International Journal of Innovative Technologies in Social Science

e-ISSN: 2544-9435

Operating Publisher
SciFormat Publishing Inc.
ISNI: 0000 0005 1449 8214

2734 17 Avenue SW,
Calgary, Alberta, T3E0A7,
Canada
+15878858911
editorial-office@sciformat.ca

ARTICLE TITLE	FOLATE AND MTHFR POLYMORPHISMS IN SLEEP, ANXIETY, AND DEPRESSION, AND VITAMIN B12 IN ANXIETY AND DEPRESSION: A REVIEW OF CURRENT EVIDENCE
DOI	https://doi.org/10.31435/ijitss.2(50).2026.5541
RECEIVED	17 February 2026
ACCEPTED	23 May 2026
PUBLISHED	01 June 2026
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FOLATE AND MTHFR POLYMORPHISMS IN SLEEP, ANXIETY, AND DEPRESSION, AND VITAMIN B12 IN ANXIETY AND DEPRESSION: A REVIEW OF CURRENT EVIDENCE

Aleksandra Sucholbiak *(Corresponding Author, Email: al.sucholbiak@gmail.com)*
Infant Jesus Clinical Hospital, Warsaw, Poland
ORCID ID: 0009-0001-2931-157X

Oskar Mikołajczyk
Infant Jesus Clinical Hospital, Warsaw, Poland
ORCID ID: 0009-0006-8470-6845

Maja Podolak
Mazovian Bródnowski Hospital, Warsaw, Poland
ORCID ID: 0009-0005-5409-8511

Radosław Jan Walkowski
Central Clinical Hospital of UCC Medical University of Warsaw, Warsaw, Poland
ORCID ID: 0009-0006-9933-6437

Irmina Grygutis
Nicolaus Copernicus Hospital in Gdańsk, Gdańsk, Poland
ORCID ID: 0009-0008-2863-3471

Urszula Jarzęcka
Medical Centre for Postgraduate Education, Professor W. Orlowski Memorial Hospital, Warsaw, Poland
ORCID ID: 0009-0009-1160-8541

Kornelia Julia Fimiarz
Nicolaus Copernicus Hospital in Gdańsk, Gdańsk, Poland
ORCID ID: 0009-0004-2867-9980

Sonia Pawełkiewicz
Nicolaus Copernicus Hospital in Gdańsk, Gdańsk, Poland
ORCID ID: 0009-0000-4087-5766

Kinga Łysak
Nicolaus Copernicus Hospital in Gdańsk, Gdańsk, Poland
ORCID ID: 0009-0008-5002-4365

ABSTRACT

One-carbon metabolism supports neuropsychiatric function by providing the substrates needed for monoamine neurotransmitter synthesis. In this review, we summarize the available evidence on selected B vitamins and methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms in relation to sleep outcomes, depression, and anxiety in adults. A PubMed search was conducted, through which 27 studies relevant to the review were identified. Observational studies suggest that the relationship between serum folate and sleep is complex, with findings remaining inconsistent across the literature. Nevertheless, improvements in sleep disturbance outcomes following L-methylfolate supplementation among individuals with MTHFR C677T/A1298C polymorphisms provide preliminary support for a link between vitamin B9 metabolism and sleep regulation. In the context of depression and anxiety, observational studies also yielded mixed results. However, interventional studies suggest that supplementation with L-methylfolate at doses exceeding 7.5 mg/day may enhance the response to antidepressant treatment in patients with depression. Furthermore, among patients with major depressive disorder and low-normal vitamin B12 levels, adjunctive therapy with weekly intramuscular injections of 1,000 µg vitamin B12 may improve treatment response. Evidence regarding the role of MTHFR polymorphisms in anxiety and depression remains inconclusive, although some studies suggest that reduced MTHFR activity may be associated with an increased risk of these conditions and that supplementation with a reduced B vitamins and micronutrient complex may offer clinical benefit. In conclusion, vitamin B status and MTHFR polymorphisms may be related to both sleep and anxiety/depressive symptoms. However, because the current evidence remains inconsistent, further large-scale longitudinal studies and well-designed randomized controlled trials are needed.

KEYWORDS

folate, MTHFR, Sleep, Anxiety, Depression, Vitamin B12

CITATION

Aleksandra Suchołbiak, Oskar Mikołajczyk, Maja Podolak, Radosław Jan Walkowski, Irmina Grygutis, Urszula Jarzęcka, Kornelia Julia Fimiarz, Sonia Pawełkiewicz, Kinga Łysak. (2026) Folate and MTHFR Polymorphisms in Sleep, Anxiety, and Depression, and Vitamin B12 in Anxiety and Depression: A Review of Current Evidence. *International Journal of Innovative Technologies in Social Science*. 2(50). doi: 10.31435/ijitss.2(50).2026.5541

1. Introduction

Folate (Vitamin B9) is an essential nutrient for central nervous system function, primarily due to its role in one-carbon metabolism (Cavalcante-Silva et al., 2025). However, since folate itself has no coenzyme activity, it must undergo a series of steps to become its primary metabolically active form, 5-methyltetrahydrofolate (5-MTHF).

The metabolic journey of folate begins with its stepwise reduction into the active coenzyme form: first to dihydrofolate (DHF) and subsequently to tetrahydrofolate (THF), both steps are catalyzed by the enzyme dihydrofolate reductase (DHFR) (He & Li, 2023; Froese, Fowler, & Baumgartner, 2019). Then THF is catalyzed by serine hydroxy methyltransferase (SHMT1) to produce 5,10-methylenetetrahydrofolate (5,10-MTHF) (Gao et al., 2018). Methylenetetrahydrofolate reductase (MTHFR) plays a key role in folate metabolism by catalyzing the conversion of 5,10-MTHF to 5-MTHF, providing the substrate for methionine synthase (MS) and subsequently methionine cycle (Froese et al. 2019). Utilizing cobalamin (Vitamin B12) as a cofactor, MS produces methionine, which is then converted in methionine cycle into S-adenosylmethionine (SAMe) (Araszkiewicz et al., 2025; Botto & Yang, 2000; Cavalcante-Silva et al., 2025), the principal methyl donor in numerous brain methylation reactions involved in the metabolism of neurotransmitters such as serotonin, dopamine, and norepinephrine (Bottiglieri, 2002; Cavalcante-Silva et al., 2025; Gao et al., 2018).

