Review

Effects of B Vitamins on Homocysteine Lowering and Thrombotic Risk Reduction - A Review of Randomized Controlled Trials in Last 10 Years

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Review

Effects of B Vitamins on Homocysteine Lowering and Thrombotic Risk Reduction - A Review of Randomized Controlled Trials in Last 10 Years

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Abstract: Homocysteine is an amino acid derived from methionine, metabolized via vitamin B6 (pyridoxine)- and vitamin B12 (cobalamin)-dependent pathways. Supplementation of B vitamins has been shown to effectively reduce plasma homocysteine levels. Previous research has also demonstrated an association between lower plasma homocysteine levels and decreased risk of myocardial infarction, stroke and venous thromboembolism. However, it remains inconclusive if supplementation of B vitamins is associated with risk reduction in thromboembolic events and confers clinical benefits. This article aims to review clinical trials in the last ten years to determine the effects of B vitamin supplementation in plasma homocysteine lowering, and evaluate its impact on reducing the risk of arterial and venous thromboembolism.

Keywords: vitamin B; homocysteine; thrombotic risk; randomized controlled trials; thrombosis; pyridoxine; folic acid; folate; cobalamin

1. Introduction

B vitamins include 8 water-soluble vitamins that consist of thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folate (B9), and cobalamin (B12) [1]. B vitamins are excreted through urine and need to be replaced daily through dairy products, leafy green vegetables or animal proteins [1]. They are cofactors for cellular pathways supporting physiological function [2]. Specifically, vitamin B6, B9, and B12 are involved in the homocysteine metabolic pathway and have previously shown to be associated with thrombotic risk reduction (Figure 1) [1,2].

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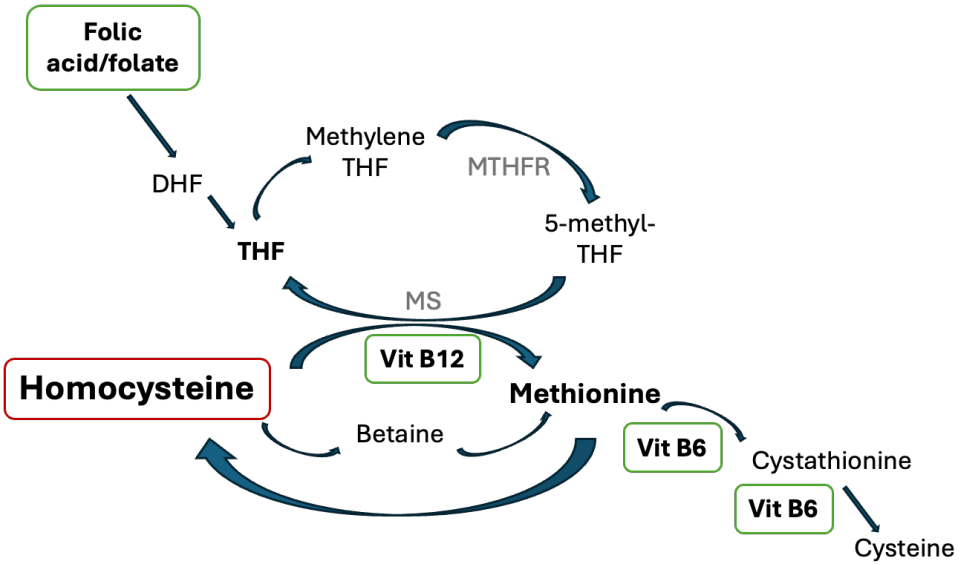

Figure 1. Overview of the homocysteine metabolism and the role of folic acid, vitamin B12, and vitamin B6. Homocysteine can be metabolized through the following three ways: homocysteine can be remethylated to methionine by methionine synthase (MS) with vitamin B12 as the cofactor of MS; homocysteine can be converted to cysteine with the presence of vitamin B6; or homocysteine is remethylated by betaine, which is derived from choline. The process of remethylation of homocysteine to methionine requires the methyl group derived from 5-methyl-tetrahydrofolate (THF) in the folate cycle. Folic acid/folate is activated to 5-methyl THF with the activity of methylene tetrahydrofolate reductase (MTHFR) [3].

