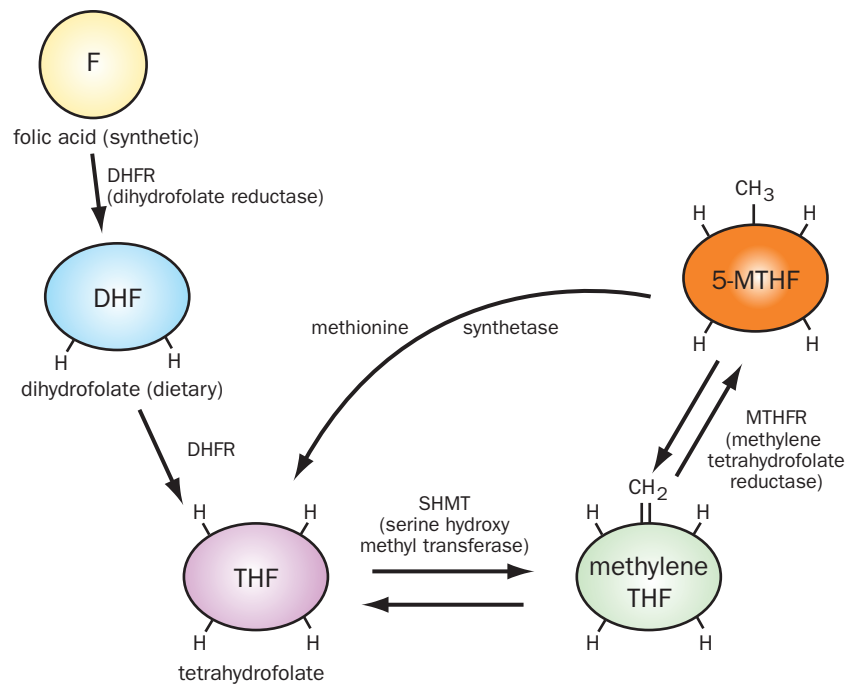
are required for dietary folate to become the metabolically active, tissue-usable forms. Folic acid is the term for the synthetic molecule, which is highly absorbed (85-95%) compared to the dietary form (50%). In either case, genetic polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) enzyme (present in approximately 60 percent of the U.S. population), which catalyzes the conversion of methylenetetrahydrofolate to the active form of the vitamin – 5-methyltetrahydrofolate (5-MTHF, L-methylfolate) – can make it difficult to convert folate, whether dietary or supplemental, to the active form (Figure 1).<sup>1</sup>

Folate is an essential nutrient involved – via its donation of a methyl group – in numerous biochemical pathways, including neurotransmitter synthesis, DNA biosynthesis, regulation of gene expression, amino acid synthesis and metabolism, and myelin synthesis and repair. It is thought that the participation of folate (specifically, 5-MTHF) in neurotransmitter synthesis is most responsible for its effects on mood and cognition.

### Low Blood Folate and Depression

Fortification of processed grains with folic acid has occurred in the United States since 1998, and since that time mean folate levels have increased; however, fortification has not abolished diseases in which folate is implicated – cardiovascular disease, depression, and neural tube defects (NTDs), although incidence of the latter has dropped significantly. This might be due to the low level of fortification or may indicate that although blood folate levels have increased, they might not be high enough to significantly impact the diseases connected to folate deficiency/insufficiency.