Dysregulation of the folate and methionine cycles has been increasingly implicated in the pathogenesis of various neuropsychiatric diseases (Gao et al., 2018). While these metabolic failures are frequently driven by nutritional deficiencies in folate or vitamin B12 (Gao et al., 2018), genetic predispositions, specifically MTHFR polymorphisms such as C677T and A1298C, represent another critical factor that can compromise these pathways. These variants are clinically significant due to their association with reduced enzyme activity

(Araszkiewicz et al., 2025; Leclerc & Rozen, 2008; Zhang et al., 2022). Namely, in the presence of 1298C allele, enzyme activity is reduced by 30%. In comparison, the 677T allele reduces activity by approximately 55% (Leclerc & Rozen, 2008). The metabolic disruptions to the folate and methionine cycles caused by these polymorphisms reduce the availability of methyl donors, which may in turn alter the synthesis of dopamine, norepinephrine, and serotonin, as well as the subsequent production of melatonin (Carmon, Amato, Patel, & Finks, 2024).

This review aims to synthesize the existing evidence regarding the associations of folate status and MTHFR polymorphisms with sleep outcomes. Furthermore, it summarizes the available evidence on B-vitamins (specifically B9 and B12) and MTHFR polymorphisms in relation to anxiety and/or depression.

2. Methodology

The PubMed database search was conducted up to 1.10.2025 using the following search term combinations: “folic acid” OR “folate’’ OR “Vitamin B9” OR “L-methylfolate” OR “MTHFR” AND “sleep”, and: “folic acid” OR “folate’’ OR “Vitamin B9” OR “L-methylfolate” OR “MTHFR” OR “cobalamin” OR “Vitamin B12” AND “depression” OR “anxiety” OR “major depressive disorder” OR “generalized anxiety disorder”. Additionally, articles referenced in the initially identified publications were screened. For the results section only studies published in English were considered. We identified 27 studies deemed relevant for inclusion. All analyzed studies were published from 2000 to 2025. From publications on sleep, only information on MTHFR polymorphisms and folate status was extracted and included in this review.

3. Results

3.1. Folate status and Sleep

Cross-sectional evidence on the association between folate status and sleep disturbances is mixed (see Table 1). Beydoun et al. (2014) reported that higher serum folate levels were associated with fewer sleep disturbances. However, findings from a more recent and larger cross-sectional study by Tu et al. (2025) suggest a more complex, U-shaped relationship between folate intake, serum folate, erythrocyte folate, and sleep disturbances. In this study, both insufficient and excessive folate levels were associated with increased risk of sleep disturbances, while moderate folate status around 439 mcg/day intake, 35 nmol/L serum, and 886 nmol/L erythrocyte folate was linked to the lowest reported risk (Tu et al., 2025).

In another large cross-sectional study, Dong and Luo (2024) identified a nonlinear association between serum folate and sleep duration. While higher folate levels initially correlated with longer sleep, this benefit reached a plateau at approximately 32 nmol/L, suggesting a physiological saturation point rather than an unlimited linear benefit (Dong & Luo, 2024). Conversely, other research has failed to establish a clear link between folate status and sleep duration (Beydoun et al., 2014). Furthermore, Beydoun et al. (2014) found no significant associations between serum folate levels and sleepiness, insomnia, and sleep apnea (see Table 1). Similarly, Cavalcante-Silva et al. (2025) reported that folate deficiency was not significantly associated with insomnia severity though they noted that the low prevalence of deficiency in their sample may have limited the study's statistical power.

While Beydoun et al. (2014) reported largely null findings regarding specific sleep disorders, Cheng et al. (2024) found that folate status, measured via whole-blood levels, was a significant predictor of sleep health in the elderly. In their matched case-control study, lower folate was tied to a higher prevalence of sleep apnea and daytime sleepiness, as well as greater difficulty falling asleep (Cheng et al. 2024). Further supporting the link between folate and sleep initiation, An, Xue, and Zhang (2023) found that lower serum folate levels increased the difficulty of falling asleep, while higher levels significantly protected against severe sleep-onset problems

Table 1. Summary of studies on folate status and sleep outcomes.

Study	Design	Sample	Assessed sleep outcomes	Significant findings on folate and sleep outcomes
Beydoun et al. (2014)	Cross-sectional	N=2,459 (NHANES 2005–2006);	- Sleep duration - Factor 1: Poor sleep-related daytime dysfunction - Factor 2: Sleepiness - Factor 3: Sleep disturbance - Overall sleep disorder - Insomnia - Sleep apnea - Restless leg syndrome - Other sleep disorders	↑ Serum folate was associated with ↓ Factor 3 ('Sleep disturbance').
An et al. (2023)	Cross-sectional	N=8,926 (NHANES 2005–2008);	Severe difficulty falling asleep	↑ Serum folate was associated with ↓ odds of severe difficulty falling asleep.
Cheng et al. (2024)	Matched case-control	N=248 (Tianjin Elderly Nutrition and Cognition);	Sleep disorders: - Sleep onset time > 30 minutes - Excessive daytime sleepiness - Sleep apnea	↓ Whole-blood folate was associated with ↑ odds of sleep disorders.
Dong and Luo (2024)	Cross-sectional	N=14,072 (NHANES 2005–2010);	Sleep duration	↑ Serum folate was associated with ↑ sleep duration, with an inverted L-shaped relationship.
Tu et al. (2025)	Cross-sectional	N=20,200 (NHANES 2007–2016);	Sleep disturbances	U-shaped association between serum folate status and sleep disturbances, with the lowest risk at moderate serum folate levels.
Cavalcante-Silva et al. (2025)	Longitudinal	Baseline: n=1,042 Follow-up: n=712 (EPISONO)	- Insomnia - Chronotype - Sleep architecture - Sleep apnea	Greater morningness was significantly associated with ↓ odds of folate deficiency and ↓ insomnia severity. *Folate deficiency was not significantly associated with insomnia severity. **Association between folate and sleep apnea not reported

EPSINO- Sao Paulo Epidemiologic Sleep Study; **NHANES**- National Health and Nutrition Examination Survey;

Table 2. Summary of selected sleep outcomes and assessment tools.