Homocysteine(Hcy) is derived from the essential amino acid methionine and is known to play a role in cellular homeostasis. Elevation of homocysteine in the plasma is linked to cardiovascular disease and venous thrombotic events [4–7]. Several studies have identified that hyperhomocysteinemia (>15 μmol/L) may negatively affect the cardiovascular system and lead to stroke, coronary artery disease, and deep vein thrombosis [8]. The positive correlation between homocysteine level and thromboembolic risk has been shown frequently when homocysteine level is above 30 μmol/L (severe/moderate hyperhomocysteinemia) [9,10]. However, the modestly elevated homocysteine showed mixed results on the effect of the cardiovascular system, and thus it may not be an independent risk factor for thromboembolism when homocysteine level is between 15 to 30 μmol/L [9]. High concentration of homocysteine in the plasma may induce oxidative damage to endothelial cells which consequently causes dysfunction of the anticoagulation system and leads to thrombotic events [11].

Vitamin B deficiency could lead to hyperhomocysteinemia, which is an independent risk factor for thrombosis events. However, results of randomized controlled trials (RCTs) of B vitamin supplementations have been inconsistent in improving clinical outcomes of arterial or venous thrombotic events. Our study aims to review currently available RCTs in the past 10 years and discuss the effects of B vitamins, mainly pyridoxine (B6), folic acid (B9), and cobalamin(B12) on clinical outcomes in reducing various arterial and venous thromboembolism events.

2. Materials and Methods

2.1. Search Strategy and Study Selection

A literature research was conducted using three databases (PubMed, Embase, and Cochrane) to identify all RCTs published between January 2014 to December 2024 using predefined search terms: (vitamin B OR folate OR folic acid OR B vitamins) AND (homocysteine OR homocysteinemia OR

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hyperhomocysteinemia) AND (thrombosis OR thrombotic OR cardiovascular event OR stroke OR cardiovascular accident OR thromboembolism).

2.2. Eligibility Criteria

Trials were eligible if they corresponded to the following characteristics:

1. Population: adult patients (greater or equal to 18 years of age).

2. Intervention: oral, enteral, or parenteral folic acid (Vitamin B9) and/or cobalamin (or Vitamin B12) and/or pyridoxine (Vitamin B6) with or without standard therapy.

3. Outcomes: Incidence of any thrombotic events including but not limited to myocardial infarction (MI), stroke or transient ischemic attack (TIA), cardiovascular accident (CVA), deep vein thrombosis (DVT), pulmonary embolism (PE). Trials reporting only biochemical outcomes or surrogate markers were excluded.

2.3. Eligibility Review and Data Abstraction

The primary citation screening was done by using the keywords listed above in the three databases. All of the authors were independently assigned to review all citations after the primary screening. Full texts of potential studies were reviewed and study details including vitamin B regimen, study methods, results on homocysteine level, and clinical outcomes were extracted.

2.4. Qualitative Analysis

For each RCT included, two reviewers independently evaluated the methodological quality, risk of bias, and synthesis of the results. Disagreements between reviewers were resolved through discussion or third-party adjudication. No quantitative analysis was performed due to the heterogenesis of the studies.