Figure 1. Formation of 5-MTHF from Folate



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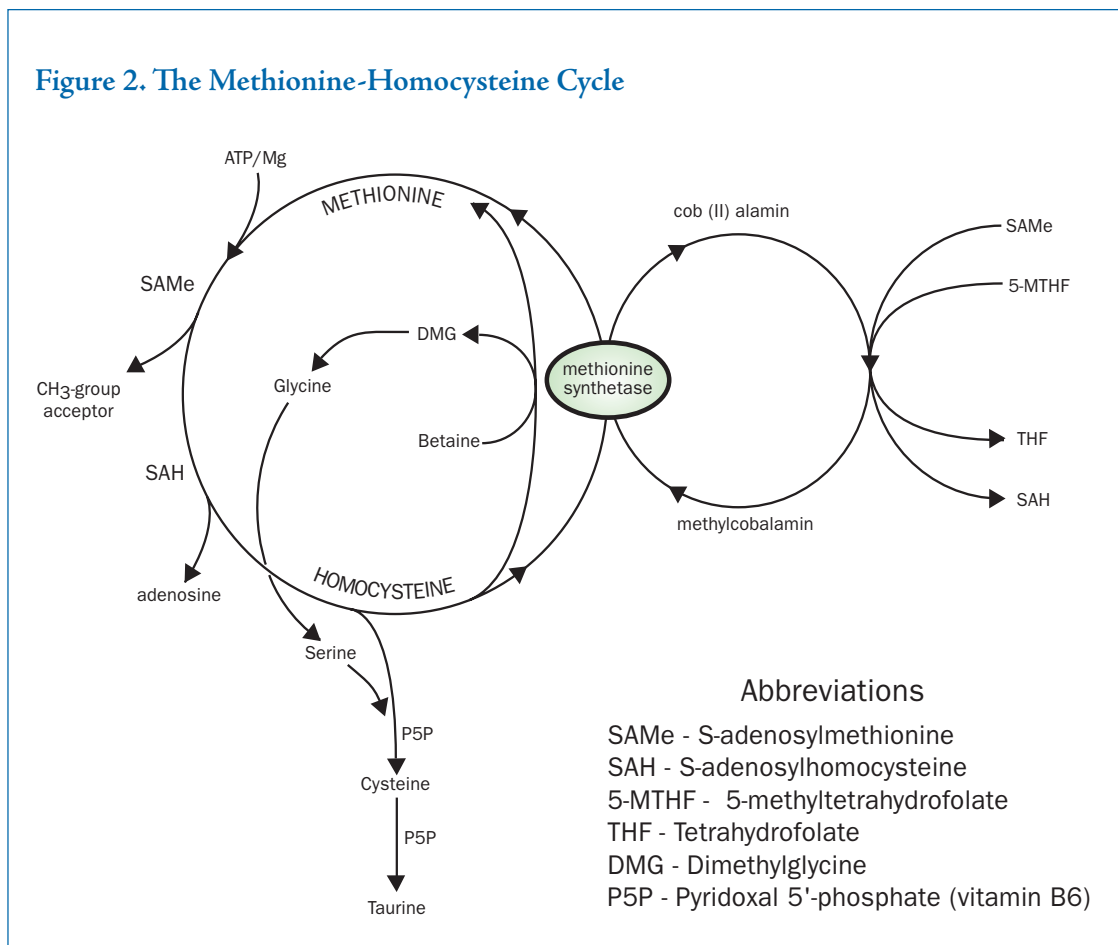
Research into the connection between folate and depression extends back to the 1960s. One of the early studies examined serum folate and vitamin B<sub>12</sub> status in 272 psychiatric in-patients. Low folate was significantly correlated to depressed patients, while patients with low B<sub>12</sub> levels had a greater association with psychosis.<sup>2</sup>

Other similar studies also found a greater number of depressed patients had folic acid deficiency than non-depressed patients.<sup>3-5</sup> These studies and others suggest folate deficiency may be present in one-third or more of individuals with major depression.

In a study of elderly Latina women, low plasma folate status was associated with a doubling of risk for depression, compared to women with the highest levels ( $p < 0.001$ ).<sup>6</sup> A 2005 Australian study of 412 people



Figure 2. The Methionine-Homocysteine Cycle



ages 60-64 found low serum folate and high plasma homocysteine were associated with increased risk of depression.<sup>7</sup>

A recent meta-analysis of 11 studies (15,315 participants) found a significant relationship between the risk of depression and low folate status.<sup>8</sup> A significant correlation between serum folate and the severity and duration of depression was found by Leavitt and Joffe in 1989. In 44 unmedicated patients with a major depressive disorder, the duration of their current episode was significantly associated with serum folate.<sup>9</sup>

### Folate and Seizure Medications

The antiseizure medication phenytoin induces a folic acid deficiency in a significant percentage of individuals treated with this drug.<sup>10</sup> Researchers studied serum folate status in 312 epileptic children and found those with depression had the lowest folate levels. Children taking phenytoin were over-represented in the

lowest serum folate group.<sup>11</sup> Rosche et al noted an incidence of depression in over 60 percent of patients with seizure disorders, as well as a lower serum folate level in patients with epilepsy, compared to controls.<sup>12</sup> In addition, their study of 46 patients with chronic seizure disorders found a significant negative correlation between serum folate and scores on the Self-Rating Depression Scale, even though patients in the lowest range were still within the range most laboratories consider normal. This finding suggests these patients may not need an outright folate deficiency to impact mood and may be more sensitive to lower folate levels than other individuals.