Study	Selected sleep outcome	Tools used to asses sleep outcomes
Beydoun et al. (2014)	Sleep disturbance	5-point Likert-scale sleep items from the NHANES CAPI sleep questionnaire
Beydoun et al. (2014)	Sleep duration	Self-reported as hours slept per night from the NHANES CAPI sleep questionnaire
Beydoun et al. (2014)	Insomnia	Self-reported from the NHANES CAPI sleep questionnaire
Beydoun et al. (2014)	Sleepiness	5-point Likert-scale sleep items from the NHANES CAPI sleep questionnaire
Beydoun et al. (2014)	Sleep apnea	Self-reported from the NHANES CAPI sleep questionnaire
An et al. (2023)	Severe difficulty falling asleep	Self-reported NHANES questionnaire item: "How often do you have difficulty falling asleep?"
Cheng et al. (2024)	Sleep disorders: sleep onset time > 30 minutes, excessive daytime sleepiness, apnea during nighttime sleep	Questionnaire-based assessment using DSM-IV criteria and EPS for daytime sleepiness
Dong and Luo (2024)	Sleep duration	Self-reported as hours slept per night using NHANES questionnaire item: "How much sleep do you usually get at night on weekdays or workdays?"
Tu et al. (2025)	Sleep disturbances	NHANES Sleep Questionnaire items: - "Have you ever been told by a doctor or other health professional that you have trouble sleeping?" - "Have you ever been told by a doctor that you have a sleep disorder?"
Cavalcante-Silva et al. (2025)	Insomnia severity	ISI
Cavalcante-Silva et al. (2025)	Sleep architecture and disorders	PSG- Polysomnography
CAPI- Computer-Assisted Personal Interview; **EPS**- Epworth Sleepiness Scale; **ISI**- Insomnia Severity Index; **NHANES**- National Health and Nutrition Examination Survey; **PROMIS-43**- Patient-Reported Outcomes Measurement Information System - 43 Profile; **PSG**- polysomnography;		

3.2. MTHFR gene polymorphisms and Sleep

We found only one paper investigating correlation between MTHFR gene polymorphisms and sleep outcomes. In a small retrospective cohort study by Carmon, Amato, Patel, and Finks (2024), daily supplementation with 5 mg L-methylfolate, which is a biologically active form of folate, led to significant improvements in sleep disturbance over eight weeks in participants with MTHFR C677T and/or A1298C polymorphisms. Enzymatic activity was classified according to the phenotype. Individuals with the 677T heterozygous genotype or the 1298CC homozygous genotype, both associated with >50% enzymatic activity, were categorized as having 'intermediate activity', whereas 677T/1298C compound heterozygotes and 677TT homozygotes, both associated with <50% enzymatic activity, were categorized as having 'low activity'. Participants with both low and intermediate phenotypes of reduced MTHFR activity experienced decreased sleep disturbance following L-methylfolate supplementation. However, individuals with polymorphisms associated with intermediate MTHFR enzymatic activity presented with greater sleep disturbance at baseline and exhibited greater improvement in symptoms from baseline than those with low enzymatic activity. Nevertheless, the authors suggest that this unexpected finding may be attributable to the small sample size which included only 10 participants (Carmon et al., 2024).

Table 3. Distribution of participants according to estimated MTHFR enzymatic activity in Carmon et al. (2024).

MTHFR Enzymatic Activity	Sample
Low Activity (< 50%)	N = 6
Intermediate Activity (> 50%)	N = 4
MTHFR- methylenetetrahydrofolate reductase; **N**- number	

3.3. Vitamin B status and MTHFR polymorphisms in anxiety and depression

Table 4. Characteristics of studies examining folate, vitamin B12, MTHFR polymorphisms, and anxiety/depression outcomes.

Study (Year)	Study design	Sample	Age
Cavalcante-Silva et al. (2025)	Longitudinal	EPISONO cohort; Baseline: n =1,042 (2007); 8-year follow-up: n = 712 (2015)	Mean age 42.34 ± 14.40 years (2007); 50.2 ± 13.4 years (2015);
Moore et al. (2019)	Cross-sectional	Community-dwelling older adults from the TUDA; n=5,186	≥60 years; Mean age: men- 73.4 years; women- 74.3 years;
Eszlari et al. (2021)	Observational GWAS	UK Biobank (primary); n = 72,621	40–69 years
		NewMood (validation); n = 1,746	18–60 years
Kendrick et al. (2008)	Longitudinal	Southampton Women's Survey; Baseline: 7,020 women; (5,051 with folate data) 2-year follow-up: 2,732;	20–34 years
Møllehave et al. (2017)	Mendelian Randomization	Health2006: n = 3,176	Health2006: 49.4 (SD 13.0) years
		Inter99: n = 1,752	Inter99: 45.6 (SD 8.1) years
Bjelland et al. (2003)	Cross-sectional	Hordaland Homocysteine Study; n = 5,948	46–49 and 70–74 years
Saraswathy et al. (2019)	Cross-sectional	Bhil indigenous population of Rajasthan, India; n = 303	25–65 years
Lok et al. (2014)	Case-control sub-study (DELTA RCT)	Recurrent MDD patients: Start: n = 172 End: n = 137	Mean age: 46.4 ± 9.5 years
		Healthy controls: n = 73	Mean age: 44.7 ± 9.4 years
Papakostas et al. (2004a)	Open-label trial followed by RCT	Open label: n = 386	Not reported in the paper.
		RCT: nonresponders n = 101 (included in biomarker analysis n = 55)	Mean age of biomarker analysis subgroup: 41.7 ± 10.6 years Mean age of those without biomarker data: 41.4 ± 10.8 years
Papakostas et al. (2004b)	Prospective continuation-phase observational, nested within an acute open fluoxetine trial	Patients with remitted MDD; n = 71	Mean age: 40.2 ± 11.1 years
Alpert et al. (2003)	Nested Observational within Multicenter RCT	Geriatric patients with MDD; n = 22; (Sertraline group: n = 12; Nortriptyline group: n = 10;)	Mean age in sertraline group: 69.1 years Mean age in nortriptyline group: 65.1 years