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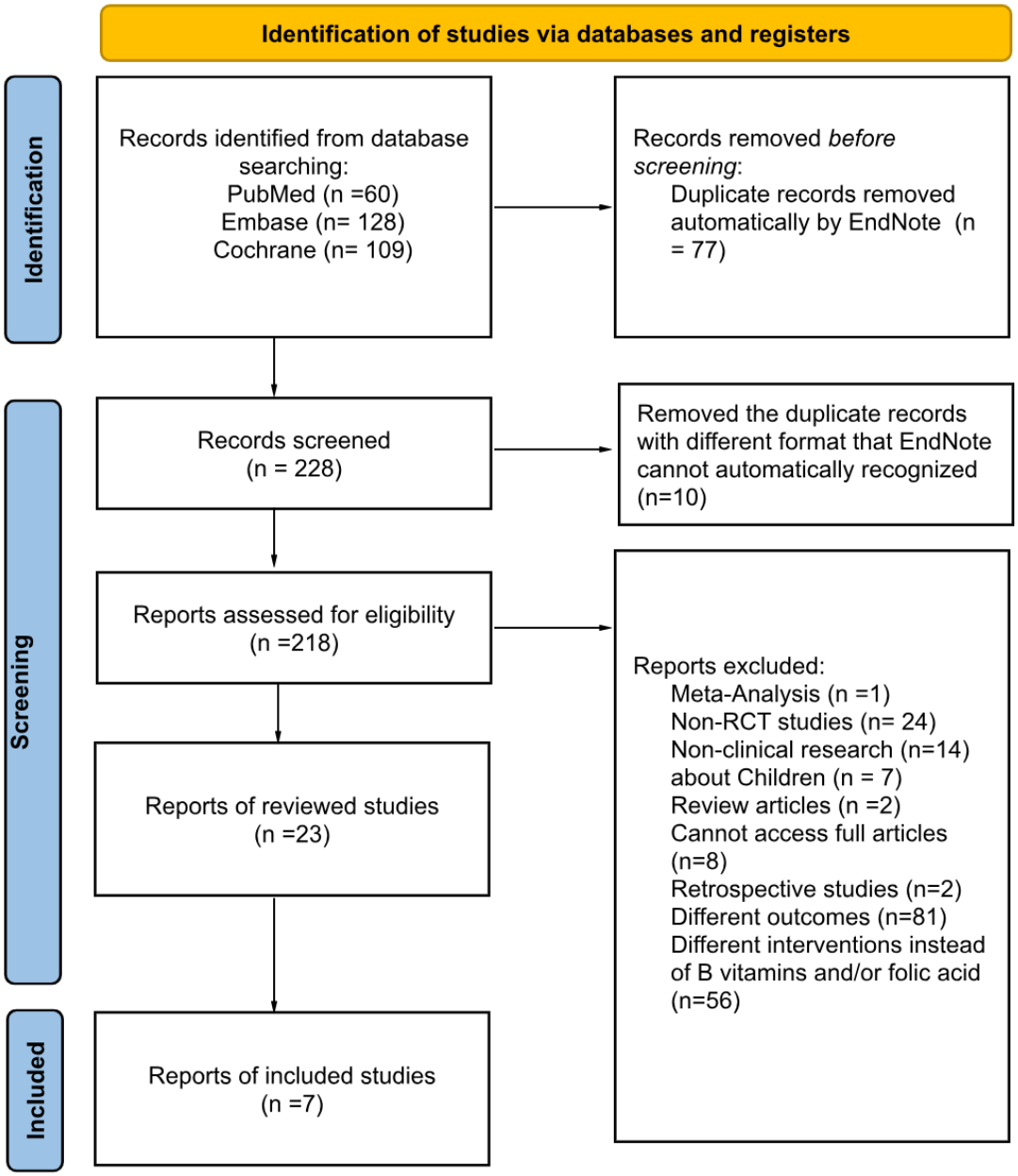

Figure 2. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection based on inclusion and exclusion criteria [12].

3. Results

3.1. Summary of Trials on Arterial Thrombosis Events

Among the 24 studies identified, 7 randomized controlled trials (RCT) met the inclusion criteria. Six of these 7 studies were based on the China Stroke Primary Prevention Trial (CSPPT) with different analysis of the effects of enalapril with or without folic acid supplementation in hypertension adults without a history of arterial thrombotic events [13]. In the CSPPT, folic acid supplementation has been shown to significantly reduce first stroke, first ischemic stroke, and composite cardiovascular events. Particularly, the risk of first stroke was significantly reduced by 73% with a subgroup with low platelets (<210 × 109/l) and high total homocysteine (tHcy) (≥15 μmol/) [14]. The effect of folic acid intervention also significantly reduced the stroke risk in patients with CC/CT MTHFR genotype [15] and in male patients with elevated serum calcium levels with increased risk of first stroke [16].

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In addition, Kotwal et al from India reported that B vitamins reduce total thrombosis, stroke and MI in soldiers in high-altitude areas [17].

However, in the post-ischemic stroke population on antiplatelet therapy, post hoc analysis of VISP trial showed higher stroke risk for patients supplemented with high-dose B vitamins therapy (vitamin B6 25 mg, vitamin B12 0.4 mg, and folic acid 2.5 mg) compared with those on low-dose therapy (vitamin B6 200 μg, vitamin B12 6 μg, and folic acid 20 μg). The increased risk was not found among those not on antiplatelets [18].

3.2. Summary of Trials on Venous Thrombotic Events

Shu et al studied the effect of folic acid on venous thromboembolic events in patients with cerebral infarction with DVT and baseline homocysteine level around 30 μmol/L for the intervention of folic acid 5mg and vitamin B12 0.25mg daily. Patients' serum folic acid and vitamin B12 levels increased with vitamin B supplementation. Homocysteine level decreased with vitamin B supplements and was negatively correlated with folic acid and vitamin B12 levels. The recurrence rate of lower limb deep venous thrombosis of the treatment group was 4.4%, which was significantly lower than that of the non-treatment group at 28.9% ($p<0.05$) [19]. Kotwal et.al also showed a numerically lower number of incidences of DVT and PE in soldiers staying in high altitude supplemented with folic acid, vitamin B6 and vitamin B12 [17].