Beginning folate supplementation in patients already on antiseizure medications can increase hepatic metabolism of the drug and cause breakthrough seizures. Therefore, the best time to begin folate supplementation in these individuals is at the same time antiseizure medication is initiated.

## Dietary Folate and Depression

In the first study to examine the association between dietary folate intake and depression, 228 (9.3%) of 2,682 middle-aged Finnish men were found to have depressive symptoms based on a standardized depression scale. Those in the lowest third of dietary folate intake had a 67-percent higher risk ( $p=0.003$ ) of being depressed than those in the highest folate intake group. No such correlations were found with intake of vitamins B<sub>1</sub>, B<sub>6</sub>, or B<sub>12</sub>.<sup>13</sup>

In subsequent studies of dietary folate intake and depressive symptoms, a French study found a significant association between high folate intake and a lower risk of depression, while a study in the Netherlands did not find any association between dietary folate intake and depression.<sup>14,15</sup> The latter study did note a higher homocysteine level in individuals with a lower dietary folate intake, which is a sensitive indicator of folate status.

## The Homocysteine, Folate, and Depression Connection

Homocysteine is an amino acid derivative from the metabolism of the sulfur-containing amino acid methionine, which is present in proteins found in meats, poultry, dairy products, eggs, and fish. Methionine is converted into S-adenosylmethionine (SAME), which participates in numerous one-carbon methylation (CH<sub>3</sub>) reactions in the body, including those that create an essential phospholipid (phosphatidylcholine) and neurotransmitters (serotonin, melatonin, epinephrine, dopamine). After donation of its methyl group, SAME becomes S-adenosylhomocysteine, then homocysteine. At this point, homocysteine must either be further metabolized via transsulfuration to become cysteine, taurine, and glutathione – a B<sub>6</sub>-dependent process – or re-methylated to become methionine again. Re-methylation is done via one of two reactions: methionine synthetase facilitates the donation of a methyl group from methylcobalamin (vitamin B<sub>12</sub>, which gets its methyl group from 5-MTHF) or betaine-homocysteine methyltransferase facilitates donation of a methyl group from betaine (trimethylglycine) (Figure 2).

Some researchers believe homocysteine is simply a marker of folate and/or B<sub>12</sub> deficiency, while others point out homocysteine causes oxidative stress resulting

in neurological and vascular damage and an interruption of the optimal biosynthesis of neurotransmitters.<sup>16</sup> Homocysteine has been the subject of a large amount of research the past 15 years, at first due to the discovery that high plasma homocysteine is related to a higher risk for cardiovascular disease, including myocardial infarction, cerebrovascular disease, and peripheral vascular disease.<sup>17</sup> More recently, investigations have been conducted into the connection between high homocysteine levels and brain dysfunction, including cognitive function, dementia, Alzheimer's disease, and depression.<sup>18</sup>

Epidemiologically, low blood levels of folate and vitamin B<sub>12</sub> and high levels of homocysteine have been correlated with depression, especially in the elderly.<sup>7,19-21</sup> A Greek study found significantly higher plasma homocysteine levels and lower B<sub>12</sub> and folate (all  $p<0.01$ ) in depressed elderly patients compared to controls. Another study of 924 men found a more than 200-percent greater risk of depression in men in the upper 20-percent of homocysteine levels compared to the lowest tertile.<sup>19</sup>