Study (Year)	Study design	Sample	Age
Carboni et al. (2021)	Two RCTs	Patients with MDD; Start (patients with analyzed biomarkers): Paroxetine study n = 489; Venlafaxine study n = 392 End: Paroxetine study n = 340; Venlafaxine study n = 251	Mean age in paroxetine study: 42.9 ± 11.19 years Mean age in venlafaxine study: 42.6 ± 11.67 years
Syed et al. (2013)	RCT	Patients with depressive symptoms and low-normal B12; Start/end: n= 73; (Study group: n = 34; Control group: n = 39;)	Mean age in study group: 37.68 ± 13.38 years Mean age in control group: 36.56 ± 12.28 years
Mech and Farah (2016)	RCT	Patients positive for MTHFR C677T or A1298C polymorphism and with MDD ± ADHD and/or GAD; Start: n = 330 End: n = 282 (Study group: 159 Control group: 123)	18–67 years; Mean age: 32 years
Papakostas et al. (2012)	Two RCTs, parallel-sequential trials	Patients with SSRI-resistant MDD; Trial 1: Start: n = 148, End: n = 119 Trial 2: Start: n = 75 End: n = 61	18–65 years; Mean age (trial 1): 47.9 years Mean age (trial 2): 48.4 years (trial 2)
Papakostas et al. (2014)	RCT using SPCD	Patients with SSRI-resistant MDD; Start: n = 75 End: n = 61	18–65 years
Bedson et al. (2014)	RCT	Patients with moderate–severe depression; Start: n = 475 End: n = 440	19–81 years
Venkatasubramanian et al. (2013)	RCT, comparative study	Female outpatients with moderate or severe depressive episodes; Start: n = 42; End: n = 30 (Group I: Start: n = 23; End: n = 15; Group II: Start: n=19; End: n = 15)	16–45 years; Mean age in Group I: 31.7 ± 7.2 years Mean age in Group II: 34.6 ± 5.7 years
Resler et al. (2008)	RCT	Depressed outpatients; Start: n = 27 (Fluoxetine + folic acid group n = 14; Fluoxetine + placebo group n = 13); End: total completers n = 20 Healthy controls, n = 15	Study group: 21–58 years, mean age 35.04 ± 2.63 years; Controls: 26–49 years, mean age 34.13 ± 2.05 years
Coppen and Bailey (2000)	RCT	Patients with MDD; Start n = 127 (Study group: Start n = 62; End n = 109 Control group: Start: n = 65; End: n = 58)	Mean age in study group: 41.9 years Mean age in control group: 44.3 years
Almeida et al. (2014)	RCT	Patients with MDD; Start: n =153 (Study group: n = 77; Control group: n = 76) End: n = 128	50–85 years

ADHD- attention deficit hyperactivity disorder; CBT- cognitive-behavioral therapy; **GWAS**- Genome-wide association study; **MDD**- major depressive disorder; **RCT**- randomized clinical trial; **SPCD**- sequential parallel comparison design;

Cavalcante-Silva et al. (2025) reported that in their longitudinal study, vitamin B12 and folate concentrations below cutoff values were associated with greater anxiety severity over an 8-year period, indicating a longitudinal relationship between poor vitamin status and anxiety symptoms.

However, in the cross-sectional study by Moore et al. (2019), the anxiety risk was not significantly correlated with low red blood cell (RBC) folate levels, and the correlation was relevant only for low plasma vitamin B6. Low folate, vitamin B6, and vitamin B2 were associated with a greater risk of depression. The authors estimated that having RBC folate concentrations in the lowest 20% was associated with an increased risk of depression by almost 80%. However, low serum total vitamin B12 did not show significant correlation to depression. In addition, consuming at least one portion per day of B-vitamin fortified food was linked to lower depression risk (Moore et al., 2019).

While worry and rumination both contribute to the pathogenesis of depression and anxiety, they appear to respond differently to folate status. Eszlari et al. (2021) demonstrated that optimal folate intake provides a protective effect against worry, the future-oriented form of perseverative negative thinking, but not against rumination (past-oriented thinking) (Eszlari et al., 2021).

Kendrick et al. (2008) found no association between low folate and depressive symptoms. Lower RBC folate was associated with baseline anxiety and depression but did not predict new depressive episodes over 2 years (Kendrick et al., 2008).

Møllehave et al. (2017) reported no evidence of a causal association between serum folate or vitamin B12 levels and risk of depression or anxiety. Although higher folate was associated with slightly increased odds of depression/anxiety in some models, the findings were inconsistent. Vitamin B12 was not consistently associated with either depression or anxiety (Møllehave et al., 2017).

Bjelland, Tell, Vollset, Refsum, and Ueland (2003) found that hyperhomocysteinemia and the MTHFR 677T/T (homozygotic) genotype were significantly associated with depression without comorbid anxiety. Plasma folate was inversely associated with depression, however, only among middle-aged women. Neither serum levels of folate nor vitamin B12 showed a significant relationship with anxiety (Bjelland et al., 2003).

Similarly, Saraswathy, Ansari, Kaur, Joshi, and Chandel (2019) found no direct association between vitamin B12 or serum folate levels with depression or generalized anxiety disorder (GAD). However, hyperhomocysteinemia was strongly associated with both conditions, corresponding to more than a threefold increased risk of depression and more than a sixfold increased risk of GAD after adjustment. The study also reported a significant negative correlation between vitamin B12 levels and homocysteine in depressed and anxious individuals. The same study also suggested that the T allele of MTHFR C677T gene polymorphism may be associated with increased risk of depression/anxiety. Nevertheless, in this study, the association was not statistically significant (Saraswathy et al., 2019).

Lok et al. (2014) reported that participants with major depressive disorder (MDD) had lower serum folate compared to controls. However, the difference was resolved after correction for demographics and became insignificant. Furthermore, the study reported that participants who were depressed during sampling had higher homocysteine and lower vitamin B6 levels than those in remission. The MTHFR C677T polymorphism was not significantly associated with recurrent MDD, although the TT genotype was more frequent in patients with MDD than controls (Lok et al., 2014).

Papakostas et al. (2004a) found that low serum folate was associated with poorer treatment response in fluoxetine-resistant MDD, with response rates of 7.1% in patients with low folate, compared with 44.7% in those with normal folate levels. In the same study, vitamin B12 and homocysteine were not significantly associated with treatment response (Papakostas et al., 2004a). In a later study, Papakostas et al. (2004b) reported that low serum folate at baseline was significantly associated with an increased risk of depressive relapse during continuation treatment with fluoxetine. Relapse was observed in 42.9% of patients with low folate levels, compared with 3.1%–3.2% of patients with normal folate levels. Similarly to the previous study, low vitamin B12 and elevated homocysteine were not significantly associated with relapse (Papakostas et al., 2004b).

In another study, Alpert, Silva, and Pouget (2003) reported that both sertraline and nortriptyline improved depressive symptoms. Although higher baseline RBC folate predicted greater improvement of both treatments, folate was a more efficient predictor in the sertraline group (Alpert et al., 2003).

