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Combined action of SAMe, Folate, and Vitamin B12 in the treatment of mood disorders: a review

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Abstract. – Mood disorders affect more than 500 million people around the world. In the last decade, their prevalence has increased, and many people suffer from nervousness, anxiety, and stress at least once in their lives. The incidence of mood disorders and anxiety increases during perimenopause or under stressful conditions. The social restrictions introduced during the COVID-19 pandemic have significantly increased the normal burden of psychological and psychic disorders. In moderate to severe cases, pharmacological treatment is currently recommended, while in mild disorders, especially in the initial phase, psychological therapy is preferable. It is known that several nutrients are crucial for brain function. Among them, folate (vitamin B9), cyanocobalamin (vitamin B12), and S-adenosyl-L-methionine (SAMe) have been shown to influence various neurobiological processes. Overall, the available evidence suggests that dietary supplementation with folic acid, vitamin B12, and SAMe can be beneficial for people with mild mood disorders.

Key Words:
SAMe, Folate, Vitamin B12, Mood disorders, Mild depression, Anxiety, Stress, Fatigue, Orodispersible formulation.

Introduction

Mood disorders, such as depression, fatigue, and anxiety disorders, are common mental health problems, that affect approximately 500 million people worldwide[1]. Compromised quality of life is a feature of mental health problems, that can have a number of negative consequences, such as absenteeism from work, lower productivity, physical comorbidities, disruption of family relationships, and high healthcare costs[2-4].

Depression is a major public health problem and the leading cause of psychiatric disability worldwide. Because of their disorder, people affected by depression suffer serious medical consequences, psychiatric comorbidities, suicidal thoughts, and psychosocial disabilities. Furthermore, depression is a condition that often becomes chronic[5,6].

Depression can arise from a combination of causes and can be subdivided in several subtypes, each phenotypically characterized by the predominance of certain symptoms. Identification of the subtype can be helpful for clinical management. Anxiety-depressive disorders, for example, are characterized by high levels of anxiety and possible risk of suicide. Mixed features may indicate bipolar disorder, and in this case, caution should be exercised in prescribing conventional antidepressants. Some subtypes show improvements only with certain drug therapies. For instance, the most effective treatment for melancholia, often characterized by anhedonia, psychomotor changes, and guilt, appears to be the administration of broad-spectrum antidepressants or agents that activate dopaminergic and noradrenergic neurotransmission[7,8].

Some people are more likely than others to develop mood disorders. For example, during bereavement, individuals may exhibit a constant longing for the deceased, frequently associated with emotional pain, rejection of death, inability to engage in future actions or relationships, and loss of identity[7]. Women have a greater risk of developing mood disorders than men, with some studies reporting a twofold increase[9].

Approximately 9.0%[1] of women worldwide suffer from depression or anxiety during their lifetime (i.e., pregnancy, childbirth, and menopause) (Table I).

During menopause, women are twice as likely to experience marked depressive symptoms, with depression scores and changes in hormone levels

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Table I. Epidemiology of mood disorders in adults[1].

Kind of mood disorders	Adult (%)	Adult Male (%)	Adult Female (%)
depression	3.4 (global) 5.2 (Italy)	2.7 (global) 3.5 (Italy)	4.1 (global) 6.9 (Italy)
anxiety	3.8 (global)	2.8 (global)	4.7 (global)

[1]global %; Italy %[12].

showing a significant relationship[10]. Stress, high body mass index, a history of depression, and low socioeconomic status are other risk factors that may contribute to the development of depression. Instead, a longer reproductive period lowers the likelihood of depression[10-12]. Prenatal depression occurs in approximately 8-30% of women and may be predictive of postpartum depression. This could lead to both impaired growth and cognitive development of the fetus or child due to fewer interactions with the mother, and the occurrence of mood disorders in the children[11].

Depressive states in children and adolescents are also relatively common. They can cause toxic substance abuse, physical illness, high risk of suicide, a propensity for misbehaviors leading to legal problems, early pregnancy, deterioration in work and school performance, and psychosocial behavior. Prevalence is estimated to be 0.4%-2.5% in children and 0.4%-8.3% in adolescents, and the etiology is not fully understood[13].

The causes of mood disorders are diverse and include both environmental and genetic factors. Among environmental causes, stressful events (including psychosocial events), stress due to childhood adversity, and life experiences in adulthood are the most important. A relationship between acute and chronic stressors and depression risk has been demonstrated. Stressful life events include chronic or life-threatening illness, financial hardship, job loss, separation, bereavement, and violence[7,8].

The SARS-CoV-2 pandemic is an example of stressful life event, affecting both adults and children[14]. Data released by Chinese authorities show that since the outbreak of the pandemic, 25% of the population suffered from symptoms, such as moderate to severe anxiety, stress-related symptoms, severe fear of infection, and post-traumatic stress disorder. People with a pre-existing pathological mental illness had a significantly high score on the total stress scale[15]. These findings support the strong negative impact of COVID-19 on the burden of individuals with pre-existing mental illness[13]. The COVID-19 epidemic had an impact also on children. A pilot study[16] carried out in Shaanxi Province among 320 adolescents and children (168 girls and 142 boys) aged 3 to 18 years showed psychological and behavioral problems, such as irritability, anxiety, distraction, and reluctance to ask about the epidemic.

Stressors can stimulate cytokines, called growth factors, and activate the release of hormones in the hypothalamus-pituitary axis, which in turn can affect depression. Violent or chronic stressful experiences trigger an inflammatory process *via* a self-reinforcing neuroendocrine cascade leading to further sensitization of individuals predisposed to depressive states[7,8].

It has also been suggested that an important factor in psychiatric disorders, particularly in bipolar mental disorder and depression, is mitochondrial dysfunction or damage to the mitochondrial electron transport chain caused by alterations in the biochemical cascade. These neuronal dysfunctions induced by stressful events interact with other environmental factors, such as smoking, alcohol consumption, altered sleep schedule, diet and exercise levels under stress, adverse childhood events, substance abuse, and trauma exposure, which, together, can cause mood disorders in physiologically susceptible and/or genetically predisposed individuals[17].

Environmental factors, particularly in minors, include episodes of child abuse or neglect. Large cohort studies[7,8] involving more than 11 million adults found that those who were abused in childhood were 2.14 times more likely to have a psychiatric diagnosis with characteristic mood disorders.