Various vitamin B supplementation strategies have been used. It ranges from folic acid with antihypertensive medication to a combination of folic acid, vitamin B12, and vitamin B6.

See Table 1 for study detailed summaries including study design, B vitamins regimen, and clinical outcomes.

Table 1. Summary of RCTs of Vitamin B Supplements to the Risk of Thrombotic Events During 2014 to 2024.

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Study	Year	Sample Size	Country	Population	Intervention and Comparison	Duration	Relevant Outcomes
Wu et al CSPPT trial	2021	20424	China	Hypertension adults without a history of stroke or MI	Enalapril 10mg and folic acid 0.8mg (single pill) vs Enalapril 10mg alone	4.5 years	Risk of first stroke: Males with baseline albumin-corrected serum calcium ≥2.43 mmol/L: 3.0% in the enoxaparin-folic acid group vs 6.5% in enalapril group (adjusted HR, 0.49; 95% CI: 0.35, 0.68). Risk reduction is 51%. Males with baseline albumin-corrected serum calcium<2.43 mmol/L: 2.6% in the enoxaparin-folic acid group vs 2.3% in enalapril group (adjusted HR, 0.49; 95% CI: 0.35, 0.68). Folic acid has no significant effects. Females with baseline albumin-corrected serum calcium ≥2.43 mmol/L: 2.6% in the enoxaparin-folic acid group vs 2.6% in enalapril group (adjusted HR, 0.99; 95% CI: 0.73,1.34). Folic acid has no significant effects. Female with baseline albumin-corrected serum calcium<2.43 mmol/L. 2.5% in the enoxaparin-folic acid group vs 3.1% in enalapril group (adjusted HR, 0.8; 95% CI: 0.58, 1.11). Folic acid has no significant effects.
Kong at al Post-hoc analysis of CSPPT	2018	10789	China	Hypertension adults without a history of stroke or MI	Enalapril 10mg and folic acid 0.8mg (single pill) vs Enalapril 10mg alone	4.5 years (median)	Risk of first stroke: Low platelet count group (<210 × 10^9/l): 1.9% in enoxaparin-folic acid group vs 4.6% in enalapril group (P <0.001) Medium to high platelet count group (≥210 × 10^9/l): 3.3% in enoxaparin-folic acid group vs 3.7% in enalapril group (P= 0.410) Risk of ischemic stroke risk: Low platelet count group (<210 × 10^9/l): 1.6% in enoxaparin-folic acid group vs 4.1% in enalapril group (P <0.001) Medium to high platelet count group (≥210 × 10^9/l): 2.9% in enoxaparin-folic acid group vs 3.1% in enalapril group (P= 0.591) Risk of hemorrhagic stroke: Low platelet count group (<210 × 10^9/l): 0.2% in enoxaparin-folic acid group vs 0.5% in enalapril group (P= 0.243) Medium to high platelet count group (≥210 × 10^9/l): 0.49% in enoxaparin-folic acid group vs 0.5% in enalapril group (P= 0.302) Subgroup analysis of joint effect of platelet and homocysteine level: Risk of first stroke risk: Low platelet count group (<210 × 10^9/l) and low tHcy (<15umol/L): 1.9% in enoxaparin-folic acid group vs 4.2% in enalapril group Low platelet count group (<210 × 10^9/l) and high tHcy (<15umol/L): 1.8% in enoxaparin-folic acid group vs 5.6% in enalapril group Medium to high platelet count group (<210 × 10^9/l) and low tHcy (>15μmol/L): 3.0% in enoxaparin-folic acid group vs 3.3% in enalapril group Medium to high platelet count group (<210 × 10^9/l) and high tHcy (>15μmol/L): 4.1% in enoxaparin-folic acid group vs 4.7% in enalapril group
Zhao at al Post-hoc analysis of CSPPT	2017	20424	China	Hypertension adults without a history of stroke or MI	Enalapril 10mg and folic acid 0.8mg (single pill) vs Enalapril 10mg alone	4.5 years (median)	There was a s significant MTHFR gene–homocysteine interaction on first stroke Risk of first stroke: CC/CT MTHFR genotype: Folic acid supplementation reduced stroke risk by 15% (HR, 0.85; 0.70–1.02) TT MTHFR genotype: Folic acid supplementation reduced stroke risk by 30% (HR, 0.70; 0.51–0.95) Among patients with CC/CT MTHFR genotype, folic acid supplementation significantly reduced stroke risk in the patients high tHcy level (tHcy>13.5μmol/L) (HR, 0.73; 95% CI, 0.55–0.97) Among patients with TT MTHFR genotype, folic acid supplementation significantly reduced stroke risk in the patients with lowest tHcy level (tHcy<12.8μmol/L) (HR, 0.44; 95% CI, 0.24–0.79)
Shu et al	2017	90	China	Patients with homocysteine cerebral infarction	Folic acid 5mg and vitamin B12 0.25mg daily vs no treatment	3 months	Vitamin B supplements significantly reduced lower limb DVT recurrence rate. Rate of recurrent DVT: 4.4% in treatment group vs 28.9% in non-treatment group (p<0.05) More stable INR, but lower PT and APTT in treatment group