In contrast, the large RCT by Carboni et al. (2021) suggested that serum homocysteine, vitamin B12, folate and RBC folate levels were not consistently associated with either MDD severity or treatment response to paroxetine or venlafaxine. In week 10 analysis of changes in Hamilton Depression Rating Scale (HAM-D)

scores, baseline biomarkers showed no significant associations overall, except for RBC folate, which was significant in the overall model of the venlafaxine study (p = 0.039) (Carboni et al., 2021).

Interestingly, supplementation studies showed mixed, though generally favorable, effects of B vitamins supplementation on depressive symptoms, particularly when these vitamins were used in active forms and as adjuncts to antidepressant therapy. Specifically, Syed et al. (2013) reported that weekly intramuscular injections of 1,000 µg vitamin B12 over 6-weeks, when used as an adjunct to SSRIs or tricyclic antidepressants (TCA), significantly enhanced treatment response in adults with MDD and low-normal B12 levels (190-300 pg/ml). Interestingly, at the three-month follow-up, 100% of participants in the treatment group showed at least a 20% reduction in HAM-D score, while in the control group 69% of participants reached the same decrease in symptom severity ($p < 0.001$). The difference remained significant even after adjustment for baseline HAM-D score (Syed et al., 2013). Furthermore, according to Mech and Farah (2016), 42% of the participants with MTHFR C677T or A1298C polymorphisms and MDD, who received a complex containing reduced B-vitamins and micronutrients (see Table 6), including 3 forms of vitamin B9 (1 mg of citrated folic acid, 2.5 mg of folinic acid, and 7 mg of l-methylfolate magnesium) and 50 µg/day adenosylcobalamin, achieved full remission after 8 weeks of treatment.

Consistent with these findings, two RCTs by Papakostas et al. (2012) demonstrated that adjunctive treatment with L-methylfolate 15 mg/day significantly improved depressive symptoms in adults with SSRI-resistant MDD. However, the authors also noted that a lower dose of L-methylfolate (7.5 mg/day) was insufficient to produce therapeutic benefits (Papakostas et al., 2012). Moreover, in a subsequent RCT Papakostas et al. (2014) found that L-methylfolate 15 mg/day was superior to placebo in SSRI-resistant MDD patients with biomarkers of inflammation, oxidative stress, or impaired metabolism.

The evidence for folic acid as an antidepressant adjunct is marked by a disconnect between small pilot studies and large-scale trials. Several small RCTs reported that even the supplementation of inactive form of folate (500 µg/day-10 mg/day), combined with fluoxetine in the dose of 20 mg/day, might be associated with improvements in depressive symptoms (Coppen & Bailey, 2000; Resler et al., 2008). However, Coppen and Bailey (2000) observed that the antidepressant benefit of folic acid augmentation (500 µg/day) was exclusive to female participants. The trial by Venkatasubramanian, Kumar, and Pandey (2013) shifted the focus from absolute efficacy to dose optimization. In a dose-comparison design, they evaluated 2 mg versus 5 mg of folic acid, reporting that the higher dose yielded significantly better outcomes (Venkatasubramanian et al., 2013).

Nevertheless, the more recent and larger RCT by Bedson et al. (2014), which included 475 adults with moderate-to-severe depression, found no significant benefit of 5 mg/day folic acid supplementation as an adjunct to various antidepressants (see Table 6). Furthermore, although Almeida et al. (2014) reported that adjunctive vitamin B complex supplementation, including folic acid, vitamin B6, and vitamin B12, added to citalopram (see Table 6) did not increase the 12-week efficacy of antidepressant treatment, they reported that the supplementation enhanced and sustained antidepressant response over one year.

Table 5. Summary of non-RCTs investigating folate, vitamin B12, MTHFR polymorphisms, and anxiety/depression outcomes.

Study (Year)	Assessment tools	Findings on Anxiety / Depression
Cavalcante-Silva et al. (2025)	BAI- anxiety	**Anxiety**: ↓ Vitamin B12 ($p = 0.044$) and folate ($p = 0.046$) levels were associated with ↑ anxiety severity.
Moore et al. (2019)	CES-D; HADS;	**Depression**: ↓ RBC folate (OR 1.79; 95% CI 1.23-2.61), plasma vitamin B6 (OR 1.45, 95% CI 1.01-2.06), and vitamin B2 (OR 1.56, 95% CI 1.10-2.00) were linked to ↑ depression risk. Total serum vitamin B12 was not correlated with depression risk. Fortified foods were linked to ↓ depression risk (OR 0.54, 95% CI 0.41-0.70). **Anxiety:** Only ↓ plasma vitamin B6 was linked to ↑ anxiety risk (OR 1.73, 95% CI 1.07-2.81).

Study (Year)	Assessment tools	Findings on Anxiety / Depression
Eszlari et al. (2021)	UK Biobank: - EPI: rumination- "Do you worry too long after an embarrassing experience?" and worry- "Are you a worrier?"; - current depression from a 4-item symptom sum; - lifetime depression by verbal interview NewMood: - BFI-44- neuroticism; - RRS; - BSI- depressive symptoms;	**Depression/anxiety:** Optimal folate intake was associated with ↓ worry, but not rumination (statistically significant, but the exact p-value not reported).
Kendrick et al. (2008)	- GHQ-12 at baseline for anxiety/depression symptoms; - GP medical records over 2 years for incident depressive symptoms	**Depression/anxiety:** ↓ RBC folate was not associated with incident depressive symptoms over 2 years. ↓ RBC folate was associated with baseline case-level symptoms of anxiety/depression (p = 0.05).
Møllehave et al. (2017)	- SCL-90-R, Depression (13 items) and Anxiety (10 items); - self-reported doctor-diagnosed depression/anxiety	**Depression/anxiety:** No significant association between serum folate or vitamin B12 levels (or genetic scores for these vitamins) and risk of depression or anxiety.
Bjelland et al. (2003)	- HADS	**Depression**: ↑ Hcy (OR 1.90; 95% CI 1.11-3.25) and the MTHFR 677T/T genotype (OR 1.69; 95% CI 1.09-2.62) were associated with ↑ depression risk without comorbid anxiety. ↓ Plasma folate was associated with ↑ depression only in middle-aged women (p = 0.02). ↓ Plasma folate and vitamin B12 were not significant overall for depression risk. **Anxiety**: None of the assessed parameters showed a significant relationship with anxiety.
Saraswathy et al. (2019)	- PHQ-9- depression; - GAD-7;	**Depression/anxiety:** No direct association of vitamin B12 or serum folate with depression/GAD. ↑ Hcy increased the risk of depression (p = 0.07) and GAD (p = 0.05). ↓ Vitamin B12 was associated with ↑ homocysteine in depressed/anxious participants (p = 0.01). MTHFR C677T: T allele showed a possible ↑ risk for depression/anxiety, but the association was not statistically significant.
Lok et al. (2014)	- SCID-I; - HDRS-17 (HAM-D); - TIC-P for antidepressant use;	**Depression**: Participants with MDD had ↓ serum folate (p = 0.025) than controls, a difference that resolved after correction for demographics (p = 0.223) Participants with MDD had ↑ homocysteine (p = 0.016) and ↓ vitamin B6 (p = 0.038) than those in remission. MTHFR C677T: Not significantly associated with recurrent MDD, although the TT genotype was ↑ in patients than controls.