Mood disorders in adults, children, and adolescents are usually treated by a combination of individual and family therapy, school-based behavioral interventions, and, in some cases, administration of antidepressants. It is estimated that even the best available evidence-based pharmacological and psychological therapies are not effective in about 60% of patients with depression[5,18], and only a subset of those affected (30-40%) achieve

symptomatic remission after appropriate treatment with a first line antidepressant[19].

The first-line treatment for mild depression in children is psychotherapy, whereas in adults, antidepressants are usually recommended for the treatment of mild and moderate-to-severe depression. Despite advances in psychopharmacological treatment, new alternative approaches are emerging. A growing number of patients who are dissatisfied with conventional therapy are seeking other treatment options[4].

Given this scenario, alternative complementary therapies have been intensively studied for their potential to provide additional benefits. Complementary, Alternative and Integrative Medicine (CAIM) includes a variety of biological and psychological treatments used in conjunction with standard medical procedures to improve patient outcomes. Integrative Psychiatry (IP) is a form of CAIM which includes treatment with nutraceuticals. These are substances registered by the United States Food and Drug Administration (FDA) as "dietary supplements" that contain ingredients, such as vitamins, amino acids, minerals, herbs or other plant extracts, and concentrates of nutrients, metabolites or constituents[4,20]. Their use is consistent with the observation that diet influences depression[9]. Clinical research on nutritional supplements as an aid in the treatment of mood disorders and depression is beginning to show promising results[5]. Current scientific evidence[21] confirms the efficacy of certain dietary supplements as adjunctive therapy for depression, and in 2015 several nutrients were described by the "International Society for Nutritional Psychiatry Research" as important for the prevention or treatment of certain mental disorders, including depression.

It is known that several nutrients are implicated in brain function. It has been proven that an adequate intake of these nutrients ensures proper neurological and psychological function and helps reduce fatigue and exhaustion. Nutrients, such as vitamin B9 (folic acid), vitamin B12 (cyanocobalamin), and S-adenosyl-L-methionine (SAMe) affect different neurobiological processes[22], and their deficiency may contribute to the development of several mood disorders. Folate and vitamin B12 deficiencies are thought to be associated with depression, reduced response to antidepressants, greater likelihood of recurrence, and longer depressive episodes[6]. As we age, vitamin B12 and folate levels decrease, and decreased levels of these molecules have been associated with several mood disorders in the elderly and in psychiatric populations[23,24].

To better understand the processes by which vitamin B12, SAMe, and folate can improve the lives of people with mild and/or moderate mood disorders, this review examines their individual roles.

SAMe

S-adenosyl-L-methionine (SAMe) was discovered by Cantoni in the 1950s as described in a review by Lu et al[25]. It is formed by the combination of L-methionine with adenosine triphosphate (ATP). Methionine is a sulfur-containing amino acid that can only be obtained from the diet[26] because the human body cannot produce it in sufficient amounts. Methionine synthesized by the body is produced from folate, vitamin B12, and homocysteine through the action of the methylenetetrahydrofolate (5-MTHF) reductase and methionine synthase[27] in a process known as the methylation cycle. 5-methyltetrahydrofolate (5-MTHF) is first converted to tetrahydrofolate (THF), and, at the same time, homocysteine is methylated to methionine, using vitamin B12 as a cofactor[21,28]. Synthesis of SAMe occurs in the cytoplasm of all cells; however, most of the synthesis and degradation takes place in hepatocytes[29], where about 70% of methionine is converted to SAMe by the enzyme methionine adenosyl transferase (MAT) in an ATP-dependent process. MAT catalyzes the transfer of the adenosyl group from ATP to the sulfur atom of L-methionine, forming a sulfonium ion[25,29], a high-energy molecule that can transfer its methyl group (-CH3) to several substrates[25,30], including DNA bases, phospholipids, proteins, free amino acids, and neurotransmitters, through a chemical reaction known as methylation.

SAMe provides methyl groups in more than 100 different reactions catalyzed by methyltransferase enzymes. Under normal conditions, most of the SAMe produced daily (about 6-8 g) is used in transmethylation reactions that donate methyl groups to various acceptors. After transfer of the methyl group, SAMe becomes S-adenosylhomocysteine (SAH), a competitive inhibitor of transmethylation reactions[25,29]. In addition to methylation reactions, SAMe also plays a role as a precursor for the amino-propylation pathway, which leads to the chemical formation of polyamines, and for the trans-sulfuration pathway, which produces glutathionine[26].

DNA methylation can activate or deactivate gene transcription, and thus control the activity of various signaling pathways[20,29]. Likewise, pro-

tein methylation is a post-translational modification that regulates enzyme activity. Phospholipid methylation is necessary to maintain cell membrane structure and thus the function of receptors localized within the lipid bilayer. Defects in methylation are implicated in the pathogenesis of central nervous system (CNS) disorders, including depression and dementia. Methylation is, therefore, one of processes that can be targeted to prevent CNS diseases, to delay their progression, or to improve therapeutic outcomes[20].

SAMe is involved in several cell membrane functions and in the synthesis of monoamines, in particular, serotonin, noradrenaline, and dopamine[2,20,31]. SAMe participates in numerous important biochemical processes and may play a role in the treatment of neurological diseases, including psychiatric disorders, due to its key role in CNS methylation[20,32].

Some studies[2,33] have reported the beneficial effects of SAMe in the treatment of depression, both as monotherapy and as an adjunct to traditional therapies. SAMe concentration in blood and cerebrospinal fluid (CSF) has been measured and its range under normal and pathological conditions has been also established. SAMe insufficiency in CSF has been found in patients with rare inherited defects in folate and methionine metabolism, as well as in more common conditions, such as depressive disorders. Studies[20,21] have shown that oral or parenteral administration of SAMe in patients with neuropsychiatric disorders increases its levels in the CSF as it crosses the blood-brain barrier.