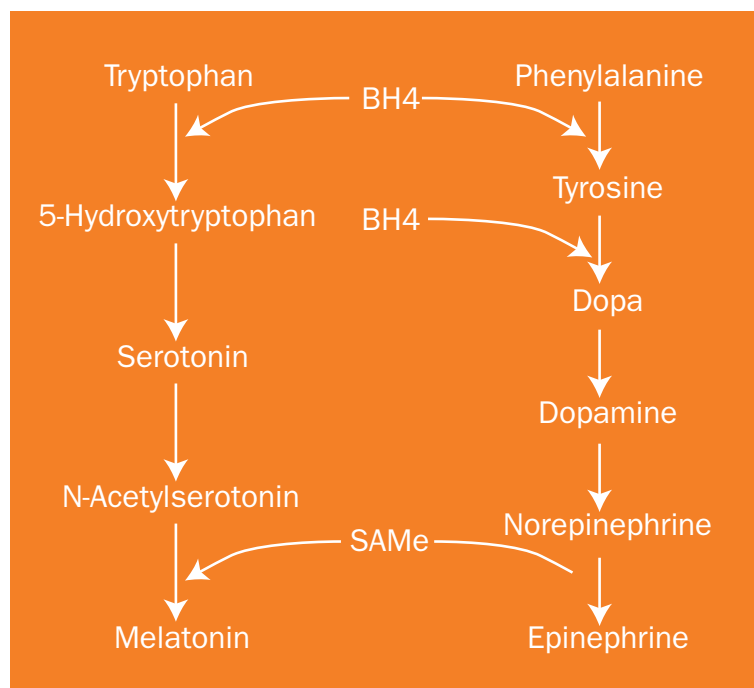
Bottiglieri et al studied 46 inpatients with severe depression and assayed their blood for homocysteine, folate, and B<sub>12</sub>.<sup>16</sup> Fifty-two percent had high homocysteine. In addition, 28 of the 46 patients were examined for cerebrospinal fluid (CSF) levels of folate, SAME, and monoamine metabolites, the latter an attempt to detect if high homocysteine inhibited the production of the neurotransmitters serotonin, epinephrine, and dopamine. Depressed patients with increased plasma homocysteine had significantly lower serum, red blood cell, and CSF folate, CSF SAME, and metabolites of all three CSF neurotransmitters.

Intervention studies in patients with cardiovascular disease and high plasma homocysteine demonstrate reliable lowering of homocysteine levels after folic acid treatment. Dosing with vitamins B<sub>6</sub> and B<sub>12</sub> has not been as predictably successful as folate intervention.<sup>22,23</sup>

Evidence appears to suggest elevated homocysteine might be due to a deficiency of dietary folate in some individuals. Decreased plasma or serum folate has been linked with depression in studies dating back 30 years. But dietary deficiency may not completely explain the low folate/high homocysteine/depression connection. A number of biochemical reactions are needed to convert dietary or supplemental folate to the active forms used in the human body. The most well known



**Figure 3. Tetrahydrobiopterin – Enzyme Cofactor for Neurotransmitter Synthesis**



genetic polymorphism of the enzymes in folate metabolism may be part of the problem. One of the last downstream folate metabolites, 5,10-methylenetetrahydrofolate, must be converted by methylenetetrahydrofolate reductase to 5-MTHF, the active folate that acts as an enzymatic cofactor in the re-methylation of homocysteine to methionine. The thermolabile variant (C677T) of the MTHFR enzyme, which results in decreased activity and thus decreased output of 5-MTHF, has been positively linked to low serum folate levels. A number of studies have examined whether this common polymorphism, present in up to 60 percent of the U.S. population, might be associated with increased risk of depression.<sup>24-26</sup> A meta-analysis of MTHFR polymorphisms and psychiatric disorders found a 36-percent greater chance of having depression in individuals who were homozygous for the (TT) enzyme variant compared to wild-type variants (CC).<sup>27</sup>

Increased homocysteine and/or decreased serum folate results in lower CSF levels of SAME.<sup>28</sup> SAME must be present as a methyl donor for both the serotonin and catecholamine pathways to function

properly. SAME has been used clinically as an antidepressant in oral, intravenous, and intramuscular dosing.<sup>28-32</sup> The antidepressant effect of SAME was significant and shown to be better than the prescription antidepressant imipramine (66% response vs. 22%, respectively).<sup>31</sup>