Study (Year)	Assessment tools	Findings on Anxiety / Depression
Papakostas et al. (2004a)	- SCID-P for diagnosis of MDD; - HAM-D-31; - HAM-D-17;	**Depression**: ↓ Serum folate associated with ↓ depression treatment response in fluoxetine-resistant MDD ($p = 0.04$). Vitamin B12 and homocysteine were not significantly associated with response.
Papakostas et al. (2004b)	- SCID-III-R for diagnosis of MDD; - HAM-D-17 for remission and relapse assessment;	**Depression**: ↓ Serum folate at baseline was significantly associated with ↑ risk of depressive relapse during continuation treatment with fluoxetine ($p = 0.004$) ↓ Vitamin B12 and ↑ homocysteine were not significantly associated with relapse of depression.
Alpert et al. (2003)	- HAM-D-24; - POMS-D;	**Depression**: ↑ Baseline folate predicted ↑ treatment response in the sertraline group ($p = 0.10$). ↑ Baseline folate predicted ↑ POMS-D outcomes with sertraline ($p = 0.05$), with no significant association in the nortriptyline group. HAM-D showed a nonsignificant trend of improvement with sertraline, no association in the nortriptyline group.
1-C-cycle- one-carbon cycle; **BAI**- Beck Anxiety Inventory; **BFI-44**- Big Five Inventory–44; **BSI**- Brief Symptom Inventory; **CES-D**- Centre for Epidemiological Studies Depression Scale; **EPI**- Eysenck Personality Inventory; **GAD**- Generalized Anxiety Disorder; **GAD-7**- Generalized Anxiety Disorder scale-7; **GHQ-12**- 12-item General Health Questionnaire; **GP**- general practitioner; **HADS**- Hospital Anxiety and Depression Scale; **HAM-D**- Hamilton Depression Rating Scale; **Hcy**- Homocysteine; **HDRS-17**- Hamilton Depression Rating Scale, 17-item; **PHQ-9**- Patient Health Questionnaire-9; **POMS-D**- Profile of Mood States – Depression subscale; **RBC**- red blood cell; **RSS**- Ruminative Response Scale; **SCID-I**- Structured Clinical Interview for DSM-IV; **SCID-III-R**- Structured Clinical Interview for DSM-III-R; **SCID-P**- Structured Clinical Interview for DSM-III-R, Patient Edition; **TIC-P**- Trimbos/IMTA Self Report Questionnaire for Costs Associated with Psychiatric Illness;		

Table 6. Summary of RCTs investigating B vitamins and depression.

Study (Year)	Intervention group	Control group	Main Findings
Carboni et al. (2021)	Paroxetine (active comparator) group and GSK372475 group. Venlafaxine (active comparator) group and GSK372475 group.	Placebo	Vitamin B12, homocysteine erythrocyte folate, and serum folate did not show consistent associations with MDD severity or antidepressant response. Baseline biomarkers in the analysis of change in HAM-D at week 10, significant only for erythrocyte folate ($p=0.039$) in overall model in venlafaxine study.
Syed et al. (2013)	Vitamin B12 (1,000 µg IM weekly × 6 weeks) + SSRI (dose equivalent of fluoxetine 20-40 mg/day) or TCA (dose equivalent of imipramine 100-250 mg/day)	Antidepressant alone (SSRI- dose equivalent of fluoxetine 20-40 mg/day or TCA- dose equivalent of imipramine 100-250 mg/day)	↓ HAM-D ($p < 0.01$)

Study (Year)	Intervention group	Control group	Main Findings
Mech and Farah (2016)	Reduced B vitamins / micronutrient combination: 25 µg/day B1, 25 µg/day B2, 25 µg/day B6, 10.5 mg/day B9, 50 µg/day B12, 25 µg/day NADH, 500 µg/day TMG, 1.5 mg/day AminoFerr®, 24 mg/day vit.C-Mg, 1 mg/day vit.C-Zn, 1 mg/day LTAMS, 20 mg/day FS-ω3;	Placebo	↓ Serum Hcy ($p < 0.001$); ↓ MADRS score ($p < 0.05$) with 42% in complete MDD remission
Papakostas et al. (2012)	L-methylfolate 7.5 mg/day or 15 mg/day + SSRI at adequate dosages (defined as ≥20 mg/day of fluoxetine, citalopram, or paroxetine; ≥10 mg/day of escitalopram; or ≥50 mg/day of sertraline) Trial 1: 7.5 mg/day for 30 days then 15 mg/day for 30 days Trial 2 tested 15 mg/day during both 30-day periods	Placebo + SSRI at adequate dosages (defined as ≥20 mg/day of fluoxetine, citalopram, or paroxetine; ≥10 mg/day of escitalopram; or ≥50 mg/day of sertraline)	↓ HAM-D and QIDS-SR scores in the L-methylfolate group vs placebo; significant improvement at 15 mg/day ($p < 0.05$), but not at 7.5 mg/day
Papakostas et al. (2014)	L-methylfolate 15 mg/day + SSRI at adequate dosages (defined as ≥20 mg/day of fluoxetine, citalopram, or paroxetine, ≥10 mg/day of escitalopram, ≥50 mg/day of sertraline); In the SPCD design, one arm received l-methylfolate in both phases, and another received placebo first then l-methylfolate.	Placebo + SSRI at adequate dosages (defined as ≥20 mg/day of fluoxetine, citalopram, or paroxetine, ≥10 mg/day of escitalopram, ≥50 mg/day of sertraline);	↓ HDRS-28 ($p = 0.017$ overall- pooled phases) The results were stronger in patients with metabolic/inflammatory markers (higher hsCRP, higher 4-HNE, lower SAM/SAH ratio, or BMI ≥ 30, and in several genetic marker subgroups).
Bedson et al. (2014)	Folic acid 5 mg/day + antidepressant in therapeutic dosages-equivalent to SSRI ≥20 mg/day or TCA ≥150 mg/day	Placebo + antidepressant in therapeutic dosages-equivalent to SSRI ≥20 mg/day or TCA ≥150 mg/day	↑ Serum folate, RBC folate ($p < 0.001$) ↓ Hcy ($p < 0.05$) ↓ BDI-II score and ↓ MADRS ($p > 0.05$)
Venkatasubramanian et al. (2013)	Group II: fluoxetine 20 mg/day + folic acid 5 mg/day	Group I: fluoxetine 20 mg/day + folic acid 1.5 mg/day	↓ HDRS ($p = 0.01$) ↓ BDI ($p = 0.01$)