SAMe has been studied in mouse models of depression[20,34] where it reduces the period of immobility during the forced swim test in a dose-dependent manner and increases concentrations of the monoamine neurotransmitters, serotonin and noradrenaline[35]. Animal studies[36] have shown that repeated administration of SAMe increases dopaminergic tone in various brain regions, including the striatum, and the density and activity of CNS beta-adrenergic receptors. Therefore, studies on central monoaminergic neurotransmitters support the proposed mechanisms for the antidepressant effects of SAMe. SAMe may also have modulatory effects on cell signaling pathways in the central nervous system. In rats, chronic treatment with SAMe resulted in high levels of calcium/calmodulin-dependent protein kinase II (CaMKII) in hippocampal synaptic vesicles, and high levels of Synapsin I in the synaptic cytosol of the hippocampus and frontal cortex. Typical antidepressants have been shown to activate CaMKII and Synapsin I, suggesting that SAMe might share a similar modulatory effect on neurotransmitter release[20,37].

SAMe has been used in European countries for more than 30 years to treat psychiatric and pathological conditions[20]. S-adenosylmethionine has several mechanisms of action that highlight its role in alleviating the clinical signs of depression. These mechanisms mainly include anti-inflammatory effects[18], such as modulation of interleukin (IL)-10, production of IL-6, modulation of the tumor necrosis factor (TNF), upregulation of protein kinase A (PKA), inhibition of pro-inflammatory mediators, modulation of the methylation state of inflammatory genes and reduction in oxidative stress[38-40].

Another possible mechanism of action is consistent with the role that SAMe plays in the release of methyl groups thereby controlling important epigenetic functions. The hypothesis of the central role of methylation in regulating gene expression/silencing implies that defects in methylation may contribute to the manifestation of mental disorders, such as depression. SAMe may ameliorate mood disorders by ramping up methylation of catecholamines, which is associated with an increase in serotonin turnover, inhibition of noradrenaline reuptake, an increase in dopaminergic activity, a decrease in secretion of prolactin, and increased conversion of phosphatidylcholine[3,41]. SAMe may also affect the noradrenergic system. In rats, increased amounts of beta-adrenergic receptors and a high affinity of alpha1-adrenergic receptors for the agonist phenylephrine were observed after SAMe administration. Thus, treatment with SAMe leads to changes in adrenergic neurotransmission that differ from those classically produced by conventional antidepressants: downregulation of beta-adrenergic receptors and upregulation of alpha-adrenergic receptors[42]. Another hypothesis concerns methylation of plasma phospholipids and associated changes in neuronal membrane that would affect the function of some membrane proteins, including monoamine receptors and transporters[21].

The use of SAMe to treat depression is safe and effective, according to a critical literature review that assessed the results of 48 studies[4,26]. SAMe has a very low-risk side effect profile, comparable to placebo[26], and has been reported to be well tolerated with no effects on weight or sexual routine[3]. However, it is not free of adverse events. The most commonly reported are mild gastro-

intestinal symptoms, such as diarrhea, nausea, vomiting, and abdominal pain[20]. Other side effects described include dizziness and sweating[43]. Occasionally, irritability, anxiety, or insomnia may occur[44]. SAMe has been shown to induce euphoria or mania in bipolar patients[45]. Compared with antidepressants, SAMe has caused weight gain and cognitive or memory impairment in dementia, Alzheimer's disease (AD), and traumatic brain injury in a few cases[20,24,38]. On the other hand, sexual dysfunction, often described for D-cycloserine (DCS) and most standard antidepressants, was not observed with SAMe. A single-site RCT in patients who were unresponsive to selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) examined whether SAMe was associated with greater improvement in sexual activity than placebo[20,46]. Compared with patients treated with placebo, patients who received additional SAMe treatment showed significantly less arousal dysfunction ($p = 0.0012$) and erectile dysfunction ($p = 0.01$), independent of treatment-related changes in depression severity[20].

SAMe has a tolerability profile that allows its use in pregnant women and during lactation. A randomized, placebo-controlled clinical trial of 30 women with postpartum depression who received 1600 mg/day of SAMe, showed a 70% reduction in depression and anxiety symptoms compared with 50% in the placebo group at day 30 after the first dose[4]. In addition, SAMe has very little risk of pharmacokinetic or pharmacodynamic interaction with other drugs. This may be of great advantage in the treatment of depressed patients who have other conditions requiring the use of medications[47].

Randomized, placebo-controlled trials have demonstrated the efficacy of SAMe therapy alone[20]. This finding was confirmed by a meta-analysis[48] approved by the Agency for Health Research and Quality in 2002, in which SAMe monotherapy outperformed placebo in the treatment of depression symptoms.

Treatment with SAMe showed superiority in outcomes over placebo and had similar effects to other antidepressants[34,48]. When used as add-on therapy in combination with other treatments (including tricyclic antidepressants: TCAs, SSRIs, and SNRIs), it proved useful not only in the treatment of depression but also for patients who did not respond to standard antidepressants[20]. A report from the Cochrane Database of Systematic Reviews concluded that SAMe was "superior to placebo when used in combination with selective serotonin reuptake inhibitor (SSRI) antidepressants"[33] but advised more rigorous studies because of the low quality of the evidence.

SAMe is also useful for the treatment of memory and cognitive impairment often associated with major depressive disorder or dementia. Other randomized, placebo-controlled trials have shown that SAMe can improve sexual dysfunction due to depression or use of conventional antidepressants. SAMe administration may also be helpful even in treating depression in patients who have other pathological conditions[38,46].

Several randomized double-blind controlled trials have compared SAMe with standard antidepressants: TCAs, nomifensine, minaprine, and escitalopram. Initial results showed that parenterally administered SAMe (150-400 mg/day) was effective or superior to TCAs (clomipramine, amitriptyline, imipramine) with fewer side effects[49-53]. Two larger studies (n = 295, n = 293)[54,55] found that intramuscularly administered SAMe (400 mg/day) was as successful as oral imipramine (150 mg/day) for a period of 4 weeks.