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Huo et al The CSPPT trial	2015	20424	China	Hypertension adults without a history of stroke or MI	Enalapril 10mg and folic acid 0.8mg (single pill) vs Enalapril 10mg alone	4.5 years (median)	Risk of first stroke: 2.7% in enalapril-folic acid group vs 3.4% in enalapril group (HR, 0.79 [95% CI, 0.68-0.93]; *P* =0 .003) Risk of first ischemic stroke: 2.2% in the enalapril–folic acid group vs 2.8% in the enalapril group; HR, 0.76; 95% CI, 0.64-0.91; *P* = 0.002) Risk of composite of first stroke and all-cause mortality: 5.4% in the enalapril–folic acid group vs 6.2% in the enalapril group (HR, 0.86; 95% CI, 0.77-0.97; *P* = 0.01)) Risk of composite cardiovascular events: 3.1% in the enalapril–folic acid group vs 3.9% in the enalapril group; HR, 0.80; 95% CI, 0.69-0.92; *P* = 0.002) Risk of hemorrhagic stroke: 0.56% in the enalapril–folic acid group vs 0.60% in the enalapril group; HR, 0.93; 95% CI, 0.65-1.34; *P* = 0.71) Risk of MI: 0.24% in the enalapril–folic acid group vs 0.23% in the enalapril group; HR, 1.04; 95% CI, 0.60-1.82; *P* =0 .89) Risk of all-cause deaths (2.9% in the enalapril–folic acid group vs 3.1% in the enalapril group; HR, 0.94; 95% CI, 0.81-1.10; *P* = 0.47)
Kotwal et al	2015	6000	India	Armed Forces personnel in the high altitude area	Vitamin B12 1000µg, B6 mg and Folic 5mg per day vs no treatment	2 years	B vitamins were effective in reducing Hcy, PAI 1, fibrinogen levels and increasing NO levels at 1 year and reducing the incidence of thrombosis at 2 years At 2 years, Incidence of total thrombotic events: 8.33% (5 events) in treatment group vs 28.33% (17 events) events in non-treatment group (relative risk = 0.29 (95% CI , 0.11-0.80) Incidence of DVT/abdominal vein thrombosis: 2 events in treatment group vs 6 events in non-treatment group Incidence of PE with or without DVT:0 event in treatment group vs 3 events in non-treatment group Incidence of stroke /cortico venous thrombosis:0 event in treatment group vs 3 events in non-treatment group Incidence of CAD/MI in age <45 years:1 event in treatment group vs 5 events in non-treatment group Incidence of pulmonary arterial hypertension: 2 events in treatment group vs 0 events in non-treatment group
Arshi et al Post hoc analysis of VISP	2015	3680	United States, Canada, Scotland	Non-Disabling post-ischemic stroke	High dose group: vitamin B6 25 mg , vitamin B12 0.4 mg, and folic acid 2.5 mg ; low dose group: vitamin B6 200 µg, vitamin B12 6 µg, and folic acid 20 µg	2 years	Risk of stroke: Patients with concurrent antiplatelets use: high-dose B vitamins therapy was associated with higher stroke risk (HR, 1.43; 95%CI, 1.02–2.01) Patients without antiplatelet use: no significant difference between high-dose and low-dose groups (HR, 0.86; 95%CI, 0.62–1.19). Risk of stroke. MI and vascular death: Patients with concurrent antiplatelets use: No significant difference between high-dose and low-dose groups (HR 0.18; 95% CI 0.90-1.54) Patients without antiplatelet use: no significant difference between high-dose and low-dose groups (HR 0.9; 95% CI 0.7-1.17)

Notes: Hcy, tHcy: homocystein, total homocysteine; MI: myocardial infarction; eGFR: estimated glomerular filtration rate; FA: Folic acid; MTHFR: methylenetetrahydrofolate reductase; CC/CT genotype: wild type (C), mutated type (T); TT-homozygous genotype, CT-heterozygous genotype, CC-noncarrier; INR, PT, APTT: international normalized ratio, prothrombin time, activated partial thromboplastin time; HR: hazard ratio; CI: confidence interval; PAI 1: plasminogen activator inhibitor-1; NO: nitric oxide; CAD: coronary artery disease; DVT: deep vein thrombosis; PE: pulmonary embolism; WENBIT: The Western Norway B Vitamin Intervention Trial; CSPPT: China Stroke Primary Prevention Trial; VISP: Vitamin Intervention for Stroke Prevention Trial.