Study (Year)	Intervention group	Control group	Main Findings
Resler et al. (2008)	Fluoxetine 20 mg/day + folic acid 10 mg/day	Fluoxetine 20 mg/day + placebo Additional healthy controls: 15 healthy subjects for comparison only	↓ HDRS between-group p value ($p = 0.04$) ↓ Serum Hcy ($p = 0.02$) Vitamin B12 did not change significantly. Lymphocyte serotonin decreased after fluoxetine in both groups, and 5-HIAA was ↓ in the folate group.
Coppen and Bailey (2000)	Fluoxetine 20 mg/day + folic acid 500 µg/day	Fluoxetine 20 mg/day + placebo	↓ HRS (overall $p < 0.05$; in women $p < 0.005$; in men not significant)
Almeida et al. (2014)	2 mg/day folic acid, 25 mg/day vitamin B6, 0.5 mg/day B12 + 20–40 mg/day citalopram	Placebo + 20–40 mg citalopram	↑ RBC folate, serum B12 and ↓ serum Hcy ($p < 0.05$); ↓ MADRS score ($p > 0.05$) ↓ Relapse risk at week 12 remission (OR 0.33, 95% CI 0.12–0.94)

4-HNE- 4-hydroxy-2-nonenal; **5-HIAA**- 5-hydroxyindoleacetic acid; **AminoFerr®**- Chelates containing amino acids for use as a dietary and/or nutritional supplement and for iron deficiency); **BDI**- Beck's Depression Inventory; **FS-ω3**- phosphatidylserine-omega-3 conjugated; **HAM-D**- Hamilton Depression Rating Scale; **Hcy**- Homocysteine; **HDRS**- Hamilton Depression Rating Scale; **HRS**- Hamilton Rating Scale for Depression; **hsCRP**- high-sensitivity C-reactive protein; **IM**- intramuscular; **LTAMS**- L-Threonic Acid Magnesium; **MADRS**- Montgomery-Asberg Depression Rating Scale; **MDD**- major depressive disorder; **NADH**- Nicotinamide adenine dinucleotide coenzyme; **QIDS-SR**- Quick Inventory of Depressive Symptomatology/Clinician Rating; **RBC**- red blood cell; **SAM/SAH ratio**- S-adenosylmethionine / S-adenosylhomocysteine ratio; **SPCD**- sequential parallel comparison design; **SSRIs**- Selective Serotonin Reuptake Inhibitors; **TCA**- Tricyclic antidepressant; **TMG**- trimethylglycine; **Vit. C-Mg**- magnesium ascorbate; **Vit. C-Zn**- zinc ascorbate;

4. Discussion

4.1. Folate status, MTHFR polimorphisms and Sleep

The reviewed studies suggest that the relationship between folate and sleep is complex, with findings remaining inconsistent across the literature.

The reported improvement in sleep disturbance following L-methylfolate supplementation, particularly among individuals with MTHFR polymorphisms at C677T and/or A1298C, provides preliminary support for the link between folate metabolism and sleep regulation (Carmon et al., 2024). In addition, the reported U-shaped association between folate levels and the prevalence of sleep disturbances suggests that both insufficient and excessive folate status may be detrimental to sleep, indicating that an optimal physiological range, rather than maximized folate concentrations, are the most beneficial (Tu et al., 2025).

In terms of insomnia, the cross-sectional study by Beydoun et al. (2014) found no significant association between serum folate status and the prevalence of insomnia symptoms. These findings are consistent with the 8-year longitudinal findings of Cavalcante-Silva et al. (2025), who found that folate deficiency did not significantly predict insomnia severity over time. However, the findings of Cavalcante-Silva et al. (2025) should also be interpreted cautiously, as the low prevalence of folate deficiency in EPSINO cohort (3% in 2007 and 7% in 2015) may have limited the statistical power to detect clinically meaningful associations, thereby potentially underestimating the true relationship.

Although Beydoun et al. (2014) did not identify a significant association between folate levels and sleep duration, Dong and Luo (2024) reported that higher serum folate concentrations were associated with longer sleep duration, following an inverted L-shaped pattern. Furthermore, evidence from An et al. (2023) and Cheng et al. (2024) raises the possibility that folate status may also be related to sleep initiation difficulties.

Taken together, these findings suggest that the association between folate and sleep parameters may depend on the specific sleep outcome assessed, as well as on population characteristics and underlying genetic variability.

4.2. B vitamins in anxiety and depression

While some longitudinal evidence suggests that folate deficiency might be associated with greater risk of anxiety (Cavalcante-Silva et al., 2025), other observational studies have not found such correlation (Bjelland et al., 2003; Moore et al., 2019; Møllehave et al., 2017; Saraswathy et al., 2019). Beyond general symptom severity, Eszlari et al. (2021) suggests a more nuanced cognitive impact, where optimal folate intake is specifically linked to reduced worry (future-oriented thinking) but shows no significant relationship with rumination (past-oriented thinking). In depression, the results are also mixed. Some studies suggest that low folate levels may increase risk of depression (Bjelland et al., 2003; Moore et al., 2019), while others do not support the association (Saraswathy et al., 2019; Kendrick et al., 2008).

The studies investigating folate status in relation to antidepressant treatment also present inconsistent findings. While Alpert et al. (2003) reported that higher baseline folate levels might be associated with greater improvement in geriatric patients treated with sertraline, the findings from RCT by Carboni et al. (2021) did not identify consistent, strong associations between folate biomarkers and either the severity of MDD or response to paroxetine and venlafaxine. However, previous evidence suggests that low folate might be associated with higher risk of depressive relapse and poorer treatment response in fluoxetine-resistant MDD (Papakostas et al., 2004b; Papakostas et al., 2004a). Nevertheless, these findings should be interpreted cautiously, as inconsistencies and methodological variability remain.