Four RCTs, including one large study (n = 281) and three smaller studies ($n \leq 30$) performed in the late 1990s and early 2000s, found oral SAMe (1600 mg/day) to be as effective as oral desipramine (250 mg/day) and oral imipramine (140-150 mg/day)[49,54,56,57]. Overall, in several controlled studies, SAMe was found to be as effective as chlorimipramine[50,51,53], imipramine[49,54-56], and nomifensine[58]. Similar results were found in an open-label study on depressed patients (n = 30) not fully responding to an SSRI or venlafaxine who were treated with oral SAMe. This study reported clinical response in 50% of patients[59]. Another small-scale, open-label study (n = 33 depressed patients not responding to antidepressant drugs) had comparable outcomes with 800 mg/day of SAMe (clinical response in 60%)[20,60]. In one RCT, outpatients with depression (n = 73) who did not respond or partially responded to SSRI or SNRI were randomized to receive up to additional 1600 mg/day SAMe or placebo for 6 weeks[20,61]. Both the percent response rate (36.1% SAMe *vs.* 17.6% placebo) and remission rate (25.8% SAMe vs. 11.7% placebo) were significantly higher in patients receiving SAMe ($p < 0.05$)[20].

The association between 200 mg of SAMe and the bacterial strain *Lactobacillus plantarum* was recently studied for the treatment of common symptoms of mild-to-moderate depression. In a 6-weeks randomized, double-blind, placebo-con-

trolled study, data were collected from subjects aged 18 to 60 years who reported signs of mild to moderate depression. The primary outcome of the study was represented by the differences between groups at baseline and after 6 weeks of treatment on the Zung Self-Rating Depression Scale (Z-SDS). The results showed that adults with subthreshold or mild-to-moderate depression treated with SAMe and *L. plantarum* supplementation had fast and clinically relevant improvements. The combination of treatments was safe and improved symptoms of anxiety and depression as well as somatic and cognitive components[62].

SAMe has been reported to produce antidepressant effects at various doses. The dose varies from 200 mg to 400 mg every 3-7 days[3,38,63], up to a limit of 800 mg twice daily[44]. However, to date, the dose-response relationship for oral administration of SAMe remains unknown.

The need to administer very high doses to achieve good efficacy when taken orally depends on the pharmacokinetics of SAMe[56]. SAMe has extremely poor bioavailability, an obstacle that can be overcome by using high doses. The reason for the poor bioavailability after oral administration is that the liver retains about 60% of the ingested dose during the first liver pass. Intramuscular administration, which initially does not involve a liver pass, achieves a significant level of efficacy at a 4-fold lower daily dose (400 mg). An oral formulation of SAMe of the orodispersible type has recently been developed and marketed. This formulation appears to enhance the absorption of SAMe in the oral cavity, particularly through the sublingual venous plexus, which opens directly into the superior vena cava, thus ensuring that the drug enters the circulation directly. Pharmacokinetic studies comparing the area under the curve (AUC) of taking a single oral dose of 200 mg of orodispersible SAMe and that of taking a similar dose of gastro-resistant tablets have shown higher bioavailability of the orodispersible formulation of SAMe, comparable to intramuscular administration[47].

The study on the orodispersible formulation of SAMe was carried out at the "Cross Research" center in Arzo, Switzerland, between 12 and 21 December 2008 in patients of both sexes, non-smokers, aged 18 to 55 years, in good health. Participants were given orodispersible SAMe tablets (119.76 mg free SAMe) or enteric coated SAMe tablets (200 mg free SAMe) to be swallowed without chewing[64]. The concentration of SAMe in the plasma tests was determined using the validated LC-MS/MS method[65], which has a sensitivity greater than 10 ng/ml. The results showed statistical differences in the plasma concentrations of SAMe when comparing the two SAMe formulations. After administration of the orodispersible tablets, SAMe reached peak blood concentration (Cmax) within 30 min to 4 hours (the median of Tmax was 1 hour and 48 min). In contrast, after administration of the enteric-coated tablets the plasma concentration of SAMe increased only by 0.1-2.6 ng/ml from baseline in the first 3 hours after ingestion. The peak blood concentration (Cmax) was reached late, between the fourth and sixth hour after administration (median Tmax: 5 hours). The maximum concentration (Cmax) of SAMe was 79.4 ± 26.1 ng/ml after administration of the orodispersible tablets and 104.3 ± 61.8 ng/ml after administration of the gastro-resistant tablets. After subtraction of the basal SAMe levels, these values decreased to 56.7 ± 22.2 and 80.9 ± 54.8 ng/ml, respectively. However, after reaching the Cmax value in blood, the SAMe concentration in blood tended to remain stable after taking the orodispersible formulation of the drug. In contrast, the same parameter showed a tendency to decrease monoexponentially in subjects who took the drug in the gastro-protected tablet formulation[64]. The effects of SAMe are shown in Table II.

Folate

The term folate, or vitamin B9, refers to a group of water-soluble compounds that are essential for the proper biosynthesis of deoxyribonucleic acid (DNA)[65,66]. In the human body, folate is mainly concentrated in the liver. The term folate refers to the vitamin in its natural form in food, while the term folic acid refers to the oxidized form that identifies the synthetic molecule[65]. Synthetic folic acid, that is converted to folate in the body, is used as dietary supplement but natural folates should be preferred, given some of the negative effects highlighted in the literature. Folic acid is converted to tetrahydrofolate (THF) by the activity of the enzyme dihydrofolate reductase, which uses nicotinamide adenine dinucleotide phosphate (NADPH) as a co-factor. THF is then converted to 5-10-methylenetetrahydrofolate (5-10-MTHF), a molecule that participates in several metabolic pathways, including DNA and methionine synthesis[65,67].

For DNA synthesis, deoxyuridine monophosphate (dUMP) gets one methyl group from 5-10-MTHF (*via* thymidylate synthase, which accepts the other methyl group) to be convert-

ed to deoxythymidine monophosphate (dTMP), allowing the cycle to continue while simultaneously regenerating dihydrofolate (DHF). Methionine is a synthetic by-product as folate lowers homocysteine levels in the blood. 5-10-MTHF donates a methyl group to the enzyme methyl-tetrahydrofolate reductase (MTHFR), becoming 5-methyl THF. Homocysteine acquires the other methyl group from 5-methyl THF *via* methionine synthase and becomes methionine. The transfer reaction of the methyl groups of 5-10-MTHF allows THF to regenerate so that the cycle can start again[65].

Folate is an essential micronutrient obtained from the diet and synthesized locally by the gut microbiome[68,69]. It is found in some food sources, such as leafy green vegetables (lettuce, spinach, asparagus, broccoli), fruit (kiwi, strawberries, and oranges), legumes (peas, beans), dried fruit (almonds and walnuts), liver and other organs, cheese, and eggs. Food preparation, cooking, and storage can destroy much of the folate because these water-soluble vitamins are sensitive to heat, light, air, and acids[65,70].