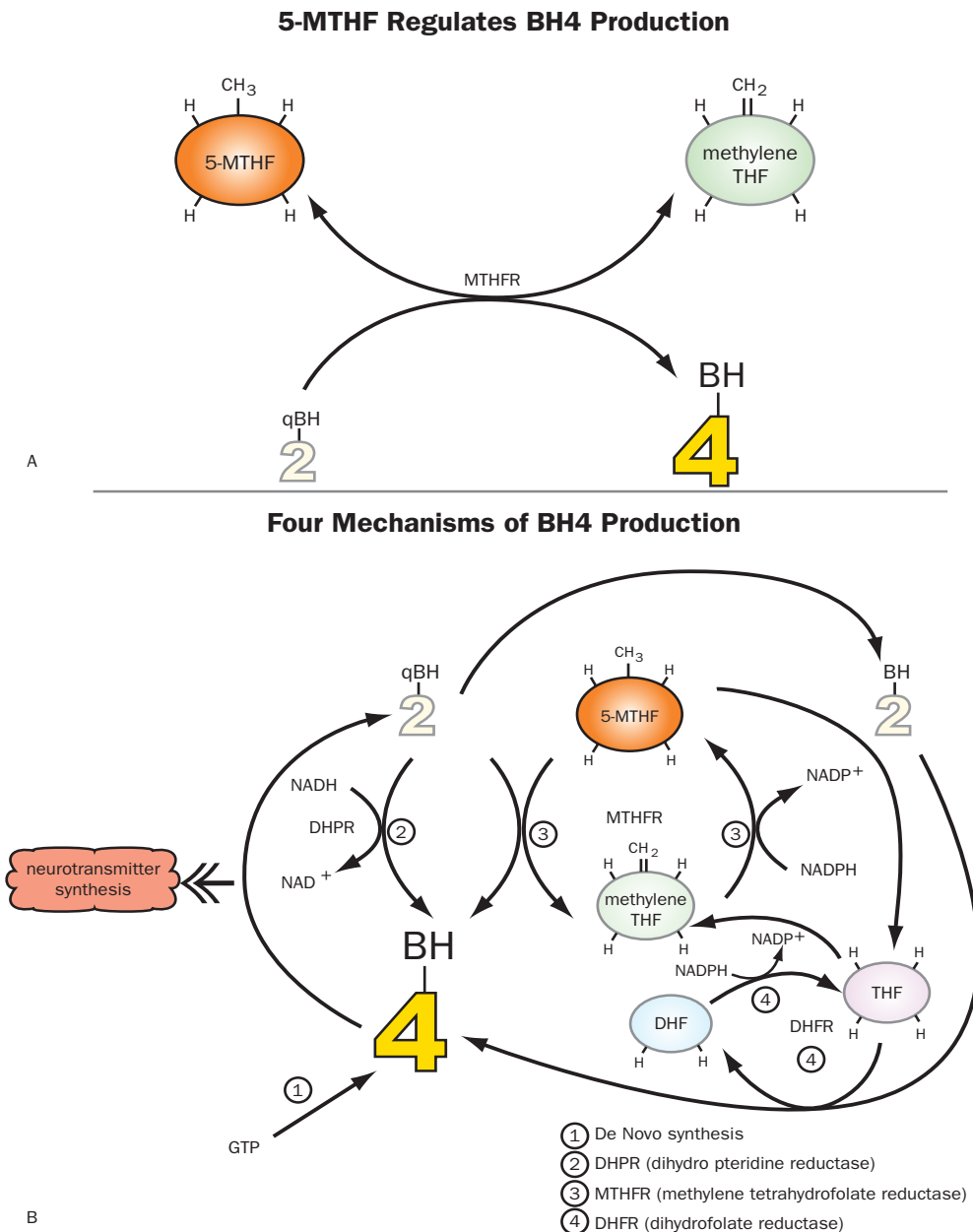
### The Tetrahydrobiopterin Connection

Tetrahydrobiopterin (BH4) is a nutrient cofactor essential to the formation of the monoamine neurotransmitters serotonin, dopamine, norepinephrine, and epinephrine. BH4 acts as a rate-limiting enzyme cofactor to the hydroxylase enzymes that metabolize tryptophan to 5-hydroxytryptophan, phenylalanine to tyrosine, and tyrosine to dopa (Figure 3). Research has demonstrated lower BH4:neopterin ratios in depressed people, which might reflect a decreased ability to convert neopterin to BH4.<sup>33-35</sup>

Folate appears to be important in regenerating BH4, which is highly susceptible to oxidation. The folate-metabolizing enzyme dihydrofolate reductase might also be involved in BH4 regeneration.<sup>36</sup> Other research suggests folate is necessary as a starting material for pterin synthesis and this may be the focus of the folate/BH4 relationship (Figure 4).<sup>34</sup>