Although observational studies generally found no correlation between vitamin B12 status and depression (Bjelland et al., 2003; Moore et al., 2019; Møllehave et al., 2017; Saraswathy et al., 2019), interventional study by Syed et al. (2013) supports the potential efficacy of intramuscular vitamin B12 supplementation as an adjunct to SSRIs or TCAs in patients with low normal vitamin B12 levels. These findings suggest that vitamin B12 supplementation may be beneficial, not only in patients with coexisting depression and vitamin B12 deficiency, but also in individuals with vitamin B12 levels in the lower normal range (Syed et al., 2013).

Furthermore, clinical trials suggest that supplementation with active forms of folate, exceeding 7.5 mg/day, can significantly enhance antidepressant response (Papakostas et al., 2012; Papakostas et al., 2014). In contrast, inactive folate supplementation did not enhance short-term antidepressant efficacy of antidepressant treatment in larger RCTs which analyzed its effectiveness as an augmentation to either citalopram (Almeida et al., 2014) or different antidepressants in therapeutic dosages, equivalent to SSRI ≥20 mg/day or TCA ≥150 mg/day (Bedson et al., 2014). These findings may suggest that the active form of vitamin B9 could be essential for clinical efficacy. On the other hand, since several smaller RCTs have reported positive results for fluoxetine supplementation with the inactive form of folate (Coppen & Bailey, 2000; Resler et al., 2008; Venkatasubramanian, Kumar, & Pandey, 2013), the question arises as to whether the inactive form may be beneficial only when combined with fluoxetine. Furthermore, since folic acid supplementation in the study by Almeida et al. (2014) was reported to sustain the response to citalopram for one year, it may be that supplementation, in the case of certain antidepressants, plays a role in maintaining treatment effects rather than in enhancing the initial response to treatment.

4.3. MTHFR polymorphisms in anxiety and depression

Evidence regarding MTHFR polymorphisms and psychiatric outcomes remains inconclusive, characterized by inconsistent findings. Early research by Bjelland et al. (2003) identified the TT genotype as a significant risk factor for depression without comorbid anxiety. Although subsequent studies by Lok et al. (2014) and Saraswathy et al. (2019) did not find a significant primary association, they nonetheless observed a trend toward increased risk for MDD/depression and anxiety among TT carriers. These findings highlight the TT genotype as a potential moderator that may exacerbate the psychiatric consequences of suboptimal folate status.

Furthermore, supplementation with a complex containing reduced B vitamins and micronutrients was reported to produce a statistically significant clinical response in patients with MTHFR C677T and/or A1298C polymorphisms and MDD (Mech and Farah, 2016).

4.4. Future directions

To further clarify the complex interplay between one-carbon metabolism and neuropsychiatric health, future longitudinal research should prioritize stable, long-term biomarkers such as erythrocyte folate or whole-blood concentrations. These measures provide a more stable assessment of tissue stores than the transient serum snapshots used in much of the current literature.

Also, further studies that integrate MTHFR genotype data are warranted. Because MTHFR polymorphisms significantly moderate folate metabolism, accounting for genetic variability is essential for defining clinically relevant deficiency thresholds and understanding the individual risk for sleep and mood disorders related to one-carbon metabolism.

Beyond observational data, there is a critical need for large-scale, RCTs to establish a causal link between folate/vitamin B12 supplementation and improvements in sleep and mood. While longitudinal studies identify associations, high-powered interventional research in diverse populations is necessary to determine the optimal vitamin B formulation, dosage and duration of treatment required to alleviate the neuropsychiatric symptoms.

Furthermore, investigations into drug-nutrient synergies are warranted to clarify whether specific classes of antidepressants derive greater adjunctive benefit from supplementation.

In addition, identifying which patient subgroups, stratified by sex, derive the most significant benefit from folate/vitamin B12 could allow clinicians to move toward targeted support.

By clarifying these variables, future studies may establish evidence-based guidelines that integrate metabolic optimization into the standard of care for neuropsychiatric disorders, potentially improving outcomes for patients who are otherwise resistant to traditional pharmacological interventions.

5. Conclusions

This review examined the potential role of one-carbon metabolism substrates in anxiety, depression, and sleep regulation. The available evidence suggests that the relationship between folate status and sleep is complex and remains inconsistent across studies. These inconsistencies may partly be explained by methodological heterogeneity, differences in population characteristics, and variations in biochemical measurements. Nevertheless, the observed improvement in sleep disturbance following L-methylfolate supplementation in individuals with MTHFR C677T and/or A1298C polymorphisms provides preliminary support for a possible role of folate metabolism in sleep regulation, warranting further investigation.

Similarly, findings regarding depression and anxiety are mixed, particularly in non-RCTs. However, RCT findings allow several patterns to be observed. The available evidence suggests that when different antidepressants are analyzed as a single group, L-methylfolate may be an effective adjunct in the treatment of depression, whereas folic acid, the inactive form of folate, appears to be ineffective. Furthermore, in adults with MDD and low normal vitamin B12 levels, weekly intramuscular injections of vitamin B12, administered adjunctively with antidepressants, may significantly enhance treatment response. In contrast, the role of active B-vitamin supplementation in anxiety has not yet been adequately explored.

Evidence on MTHFR polymorphisms, particularly C677T, also remains inconclusive, although the TT genotype may be associated with an increased vulnerability to depression. Consequently, patients with MDD who carry MTHFR polymorphisms may benefit from supplementation with reduced (active) B-vitamin forms, which may partially bypass impaired folate metabolism, though evidence is still evolving and not universal.

Overall, discussed vitamin B status, together with MTHFR polymorphisms, may be associated with sleep, anxiety, and depressive symptoms. Nevertheless, the current body of evidence is not yet definitive, and further large-scale longitudinal studies and well-designed RCTs are needed to clarify these relationships. Furthermore, future studies should determine whether treatment efficacy in sleep problems, depression, and anxiety depends on the vitamin B formulation used, clarify which patient groups may benefit most from supplementation, and investigate whether certain classes of medication may derive greater benefit from supplementation.

Conflicts of Interest: No conflicts of interest to declare.

Declaration of the use of generative AI and AI-assisted technologies in the writing process: In preparing this work, the authors used ChatGPT for the purpose of enhancing language clarity, improving readability, text formatting, and basic data analysis. After using this tool/service, the authors have reviewed and edited the content as needed and accepted full responsibility for the substantive content of the publication.

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