The recommended intake for the general population is 320 µg/day. However, in women the daily requirement for folate increases during pregnancy and breastfeeding. In women of childbearing age, in women planning or not ruling out pregnancy, and in women who are currently pregnant, the recommended intake is 520 µg/day, and during breastfeeding it is 450 µg/day (to replenish amounts lost with breast milk)[71].

Folate deficiency can cause macrocytic megaloblastic anemia, which may be due to a history of alcoholism, a predisposition to difficult absorption, hemolytic anemia, or increased requirements during pregnancy[65].

In recent years, numerous studies have found severe folate deficiencies in psychiatric populations, particularly in depressed individuals. The first major study describing folate deficiency in psychiatric patients involved determination of serum folate levels in 423 patients admitted to a psychiatric center[72]. Further scholars[28] found that folate deficiency was very common in depressed patients (29-30%), in organic psychosis (24%), and in people with schizophrenia (20%).

Most of the early studies were based on assessment of serum folate levels. However, more recent analyses[5,73,74] have used tests on red blood cells, that specifically identify intracellular deposits and have shown that up to 30% of people with major depression are folate deficient. In depressed subjects, investigators found a folate concentration in red blood cells below 150 µg/L and an increase in total plasma homocysteine in 52% of patients. This suggests that folate concentration in red blood cells is significantly correlated with plasma homocysteine[73]. Folate deficiency is consistently associated with a high risk of depression, more severe depressive symptoms, longer depressive episodes, and an increased risk of relapse[5,75-77].

Some studies on the relationship between depression and folate deficiency, carried out mainly in epileptic patients, have shown that anticonvulsant therapy leads to low serum folate concentrations, making psychological symptoms, such as depression and psychosis more common. A study[44] conducted in the United States on 213

Table II. Mode of action of SAMe.

Action of SAMe	Consequences
Synthesis of monoamines[2]	Increase of serotonin, noradrenaline, and dopamine, which improves mood disorders
Methylation in the CNS32	Donor of methyl groups in more than 100 distinct reactions catalyzed by methyltransferase enzymes
Transmethylation reactions[25,29]	SAMe is converted into S-adenosylhomocysteine (SAH)
Precursor of the aminopropylation pathway[26]	Synthesis of polyamines, the transulfuration pathway, and glutathione
Anti-inflammatory effects[3,41]	Modulation of inflammatory mediators (IL-10; IL-6; TNF), with a marked effect on reducing inflammatory phenomena
Donation of methyl groups[42]	Greater methylation of catecholamines, serotonin turnover, inhibition of noradrenaline reuptake, increase in dopaminergic activity, decrease in secretion of prolactin and increased conversion of phosphatidylcholine
Influence on the noradrenergic system[42]	Increase in the quantity of beta-adrenergic receptors and in the affinity of alpha1-adrenergic receptors for the agonist of phenylephrine
Methylation of plasma phospholipids[21]	Alteration of the fluidity of the neuronal membrane
Effect on the functionality of some membrane proteins[21]	Effect on monoamine receptors and transporters

subjects suffering from depression found that the response to standard antidepressant therapy was reduced in patients with low levels of folate. A statistical association between depression and folate levels was confirmed in a study of the general population involving 301 depressed subjects, 121 with dysthymia and 2256 without depression[44].

The mechanisms underlying the relationship between folate and depression are diverse. In the body, folate participates in numerous reactions including the production of neurotransmitters involved in depression, such as dopamine, norepinephrine, and serotonin. Folate, along with S-adenosylmethionine (SAMe), affects the rate of tetrahydrobiopterin, an antioxidant cofactor in the synthesis of these neurotransmitters. This means that a folate deficiency lowers dopamine, serotonin, and norepinephrine levels, providing a neurochemical basis for the manifestation of depression[5].

Another mechanism concerns the correlation between folate and homocysteine. Elevated blood homocysteine levels appear to be associated with several psychiatric and neurodegenerative disorders, such as schizophrenia, depression, Parkinson's disease, and Alzheimer's disease[28,78]. Reduced folate levels correspond with high homocysteine levels, and some studies have shown that high homocysteine levels may be correlated with more severe symptoms of depression[73] due to toxicity to neuronal cells[28,79].

Homocysteine causes the formation of neurotoxic molecules, such as cysteine sulfinic acid and homocysteic acid, which in turn act as agonists with excitotoxic effects on dopaminergic neurons and on the N-methyl-D-aspartate (NMDA) receptor[13,80]. Hyperhomocysteinemia causes increased oxidative stress and lesions in the vascular endothelium[13].

Genetic studies carried out on the gene encoding methylenetetrahydrofolate reductase (MTHFR), whose role is to metabolize folate, have highlighted how subjects carrying the C677T TT genotype have an approximately 1.4 times greater probability of having depressive diseases, compared to subjects with the C677T CC genotype[5,81].

The Cochrane Collaboration published a systematic review in 2003[82] suggesting that folate can have a role as an adjunct to other depression therapies. These results are confirmed by a naturalistic study[82] in which 67.9% of subjects responded positively to treatment with L-methylfolate and showed a reduction in patient health questionnaire scores of approximately 50%. Self-reported adherence to a dose of 7.5 mg or 15 mg was 90%, suggesting high tolerability of L-methylfolate. In patients who had only a partial response or no response to SSRIs, the additional administration of 15 mg of L-methylfolate was found to reduce the mean Hamilton Depression Rating Scale score by 2.6 points[82].

A landmark double-blind, randomized, placebo-controlled trial examined the effect of daily folic acid supplementation of 200 mg compared with placebo on improving affective morbidity in a cohort of 75 subjects treated with lithium. In this study, it was observed that those who had the highest plasma folic acid concentration showed significant improvement in their affective state. Based on these results, daily folic acid supplementation of 300-400 mg/day is recommended as it may be beneficial during long-term treatment with lithium[44].