Table 2. Summary of the Thrombotic Outcomes of Table 1*.

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Intervention	Arterial Thrombotic Events			Venous Thrombotic Events	Composite Thrombosis^	Safety
	 First Stroke	 Recurrent stroke	 Composite CV endpoints	 DVT	 Composite Thrombotic Events	 Hemorrhagic stroke
FA	Kong et al-pts w/low plt; Zhao et al; Wu et al		Huo et al			Huo et al
FA+B12				Shu et al		
FA+B6+B12		Arshi et al-pts w/ antiplatelet, high dose vs low dose†	Arshi et al		Kotwal et al	

Notes: ^Thrombosis outcome is a composite outcome of arterial and venous thrombotic event, including deep vein thrombosis, pulmonary embolism, stroke, coronary artery disease/myocardial infarction in young (< 45 years), and pulmonary artery hypertension.†High dose: 25 mg B6, 0.4 mg B12, and 2.5 mg folic acid; low dose: 200 μg B6, 6 μg B12, and 20 μg folic acid. Results showed significant decreased risk; Results showed no significant changes; Results showed significant increased risk; Results not reported. FA: folic acid; B6: vitamin B6; B12, vitamin B12; DVT: deep venous thrombosis; CV: cardiovascular; Composite CV endpoints: myocardial infarction, cardiovascular death, and stroke; Pts: patients; w/: with; plt: platelet.

Table 3. Change of Serum Homocysteine, Folic Acid and Vitamin B12 Levels

		Homocysteine	Folic acid	Vitamin B12
Shu et al	Baseline	30.13 ± 1.84 μmol/L	7.25 ± 2.35 μg/L	323.52 ± 93.76 μg/L
	After treatment	10.45 ± 2.62 μmol/L	15.13 ± 5.23 μg/L	645.92 ± 102.48 μg/L
Huo et al	Baseline	12.5(10.5-15.5) μmol/L	8.1(5.6-10.4) ng/mL	379.6(314.3-475.2)pg/mL
	After treatment	N/A	19.9(14.7-23.3) ng/mL	N/A
Kotwal et al	Baseline	8.19 ± 2.6 μmol/L	10.32 ± 2.43 μg/L	279.6 ± 20.72 μg/L
	After treatment	10.99 ± 2.15 μmol/L	32 ± 2.8 μg/L	520 ± 38.8 μg/L
Arshi et al	Baseline	13.4 μmol/L	27 mmol/L	375 pmol/L
	After treatment	11.1 μmol/L*	80 mmol/L*	695 pmol/L*

* Decrease in high-dose group at 2 years, visually estimate from graph.

4. Discussion

4.1. Effects of Vitamin B Supplements on Arterial Events

The possible role of folic acid in reducing the risk of stroke through lowering homocysteine levels has been investigated. One of the studies from CSPPT [13] has demonstrated that compared to the sole use of antihypertensive medication, the combination of blood pressure drug and folic acid could reduce the risk of stroke by 21%. In a following study [14], the risk factor of primary stroke prevention through folic acid has been identified. As a comparison, the previous clinical studies VISP

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trial [20] and VITATOPS [21] showed no statistically significant results in reducing the primary outcome of recurrent stroke with high-dose folic acid, vitamin B6 and B12 (2.5mg, 25 mg, and 0.4 mg) group compared to low-dose (20 μg, 200 μg, and 6 μg) group. They also showed no differences in reducing the composite outcomes of recurrent stroke or TIA when comparing folic acid with placebo, respectively [20,21].