Folate and BH4 share a second biochemical pathway. In vascular endothelial cells, endothelial nitric oxide synthase (eNOS) is the enzyme responsible for creating nitric oxide. BH4 is the nutrient cofactor for this enzyme. It has been demonstrated that folate, in the form of 5-MTHF, regenerates oxidized BH4,<sup>37</sup> and in the absence of an adequate amount of BH4, 5-MTHF “stands in” for BH4 at the enzyme level.<sup>38</sup> The chemical structures of BH4 and 5-MTHF are similar enough that eNOS will accept 5-MTHF as a substitute cofactor (Figure 5).<sup>39</sup> A similar mechanism might be at play in the antidepressant effect of folate. This author hypothesizes that 5-MTHF might substitute for BH4 in the hydroxylase enzymes involved in monoamine neurotransmitter synthesis as it does with eNOS. Even if this does not turn out to be the case, 5-MTHF does appear to significantly influence BH4 levels and activity (Figure 6).

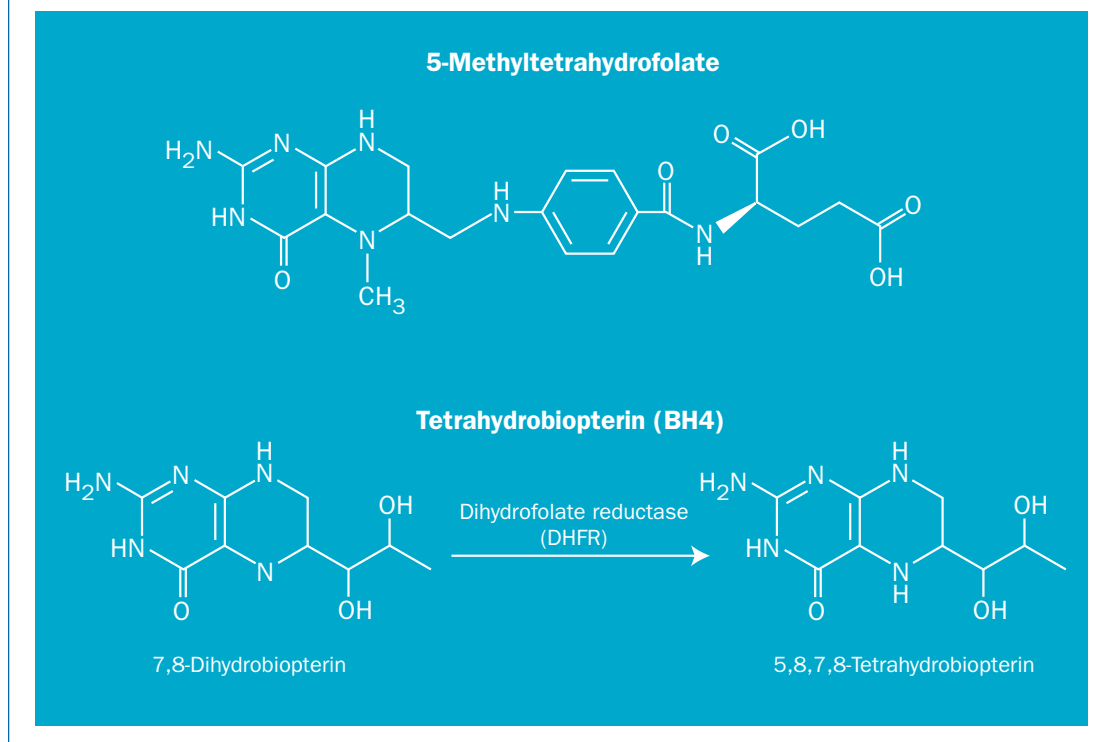
Figure 4. Tetrahydrobiopterin Production and Regulation



BH4=tetrahydrobiopterin; 5-MTHF=5-methyltetrahydrofolate; H=hydrogen; CH<sub>3</sub>=methyl group; CH<sub>2</sub>=methylene; THF=tetrahydrofolate; MTHFR=methylene tetrahydrofolate reductase; qBH=quinonoid dihydropterin; NADH=nicotinamide adenine dinucleotide; NADP=nicotinamide adenine dinucleotide phosphate; NAD=nicotinamide adenine dinucleotide (oxidized); NADPH=nicotinamide adenine dinucleotide phosphate (reduced); DHF=dihydrofolate; DHFR=dihydrofolate reductase; GTP=guanosine triphosphate.