Failure to respond to antidepressants has also been associated with low folate levels. In a trial carried out in 127 subjects comparing treatment with fluoxetine plus a daily folate supplement of 400 mg with treatment with fluoxetine plus placebo, fluoxetine plus folate was found to be effective in 94% of subjects compared with 61% of subjects receiving fluoxetine and placebo. More specifically, patients in the supplemented group showed a statistical increase in plasma folate concentration as well as a reduction in homocysteine. The mean Hamilton Rating Scale score was 6.8 (SD 4.1) in the group treated with fluoxetine plus folate, compared to 11.7 (SD 6. 7) in those receiving fluoxetine plus placebo ($p < 0.001$)[83].

Another study examined the effect of 15 to 30 mg/day of folinic acid in patients with heavy depression who had a poor response to SSRIs. Additional treatment with folinic acid resulted in a significant improvement in response (HAM -D-17 scores showed a reduction of more than 50%) and in SSRI-refractory depression. The studies also examined the efficacy of 5-MTHF as adjunctive therapy in SSRI-resistant depression. In a randomized, double-blind study it was observed that 15 mg of L-methylfolate produced a significant improvement in response rate and a positive change in HAM-D score compared with placebo, with no significant side effects[44].

Long-term folate supplementation appears to have positive results in reducing the occurrence, severity, and recurrence of depressive symptoms[84]. Although folic acid supplementation may interfere with the effectiveness of other psychotropic drugs[85], folate is a safe supplement for most people[86].

Particular caution should be exercised in people who have or are suspected of having neoplasms. Although there is no compelling evidence in the literature, folic acid supplementation should be avoided in cancer patients and survivors and in highly predisposed and susceptible individuals at risk for developing cancer. Furthermore, folic acid supplementation should not be routinely recommended for individuals aged ≥50 years and with a high prevalence of precursor lesions[87,88].

A recent meta-analysis which considered 16 randomized controlled trials reported data on 1,520 patients and examined the tolerability and effectiveness of folate as an additional therapy for psychiatric syndromes. The results showed that folate was effective, as was adjunctive methylfolate, in improving depressive symptoms[6,89].

In addition, after an average of 15.2 weeks of treatment, remission of symptoms occurred in 47.8% of patients in the folate group, compared with 26.6% in the placebo group. Subgroup analyses revealed that the large improvements with supplemental folate in the treatment of depressive symptoms were smaller in the female-dominated trials (≥60%). The efficacy of supplemental folate in treating mental illness as a function of patient gender is still controversial[68]. The benefit of folic acid supplementation also seems to be related to the age of the patient. One study[90] showed that the effect of folate against depressive symptoms was greatly reduced in female patients older than 35 years. Similarly, a meta-analysis[68] showed that the effect on depressive symptoms disappeared in patients older than 42.1 years, probably due to reduced absorption of folate in the intestine of older patients.

Many scholars[91] show a correlation between psychiatric disorders, folate, and homocysteine levels in adults, but there are very few studies in children and adolescents. The literature on childhood suggests that alterations in folate and homocysteine metabolism can have a role in the pathophysiology of autism and attention deficit hyperactivity disorders. A cross-sectional study[92] of adolescents showed that intake of various B vitamins, including vitamin B9, was correlated with a low incidence of depression. Another study found that adding L-methylfolate (LM) to antidepressant therapy could be effective and safe for treatment-resistant depression in children. The study involved 10 patients (mean age 14.4 ± 2.8 years) with treatment-resistant depression, who were predominantly female (80%) Caucasian (90%) and reported an average of three psychiatric diagnoses of comorbidities and three psychotropic drug failures. Most subjects (80%) also had one of the two MTHFR gene variants, that caused reduced MTHFR activity. Results showed that anxiety, depression, and irritability improved in 80% of patients and LM was well tolerated. Thus, adjunctive LM may be an effective and safe strategy for the treatment of treatment-resistant depressive disorders in pediatric patients[6]. The effects of folate are listed in Table III.

Vitamin B12

Vitamin B12, also known as cobalamin, belongs to the B vitamins. It can occur in various forms such as cyanocobalamin, methylcobalamin, hydroxocobalamin, and deoxyadenosylcobalamin. Carrier proteins known as transcobalamines (TC) bind vitamin B12 in the serum. Most of the vitamin is bound to the inactive form of the TCI carrier (also known as haptocorrin). The active form of the carrier protein for vitamin B12 is transcobalamin II (TCII), which binds nearly 20% of the circulating vitamin[93,94]. From a dietary perspective, vitamin B12 is a micronutrient. The recommended daily intake for women and men is 2 μg, which increases to 2.2 μg during pregnancy and 2.4 μg during lactation[71]. The organism absorbs vitamin B12 through a special mechanism. The vitamin is released from food due to the acidic environment of the stomach. Once released, vitamin B12 combines with another specific protein and migrates to the small intestine. Here, the

Table III. Folate mode of action.

Action of folate	Consequences
Production of new cells[65]	Normal levels of red blood cells
Alteration of homocysteine levels[65]	Reduction of cardiovascular risk
Production of dopamine, norepinephrine, and serotonin neurotransmitters[65,67]	Effects at the level of the CNS
Influence on the rate of production of tetrahydrobiopterin[5]	Antioxidant cofactor in the synthesis of these neurotransmitters

complex is broken down by pancreatic enzymes and alkaline pH. Some specialized gastric cells release an intrinsic factor (IF) which binds to vitamin B12 in the terminal part of the ileum where the vitamin B12-IF complex is absorbed. This process occurs via a receptor-based mechanism mediated by the presence of pancreatic calcium. Approximately 1% is instead taken up by passive absorption. Typically, vitamin B12 deficiency is caused by malabsorption and is not due to a deficiency in the diet[95].

Vitamin B12 occurs in the human body in two active forms: methylcobalamin and 5-deoxyadenosyl cobalamin. Methylcobalamin is important for the activity of methionine synthase, the synthesis of which depends on methylation. The enzyme methionine synthase is involved in the synthesis of methionine from homocysteine. The other form, 5-deoxyadenosyl cobalamin, is needed to convert L-methylmalonyl-CoA to succinyl-CoA[95].

In vitamin B12 deficiency, the substrates of the two vitamin B12-dependent reactions accumulate, resulting in increased plasma levels of methylmalonic acid and homocysteine. The main problems associated with vitamin B12 deficiency are megaloblastic anemia due to inhibition of DNA synthesis and neurological manifestations, since vitamin B12 plays a crucial role in the homeostasis of the nervous and blood systems[96].