Sub analysis of previous studies pointed to a reverse association between B vitamins supplementation and antiplatelet use and in reducing the risk of stroke. One of the post-hoc analyses of the VISP trial showed that high-dose folic acid with vitamin B6 and B12, compared to low-dose, may increase the risk of recurrent stroke in patients with the concurrent use of antiplatelets (hazard ratio (HR), 1.43; 95% confidence interval (CI), 1.02–2.01) [18]. However, among patients who were not on antiplatelets, a trend of decreased risk was identified with the high dose of folic acid. Similar trends were reported in one of the post-hoc analyses of VITATOPS trial that unlike patients who were not the recipients of any antiplatelets, patients with the baseline use of antiplatelets did not benefit from the treatment of B vitamins regarding the primary or secondary outcomes of stroke, MI, or cardiovascular death [22]. The aforesaid evidence indicates the baseline use of antiplatelets may be used as a predictor of the efficacy of B vitamins and antiplatelet-naive patients may be a targeted population of B vitamins treatment in the prevention of stroke and other cardiovascular thrombotic events. As the current results are from post-hoc analysis, more prospective studies are warranted to investigate the correlation between antiplatelets, B vitamins, and the risk of stroke.

4.2. Effects of Vitamin B Supplements on Venous Thrombotic Events

Studies on risk reduction of VTE through homocysteine lowering by daily supplementation of B vitamins were conflicting. While Shu et al. [19] found positive effects of B vitamins in reduced lower limb DVT recurrence rate, VITRO study found that homocysteine lowering by B vitamins did not prevent any recurrent VTE [23]. In the VITRO study, adult patients with a first confirmed deep vein thrombosis (DVT) or pulmonary embolism (PE) with a homocysteine level above the 75th percentile of the normal value were randomized to daily supplementation of 5mg folic acid, 50mg pyridoxine, and 0.4mg cyanocobalamin) or placebo. Patients were followed for 2.5 years. The number of recurrent VTE was 12.2% in the B vitamin group vs. 14.4% in the placebo group [23].

Since 2014, there's only one study that specifically investigated combination B supplements reducing the risk of deep vein thrombosis (DVT) through serum homocysteine lowering. However, previous meta-analysis also has shown that reduced levels of folic acid and vitamin B12 could be an independent risk factor for venous thrombosis regardless of homocysteine level [24]. Cattaneo et al also found the correlation between low vitamin B6 levels and the risk of DVT is independent of fasting tHcy levels [25]. Another study found there was a significantly reduced vitamin B6 level among patients with unprovoked VTE compared to healthy volunteers ($p<0.009$) [26]. There were no significant differences in terms of tHcy level, folic acid level, and vitamin B12 level. These findings suggest that B vitamins may play a protective role in the prevention of venous thrombosis independent of the homocysteine pathway. Further randomized controlled studies are needed to support these benefits.

4.3. Effects of Vitamin B Supplements on Other Vascular Outcomes

Other than DVT/PE, ischemic stroke, and MI, B vitamins also have effects on vascular endothelial function. For example, Chambers et al demonstrated oral folic acid and vitamin B12 supplementations improved vascular endothelial function in patients with coronary heart disease. The mechanism is thought to be via reducing homocysteine levels in the body [27]. Similarly, Menzel et al also demonstrated that B vitamins could reduce the deterioration of endothelial function in addition to reducing blood pressure and tHcy levels [28]. Zamani et al published a systematic review in 2023 on folic acid's effect on endothelial function [29]. The meta-analysis suggested that folic acid supplementation may improve endothelial function by increasing flow-mediated dilation (FMD) and FMD% levels.

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Low vitamin B6 and folic acid levels along with elevated homocysteine levels are independent risk factors for retinal vein occlusion (RVO) [30]. Meng et al published a study in 2018 and demonstrated that the prevalence of retinal atherosclerosis (RA) was 77.6% in patients with hypertension and diabetes, and folic acid supplementation was associated with reduced RA in female patients with hyperhomocysteinemia [31]. Hodis et al showed high dose vitamin B supplementation reduced the progression of early-stage subclinical atherosclerosis (carotid artery intima-media thickness) in well-nourished individuals at low risk of cardiovascular disease with a fasting homocysteine level of >9.1 μmol/L [32]. Vitamin B supplementation could improve arterial function in vegetarians with subnormal vitamin B12 levels [33].

4.4. Effects of Vitamin B Supplements and tHcy Lowering

In the CSPPT trial, folic acid supplementation was also shown to decrease tHcy level and the degree of reduction was affected by sex, MTHFR C677T genotypes, baseline folate, tHcy, estimated glomerular filtration rate levels, and smoking status [34]. Homocysteine-lowering response by genotype was eliminated when plasma folate levels reached ≈15 ng/mL or higher [35].