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Figure 5. Structure Comparison of 5-MTHF and BH4



Supplementation with 5-MTHF is significantly more effective than supplementation with folic acid at raising plasma 5-MTHF levels. After supplementation of 24 individuals with 5 mg folic acid or 5 mg 5-MTHF, Willems et al found a seven-fold higher peak concentration of plasma 5-MTHF in the 5-MTHF group, compared to folic acid ( $p < 0.001$ ). No significant difference was noted between patients with the TT MTHFR genotype ( $n = 12$ ) compared to the CC genotype ( $n = 12$ ).<sup>40</sup>

Vitamin C (ascorbic acid) is another cofactor in the rate-limiting hydroxylase enzymes involved in monoamine neurotransmitter synthesis. This essential antioxidant is both a cofactor at the enzyme level and a stabilizer of BH4, which prevents oxidation of BH4 and increases BH4 levels. It appears intracellular BH4 levels are critically dependent on cellular levels of ascorbate.<sup>41,42</sup>

## Clinical Intervention Studies Using Folates

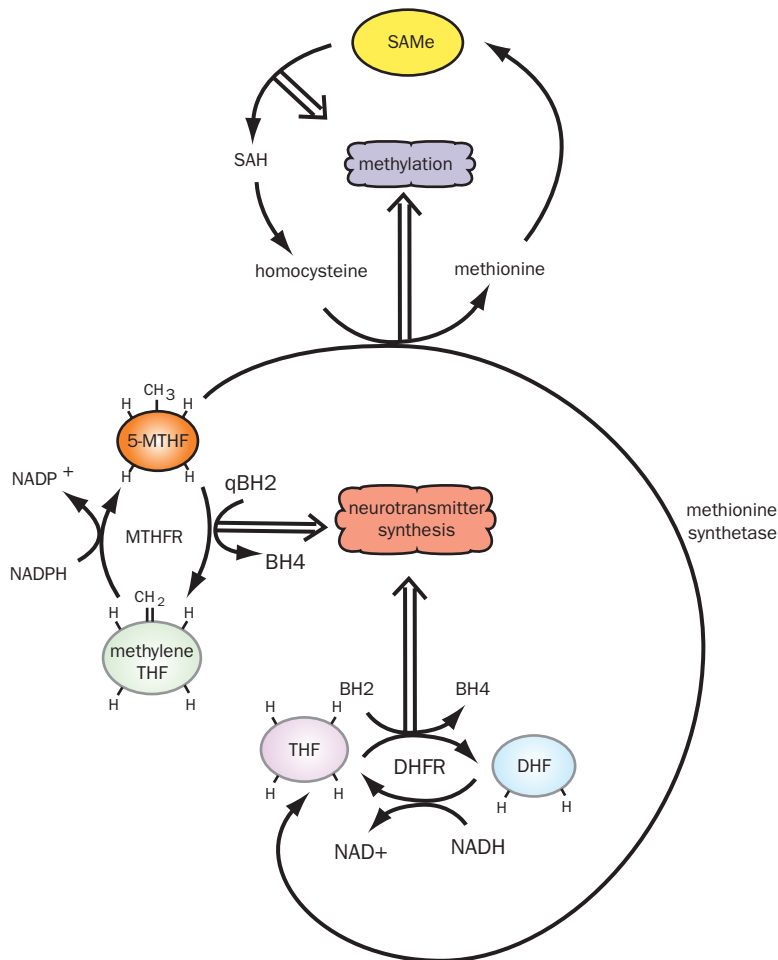
As described above, low folate levels are common in individuals with major depressive disorder. The commonsense approach would be to raise folate levels via supplementation with folic acid or the active metabolite 5-MTHF.

A recent animal study found administration to mice of either folic acid or folinic acid had an antidepressant effect as a stand-alone treatment.<sup>43</sup>

A six-week, open trial of high-dose 5-MTHF (50 mg daily) was conducted in 20 depressed elderly patients. Of the 16 patients who completed at least four weeks of the study, a significant improvement in depressive symptoms was seen in 81 percent.<sup>44</sup>

Passeri et al studied the effect of high-dose 5-MTHF (50 mg daily) in 96 depressed elderly patients with concomitant dementia, but with normal folate levels at baseline. After eight weeks' treatment with 5-MTHF in addition to the psychotropic medications being taken at baseline, the 5-MTHF group exhibited a significant