Vitamin B12 deficiency leads to neurological dysfunctions such as myelinopathies and neuropathies, as well as neuropsychiatric disorders. Neurological problems have been reported in 20-30% of cases that were vitamin B12 deficient and are likely related to progressive neuronal degeneration caused by inhibition of methionine synthase. In addition, vitamin B12 deficiency has been associated with cognitive and mental impairments, such as memory loss, irritability, dementia, and depression[93].

Several studies have shown an association between vitamin B12 deficiency and psychiatric disorders such as phobias, panic psychosis, bipolar disorder, and depression. A psychiatric diagnosis may be preceded by symptoms, such as negativity, irritability, agitation, confusion, poor concentration, amnesia, disorientation, and insomnia[44].

Psychiatric symptoms associated with vitamin B12 deficiency can be difficult to confirm because mental disorders can occur without hematologic or neurologic manifestations[95]. In a study[93] of older people involving 3,884 subjects, it was found that individuals with vitamin B12 deficiency had a 70% higher risk of developing depression than those with normal vitamin B12 levels.

A 2018 observational study examining the relationship between depression and vitamins found that women with lower serum levels of vitamin B12 were at greater risk than men. This puts pregnant women at high risk, as they are more likely to develop symptoms of depression. Another analysis[97], conducted as part of the National Health and Nutrition Examination Survey using the Patient Health Questionnaire Score-9 (PHQ-9), found that pregnant women have a 3.82-fold higher risk of developing depression. Interestingly, due to the increased need during pregnancy, there is an association between postpartum depression and vitamin B12 deficiency. A study of 1,570 women aged 18 to 50 years who were screened for postpartum depression 6 weeks after delivery showed that levels of vitamin B12 were significantly lower in women with postpartum depression. Multivariate analysis indicated that vitamin decline was correlated with postpartum depression[98]. These results have been confirmed by other studies[99] in which a lower concentration of vitamin B12 was observed in women with depressive symptoms on the seventh day after delivery. The same observations have been made in studies of prenatal depression. In a pilot study conducted in 108 women to examine vitamin B12 levels, total folate, and hyperhomocysteinemia in the 24-48 hours after delivery, the authors observed higher hyperhomocysteinemia in depressed women than in women who were not depressed. Of the 108 patients, 28 women were also examined at 6 weeks postpartum, and analogous conclusions were drawn[100].

Vitamin B12 levels have also been studied in other subpopulations. For example, a study[101] on adolescents and children found that low levels of vitamin B12 and high levels of plasma homocysteine can promote obsessive-compulsive disorders. Several cross-sectional studies of elderly community residents suggest that depression is related to vitamin B12 deficiency. In a Dutch study, 278 subjects over 55 years of age who suffered from depression had low plasma vitamin B12 levels. Elderly Chinese patients (n = 669) had reduced levels of vitamin B12 (<180 pmol/L) and depressive symptoms, independent of folate or homocysteine levels. These findings are consistent with a previous study conducted in 700 elderly women who were twice as likely to experience acute depression with high levels of methylamalonic acid (MMA: a marker of vitamin B12 deficiency)[44]. Melancholic depressive symptoms are more like-

ly to be associated with low plasma vitamin B12 levels, as reported in a randomized controlled trial showing that low vitamin B12 levels were observed in 22% of the depressed population in the sample studied[97].

Most studies suggest that vitamin B12 supplementation may have a function in treating depression[44]. In a case report of a woman (52 years old) suffering from depression, catatonia, and cognitive dysfunction, without hematologic or neurologic abnormalities, a vitamin B12 level of 150 pg/ml (reference range 190 pg/ml -1190 pg/ml) was found. After treatment of vitamin B12 deficiency, there was complete remission of symptoms without concomitant administration of psychotropic drugs[95].

An American study[102] conducted in 35,053 elderly people found that vitamin B12 supplementation helped reduce the occurrence of depressive symptoms over an average of 7.2 years. Specifically, the study highlighted that supplementation with 10 μg of vitamin B12 reduced the likelihood of developing depression by 2%. The conclusions of this study are consistent with a randomized controlled trial published in 2012 which observed that daily oral administration of 100 μg vitamin B12 and 400 μg folic acid improved cognitive function[103].

These results were confirmed by an open label, randomized, controlled trial in which 199 depressed patients were studied. In total, 73 patients had low vitamin B12 levels. 39 patients were assigned to a control group receiving only tricyclic antidepressants, and 34 patients were assigned to a treatment group receiving an additional injectable dose of 1000 μg vitamin B12 per week for 6 weeks. After three months of treatment, 100% of patients reported that their HAM-D score decreased by at least 20%, while only 69% had a decrease in HAM-D score of at least 20% in the control arm. Thus, supplementation of antidepressants with vitamin B12 resulted in significant relief of depressive symptoms[104].

Several clinical cases show that people with vitamin B12 deficiency do not respond to antidepressants but have marked clinical improvement after vitamin B12 supplementation. However, there are currently no recommended guidelines for prophylactic use of vitamin B12 in the treatment of depression[44], although it has been shown to be useful in preventing recurrence after depressive symptoms have resolved[105]. Adverse effects rarely occur with vitamin B12 use, so physicians may prescribe vitamin B12 to patients with depression or those with low serum vitamin B12 levels or elevated MMA, given the low risk profile. The effects of vitamin B12 are summarized in Table IV.

Combination of SAMe, Folate and vitamin B12

The combination of SAMe, vitamin B12, and folic acid is likely to provide a synergistic effect in the treatment of mild to moderate mood disorders with a high safety profile and no adverse effects. This is a major advantage over other supplements containing, for example, hypericum, a compound known to interact with several classes of drugs or containing 5-hydroxy-tryptophan deriving from *Griffonia Simplicifolia*, which can interfere with classical antidepressants.

SAMe acts on mood disorders and related symptoms, such as fatigue and stress due to the crucial role it plays in the methylation cycle. This role is essential for methylation in the CNS and for the synthesis of monoamines such as noradrenaline, serotonin, and dopamine[20]. Similarly, vitamin B12 and folic acid are deficient in people with mood disorders, suggesting that deficiency of these two substances can alter some biochemical reactions related to methylation.