4.5. Potential Cofounders on Clinical Trial Outcomes

4.5.1. Dietary Fortification and Nutritional Deficiencies

Mandatory fortification of grains in the US may be one of the reasons that causes the mixed results regarding reducing stroke risk between trials from North America and the rest of the world. Since mandatory fortification, the mean population tHcy level was lowered from 10.1 to 9.4 μmol/L($p<0.001$) [36]. Most of Europe and China do not mandate fortification [wald et al]. The meta-analysis of folic acid and stroke in non-mandatory fortification areas [37] showed a modest reduction of future strokes with the use of folic acid (RR, 0.85; 95% CI, 0.77 to 0.95) [38], which suggests that the benefits in prevention of thromboembolism may only apply in population with very high baseline homocysteine due to lack of mandatory fortification. In places with high prevalence of vegetarianism, deficiency of Vitamin B12 is also more common.

4.5.2. Concurrent Medication Treatment

Several drugs could decrease the absorption of vitamin B6,9 and 12. For example, antiepileptics and sulfasalazine can reduce the absorption of folic acid. Isoniazid, cycloserine, penicillamine, hydralazine, levodopa, and some anticonvulsants could affect vitamin B6 absorption. Proton pump inhibitors, H2 receptor antagonists, colchicine, and metformin could lead to vitamin B12 malabsorption. Medications can also cause hyperhomocysteinemia, for example, metformin, methotrexate, niacin, and cholestyramine [39].

4.5.3. Genetic Mutations

MTHFR gene mutation is associated with reduced enzymatic efficiency and increased homocysteine levels. One of the common genetic variants MTHFR 677C→T has been identified as one of the causes of hyperhomocysteinemia [40]. As the genotype C677T (heterozygous) was associated with mildly increased homocysteine level, homozygous T677T polymorphism elevated homocysteine level by 25% compared to the CC genotype (non-carriers) [41]. Homozygous TT genotype has been more frequently found in the Chinese populations than in other populations. This genotype is associated with a 13% increase in the risk of any type of stroke (adjusted odd ratio 1.13, 95% CI 1.09–1.17) when compared to noncarriers [42]. This genetic distribution perhaps leads to a higher risk of hyperhomocysteinemia and a more significant efficacy of folic acid supplementation among Chinese in the prevention of stroke. One of the CSPPT trial post-hoc analysis focused on the MTHFR mutation subgroup and identified folic acid benefited most in patients with TT genotype

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and low platelet count with a risk reduction of 66% (HR 95%CI, 0.15-0.81, Number Needed to Treat = 27) [43].

4.5.4. Safety of Vitamin B Supplement

Though B vitamins are water-soluble vitamins with a wide therapeutic index, over-supplementing B vitamins could also lead to negative clinical outcomes. For example, over supplementation of vitamin B6 could lead to peripheral neuropathy and the recommended upper limit is 100mg/day. Interestingly, in 2020, Flores-Guerrero et al performed a prospective population-based cohort study that demonstrated higher levels of plasma concentrations of vitamin B12 was associated with increased risk of all-cause mortality after adjusting for age, sex, renal function, and other clinical and laboratory variables. Caution should be taken when considering vitamin B12 supplementation in the absence of vitamin B12 deficiency [44]. The DIVINe trial (Diabetic Intervention with Vitamins to Improve Nephropathy) also concluded that the cyanocobalamin may be harmful for participants with impaired renal function [45]. Hence, the potential harmful effect with over-supplementation in the general population and supplementation in the renal impairment group can mitigate the potential beneficial effects.

4.6. Limitations and Further Research

Our review is limited by narrative nature that no quantitative analysis was performed due to heterogeneity of trial designs. Our literature search included RCTs in the last 10 years (2014 to 2024). We did not include RCTs prior to 2014 as the management of patients with MI, stroke, PE and DVT have been vastly improved considering recent medical advancements. Hence, comparing outcome measures are no longer comparable and relevant when looking at data from older days. The fact that there are only 7 published RCTs in the time span of 10 years with clinical outcomes further solidified that new trials are much needed in regard to the effect of vitamin B supplements in reducing thrombotic events.

Well-designed clinical trials are needed with the considerations of patients' baseline social and demographic information, such as baseline vitamin B and homocysteine level, smoking and alcohol use, and current medication use. Underlying conditions such as hypercholesterolemia and obesity, genetic mutations also need to be considered for a more homogenous cohort that may yield definitive results.

5. Conclusions

This review investigated the effect of B vitamin supplementation on thrombotic risks by analyzing clinical trials from the last ten years. Limited studies were found with conflicting results in thrombotic risk reduction with supplement of B vitamins. Thus, more clinical trials are needed to determine a clearer correlation between B vitamin supplementation and the risk of thrombosis.

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