**Figure 6. Actions of 5-MTHF and SAMe in Methylation and Neurotransmitter Synthesis**



SAMe=S-adenosyl-methionine; SAH=S-adenosylhomocysteine; H=hydrogen; CH<sub>3</sub>=methyl group; 5-MTHF=5-methyltetrahydrofolate; CH<sub>2</sub>=methylene; NADP=nicotinamide adenine dinucleotide phosphate; qBH<sub>2</sub>=quinonoid dihydropterin; MTHFR=methylene tetrahydrofolate reductase; BH<sub>4</sub>=tetrahydrobiopterin; NADPH=nicotinamide adenine dinucleotide phosphate (reduced); CH<sub>2</sub>=methylene; BH<sub>2</sub>=biopterin (oxidized); THF=tetrahydrofolate; DHFR=dihydrofolate reductase; DHF=dihydrofolate; NAD=nicotinamide adenine dinucleotide (oxidized); NADH=nicotinamide adenine dinucleotide (reduced).

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( $p < 0.05$ ) reduction in the Hamilton Depression Rating Scale (HDRS). When patients taking 5-MTHF were compared to a group taking the antidepressant drug trazodone, the results were equivalent.<sup>45</sup>

As seen in other studies, Godfrey et al found 33 percent of 123 patients participating in a six-month, double-blind, placebo-controlled study of psychiatric disorders had borderline or frank folate deficiency (red cell folate  $< 200$  mcg/L). Patients being treated for major depression and schizophrenia were given 15 mg 5-MTHF daily in addition to standard psychotropic medications. The treatment resulted in significant improvements in the HDRS and in what the authors termed "social recovery."<sup>46</sup>

### Augmentation of Antidepressant Therapy With Folate

A significant percentage of people treated with antidepressant medications do not respond with remission of their depressive symptoms. This is often responded to with an increase in drug dosage, switch to a different drug within the same class, or switch to a drug in a different class.

Depressed individuals with low serum folate levels are significantly less likely to respond favorably to the SSRI drug fluoxetine<sup>47,48</sup> and more likely to relapse during treatment with fluoxetine.<sup>49</sup> These studies did not find a correlation between response to the drug and the subjects' homocysteine or B<sub>12</sub> levels.

In a group of depressed geriatric patients, lower serum folate was associated with a worse response to either an SSRI drug (sertraline) or a tricyclic antidepressant (nortriptyline). Conversely, higher baseline folate was associated with a greater response to either drug.<sup>50</sup>



Folic acid (500 mcg/day) was given to 62 depressed men and women along with 20 mg fluoxetine. Another group was given placebo with fluoxetine. In the folic acid plus fluoxetine group, women had a good response – 94 percent were considered responders, compared to 61 percent of women taking fluoxetine and placebo.<sup>51</sup>

A group of 22 depressed adults non-responsive to an SSRI drug were given 15-30 mg folinic acid (5-formyltetrahydrofolate, an intermediate metabolite of folic acid metabolized to 5-MTHF) for eight weeks. Folate levels rose significantly and 31 percent of study participants had a 50-percent reduction in HRDS scores. Nineteen percent achieved remission of depression symptoms.<sup>52</sup>

## Discussion

Folate deficiency is found in approximately one-third of depressed individuals. From the research it appears a folate level in the normal range might still be inadequate for the purpose of methyl donation and neurotransmitter synthesis. Therefore, the number of depressed people who have an *insufficient* amount of folate might be significantly greater. The level of homocysteine in the plasma is also an indicator of folate status, although homocysteine itself, being a pro-oxidant and known to damage neurons, might contribute to the problem. Since the treatment for high homocysteine is folic acid, folinic acid, or the active methyl donor 5-MTHF, the contribution of homocysteine to the disease process is a moot point. In the end, the patient needs folate. The contribution of BH4 to depression is fascinating and significant, and needs more research. From what is currently known, protecting BH4 from oxidation with ascorbic acid and 5-MTHF, recycling it from its oxidized form, and possibly substituting for it with 5-MTHF is important in individuals with depression and might be at least one of the mechanisms for the positive outcomes with folate supplementation in depression.

Low serum folate may also contribute to patients not responding to antidepressant medication. Assessing the serum or red blood cell folate level prior to putting a patient on an antidepressant medication, then correcting a deficiency, might obviate the need for the antidepressant. If symptoms persist after correcting a folate deficiency it would be prudent to continue dos-

ing 5-MTHF at a higher level (5-10 mg), whether the patient takes an antidepressant or not, to increase the chance of recovery.

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