Considering their individual functions, the synergistic action of the three components examined can restore the levels of endogenous SAMe and/or folic acid and vitamin B12 in people with mood disorders who have reduced plasma concentration

Table IV. Mode of action of vitamin B12.

Action of vitamin B12	Consequences
Mandatory for the activity of the enzyme methionine synthase[95]	Essential for the activity of methionine
Conversion of homocysteine into methionine[95]	Reduction of homocysteine levels and cardiovascular risk
Proper formation of red blood cells[96]	Correct function of red blood cells
Normal function of nerve cells[96]	Correct functioning of neuronal circuits

of these substances. Furthermore, the presence of vitamin B12 and folic acid in the formulation, induces the body to produce new endogenous SAMe (methylation cycle) since folic acid and vitamin B12 are cofactors in the reactions that generate SAMe (Figure 1).

Conclusions

Mood disorders, including major depressive disorders, represent one of the most important therapeutic challenges of our millennium. Over the past decade, the prevalence of these disorders has increased, and this trend has intensified in the current pandemic period COVID-19. Because of the varied etiology and multifaceted nature of these pathologies, treatment of the associated symptoms is often complex. Conventional antidepressant treatment options may not always be appropriate for the needs of all patients affected by depression because they do not directly address hidden pathogenic factors, that include nutritional deficiencies, oxidative stress, inflammation, neuroprotection, and neurogenesis. Furthermore, there are limited treatment options for patients with mood disorders who do not respond to conventional therapy.

Nutrition is the focus of a new field, nutritional psychiatry, which aims to identify the dietary components of particular importance to mental health. Thus, targeting dietary imbalances by prescribing dietary modification/supplementation with medical foods and supplements may offer a variety of complementary strategies for treating patients who do not respond adequately to antidepressants and mood stabilizers. Consistent with this approach, the articles summarized

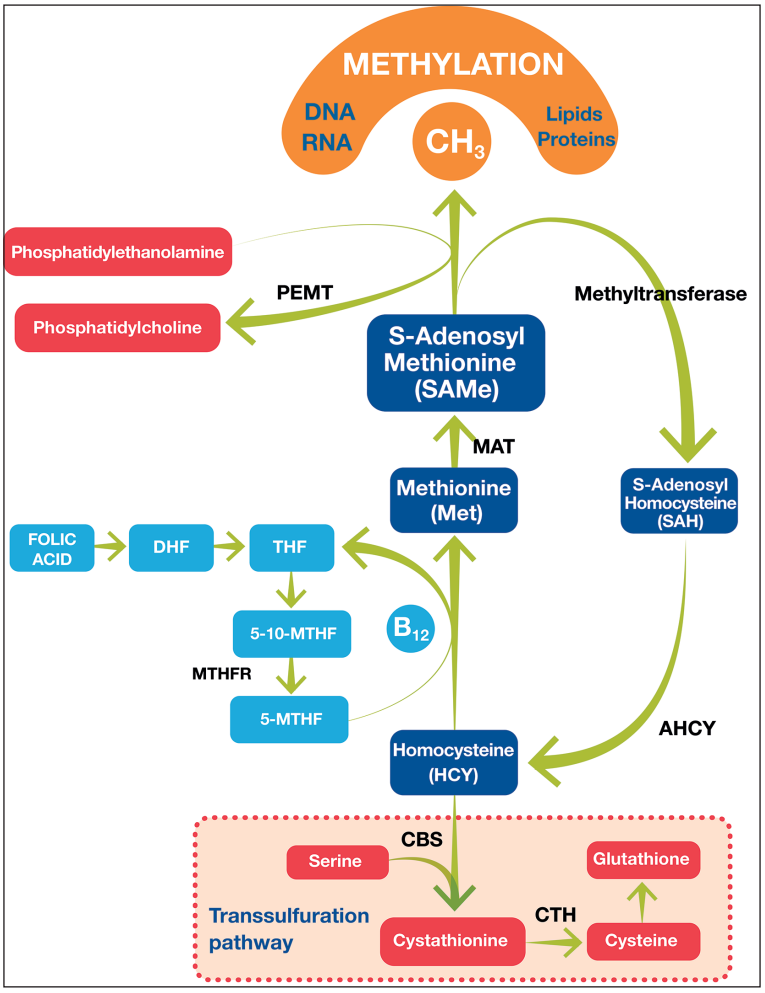

Figure 1. SAMe, folic acid, and vitamin B12: the cycle.

in this literature review agree, although not always at a highly homogeneous level, that folic acid, vitamin B12, and SAMe, taken as dietary supplements, may be beneficial in managing the symptoms of patients with depressive and mood disorders.

Supplementation with folic acid, vitamin B12, and SAMe may be related to the severity of the disorder: less severe, mildly symptomatic, and borderline disorders could be treated by oral supplementation with SAMe, vitamin B12, and folic acid alone. This approach could be effective also in the treatment of early-stage depressive disorders and/or transient conditions. Other disorders that might benefit from supplementation with SAMe, folic acid, and vitamin B12 as a first-line treatment include subthreshold depression, seasonal mood disorders, anxious-depressive syndromes, and those disorders associated with the current COVID-19 pandemic, a persistent, global, and pervasive event that has been shown to cause a set of interrelated mental disorders, including sleep and mood disorders, and post-traumatic stress syndromes. The initial additional treatment not only relieves symptoms with little or no side effects, but may also enhance subsequent pharmacological treatment, if the symptoms worsen[38]. The available experimental evidence has generally demonstrated a favorable safety profile for these compounds. The mechanism of action of these substances, the clinical findings, and their favorable safety profile suggest that early and prompt dietary modification/supplementation with SAMe, vitamin B12, and folic acid can be a useful and safe option to curb symptoms such as mood swings, stress, and fatigue, and may also improve and prolong the efficacy of conventional pharmacological treatment. Taken together, the safety and efficacy of these compounds could influence clinical decision making in psychiatric and psychological disorders.

To improve the results in terms of speed of efficacy, it is advisable to use orodispersible mixtures. Indeed, this product is known to have a better bioavailability, which corresponds to a higher efficacy of the components and a faster effect, since the orodispersible formula has the property of quickly entering the bloodstream. However, given the heterogeneity of the studies reviewed here and methodological concerns, further controlled clinical trials are needed to validate the findings and to explore the full range of potential benefits or adverse effects of these compounds.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

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